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МОНОГРАФИИ

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Нанотехнологии за биомедицинска диагностика.

Част I: Квантови точки.

(монография)

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Въведение

Наномедицината и нанофармацевтиката са едни от най-модерните и най-бързоразвиващите се клонове на съвременната биомедицинска наука. Нанотехнологиите навлизат в биологията, фармацията и медицината в самото начало на настоящото столетие. Първите работи в тази област се появяват едва преди около 10-тина години. Терминът „фармацевтична нанотехнология“ се среща за първи път в PubMed през 1999 в *Annals of Pharmacotherapy*. От 1999 досега броят на публикациите в тази област расте експоненциално – от едва 8 за периода 1999-2001, на 350 за периода 2002-2004, и над 700 за периода 2005-2008. През 2004, *International Journal of Pharmaceutics* открива нова секция в своето съдържание: „*Pharmaceutical nanotechnology*“. Оттогава „фармацевтичната нанотехнология“ става една от основните области, залегнали в тематиката на фармацевтичните журналы.

Понастоящем нанофармацевтичните технологии се развиват основно в две направления: (1) разработване на нанофармацевтици за терапевтични цели (лекарствени препарати и преносители на лекарства); и (2) НАНОПРОБИ за диагностични цели – инертни или биологично-активни наноструктури с контрастни свойства. Последните се разработват за нуждите на образната диагностика – магнитно-резонансна томография (MRI), позитронно-емисионна томография (PET), оптично визуализиране, рентгенография и компютърна томография (СТ), с цел да се повиши чувствителността и/или специфичността на съответния метод.

Големите очаквания към нанопробите са свързани с факта че техните контрастни свойства са в пъти, а понякога и на порядък по-големи от контрастните свойства на конвенционалните контрастни вещества. В по-далечно бъдеще се очаква обединяване на тези две направления, с оглед на развитието и целите на една нова област в медицината, наречена „наномедицина“. Очаква се това да стане на базата на разработване на многофункционални (мултифункционални) наночастици – носители едновременно на терапевтични, диагностични (или сензорни) и прицелни свойства. Поради тази причина, настоящото разделяне на нанофармацевтиците на такива за терапевтични и за диагностични цели е до голяма степен условно, още повече, че принципните изисквания към тяхната структура и свойства се

припокриват значително, независимо от сферата им на приложение. Освен това, както ще бъде показано в настоящата монография, наночастици, изградени основно от един материал могат както да проявяват високи контрастни свойства, така и да служат за терапевтични цели (като например, за фотосенсибилизация на туморни клетки).

Понастоящем, обаче, нанопробите с контрастни свойства и възможности за клинично приложение са все още в началото на своето развитие. Тяхното разработване започва на основата на наноколоидните системи, известни от много десетилетия. Трябваше, обаче, да се извърви дълъг път на тяхното усъвършенстване, за да се очертае тенденция за превръщането на наночастиците от колоидните разтвори в нанопроби с ясно дефинирани и програмируеми размери и свойства, имащи поведение във водни разтвори по-скоро на големи молекули, каквито са биомакромолекулите, а не просто късчета твърда материя с ниска коливна стабилност. За тази цел първо трябваше да се намерят условията за получаване на хомогенни наноструктури: (i) с малки размери – от порядъка на 5-10 до максимум 50-60 nm (каквито са размерите на биомакромолекулите); (ii) еднакви по форма и размери частици – с много висока хомогенност на размера; (iii) с висока разтворимост във водни разтвори и решаването им с помощта на флуоресцентни наночастици (квантови точки).

ГЛАВИ ОТ КНИГИ

In: “Free Radicals: Chemistry, Pathology and Medicine” (eds. C. Rice-Evans, T. Dormandy), Richelieu Press, London, 1988, p. 417-438.

Protein kinase C participates in the regulation of lipid peroxidation in biological membranes.

[Kagan V](#), [Bakalova R](#), [Serbinova E](#), [Koynova G](#), [Baldenkov G](#), [Tkachuk V](#), [Stoytchev Ts](#).

(Статията включва оригинални резултати.)

Introduction

It is now accepted that low steady-state concentrations of lipid peroxidation (LPO) products play an essential role in cell metabolism and are necessary for the physiological modifications of biomembranes. Excessive generation of the products, on the other hand, underlies membrane damage in many pathological conditions. In other words, destruction of membrane structures in many pathological states is probably due not to the appearance of new pathobiochemical pathways, but to the uncontrolled increase or imbalance of normal reactions.

The main components of the antioxidant system which controls the intensity of LPO reactions at a normal steady-state level are well known: they include water-soluble and lipophilic free radical scavengers, transition metal chelators and specific antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase). Recently, the participation of calcium ions in this regulation has been demonstrated, pointing to the possible role of secondary messengers.

It is well established that modifications of the physico-chemical properties of biomembranes due to the action of activated oxygen species and LPO products can lead to severe disturbances of calcium homeostasis and to changes in specific receptor-enzymic complexes (adenylate cyclase, guanylate cyclase, phosphatidylinositol specific phospholipase C). However, it is still unclear what pathways produce secondary messengers in LPO reactions in the cell and how

important these are in coordination the activity of different components of the antioxidant system.

The present experiments were designed with these questions in mind. The findings illustrate the influence of the effectors of the two main regulatory systems – cAMP-dependent and the other phosphatidylinositol-dependent – on LPO reactions in membrane structures of liver and heart. For this purpose we used forskolin (FK), an activator of the adenylate cyclase system, as well as phorbol-12-myristate-13-acetate (PMA), a structural analogue of diacylglycerol and an activator of protein kinase C (PK-C), which participates in phosphatidylinositol-dependent transmembrane signal transduction.

In: "Biological Oxidation Systems" (eds. C. Reddy, G.A. Hamilton, M.K. Madyastha), Academic Press, New York, 1990, p. 889-908.

(Статията включва оригинални резултати.)

Mechanisms of Vitamin E Control of Lipid Peroxidation: Regeneration, Synergism, Asymmetry, Migration and Metal Chelation

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Abstract

The very high efficiency of vitamin E in preventing and inhibiting lipid peroxidation in biomembranes (where its molar concentration within the lipid bilayer is less than 0.1 mol%) is due to its multifunctional antioxidant properties. Data is presented which show that: (i) α -tocopherol is non-uniformly distributed within the membrane lipid bilayer, being preferentially located in the regions enriched with polyenoic lipids (the main substrates of lipid peroxidation). (ii) α -tocopherol local deficiency in membrane monolayers appearing in the course of oxidative stress may be replenished by means of its intermembrane exchange: this process is accelerated by accumulation of lipid peroxidation products and membrane disordering. (iii) Chromoxy radicals can be reduced to form chromanols by NAD(P)H-dependent enzymes in microsomes and mitochondria. In this regeneration, GSH acts synergistically; recycling of chromoxy radicals also occurs non-enzymatically by ascorbate. (iv) Water-soluble antioxidants (e.g. carnosine) and protein-kinase C-dependent inhibitors of lipid peroxidation (activated by phorbol-12-myristate-13-acetate) synergistically increase α -tocopherol antioxidant potency. (v) α -tocopherol within the lipid bilayer chelates iron (necessary for propagation of lipid peroxidation), thus hydroperoxide cleavage sites are near to α -tocopherol-rich membrane domains.

In: "Membrane Lipid Oxidation" (ed. C. Vigo-Pelfrey), CRC Press, Boca-Raton, Florida, 1990, p. 192-208.

Lipid peroxidation in the tumor cells and tissues of tumor-bearing animals.

Kagan V, Bakalova R, Karakashev P.

(Статията включва оригинални резултати.)

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- III. Possible mechanisms of the resistance of tumor cells to oxidative stress
 - A. Decrease generation of activated oxygen species in tumor cells
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 - D. Possible participation of protein kinase C in the inhibition of lipid peroxidation in tumor cells
- IV. Toxic effects of lipid peroxidation products on tumor cells
- V. Tumor-host relations and accumulation of lipid peroxidation products in the organs of tumor-bearing animals

References

In: "Vitamin E in Health and Disease: Biochemistry and Clinical Applications" (eds. L. Packer, J. Fuchs), Marcel Dekker, New York, 1992, p. 171-178.

(Статията включва оригинални резултати.)

Intermembrane Transfer of α -Tocopherol and Its Homologs

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INTRODUCTION

Vitamin E is the term used for eight naturally occurring fat-soluble nutrients called tocopherols. α -Tocopherol has the highest biological activity and predominates in many species (1,2). In humans vitamin E is the most important lipid-soluble antioxidant, and its deficiency may cause neurological dysfunction, myopathies, and diminished erythrocyte life span (3-5). Most of vitamin E is located in the mitochondria and in the endoplasmic reticulum, whereas little is found in cytosol and in peroxisomes (6). In membranes vitamin E is not uniformly distributed but forms clusters in the lipid bilayer and is preferentially concentrated in domains rich in polyenoic phospholipids (7).

α -Tocopherol interacts with lipid alkoxyl and peroxy radicals, acting as a chain-breaking antioxidant (8,9). This antioxidant function of vitamin E results in its consumption. Thus oxidative stress is usually accompanied by a loss of vitamin E (10). This may cause a localized antioxidant deficiency in the membrane: domains rich in enzymic or nonenzymic generators of active oxygen species and lipid radicals may become tocopherol-deficient microenvironments. This "local E hypovitaminosis" in the membrane may be overcome by (1) redistribution of endogenous tocopherols from regions with a high content of vitamin E to those poor in tocopherols, or (2) supplementation with exogenous vitamin E, in particular liposome-incorporated vitamin E. In both cases, the rate of inter- and intra-membrane exchange of vitamin E molecules may be crucial for the replenishment of vitamin E loss.

The efficiency of trans-bilayer tocopherol migration ("flip-flop") is very low (7,11,12). The replenishment of E-deficient membrane domains may be brought about by either lateral diffusion or intermembrane transfer and exchange of tocopherols. Since the rate of the lateral diffusion of tocopherols is sufficiently high (13), this process may not be limiting. However, opinions differ concerning the intermembrane transfer and exchange of tocopherols. It has been suggested that intermembrane exchange is only efficient in the presence of tocopherol binding proteins (14-16).

Here, the results of the studies on the intermembrane exchange of tocopherols between tocopherol-loaded (donor) and tocopherol-free (acceptor) liposomes, as well as between donor liposomes and microsomal membranes as acceptors, are reported.

In: "Free Radicals in the Brain: Aging, Neurological and Mental Disorders" (eds. L. Packer, L. Prillipko, Y. Christen), Springer-Verlag, Berlin, 1992, p. 49-61

Antioxidant protection of the brain against oxidative stress.

[Kagan V](#), [Bakalova R](#), [Koynova G](#), [Tyurin V](#), [Serbinova E](#), [Petkov VV](#), [Petkov VD](#), [Staneva D](#), [Packer L](#).

(Статията включва оригинални резултати.)

Abstract

Neural membranes are highly susceptible to oxidative damage and uncontrolled free radical oxidation is suggested to be involved in many neural and mental disorders. Vitamin E is known to be the major chain-breaking lipid-soluble antioxidant in the blood. This statement also holds true for neural membranes. Under physiological conditions low concentrations of vitamin E are sufficient to prevent membrane oxidative damage due to a unique ability of vitamin E molecules (tocopherols and tocotrienols) to act as membrane free radical harvesting centers by the mechanism of vitamin E regeneration (reduction of vitamin E radical) by other intracellular reductants (NADPH- and NADH-dependent electron transport, vitamin C, reduced thiols). Vitamin E can synergistically interact with an unidentified protein kinase C-dependent mechanism, thus enhancing the overall antioxidant protection of brain membranes. Oxidative insult may result in a depletion of vitamin E in brain membranes followed by an accumulation of lipid peroxidation products. Enrichment of brain membranes with vitamin E by dietary supplementation provides for a higher protection of brain membranes against free radical oxidation. Thus the maintenance of a sufficient vitamin E concentration in the membranes of neural cells may be crucial for their resistance to oxidative stress. Ubiquinol, another lipid-soluble antioxidant, is less efficient than vitamin E (alpha-tocopherol as an inhibitor of lipid peroxidation in brain membranes).

In: "Encyclopaedia of Molecular Cell Biology and Molecular Medicine" (ed. R.A. Meyers), Wiley-VCH Verlag GmbH, Weinheim, Germany, 2005, Vol. 8, p. 263-287.

Microarray-Based Technology: Basic Principles, Advantages and Limitations
[Bakalova R](#), [Ewis A](#), [Baba Y](#).

Abstract

In April 2003, 50th anniversary year of the discovery of the double-helical structure of DNA, a high-quality and comprehensive sequencing of the human genome was completely accomplished, and a revolution in molecular cell biology and molecular medicine has begun. Many complex questions bring up (e.g., how genes contribute to normal human development, individual variability, and common diseases) and they need complex answers. It was necessary to develop techniques which permit complex answers. Microarray technology is a revolution technology allowing the simultaneous assessment of the transcription of tens of thousands of genes rapidly, as well as of their relative expression between normal and injured cells. There is widespread hope that microarrays will significantly impact on our ability to explore the genetic changes associated with etiology and development of many diseases, and to discover new biomarkers for disease diagnosis and prognosis prediction, and new therapeutic tools. The present article provides an overview of microarray technology with accents on its recent advantages and limitations. The first chapter is focused on the basic principles of microarrays, array platforms and fabrication, advantages and restrictions of cDNA-based (spotted) and oligonucleotide-based arrays (GeneChips and Codelink). The second chapter describes the crucial points in microarray study design (e.g., choice of reference source, sample preparation and preservation, labelling and hybridization). Finally, a brief description of microarray data normalization, mining and validation is given in the end of the review, transferring to several basic web-sites and software with detailed explanation of this most underappreciated challenge facing researchers working on microarray projects.

In: "Non-viral Gene Therapy: Gene Design and Delivery" (eds. K. Taira, K. Kataoka, T. Niidome), Springer-Verlag, Tokyo, 2005, p. 187-197.

(Статията включва оригинални резултати.)

Controlled Intracellular Localization of Oligonucleotides by Chemical Conjugation

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HIDEKI OHBA², and MASAYUKI FUJII^{1,3}

1 Introduction

Artificial control of genetic expression by oligonucleotides is a powerful tool for biological studies and medical therapies. Nucleic-acid drugs, such as antisense oligonucleotides, ribozymes, decoys and siRNAs, have attracted much attention and have been intensively studied for the past two decades (Crooke et al. 2004; Tung et al. 2000; Fischer et al. 2001; Eisele et al. 1999; Zubin et al. 2002; Antopolsky et al. 1999). Difficulties in using oligonucleotides as therapeutic agents involve their transport through the cell membrane, delivery and localization in the targeted cellular structure, and targeting of the specific mRNA or DNA sequence with sufficient affinity and specificity. For these reasons, DNA-peptide conjugates have been attracting intensive attention as alternative and advanced materials for the technology of genetic medicines and novel functional nucleic acids (Stetsenko et al. 2000, 2002; Soukchareum et al. 1995; Haralambidis et al. 1987; Antopolsky et al. 2002). In this chapter, the cellular uptake and controlled localization of oligonucleotide-peptide conjugates are discussed.

2 Synthesis of Oligonucleotide-Peptide Conjugates by Solid-Phase Fragment Condensation

Synthetic methods of oligonucleotide-peptide conjugates so far studied can be classified into two categories: solution-phase synthesis and solid-phase synthesis. The former mostly involves coupling procedures of oligonucleotide and peptide fragments using small linker molecules having two different functionalities (Antopolsky et al. 1999; Stetsenko et al. 2000).

ОРИГИНАЛНИ СТАТИИ В ЧУЖДИ ПЕРИОДИЧНИ ИЗДАНИЯ

[Dokl Akad Nauk SSSR](#). 1987;295(3):728-31.

[Mechanisms of biomembrane stabilization with alpha-tocopherol. The role of the isoprenoid chain in the inhibition of lipid peroxidation].

[Article in Russian]

[Kagan VE](#), [Serbinova EA](#), [Bakalova RA](#), [Stoichev TsS](#), [Erin AN](#).

No abstract available.

PMID: 3622237 [PubMed - indexed for MEDLINE]

[Biull Eksp Biol Med](#). 1987 Sep;104(9):304-6. Impact factor – 0.279

[Stabilizing effect of hydroxybenzimidazole and its derivatives on biological membranes during activation of lipid peroxidation].

[Article in Russian]

[Bakalova R](#), [Davitashvili NG](#), [Stoliarova LG](#), [Smirnov LD](#), [Erin AN](#).

Abstract

Inhibition of lipid peroxidation (LPO) by oxybenzimidazole (OBI) and its derivatives-alkyloxybenzimidazole (AOBI) and alkylethoxybenzimidazole (AEBI) was studied in liver microsomes and brain synaptosomes. It has been shown that both OBI and AOBI strongly inhibit LPO in microsomes and not synaptosomes. AEBI failed to inhibit LPO in microsomes. AOBI is more potent than OBI both in ascorbate- and NADPH-dependent LPO of microsomes. An antioxidant effect of both compounds is more marked in ascorbate-dependent LPO. The investigation of the possible use of AOBI for the protection of liver membranes in various pathological conditions associated with LPO activation seems promising.

PMID: 3663915 [PubMed - indexed for MEDLINE]

[Free Radic Res Commun](#). 1988;4(5):277-81. Impact factor – 2.805

The role of secondary messengers in the regulation of lipid peroxidation in rat liver microsomes.

[Baldenkov GN](#), [Serbinova EA](#), [Bakalova RA](#), [Tkachuk VA](#), [Kagan VE](#), [Stoytchev TsS](#).

Abstract

The effect of phorbol-12-myristate-13-acetate (PMA), an activator of protein kinase C (PK-C) on lipid peroxidation (LPO) in rat liver homogenates and microsomes was studied. PMA (10^{-10} to 10^{-6} M) produced a concentration-dependent inhibition of LPO, which was greatly decreased by polymyxin B (PxB) (an inhibitor of PK-C). The non-active analogue of PMA, 4 alpha-phorbol-12,13-didecanoate (4 alpha-PDD) exerted no inhibitory effect. The adenylate cyclase activator, forskolin (FK) (10^{-6} M) abolished the inhibitory effect of PMA on LPO. PMA and FK did not inhibit LPO in liposomes. It is suggested that LPO in biomembranes could be regulated by PK-C, whose inhibitory effect might be prevented by cAMP-dependent protein kinases.

PMID: 3234856 [PubMed - indexed for MEDLINE]

[Biull Eksp Biol Med](#). 1990 Jan;109(1):37-9. Impact factor – 0.279

[Intermembrane transport and antioxidant action of alpha-tocopherol in liposomes].

[Article in Russian]

[Rangelova DS](#), [Zhelev Zh](#), [Bakalova RA](#), [Tiurin VA](#), [Denisova NA](#), [Serbinova EA](#), [Paker L](#), [Kagan VE](#).

Abstract

Studies were made of the ability of alpha-tocopherol, incorporated into unilamellar liposomes from saturated or unsaturated phospholipids (donor liposomes) to inhibit the accumulation of lipid peroxidation (LPO) products in unilamellar liposomes from rat cerebral cortex lipids (acceptor liposomes) in the presence of LPO inducer (Fe + ascorbate). With the molar alpha-tocopherol: phospholipids ratios from 1:1000 to 1:100 in donor liposomes, obtained through sonication of lipid dispersions, alpha-

tocopherol was incorporated into both monolayers of liposomes and was distributed in monomeric form without forming clusters. Based on the dependencies of LPO inhibition on the alpha-tocopherol concentrations, we chose the ones that completely prevented the accumulation of LPO products in donor liposomes. Under these conditions LPO inhibition in mixtures of donor and acceptor liposomes was fully determined by the antioxidant effect of alpha-tocopherol in acceptor liposomes due to its intermembrane transfer. The efficiency of the "intermembrane" antioxidant action of alpha-tocopherol increased in the course of pre-incubation of donor and acceptor liposomes (up to 60 min) and this increase was more pronounced when the donor liposomes contained unsaturated phospholipids. Evidence was obtained that the intermembrane transfer of alpha-tocopherol did not result from the fusion of donor and acceptor liposomes during pre-incubation.

PMID: 2334795 [PubMed - indexed for MEDLINE]

[Biull Eksp Biol Med.](#) 1990 Nov;110(11):480-3. Impact factor – 0.279

[The mechanisms of the antioxidant action of shielded phenols in biological membranes. The effects of 4-methyl-2,6-di-tert-butylphenol (ionol) derivatives on luminol-dependent chemiluminescence].

[Article in Russian]

[Kharfuf M](#), [Serbinova EA](#), [Bakalova RA](#), [Savov VM](#), [Kagan VE](#).

Abstract is not available.

PMID: 2083327 [PubMed - indexed for MEDLINE]

[Arch Biochem Biophys.](#) 1990 Jul;280(1):147-52.

Intermembrane transfer and antioxidant action of alpha-tocopherol in liposomes.

