Do Mistletoe (*Viscum album* L.) Lectins Influence Isometric Contraction of Non-diseased Human Mesenteric Arteries *ex vivo*?

Daniela Z. Dimitrova[1*], Biliana Nikolova[1], Vanya Bogoeva[2], Bozhil Robev[3], Iana Tsoneva[1], Stanislav Dimitrov[4], Boris Kadinov[5]

[1]*Department of Electroinduced and Adhesive Properties*
Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences
Sofia 1113, Bulgaria
E-mail: daniadim@yahoo.com

[2]*Department of Molecular Biology of Cell Cycle*
Institute of Molecular Biology "Acad. Roumen Tsanev", Bulgarian Academy of Sciences
Sofia 1113, Bulgaria
E-mail: bogoevaviva7@gmail.com

[3]*Hematology Clinic, Department of Medical Oncology*
University Multi-Profile Hospital for Active Treatment (UMHAT) "St. Ivan Rilski"
Sofia 1606, Bulgaria
E-mail: bostro@abv.bg

[4]*Department of Anesthesiology and Intensive Care*
Military Medical Academy
Sofia 1606, Bulgaria
E-mail: anestezist@abv.bg

[5]*Department of Synaptic Signalisation and Communications*
Institute of Neurobiology, Bulgarian Academy of Sciences
Sofia 1113, Bulgaria
E-mail: kadinovb@bio.bas.bg

[*]*Corresponding author*

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Abstract: *Mistletoe (Viscum album L., VA) lectins (MLs) are plant lectins with potent anticancer activity. Although wide use of VA extracts in curing cancer, the effects of purified MLs on human vasculature in term of possible side effect of the lectin has not yet been reported. The present study was aimed to investigate isometric contractions of isolated human mesenteric arteries during MLs application. The contractile response of arteries was studied using Mulvany-Halpern myograph and the isometric contractions under MLs' treatment were examined in artery segments with either intact endothelium or after endothelium removal. Furthermore, the effect of the lectin was assessed in arterial preparations in basal tension, in arteries precontracted with 42 mM KCl as a depolarizing stimulus or endothelin-1 (ET-1) as a potent receptor-operated agonist of vascular smooth muscle contraction. The results showed that MLs (1 to 100 nM) failed to affect the high K^+-induced contractions of both endothelium-intact and endothelium-denuded arteries. The contractions of tissue preparations without endothelium in basal tone or after ET-1 (1 nM) treatment were also not affected by the application of MLs. The observed mild effect of MLs on the contractility of human vasculature may potentially be beneficial with MLs-based anticancer therapy without vascular side effects.*

Keywords: *Mistletoe lectins, Human mesenteric artery, Contraction, ex vivo, Cancer treatment.*

Introduction

Recently the extracts of plant *Viscim album* L. (VA) or European mistletoe are among the most prescribed and utilised therapeutic agents in complementary and alternative medicine therapies for cancer in Europe, particularly in German-speaking countries [1, 20, 22]. The most being commercialised adjuvant anticancer drugs are known as Iskador AG (Arlesheim, Switzerland of total mistletoe lectin concentration 287 ng/ml) [16, 30]. Published papers have reported a variety of biological effects of VA extracts and among them: antitumor activity and immune system stimulating properties [8]. Preclinical research and clinical trials on the VA-based cancer treatment also exist [14, 17, 20, 22]. While clinical evaluations of VA preparations as perspective anticancer agents in adjuvant cancer therapy have expanded [10], scientific data concerning possible side effects of the VA extracts and their ingredients are insufficient. In this context, cardiovascular system and particularly blood vessels represent such a common target for undesired actions of many drugs, including anticancer ones during cancer medication treatment [26]. Furthermore, it is well established that the alterations in the artery contractility coincide with vasospasm for example hence leading to live-threatening conditions [9]. Therefore, the scientific area of physiology and pathophysiology of blood vessels attracts continuously the scientific interest the research efforts in term of the importance of this topic of research. In the existing scientifically based evidences of concerning the impact of VA compounds on cardiovascular system, only few papers reported the effect of VA extracts or the plant's ingredients on contractile behavior of arterial blood vessels.

