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Cardioprotective Effects of HMG-Co-A Reductase Inhibitors: Role of the Mechanisms of Preconditioning

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Abstract: Statins exhibit dose-dependent cardioprotective effect in the simulation of coronary occlusion infarction in rats. In the implementation of cardioprotective effects mechanisms of pharmacological preconditioning have an essential value as evidenced by removal of the effects during blockade of K^+-ATP channels with glibenclamide and blockade of iNOS with aminoguanidine.

Key words: Endothelial dysfunction, preconditioning, statins, simvastatin, atorvastatin, rosuvastatin, endotoxin

INTRODUCTION

HMG-Co-A reductase inhibitors are among the most widely used drugs in the treatment of hypercholesterolemia and the prevention of coronary heart disease. Evidence-based medicine found their important place in primary and secondary prevention of cardiovascular continuum (Ravingerova *et al*., 2015; Pokrovskiy *et al*., 2014; Chang *et al*., 2015). Many studies suggest that inhibitors of HMG-CoA-reductase have cholesterol independent pleiotropic effects (Pokrovskiy *et al*., 2014; Chang *et al*., 2015). One such effect is preconditioning-like effect during ischemia-reperfusion of cerebral, coronary and mesenteric vessels (Pokrovskiy *et al*., 2014; Chang *et al*., 2015).

The aim of this study was to investigate the cardioprotective activity of a wide range of doses of simvastatin, atorvastatin and rosuvastatin as well as the evaluation of the contribution of K^+-ATP channels and iNOS in the protective effects of statins.

MATERIALS AND METHODS

The experiments were performed on male Wistar albino rats weighing 200-250 g. The drugs were administered to rats intragastrically 1 time a day for 30 min before the simulation of Myocardial Infarction (MI). MI reproduced on anesthetized animals by ligation of the descending branch of the left coronary artery at the level of the lower edge of the left atrial appendage. After 4 days after coronary occlusion the animals were euthanized. To determine the size of area of myocardial necrosis clearly visible uncolored ischemic site and the intact site were excised and placed in two separate sample bottles containing phosphate buffer (pH 7.4) and 1 mg mL^{-1} of triphenyl tetrazolium bromide. The weight ratio of tissue sites and the buffer was 1:9. Sample bottles were placed into a thermostat and incubated for one hour at 37°C to form a red formazan. Sizes of necrosis area were calculated based on the difference between the content of the dye in the intact and ischemic areas (Mikhin *et al*., 2011). Animals were divided into experimental groups:

- Control (MI) (n = 10)
- MI+Simvastatin (S) 2.2 mg kg^{-1} (n = 10), 4.3 mg kg^{-1} (n = 10), 8.5 mg kg^{-1}(n = 10)
- MI+Atovastatin (A) 1.1 mg kg^{-1}(n = 10), 2.2 mg kg^{-1} (n = 10), 4.3 mg kg^{-1}(n = 10)
- MI+Rosuvastatin (R) 2.2 mg kg^{-1}(n = 10), 4.3 mg kg^{-1} (n = 10), 8.5 mg kg^{-1}(n = 10)
- MI+Nanoparticulated Rosuvastatin (NR) 3 mg kg^{-1} (n = 10), 6.3 mg kg^{-1}(n = 10), 11.6 mg kg^{-1}(n = 10)
- MI+Simvastatin 8.5 mg kg^{-1}+glibenclamide 5 mg kg^{-1} (n = 10)
- MI+Atovastatin 4.3 mg kg^{-1}+glibenclamide 5 mg kg^{-1} (n = 10)
- MI+Rosuvastatin 8.5 mg kg^{-1}+glibenclamide 5 mg kg^{-1} (n = 10)
- MI+Nanoparticulated Rosuvastatin+glibenclamide 5 mg kg^{-1} (n = 10)
- MI+Simvastatin 8.5 mg kg^{-1}+aminoguanidine 40 mg kg^{-1} (n = 10)
- MI+Atovastatin 4.3 mg kg^{-1}+aminoguanidine 40 mg kg^{-1} (n = 10)

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Table 1: Effects of HMG-Co-A reductase inhibitor Simvastatin, Atorvastatin, Rosuvastatin and Nanoparticulated Rosuvastatin on the area of necrosis in the simulation of coronary occlusion infarction in rats (M±m in % from weight of the left ventricle; n = 10)

