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New possibility of application of L-arginine and L-Norvaline in surgery.

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ABSTRACT

The experiment was performed on white rats of Wistar line. The influence of L-arginine and L-Norvaline on the condition of the ischemic soft tissues in comparison with the effect of the late phase of ischemic preconditioning was studied. Selection of optimal drug doses was produced. The role of inducible NO synthase was determined in the resulting effects. Studies were conducted on the model of the isolated skin flap on the supply leg. Skin flap was made on the second day of the experiment. Distant ischemic preconditioning was performed by 30 minutes of tourniquet on the upper third of the left thigh on the first day of the experiment, and then every 48 hours. L-arginine was administered at a daily dose of 20 mg/kg, 200 mg/kg, 500 mg/kg; L-Norvaline 10 mg/kg, 100 mg/kg, 250 mg/kg according to the same scheme. Blockade of inducible NO-synthase was modelled by the introduction of aminoguanidine. The skin flap of survival rate was assessed on day 6 of the experiment. Found that L-arginine and L-Norvaline, as well as remote ischemic preconditioning, increase the survival of isolated skin flap on the supply leg. L-Norvaline in a daily dose of 10 mg/kg, 100 mg/kg ,250 mg/kg has the same effect, L-arginine has the best effect at a dose of 200 mg/kg. In the implementation of the obtained results the inducible NO-synthase plays an important role. The results allow to speak about possibility of the initiation of the preconditioning of the studied drugs, as well as to conduct further studies of the effects of pharmacological preconditioning with L-arginine and L-Norvaline in surgery.

Keywords: rats, ischemic, remote ischemic preconditioning, pharmacological preconditioning, L-arginine, L-Norvaline, inducible NO-synthase.

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INTRODUCTION

Every living organism has complex mechanisms of survival and recovery. The task of scientists was to study and find ways to activate them. In this regard, the phenomenon of ischemic preconditioning deserves detailed study. Numerous works devoted to the study of the effects of early phase of preconditioning on the heart and brain [1]. However, the implementation mechanism of the phenomenon can be considered as a universal method of struggle against ischemia and its consequences [2]. In addition, the greatest interest is to study the effects of the late phase of preconditioning, which is based on genomic, reprogramming cells. Undoubtedly relevant is the search of drugs able to activate different stages of the process of preconditioning [3].

The present research explores the possibility of using L-arginine and L-Norvaline for pharmacological preconditioning in surgery.

METHODS

The experiment was performed on 110 white Wistar rats weighing 220-250 g. The study of the effect of remote ischemic preconditioning, L-arginine and L-Norvaline on the condition of ischemic soft tissues, the selection of optimal drug doses, as well as defining the role of inducible NO-synthase in the obtained effects were carried out on the model of the isolated skin flap on the supply leg. Flap all animals made on the second day of the experiment under anesthesia in the abdominal wall at 1 – cm base, 4 cm length, was isolated in a sterile plastic bag, the wound sutured nodal seam. Assessment of survival was made by measuring the area of surviving tissue on the 6th day of the experiment. [4]. All animals were divided into 7 groups: I – control with the modeling of the skin flap and by intragastric administration of starch (n=10); II – modeling of the skin flap and the holding of remote ischemic preconditioning (n=10), III – modeling of the skin flap and the introduction of the L-arginine: III And a daily dose of 20 mg/kg (n=10), III – 200 mg/kg (n=10), III – 500 mg/kg (n=10) ; IV – modeling of the skin flap and the introduction of L-Norvaline: IV– in a daily dose of 10 mg/kg (n=10), IV - 100 mg/kg (n=10), IV – 250 mg/kg (n=10); V - modeling of the skin flap and the holding of remote ischemic preconditioning on the background of blockade of inducible NO-synthase; VI – modeling of the skin flap and the introduction of L-arginine at a daily dose of 200 mg/kg, on the background of blockade of inducible NO-synthase; VII – modeling of the skin flap and the introduction of L-Norvaline in a daily dose of 10 mg/kg on a background of blockade of inducible NO-synthase. Starch in group I, the preparations in groups III, IV, VI and VII were injected according to the same pattern – on the first day of the experiment, and then every 48 hours, distant ischemic preconditioning in group II and V were performed 30 minutes tourniquet on the upper third of the left thigh according to the same scheme [4]. Blockade of inducible NO-synthase was performed by the animal V, VI and VII groups intraperitoneal introduction of aminoguanidine, in a daily dose of 100 mg/kg, daily, throughout the experiment [5].

RESEARCH RESULTS

On the sixth day of the experiment in the control group, the area of surviving tissue graft amounted to 1.59±0.03 sq.cm. The conduct of remote ischemic preconditioning contributed to a significant increase in the survival of skin flap in comparison with the control group – to a value of 2.36±0.09 sq.cm. ($p<0.05$). The introduction of drugs in all used doses also contributed to a significant increase in the survival of skin flap in comparison with the control group, group III A 2.00±0.06 sq.cm. ($p<0.05$) in the group III V to 2.41±0.05 sq.cm. ($p<0.05$), III C to 1.90±0.06 sq.cm. ($p<0.05$) in group IV A to 2.70±0,07 sq.cm. ($p<0.05$), in group IV V to 2.63±0.06 sq.cm. ($p<0.05$), IV C up to 2.64±0.04 sq.cm. ($p<0.05$).