Among these actions, a vasodilatory effect of VA extracts was observed [5, 13, 18, 27] and one study showed a slight contractile effect of VA compounds on noradrenaline-preconstricted rat aortic preparations [3]. Concerning the other influences of VA extract on blood vessels, a clear anti-angiogenic effect has been ascertained [6, 12] and the observed effect generally was attributed to the cytotoxic (damaging) action of VA on the endothelial cells [6]. Since the lectin's fraction of the mistletoe extract (MLs) exhibited remarkable cancer cell cytotoxicity and immunomodulatory activities as well, MLs have been the most thoroughly investigated ingredients of VA extract. For example, the galactoside-specific lectin *Viscum album* agglutinin-I (VAA-I) possesses valuable bioactive properties as immunomodulatory [7] and antitumor [15, 24] and this particular lectin might be of great interest for the potential future use for cancer therapy/treatment or against cancer diseases.

Examining the literature concerning the effect of lectins on both healthy and tumor vasculatures, the cytotoxic action of MLs on endothelium of tumor vessels resulting in a disturbance of tumor angiogenesis was found to be reported [4, 21]. More recently, a poorly developed vascularisation in mouse tumor due to the diet containing purified VAA-I have been demonstrated [23]. It could be acknowledged that observed anti-angiogenic action of MLs contributes to overall anti-tumor effects of the compound in term of use of VA lectins in cancer therapy, especially in the modern blood vessels-targeted cancer treatment.

In summary, despite of wide use of VA extracts and mistletoe-derived lectins in curing cancer as well as the importance of the topic of research involving the evaluation of possible cardiovascular side effects exerted by MLs, no one research focused on artery physiology upon MLs treatment has been conducted to date.

The aim of the present study was therefore to investigate the isometric contractile responses of arteries isolated from human mesentery and subjected to the MLs application *ex vivo*.

Materials and methods

Contraction study

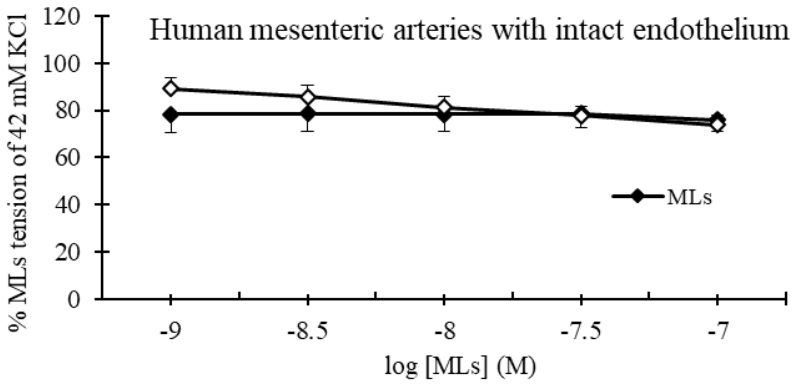
Experimental procedures with human mesenteric artery were performed in accordance with the Declaration of Helsinki. The experimental design was ethically approved and oral informed consents were obtained from participating patients. Non-diseased mesenteric arterial segments were isolated from patients ($n = 11$, aged 41-66 years) undergoing elective abdominal surgery for non-inflammatory conditions (the majority: colorectal carcinoma). The research was conducted using the method of wire myography of small blood vessels [19]. Briefly, artery ring preparations were mounted on Mulvany-Halpern myograph (Model 410A, DMT, Denmark) for recording the isometric contraction. Endothelium either was left intact or was removed. The experiments were done in aerated modified physiological salt solution (PSS) consisting of (in mM) 120 NaCl, 4.5 KCl, 1.0 $MgSO_4$, 25 $NaHCO_3$, 1.2 NaH_2PO_4, 0.025 EDTA, 2.5 $CaCl_2$, 5.5 glucose, pH 7.4 at 37 °C. The experimental design included normalisation procedures of the vessels strips and consequent incubations of the arterial segments with 125 mM K^+-containing PSS (twice) and histamine (100 nM). Further contractions were induced by either 42 mM KCl or 1 nM ET-1 followed by an application of cumulative doses of MLs (1, 3, 10, 30 and 100 nM) or the vehicle (i.e., PSS) of the substance. At the end of each experiment, arterial strips were contracted with high K^+-containing PSS to in order confirm the viability of the artery preparations. The lectin (mixture of ML I, ML II and ML III) from *Viscum album* L. (European mistletoe) was purchased by Sigma-Aldrich and was dissolved before experiments with phosphate-buffered saline (PBS) in accordance with the supplier's recommendations.