[Kagan VE](#), [Bakalova RA](#), [Zhelev ZZ](#), [Rangelova DS](#), [Serbinova EA](#), [Tyurin VA](#), [Denisova NK](#), [Packer L](#).

Abstract

Intermembrane transfer and exchange of tocopherol are not well understood. To study this we tested the ability of alpha-tocopherol containing unilamellar donor liposomes to inhibit the accumulation of lipid peroxidation products in acceptor liposomes. With molar ratios of alpha-tocopherol:phospholipids from 1:100 to 1:1000 in donor liposomes prepared by sonication of lipid dispersions, alpha-tocopherol was incorporated into both monolayers and was homogenously distributed in monomeric form without forming clusters in the liposomes. Concentrations of alpha-tocopherol which completely prevented the peroxidation of lipids were chosen for donor liposomes. Hence inhibition of lipid peroxidation in mixtures of donor and acceptor liposomes was determined by the antioxidant effect of alpha-tocopherol in acceptor liposomes which resulted from intermembrane transfer and exchange of alpha-tocopherol. Evidence was obtained that this was not due to fusion of donor with acceptor liposomes. The efficiency of the "intermembrane" antioxidant action of tocopherol was more pronounced when donor liposomes contained unsaturated phospholipids, indicating that the presence of unsaturated fatty acids in the outer monolayer phospholipids facilitates intermembrane tocopherol exchange.

PMID: 2353816 [PubMed - indexed for MEDLINE]

[Biochem Pharmacol.](#) 1990 Dec 1;40(11):2403-13. Impact factor – 4.889

Mechanisms of stabilization of biomembranes by alpha-tocopherol. The role of the hydrocarbon chain in the inhibition of lipid peroxidation.

[Kagan VE](#), [Serbinova EA](#), [Bakalova RA](#), [Stoytchev TS](#), [Erin AN](#), [Prilipko LL](#), [Evstigneeva RP](#).

Abstract

The effects of alpha-tocopherol and its homologues with different chain lengths (6-hydroxy-chromanes: C1, C6, C11) on lipid peroxidation in natural membranes (liver microsomes and mitochondria, brain synaptosomes) and liposomes were studied. It was shown that the antioxidant activity of alpha-tocopherol homologues decreased in the order: C1 greater than C6 greater than C11 greater than alpha-tocopherol (C16). Using fluorescent measurements, the possible reasons underlying these differences were investigated: (i) the distribution between the aqueous media and nonpolar phase of the membrane, which predetermines the binding of alpha-tocopherol homologues to membranes; (ii) the incorporation of alpha-tocopherol homologues into lipid bilayer; (iii) non-uniform distribution (formation of the clusters) of tocopherol homologues in the lipid bilayer; and (iv) transbilayer mobility of alpha-tocopherol homologues and accessibility of the inhibitors for radical-generating centres under enzymically and non-enzymically induced lipid peroxidation. It was demonstrated that: (i) binding of C1 with membranes was less efficient than that of longer-chain homologues (C6, C11, C16); (ii) the level of incorporation of alpha-tocopherol homologues into membranes decreased in a succession alpha-tocopherol C11 greater than C6 greater than C1; (iii) all alpha-tocopherol homologues existed in the lipid bilayer not only in a monomeric form but also associated in clusters thus decreasing the efficiency of radical scavenging; (iv) the short-chain alpha-tocopherol homologue, C1, exhibited a high transbilayer mobility whereas the long-chain one, C16, underwent no transbilayer migration within tens of minutes. The inhibiting effect of alpha-tocopherol esters and C1-acetate was predetermined by their hydrolysis in biomembranes; a strong correlation exists between the rate of the ester hydrolysis and their antioxidant activity in the membrane. In liposomes, in which the esterase activity was absent, alpha-tocopherol esters and C1-acetate exhibited very low lipid peroxidation inhibition.

PMID: 2268364 [PubMed - indexed for MEDLINE]

[Biull Eksp Biol Med](#). 1991 Nov;112(11):482-5. Impact factor – 0.279

[The efficiency of the action of alpha-tocopherol and its homologs on luminol-dependent chemiluminescence induced by (Fe²⁺ + NADP.H) and (Fe²⁺ + ascorbate) systems in rat liver microsomes].

[Article in Russian]

[Bakalova R](#), [Sokolova Ts](#), [Ribarov S](#), [Kagan V](#).

Abstract

The effects of alpha-tocopherol (C16) and its homologues with different chain length (6-hydroxychromanes-C1, C6, C11) on lipid peroxidation induced luminol-dependent chemiluminescence in rat liver microsomal suspensions were studied. It was shown that C1, C6 and C11 inhibited the (Fe²⁺ + ascorbate)-and (Fe²⁺ + NADP.H)-induced chemiluminescence. The inhibitory effect was decreased in the order: C1 C6 C11, C16 was not influenced chemiluminescence. The possible reason underlying these differences was discussed: different efficiency of interaction of C16 and its homologues with hydroxyl and superoxide radicals, which initiate the luminol-dependent chemiluminescence. It was concluded that C16 (in concentration below 0.5 mM) was not interacted with hydroxyl and superoxide free radicals, generated in

microsomal suspensions under (Fe(2+) + ascorbate)- and (Fe(2+) + NADP.H)-dependent lipid peroxidation.

PMID: 1810482 [PubMed - indexed for MEDLINE]

[Biull Eksp Biol Med.](#) 1992 Feb;113(2):132-4. Impact factor – 0.279

[Activation of lipid peroxidation and changes in vitamin E contents in the lungs under oxidative stress].

[Article in Russian]

[Kovacheva-Ivanova S](#), [Bakalova R](#), [Kagan V](#), [Georgiev G](#).

Abstract

The lipid peroxidation (LPO) of the lung tissue and the bronchoalveolar lavage in rats under the influence of immobilization has been investigated. The effects accompanying the development of oxidative stress in animals--an increase in the content of conjugated dienes and fluorescent LPO products in biological objects and a strong decrease in the content of vitamin E in the lung tissue were registered.

PMID: 1611051 [PubMed - indexed for MEDLINE]

[Gen Physiol Biophys.](#) 1994 Dec;13(6):469-82. Impact factor – 1.146

Immobilization stress enhances lipid peroxidation in the rat lungs. Materials and methods.

[Kovacheva-Ivanova S](#), [Bakalova R](#), [Ribavov SR](#).

Abstract

The present work was carried out to study the involvement of lipid peroxidation in immobilization-induced damage of the rat lung. Thirty-hour immobilization stress was found to result in a marked morphological alteration of the lung ultrastructure and in significant increases of both acid and alkaline phosphatase for immobilization times exceeding 12 and 24 hours respectively. Also, increased concentrations of conjugated dienes and fluorescent products of lipid peroxidation were measured in the lungs of rats immobilized over 12 h. Immobilization stress was followed by significant changes in the fatty acid contents of lung phospholipids. The levels of polyunsaturated fatty acids C-18:2 (linoleic acid) and C-20:4 (arachidonic acid) were decreased even during the alarm phase. The contents of monounsaturated fatty acids did not change, while those of saturated fatty acids slightly increased. The involvement of lipid peroxidation in immobilization-induced damage of the rat lung was indirectly supported by the observation of decreased levels of vitamin E at 12 h immobilization. All the above data suggest that lipid peroxidation is somehow involved in the immobilization-induced damage of the rat lung. The observed changes in lipid peroxidation preceded the immobilization stress-induced damage of the lung cell membranes. Therefore, it seems likely that lipid peroxidation is the cause, rather than a consequence of the stress-altered lung structure.

PMID: 7797054 [PubMed - indexed for MEDLINE]

[Eur J Anaesthesiol.](#) 1995 Mar;12(2):155-62. Impact factor – 1.679

Nitrous oxide with fentanyl and droperidol minimizes lipid peroxidation in the liver.

[Chinev S](#), [Bakalova R](#), [Peneva V](#), [Uzunova P](#), [Galabova T](#), [Sokolova Z](#), [Ribarov S](#).

Abstract

We have studied the level of lipid peroxidation in the liver of rats exposed to nitrous oxide plus oxygen or injected with droperidol or fentanyl. The effect of nitrous oxide used in combination with droperidol and fentanyl was also investigated. All the tested anaesthetics caused lipid peroxidation in the rat liver. It seems likely, however, that the mechanism by which droperidol and fentanyl initiate lipid peroxidation differs from that which nitrous oxide uses. Free radical products and/or activated oxygen species are produced during fentanyl and droperidol metabolism in the liver. However, nitrous oxide is not metabolized in the liver and probably undergoes one electron reduction outside the liver thus producing free radical products and/or activated oxygen species which are able to diffuse and initiate lipid peroxidation in the liver. It was also found that the level of lipid peroxidation in the liver of rats injected with droperidol and fentanyl and then exposed to nitrous oxide was low and close to that of the control animals. We suggest that, when used in combination, the products generated outside the liver as a result of nitrous oxide metabolism are transported to the liver and take part in reactions with the products of the metabolism of droperidol and fentanyl, thus decreasing the concentration of the species able to initiate lipid peroxidation.

PMID: 7781635 [PubMed - indexed for MEDLINE]

[Gen Physiol Biophys](#). 1996 Dec;15(6):463-75. Impact factor – 1.146

Oxidation of low density lipoproteins leads to disturbance of their binding with alpha-tocopherol.

[Bakalova RA](#), [Goudev AR](#), [Zhelev ZZ](#), [Nachev C](#), [Ribarov SR](#).

Abstract

The dynamics of binding of exogenous alpha-tocopherol (alpha-T) added to native or oxidatively modified LDLs (LDLs or oxLDLs) were investigated. Venous blood from 31 clinically healthy blood donors (15 males and 16 females) was used. LDLs were isolated by density gradient ultracentrifugation. LDLs were oxidized in vitro by CuSO₄. LDLs or oxLDLs were enriched with exogenous alpha-T (initial concentrations: 0; 10; 20; 50; or 100 nmol per mg protein). The contents of alpha-T in LDLs or in oxLDLs were measured by HPLC. Lag-phase of LDL oxidation before or after saturation with alpha-T was recorded. Correlation analysis of the lag-phase of LDL oxidation and alpha-T content in LDLs was carried out by the method of Esterbauer et al. The experimental results demonstrated that: (i) alpha-T was incorporated into native LDLs to a higher extent as compared to oxLDLs. (ii) A saturation of LDLs and oxLDLs with alpha-T was observed. (iii) A positive correlation was observed between the duration of the lag-phase of LDL oxidation in vitro and the content of alpha-T in LDLs. (iv) Based on LDL saturation with alpha-T, the persons could be classified in two groups: LDLs from group I of 26 persons were found to incorporate exogenous alpha-T to the extent of 1.8 to 3 times its initial concentration; LDLs from group II of 5 persons incorporated little or no exogenous alpha-T. In the first group, oxidation of LDLs lead to a considerable decrease in alpha-T dependent variable k and to a moderate reduction of alpha-T-independent variable a in the equation of Esterbauer et al.: lag-phase = $k \cdot [\text{alpha-tocopherol}] + a$. In the second group, oxidation of LDLs lead to insignificant changes in k , as well as in a . (v) According to the levels of k and a the native LDLs from the second group of 5 persons were very close to oxLDLs from the first group of 26 persons. Presumably, native LDLs from the second group of persons were initially

oxidatively modified, and probably this will be a risk group in relation to atherogenic disorders.

PMID: 9248832 [PubMed - indexed for MEDLINE]

[Eur J Anaesthesiol](#). 1998 Nov;15(6):686-94. Impact factor – 1.679

Lipid peroxidation in rat lung induced by neuroleptanalgesia and its components.

[Chinev S](#), [Bakalova R](#), [Kovacheva S](#), [Ribarov SR](#).

Abstract

The aim of the present work was to determine the likelihood of lipid peroxidation in the lungs of rats subjected to neuroleptanalgesia and its components. In particular, the effect of fentanyl, droperidol, a nitrous oxide/oxygen mixture when used separately or in combination, on the lung level of lipid peroxidation was investigated. The in vitro antioxidant properties of fentanyl and droperidol were also tested. Lipid peroxidation was evidenced by the endogenously generated conjugated dienes and fluorescent products of lipid peroxidation and the decrease in lung vitamin E content. It was found that fentanyl and droperidol, used separately or in combination, did not induce lipid peroxidation in the rat lung, while the exposure of rats for 120 min to a nitrous oxide/oxygen mixture (2:1 v/v) led to well-expressed peroxidation. The (N₂O + O₂)-pro-oxidant action was significantly inhibited in rats previously injected with fentanyl and/or droperidol. The results show that the application of fentanyl, droperidol and (N₂O + O₂), as in neuroleptanalgesia, ensures minimal lipid peroxidation in the lung. In addition, we found that fentanyl and droperidol were able to inhibit the Fe(2+)-catalysed lipid peroxidation in lung homogenate. We speculate that the inhibitory effect of fentanyl and/or droperidol on the (N₂O + O₂)-induced lipid peroxidation in the rat lung may be caused directly by their antioxidant properties. However, another explanation seems to be possible. The free radicals that are produced during the metabolism of fentanyl and droperidol may react with the radicals generated during the one-electron reduction of nitrous oxide. Such reactions will obviously reduce the free radical concentration in the organism and, hence, the likelihood of initiating lipid peroxidation.

PMID: 9884854 [PubMed - indexed for MEDLINE]

[Gen Physiol Biophys](#). 1999 Mar;18(1):87-97. Impact factor – 1.146

Serum level of IgG autoantibodies against oxidized low density lipoproteins and lag-phase of serum oxidation in coronary heart disease--inverse correlation.

[Bakalova R](#), [Zhelev Z](#), [Goudev A](#), [Ribarov S](#), [Nachev C](#).

Abstract

High affinity IgG autoantibodies (ABs) against oxLDLs and lag-phase of serum oxidation were tested in patients with coronary heart disease (CHD). Fifty one (37 M/14 F) patients with CHD defined as Q-wave myocardial infarction and/or stenosis of more than 50% and 51 (34 M/17 F) healthy blood donors as controls participated in this study. LDLs were isolated by gradient ultracentrifugation and oxidized with CuSO₄. The modified LDLs (oxLDLs) or native LDLs (nLDLs) were used as antigens in an enzyme immunoassay (ELISA) to detect IgG ABs in both groups. The serum was oxidized by CuSO₄ and the oxidation was monitored spectrophotometrically at $\lambda = 234$ nm to follow the formation of conjugated dienes. The lag-phase (in minutes) is the interval between the addition of CuSO₄ to the serum and the beginning of extensive oxidation (increasing absorbance at 234 nm).

The concentrations of total cholesterol, triglycerides, HDL-cholesterol, apo-A and apo-B were measured as well. The mean level of ABs against oxLDLs (expressed as optical density units) was 0.590 +/- 0.330 in CHD-patients vs 0.244 +/- 0.200 in controls ($p < 0.001$). The lag-phase in minutes was 47.00 +/- 27.19 in CHD-patients and 80.23 +/- 26.30 in controls ($p < 0.001$). A negative correlation between ABs levels and lag-phase was established in CHD-patients ($r = -0.69$, $p < 0.001$) and controls ($r = -0.62$, $p < 0.001$). A poor correlation was established between ABs levels or lag-phase, on one hand, and other measured parameters. In conclusion, the lag-phase of serum oxidation by Cu^{2+} could be informative for LDL susceptibility to modification and the extent of consequent humoral immune response.

PMID: 10378123 [PubMed - indexed for MEDLINE]

[Gen Physiol Biophys](#). 2000 Mar;19(1):103-13. Impact factor – 1.146

Relationships between serum levels of autoantibodies against oxidized low density lipoproteins, lipid-soluble antioxidants and apolipoprotein B in patients with coronary heart disease.

[Bakalova RA](#), [Hadzhimitova V](#), [Ribarov S](#).

Abstract

High affinity IgG autoantibodies against oxidized low density lipoproteins (oxLDLs), apolipoprotein B and lipid-soluble antioxidants--alpha-tocopherol and beta-carotene, were tested in patients with coronary heart disease. Correlation relationships between these parameters were analysed. Fifty one patients with coronary heart disease (37 males/14 females) defined as Q-wave myocardial infarction and/or stenosis of more than 50%, and 51 healthy blood donors (34 males/17 females) as controls participated in this study. LDLs were isolated by density gradient ultracentrifugation and oxidized with Cu^{2+} . OxLDLs or native LDLs (nLDLs) were used as antigens in enzyme immunoassay (ELISA) to detect IgG autoantibodies in the serum. The contents of alpha-tocopherol and beta-carotene were measured by HPLC. Apolipoprotein B was determined by immunoturbidimetry. Correlation analysis of the parameters was carried out by Spearman's test. Alpha-tocopherol was decreased significantly in the serum of patients with coronary heart disease (2.96+/-1.63 nmol/mg serum protein vs 6.23+/-2.28 nmol/mg serum protein in Control group) ($p < 0.01$). Also, the serum level of beta-carotene was decreased in patients with coronary heart disease (174.0+/-95.7 pmol/mg serum protein vs 313.2+/-141.5 pmol/mg serum protein in Control group) ($p < 0.01$), while apolipoprotein B was increased significantly (1.20+/-0.34 g/l in patients with coronary heart disease vs 0.86+/-0.23 g/l in Control group) ($p < 0.001$). In a previous study we established that the mean serum level of IgG autoantibodies against oxLDLs (expressed in optical density units) was about 2.5 times higher in patients with coronary heart disease as compared to control subjects ($p < 0.001$). A good positive linear correlation was observed between alpha-tocopherol and apolipoprotein B levels in Control group ($r = 0.78$, $p < 0.001$), as well as in the group of patients with coronary heart disease ($r = 0.42$, $p < 0.001$). Poor nonsignificant correlations were established between all another measured parameters. In conclusion, the lipid-soluble antioxidants--alpha-tocopherol and beta-carotene, are not informative with respect to the susceptibility of the serum to oxidative modifications and as to the extent of the subsequent humoral immune response. Presumably, the reduction of the correlation coefficient between apolipoprotein-B and alpha-tocopherol in patients with coronary heart disease in comparison with control subjects could provide indirect information on modifications of apolipoprotein-B and

on a decrease of its susceptibility to interact with this major lipid-soluble antioxidant in atherogenesis.

PMID: 10930142 [PubMed - indexed for MEDLINE]

[Methods Find Exp Clin Pharmacol](#). 2000 Jun;22(5):267-9. Impact factor – 1.037

Determination of malondialdehyde in biological samples by solid-phase extraction and high-performance liquid chromatography.

[Bakalova R](#), [Mileva M](#), [Kotsev C](#), [Bardarov V](#), [Ribarov S](#).

Abstract

An analytical procedure for determination of malondialdehyde in tissue homogenates and blood serum was developed. A reaction with 2,4-dinitrophenylhydrazine is used followed by cleaning up of the derivative by solid-phase extraction. The samples were analyzed by isocratic high-performance liquid chromatography (HPLC) using a narrow-bore HPLC-column. A good separation of the 1-pyrazole peak from that of 2,4-dinitrophenylhydrazine was observed. A high linear dependence was established by the concentration of 1-pyrazole in the range of 10-5000 ng/ml. The detection limit of the method applied for tissue homogenates and blood serum was approximately 10 ng/ml or lower, and RSD of the method was 9% (n = 8). The peak of 1-pyrazole for these samples was well separated from the other matrix peaks. Experiments carried out evaluated that the solid-phase extraction might be an effective step of the sample preparation, significantly increasing the selectivity of the analysis and the life-time of the column. The method seems to be applicable for determination of malondialdehyde in different biological samples.

PMID: 11031725 [PubMed - indexed for MEDLINE]

[Toxicol Lett](#). 2000 Apr 3;114(1-3):39-45. Impact factor – 3.581

Effect of vitamin E on lipid peroxidation and liver monooxygenase activity in experimental influenza virus infection.

[Mileva M](#), [Tancheva L](#), [Bakalova R](#), [Galabov A](#), [Savov V](#), [Ribarov S](#).

Abstract

Influenza virus infection was associated with development of oxidative stress in liver of mice, viz. increase in amount of lipid peroxidation products, decrease in cytochrome P-450 and NADP. H-cytochrome c-reductase activity, and inhibition of liver monooxygenases (aniline hydroxylase, ethylmorphine-N-demethylase, amidopyrine-N-demethylase and analgin-N-demethylase). These effects were most pronounced on the 7th day after virus inoculation as compared to the 5th one. Supplementation of mice with vitamin E before virus inoculation leads to liver protection against oxidative stress and toxicosis. A marked decrease of lipid peroxidation products and an increase of cytochrome P-450 and activities of monooxygenases was established. The stabilizing effect of vitamin E was dose-dependent and was most pronounced on the 5th day after virus inoculation as compared to the 7th one.

PMID: 10713467 [PubMed - indexed for MEDLINE]

[Jpn J Physiol. \(J Physiol Sci\)](#) 2001 Apr;51(2):201-8. Impact factor – 1.356

Frequency dependence of local cerebral blood flow induced by somatosensory hind paw stimulation in rat under normo- and hypercapnia.

