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3-(2,2,2-Trimethylhydrazinium) Propionate: New Concept Of Realization Of Cardioprotective Effect.

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ABSTRACT

In the work we have estimated influence of 3-(2,2,2-trimethylhydrazinium) propionate on the area of necrotizing myocardium or left ventricle and the level of troponin of blood serum at acute coronary occlusion (60 min) with further reperfusion (90 min) in narcotized rabbits. It has been demonstrated that distant and pharmacological preconditioning with 3-(2,2,2-trimethylhydrazinium) propionate helps degrade necrotized area by 3 and 1.5 times, respectively. We have identified participation of ATP-sensitive potassium channels (P_{ATP}-channels) of endothelial and inducible NO-synthase in realization of cytoprotective effect of distant preconditioning and 3-(2,2,2-trimethylhydrazinium) propionate, which is indicative of realization of mechanism of pharmacological preconditioning in its cardioprotective effect.

Keywords: preconditioning, ischemia/reperfusion, P_{ATP}-channels – dependant channels, 3-(2,2,2-trimethylhydrazinium) propionate.

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INTRODUCTION

There are enough studies, dedicated to application of 3-(2,2,2-trimethylhydrazinium) propionate (Mildronat®) at cardiovascular diseases. It is commonly known that 3-(2,2,2-trimethylhydrazinium) propionate leads to limitation of fatty acids’ flow through mitochondria membrane and protects the cells from the death in conditions of hypoxia [1]. However, the question regarding its possibility of preconditioning myocard hasn’t been studied yet.

THE METHOD

Study of survivability of ischemic myocard was performed for 66 rabbits with the weight of 2-2.5 kg. Influence of 3-(2,2,2-trimethylhydrazinium) propionate on the size of the necrosis zone was performed on the model of coronary occlusive myocardial infarction. Myocardial infarction was reproduced in narcotized animals (chloral hydrate, 300 mg/kg), which had their breathing controlled, by means of banding ramous descendens of left coronary artery on the level of lower edge of left atrial appendage (60 min) with further reperfusion (90 min). Cross sections of myocard were performed every 0.8 cm, starting from the level of 0.8 cm lower than applied threads. Myocard’s sections were placed into container with phosphate buffer (pH 7.4) and 1 mg/ml of triphenyl tetrazolium chloride for formation of red formazan [2, 3, 4, 5]. Calculation of areas of intact and necrotized myocard of left ventricle were performed on each of four sections with the help of pixel-by-pixel analysis in Adobe Photoshop 9.0. The level of Troponin I (TnI) was defined on immunofluorescence apparatus Triage MeterPro (Biosite, USA).

Ischemic preconditioning was performed by means of stopping blood flow for 5 minutes in ligated coronary artery 30 minutes prior to total ischemia of myocard. Distant ischemic preconditioning was performed by application of tourniquet on the upper third part of thigh for 10 minutes, with further 20-minute reperfusion and reproduction of infarct model for 60 minutes.

Pharmacological preconditioning was performed via intravenous injection of 3-(2,2,2-trimethylhydrazinium) propionate into marginal ear vein of rabbits (Mildronat®, produced by “GRINDEX”, Latvia) in the dose of 90 mg/kg.

With the purpose of hypothesis’ confirmation regarding realization of preconditioning effects through activation of nitrogen oxide’s synthesis system and P_ATP-channels, the animals were injected with L-NAME in the dose of 25 mg/kg (blocking agent of inducible (iNOS) and endothelial (eNOS) NO-synthetase), glibenclamide (blocking agent of P_ATP-channels) in the dose of 0.4 mg/kg, amino guanidyn (blocking agent of inducible (iNOS) in the dose of 100 mg/kg [6,7].

RESULTS OF THE STUDY

Modeling of ischemia / reperfusion of coronary artery led to development of myocard’s necrosis, the size of which was 27.3±1.2 % from the total mass of left ventricle (Table 1).

Ischemic preconditioning (5 min) 30 minutes prior to coronary occlusion helped to degrade necrotized area by 3 times up to 11.8±0.8% Table 1).

Distant ischemic preconditioning. I.V. injection of 3-(2,2,2-trimethylhydrazinium) propionate in the dose of 90 mg/kg and their combination 30 minutes prior to modeling of coronary occlusive myocard infarct led to significant decrease of the area of necrotized myocard and equaled 9.05±0.6, 20.2±1.0 and 11.69±0.58%, respectively. Similar tendency was observed in dynamics of TnI in blood plasma (Table 1).

Non-selective blocking of NO-synthase via I.V. injection of L-NAME and selective blocking of inducible NO-synthase (iNOS) via I.V. injection of aminoguanidin, secondary to modeling of ischemia/reperfusion of propionate and distant ischemic preconditioning (Table 1).