Groups	The size of necrosis area (%)
Control (MI)	12.71±0.59
MI+Simvastatin (S) 2.2 mg kg^{-1} (n = 10)	11.00±0.35*
4.3 mg kg^{-1} (n = 10)	10.20±0.50*
8.5 mg kg^{-1} (n = 10)	9.75±0.36*
MI+Atovastatin (A) 1.1 mg kg^{-1} (n = 10)	11.01±0.37*
2.2 mg kg^{-1} (n = 10)	10.11±0.49*
4.3 mg kg^{-1} (n = 10)	9.53±0.30*
MI+Rosuvastatin (R) 2.2 mg kg^{-1} (n = 10)	11.00±0.35*
4.3 mg kg^{-1} (n = 10)	10.20±0.50*
8.5 mg kg^{-1} (n = 10)	9.75±0.35*
MI+Nanoparticulated Rosuvastatin (NR) 3 mg kg^{-1} (n = 10)	9.90±0.42*
6.3 mg kg^{-1} (n = 10)	9.10±0.47*
11.6 mg kg^{-1} (n = 10)	8.73±0.36*
MI+Simvastatin 8.5 mg kg^{-1}+glibenclamide	12.56±0.49
MI + Atovastatin 4.3 mg kg^{-1}+ glibenclamide 5 mg kg^{-1} (n=10)	13.20±0.56
MI+Rosuvastatin 8.5 mg kg^{-1}+glibenclamide 5 mg kg^{-1} (n = 10)	12.75±0.43
MI+Nanoparticulated Rosuvastatin+glibenclamide 5 mg kg^{-1} (n = 10)	12.32±0.41
MI+Simvastatin 8.5 mg kg^{-1}+aminoguanidine 40 mg kg^{-1} (n = 10)	12.00±0.41
MI+Atovastatin 4.3 mg kg^{-1}+aminoguanidine 40 mg kg^{-1} (n = 10)	11.95±0.39
MI+Rosuvastatin 8.5 mg kg^{-1}+aminoguanidine 40 mg kg^{-1} (n = 10)	12.50±0.54
MI+Nanoparticulated Rosuvastatin+aminoguanidine 40 mg kg^{-1} (n = 10)	12.00±0.43

*p<0.05 in comparison with control

- MI+Rosuvastatin 8.5 mg kg^{-1}+aminoguanidine 40 mg kg^{-1}(n = 10)
- MI+Nanoparticulated Rosuvastatin+aminoguanidine 40 mg kg^{-1} (n = 10)

The reliability of absolute parameters changes are determined by the difference method of variational statistics with finding of the average values (M), average error (±m) and error probability (p) by the student. Differences were evaluated as valid when $p<0.05$. Statistical calculations were performed using the Microsoft Excel.

RESULTS

Ligation the descending branch of the left coronary artery in rats in a group of control animals led to the development of myocardial necrosis which amounted to 12.71±0.59% of total myocardium. The use of drugs in the above doses resulted in a statistically significant reduction of necrosis area relative to the control animal group (Table 1).

The use of of HMG-Co-A reductase inhibitors Simvastatin, Atorvastatin, Rosuvastatin and Nanoparticulated Rosuvastatin exerted a dose-dependent cardioprotective action. Thus studied statins appeared about equally effective.

The blockade of K^+-ATP channels with glibenclamide (5 mg kg^{-1}) completely removes the cardioprotective effects of HMG-Co-A reductase inhibitors Simvastatin, Atorvastatin, Rosuvastatin and Nanoparticulated Rosuvastatin in modeling of coronary occlusion infarction in rats (Table 1).

Use of aminoguanidine (40 mg kg^{-1}) for blockade of iNOS also completely eliminates the cardioprotective effects of studied statins. This shows the involvement of the effects participating in implementation of mechanisms of pharmacological preconditioning as «first window» (blockade of K^+-ATP channels with glibenclamide (5 mg kg^{-1}) and the «second window» (blockade of iNOS with aminoguanidine 40 mg kg^{-1}).