Analysis of data and statistics

The recorded artery wall tension (in mN/mm) upon MLs stimulation are presented as a percentage of maximal 42 mM KCl or 1 nM ET-1-elicited isometric contractions. Moreover, data on the figures are expressed as means ±SEM and *n* indicates the number of used human mesenteric artery segments. Statistical comparison between the data yielded by arterial contractions of treated and non-treated artery segments was performed by means of one-way ANOVA with Bonferroni's correction ($p < 0.05$).

Results and discussion

Human mesenteric arteries contractile function *ex vivo* under MLs treatment were investigated in different conditions (i.e. endothelium intact- and endothelium denuded- preparations as well as precontractions of the isometric artery ring preparations with different agonist of the artery contraction: 42 mM KCl and 1 nM ET-1) in order to elucidate the effect of MLs on healthy human mesenteric arteries in detail. Under the experimental condition using precontraction with 42 mM KCl of endothelium-intact human mesenteric artery ring preparations, logarithmically increasing doses of MLs (1, 3, 10, 30 and 100 nM) did not change (potentiate or attenuate) the contractile response of the investigated human mesenteric artery preparations (Fig. 1a). In Fig. 1 with solid diamonds is denoted tension developed under stimulation with MLs in increasing concentrations (1, 3, 10, 30 and 100 nM) presented as a percentage of maximal 42 mM KCl-evoked contraction. Open diamonds denote control experiments, in which PBS (vehicle of the substance) was applied in corresponding volumes. Statistical differences between treated with lectin and non-treated preparation were determined using ANOVA test with Bonfferoni's correction at $p < 0.05$.



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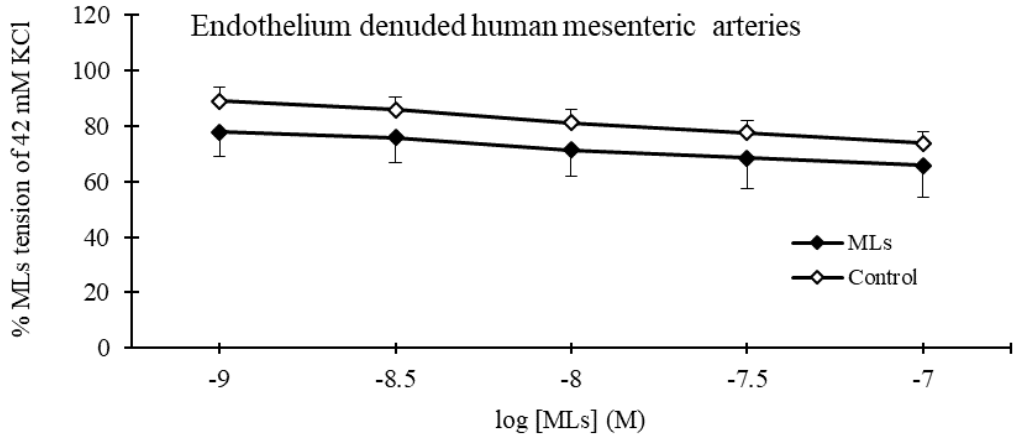