[Bakalova R](#), [Matsuura T](#), [Kanno I](#).

Abstract

We measured the field potential and the changes in local cerebral blood flow (LCBF) response during somatosensory activation (evoked LCBF) in alpha-chloralose--anesthetized rats by laser-Doppler flowmetry under normocapnia ($\text{PaCO}_2=34.3\pm 3.8$ mmHg) and hypercapnia ($\text{PaCO}_2=70.1\pm 9.8$ mmHg). Somatosensory activation was induced by electrical stimulation (0.2, 1, and 5 Hz with 1.5 mA for 5 s) of the hind paw. The neuronal activity of the somatosensory area of the hind paw was linear to the stimulus frequency, and there was no significant difference in the neuronal activity between hypercapnia and normocapnia. The baseline level of LCBF under hypercapnia was about 72.2% higher than that under normocapnia ($p<0.01$). The absolute response magnitude under hypercapnia was greater than that under normocapnia ($p<0.05$). The evoked LCBF under both conditions showed a frequency-dependent increase in the 0.2 to 5 Hz range, and the difference in the absolute response magnitude at the same stimulus frequency between normocapnia and hypercapnia became large with increasing stimulus frequency ($p<0.05$). On the other hand, after normalization to each baseline level there was no significant difference in the response magnitude of the normalized evoked LCBF between normocapnia and hypercapnia, indicating that the normalized evoked LCBF reflects neuronal activity even when the baseline LCBF was changed by the PaCO_2 level. The peak time and termination time of LCBF response curves with respect to the graded neuronal activity at 1 and 5 Hz stimulation increased significantly under hypercapnia, compared with those under normocapnia ($p<0.05$), although the rise time of 0.5 s was nearly constant. In conclusion, the results suggest a synergistic effect of the combined application of graded neuronal stimuli and hypercapnia on the LCBF response.

PMID: 11405913 [PubMed - indexed for MEDLINE]

[Arch Toxicol.](#) 2002 Mar;76(2):96-103. Epub 2002 Feb 6. Impact factor – 4.041

Effect of immobilization, cold and cold-restraint stress on liver monooxygenase activity and lipid peroxidation of influenza virus-infected mice.

[Mileva M](#), [Bakalova R](#), [Tancheva L](#), [Galabov S](#).

Abstract

The present study provides a direct experimental evidence that the combination of influenza A/Aichi/2/68 (H3N2) infection with different models of "oxidative stress", such as immobilization, cold and cold-restraint, is associated with graduated oxidative disturbances in the liver of mice, despite the absence of virus and inflammation in this tissue. It was found that experimental influenza virus infection is accompanied with a significant increase of lipid peroxidation products, a decrease of natural antioxidants (vitamin E, glutathione) and cytochrome P-450, an inhibition of cytochrome c reductase and liver monooxygenases (analgin- N-demethylase and amidopyrine- N-demethylase). Immobilization and cold stress, applied separately or in combination (cold-restraint), did not influence significantly any of the analysed parameters compared to those of the control group of non-infected mice. Preliminary exposure of mice to immobilization or cold stress and subsequent inoculation of influenza virus resulted in a significant increase of lipid peroxidation products and a significant decrease of vitamin E and reduced glutathione, compared with levels in control (non-infected) animals. Compared to influenza virus-infected and non-stressed animals, the changes in all these parameters were negligible. Immobilization or cold stress, applied in combination with influenza virus infection, partially prevented the suppressive

effect of influenza virus on cytochrome P-450 and liver monooxygenases. A tendency towards normalization of these parameters to the control levels was observed. However, after application of cold-restraint plus influenza virus infection, the level of cytochrome P-450 and activity of cytochrome c reductase stayed markedly lower than in infected and non-stressed animals. The activities of liver monooxygenases were slightly increased compared with those of infected and non-stressed animals, but stayed relatively low compared to control (non-infected) mice. Combination of cold-restraint and influenza virus infection resulted in a greater synergistic increase of lipid peroxidation products and a greater synergistic decrease of vitamin E and reduced glutathione compared to controls, as well as to influenza virus-infected and non-stressed animals.

PMID: 11914779 [PubMed - indexed for MEDLINE]

[Comp Immunol Microbiol Infect Dis.](#) 2002 Jan;25(1):1-11. Impact factor – 3.607
Effect of vitamin E supplementation on lipid peroxidation in blood and lung of influenza virus infected mice.

[Mileva M](#), [Bakalova R](#), [Tancheva L](#), [Galabov A](#), [Ribarov S](#).

Abstract

The influenza virus infection (A/Aichi/2/68) was associated with development of oxidative stress in lung and blood of mice, accompanied by an increase in levels of lipid peroxidation products (conjugated dienes and total malondialdehyde) and a decrease in endogenous amounts of natural antioxidant vitamin E. These effects were most pronounced on the 5th day after virus inoculation, in comparison with those on the 7th. Supplementation of mice with exogenous vitamin E before virus inoculation lead to lung and blood protection against lipid peroxidation. A marked decrease in lipid peroxidation products and an increase in vitamin E content was established in blood and lung on the 5th and 7th day after virus inoculation. The stabilizing effect of vitamin E is dose-dependent in blood and dose-independent in lung, and was most pronounced on the 5th day after virus inoculation in comparison with the 7th day.

PMID: 11831742 [PubMed - indexed for MEDLINE]

[Methods Find Exp Clin Pharmacol.](#) 2002 Nov;24(9):559-64. Impact factor – 1.037
Laser-Doppler imaging of activation-flow coupling in the somatosensory cortex: normalization of signal when the baseline changes significantly.

[Zhelev Z](#), [Bakalova R](#).

Abstract

This study describes two approaches used to normalize the laser-Doppler flowmetry (LDF) signal, corresponding to the regional cerebral blood flow (rCBF) response after electrical hind-paw stimulation. The first approach divides the LDF signal to the baseline and subsequently integrates the response curve from the rise point to the termination point (defined formally as "normalized rCBF response", and the second subtracts the baseline from the LDF signal and subsequently integrates the response curve from the rise point to the termination point (defined as "absolute rCBF response"). Both parameters are given in arbitrary units. A comparative analysis of the changes in the "normalized" and "absolute" LDrCBF response is presented both for when the baseline does not change significantly, and for when the baseline changes significantly under the influence of different factors. In summary, when the

baseline changes significantly it is preferable to normalize the LDrCBF response towards the baseline by subtraction, not by division.

PMID: 12616701 [PubMed - indexed for MEDLINE]

[Prostaglandins Leukot Essent Fatty Acids](#). 2002 Dec;67(6):379-88. Impact factor – 1.653

Cyclooxygenase-pathway participates in the regulation of regional cerebral blood flow in response to neuronal activation under normo- and hypercapnia.

[Bakalova RA](#), [Matsuura T](#), [Kanno I](#).

Abstract

The present study was designed to investigate whether cyclooxygenase products are involved in the regulation of the regional cerebral blood flow, evoked by somatosensory activation (evoked rCBF) under normo- and hypercapnia. Indomethacin (IMC) was used as cyclooxygenase inhibitor. It was applied intravenously (i.v., 10 mg/kg/h) in two experimental protocols-before hypercapnia (i) and after hypercapnia (ii). Somatosensory activation was induced by electrical hind paw stimulation (5 Hz frequency, 5 s duration, 1.5 mA). The evoked rCBF-response was measured in alpha-chloralose anesthetized rats using laser-Doppler flowmetry. IMC abolished completely the effect of hypercapnia on the baseline level of CBF. The drug reduced significantly evoked rCBF-response also. The inhibitory effect of IMC on evoked rCBF-response is better expressed under normocapnia (approximately 70%) than that under hypercapnia (approximately 40%). After IMC application, the normalized evoked rCBF curves peaked earlier as compared to that before its application ($P < 0.05$), although the rise time of 0.5 s was nearly constant regardless of stimulus frequency. In conclusion, the results suggest a participation of IMC-sensitive and cyclooxygenase-dependent mechanisms in the regulation of evoked rCBF, induced by somatosensory stimulation.

PMID: 12468258 [PubMed - indexed for MEDLINE]

[Exp Biol Med \(Maywood\)](#). 2002 Jul;227(7):465-73. Impact factor – 2.954

The cyclooxygenase inhibitors indomethacin and Rofecoxib reduce regional cerebral blood flow evoked by somatosensory stimulation in rats.

[Bakalova R](#), [Matsuura T](#), [Kanno I](#).

Abstract

The present study was designed to investigate whether administration of indomethacin (IMC), a non-selective cyclooxygenase (COX-1 and COX-2) inhibitor, and Rofecoxib, a highly selective COX-2 inhibitor, affect the regulation of regional cerebral blood flow response evoked by somatosensory activation (evoked rCBF). IMC and Rofecoxib were applied intravenously (6.25 and 3 mg/kg/hr, respectively). Somatosensory activation was induced by electrical hind paw stimuli of 0.2, 1, and 5 Hz (5-sec duration, 1.5 mA). The evoked rCBF was measured in alpha-chloralose anesthetized rats using laser-Doppler flowmetry. Before and after drug application, the evoked rCBF showed a frequency-dependent increase in the range of 0.2-5 Hz stimulation. IMC reduced significantly (about 50%-60%) evoked rCBF in response to all frequencies of hind paw stimulation ($P < 0.05$). Rofecoxib reduced significantly (about 50%) evoked rCBF in response to 1 and 5 Hz stimulation ($P < 0.05$), but did not affect evoked rCBF at 0.2 Hz. After IMC or Rofecoxib application, the normalized evoked rCBF curves peaked earlier as compared with that before their

application ($P < 0.05$), although the rise time of 0.5 sec was nearly constant regardless of the stimulus frequency. The termination time of evoked rCBF curves was changed significantly after IMC application at 0.2 Hz stimulation ($P < 0.05$), but was not affected after Rofecoxib application. Neither COX inhibitor significantly affected the baseline level of CBF. The results suggest a participation of COX products in the regulation of evoked rCBF in response to somatosensory stimulation in the brain.

PMID: 12094010 [PubMed - indexed for MEDLINE]

[Cancer Lett.](#) 2002 Oct 28;184(2):207-14. Impact factor – 4.864

Fractionation of normal and leukemic T-cells by lectin-affinity column chromatography.

[Ohba H](#), [Bakalova R](#), [Moriwaki S](#), [Nakamura O](#).

Abstract

A method for rapid fractionation of normal and leukemic T-cells (Jurkat, RPMI-8402, MOLT-4), using lectin-affinity column chromatography, is described. CNBr-activated Sepharose 6MB was used as a non-mobile phase. The gel was covalently conjugated with Dolichos biflorus agglutinin (DBA) over 24 h. The normal cells were eluted by phosphate buffered saline (Ca(2+) and Mg(2+) free), while the leukemic T-cells, interacting with DBA, were removed by N-acetyl-D-galactosamine or by low-concentrated acetic acid as a mobile phase. The cell fractions were detected spectrophotometrically at 600 nm. The rate of cell elution decreased in the order: normal > leukemic T-cells. The viability and the type of separated T-cell fractions were characterized by flow cytometry, using adequate fluorescent antibodies. The interactions between leukemic T-cells and DBA-saturated Sepharose beads were examined by fluorescent microscopy, using fluorescent isothiocyanate-DBA as a fluorescent marker.

PMID: 12127693 [PubMed - indexed for MEDLINE]

[Cancer Lett.](#) 2003 Mar 20;192(1):59-65. Impact factor – 4.864

Purification of normal lymphocytes from leukemic T-cells by lectin-affinity adsorbents - correlation with lectin-cell binding.

[Bakalova R](#), [Ohba H](#).

Abstract

Utilization of leukemic T-cells from normal ones, using lectin-affinity adsorbents, is described. CNBr-activated Sepharose 6MB was covalently coupled to Soybean (SBA) or Dolichos Biflorus Agglutinins (DBA), then serves as an affinity probe for separation of leukemic T-cells from normal lymphocytes. The normal lymphocytes were removed almost completely by phosphate buffered saline (Ca(2+) and Mg(2+) free) (PBS(-)) from lectin-affinity column. More than 80% of the leukemic T-cells were retained on the lectin-affinity adsorbent, whereas another 10-15% were easily removed by PBS(-). There was a very good linear correlation between percent of cells, retained on the lectin-affinity adsorbent and percent of cells, interacting with the respective free lectin ($r=0.97$ for SBA, and $r=0.93$ for DBA). The viability of normal lymphocytes was not influenced after passing through the columns. In the case of leukemic T-cells - about 90% of the easily removed cells were dead, and another 10% were viable cells, non-interacting with DBA or SBA.

PMID: 12637153 [PubMed - indexed for MEDLINE]

[Biomed Chromatogr.](#) 2003 Jun;17(4):239-49. Impact factor – 1.545

Interaction of soybean agglutinin with leukemic T-cells and its use for their in vitro separation from normal lymphocytes by lectin-affinity chromatography.

[Bakalova R, Ohba H.](#)

Abstract

A procedure for separation of leukemic T-cells from normal lymphocytes, using lectin-affinity column chromatography, is described. CNBr-activated Sepharose 6MB was used as a non-mobile phase. The gel was covalently coupled with soybean agglutinin (SBA), then served as an affinity probe for fractionation of mixture of normal lymphocytes and leukemic cells. Leukemic cell lines, derived from acute lymphoblastic leukemia (Jurkat, MOLT-4, RPMI-8402), were tested. The elution of normal lymphocytes was carried out by PBS(-). The leukemic T-cells, interacting with SBA, were removed by N-acetyl-D-galactosamine or low-concentration acetic acid. The type and viability of the separated cell fractions were analyzed by flow cytometry and fluorescent microscopy, using adequate fluorescent antibodies. The interaction of leukemic T-cells with free SBA, as well as with SBA-conjugated Sepharose beads, was examined fluorimetrically and visualized by fluorescent microscopy, using FITC-SBA as a marker. The rate of cell elution on SBA-affinity column decreased in order: normal > leukemic T-cells. Both normal lymphocytes and leukemic T-cells were removed in a mixture from SBA-free Sepharose 6MB by PBS(-) and were not fractionated discretely. The leukemic T-cells specifically interacted with SBA as well as with SBA-affinity adsorbent. In contrast, the normal lymphocytes did not interact with free SBA as well as with SBA-conjugated Sepharose beads in the concentrations applied. The method potentially combines a discrete cell fractionation with manifestation of a specific target cytotoxicity of SBA against leukemic T-cells, without any influence on normal lymphocytes.

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PMID: 12833389 [PubMed - indexed for MEDLINE]

[Biochim Biophys Acta.](#) 2003 Jan 20;1619(2):144-50. Impact factor – 4.663

Cytoagglutination and cytotoxicity of Wheat Germ Agglutinin isolectins against normal lymphocytes and cultured leukemic cell lines--relationship between structure and biological activity.

[Ohba H, Bakalova R, Muraki M.](#)

Abstract

The relationships between degree of lectin-cell binding, cytotoxicity and cytoagglutinating activity of three Wheat Germ Agglutinin isolectins (WGA-1, WGA-2, WGA-3) against normal lymphocytes and cultured leukemic cell lines (Jurkat, MOLT-4, Raji, Daudi, K-562) were studied. All WGA-isolectins interacted in a similar degree with normal lymphocytes, while in the case of leukemic cells, the degree of isolectin-cell binding increased in the order: WGA-1 < or = WGA-3 < WGA-2 at isolectin concentrations 0.5 microM and higher, and WGA-3 < WGA-2 < or = WGA-1 at 0.25 microM isolectin concentration. The WGA interacted in higher degree with Jurkat, Raji, Daudi and K-562, followed by MOLT-4 and normal lymphocytes. The velocity of cytoagglutination in the presence of 0.25 microM WGA-isolectins increased in the order: WGA-3 < WGA-2 < or = WGA-1, and was better expressed in Jurkat, Raji, Daudi and K-562, followed by MOLT-4 and normal lymphocytes. The cytotoxicity of isolectins was very well expressed against Jurkat, MOLT-4, Raji and

Daudi, and less expressed against K-562 and normal lymphocytes. In the case of leukemic cells, the cytotoxic effect of WGA-isolectins increased in the order: WGA-3<WGA-2=WGA-1. A very good positive correlation was determined between velocity of cytoagglutination and degree of lectin-cell binding ($r=0.77$, $P<0.001$). A good inverse correlation was found between cytotoxicity and degree of lectin-cell binding ($r=-0.34$, $P<0.001$), and poor correlation was observed between cytotoxicity and cytoagglutinating activity of WGA-isolectins ($r=0.16$, $P<0.01$). The results suggest that the WGA-isolectins, structurally distinguishable in only several amino acid sequences, interacted in different degrees with leukemic cells and manifested different cytoagglutinating and cytotoxic activity.

PMID: 12527110 [PubMed - indexed for MEDLINE]

[Cell Biol Toxicol.](#) 2003 Feb;19(1):3-12. Impact factor – 2.056

Effect of phenothiazines on protein kinase C- and calcium-dependent activation of peritoneal macrophages.

[Hadjimitova V](#), [Bakalova R](#), [Traykov T](#), [Ohba H](#), [Ribarov S](#).

Abstract

The effects of some phenothiazines (promethazine, PMZ; chlorpromazine, CPZ; levomepromazine, LVPZ; thioridazine, TRDZ; trifluoperazine, TFPZ) on the activation and viability of rat peritoneal macrophages were investigated. The macrophage activation was estimated by measuring of luminol-dependent chemiluminescence, induced by phorbol-12-myristate-13-acetate (PMA) (a protein kinase C activator) or calcium ionophore A23187. The viability of macrophages was determined using ATP bioluminescence as a criterion of cell viability. It was observed that all drugs, in concentrations higher than 1 micromol/L, markedly decreased the chemiluminescent index of PMA-activated or A23187-activated macrophages. The inhibitory effect was dose-dependent. It was better expressed in the case of CPZ, followed by TFPZ and TRDZ, and less expressed in the case of PMZ and LVPZ. The suppression of chemiluminescence of PMA-/A23187-activated macrophages by phenothiazines was not a result of their cytotoxic effect. Moreover, it was found that all drugs dose-dependently enhanced the viability of macrophages, estimated by ATP production. The inhibitory effects of phenothiazines on the chemiluminescence of PMA-/A23187-activated macrophages were greater than their ability to decrease KO₂-induced chemiluminescence as a result of interaction with superoxide radicals. It may be supposed that the inhibitory effect of phenothiazines on PMA-/A23187-induced chemiluminescence of macrophages is a result not only of interaction between drugs and superoxide radicals, generated during the "oxidative burst" of activated cells. Presumably the drugs have an immunomodulating effect on rat peritoneal macrophages.

PMID: 12661983 [PubMed - indexed for MEDLINE]

[Cancer Chemother Pharmacol.](#) 2003 Jun;51(6):451-8. Epub 2003 Apr 15. Impact factor – 2.759

Relationships between degree of binding, cytotoxicity and cytoagglutinating activity of plant-derived agglutinins in normal lymphocytes and cultured leukemic cell lines.

[Ohba H](#), [Bakalova R](#).

Abstract

PURPOSE: To clarify the relationships between the degree of lectin-cell binding, cytotoxicity and cytoagglutinating activity of plant-derived lectins in normal lymphocytes and cultured leukemic cell lines.

METHODS: Plant lectins with different quaternary structures and saccharide specificity were used: Dolichos biflorus agglutinin (DBA), Soybean agglutinin (SBA) and Wheat germ agglutinin (WGA). The leukemic cell lines used were: Jurkat, MOLT-4, RPMI-8402, HPB-ALL, CCR-HSB-2 and BALL-1 (derived from acute lymphoblastic leukemia); Raji and Daudi (derived from Burkitt's lymphoma); K-562 (derived from myelogenous leukemia). The lectin-cell binding was detected microscopically and fluorimetrically using FITC-conjugated lectins. Cytotoxicity was estimated by the CellTiter-Glo luminescent cell viability assay, and cytoagglutinating activity by a spectrophotometric method.

RESULTS: The binding of DBA and SBA to normal lymphocytes was negligible, while their binding to leukemic cells increased markedly with increasing lectin concentration. Analogous results were obtained for WGA. However, it was found that WGA also interacted to a significant degree with normal lymphocytes. The degree of lectin-cell binding increased in the order: DBA<SBA<WGA. The cytoagglutinating activity and cytotoxicity of lectins increased in the same order. DBA did not exhibit a cytotoxic effect against normal or leukemic cells, and showed a poor cytoagglutinating activity only in MOLT-4, CCR-HSB-2 and BALL-1 cells. SBA exhibited poor cytotoxicity against Jurkat, RPMI-8402, HPB-ALL and CCR-HSB-2 cells, but a well-defined cytotoxicity against Raji and Daudi cells. SBA showed poor cytoagglutinating activity in leukemic cells. In contrast, WGA at concentrations higher than 0.05 microM showed high cytotoxicity against all leukemic cell lines tested as well as against normal lymphocytes. WGA also showed a well-expressed cytoagglutinating effect in all cell lines except normal lymphocytes. There was a moderate inverse correlation between cell viability and the velocity of cytoagglutination ($r=-0.56$, $P<0.001$), and a good correlation between cell viability and the degree of lectin-cell binding ($r=-0.75$, $P<0.001$). There was a low positive correlation between the velocity of cytoagglutination and the degree of lectin-cell binding ($r=0.43$, $P<0.001$).