The results showed that L-arginine and L-Norvaline, in all applied doses, as well as remote ischemic preconditioning increases survival of isolated skin flap on the supply leg.

The choice of drugs in the current study due to their ability to stimulate the synthesis of nitric oxide, as it is the stimulation of synthesis of nitric oxide under the action of ischemia and subsequent reperfusion is considered the most significant mechanism of ischemic adaptation [6].

We should also focus on "the hypothesis of nitric oxide" offered by group of scientists headed by R. Bolli in 1998 [6]. Its main provisions are as follows.

- Nitric oxide plays a key role in the initiation and mediation of the phenomenon of "second protective" window (late phase of preconditioning);
- In the role of a trigger of the "second protective window" nitric oxide acts in the first transient ischemic episode, when it causes the increased formation of NO (presumably due to endothelial NO-synthase) and superoxide dismutase. Interacting, NO and O_2 form peroxynitrite. Peroxynitrite, in turn, activates the ε-isoform of protein kinase C. Stimulation of its can also be and other active forms of oxygen are O_2 derivatives;
- Activation of the ε-isoforms of protein kinase C triggers complex signaling cascade that includes activation of various tyrosine kinases, mitogenactivated protein kinases and transcription factors. These mechanisms lead to an increase in gene transcription induced NO-synthase and, as a consequence, to increase activity of this enzyme, the time interval of the delayed phase of the protection of ischemic preconditioning (day 2, following the original terminology R. Bolli).

In the development of ischemic preconditioning have the value of two different isoforms NO-synthases: calcium-dependent endothelial involved in the early phases of the process, and calcium-dependent induced by generating nitric oxide for myocardial protection at a later date. It is revealed that in the early stages of the formation of late phase ischemic preconditioning nitric oxide is synthesized by the activation of endothelial and later (after 24 hours) is due to inducible NO-synthase.

The authors of "hypothesis of nitric oxide" believe that the increased activity of inducible NO synthase logged in 24 to 72 hours initiated after the ischemic episode, is responsible for the implementation of the protective effects of the "second window" of preconditioning. It should again be emphasized that, according to R. Bolli, species are also the trigger for the development of the "second window" reactive oxygen, some of them react with nitric oxide to form other free radicals such as peroxynitrite [7]. Nitroxyl, another single-electron-derived nitric oxide, provides protection similar to preconditioning and greater than nitric oxide. That is, reactive NO is required for protection from ischemic damage. At the same time in high concentrations nitroxyl and peroxynitrite are able to cause damage. What dose of donators of nitrogen oxide and determines the possibility of applying for pharmacological preconditioning

In the present study, we attempted to simulate the activation of preconditioning, in particular its later phase. The drugs were administered before the simulation of a pathology that involves education at the time of operation of nitroxyl and peroxynitrite and the activation of protein kinase C. The choice of doses was carried out according to the literature dedicated to experimental studies on animals instructions use of drugs, as well as conversion doses (mg/kg or mg/sq. m.) for rats and humans depending on the body weight (E. J. Freireich, 1966). It is known that the daily routine leads to a gradual lifting effect. Given that the preconditioning effect begins to weaken after 48 hours [8], we selected scheme is the introduction of daily doses through the corresponding time period.

L-Norvaline in a daily dose of 10 mg/kg, 100 mg/kg, 250 mg/kg has an equivalent effect in the model of isolated skin flap on the supply leg. Significant differences between the values in the subgroups of group IV was not detected. In the group with L-arginine best effect is achieved when using the drug in a daily dose of 200 mg/kg, with dose reduction to 20 mg/kg and increasing up to 500 mg/kg, the survival rate of the isolated skin flap on the supply leg was significantly decreased. The obtained results due to the different mechanism of action of drugs. L-arginine known as the precursor to nitric oxide, deficiency and excess of it leads to less severity of the resulting effect. L-Norvaline is a selective inhibitor of arginase. The results indicate bosonization effect of the drug.

It is proved that the development effects of the late phase of preconditioning play an important role: inducible NO-synthase, cyclooxygenase of second type, where the manganiferous superoxide dismutase, aldose reductase, activating the synthesis of heat shock proteins, which are involved in stabilization of the cytoskeleton. In mechanisms of signal transduction for the late phase of ischemic preconditioning is the significance of activation of protein kinase C, mitogen activated protein kinases and tyrosine kinases [9]. These enzymes mediate a number of effects of late ischemic preconditioning, including reducing the level of apoptosis and change the intensity of the inflammatory response through modulation of the synthesis of proinflammatory factors. The induction of ischemic tolerance is accompanied by significant changes in gene expression. This suggests that preconditioning stimulates a fundamental genomic reprogramming of cells that forms cytoprotection and recovery.