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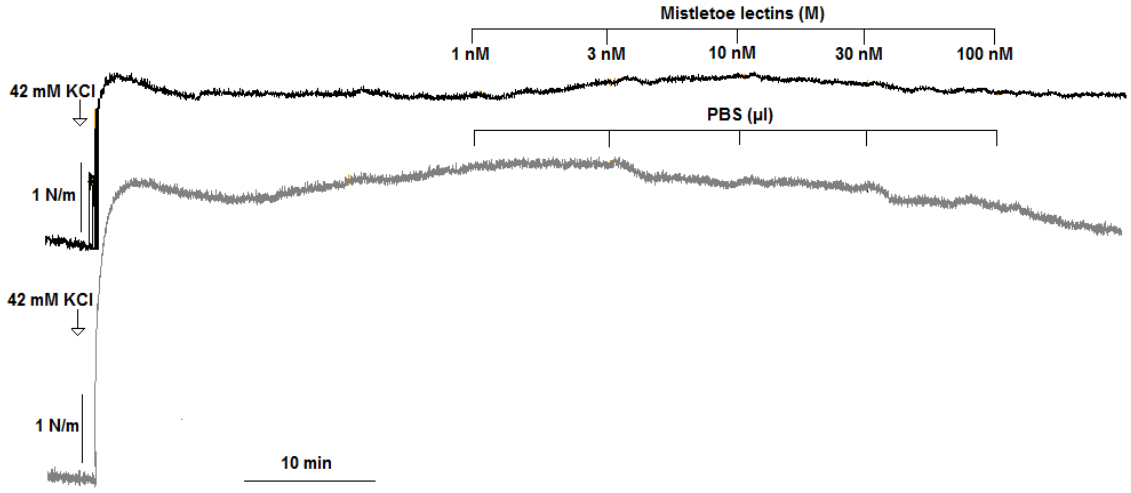


c)

Fig. 1 Effect of MLs on contraction of human mesenteric arterial segments precontracted with 42 mM KCl with a) intact endothelium (six MLs-treated and six control preparations); b) endothelium denuded (six MLs-treated and six control preparations); c) Representative original traces of the contraction time-course of an artery ring preparation with intact endothelium precontracted with 42mM KCl and exposed to increasing concentration series of MLs (black line) and of a single artery preparation with corresponding volumes of PBS applied (grey line).

In addition to quantitative presentation of results, the unresponsiveness of the investigated vessels to MLs *ex vivo* could be observed on a representative original trace where the time course of the isometric contractions (recorded as wall tension in N/m) during a single experiment were not significantly different for the treated with MLs and the control mesenteric segments (Fig. 1c). Even single dose of the lectin (1 μM) applied in some of experiments was not able to affect the contraction of experimental arterial strips comparing to the control ones (data not shown). Moreover, arterial rings from human mesentery subjected to endothelium removal in the condition of high KCl-containing medium (i.e., 42 mM-containing PSS) also did not respond with a change of isometric contraction to MLs application (Fig. 1b).

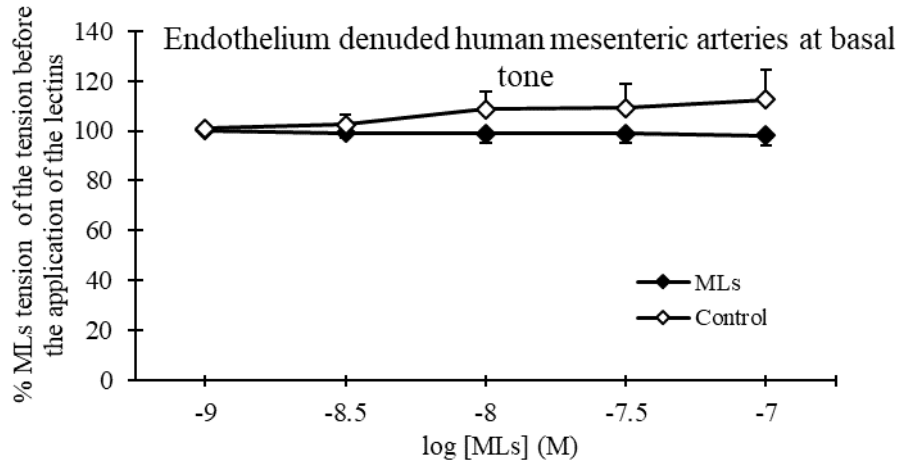
Contraction responses to exogenously applied MLs (1 nM, 3 nM, 10 nM, 30 nM and 100 nM) on basal tension of endothelium denuded arterial strips were also examined. As shown on Fig. 2a, neither an increase nor a decrease in basal tone was observed in the isolated artery from human mesentery during the stimulation with MLs. Dose-response curves in Fig. 2 were constructed from the mean values of the measurements of tension (N/m). Solid diamonds: tension developed under stimulation with MLs in increasing concentrations (1, 3, 10, 30 and 100 nM). Open diamonds: control experiments, in which PBS (vehicle of the substance) was applied in corresponding volumes. Bars are SEM. ANOVA test with Bonfferoni's correction was performed at $p < 0.05$.