CONCLUSION: The results suggest that the lectins that bound most strongly to leukemic cells expressed higher cytotoxic and cytoagglutinating activities.

PMID: 12695857 [PubMed - indexed for MEDLINE]

[Nucleic Acids Res Suppl.](#) 2003;(3):237-8. Impact factor – 7.836

Control of intracellular delivery and inhibition of genetic expression by DNA-peptide conjugates.

[Kubo T](#), [Anno Y](#), [Yano M](#), [Takamori K](#), [Bakalova R](#), [Ohba H](#), [Fujii M](#).

Abstract

Various types of DNA-peptide conjugates were synthesized by solid phase fragment condensation (SPFC). DNA-LNS (nuclear localizing signal) peptide conjugate was proved to be delivered and localized into cellular nucleus and exhibited higher antisense inhibitory effect against telomerase than antisense phosphorothioate DNA. In contrast, DNAzymes conjugated with NES (nuclear export signal) peptide was shown to be taken up and localized in cytoplasm. Inhibitory effect of the conjugate DNAzyme against BCR-ABL tyrosine kinase was evaluated to be more significant than the native DNAzyme.

PMID: 14510468 [PubMed - indexed for MEDLINE]

[Nucleic Acids Res Suppl.](#) 2003;(3):177-8. Impact factor – 7.836

Conjugate DNazymes.

[Kubo T](#), [Takamori K](#), [Bakalova R](#), [Ohba H](#), [Fujii M](#).

Abstract

Conjugate DNazymes were synthesized by solid phase fragment condensation and their biological properties were characterized. They have increased affinity to target RNA, enhanced stability against DNase 1 digestion and comparable or higher RNA cleaving activity compared with native and also phosphorothioate DNA zymes. It was also demonstrated that conjugate DNazymes could inhibit BCR-ABL tyrosine kinase in cellular lysis of human leukemia cell line. Consequently DNazymes can be expected to act effectively in cellular system and also in vivo system.

PMID: 14510438 [PubMed - indexed for MEDLINE]

[Nucleic Acids Res Suppl.](#) 2003;(3):179-80. Impact factor – 7.836

Antisense effects of DNA-peptide conjugates.

[Kubo T](#), [Bakalova R](#), [Ohba H](#), [Fujii M](#).

Abstract

Antisense conjugate DNA covalently bound to functional amines, sugars or peptides such as nuclear localization signals (NLS), nuclear export signals (NES) and artificial amphiphilic alpha-helical and beta-sheet peptides were synthesized by solid phase fragment condensation (SPFC). Inhibitory effects on human telomerase of synthesized antisense conjugate DNAs were evaluated by TRAP assay. Antisense conjugate DNAs showed several times higher inhibitory effects on telomerase activity compared to native antisense DNA in cell lysis solutions. Antisense conjugate DNAs showed comparable or higher as same as inhibitory activity compared with antisense phosphorothioate DNA (S-DNA). Interestingly, antisense conjugate DNA showed much higher inhibition against telomerase activity with native antisense DNA or antisense phosphorothioate DNA in cellular system. We observed enhanced cellular uptake and positively controlled intracellular localization of conjugate DNAs by use of confocal fluorescent microscope.

PMID: 14510439 [PubMed - indexed for MEDLINE]

[Toxicol In Vitro.](#) 2004 Dec;18(6):765-71. Impact factor – 2.546

Hot-compressed-water decomposed products from bamboo manifest a selective cytotoxicity against acute lymphoblastic leukemia cells.

[Ando H](#), [Ohba H](#), [Sakaki T](#), [Takamine K](#), [Kamino Y](#), [Moriwaki S](#), [Bakalova R](#), [Uemura Y](#), [Hatate Y](#).

Abstract

We examined the effect of hot-compressed-water (HCW) extracted and fractionated bamboo products (named as fractions A and B) on the viability of human cultured cell lines, derived from leukemia patients and human peripheral blood lymphocytes, obtained from normal adults. Fraction A was composed of xylose, xylooligosaccharides and water-soluble lignin, determined by high-performance anion exchange chromatography and spectrophotometry. Fraction B was composed of glucose and celooligosaccharides. It was found that Fraction B expressed a negligible

cytotoxic effect against leukemia cells, while Fraction A reduced markedly (in a dose-dependent manner) the viability of leukemia cell lines, derived from acute lymphoblastic leukemia (ALL)--Jurkat and MOLT-4. Fraction A did not influence the viability of leukemia cells, derived from myelogenous leukemia (ML-2) or lymphoma (SupT-1), as well as the viability of normal lymphocytes. Furthermore, microscopic examination of ALL-derived cells treated with Fraction A showed typical apoptotic morphological changes such as a condensation of nucleus and membrane blebbing, as well as phosphatidylserine (PSer) exposure on the cell surface. The effect of decomposed products of commercially available xylan against ALL-derived Jurkat cells was significantly lower than that of Fraction A. These results suggest that the cytotoxic effect of Fraction A may be attributed to apoptosis, induced by xylooligosaccharides and it is specific for ALL-derived cells. We speculate that the water-soluble lignin is an important factor, potentiating the cytotoxic effect of xylan in HCW-extracts from bamboo.

PMID: 15465641 [PubMed - indexed for MEDLINE]

[Luminescence](#). 2004 Nov-Dec;19(6):319-21. Impact factor – 1.395

Chemiluminescent analysis of the antioxidant and immunomodulation effects of several psychotropic drugs on peritoneal macrophages.

[Hadjimitova V](#), [Traykov T](#), [Bakalova R](#), [Petrova V](#), [Lambev I](#), [Ohba H](#), [Ishikawa M](#), [Baba Y](#).

Abstract

The present study describes the application of several chemiluminescent (CL) methods for evaluation of antioxidant and immunomodulation effects of psychotropic drugs upon phagocytes: KO₂-induced luminal-dependent CL for detection of superoxide anion radicals in a pure chemical system; PMA- and A23187-induced CL of peritoneal macrophages for detection of free radicals in cell suspension; and CL, produced by the luciferase-catalyzed luciferin + ATP reaction, for evaluation of cell viability before and after drug application. These methods provide also a way to investigate the location of drug action. It was found that the psychotropic drugs influence the 'oxidative burst' of macrophages through two mechanisms: by expression of drug antioxidant properties and/or by a direct immunomodulation effect.

PMID: 15558671 [PubMed - indexed for MEDLINE]

[Cancer Chemother Pharmacol](#). 2004 Aug;54(2):161-72. Epub 2004 Apr 23. Impact factor – 2.759

Atypical protein-kinase C-zeta, but neither conventional Ca²⁺ -dependent protein-kinase C isoenzymes nor Ca²⁺ -calmodulin, participates in regulation of telomerase activity in Burkitt's lymphoma cells.

[Bakalova R](#), [Ohba H](#), [Zhelev Z](#), [Kubo T](#), [Fujii M](#), [Ishikawa M](#), [Shinohara Y](#), [Baba Y](#).

Abstract

PURPOSE: To clarify the role of the pathways dependent on protein-kinase C (PK-C) and Ca²⁺/calmodulin (CaM) in the regulation of telomerase activity in Burkitt's lymphoma cells.

METHODS: Burkitt's lymphoma cells (Raji and Daudi) were treated with the PK-C inhibitor, bisindolylmaleimide (BIM), or the CaM inhibitor, trifluoperazine (TFPZ), in a dose-dependent manner and in a time-dependent manner. The activities of PK-C isoenzymes were analyzed fluorimetrically using POLARIS assay kits. CaM-kinase II

activity was analyzed radiographically, using CaMK-II immunoprecipitation kinase assay kits. Telomerase activity was detected by a conventional telomeric repeat amplification protocol and Stretch PCR. The level of catalytic subunit of telomerase (hTERT) in drug-treated and nontreated cells was analyzed by flow cytometry using anti-hTERT antibody labeled with ZenonAlexa Fluor-488 IgG. Apoptosis was estimated in terms of phosphatidylserine exposure on the cell surface and DNA fragmentation.

RESULTS: It was found that BIM inhibited telomerase activity and this process preceded apoptosis. The subsequent addition of exogenous PK-C (mixture of isoenzymes) to the cell lysates restored telomerase activity if incubation of cells with BIM was up to 24 h. Using PK-C isoenzymes, it was established that atypical PK-Czeta, but not conventional Ca²⁺-dependent PK-Calpha, PK-Cbeta or PK-Cgamma, is responsible for the reactivation of telomerase in BIM-treated cells. BIM also showed a well-expressed cytotoxicity against intact leukemia cells. In contrast, the CaM inhibitor TFPZ showed the same cytotoxic effect without any influence on telomerase activity during incubation for 24 h with leukemia cells. After incubation for 48 h, TFPZ markedly suppressed telomerase activity. However, the effect followed apoptosis and appeared to be a result of cell death. The addition of exogenous CaMK-II to the cell lysates obtained from TFPZ-treated cells did not reactivate telomerase.

CONCLUSION: The present study confirmed the participation of atypical PK-Czeta, but not conventional Ca²⁺-dependent PK-C isoenzymes (alpha, beta, gamma) nor the Ca²⁺/CaM-dependent pathway, in the regulation of telomerase activity in Burkitt's lymphoma cells.

PMID: 15106017 [PubMed - indexed for MEDLINE]

[Cancer Chemother Pharmacol.](#) 2004 Mar;53(3):267-75. Epub 2003 Dec 9. Impact factor – 2.759

Phenothiazines suppress proliferation and induce apoptosis in cultured leukemic cells without any influence on the viability of normal lymphocytes. Phenothiazines and leukemia.

[Zhelev Z](#), [Ohba H](#), [Bakalova R](#), [Hadjimitova V](#), [Ishikawa M](#), [Shinohara Y](#), [Baba Y](#).

Abstract

PURPOSE: The purpose of the present study was to investigate the effects of phenothiazines (at clinically relevant doses) on the viability and proliferation of leukemic cell lines and normal lymphocytes, and to investigate the possibility of specific induction of apoptosis in leukemic cells.

METHODS: Phenothiazines with different chemical structure and hydrophobicity were used: chlorpromazine (CPZ); levomepromazine (LVPZ); prometazine (PMZ); trifluoperazine (TFPZ); thioridazine (TRDZ). The leukemic cell lines used were: Daudi and Raji (derived from Burkitt's lymphoma), K-562 (derived from myelogenous leukemia), and BALL-1, MOLT-4, HPB-ALL and CCRF-HSB-2 (derived from acute lymphoblastic leukemia). The cytotoxicity of the phenothiazines was determined by a CellTiter-Glo luminescent cell viability assay, using ATP bioluminescence as a marker of cell viability as well as a marker of mitochondrial activity. The proliferation of leukemic cells was determined using a CellTiter-AQ cell proliferation assay which is based on the reduction of a methyl-tetrazolium compound to the formazan product. Apoptosis induction was estimated using phosphatidylserine

(PSer) translocation to the cell surface and DNA fragmentation as characteristics of the process.

RESULTS: Phenothiazines (at concentrations in the range 0.1-10 micro M) did not affect the viability of normal lymphocytes during a 24-h incubation. Moreover, about 15-20% increase in ATP bioluminescence was observed in normal cells during treatment with 40 micro M phenothiazines. In contrast, the phenothiazines manifested strong cytotoxicity and antiproliferative activity against leukemic cells. The most powerful drugs were TFPZ and TRDZ, followed by CPZ. They showed a significant cytotoxic effect against leukemic cells even at 5-10 micro M. The most sensitive cell lines were MOLT-4 and Raji, and the most resistant were HPB-ALL and CCRF-HSB-2. All phenothiazines induced PSer exposure on the surface of leukemic cells, but not of normal lymphocytes. TFPZ, TRDZ and CPZ also induced DNA fragmentation in almost all leukemic cell lines during a 48-h incubation. The strongest apoptotic agent was TRDZ. The apoptosis induction was not accompanied by a significant release of cytochrome c from the mitochondria into the cytoplasm of native cells. Moreover, the drugs markedly suppressed Ca(2+)-induced cytochrome c release in isolated mitochondria of leukemic cells.

CONCLUSIONS: The results suggest that in clinically relevant doses (up to 20 micro M) some phenothiazines (TFPZ, TRDZ, CPZ) expressed a selective cytotoxicity and antiproliferative activity, and induced apoptosis in leukemic cells without any influence on the viability of normal lymphocytes. It is considered that the mechanism of apoptosis induction in phenothiazine-treated leukemic cells is associated with inhibition of mitochondrial DNA polymerase and decreased ATP production, which are crucial events for the viability of cancer cells.

PMID: 14663628 [PubMed - indexed for MEDLINE]

[Toxicol Appl Pharmacol](#). 2004 Mar 1;195(2):182-93. Impact factor – 3.993

Plant-derived abrin-a induces apoptosis in cultured leukemic cell lines by different mechanisms.

[Ohba H](#), [Moriwaki S](#), [Bakalova R](#), [Yasuda S](#), [Yamasaki N](#).

Abstract

Abrin-a consists of A-chain with N-glycosidase activity, which inhibits protein synthesis, and lectin-like B-chain responsible for binding with cell-surface receptors and penetrating of abrin-a molecule into the cells. As a lectin component, the B-chain can also participate in cell signal transduction. It has been reported that abrin induces apoptosis, but the molecular mechanism(s) of this induction have been obscure and several alternative variants have been discussed. The present study demonstrates that abrin-a induces apoptosis in human cultured cell lines, derived from acute lymphoblastic leukemia (ALL) (Jurkat, CCRF-CEM, MOLT-4, HPB-ALL). The apoptosis was estimated by: phosphatidylserine (PSer) exposure at the cell surface, activation of caspase cascade, and DNA fragmentation. The penetrating of abrin-a into the cells was detected by fluorescent confocal microscopy, using fluorescein isothiocyanate (FITC) as a fluorescent marker. It was established that the effect of abrin-a on the apoptosis induction in leukemic cells was dose- and time-dependent. The process was initiated 1 h after abrin-a application (before its penetrating into the cells) and was characterized with PSer translocation from the inner to the outer monolayer of plasma membrane, caspase activation on the first to second hour after beginning of treatment, with maximum on the third to fourth hour, and DNA fragmentation on the fourth to sixth hour, depending of the cell line. The exposure of

PSer on the cell surface was detected in Jurkat, CCRF-CEM, and MOLT-4 cells. In HPB-ALL, no significant changes in PSer exposure on the cell surface was observed. Activation of caspase-3, -8, and -9 was detected in Jurkat, MOLT-4, and HPB-ALL. Surprisingly, the activity of caspase-3 increased on the first hour after beginning of treatment, while the activity of caspase-8 and -9 began to increase on the second hour. In CCRF-CEM, activation of caspases was not measured, but the apoptosis progressed to DNA fragmentation in a dose- and time-dependent manner. DNA fragmentation was also detected in Jurkat, but not in MOLT-4 and HPB-ALL cells. It seems that the mechanisms of abrin-a-induced apoptosis are different and the progress of apoptosis depends of the cell line. There was a very good positive correlation between the agglutinating activity of abrin-a and development of apoptosis to DNA fragmentation. The time-dependent effects of abrin-a on apoptosis as well as its time-dependent penetration into the cells suggest that the B-chain probably triggers the apoptosis, while the A-chain and breakage of the disulfide bond are responsible for its progress.

PMID: 14998684 [PubMed - indexed for MEDLINE]

[FEBS Lett.](#) 2004 Apr 23;564(1-2):73-84. Impact factor – 3.601

Antisense inhibition of Bcr-Abl/c-Abl synthesis promotes telomerase activity and upregulates tankyrase in human leukemia cells.

[Bakalova R](#), [Ohba H](#), [Zhelev Z](#), [Kubo T](#), [Fujii M](#), [Ishikawa M](#), [Shinohara Y](#), [Baba Y](#).

Abstract

Clinical studies in chronic myelogenous leukemia demonstrate that the overexpression of Bcr-Abl tyrosine kinase is usually accompanied by relatively low telomerase activity in the chronic phase, which reverts to a high activity in blast crisis. The present study was designed to investigate the cross-talk between both enzymes, using Bcr-Abl-positive K-562 and Bcr-Abl-negative Jurkat cell lines, treated with antisense oligodeoxyribonucleotides (ODNs) against Bcr-Abl/c-Abl mRNA. The decreased amount and enzyme activity of Bcr-Abl/c-Abl provoked telomerase activation in both cell lines. After short-term treatment with anti-Bcr-Abl/c-Abl ODNs (6 days), no variations in hTERT and phospho-hTERT were detected. The decreased amount of Bcr-Abl/c-Abl was accompanied by: alterations in telomeric associated proteins-overexpression of tankyrase and decreased amount of TRF1/Tin2, cell growth arrest of K-562 cells, reaching a plateau after 6 days treatment, and increased proliferating activity of Jurkat cells. No changes in telomere length were detected after short-term treatment. In contrast, after long-term treatment with anti-Bcr-Abl/c-Abl ODNs (36 days), a significant elongation of telomeres and enhancement of hTERT were established, accompanied by an increased proliferating activity of both cell lines. These data provide evidence that the inhibition of Bcr-Abl or c-Abl synthesis keeps a potential to restore or induce cell proliferation through telomere lengthening control and telomerase activation.

PMID: 15094045 [PubMed - indexed for MEDLINE]

[FEBS Lett.](#) 2004 Jul 16;570(1-3):195-204. Impact factor – 3.601

Suppression of bcr-abl synthesis by siRNAs or tyrosine kinase activity by Glivec alters different oncogenes, apoptotic/antiapoptotic genes and cell proliferation factors (microarray study).

[Zhelev Z](#), [Bakalova R](#), [Ohba H](#), [Ewis A](#), [Ishikawa M](#), [Shinohara Y](#), [Baba Y](#).

Abstract

Short 21-mer double-stranded/small-interfering RNAs (ds/siRNAs) were designed to target bcr-abl mRNA in chronic myelogenous leukemia. The ds/siRNAs were transfected into bcr-abl-positive K-562 (derived from blast crisis chronic myelogenous leukemia), using lipofectamine. Penetrating of ds/siRNAs into the cells was detected by fluorescent confocal microscopy, using fluorescein-labeled ds/siRNAs. The cells were treated with mix of three siRNA sequences (3 x 60 nM) during 6 days with three repetitive transfections. The siRNA-treatment was accompanied with significant reduction of bcr-abl mRNA, p210, protein tyrosine kinase activity and cell proliferation index. Treatment of cells with Glivec (during 8 days with four repetitive doses, 180 nM single dose) resulted in analogous reduction of cell proliferation activity, stronger suppression of protein tyrosine kinase activity, and very low reduction of p210. siRNA-mix and Glivec did not affect significantly the viability of normal lymphocytes. Microarray analysis of siRNA- and Glivec-treated K-562 cells demonstrated that both pathways of bcr-abl suppression were accompanied with overexpression and suppression of many different oncogenes, apoptotic/antiapoptotic and cell proliferation factors. The following genes of interest were found to decrease in relatively equal degree in both siRNA- and Glivec-treated cells: Bcl2, Bcl2l1 and Bcl2l2 proto-oncogene, chromatin-specific transcription elongation factor FACT 140-kDa subunit mRNA, gene encoding splicing factor SF1, and mRNA for Tec protein tyrosine kinase. siRNA-mix and Glivec provoked overexpression of the following common genes: c-jun proto-oncogene, protein kinase C- α , pvt1 oncogene homologue (myc activator), interleukin-6, 1-8D gene from interferon-inducible gene family, tumor necrosis factor receptor superfamily (10b), and STAT-induced STAT inhibitor.

PMID: 15251464 [PubMed - indexed for MEDLINE]

[Cancer](#). 2004 Sep 15;101(6):1390-403. Impact factor – 5.131

Inhibition of bcr-abl and/or c-abl gene expression by small interfering, double-stranded RNAs: cross-talk with cell proliferation factors and other oncogenes.

[Ohba H](#), [Zhelev Z](#), [Bakalova R](#), [Ewis A](#), [Omori T](#), [Ishikawa M](#), [Shinohara Y](#), [Baba Y](#).

Abstract

BACKGROUND: Short, 21-mer, double-stranded/small interfering RNAs (ds/siRNAs) were designed to target bcr-abl mRNA in chronic myelogenous leukemia (CML) with a potential also to target c-abl mRNA.

METHODS: ds/siRNAs were transfected into bcr-abl-positive K-562 cells (derived from blast-crisis) or bcr-abl-negative/c-abl-positive Jurkat cells (derived from acute lymphoblastic leukemia) using lipofectamine. ds/siRNAs intracellular uptake was detected by fluorescent confocal microscopy using fluorescein-labeled ds/siRNAs. The treatment was performed over 6 days with repetitive siRNA transfections. Efficiency of the siRNAs was determined 24 hours after single siRNA transfection and 6 days after repetitive siRNA transfections.

