Trehalose Lipid Biosurfactant Reduced Cancer Cell Viability but Did not Affect the Isometric Contraction of Rat Mesenteric Arteries *in vitro*

Boris Kadinov[1#], Biliana Nikolova[2#], Iana Tsoneva[2], Severina Semkova[2], Lyudmila Kabaivanova[3], Daniela Dimitrova[2*]

[1]*Institute of Neurobiology*
Bulgarian Academy of Sciences
Acad. G. Bonchev Str., Bl. 23
1113 Sofia, Bulgaria
E-mail: kadinovb@bio.bas.bg

[2]*Institute of Biophysics and Biomedical Engineering*
Bulgarian Academy of Sciences
Acad. G. Bonchev Str., Bl. 21
1113 Sofia, Bulgaria
E-mails: nikolova@bio21.bas.bg, itsoneva@bio21.bas.bg, severina@bio21.bas.bg, daniadim@yahoo.com

[3]*The Stefan Angeloff Institute of Microbiology*
Acad. G. Bonchev Str., Bl. 26
1113 Sofia, Bulgaria
E-mail: lkabaivanova@yahoo.com

[*]*Corresponding author*

[#]*B.K. and B.N. participate equally in the present work*

Received: March 26, 2019 **Accepted: November 21, 2019**

Published: March 31, 2020

Abstract: *Trehalose lipid biosurfactant from Nocardia farcinica strain is a naturally derived substance with potent anticancer activity. The increasing interest in naturally derived substances-based modality of cancer treatment requires investigations of the possible adverse effects of these substances, including the effects on vasculature. Therefore the present study was designed to investigate the effect of Trehalose lipid on isometric contraction of isolated rat mesenteric arteries. The contractile responses of arteries under Trehalose lipid was studied using wire myography for small blood vessels. The isometric contractions of rat mesenteric artery rings with intact endothelium were examined. The effect of this biosurfactant was assessed in arteries precontracted with 42 mM KCl as a vascular smooth muscle depolarizing stimulus. The results showed that Trehalose lipid (75 μM) failed to change high K^+-induced contractions. The observed lack of effect of Trehalose lipid biosurfactant on the contractility of rat mesenteric arteries in vitro together with finding of reduced cancer cells viability makes it to be a suitable for potential medical application.*

Keywords: *Trehalose lipid, Breast cancer cells, Mesenteric artery, Isometric contraction, Vascular smooth muscle, Rat.*

Introduction

Biosurfactants produced by microorganisms are promising molecules due to their structural novelty, surface activity, versatility, and diverse properties for many biomedical applications. Various Trehalose containing glycolipids playing role of biosurfactants are known to be produced by microorganisms belonging to mycolates group such as *Arthrobacter*, *Nocardia*,

Rhodococcus and *Gordonia*. From the biomedical point of view, Trehalose lipid biosurfactants have been shown to exert an impressive number of physiological and pathophysiologically-related properties such as antimicrobial, antiviral, anti-adhesive, anticancer or immunomodulating properties [6].

The anti-tumor potential of these molecules is being studied, although results are still scarce and few data are available regarding the mechanisms underlying such activity [1, 5].

The biosurfactants such as Trehalose lipid can act as therapeutic agents due to their functions in cell membrane interactions. Trehalose lipids possess antitumor activity on hematopoietic human cell lines and the antiproliferative effect is dose dependent [3].

During chemotherapy the antitumor drugs for solid tumors are injected predominately in the blood. While evaluations of Trehalose lipid biosurfactant from *Nocardia farcinica* strain as a perspective anticancer agent have expanded [3], scientific data concerning possible side effects or its toxicity were almost completely lacking. The cardiovascular system and particularly blood vessels represent such a common target for undesired actions of many drugs, including anticancer ones during cancer medication treatment [12]. Furthermore, it is well established that the alterations in the artery contractility coincide with vasospasm for example hence leading to life-threatening conditions [7]. The investigation of the vasomotor properties of arteries upon treatment with a substance of interest as a sign of the toxicity of such a compound is already implemented in similar experimental design [13].

Therefore, the scientific area of physiology and pathophysiology of blood vessels continuously attracts the scientific interest and the research efforts in terms of the importance of this topic. Moreover, the investigation dedicated exclusively to mesenteric artery blood vessels, could contribute sufficiently to the impact of every research using these arteries as objects at least due to the fact that mesentery was designated as an organ fairly recently [4].