Similarly, MLs at logarithmically increasing concentrations (1, 3, 10, 30 and 100 nM) added 40 min after a single application of ET-1 (1 nM), did not influence the time-course of agonist-developed wall tension in vessels without intact endothelium (Fig. 2b).

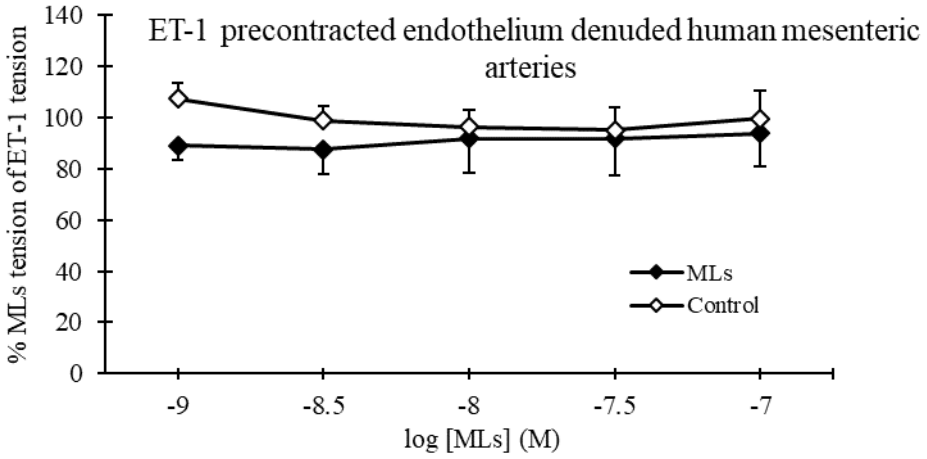
Using the previously published results demonstrating either dilatation or contraction of arteries under incubation with VA extract and the findings in the present study showing no effect of MLs alone on human mesenteric artery *ex vivo*. It could be suggested that MLs could not be attributed to vasoactive properties of VA whole extract. One of the possible causes for the unresponsiveness observed in the isolated human mesenteric arteries to MLs might be the insufficient time of incubation of arterial preparations with the lectin leading to possible effects on contractility of the investigated vessels. Indeed, as has been previously shown, significantly longer than our exposure in *in vitro* conditions of cell culture for obtaining cytotoxic or cytostatic effects of VAA-I was reported [11, 28]. Here it should be mentioned that for the present experiments the time of incubation and the concentrations concerning MLs application were selected in the manner allowing to avoid a direct toxic effect of the lectin on the vessels preparation as well as to be enough effective in exerting a vascular effect.

The concentration range of MLs used in the present experimental design was consistent within the concentration ranges normally utilised in previous *in vitro* experiments [2, 11, 28]. Emphasising on the fact, that such concentrations exerted a cytotoxic effect on the cells as was shown in the mentioned research and taking into account that MLs concentration used in the present study failed to influence the vitality of mesenteric arterial preparations, it could be concluded that the used concentration of MLs was not responsible by itself for the observed lack of effect of the lectin on the contraction of human mesenteric artery.

The unchanged contractility of investigated blood vessels upon lectin stimulation may be explained by an alteration of the capabilities of MLs to penetrate artery tissues. But most probably, this was not the reason of the observed effects because a good potency of MLs for uptake into the eukaryotic cells has been demonstrated previously [12].



a)



b)

Fig. 2 Effect of MLs on contraction of human mesenteric endothelium denuded arterial segments in: a) basal tone (five MLs-treated and five control preparations) and b) on the contraction of human mesenteric endothelium denuded arterial segments precontracted with 1 nM ET-1 (seven MLs-treated and six control preparations).