RESULTS: Two of the designed ds/siRNAs decreased the target mRNA levels markedly (determined by reverse transcriptase-polymerase chain reaction analysis) and bcr-abl/c-abl oncoproteins (determined by flow cytometry using Fluor-488-labeled, anti-c-abl antibody as well as by Western blot analysis). These sequences also inhibited protein tyrosine kinase activity significantly and suppressed cell proliferation. One of the three selected ds/siRNAs expressed only slight effects on the bcr-abl/c-abl mRNA in K-562 cells (but not on the oncoprotein level), on protein

tyrosine kinase activity, and on cell proliferation. The combination of the three ds/siRNA constructs provoked stronger decreases in bcr-abl/c-abl mRNAs and their respective oncoproteins and produced the strongest suppression of cell proliferation.

CONCLUSIONS: The cross-talk between siRNA interference of bcr-abl oncogene and the expression of several apoptotic/antiapoptotic factors, cell proliferation factors, and other oncogenes exists and it was determined by microarray analysis in K-562 cells that were treated over 6 days.

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PMID: 15368327 [PubMed - indexed for MEDLINE]

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Synthesis, Biological Properties and Antisense Effects of Oligonucleotide-Petide Conjugates

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Abstract: In order to improve the biological and pharmacological properties of antisense oligonucleotides, we have been recently focussed our efforts on synthesis of DNA-peptide conjugates and biological evaluation of them. Oligonucleotides can be covalently linked to peptides composed of any sequence of amino acids by SPFC [1]. The peptides incorporated into the conjugates include nuclear localizing signals (NLS), nuclear export signals (NES), membrane fusion domain of some viral proteins and some designed cationic α -helical or β -sheet peptides with amphipathic character. Some polyamines and sugars were also conjugated with oligonucleotides by SPFC in good yields. Evaluation of biological properties of DNA-peptide conjugates indicated that (a) the conjugates could bind to target RNA and dsDNA with increased affinity, (b) the conjugates were more resistant to cellular nuclease degradation, (c) the conjugates-RNA hybrids could activate RNase H as effective as native oligonucleotides, (d) the conjugates with fusion peptides showed largely enhanced cellular uptake, (e) the conjugates with NLS could be predominantly delivered into cell nucleus, (f) the conjugates with NES could be localized in cytoplasm. As a result, antisense oligonucleotides conjugated with NLS could inhibit human telomerase in human leukemia cells much more strongly than phosphorothioate oligonucleotides.

[Org Biomol Chem](#). 2005 Sep 21;3(18):3257-9. Epub 2005 Aug 15. Impact factor – 3.451

Controlled intracellular localization and enhanced antisense effect of oligonucleotides by chemical conjugation.

[Kubo T](#), [Zhelev Z](#), [Bakalova R](#), [Ohba H](#), [Doi K](#), [Fujii M](#).

Abstract

Oligonucleotides can be covalently linked to peptides composed of any sequence of amino acids by solid phase fragment condensation. The peptides incorporated into the conjugates include nuclear localizing signals (NLS), nuclear export signals (NES), membrane fusion domain of some viral proteins and some designed peptides with amphipathic character. Evaluation of biological properties of DNA-peptide conjugates indicated that (a) the conjugates could bind to target RNA and dsDNA with increased affinity, (b) the conjugates were more resistant to cellular nuclease degradation, (c) the conjugate-RNA hybrids could activate RNase H as effectively as native oligonucleotides, (d) the conjugates with fusion peptides showed largely enhanced cellular uptake, (e) the conjugates with NLS could be predominantly delivered into the cell nucleus, (f) the conjugates with NES could be localized in the cytoplasm. As a result, antisense oligonucleotides conjugated with NLS could inhibit human telomerase in human leukemia cells much more strongly than phosphorothioate oligonucleotides.

PMID: 16132084 [PubMed - indexed for MEDLINE]

[Bioorg Med Chem Lett](#). 2005 Jan 3;15(1):167-70. Impact factor – 2.661

Efficient cleavage of RNA, enhanced cellular uptake, and controlled intracellular localization of conjugate DNAzymes.

[Kubo T](#), [Takamori K](#), [Kanno K](#), [Bakalova R](#), [Ohba H](#), [Matsukisono M](#), [Akebiyama Y](#), [Fujii M](#).

Abstract

Conjugate DNAzymes with polyamines and peptides were successfully prepared by solid phase fragment condensation (SPFC) and showed up to 4.2 times higher catalytic efficiency ($k(\text{cat})/K(\text{m})$) and enhanced tolerance against DNase I digestion. To be pointed out, intracellular localization of DNAzymes could be controlled by conjugated with naturally occurring signal peptides responsible for nuclear cytoplasmic transport of proteins.

PMID: 15582433 [PubMed - indexed for MEDLINE]

[Electrophoresis](#). 2005 Aug;26(15):3021-4. Impact factor – 3.569

Nonradioactive telomerase activity assay by microchip electrophoresis: privileges to the classical gel electrophoresis assay.

[Zhelev Z](#), [Bakalova R](#), [Ewis A](#), [Ohba H](#), [Ishikawa M](#), [Baba Y](#).

Abstract

The present study accents on the privileges of microchip-based electrophoresis to the conventional gel electrophoresis in separation of telomerase repeat amplification protocol/polymerase chain reaction (PCR) ladder products obtained in telomerase-catalyzed reaction in cancer cells. We try to clarify the interpretation of the results obtained by both electrophoretic procedures and to avoid misinterpretation as a result of PCR-dependent artefacts.

PMID: 16078194 [PubMed - indexed for MEDLINE]

[FEBS Lett](#). 2005 Nov 7;579(27):6128-34. Epub 2005 Oct 11. Impact factor – 3.601

The NK-lysin derived peptide NK-2 preferentially kills cancer cells with increased surface levels of negatively charged phosphatidylserine.

[Schröder-Borm H](#), [Bakalova R](#), [Andrä J](#).

Abstract

The NK-lysin derived peptide NK-2 is a potent antibacterial, but non-toxic to a human keratinocyte cell line and of low hemolytic activity. Its target selectivity is based upon a strong binding preference to membranes containing anionic phospholipids, which are normally not found on the surface of human cells. Here, we analyzed the interaction of NK-2 with normal human lymphocytes and seven different human cancer cell lines and demonstrate that some of these cells expose negatively charged surface phosphatidylserine (PS), which presumably facilitates killing of the cells by NK-2. This is underlined by the specific intercalation of the peptide into PS-containing liposomes analyzed by fluorescence-resonance energy transfer spectroscopy.

PMID: 16269280 [PubMed - indexed for MEDLINE]

[J Biochem Biophys Methods](#). 2007 Apr 10;70(3):503-6. Epub 2006 Sep 16. Impact factor – 1.808

Dual-labeled telomere sensing probes for quantification of telomerase activity assay.

[Bakalova R](#), [Zhelev Z](#), [Kubo T](#), [Mileva M](#), [Ohba H](#).

Abstract

The present study describes an empirically discovered phenomenon that might be useful for development of a sensitive and rapid methodology for quantification of telomerase activity assay with simple data acquisition and possibility for calculation of telomerase product in absolute units. The method is based on the design and application of two single-stranded telomere sensing probes consisting of dual-labeled 16-mer oligonucleotides (fluorescent Cy3/Cy3-labeled and non-fluorescent IowaBlack/BHQ-labeled) that can simultaneously hybridize on the primary product of the telomerase reaction.

PMID: 17055587 [PubMed - indexed for MEDLINE]

[Oligonucleotides](#). 2007 Winter;17(4):445-64. Impact factor – 2.986

Modified 27-nt dsRNAs with dramatically enhanced stability in serum and long-term RNAi activity.

[Kubo T](#), [Zhelev Z](#), [Ohba H](#), [Bakalova R](#).

Abstract

The present study describes improved properties of 27-nt dsRNAs over 21-nt siRNAs, and accents on the possibility to use their modifications and conjugates for direct long-term gene silencing in viable cells and animals, avoiding conventional transfectants. Using a Renilla Luciferase gene-silencing system and cultured cell lines, we established that 27-nt dsRNAs possessed about three to five times higher "long-term" RNAi activity than 21-nt siRNAs and 21-nt dsRNAs. Moreover, if RNA duplexes were preincubated with cell-cultured medium for several hours before their transfection in cells, 21-mer completely lost its RNAi effect, while 27-mer, its amino modifications, thiol modifications, and cholesterol conjugates manifested a strong gene silencing. In attempts to clarify the reason(s) for the higher RNAi activity of 27-nt dsRNAs, we found that they were approximately 100 times more stable than 21-nt siRNA and 21-nt dsRNA in cell-cultured medium supplemented with 10% inactivated serum, approximately 50 times more stable in 90% inactivated serum, and approximately six times more stable in active serum. The 5' sense modification was

selected as the most stable, accessible to Dicer, and with highest RNAi potential. The RNAi activity of 5' sense modifications was higher even than the activity of nonmodified 27-nt dsRNA. The 5' sense amino modification also did not influence the activity of 21-nt siRNA, right overhang 25/27-nt (R25D/27), and 25D/27-nt RNAs. The stability of 5' sense modified R25D/27-nt and 25D/27-nt RNAs in serum was lower than that of blunt 27-nt dsRNA. However, these asymmetric RNAs were more active than modified and nonmodified blunt 27-nt dsRNAs, which demonstrates the superiority of the asymmetric design. The 5' sense modifications were considered as most appropriate for conjugation with small signal molecules to facilitate the intracellular delivery of RNA duplex, to preserve its RNAi capacity, and to ensure a possibility for rapid long-term gene silencing in viable cells and animals. The 5' sense conjugation with cholesterol approved this assumption.

PMID: 17894530 [PubMed - indexed for MEDLINE]

[Methods Find Exp Clin Pharmacol](#). 2007 Jul-Aug;29(6):417-21. Impact factor – 1.037
RNA interference--about the reality to be exploited in cancer therapy.
[Bakalova R](#).

Abstract

The discovery of RNA interference (RNAi) in mammalian cells raises the expectations of gene therapy, especially in cancer. However, it is too early to say how great this promise may be because of many disputable problems including intracellular delivery of siRNA, the transient nature of RNAi and potential side effects after long-term treatment. The present microarray study demonstrates that the RNAi of one oncogene (encoding bcr-abl fusion protein in chronic myelogenous leukemia) triggers an overexpression of other "sleeping" oncogenes, antiapoptotic genes and factors, preserving the immortalization of bcr-abl-positive leukemia cells.

PMID: 17922071 [PubMed - indexed for MEDLINE]

[Biochem Biophys Res Commun](#). 2008 Jan 4;365(1):54-61. Epub 2007 Oct 29. Impact factor – 2.595

Chemically modified symmetric and asymmetric duplex RNAs: an enhanced stability to nuclease degradation and gene silencing effect.

[Kubo T](#), [Zhelev Z](#), [Ohba H](#), [Bakalova R](#).

Abstract

The present study accents on the relationship between dicing, nuclease stability, and RNAi activity of various types of chemically modified symmetric and asymmetric dsRNAs, covalently bound with amino-groups or cholesterol at one or both terminals. All modified dsRNAs were subjected to cleavage by recombinant Dicer enzyme. They possessed a high resistance to nuclease degradation in cell cultured medium and an excellent RNAi activity in viable cells. The best stability and RNAi activity was detected for 5'-sense amino-modified RNAs. These modifications manifested also a high long-term gene silencing effect within seven days post-transfection, while the RNAi activity of the native 21nt siRNA expired within two days. The conjugation of dsRNA with cholesterol at 5'-sense end resulted in easy intracellular delivery without transfection reagents. After a direct transfection in cells, the cholesterol-conjugated 27nt dsRNA possessed a higher RNAi activity than cholesterol-conjugated 21nt siRNA.

PMID: 17971296 [PubMed - indexed for MEDLINE]

[Jpn. J. Appl. Phys.](#), 2008, 47(2): 1346–50. Impact factor – 1.018

Highly efficient gene suppression by chemically modified 27 nucleotide double-stranded RNAs

[Takanori K](#), [Zhelev Z](#), [Bakalova R](#), [Ohba H](#)

Abstract

RNA interference (RNAi) technology, described by Fire and Mello in 1998, is a powerful tool for the suppression of gene expression in mammalian cells. RNAi technology has several advantages over other chemical and genetic drugs. However, several problems in RNAi technology, such as cellular delivery, nuclease stability, and side effects, should be solved before applying it in the clinic. In this study, we focused on the development of novel chemically modified 27 nucleotide (nt) doublestranded RNAs (dsRNAs) with improved biological properties. Our chemically modified 27 nt dsRNAs exhibited an enhanced RNAi activity and a markedly increased stability in cell culture medium (containing 10% serum) in comparison with widely used 21 nt siRNAs and recently reported nonmodified 27 nt dsRNAs. The chemically modified 27 nt dsRNAs also exhibited a strong high long-term gene silencing effect after the 7 d treatment of viable cells. The chemically modified 27 nt dsRNAs in specific positions could be processed to 21 nt siRNAs by a recombinant Dicer enzyme. We suggested that the chemically modified 27 nt dsRNAs could be used for therapeutic applications (as genetic drugs) and bioanalyses. [DOI: 10.1143/JJAP.47.1346]

[Chem Commun \(Camb\)](#). 2009 Jan 7;(1):53-5. Epub 2008 Nov 13. Impact factor – 5.787

Nitroxyl radicals as low toxic spin-labels for non-invasive magnetic resonance imaging of blood-brain barrier permeability for conventional therapeutics.

[Zhelev Z](#), [Bakalova R](#), [Aoki I](#), [Matsumoto K](#), [Gadjeva V](#), [Anzai K](#), [Kanno I](#).

Abstract

The present study describes a novel non-radioactive methodology for in vivo non-invasive, real-time imaging of blood-brain barrier (BBB) permeability for conventional drugs, using nitroxyl radicals as spin-labels and magnetic resonance imaging (MRI).

PMID: 19081996 [PubMed - indexed for MEDLINE]

[Mol Pharm](#). 2009 Mar-Apr;6(2):504-12. Impact factor – 5.400

Nitroxyl radicals for labeling of conventional therapeutics and noninvasive magnetic resonance imaging of their permeability for blood-brain barrier: relationship between structure, blood clearance, and MRI signal dynamic in the brain.

[Zhelev Z](#), [Bakalova R](#), [Aoki I](#), [Matsumoto K](#), [Gadjeva V](#), [Anzai K](#), [Kanno I](#).

Abstract

The present study describes a novel nonradioactive methodology for in vivo noninvasive, real-time imaging of blood-brain barrier (BBB) permeability for conventional drugs, using nitroxyl radicals as spin-labels and magnetic resonance imaging (MRI). Two TEMPO-labeled analogues (SLENU and SLCNUgly) of the anticancer drug lomustine [1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea] were

synthesized, using a substitution of the cyclohexyl part with nitroxyl radical. Nonmodified nitroxyl radical TEMPOL was used for comparison. The nitroxyl derivatives were injected intravenously in healthy mice via the tail vein, and MR imaging of the brain was performed on a 7.0 T MRI. The MRI signal dynamic of SLENU and SLCNUgly followed the same kinetics as nonmodified TEMPO radical. SLENU and SLCNUgly were rapidly transported and randomly distributed in the brain tissue, which indicated that the exchange of cyclohexyl part of lomustine with TEMPO radical did not suppress the permeability of the anticancer drug for BBB. The selected nitroxyl derivatives possessed different hydrophobicity, cell permeabilization ability, and blood clearance. Based on these differences, we investigated the relationship between the structure of nitroxyl derivatives, their half-life in the circulation, and their MRI signal dynamic in the brain. This information was important for estimation of the merits and demerits of the described methodology and finding pathways for overcoming the restrictions.

PMID: 19718801 [PubMed - indexed for MEDLINE]

[Gen Physiol Biophys](#). 2009 Dec;28(4):356-62. Impact factor – 1.146

EPR signal reduction kinetic of several nitroxyl derivatives in blood in vitro and in vivo.

[Zhelev Z](#), [Matsumoto K](#), [Gadjeva V](#), [Bakalova R](#), [Aoki I](#), [Zheleva A](#), [Anzai K](#).

Abstract

The present study is focused on the mechanism(s) of electron-paramagnetic resonance (EPR) signal reduction kinetic of several nitroxyl radicals and nitroxyl-labeled anticancer drugs in physiological solutions in the context of their application for evaluation of oxidation/reduction status of blood and tissues--an important step in biomedical diagnostics and planning of therapy of many diseases. The nitroxyl derivatives were characterized with different size and water-solubility. Some of them are originally synthesized. In buffer, in the absence of reducing and oxidizing equivalents, the EPR signal intensity of all nitroxyls was constant with the time. In serum and cell cultured medium, in an absence of cells and in a negligible amount of reducing and oxidizing equivalents, there was no significant EPR signal reduction, too. In vitro (in freshly isolated blood samples), the EPR signal intensity was characterized with slow decrease within 30 min, presumably as a result of interaction between the nitroxyl derivative and blood cells. The EPR spectrum of hydrophobic nitroxyls showed a slight anisotropy in cell-containing solutions and it did not change in non-cell physiological solutions. This suggests for a limited motion of more hydrophobic nitroxyls through their preferable location in cell membranes. In vivo (in the bloodstream of mice under anesthesia), the EPR signal reduction kinetic was characterized by two phases: i) a rapid enhancement within 30 s as a result of increasing of nitroxyl concentration in the bloodstream after its intravenous injection, followed by ii) a rapid decrease (approximately 80-100%) within 2-5 min, presumably as a result of transportation of nitroxyl in the tissues. The hydrophobic nitroxyls were characterized with stronger and faster decrease in EPR signal intensity in the blood in vivo, as a result of their higher cell permeability, rapid clearance from the bloodstream and/or transportation in the surrounding tissues. The hydrophilic nitroxyls persist in the bloodstream (in their radical form) for a comparatively long time. The data suggest that the hydrophobic cell-permeable nitroxyl derivatives are most appropriate for evaluation of cell and tissue oxidation/reduction status, while the hydrophilic nitroxyls (impermeable for cell membranes or with very slow cell

permeability) are most appropriate for evaluation of oxidation/reduction status of blood using EPR imaging.

PMID: 20097958 [PubMed - indexed for MEDLINE]

[Neurosci Res.](#) 2009 Sep;65(1):64-70. Epub 2009 May 23. Impact factor – 2.096

Effect of cyclooxygenase-2 on the regulation of cerebral blood flow during neuronal activation in the rat.

[Matsuura T](#), [Takuwa H](#), [Bakalova R](#), [Obata T](#), [Kanno I](#).

Abstract

The present study was designed to clarify the precision of the main approach for investigating the regulation of local cerebral blood flow (CBF) response to neuronal activation in the brain (neurovascular coupling). In this study, we examined the effects of NS-398, a highly selective cyclooxygenase-2 inhibitor, on the physiological variables, baseline CBF, and local CBF response during rat somatosensory neuronal activation by laser-Doppler flowmetry. Blood pressure and heart rate were significantly decreased 3h after i.v. infusion of NS-398. Baseline CBF and local CBF during somatosensory activation gradually decreased with an increase in time of NS-398 infusion up to 3h, although neuronal activity in the somatosensory area was almost constant during the infusion. The results suggest that cyclooxygenase-2 participates in the regulation of local CBF during neuronal activation in rats. The present study also revealed the potential side-effects of dimethylsulfoxide, a solvent of NS-398, on neurovascular coupling.

PMID: 19467272 [PubMed - indexed for MEDLINE]

[Mol Biosyst.](#) 2010 Dec;6(12):2386-8. Epub 2010 Oct 8. Impact factor – 3.825

Imaging of cancer by redox-mediated mechanism: a radical diagnostic approach.

[Zhelev Z](#), [Bakalova R](#), [Aoki I](#), [Gadjeva V](#), [Kanno I](#).

Abstract

The present study describes a new diagnostic approach for carcinogenesis based on the different tissue redox activity of normal and cancer-bearing mammals and its visualization and estimation by cell permeable and DNA annealing probe (nitroxide-labeled nitrosourea) and magnetic resonance imaging.

PMID: 20936212 [PubMed - indexed for MEDLINE]

[J Physiol Sci.](#) 2010 Nov;60(6):399-406. Epub 2010 Oct 7. Impact factor – 1.356

Contribution of nitric oxide to cerebral blood flow regulation under hypoxia in rats.

[Takuwa H](#), [Matsuura T](#), [Bakalova R](#), [Obata T](#), [Kanno I](#).

