

Statement

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About: PhD thesis of Desislava Lazarova

Title: Contrast-enhanced magnetic resonance techniques of pathologies, based on cellular redox-imbalance

The subject of the thesis is very actual and relevant. The aim of the study is to develop visualization and analytical approaches, both in vitro (at cellular level) and in vivo (tissue redox-status), in pathologies accompanied by redox disbalance and oxidative stress. The mito-TEMPO spin-probe, which penetrates through the cell membranes, was used. The probe is localized mainly in the mitochondria, a major endogenous source of reactive oxygen species and an inducer of oxidative stress in various pathologies.

The presented thesis is written on 95 standard pages, contains 39 figures, and divided by standard sections: Introduction, Review of Literature, Aims and Tasks, Materials and Methods, Results and Discussion, Conclusions, Contributions, References. The work is well balanced; the largest section is the Results and Discussion.

The aim of this dissertation is to develop contrast-enhanced magnetic resonance methods for visualizing pathologies, accompanied by disorders in the cellular redox-status. In order to achieve this purpose, precise and clearly formulated tasks are set.

For the study, wide methodological approaches are used. The methods are described in details in Material and Methods chapter. For the purposes of the studies, model systems, cell lines were used, comparing the redox-status of normal and cancer cells of the same origin but different differentiation level: FHC, HT29, HCT116 and Colon26; as well as two experimental animal models (renal dysfunction and Parkinson's disease model). The PhD student has analyzed and interpreted the results thoroughly, taking into account the advantages and disadvantages of the used spin-probe and magnetic resonance imaging techniques.

The results obtained during this study are summarized as contributions with an original character.

1. A new methodical approach has been developed by using EPR spectroscopy to identify isolated cultured cell lines of the same origin, but with different proliferative activity. The method is based on the dynamics of the EPR contrast of mito-TEMPO in the cell suspensions and allows the assessment of the level of intracellular superoxide in absolute units.
2. A new methodical approach has been developed for early diagnosis of renal damage *in vivo*, based on changes in tissue redox-status induced by hypercholesterolemia. The method is based on the dynamics of the MRI-contrast of mito-TEMPO nitroxide radical in the renal cortex at the development of glomerulosclerosis and the increase of oxidative capacity of the cells in this area.
3. The possibility of using mito-TEMPO as a new contrast agent for the early diagnosis of renal damage by nitroxide-enhanced urography has been demonstrated.

The results presented in this dissertation are published in 6 scientific articles (4 with impact factor and 2 without), in two of the articles the PhD student is a first author. The results are also presented at 4 scientific forums. The total impact factor of the publications is 7.249. Noticed citations are 73.

Conclusion

The thesis of Desislava Lazarova is written in correct and pure Bulgarian language, balanced and structured very well. The PhD student has done a great deal of experimental work. The experiments on the dissertation are logically planned, precisely realized and analyzed. The content of the autoreferate correspond to the dissertation.

The number of published papers, congress presentations and citations of the results of thesis cover and even exceed the criteria to obtain doctor's degree, according to the rules of the Sofia University "St. Kl. Ohridski".

All mentioned above, as well as my personal impressions from the PhD student, gives me a good reason to recommend to the scientific jury to award to Desislava Lazarova the educational and scientific degree "doctor", in the professional field 4.1 Physical Sciences, scientific specialty: Biophysics.

March 25th 2019

assoc. prof. Biliانا Nikolova PhD