Hence, the present study was aimed to examine the complex interaction between Trehalose lipid biosurfactant from *Nocardia farcinica* strain and vascular smooth muscle contraction machinery during isometric contraction of isolated rat mesenteric artery in order to ensure its proper biomedical application.

Materials and methods

Trehalose lipid biosurfactant was isolated from *Nocardia farcinica* strain BN26. Isolation, purification and characterization were previously described [3].

Cell line

The breast cancer cell line MDA-MB231 was cultivated in RPMI-1640 medium (Invitrogen, Karlsruhe, Germany) supplemented with 10% fetal calf serum (FCS) and 2 mM L-glutamine. The cells were maintained at 37 °C in an incubator with humidified atmosphere containing 5% CO_2 and were routinely passaged when 80-85% of cells were confluent using 0.25% trypsin and 0.02% EDTA (Invitrogen, Karlsruhe, Germany).

Cell viability assay

The cytotoxicity effect of Trehalose lipid was assessed by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) test. Cells were seeded at concentration 1×10^5 cells/ml 24 h prior treatment for the adhesion. Therechalose lipid in corresponding concentrations

(15 μM; 25 μM; 35 μM; 45 μM; 50 μM; 60 μM) was added. Cells were exposed to the substance for 24 h. Every treatment point was triplicated. After Trehalose lipid treatment MTT solution (10 mg/ml) was added to each well. The plates were incubated 3 h at 37 °C and the formed formazan crystals were dissolved with 110 μl of 5% formic acid in 2-propanol. Absorption was measured at 580 nm. The controls were treated in the same way without Trehalose lipid.

Contraction study

Procedures with rat mesenteric artery were performed in accordance with the Declaration of Helsinki and were approved by Ethics Committee of the Institute of Neurobiology, Bulgarian Academy of Sciences. Arterial segments were isolated from rat mesentery (male matured rat, six rats, $n = 6$). Contractile responses of the artery preparations were registered using the precise method of myography of small artery by Mulvany-Halpern wire myograph (Model 410 A, DMT, Denmark) [10]. Segments of rat mesenteric artery with intact endothelium and perivascular nerves suspended in modified physiological salt solution were normalized and contracted twice by 125 mM KCl-containing physiological salt solution (PSS), the last consisting of (in mM): 120 NaCl, 4.5 KCl, 1.0 MgSO4, 25 NaHCO3, 1.2 NaH2PO4, 0.025 EDTA, 2.5 $CaCl_2$, 5.5 glucose; pH 7.4 at 37 °C). An application of 25 μl (final concentration 75 μM) of Trehalose lipid or the its vehicle – DMSO (25 μl) were added to the organ bath on the steady-state contractile response elicited by 42 mM KCl-containing physiological salt solution approximately for one hour. The concentration of Trehalose lipid chosen for the *in vitro* contraction experiments was in accordance with the previously published data reporting the concentration of DMSO below 1% in the organ bath as suitable for revealing the proper contraction properties of arteries [11]. At the end of each experiment, arterial strips were contracted with noradrenaline (10^{-5} M) in order to confirm the viability of the artery preparations. The measurements were done Trehalose lipid application on 42 mM KCl-containing PSS-developed isometric contraction at 10, 20 and 30 minutes.

Analysis of data and statistics

Measurements of Trehalose lipid-developed tension are presented as a percentage of 42 mM KCl-elicited isometric contractions (in mN/mm). Data on the figures are expressed as means ± SEM and n indicates the number of isolated rat mesenteric artery segments. The results were compared statistically by means of one-way ANOVA with Bonferroni correction; $p < 0.05$ was considered to be significant.

Results and discussion

The cytostatic effect of Trehalose lipid was assessed by MTT. High metastatic breast cancer cell line MDA-MB 231 was treated for 24 h with 15 μM; 25 μM; 35 μM; 45 μM; 50 μM; 60 μM, respectively. The response was dose dependent upon the applied treatment (Fig. 1). Calculated IC_{50} = 46.919 μM.

For the second part of experiments choose concentration was 75 μM, almost two times higher than calculated IC_{50}. Different exposure time during the contractile response experiments (up to 1 h) was reason for increase of the concentration.