Abstract

This study was designed to clarify whether nitric oxide (NO) participates in the regulation of local cerebral blood flow (CBF) during hypoxia (inhalation of 15% O₂ in N₂). The CBF response to hind-paw stimulation (evoked CBF) of Sprague-Dawley (SD) rats was measured by laser-Doppler flowmetry. Physiological variables, such as heart rate, mean blood pressure, and PaCO₂ during hypoxia, were identical to those under normoxic conditions. Hypoxia increased the baseline CBF (17.5 ± 14.3%) and the normalized peak amplitude of evoked CBF (31.1 ± 18.5%) relative to those during normoxia. When an NOS inhibitor was infused intravenously, these

differences were abolished in both the baseline CBF or evoked CBF between normoxic and hypoxic conditions, whereas the heart rate decreased and the mean blood pressure increased during hypoxia in comparison with these during normoxia. The field potential was constant under all experimental conditions. These results suggest that NO plays a major role in the regulation of baseline and evoked CBF during hypoxia.

PMID: 20927617 [PubMed - indexed for MEDLINE]

[Int J Nanomed.](#) 2011; 6:1–14. Impact factor – 4.976

Chemical nature and structure of organic coating of quantum dots is crucial for their application in imaging diagnostics

[Bakalova R](#), [Zhelev Z](#), [Kokuryo D](#), [Spasov L](#), [Aoki I](#), [Saga T](#)

Abstract

Background: One of the most attractive properties of quantum dots is their potential to extend the opportunities for fluorescent and multimodal imaging in vivo.

Methods: We compared quantum dots coated with non-crosslinked amino-functionalized polyamidoamine (PAMAM) dendrimers, quantum dots encapsulated in crosslinked carboxyl-functionalized PAMAM dendrimers, and silica-shelled amino-functionalized quantum dots. A multimodal fluorescent and paramagnetic quantum dot probe was also developed and analyzed. The probes were applied intravenously in anesthetized animals for visualization of brain vasculature using two-photon excited fluorescent microscopy and visualization of tumors using fluorescent IVISR imaging (Caliper Life Sciences, Hopkinton, MA) and magnetic resonance imaging.

Results: Quantum dots coated with non-crosslinked dendrimers were cytotoxic. They induced side effects in vivo, including vasodilatation with a decrease in mean arterial blood pressure and heart rate. The quantum dots penetrated the vessels, which caused the quality of fluorescent imaging to deteriorate. Quantum dots encapsulated in crosslinked dendrimers had low cytotoxicity and were biocompatible. In concentrations 0.3 nmol quantum dots/kg bodyweight, these nanoparticles did not affect blood pressure and heart rate, and did not induce vasodilatation or vasoconstriction. PEGylation (PEG [polyethylene glycol]) was an indispensable step in development of a quantum dot probe for in vivo imaging, based on silica-shelled quantum dots. The non-PEGylated silica-shelled quantum dots possessed low colloidal stability in high-salt physiological fluids, accompanied by rapid aggregation in vivo. The conjugation of silica-shelled quantum dots with PEG1100 increased their stability and half-life in the circulation without significant enhancement of their size. In concentrations 2.5 nmol/kg bodyweight, these quantum dots did not affect the main physiological variables. It was possible to visualize capillaries, which makes this quantum dot probe appropriate for investigation of mediators of vasoconstriction, vasodilatation, and brain circulation in intact animals in vivo. The multimodal silica-shelled quantum dots allowed visualization of tumor tissue in an early stage of its development, using magnetic resonance imaging.

Conclusion: The present study shows that the type and structure of organic/bioorganic shells of quantum dots determine their biocompatibility and are crucial for their application in imaging in vivo, due to the effects of the shell on the following properties: colloidal stability, solubility in physiological fluids, influence of the basic physiological parameters, and cytotoxicity.

[Mol Pharm.](#) 2011 Jul 11. [Epub ahead of print] Impact factor – 5.400

Nitroxide derivatives for imaging of hypercholesterolemia-induced kidney dysfunction and assessing the effectiveness of anti-lipidemic drugs.

[Tomizawa A](#), [Hadjidekov G](#), [Ishii I](#), [Bakalova R](#), [Zhelev Z](#), [Aoki I](#), [Saga T](#), [Kitada M](#).

Abstract

The present study was designed to clarify the possibility for application of nitroxide derivatives in magnetic resonance imaging (MRI) of hypercholesterolemia-mediated renal dysfunction in mice, as well as to assess the effectiveness of anti-lipidemic drugs (cholestyramine and ezetimibe). The mice were separated in four groups: (i) on a normal diet (ND) without medication (control); (ii) on a high cholesterol diet (CD) without medication; (iii) CD mice receiving cholestyramine; and (iv) CD mice receiving ezetimibe. In CD mice without medication, a hypercholesterolemia was developed, detected by the increasing of total plasma cholesterol and non-HDL cholesterol, and decreasing of HDL cholesterol. The hypercholesterolemia compromised renal function: blood urea nitrogen, creatine and uric acid increased significantly, accompanied with development of glomerulosclerosis, enhancement of the amount of neutrophils and overexpression of metalloproteinase-9. The mice were subjected to anesthesia and MR imaging was performed on 7 Tesla magnet (T1-weighted incoherent gradient-echo sequence; fast low-angle shot). The region-of-interest was selected within the kidney. The images were obtained before and after injection of contrast probe [carbamoyl-PROXYL (CMP) or Gd-DTPA]. In the kidney of ND mice, the MRI signal intensity increased after injection of CMP, reached a maximum (very well-defined renal filtration peak) and decreased to the baseline level within 14 min. In kidney of CD mice, the CMP-mediated enhancement of MRI signal was not detected. Anti-lipidemic drugs partially abolished the effect of hypercholesterolemia on CMP-enhanced MRI in the kidney. The kinetic curves of Gd-enhanced MRI signal had also different profiles in the kidney of ND and CD mice. They were similar to the profiles of the kinetic curves, obtained from MR urography of healthy human and human with renal pathology, respectively. The present study suggests that CMP is suitable MRI contrast probe for visualization of hypercholesterolemia-induced renal dysfunction in intact animals and the assessment of the efficacy of anti-lipidemic drugs. The probe was applied in concentration, which was 3 times lower than the LD50 for intravenous administration in mice. Since the probe is excreted by the kidney, it could be considered harmless for mammals in the selected dose and appropriate candidate for translational research.

PMID: 21744874 [PubMed - as supplied by publisher]

[Biochim Biophys Acta](#). 2011 Jun 29. [Epub ahead of print] Impact factor – 4.663

Carbamoyl-PROXYL-enhanced MRI detects very small disruptions in brain vascular permeability induced by dietary cholesterol.

[Tomizawa A](#), [Ishii I](#), [Bakalova R](#), [Zhelev Z](#), [Aoki I](#), [Shibata S](#), [Kitada M](#).

Abstract

BACKGROUND: Gd-DTPA-enhanced magnetic resonance imaging (MRI) is a conventional method for non-invasive investigation of blood-brain-barrier (BBB) permeability in animal models. It allows the visualization of serious injury to the BBB. We developed a novel approach for detecting very small disruptions in BBB permeability induced by dietary cholesterol by using carbamoyl-PROXYL (CMP) as an MRI contrast probe.

METHODS: Mice were separated into two groups: normal diet (ND-mice) and high cholesterol diet (CD-mice). MRI-signal dynamics, plasma cholesterol, matrix metalloproteinase (MMP-9, MMP-2), and the white blood cell profile were analyzed. For the MRI analysis, two regions-of-interest (ROI) were selected: brain (ROI-1) and surrounding area (ROI-2).

RESULTS: In the ROI-2 of ND-mice, CMP- or Gd-enhanced MRI-signal followed typical kinetics with a half-life of signal decay ($\tau(1/2)$) ~8 or ~15min, respectively. In CD-mice, the MRI-signal increased continuously without decay. In the ROI-1 of ND- and CD-mice, MRI-signal enhancement was not detected by Gd-DTPA. In the ROI-1 of ND-mice, CMP-induced MRI-signal enhancement was negligible, while in CD-mice, it was significant ($\tau(1/2)$ >15min). Hypercholesterolemia increased the plasma levels of MMP-9 and neutrophils.

CONCLUSIONS: Hypercholesterolemia increases vascular permeability, which is mediated by MMP-9 and neutrophils.

GENERAL SIGNIFICANCE: Even very small disruptions in brain vascular permeability could be detected by CMP-enhanced MRI but not by Gd-DTPA-enhanced MRI.

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ОРИГИНАЛНИ СТАТИИ В БЪЛГАРСКИ ПЕРИОДИЧНИ ИЗДАНИЯ

[Хирургия \(София\)](#). 1993; 47(6):9-11.

Индукция на липидна пероксидация в белите дробове при халотанова анестезия.

[Ковачева С](#), [Хинев С](#), [Бакалова Р](#), [Рибаров С](#).

Резюме

Проучена е експериментално възможността на халотановата анестезия да индуцира свободно-радикални процеси в белите дробове на плъхове.

Изследвани са бели мъжки плъхове Wistar, разделени в 5 групи, експонирани по 4 часа, както следва: I (контролна) – на атмосферен въздух; II – на райски газ + кислород (2:1); III – на кислород (100%); IV – на халотан (1 об.%) + кислород (100%); и V – на халотан (1 об.%) + (райски газ + кислород; 2:1).

Установено е повишено съдържание на конюгираните диени и липофусцин-подобните флуоресцентни продукти във всички експериментални групи (II-V), което свидетелства за интензифициране на процесите на липидна пероксидация в белодробната тъкан. Допълнително доказателство за този извод е наблюдаваното намалено съдържание на алфа-токоферол във всички експериментални групи. Най-съществени са измененията в групите, експонирани на кислород (100%) и халотан (1 об.%) + кислород (100%).

Направено е предположение, че интензификацията на процесите на липидна пероксидация при използваните инхалационни въздействия може да обясни поне частично увреждането на белодробната тъкан при т.нар. „шоков бял дроб”.

[Хирургия \(София\)](#). 1993; 47(6):14-16.

Индукция на липидна пероксидация в черен дроб при халотанова анестезия.

[Хинев С](#), [Бакалова Р](#), [Пенева В](#), [Узунова П](#), [Ковачева С](#), [Гълъбова Т](#), [Соколова Ц](#), [Рибаров С](#).

Резюме

Изследвано е нивото на липидна пероксидация в черен дроб на плъхове, подложени на анестезия с халотан + райски газ + кислород, както и на плъхове, експонирани поотделно на всеки един от компонентите, влизащи в състава на тази анестезия. Установено е, че приложени поотделно, инхалаторните анестетици и кислородът предизвикват значително увеличаване на конюгираните диени и липофусцин-подобните флуоресцентни продукти, като най-силно изразен е ефектът на халотана, следван от кислорода и райския газ. Влиянието на последния върху нивото на липидната пероксидация е статистически недостоверно. По отношение на ТБК-активните продукти статистически значими разлики спрямо контролата не се регистрират. Приложени в комбинация халотан + райски газ + кислород, анестетиците предизвикват по-слабо повишаване на диеновите конюгати и липофусцин-подобните флуоресцентни продукти в сравнение с халотана и кислорода, приложени поотделно.

Представените резултати показват, че уврежданията на черния дроб след халотанова анестезия включват, поне частично, индукция на липидна пероксидация в хепатоцитните мембрани.

[Анестезиология и интензивно лечение](#). 1994; 21(2):9-15.

Промени в липидния профил на кръвната плазма и индукция на липидна пероксидация в условия на обща анестезия.

[Хинев С](#), [Бакалова Р](#), [Рибаров С](#).

Резюме

Изследвани са промените на липидния профил на кръвната плазма при пациенти в условия на обща анестезия (халотанова анестезия или невролептаналгезия). Кръвните проби са анализирани 48 часа преди анестезията, непосредствено след прекратяване на анестезията и 24 часа след прекратяването ѝ. Оценено е нивото на общите липиди, фосфолипидите и свободните мастни киселини. Анализирано е съдържанието на продуктите на липидна пероксидация (МДА-Шифови бази) в кръвната плазма. И при двете форми на анестезия се регистрира намаляване на количеството на общите липиди и фосфолипидите в плазмата през изследвания период от време. Непосредствено след прекратяване на анестезията рязко се покачва нивото на свободните мастни киселини и продуктите на липидна пероксидация над общоприетите референтни стойности. Наблюдаваните промени се интерпретират в два аспекта: като следствие от приложената анестезия и като следствие от стреса, в който се намират пациентите преди и след операция.

[Khirurgiiia \(Sofia\)](#). 1995;48(5):23-5.

[The lipid peroxidation level and antioxidant status of the plasma in patients operated on under propofol (Diprivan) anesthesia].

[Article in Bulgarian]

[Khinev S](#), [Dafinova K](#), [Tenchova V](#), [Bakalova R](#).

Abstract

It has been investigated the level of lipid peroxidation and antioxidant status of blood plasma from patients operated under general anaesthesia with propofol (Diprivan).

The following parameters have been registered: TBA-reactive substances (TBARS) and fluorescent lipofuscine-like lipid peroxidation products, before, directly after and 24 hours after anaesthesia. It was not shown statistically significant changes of TBARS before and after anaesthesia. The levels of TBARS were initially higher in comparison of referent levels. The fluorescent lipofuscine-like products were increased weakly after anaesthesia directly but the changes were not statistically significant, too. The antioxidant capacity of blood plasma was decreased weakly 24 hours after anaesthesia, in comparison of the levels, registered before anaesthesia. The good correlation between fluorescent products and antioxidant status of plasma was observed.

PMID: 8648960 [PubMed - indexed for MEDLINE]

[Acta Physiol Pharmacol Bulg.](#) 1995;21(4):81-5.

Oxidative modifications of blood serum in humans with coronary artery disease.
[Bakalova R](#), [Goudev A](#), [Zhelev Z](#), [Nachev C](#).

Abstract

Oxidative modifications of blood serum in humans with and without coronary artery disease were investigated. Four parameters were analyzed: the intensity of serum fluorescence, which is indicative of the content of lipofuscine-like lipid peroxidation products; the content of thiobarbituric acid-reactive substances; the lag-phase of serum oxidation by azo-compounds; and the content of lipophilic natural antioxidants- α -tocopherol, beta-carotene and ubiquinol-9(10). It was found that coronary artery disease resulted in a significant increase of serum fluorescence and the content of TBARS. The atherogenic disorders in humans with coronary artery disease drastically decreased the lag-phase of serum oxidation in the presence of 2,2'-azo-bis-(2-amidinopropane) dihydrochloride. The oxidative modifications of serum were in close correlation with the balance of natural lipophilic antioxidants in blood serum, i.e. α -tocopherol, ubiquinols and beta-carotene. The contents of all antioxidants tested in serum were significantly decreased in patients with coronary artery disease.

PMID: 8830879 [PubMed - indexed for MEDLINE]

[Анестезиология и интензивно лечение.](#) 1996; 23 (2):27-30.

Влияние на многокомпонентната съвременна анестезия върху фармакометаболизиращата функция на черния дроб на пациенти по време на обща анестезия и в ранния следоперативен период.

[Хинев С](#), [Дафинова К](#), [Бакалова Р](#), [Иванов А](#).

Резюме

Целта на настоящото изследване е да се проследи нивото на серумните ензимни активности и антипириновия тест в урината на пациенти с коремни операции под обща анестезия и да се обективизира влиянието на анестетиците върху фармакометаболизиращата функция на чернич дроб.

[Хигиена и здравеопазване.](#) 1996; 39(4):16-19.

Промени в активността на чернодробните монооксигенази под влияние на някои анестетици.

[Хинев С](#), [Бакалова Р](#), [Михайлова А](#).

Резюме

Изследвано е влиянието на широко използваните в анестезиологичната практика анестетици: халотан, двуазотен окис, фентанил и дроперидол, върху активността на чернодробните монооксигенази. Експонирането на плъхове на халотан и/или двуазотен окис е осъществено в специални инхалаторни камери, при динамичен режим на третиране в продължение на четири часа. Фентанилът (0.03 мг/кг) и дроперидолът (2.5 мг/кг) са инжектирани интраперитонеално. Микрозомалната фракция е изолирана непосредствено след третирането. Активността на чернодробните монооксигенази е оценена по съдържанието на цитохром P-450 и цитохром б5, както и по скоростта на биотрансформация на аминопирин и анилин. Установено е инхибиране на аминопирин-деметилазата при изолираното прилагане на халотан и двуазотен окис. Този ефект се засилва при комбинираното прилагане на тези два анестетика. Анилин-хидроксилазната активност е потисната единствено при групата, експонирана на двуазотен окис. При изолираното прилагане на фентанил и дроперидол се регистрира инхибиране на оксидазите със смесена функция, докато при комбинираното им използване промени не се наблюдават. Отклонение в съдържанието на цитохромите P-450 и б5 не се регистрират. Получените резултати позволяват оптимизиране на използваните схеми в анестезиологичната практика.

[Khirurgija \(Sofia\)](#). 1996;49(2):27-9.

[The induction of lipid peroxidation in the serum of patients with acute peritonitis].

[Article in Bulgarian]

[Khinev S](#), [Dafinova K](#), [Koïnova G](#), [Dimitrov P](#), [Ivanov A](#), [Bakalova R](#).

Abstract

The level of lipid peroxidation products in the sera of patients with acute peritonitis and clinically healthy blood donors are studied. An increase in the level of TBA-reactive substances is documented in the acute peritonitis group--twice as high as compared to clinically healthy blood donors. Changes in the patients in toxic and terminal phases of peritonitis are rather significant. Following hemofiltration, the serum lipid peroxidation products are decreased in comparison with the pre-hemofiltration state.

PMID: 8992057 [PubMed - indexed for MEDLINE]

[Acta Physiol Pharmacol Bulg](#). 2000;25(1):19-26.

Pharmacodynamic of the antioxidant action of alpha-tocopherol and its derivatives in liver, brain, heart and skeletal muscles.

[Bakalova R](#), [Mileva M](#), [Kutsev C](#), [Zlateva G](#), [Ribarov S](#).

Abstract

The aim of the present work was to determine the pharmacodynamics of antioxidant effect of alpha-tocopherol and its derivatives (alpha-tocopheryl esters and chromanols with different chain-length) in the animal tissues, as well as the role of cytochrome P-450 in biotransformation of these compounds. Alpha-tocopherol and its derivatives were injected intraperitoneally in rats or mice in a single dose of 100 mmol per kg b.w. The animals were sacrificed at different time intervals (0, 1, 2, 4, 8, 12, 24, 36 hours) and the liver, heart, brain and skeletal muscles were removed, homogenized and incubated with lipid peroxidation (LPO) inducers (Fe²⁺ + ascorbate). LPO was evidenced by the generated malone dialdehyde (MDA). Data were expressed as

percentage of LPO inhibition by alpha-tocopherol or its derivatives as compared to control group. The kinetic curves of the inhibitory action of alpha-tocopherol and its derivatives on LPO were characterized by three phases: a phase of increasing antioxidant activity, a phase of maximal antioxidant activity (about 60-95% LPO inhibition), and a phase of decreasing antioxidant activity. Alpha-tocopheryl esters possessed dynamics of antioxidant action the same as alpha-tocopherol. Therefore the hydrolysis of alpha-tocopheryl esters in animal organism is not a limiting factor for their antioxidant effect. The alpha-tocopherol derivatives with short chain-length (C1, C6) had a shorter half-life in animal tissues as compared to alpha-tocopherol or its esters. In vitro experiments showed that C1 and C6 are substrates of cytochrome P-450. In contrast, alpha-tocopherol and its esters did not bind to cytochrome P-450 even at concentrations as high as 10 mmol/l. Apparently, C1 and C6 underwent biotransformation and were excreted more quickly from the organism.
PMID: 11140188 [PubMed - indexed for MEDLINE]

[Pharmacia \(Bulgaria\)](#). 2005; 52(1-2):3-6.

Potential of DNA-sugar conjugates as antisense-based drugs: intracellular uptake, stability of sense-antisense duplexes, resistance against nuclease degradation, and antisense effect

[Bakalova R](#), [Kubo T](#), [Mileva M](#), [Zhelev Z](#), [Ohba H](#), [Fujii M](#)

Abstract

The present study describes the possibility for carbohydrate receptor-mediated antisense oligo-DNA transfer into the cells. Oligo-DNAs were covalently conjugated at 5'-end with sugar (galactosamine). The synthesis was carried out by solid-phase fragment condensation. Using FITC-labeled DNA-sugar conjugates it was detected that they were delivered into the cells rapidly and were localized into the high density sites of the cytoplasm. The described modification of antisense oligo-DNAs with sugar was found to improve their biochemical and pharmacological properties. The binding affinity of oligo-DNAs to the respective target sense sequences enhanced significantly after conjugation with sugar, estimated by their melting temperatures. DNA-sugar conjugates were found to be more resistant to nuclease degradation in serum in comparison with the respective non-conjugated oligo-DNAs. The sequences of oligo-DNA were selected to be antisense against RNA-template of human telomerase. The presence of sugar enhanced the specific antisense activity of oligo-DNAs, resulting in stronger reduction of the activity of respective sense target (telomerase). In telomerase activity assay it was established that ID₅₀ was ~40-50 nM for DNA-galactosamine conjugates vs ~350-400 nM for the respective non-conjugated oligo-DNA sequences.