We investigated the role of inducible NO-synthase in the implementation of the anti-ischemic effects of the methods used. Introduction aminoguanidine animals V, VI and VII groups led to the complete leveling of the earlier achievements. Blockade of inducible NO-synthase in animals of group V who underwent remote ischemic preconditioning contributed to reducing the survival rate of the isolated skin flap on the supply leg, was earlier to the value of 1.61±0.02 sq. cm., not having significant difference from indicator in control group (1.59±0.03 sq. cm.) (p=0.550). A similar picture was observed in groups VI and VII with L-arginine 1.57±0.04 sq. cm. (p=0.684) and L-Norvaline 1.62±0.05 sq. cm. (p=0.541). The results obtained in these experimental groups, indicates a significant role of inducible NO-synthase in the implementation of the anti-ischemic effect of remote ischemic preconditioning and L-arginine and L-Norvaline. Studied the results are consistent with the literature data [10 - 14].

CONCLUSION

L-arginine and L-Norvaline along with the distant ischemic preconditioning increases the survival rate of the isolated skin flap on the supply leg. And L-Norvaline in a daily dose of 10 mg/kg, 100 mg/kg, 250 mg/kg has an equivalent of anti-ischemic effect on the model used, L-arginine has the best effect at a dose of 200 mg/kg. In the implementation of the obtained results the inducible NO-synthase plays an important role. The results allow to speak about possibility of the initiation of the preconditioning of the studied drugs, as well as to conduct further studies of the effects of pharmacological preconditioning with L-arginine and L-Norvaline in surgery.

REFERENCES

[1] Bokeriya, L. A., Chicherin, I. N., 2007. The nature and clinical significance of "new ischemic syndromes". - M.: Scientific Center for Cardiovascular Surgery named after A. N. Bakulev Russian Academy of Medical Sciences. - 302 p.

[2] Kolesnik, I. M., 2010. The effect of remote preconditioning and recombinant erythropoietin on the survival of tissues and neovasculgen (experimental study): Medical Sciences: 14.03.06, 14.01.17 . Kursk. 126 p.

[3] Sommer, C., 2008. Ischemic preconditioning: postischemic structural changes in the brain . J. Neuropathol. Exp. Neurol., 67: 85–92.

[4] Kolesnik, I.M, Pokrovskiy, M.V, Lutcenko, V.D, and Pokrovskaya, T.G. , 2015. Experimental Study of ATP-Dependent Potassium Channels Activators Using Possibility in Surgery. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 4: 95 – 98.

[5] Hao, W., Wu, X.Q., Xu ,R.T., 2009. The molecular mechanism of aminoguanidine - mediated reduction on the brain edema after surgical brain injury in rats . Brain. Res., 28:156-161.

[6] Bolli, R., 1998. The nitric oxide hypothesis of late preconditioning .Basic. Res. Cardiol., 93: 325-338.

[7] Bolli, R., 1998. Causative role of oxyradicals in myocardial stunning: a proven hypothesis .Basic. Res. Cardiol., 93:156-162.

[8] Dirnagl, U., Becker, K., Meisel, A., 2009. Preconditioning and tolerance against cerebral ischaemia from experimental strategies to clinical use . Lancet., 8(4): 398–412.

[9] Perez-Pinzon, M.A., Dave, K.R., Raval, A.P., 2005. Role of reactive oxygen species and protein kinase C in ischemic tolerance in the brain . Antiox Redox Signal., 7: 1150–1157.

[10] Ivlitskaya, I.L., Korokin, M.V., Loktionov, A.L.,2016. Pharmacological efficiency of statins and L-norvalin at an endotoxin-induced endothelial dysfunction .Research result: pharmacology and clinical pharmacology.,2(2): 25-35.

[11] Shakhno, E.A., Savitskaya, T.A., Pokrovskaya , T.G., 2016. Use of L-arginine immobilised on activated carbon for pharmacological correction of endothelial disfunction . Research result: pharmacology and clinical pharmacology., 2(1): 30-35.

[12] Shabelnikova, A.S., 2016. Correction of ischemic damage to the retina on application of pharmacological preconditioning of recombinant erythropoietin . Research result: pharmacology and clinical pharmacology.,2(2) :67-90.

[13] Rajkumar, D.S.R., Gudyrev, O.S., Faitelson, A.V., 2016. Study of the influence of L-norvaline, rosuvastatin and their combination on the level of microcirculation in bone tissue in experimental osteoporosis and fractures on its background . Research result: pharmacology and clinical pharmacology., 2(1): 20-24.

[14] Denisyuk, T.A., Lazareva, G.A., Provotorov, V.Yu., Shaposhnikov, A.A., 2016. Endothelium and cardioprotective effects of HMG-Co-Areductase in combination with L-arginine in endothelial dysfunction modeling. Research result: pharmacology and clinical pharmacology., 2(1): 4-8.