The results demonstrated that Trehalose lipid (75 μM) did not potentiate or attenuate the contractile responses of the endothelium-intact rat mesenteric arteries in the conditions of depolarization (by 42 mM KCl) of vascular smooth muscle (Fig. 2 and Fig. 3). Moreover, the observed slight relaxation of isometric artery preparations in the medium of Trehalose lipid

plus DMSO, as well as the DMSO alone-treated mesenteric segments is completely due to the vehicle of the Trehalose lipid – DMSO.

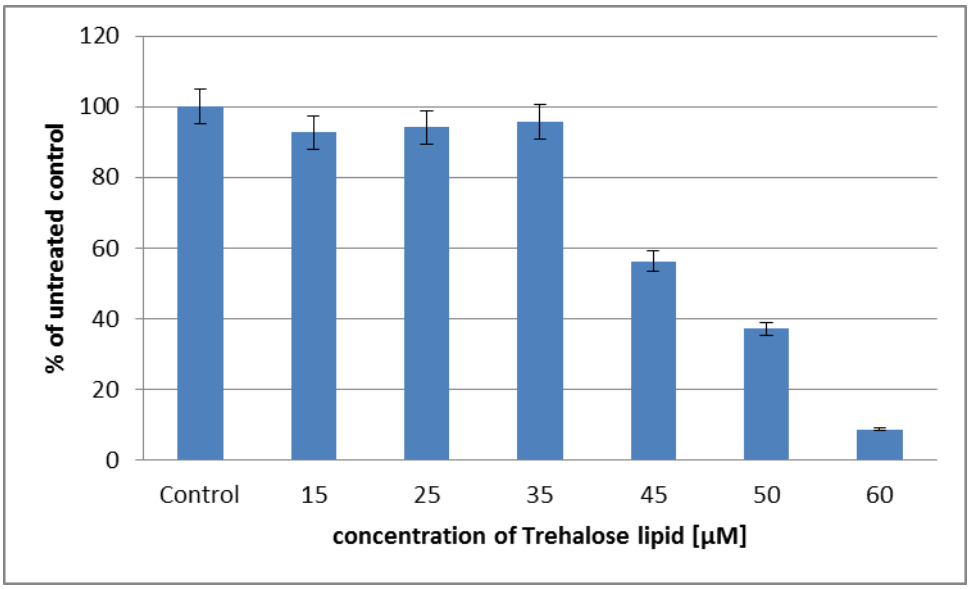

Fig. 1 *In vitro* cytostatic effect of Trehalose lipid on MDA-MB 231 cell line viability, 24 h after treatment. The data are averaged from three independent experiments. Bars – SD (standard deviation).