In conclusion, the synthesized oligo-DNA-sugar conjugates can be considered as promising chemical modification of antisense DNAs, enhancing their possibility for *in vivo* application.

[Trakia J Sci](#). 2010; 8(2):1-5.

Magnetic resonance imaging of brain neuroblastoma based on nitroxide redox cycle

[Zhelev Z](#), [Bakalova R](#), [Shibata S](#), [Gadjeva V](#), [Spasov L](#), [Aoki I](#)

Abstract

In the present study we propose a new diagnostic methodology for non-invasive imaging of tissue red/ox activity in intact healthy and cancer-bearing mammals, which allows a differentiation of cancer development from normal (healthy) condition. The method is based on red/ox cycle of cellpermeable nitroxide radicals and their MRI contrast properties, which makes them useful molecular sensors for tissue red/ox activity. The nitroxide radical (which is characterized by T1 contrast) participates in electron-transfer reactions with the intracellular reactive oxygen species and reducing equivalents with formation of non-contrast intermediate products, which is accompanied with MRI signal decay. The half-life of MRI signal decay ($T_{1/2}$) was used as a marker of tissue red/ox activity to the nitroxide probe. The experiments were conducted on healthy and cancer-bearing mice. The mice were under anesthesia during the MRI measurements. All measurements were conducted on 7 Tesla MRI. In healthy mice, the half-life of MRI signal decay in the selected regions of interest (ROI; brain and surrounding tissues) was considered as a reference steady-state value, which is indicative of tissue red/ox activity in norm. In cancer-bearing mice, the half-life of MRI signal decay in the same or similar ROI was markedly different from this reference value. The results demonstrated that the normal (healthy) tissues possessed a significantly higher reduction activity to the nitroxide probe in comparison with cancer tissue, which could be an appropriate diagnostic marker for carcinogenesis.

ОБЗОРНИ СТАТИИ В ЧУЖДИ ПЕРИОДИЧНИ ИЗДАНИЯ

[Lancet](#). 2002, 359(9319): 1776-7. Impact factor – 33.633

Decrease of resistance to imatinib in leukaemia

[Bakalova R](#), [Zhelev Z](#), [Ohba H](#)

James Griffin's Feb 9 Commentary¹ about resistance to targeted therapy in leukaemia, in response to the report by Nikolas von Bubnoff and colleagues (Feb 9, p 487), puts forward an exceptionally important question about the effectiveness of imatinib (STI571, a BCR-ABL tyrosine kinase inhibitor) for chronic myeloid, Philadelphia-chromosome-positive, and acute lymphoblastic leukaemia. He improves our understanding of the necessity for new strategies for disease control and treatment, including combined application of different substances with different contact sites.

Kano and colleagues have reported a synergistic in-vitro effect of imatinib with several agents against Philadelphia-chromosome-positive leukaemia, such as recombinant and natural alfa interferons, as well as additive effects with cytarabine, homoharringtonine, etoposide, doxorubicin, and vin-cristine. Their findings suggest that imatinib combined with other conventional treatment agents, except methotrexate, can be advantageous for Philadelphia-chromosome-positive leukaemias. Kano and colleagues recommend a combined administration of imatinib and alfa interferons or vincristine as highly effective against these leukaemias. However, given possible discrepancies between in-vitro studies and clinical trials, these findings provide only useful information for the establishment of clinical protocols involving imatinib.

Several signalling cascades are involved in expression of the BCR-ABL tyrosine kinase, all of which drive leukaemogenesis—eg, imatinib has no effect on interleukin 3 and cell signalling dependent on granulocyte macrophage colony stimulating factor, and does not prevent activation of the other Jak/Stat pathways. Thus, all inhibitors of Jak/Stat pathways are also potentially candidates for combined treatment with imatinib.

[Lancet](#). 2022, 360(9331): 487. Impact factor – 33.633

Effect of vitamins E and C on transplant-associated atherosclerosis

[Zhelev Z](#), [Bakalova R](#)

James Fang and colleagues¹ show that although vitamins C and E reduce the progression of transplant-associated atherosclerosis, they do not affect the frequency of episodes of acute parenchymal rejection. They suggest that transplant-associated atherosclerosis and parenchymal rejection are mediated by different parts of the immune system, and that treatment that eliminates transplant-associated atherosclerosis may not affect acute parenchymal rejection. We think that a possibility for cross-talk between the two mechanisms exists, and that vitamins C and E may act as mediators of both. Unfortunately, dependent on doses, opposite and paradoxical effects can arise.

Vitamins C and E are powerful antioxidants, more so in combination, but they also have immunostimulating effects; therefore, it is not surprising that they do not prevent parenchymal rejection in the host. Moreover, they can affect host immune reactivity by different and opposite mechanisms, and the final effect is unpredictable. At least three important cross-linked targets for vitamins C and E exist in the area of graft: macrophage activation; endothelial-cell activation and cytokine expression; and nitric-oxide (NO) and prostanoids synthesis and regulation of microcirculation.

Vitamin E inhibits protein-kinase C-dependent oxidative burst of macrophages, and thus suppresses macrophage-mediated oxidative stress. Vitamin C regenerates vitamin E from tocopheroxyl-radical, which keeps its steady-state concentration in the host. Both mechanisms are widely discussed by Fang and colleagues. However, the two vitamins have many other effects on the host immune system. Supplementation with vitamins C and E increases cytokine production in healthy adults. The same effect might occur in transplant recipients.

[Biochem Pharmacol](#). 2003 Nov 15;66(10):1879-84. Impact factor – 4.889

Cross-talk between Bcr-Abl tyrosine kinase, protein kinase C and telomerase-a potential reason for resistance to Glivec in chronic myelogenous leukaemia.

[Bakalova R](#), [Ohba H](#), [Zhelev Z](#), [Ishikawa M](#), [Shinohara Y](#), [Baba Y](#).

(Статията включва оригинални резултати.)

Abstract

To prevent the resistance to Glivec in patients with chronic myelogenous leukaemia (CML), it is necessary to get a good understanding of its potential mechanisms. The present hypothesis accents on the mechanisms whereby Bcr-Abl tyrosine kinase remains inhibited by Glivec, but alternative signalling pathways become activated—the potential reason associates with activation of telomerase after long-term treatment with Glivec and recovery of cell proliferation and immortality. The hypothesis is based on the observations about differences in telomere dynamics and telomerase activity between chronic and blast phases of CML patients, as well as about the potential effect of Glivec on the cross-talk between telomerase, Bcr-Abl tyrosine kinase and protein kinase C family-key enzymes in CML. It proceeds from recently published data, demonstrating that protein kinase C activates and c-Abl tyrosine kinase inhibits telomerase. During optimization of chemical structure, Glivec loses its effect on protein kinase C and enhances the effect on Bcr-Abl tyrosine kinase,

resulting in a high potential to activate telomerase indirectly through its effect on both kinases. Experimental preclinical data are given in confirmation of this hypothesis.
PMID: 14599545 [PubMed - indexed for MEDLINE]

[Expert Rev Mol Diagn.](#) 2005 May;5(3):315-28. Impact factor – 4.652

A history of microarrays in biomedicine.

[Ewis AA](#), [Zhelev Z](#), [Bakalova R](#), [Fukuoka S](#), [Shinohara Y](#), [Ishikawa M](#), [Baba Y](#).

Abstract

The fundamental strategy of the current postgenomic era or the era of functional genomics is to expand the scale of biologic research from studying single genes or proteins to studying all genes or proteins simultaneously using a systematic approach. As recently developed methods for obtaining genome-wide mRNA expression data, oligonucleotide and DNA microarrays are particularly powerful in the context of knowing the entire genome sequence and can provide a global view of changes in gene expression patterns in response to physiologic alterations or manipulation of transcriptional regulators. In biomedical research, such an approach will ultimately determine biologic behavior of both normal and diseased tissues, which may provide insights into disease mechanisms and identify novel markers and candidates for diagnostic, prognostic and therapeutic intervention. However, microarray technology is still in a continuous state of evolution and development, and it may take time to implement microarrays as a routine medical device. Many limitations exist and many challenges remain to be achieved to help inclusion of microarrays in clinical medicine. In this review, a brief history of microarrays in biomedical research is provided, including experimental overview, limitations, challenges and future developments.

PMID: 15934810 [PubMed - indexed for MEDLINE]

[Cell Mol Neurobiol.](#) 2007 May;27(3):359-65. Epub 2006 Dec 21. Impact factor – 2.423

Ultra-fast biosensors and multi-photon microscopy in the future of brain studies.

[Bakalova R](#).

Abstract

The direct, highly selective and sensitive real-time imaging of neuro- and biochemical mediators is the only way to clarify precisely the chemistry of the brain and to discover the key molecular targets involved in regulation of brain homeostasis. To realize that, we need: high-speed deep-tissue imaging techniques with high spatial and temporal resolution; and ultra-fast and highly selective molecular sensors, giving a possibility to monitor target molecules directly in their physiological environment; in addition, these molecular sensors have to be comparatively small and permeable for blood-brain barrier, to be applicable in brain studies. The present view accents on the perspectives for development of direct approach for investigation of function/flow coupling phenomenon in the brain, based on the current progress in development of ultra-fast molecular sensors for direct visualization of biochemical mediators (e.g., nitric oxide, Ca ions), and high-speed two-photon/multi-photon deep-tissue imaging.

PMID: 17186362 [PubMed - indexed for MEDLINE]

[Brain Res Bull.](#) 2007 Jun 15;73(1-3):150-3. Epub 2007 Mar 15. Impact factor – 2.498

Fluorescent molecular sensors and multi-photon microscopy in brain studies.

[Bakalova R](#).

Abstract

To clarify the brain phenomena, to prove directly the major biochemical pathways in cerebral tissue, and to discover the crucial steps in brain pathology, it is necessary to develop a high speed deep-tissue imaging techniques with high spatial and temporal resolution, and ultra-fast and highly selective molecular sensors, giving a possibility to monitor target molecules directly in their physiological environment. This technical comment accents on the perspectives for development of direct approach for investigation of function/flow coupling phenomenon and zinc transport into the brain, based on the current progress in development of ultra-fast molecular sensors for direct visualization of biochemical mediators and neurotransmitters, and high speed multi-photon deep-tissue imaging.

PMID: 17499649 [PubMed - indexed for MEDLINE]

[Drug Delivery Systems](#). 2008; 23(1):61-68.

Magnetic resonance molecular imaging using drug delivery systems.

[Article in Japanese]

[Aoki I, Bakalova R.](#)

Abstract

MRI has provided high tissue contrast and spatial resolution non-invasively and is widely used in the clinical field. High field MR imaging, which provides a high signal-to-noise ratio and frequency resolution, is expected to allow imaging of specific molecular events. Examples include the efficiency of drug development, visualization of regeneration or migration treatment, and oncology or neuroscience research and diagnostics. Drug delivery systems are a key technology for the application of MRI to “molecular imaging”.

ОБЗОРНИ СТАТИИ В БЪЛГАРСКИ ПЕРИОДИЧНИ ИЗДАНИЯ

[Анестезиология и интензивно лечение](#). 1994; 21(2):35-44.

Инхалаторните анестетици – метаболизъм и чернодробна токсичност. I. Метаболизъм и токсичност на халотана.

[Бакалова Р, Хинев С, Рибаров С.](#)

Резюме

В работата са разгледани основните пътища на биотрансформация на инхалаторните анестетици в черния дроб. Представени са механизмите на халотановия метаболизъм от чернодробната монооксигеназна система и възможностите за генериране на продукти със свободнорадикалова природа в хода на този метаболизъм. Лансира се схващането, че основна роля в хепатотоксичността на халотана играят индукцията на микрозомалната монооксигеназна система на черния дроб и генерирането на свободнорадикални метаболити, индуциращи процеси на липидна пероксидация в хепатоцитите.

[Анестезиология и интензивно лечение](#). 1995; 22(2):18-23.

Инхалаторните анестетици – метаболизъм и чернодробна токсичност. II. Индукция на липидна пероксидация в черния дроб при халотанова анестезия в условия на хипер-, нормо- и хипоксия.

[Хинев С, Бакалова Р, Пенева В, Дафинова К, Рибаров С.](#)

Резюме

Разгледани са възможностите за индукция на липидна пероксидация в черния дроб под въздействие с халотан в условия на нормо-, хипо- и хипероксия. Проследено е съдържанието на конюгираните диени, флуоресцентните липофусцин-подобни флуоресцентни продукти, ТБК-активните продукти и природния антиоксидант витамин Е в черния дроб на плъхове, експонирани на 1 об.% халотан в присъствие на 33% и 100% кислород в газовата смес, както и в присъствие на 67% двуазотен окис. Установено е, че намаляването на кислородното съдържание от 100% на 33% води до понижаване на нивото на продуктите на липидна пероксидация в черния дроб и дава възможност да се отдиференцира хепатотоксичния ефект на халотана от този на високото кислородно съдържание.

[Какво ново в липидологията.](#) 1995; 2(2):4-6.

Ролята на антиоксидантите в профилактиката и лечението на сърдечно-съдовите заболявания

[Бакалова Р.](#)

(Статията включва оригинални резултати.)

(No abstract available.)

[Актуална липидология.](#) 1997; (2):21-30.

Участие на свободно-радикалните процеси в атерогенезата.

[Бакалова Р.](#), [Хаджимитова В.](#), [Милева М.](#), [Рибаров С.](#)

(Статията включва оригинални резултати.)

Резюме

Епидемиологичните проучвания през последните няколко години доказаха по безспорен начин участието на свободно-радикалните процеси в атерогенезата. Настоящата работа обобщава известните в литературата факти и данни от собствени изследвания, свързани с окислителното модифициране на ЛНП като главен рисков фактор за атерогенеза. Акцентът е поставен върху две основни причини, отговорни за окислителното модифициране на ЛНП:

- прооксидантния статус на плазмата, обусловен от: повишен синтез на катехоламини в кръвта; конверсия на ксантин-дехидрогеназата в ксантин-оксидаза; активиране на липоксигеназите и фосфолипаза А2; активиране на липидната пероксидация и свободно-радикалното окисление на белтъците и нуклеиновите киселини;
- намаления антиоксидантен капацитет на окислително модифицираните ЛНП (оксЛНП) и вероятността за генетична детерминираност на процеса.

Проведен е сравнителен анализ на прооксидантния/антиоксидантния баланс на нативни ЛНП и оксЛНП, изолирани съответно от клинично здрави кръводарители и от пациенти с атеросклероза.

Показано е, че свободните мастни киселини нарастват до 170% в оксЛНП, количеството на наситените и мононенаситените мастни киселини практически не се изменя, докато полиненаситените мастни киселини – основният субстрат на липидната пероксидация, намаляват значително при оксЛНП. В оксЛНП се наблюдава също натрупване на конюгирани диени, оксистероли, ТБК-активни продукти и флуоресцентни липофусцин-подобни продукти на липидната пероксидация.

Анализът на липидния профил доказва ускорено развитие на свободно-радикални процеси в оксЛНП – фосфатидилхолинът намалява до 50-60%, лизофосфатидилхолинът и триглицеридите значително нарастват.

Наблюдава се рязко намаляване или пълно изчерпване на природните липорастворими антиоксиданти в оксЛНП – токофероли, токотриеноли, убихиноли, каротеноиди и др.

На базата на проведения сравнителен анализ е направен опит да се обоснове необходимостта от определяне на антиоксидантния капацитет на ЛНП като възможен критерий за оценка на целесъобразността и изхода от антиоксидантната профилактика и терапия на атеросклерозата.

ОРИГИНАЛНИ СТАТИИ В СБОРНИЦИ ОТ НАУЧНИ ФОРУМИ

In: “Proceedings of the First Bulgarian International Symposium on Cardiovascular Diseases and Paediatric Traumatology”, September 11, 1995, Sofia, Bulgaria, Ed. by MSCJ, Arlington, USA, 1995, Vol. I, p. 10-17.

Computer correlative analysis and mathematical modelling of antioxidant status and level of lipid peroxidation in plasma lipoproteins in atherogenesis.

[Bakalova R](#), [Goudev A](#), [Zhelev Z](#).

Abstract

(1) Analysis of lipid peroxidation products in blood plasma and plasma lipoproteins from clinically healthy humans and humans with coronary artery disease (CAD): lipid and fatty acid hydroperoxides, TBA-reactive substances, fluorescent lipofuscin-like products, volatile hydrocarbons, products causing superweak or luminol-dependent chemiluminescence. Correlative analysis of the results.

(2) Analysis of some natural prooxidants and antioxidants in blood plasma and plasma lipoproteins from clinically healthy humans and humans with CAD: vitamin E, beta-carotene, ubiquinol, antioxidant enzymes, leukotriens, prostaglandins, cholesterol, fatty acid and phospholipid profile. Correlative analysis of the results.

(3) Establishment of a mathematical model, employing experimental measurement of a limited number of parameters to estimate antioxidant status of blood plasma and plasma lipoproteins.

In: “Proceedings of the International Symposium on Single-Molecule Bioanalysis and Nano-Biodevice”, March 11-12, 2003, Takamatsu, Japan, 2003, Vol. I, p. 1-2.

Application of single molecules in separation of cell populations for the necessities of cancer and neurodegenerative therapy.

[Bakalova R](#), [Ohba H](#).

No abstract available.

In: “Proceedings of the 2nd Symposium of the International Society of Rare Sugars”, May 27-29, 2004, Takamatsu, Japan, 2004, p. 189-195.

Effects of rutin and quercetin on the “oxidative stress”, induced by influenza virus infection in mice alveolocytes.

[Mileva M](#), [Bakalova R](#), [Zlateva G](#), [Galabov A](#), [Ohba H](#), [Ishikawa M](#), [Baba Y](#).

Abstract

The present study was designed to investigate the effect of two flavonoids – quercetin (aglicone) and its sugar-containing homologue rutin (quercetin-3-rutinoside), on the “oxidative stress” in alveolocytes, isolated from influenza virus inoculated mice. The

results demonstrated that influenza virus infection A/Aichi/2/68 (H3N2) was accompanied with a significant increase of the endogenous lipid peroxidation products and a decrease of natural antioxidants vitamin E in mice alveolocytes. Influenza virus sensitized the cells against exogenous prooxidant agents as xanthine-xanthine oxidase, free Fe^{2+} ions, and (Fe^{2+} + ascorbate). Supplementation of mice with quercetin or rutin separately or in combination protected the alveolocytes against lipid peroxidation induction and enhancement of virus titer in the lung of infected mice. Moreover, both substances increased the resistance of alveolocytes against exogenous prooxidant agents. Applying separately, rutin demonstrated most well expressed antioxidant effect than quercetin. A combination of both flavonoids manifested a synergistic antioxidant activity. It was established that the sugar part of rutin molecule plays an important role in the protection of alveolocytes against influenza virus-induced “oxidative stress”, as well as in the enhancement of the cell resistance against exogenous prooxidants. In this context, rutin-rich plant products can be considered as a promising source with considerable anti-inflammation and antioxidant efficiency.

In: “Proceedings of the Third International Symposium on Single-Molecule Bioanalysis and Nano-biodevice (SMBN2004)”, November 24-26, 2004, Takamatsu, Japan, 2004, p. 74-75.

Possibility for quantification of non-radioactive telomerase activity assay by microchip electrophoresis

[Zhelev Z](#), [Ewis A](#), [Bakalova R](#), [Dang F](#), [Ohba H](#), [Ishikawa M](#), [Shinohara Y](#), [Baba Y](#).

Abstract

The present study describes rapid and sensitive microchip-based electrophoretic procedure for separation of TRAP/PCR ladder products obtained in telomerase-catalyzed reaction in leukemia cells (K-562 line). The method gives a possibility for quantification of the non-radioactive telomerase activity assay.

In: “Proceedings of the Third International Symposium on Single-Molecule Bioanalysis and Nano-biodevice (SMBN2004)”, November 24-26, 2004, Takamatsu, Japan, 2004, p. 24-25.

Microarray-based technology for discovery of multidrug resistance factors in gene therapy of cancer

[Bakalova R](#), [Zhelev Z](#), [Ewis A](#), [Ohba H](#), [Ishikawa M](#), [Shinohara Y](#), [Baba Y](#).

Abstract

The development of antisense therapeutic strategy and RNA interference enhanced the expectations to decide the problems of gene therapy, especially in cancer. The target specificity of antisense-based drugs and siRNAs is beyond any doubt, but many queries exist about their side-effects during long-term treatment and the possibility for development of multidrug resistance (MDR) as in the case of conventional chemotherapeutics. In this context, microarray technology gives a possibility to discover rapidly all upregulated MDR-factors during antisense and conventional therapy of cancer, as well as to prognosticate the risk for development of drug resistance. The present study is only a particular case, but it clearly demonstrates that RNA interference of one oncogene has a potential to switch on overexpression of other oncogenes, antiapoptotic genes and factors that can be responsible for recovery and preservation of proliferation and immortalization of cancer cells.