Blocking P_ATP-channels with the help of glibenclamide on the background of modeling of myocard ischemia/reperfusion fully cancel cardioprotective effects, as well as an application of 3-(2,2,2-
trimethylhydrazinium) propionate and distant ischemic preconditioning in regards to necrosis zones and Tnl level (Table 1).

Table 1: Influence of 3-(2,2,2-trimethylhydrazinium) propionate (90 mg/kg I.V.) and distant ischemic preconditioning on the area of necrotizing myocardium of left ventricle and the level of troponin of blood serum at acute coronary occlusion (60 min) with further reperfusion (90 min) in narcotized rabbits (M±m; in % of left ventricle’s weight; n=6).

<table>
<thead>
<tr>
<th>N n/ n</th>
<th>Experimental group</th>
<th>% of necrosis</th>
<th>The level of Tnl in plasma (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>CO/ reperfusion (control)</td>
<td>27.3±1.2</td>
<td>16.3 ±1.2</td>
</tr>
<tr>
<td>2.</td>
<td>CO / reperfusion + IP</td>
<td>11.8±0.8*</td>
<td>5.4±1.4*</td>
</tr>
<tr>
<td>3.</td>
<td>CO / reperfusion + DIP</td>
<td>9.05±0.6*</td>
<td>4.6±0.8*</td>
</tr>
<tr>
<td>4.</td>
<td>CO / reperfusion + 3-(2,2,2-trimethylhydrazinium) propionate</td>
<td>20.2±1.0*</td>
<td>12.2±1.1*</td>
</tr>
<tr>
<td>5.</td>
<td>CO / reperfusion + 3-(2,2,2-trimethylhydrazinium) propionate + DIP</td>
<td>11.69±0.5*</td>
<td>6.4 ±2.9*</td>
</tr>
<tr>
<td>6.</td>
<td>CO / reperfusion + L-NAME (25 mg/kg/day) + DIP</td>
<td>25.3±0.8#</td>
<td>15.9±0.9#</td>
</tr>
<tr>
<td>7.</td>
<td>CO / reperfusion + L-NAME (25 mg/kg/day) + 3-(2,2,2-trimethylhydrazinium) propionate</td>
<td>23.6±1.3#</td>
<td>16.3 ±1.7#</td>
</tr>
<tr>
<td>8.</td>
<td>CO / reperfusion +aminoguanidine (100 mg/kg) + DIP</td>
<td>22.8±2.0#</td>
<td>14.6 ±1.8#</td>
</tr>
<tr>
<td>9.</td>
<td>CO / reperfusion +aminoguanidine (100 mg/kg)+ 3-(2,2,2-trimethylhydrazinium) propionate</td>
<td>26.3±1.3#</td>
<td>17.3 ±1.2#</td>
</tr>
<tr>
<td>10.</td>
<td>CO / reperfusion + glibenclamide (0.4 mg/kg) + DIP</td>
<td>24.2±1.5#</td>
<td>15.3±1.3#</td>
</tr>
<tr>
<td>11.</td>
<td>CO / reperfusion + glibenclamide (0.4 mg/kg)+3-(2,2,2-trimethylhydrazinium) propionate</td>
<td>32.6±1.8#</td>
<td>17.7 ±0.9#</td>
</tr>
</tbody>
</table>

Note: CO / reperfusion is the coronary occlusion of ramus descendens of left coronary artery on the level of the ear (60 min) with further reperfusion (90 min); IP is ischemic preconditioning (5 min); DIP is distant ischemic preconditioning (10 min) of the upper third of thigh; *p < 0.05 means in comparison with control; # p < 0.05 05 means in comparison with the series without pharmacological analyzer.

Thus, in the course of implemented investigation we have discovered that IP, DIP and 3-(2,2,2-trimethylhydrazinium) propionate have comparable cardioprotective effect on the model of ischemia/reperfusion in narcotized rabbits.

P_{ATP}-channels are effector mechanisms in realization of anti-ischemic effect of DIP and 3-(2,2,2-trimethylhydrazinium) propionate. NO acts like a trigger of IP. In both cases its synthesis is performed via activation of iNOS and eNOS [8, 9, 10]. 3-(2,2,2-trimethylhydrazinium) propionate, when competing for receptors of gamma-butyrobetaine, leads to reduction of carnitine concentrations. At this, its level increases. As a result of affinity of GBB with acetylcholine, activation of endothelial acetylcholine receptors occurs, which leads to induction of NO synthesis [11].

CONCLUSIONS

- 3-(2,2,2-trimethylhydrazinium) propionate provides significant protective effect on ischemia/reperfusion models in narcotized rabbits, reducing necrosis zone and Tnl concentrations in blood plasma by 1.5 times.
- P_{ATP}-Channels and iNOS take part in realization of cardioprotective effects of 3-(2,2,2-trimethylhydrazinium) propionate, as evidenced by cancellation of protective effect when glibenclamide and aminoguanidine are injected.

REFERENCES