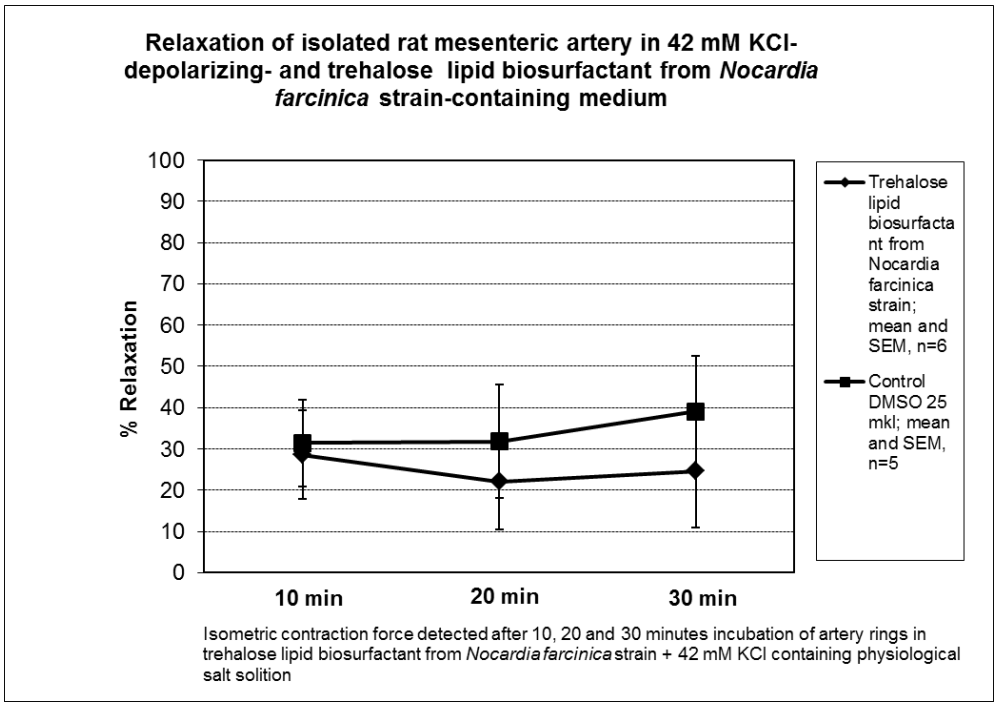

Fig. 2 Effect of Trehalose lipid biosurfactant from *Nocardia farcinica* strain on isometric contraction of rat mesenteric arterial segments with intact endothelium precontracted with 42 mM KCl-containing PSS. Dose-response curves were constructed from the mean values of the measurements of tension (mN/mm). Squares: tension developed under stimulation with Trehalose lipid biosurfactant from *Nocardia farcinica* strain. Diamonds: control experiments, in which DMSO (vehicle of the substance) was applied in corresponding volumes. Bars are SEM; ANOVA test with Bonferroni correction was performed at $p < 0.05$.

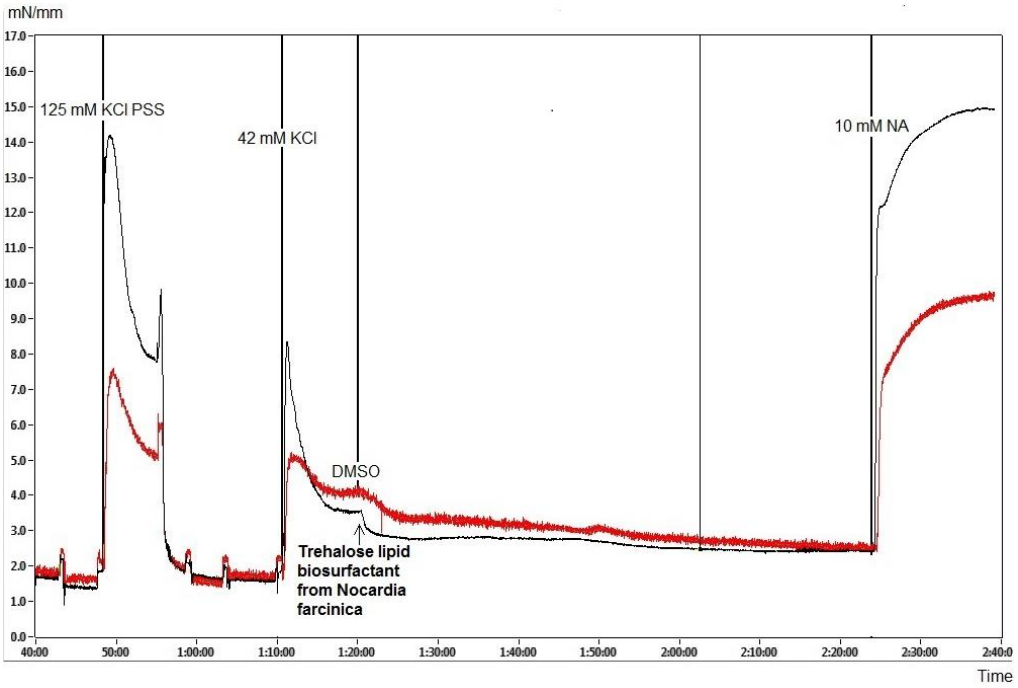

Fig. 3 Typical original traces exemplifying the effect of Trehalose lipid biosurfactant from *Nocardia farcinica* strain on rat mesenteric artery contraction *in vitro*.
Black line: time course of artery isometric contraction upon consequent stimulation with 125 mM KCl-containing PSS, 42 mM KCl-containing PSS, Trehalose lipid biosurfactant from *Nocardia farcinica* strain-containing PSS and 10 mM noradrenaline (NA)-containing bath solution. Grey line: time course of artery isometric contraction upon consequent stimulation with 125 mM KCl-containing PSS, 42 mM KCl-containing PSS, DMSO-containing PSS and 10 mM noradrenaline (NA)-containing bath solution.