DISCUSSION

At the present time, we discussed the following possible mechanisms of «ischemic preconditioning»:

- Endothelial release of bradykinin and the subsequent stimulation of the formation of Nitric Oxide (NO). Nitrogen oxide activates guanylate cyclase which results in increased levels of cyclic cGMP in turn this inhibits the entry of calcium ions through calcium L-type channels. These changes lead to the dilatation of the coronary arteries and their branches and to improve portability of myocardial ischemia
- Stimulation of the A1-adenosine receptors by adenosine which is formed by the hydrolysis of ATP. Simultaneously, adenosine acts on the G-protein and a phospholipase, moreover there is movement of protein kinase C from cytosol to sarcolemma
- As a result of these mechanisms opens ATP-sensitive K^+ channels, shortened the action potential and decreases Ca^{2+} entry into the cell

- As a result myocardial contractility decreases, energy consumption and consumption of ATP (Skyschally *et al*., 2008; Matsumura *et al*., 1998) reduced
- Stimulation of adrenergic receptors with catecholamine which leads to increased amounts of G-protein and an increase in the activity of phospholipase and then develops increased production of diacylglycerol and inositol triphosphate, transformation of protein kinase C and of opening of ATP-dependent K^+ channels and further activation of the same metabolic adaptation mechanisms described above (during the activation of adenosine receptors)
- Excitation of muscarinic M2-receptors with acetylcholine it increases the synthesis of endothelial Nitric Oxide (NO) with subsequent accumulation of cGMP which on the one hand causes vasodilation other-opens K^+ channels and blocks the flow of Ca^{2+} through calcium channels, resulting in decrease of myocardial contractility and energy needs
- A kind of «useful» effect of small amounts of oxygen free radicals produced in the myocardium; the consequences of this effect is the opening of ATP-sensitive K^+ channels and increased formation of myocardial adenosine which ultimately leads to coronary vasodilation and cardioprotective effect

However, it should be noted that the cardioprotective effect of small amounts of oxygen free radicals is not universally accepted and needs further study.

From the description of the above mechanisms of development of the phenomenon of intermittent ischemia or metabolic adaptation it can be seen that the most important mechanism is the opening of ATP-sensitive K^+ channels in the myocardium. Mechanism of development of the «second protective window» generally corresponds to the above-described. Due to stimulation of myocardial cellular receptors occur activation of protein kinase C and its movement not only to the membrane but also to the cell nucleus. In the nucleus, protein kinase C can express proteins are involved in gene transcription, leading to the synthesis of effector proteins, stress proteins of heat shock and superoxide dismutase. Thus, intermittent ischemia or metabolic adaptation are methods of protecting the myocardium from ischemic damage, however, the protective effect of preconditioning has its limitations, after prolonged repetitive episodes of myocardial ischemia protective effect is exhausted (Kolesnik *et al*., 2010).

The study of the pathophysiological bases of the phenomenon of intermittent ischemia emit 3 types of cardioprotection (Hattori *et al*., 2002; Swaminathan *et al*., 2010):

- Endogenous cardioprotection (the phenomenon of short episodes of ischemia, the effect of the «second window», hypoxia, the effect of catecholamines, tachycardia, increased vagal tone)
- Metabolic cardioprotection (infusions of glucose-insulin mixture)
- Pharmacological cardioprotection (use of adenosine, nitric oxide, the openers of K^+ ATP channels, beta adrenoblockers, calcium antagonists, angiotensin converting enzyme inhibitors)

On the basis of the experimental data suggests that statins may be assigned to a group of drugs with pleiotropic properties of pharmacological preconditioning (Chang *et al*., 2015; Vale *et al*., 2011).

CONCLUSION

Simvastatin, Atorvastatin, Rosuvastatin and Nanoparticulated Rosuvastatin exhibit dose-dependent cardioprotective effect in the simulation of coronary occlusion infarction in rats. The blockade of K^+-ATP channels with glibenclamide completely removes the cardioprotective effects of Simvastatin, Atorvastatin, Rosuvastatin and Nanoparticulated Rosuvastatin in modeling of coronary occlusion infarction in rats. Use of aminoguanidine for blockade of iNOS also completely eliminates the cardioprotective effects of Simvastatin, Atorvastatin, Rosuvastatin and Nanoparticulated Rosuvastatin. This shows the involvement of the effects participating in implementation of mechanisms of pharmacological preconditioning.

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