As shown in this study, endothelium also did not exert any influence on the vascular contraction of studied arteries upon stimulation with MLs although it is thought that this layer of the arterial wall plays an active role in the maintenance of many physiological and pathophysiological functions in arteries [29]. An interesting conclusion could be drawn comparing the present results and one recent study reported an endothelial cytotoxicity of VA extract [6]: beyond the observed lack of effect of the lectin on the contraction of arteries with intact endothelium and the unaffected viability of the preparation upon MLs treatment here, we implicitly suggest that ML alone is not such ingredient in VA extract that is responsible for potential damages in the endothelial layer of artery during anticancer therapy using VA extracts.

High K^+-solutions and ET-1 are recognized as potent stimuli of arterial smooth muscle contraction utilizing different cellular signaling pathways leading to contraction [25]. As was demonstrated by our results, the application of MLs did not affect both the depolarisation-triggered and ligand-receptor-activated signaling in vascular smooth muscle cells of human mesenteric arteries in vitro. We suppose that this phenomenon might be due to either the lack of intrinsic capability of MLs to exert a direct contractile effect of precontracted vessel or could depend on an unresponsiveness of human arteries from mesenteric bed to these particular lectins.

Conclusion

To our knowledge, this is the first study investigating the vasoactive effect of MLs on the contractile properties of human mesenteric artery *ex vivo*. It could be proposed that the observed mild effect of the lectins on human vasculature, particularly from mesenteric bed could favor the implementation of MLs in cancer treatment modality without life-threatening vascular complications. Effects of MLs, as well as the mistletoe compounds containing commercial product Iskador AG, alone or in combination with the classical chemotherapy agents 5-fluorouracil and irinotecan on proper function of mesenteric artery, will be subject of the future study.

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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, molecular biology and ecology.

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, Bulgarian Academy of Sciences. She received her Ph.D. degree in 2001 in the same institute. 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.

Assoc. Prof. Vanya Bogoeva, Ph.D.
E-mail: bogoevaviva7@gmail.com

Vanya Bogoeva works as an Associate Professor at Institute of Molecular Biology "Acad. Roumen Tsanev". She had specializations in Italy, Check Republic, Norway and France. Her research experience is focused on cancer research, protein study, photophysical studies. She has been a principal investigator of an ICGEB project (2008-2010) and French-Bulgarian project (2017-2019). She participated in a bilateral Austrian-Bulgarian project and 4 projects, financed by the National Science Fund of Bulgaria. She is a leader of a project of a young scientist (2016-2017). Assoc. Prof. Bogoeva has 19 scientific publications in referred journals (IF 43), including: Proteomics, BBA, Nanotoxicology, etc.

Bozhil Robev, MD, Ph.D.
E-mail: bostro@abv.bg

Bozhil Robev has graduated Medicine at the Higher Medical Institute in Stara Zagora with excellent results. He has headed the Department of Medical Oncology at the University Hospital "St. Ivan Rilski", Sofia since 2019. Dr. Robev participates in many national, regional and international forums in the field of medical oncology. In 2019 he received his Ph.D. degree on the topic "Comparative clinical study of the effect of combination chemotherapy with cisplatin on the plasma antioxidant capacity of patients with lung cancer". Dr. Robev's research interests are in the field of oncopharmacology, oncogenetics, neuroedocrine tumors and tumors of the genitourinary system.

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

Iana Tsoneva has been a head of Department of Electroinduced and Adhesive Properties until 2015 at the Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, Sofia, Bulgaria. She received her Ph.D. degree in 1976, Associate Professor in 1990, D.Sc. in 2005 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.

Staniaslav Dimitrov, MD
E-mail: anestezist@abv.bg

Dr. Stanislav Dimitrov, MD, is a specialist in Anesthesiology and Intensive Care Medicine and works at Department of Anesthesiology and Intensive Care, Military Medical Academy, Sofia. He had a specialization in Israel. His professional and research experience is focused on intensive care medicine and smooth muscle physiology. Dr. Dimitrov has numerous publications in referred scientific journals.

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 the Institute of Physiology, Bulgarian Academy of Sciences. He received his Ph.D. degree in 2014 in the Institute of Neurobiology, Bulgarian Academy of Sciences. 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.

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