Recently, glycolipid biosurfactants including Rhamno lipid, Trehalose lipid, Succinoil-trehalose lipid have been shown to have effects on cancer cells [2, 3, 8]. It was found that Rhamnolipids are less effective compared to Trehalose lipid [2, 3]. As it is shown (Fig. 1) the anticancer effect of Trehalose lipid is in micromolar concentrations. Our data about reduced cell viability of breast cancer cells (MDA-MB231) as well as the lack of data about vascular toxicity of Trechlose lipid isolated from *Nocardia farcinica* encouraged us to conduct the present study. This is the first study investigating the effect of biosurfactants on cardiovascular system *in vitro*. A lack of toxicity on vascular smooth cell contractility was found thus suggesting the potential of the use of Trechlose lipid in the field of biomedical applications. These findings are in concert with mainstream scientific opinions pointing out that biosurfactant are generally considered safer than synthetic pharmaceuticals, due to their biological origin [12]. The present results showing the non-toxic effect of Trehalose lipid are in concert with previously published data [9, 12]. Nevertheless, the data demonstrating some level of toxicity of Treholose lipid-containing biosurfactant also exist in the literature [6]. This suggests the need of further thorough investigation of every different type of toxicity of Trehalose lipids with a potential to be involved in biomedical treatment protocols.

Conclusion

This is the first study in the literature investigating the role of Trehalose lipid on arterial smooth muscle function thus contributing to the scientific knowledge of crosstalk of this perspective antitumor substance and cardiovascular system. The obtained results could be aimed at the development of adequate disease treatment.

Acknowledgements

This study was supported by the National Scientific Fund of Bulgaria by contract DN11/2, 14.12.2017 to B.N.

References

1. Akiyode D., J. George, G. Boateng, G. T. M. Getti (2015). Application of Biosurfactants in Cancer Therapy, Ann Oncol, 26 (Supplement 2), ii28-ii30.
2. Christova N., B. Tuleva, A. Kril, M. Georgieva, S. Konstantinov, et al. (2013). Chemical Structure and *in vitro* Antitumor Activity of Rhamnolipids from *Pseudomonas aeruginosa* BN10, Appl Biochem Biotechnol, 170, 676-89.
3. Christova N., S. Lang, V. Wray, K. Kaloyanov, S. Konstantinov, et al. (2015). Production, Structural Elucidation, and *in vitro* Antitumor Activity of Trehalose Lipid Biosurfactant from *Nocardia farcinica* Strain, J Microbiol Biotechnol, 25, 439-447.
4. Coffey J. C., P. O'Leary (2016). The Mesentery: Structure, Function, and Role in Disease, Lancet Gastroenterol Hepatol, 1, 238-247.
5. Duarte C., E. J. Gudiña, C. F. Lima, L. R. Rodrigues (2014). Effects of Biosurfactants on the Viability and Proliferation of Human Breast Cancer Cells, AMB Express, 4, 40 http://www.amb-express.com/content/4/1/40.
6. Franzetti A., I. Gandolfi, G. Bestetti, T. J. P. Smyth, I. M. Banat (2010). Production and Application of Trehalose Lipid Biosurfactants, Eur J Lipid Sci Technol, 112, 617-627.
7. Herrmann J., A. Lerman (2012). Coronary Artery Disease: New Approaches without Traditional Revascularization, Barsness, Holmes, G. D., Ed., Springer-Verlag London Limited, 21-28.
8. Isoda H., D. Kitamoto, H. Shinmoto, M. Matsumura, T. Nakahara (1997). Microbial Extracellular Glycolipid Induction of Differentiation and Inchibition of Protein Kinase C Activity of Human Promyelocytic Leukaemia Cell Line HL60, Biosci Biotechnol Biochem, 61, 609-614.
9. Marques A. M., A. Pinazo, M. Farfan, F. J. Aranda, J. A. Teruel, et al. (2009). The Physiochemical Properties and Chemical Composition of Trehalose Lipids Produced by *Rhodococcus erythropolis* 51T7, Chem Phys Lipids, 158, 110-117.
10. Mulvany M. J., W. Halpern (1977). Contractile Properties of Small Arterial Resistance Vessels in Spontaneously Hypertensive and Normotensive Rats, Circ Res, 41, 19-26.
11. Pitts L., A. Young, J. McCulloch, E. MacKenzie (1986). Vasomotor Effects of Dimethyl Sulfoxide on Cat Cerebral Arteries *in vitro* and *in vivo*, Stroke, 17, 483-487.
12. Rodrigues L., I. M. Banat, J. Teixeira, R. Oliveira (2006). Biosurfactants: Potential Applications in Medicine, J Antimicrob Chemoth, 57, 609-618.
13. Suresh V., A. Khavandi, H. Hancock (2004). 5-Fluorouracil Induced Coronary Artery Vasospasm, Heart, 90, 439.
14. Vukova T., S. Dimitrov, H. Gagov, D. Dimitrova (2016). In Focus: Fe_3O_4 Nanoparticles and Human Mesenteric Artery Interaction *in vitro*, Nanomedicine, 11, 921-932.