[Nucleic Acids Symp Ser \(Oxf\)](#). 2004;(48):99-100.

Precisely controlled intracellular delivery of DNA-peptide conjugates.

[Kubo T](#), [Kanno K](#), [Rumiana B \(Bakalova R\)](#), [Ohba H](#), [Fujii M](#).

Abstract

Precisely controlled intracellular delivery of oligonucleotides was achieved by conjugation with signal peptides and amphiphilic designed peptides. Localization in the cellular nucleus was accelerated by nuclear localizing signals derived from naturally occurring viral proteins and arginine rich designed peptides, and cytoplasmic localization was enhanced by nuclear export signals and membrane fusion peptides.

PMID: 17150497 [PubMed - indexed for MEDLINE]

[Nucleic Acids Symp Ser \(Oxf\)](#). 2004;(48):303-304.

Control of intracellular delivery of oligonucleotides by signal peptides and genetic expression in human cells.

[Kubo T](#), [Kanno K](#), [Ohba H](#), [Rumiana B \(Bakalova R\)](#), [Fujii M](#).

Abstract

In the present study, membrane permeability and intracellular localization of oligonucleotide (ODN) conjugated with naturally occurring functional peptides or designed peptide were investigated, as well as antisense properties of them to inhibit of telomerase activities. All conjugate antisense ODNs showed higher membrane permeability and nuclease resistance than natural ODN. Intracellular localization of ODN could be precisely controlled by conjugation with functional peptides. Conjugate antisense ODNs indicated thousand fold higher inhibitory effects than natural ODN in cellular extract and 95% suppression in human leukemia cells.

PMID: 17150599 [PubMed - indexed for MEDLINE]

[Nucleic Acids Symp Ser \(Oxf\)](#). 2005;(49):333-4.

Suppression of bcr/abl chimeric gene by conjugate DNA enzymes in human cells.

[Takamori K](#), [Kubo T](#), [Zhelev Z](#), [Rumiana B \(Bakalova R\)](#), [Ohba H](#), [Doi K](#), [Fujii M](#).

Abstract

Conjugate DNAzymes with polyamines and peptides were successfully prepared by solid phase fragment condensation (SPFC) and showed up to 4.2 times higher catalytic efficiency (kcat/Km). Intracellular localization of DNAzymes could be controlled by conjugated with naturally occurring signal peptides which are responsible for nuclear cytoplasmic transport of proteins. Suppression of bcr/abl chimeric gene on Philadelphia chromosome by conjugate DNA enzymes were largely enhanced in human leukemia cells.

PMID: 17150769 [PubMed - indexed for MEDLINE]

[Nucleic Acids Symp Ser \(Oxf\)](#). 2005;(49):337-8.

Highly sensitive inhibition of hTERT mRNA expression and telomerase activity by DNA-signal-peptide conjugates.

[Kubo T](#), [Bakalova R](#), [Zhelev Z](#), [Ohba H](#), [Fujii M](#).

Abstract

In the present study, we investigated the antisense properties of conjugate oligonucleotides (ODNs) inhibiting human telomerase activity. Conjugate oligonucleotides assembled with signal peptides, artificially designed peptides, amines and sugars were synthesized by solid phase fragment condensation (SPFC) in sufficient yields. Conjugate ODNs showed a high resistance to nuclease degradation and sufficient binding affinity to target RNA, comparatively rapid and sufficient intracellular delivery and specific localization controlled by signal peptides (nuclear localization signals, NLS; nuclear export signals, NES). ODN-NLS conjugates demonstrated high antisense inhibitory effects against human telomerase activity into the nucleus (e. g, phosphorothioate conjugate inhibited the telomerase activity over 95%), whereas ODN-NES conjugates inhibited target mRNA expression into the cytoplasm.

PMID: 17150771 [PubMed - indexed for MEDLINE]

[Nucleic Acids Symp Ser \(Oxf\)](#). 2007;(51):407-8.

Enhancement of gene silencing potency and nuclease stability by chemically modified duplex RNA.

[Kubo T](#), [Zhelev Z](#), [Bakalova R](#), [Ohba H](#).

Abstract

In this study, we describe a development of chemically modified dsRNAs with improved biological properties. These dsRNAs possess an enhanced RNAi activity and a dramatically increased stability in cell cultured medium (containing 10% serum) in comparison with widely used 21nt siRNA. The chemically modified dsRNAs manifested a high longterm gene suppression after one week post-transfection, whereas 21nt siRNA showed a high RNAi activity only after 48 h cell transfection.

PMID: 18029759 [PubMed - indexed for MEDLINE]

In: "Proceedings of the 15th International Conference on Bioluminescence & Chemiluminescence" (eds. X. Chen, X-L. Yang, X-R. Zhang, Z.J. Cui, L.J. Kricka, P.E. Stanley), World Scientific, 2009, 189-192.

Luminol-dependent chemiluminescence increases with formation of phenothiazine cation radicals by horseradish peroxidase

[Hadjimitova V](#), [Traykov T](#), [Bakalova R](#).

Abstract

The introduction of phenothiazines into a HRP-H₂O₂-luminol system causes a strong increase in chemiluminescence. This investigation includes: chlorpromazine, levomepromazine, trifluoperazine, thioridazine and promethazine, in concentrations between 5.10⁻⁸ and 5.10⁻⁵ mol/L. The results show that all substances increase chemiluminescence up to eight times above the control. As a result of the processes occurring in the samples, cation radicals of phenothiazines are generated. Their presence is demonstrated through their characteristic absorption in the visual range. The radicals' concentration was proportional to the chemiluminescence increase. Chlorprothixene's structure is very similar to that of chlorpromazine, but doesn't allow the formation of a radical as in the case of chlorpromazine. Putting chlorprothixene instead of chlorpromazine into the system does not cause a chemiluminescence increase.

We conclude that the chemiluminescence increase is related to the formation of phenothiazine radicals, as there is a relationship between the increase in

chemiluminescence and the type of cation radicals, and a small difference in structure inhibits the process.

In: "Proceedings of the 15th International Conference on Bioluminescence & Chemiluminescence" (eds. X. Chen, X-L. Yang, X-R. Zhang, Z.J. Cui, L.J. Kricka, P.E. Stanley), World Scientific, 2009, 193-196.

Variety of chemiluminescent methods for antioxidant activity investigation of
[Hadjimitova V](#), [Traykov T](#), [Bakalova R](#).

Abstract

In order to investigate antioxidant activity (AOA) of ethanol extract from *Crataegus Oxicantha* leaves and flowers, we used three methods based on luminol-dependent chemiluminescence (CL). We used CL detection of hydroxyl radicals ($\bullet\text{OH}$), generated by a H_2O_2 -(Fe^{2+} -EDTA) system, hypochlorite, generated by NaOCl, and decomposition of H_2O_2 by a HRP-luminol system. The combination of these three methods, based on a common amplifier of CL, allows for a comparison to be made of the AOA of hawthorn, exhibited with regard to the three most common *in vivo* reactive oxygen species. We found out that the extract from *Crataegus Oxicantha* shows very strong antioxidant properties. Its strongest AOA is exhibited in HRP- H_2O_2 , followed by scavenger activity with regard to $\bullet\text{OH}$ and hypochlorite. C-50 values obtained by the three systems are respectively <10 mg/L, 90 mg/L and 120 mg/L. We found out that the results observed are not due to a quenching effect, by using an alternative photometric method for $\bullet\text{OH}$ detection.

РЕЗЮМЕТА В СПИСАНИЯ С ИМПАКТ ФАКТОР

[Eur Heart J](#). 1995; 16:S230. Impact factor – 2.153

IgG autoantibodies against oxidized low-density lipoproteins in coronary artery disease patients – do they have diagnostic value?.

[Goudev A](#), [Nachev Ch](#), [Dishlyanova B](#), [Bakalova R](#), [Kehayov I](#), [Runev N](#), [Kyurkchiev S](#).

Abstract

Autoantibodies (AB's) against oxidized LDL (oxiLDL) are known to be common finding in patients with atherosclerosis. It has been presumed that anti-oxiLDL AB's might have a predictive value in patients with carotid atherosclerosis. In the present study high affinity auto-antibodies of IgG class reacting against oxiLDL were tested in CAD patients.

Methods: LDL was isolated by gradient ultracentrifugation and oxidized by treatment with CuSO_4 . The modified *in vitro* LDL was used as an antigen in an enzyme immunoassay (ELISA). Sera from 51 patients (37 male/14 female) with CAD, determined as Q-wave myocardial infarction and/or stenosis of more than 50% from coronarography, and from 51 (34 male/17 female) healthy blood donors as controls, were tested for the presence of IgG AB's against oxiLDL. The sera from both groups were also tested against native LDL. The concentration of total cholesterol, triglycerides, HDL-C, LDL-C, Apo-A and Apo-B was measured as well.

Results: The mean AB's level against modified with CuSO_4 LDL, expressed in optical density units, was 0.594 ± 0.350 in cases and 0.211 ± 0.139 in controls ($p < 0.001$). There was practically no reaction against native LDL. Poor correlation was established between AB's level and the others lipid parameters. Five from 9 patients

with familial hypercholesterolemia (FH) in this study had AB's level in the highest quintal and none of them in the lowest one.

Conclusion: Level of AB's of IgG class reacting against oxLDL in CAD patients was significantly higher than control subjects. Patients with FH have the highest level of AB's. This suggests that antibodies against oxLDL could be used as a marker of atherosclerotic damage of coronary arteries.

[Eur Heart J](#). 1996; 17:S192. Impact factor – 2.153

IgG autoantibodies against oxidized low-density lipoproteins in coronary artery disease patients – do they have diagnostic value?.

[Goudev A](#), [Bakalova R](#), [Runev N](#), [Ganev V](#), [Kyurkchiev S](#), [Kehayov I](#), [Nachev Ch](#).

Abstract

Autoantibodies (AB's) against oxidized LDL (oxLDL) might have a predictive value for further events in patients with carotid atherosclerosis, CHD and PAD. Low serum antioxidant capacity is one of the discussed reasons for accelerated atherosclerosis. In the present study high affinity IgG AB's against oxLDL and lag-phase of serum oxidation were tested in CHD patients and controls.

Methods: Thirty seven (25M/12F) patients with CAD defined as Q-wave M and/or stenosis of more than 50% and 44 (30M/14F) healthy blood donors as controls participated in this study. LDL was isolated by gradient ultracentrifugation and oxidized with CuSO₄. The modified LDL and native LDL were used as antigens in an enzyme immunoassay (ELISA) for detection of IgG AB's in both groups. The serum (1 mg protein/ml) was treated with 46 mM CuSO₄ and the oxidation monitored spectrophotometrically at $\lambda=234$ nm to follow the formation of conjugated diens. The lag-phase (in minutes) is the interval between the addition of CuSO₄ and the beginning of the extensive oxidation. The concentrations of total cholesterol, triglycerides, HDL-cholesterol, Apo-A and Apo-B were measured as well.

Results: The mean AB's level against oxLDL expressed in optical density units was 0.650 +/- 0.330 in CHD patients vs. 0.230 +/- 0.170 in controls (p<0.001). Lag-phase in minutes was 49.24 +/- 28.07 in CHD patients and 77.95 +/- 27.84 in controls (p<0.001). A negative correlation between AB's level and lag-phase was established in CHD patients (r=-0.73, p<0.001) and controls (r=-0.63, p<0.001). A poor correlation was established between AB's level, lag-phase and other measured parameters.

Conclusion: In this study IgG AB's level against oxLDL was significantly higher and lag-phase of serum oxidation was shorter in CHD patients vs. controls. A strong negative correlation between IgG AB's against oxLDL and lag-phase oxidation with CuSO₄ was found in both groups. Lag-phase of serum oxidation could be informative for the LDL susceptibility to modification and the extent of consequent humoral immune response.

[Eur J Pharm Sci](#). 1998; 6:S83. Impact factor – 3.291

Captopril protects against lens lipid peroxidation on diquat-induced cataract in rabbits.

[Mehandjiev D](#), [Mileva M](#), [Bakalova R](#).

Abstract

LPO is considered as a triggering mechanism of cataractogenesis. The goal of present study was to estimate the level of LPO in rabbit eyes after intravitreal injection of a single dose of 150 mmol/l diquat in 30 μ mol/l 0.9% NaCl, in both eyes of each animal. The right eyes were treated with topical Captopyrl solution (1% in 0.9% NaCl), five times daily. The left eyes were treated with the diluent as autocontrol. From slit-lamp biomicroscopic observation, the grading of lenses opacities was evaluated by 7 grade scale: 0 – normal lens; to 6 – complete opacity. At the end of the 10th weeks, the left lenses of the animals reached to 4-6 grade of opacity. The lenses of the Captopyrl-treated right eyes reached to 0-2 grade of opacity. After CO₂ exposure, the animals were scarified and the tissue was used for biochemical analysis.

MDA in lenses of Captopyrl-treated eyes was 150% lower than the left autocontrol eyes. The level of conjugated diens in right lenses was 166% lower than the left ones. In aqueous humor of the left eyes, the level of conjugated diens was 327% lower in comparison with the right eyes. Similar results were obtained for the lipofuscine-like fluorescent products and trienes – 135% and 143% lower, respectively.

[Luminescence](#). 2004; 19(3):192. Impact factor – 1.395

Potential mechanism of the enhancement of photoluminescence of CdSe quantum dots under UV-irradiation: role of free Cd and Se ions

[Zhelev Z](#), [Jose R](#), [Bakalova R](#), [Nagase T](#), [Ohba H](#), [Ishikawa M](#), [Shinohara Y](#), [Baba Y](#).

Abstract

The present study describes an enhancement of the photoluminescence of CdSe quantum dots under long-term UV-irradiation in organic and aqueous solutions. The photoenhancement effect followed a multiexponential kinetics and was found to depend on several factors: intensity of UV-light, polarity of the solvent, presence of capping agents on the nanocrystal surface, and presence of free Cd and Se ions in the solution.

High intensity UV-irradiation provoked a rapid enhancement of the photoluminescence of CdSe nanocrystals, reaching the maximum with subsequent photoluminescence decay. Obviously, two antagonistic processes take place on the nanocrystal surface and influence its photoluminescence quality – the first is responsible for the photoenhancement effect, and the other is UV-mediated destruction of the nanocrystals (approved by decrease of their characteristic absorbance spectra and blue-shifting of both, fluorescent and absorbance spectra).

Low intensity UV-light provoked a comparatively slow enhancement of the photoluminescence of CdSe nanocrystals, reaching saturation after 5-6 hours irradiation in organic solvents (butanol and chloroform). In aqueous solution, the UV-induced photoluminescence dynamics followed almost linear increase during 9 hours irradiation. The photoenhancement effect was reversible or irreversible depending of the additional ingredients. The role of free Cd and Se in these processes was clarified. The results are discussed in the context of UV-induced liberation of free Cd and Se ions from the nanocrystal surface and their hypothetical reversible deposition with trapping of the surface holes and influencing the efficiency of radiative versus nonradiative exciton decay during the enhancement of photoluminescence.

[Luminescence](#). 2004; 19(3):132. Impact factor – 1.395

Application of highly luminescent quantum dot bioconjugates in protein imaging: quantum dot-based immunoblot analysis

[Bakalova R](#), [Zhelev Z](#), [Ohba H](#), [Ishikawa M](#), [Baba Y](#).

Abstract

The highly luminescent semiconductor quantum dots (QDots) have attracted a great interest for life science research, because of their potential to be used as new fluorescent probes in cell and protein imaging techniques. The interest to QDots is based on their higher brightness than the conventional fluorophores and privilege for single-source excitation for all colors.

The present study describes a synthesis of highly luminescent Qdot-bioconjugates and development of method for Qdot-based Western blot analysis. Water-soluble CdSe quantum dots (~2-3 nm) were conjugated with several antibodies - anti-c-abl, anti-lamin A/C and anti- β -actin, and were applied for immunoblot analysis of the respective proteins in leukemia cells (K-562 - derived from chronic myelogenous leukemia, and Jurkat – derived from acute lymphoblastic leukemia). In Qdot-based Western blot analysis we used only a primary antibody and the photoluminescence of Qdot-antibody conjugates, retained on PVDF-membrane, was detected by ChemImager. The described procedure avoided the application of secondary antibody and subsequent HRP-catalyzed enzyme reaction with formation of luminescent product, which often compromises the results after ensuring saturation. The sensitivity of Qdot-based immunoblot analysis is about 3 times higher than that of the conventional Western blot procedure. However, the photobleaching of Qdot-labeled blots was faster and the stability of Qdot-antibody blotted membranes was lower than in conventional immunoblot procedure. To ensure higher efficiency and to guarantee comparatively high stability of Qdot-blot photoluminescence, biotinilated antibodies were covalently conjugated with Qdot and sandwich-type avidin-biotin assay system was additionally applied, using Qdot-avidin and Qdot-biotin conjugates.

[Luminescence](#) .2004; 19(3):142. Impact factor – 1.395

Chemiluminescent analysis of the antioxidant and immunomodulation effects of several psychotropic drugs on peritoneal macrophages.

[Hadjimitova V](#), [Traikov T](#), [Bakalova R](#), [Petrova L](#), [Lambev I](#), [Ishikawa M](#), [Baba Y](#).

Abstract

The ability of peritoneal macrophages to produce superoxide radicals and to induce luminol-dependent chemiluminescence was used to test the antioxidant and immunomodulating effects of several psychotropic drugs: three-cycled antidepressants (imipramine, IMI; amitriptyline, AMI), phenothiazines (chlorpromazine, CPZ) and thioxanthenes (chlorprothixene, CPX). The induction of luminol-dependent chemiluminescence was carried out by activation of protein kinase C- or calmodulin-dependent “oxidative burst” of macrophages, using phorbol-12-myristate-13-acetate (PMA) and calcium ionophore A23187, respectively. The inhibitory effect of CPZ on the PMA-/A23187-induced chemiluminescence was higher than the ability of the drug to decrease KO₂-induced chemiluminescence in a pure chemical system, as a result of its scavenger activity against superoxide radicals only. Presumably, the inhibitory effect of CPZ on the PMA-/A23187-induced macrophage chemiluminescence was also a result of its immunomodulating activity. In contrast, the antidepressants (IMI, AMI) manifested a weak effect on the luminol-dependent chemiluminescence of the macrophages and did not express any effect on KO₂-induced chemiluminescence.

It was also observed that the suppression of the macrophage chemiluminescence by all the investigated drugs was not a result of their toxicity. Moreover, it was

established that all drugs dose-dependently enhanced the macrophage ATP bioluminescence, which is an indirect evidence for immunomodulation.

[Luminescence](#). 2008; 23:70-71. Impact factor – 1.395

Luminol-dependent chemiluminescence increases with formation of phenothiazine cation radicals by horseradish peroxidase

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The introduction of phenothiazines into a HRP-H₂O₂-luminol system causes a strong increase in chemiluminescence. This investigation includes chlorpromazine, levomepromazine, trifluoperazine, thioridazine and promethazine, in concentrations of 5.10⁻⁸–5.10⁻⁵ mol/L. The results show that all these substances increase chemiluminescence up to eight times above the control. As a result of the processes occurring in the samples, cation radicals of phenothiazines are generated. Their presence is demonstrated through their characteristic absorption in the visual range. The radicals' concentration was proportional to the chemiluminescence increase. Chlorprothixene's structure is very similar to that of chlorpromazine but does not allow the formation of a radical, as in the case of chlorpromazine. Putting chlorprothixene instead of chlorpromazine into the system does not cause a chemiluminescence increase. We conclude that the chemiluminescence increase is related to the formation of phenothiazine radicals, as there is a relationship between the increase in chemiluminescence and the type of cation radicals, and a small difference in structure inhibits the process.

[Luminescence](#). 2008; 23:71. Impact factor – 1.395

Variety of chemiluminescent methods for antioxidant activity investigation of *Crataegus oxicantha* extract

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In order to investigate the antioxidant activity (AOA) of ethanol extract from hawthorn (*Crataegus oxicantha*) leaves and flowers, we used three methods based on luminol-dependent chemiluminescence (CL). We used CL detection of hydroxyl radicals ($\cdot\text{OH}$), generated by a H_2O_2 -(Fe^{2+} -EDTA) system, hypochlorite generated by NaOCl, and decomposition of H_2O_2 by a HRP-luminol system. The combination of these three methods, based on a common amplifier of CL, allows for a comparison to be made of the AOA of hawthorn exhibited with regard to the three most common *in vivo* reactive oxygen species. We found that the extract from *C. oxicantha* showed very strong antioxidant properties. Its strongest AOA was exhibited in HRP- H_2O_2 , followed by scavenger activity with regard to $\cdot\text{OH}$ and hypochlorite. C-50 values obtained by the three systems were, respectively, <10 mg/L, 90 mg/L and 120 mg/L. By using an alternative photometric method for $\cdot\text{OH}$ detection, we found that the results observed were not due to a quenching effect.