Boris Kadinov, Ph.D.
E-mail: kadinovb@bio.bas.bg

Boris Kadinov graduated Sofia University "St. Kliment Ohridski", Faculty of Biology, specialization Zoology in 1998. Since 1998 he is working in Institute of Physiology, Bulgarian Academy of Sciences. He received his Ph.D. Degree in 2014 in Institute of Neurobiology, BAS. His scientific interests are in the field of electrophysiology of smooth muscle, physiology of visceral muscles, wire myography of vessels, oxidative stress, endogenous carbon monoxide and gaseous neurotransmitters.

Assoc. Prof. Biliana Nikolova, Ph.D.
E-mail: nikolova@bio21.bas.bg

Biliana Nikolova graduated Sofia University "St. Kliment Ohridski", Faculty of Biology, specialization Biochemistry and Microbiology in 1992. Since 1992 she is working in Institute of Biophysics, later in Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences. She received her Ph.D. degree in 2001 in Institute of Biophysics. Since 2012 she is an Associate Professor in the Institute of Biophysics and Biomedical Engineering. Her scientific interests are in the field of electroporation, electroloading, drug delivery systems and electrochemotherapy.

Prof. Iana Tsoneva, D.Sc.
E-mail: itsoneva@bio21.bas.bg

Prof. Iana Tsoneva has Ph.D. Degree in 1976 and D.Sc. in 2005. Since 1990 she is an Associate Professor and Professor – since 2006. Her interests are in the field of electroporation; influence of the electrical field on the membrane permeability; electrotransfer of macromolecules in cancer and normal cells; electrical properties of biological membranes; electrochemotherpy; electroinduced changes in cell adhesion – adhesion; cell proliferation, and viability in normal and neoplastic cells. She is an author of more than 70 papers in the field of electroindiced effect.

Senior Assist. Prof. Severina A. Semkova, Ph.D.
E-mail: severina@bio21.bas.bg

Severina Semkova received B.Sc. Degree in 2011; M.Sc. Degree in Biophysics in 2013 from Sofia University "St. Kliment Ohridski", Faculty of Biology, specialization Molecular Biology –. She received her Postgraduate Qualification "Teacher in Biology" in 2013. In 2017 she received Ph.D. Degree in the Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences. Her scientific topics are in anticancer drugs, electrochemotherapy, fluorescent imaging, nanoparticles and drug delivery.

Assoc. Prof. Lyudmila Kabaivanova, Ph.D.
E-mail: lkabaivanova@yahoo.com

Lyudmila Kabaivanova graduated from Sofia University "St. Kliment Ohridski", Faculty of Biology, with M.Sc. Degree in Biochemistry and Microbiology. She received her Ph.D. Degree in Microbiology at The "Stephan Angeloff" Institute of Microbiology, Bulgarian Academy of Sciences. Currently, she is an Associate Professor, Scientific secretary at the same institute and Head of Department "Applied Microbiology". Her scientific interests are in the field of microbiology and biotechnology, obtaining of bioactive substances from bacteria and algae with different applications.

Senior Assist. Prof. Daniela Dimitrova, Ph.D.
E-mail: daniadim@yahoo.com

Daniela Dimitrova graduated Sofia University "St. Kliment Ohridski", Faculty of Biology, specialization Ecology in 1991. Since 1995 she is working in Bulgarian Academy of Sciences at Institute of Physiology, Institute of Molecular Biology, Institute of Experimental Morphology and Anthropology, Institute of Biophysics and Institute of Biophysics and Biomedical Engineering. She received her Ph.D. Degree in 2010 in Physiology. Since 2011 she is a Senior Assistant Professor at the same institute. Her scientific interests are in the field of physiology of smooth muscle, artery physiology, ion channels, and molecular biology.