ORIGINAL ARTICLE

Potentiating Interaction of Ethoxydol and Rosuvastatin in an Experimental Model of Langendorf-isolated Rat Heart Total Ischemia-reperfusion

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Abstract

Introduction: Ethoxidol is a promising cardioprotective drug, the pharmacological activity of which is based on antihypoxic and antioxidant action. **Aim and Objective:** This study aims to study the cardioprotective activity of ethoxydol in monotherapy and combination of ethoxidol and rosuvastatin using Langendorf-isolated rat heart model. **Materials and Methods:** Langendorf-isolated hearts were divided into four groups: (I) Control, (II) ethoxidol in a small dose (1×10^{-4} g/l), (III) ethoxydol in a large dose (3.8×10^{-4} g/l), and (IV) combined use of a large dose of ethoxidol (3.8×10^{-4} g/l) and rosuvastatin (4×10^{-5} g/l). Ischemic lesions were modeled with a 60-min pause in heart perfusion. For the study of cardioprotective activity, the contractile function of the heart and NADPH activity of myocardiocytes was evaluated by staining sections with triphenyltetrazolium chloride. **Results:** The dose-dependent pharmacological efficacy of ethoxydol was established. In addition, the combination of ethoxydol at a concentration of 3.8×10^{-4} g/l with rosuvastatin (4×10^{-5} g/l) had the greatest effect on the reduction of contractility during the reperfusion period. **Conclusion:** The study of cardioprotective activity showed that *in vitro* ethoxydol at a dose of 3.8×10^{-4} g/l can significantly improve the morphofunctional state of cardiomyocytes, which is manifested in an increase in the proportion of postischemic cardiac resumption, reduction of ischemic contracture, and recovery of contractility during the reperfusion period.

Key words: Ethoxydol and Rosuvastatin, Langendorf-isolated, Ischemia-reperfusion, myocardiocytes

INTRODUCTION

Hypoxia is one of the universal pathological processes in many diseases and critical conditions.[1] Therefore, issues related to hypoxia go far beyond the pathophysiology, become a social problem, and require the development of new effective methods and pharmacological tools that facilitate the body's adaptation to hypoxia, or prevent its development, accelerating the normalization of function in the posthypoxic period.[2-9] One of the promising drugs with antihypoxic and antioxidant orientation of action is ethoxydol. The possibility of implementing the cardioprotective effect of ethoxidol in ischemic myocardial injuries, as well as the pharmacological interaction with a group of HMG-CoA reductase inhibitors, requires experimental verification.

MATERIALS AND METHODS

The experiments were carried out in accordance with the requirements of GOSTISO/IEC 1704-2009, GOST R ISO

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5725–2002, and the rules of laboratory practice approved by Order No 708n of the Ministry of Health and Social Development of the Russian Federation on August 23, 2010, in compliance with The European Convention for the Protection of Vertebrates Used for Experiments or Other Scientific Purposes (Directive 2010/63/EU). The experiments were conducted in accordance with The Guidelines for Pre-clinical Study of Medicinal Products (2012).

The initial number of animals in the groups varied and depended on the restoration of contractility in the reperfusion period. The total number of hearts that restored work in each group was 10. The animals were represented by the following groups: (I) Control - reperfusion with a standard Krebs-Henseleit solution, (II) ethoxidol in a small dose - reperfusion with Krebs-Henseleit solution containing 1×10^{-4} g/l of ethoxidol, (III) ethoxydol in a large dose - reperfusion with a solution Krebs-Henseleit containing 3.8×10^{-4} g/l ethoxydol, and (IV) combined use of a large dose of ethoxydol and rosuvastatin - reperfusion with Krebs-Henseleit solution containing 3.8×10^{-4} g/L ethoxydol and 4×10^{-5} g/l rosuvastatin. Hearts were removed from animals under combined anesthesia (Xyla premedication 0.5 ml/kg, basic anesthesia - Zoletil [50 mg/kg]), the aorta was cannulated and immediately connected to a perfusion unit. The perfusion unit was a standard 4-heart unit for perfusion of Langendorf-isolated heart produced by Cardioprotect LLC, St. Petersburg.

In the perfusion column was a standard Krebs-Henseleit solution of the following composition (mmol): NaCl - 118.5, KCl - 4.7, $MgSO_4/7H_2O$ - 1.2, KH_2PO_4 - 1.2, $CaCl_2$ - 1.5, glucose - 11.1, and $NaHCO_3$ - 25.0. Oxygenation was carried out with a mixture of carbon dioxide and oxygen, the pH and the temperature of the solution were monitored with a pH meter and were 7.4 ± 0.2 at 37°C.

The contractile function of the heart was recorded using a polyethylene canister inserted into the left ventricle cavity and connected by a tube to a pressure sensor embedded in the PhysExpBlackBox software and hardware system (manufactured by Cardioprotect LLC, St. Petersburg). With the help of the "PhysExp" program, all rats were registered with indicators of contractility: Intraventricular pressure (IVP, mmHg), heart rate (HR, beats/min), maximum speed of contraction (+dp/dt, mm hg/s), and the maximum rate of myocardial relaxation (-dp/dt, mm hg/s).

To wash the heart, the heart was perfused with a standard Krebs-Henseleit solution for 20 min, after which a total 60-min ischemia was simulated by discontinuing the perfusion. Reperfusion was carried out for 120 min, after which staining was carried out according to a standard procedure with 1% triphenyltetrazolium chloride.[10] The area of necrosis of sections was analyzed in the program Adobe Photoshop CS. Statistical processing of the results included the calculation of arithmetic averages (M), standard error of the mean. The significance of change was determined using the Mann–Whitney *U*-test.

RESULTS

Investigation of myocardial functional activity after ischemia simulation

After 20 min of perfusion with a standard Krebs-Henseleit solution that does not contain the test substances, the parameters of the hearts (HR, IVP, contraction rate, and relaxation rate) in the control and experimental groups had no significant differences. This indicates the same initial conditions of the hearts before ischemia and subsequent perfusion of the studied substances.

After a 60-min total ischemia, heart reperfusion was activated. During reperfusion, the resumption of work occurred within 20–40 min or did not occur at all. The ratio (%) of the resumed hearts is shown in Table 1.

The recovery of the hearts' work increases with reperfusion with Krebs-Henseleit solution containing ethoxydol at a concentration of 1×10^{-4} - in comparison with the control group data by 15%, at a concentration of 3.8×10^{-4} g/l - by 38%; a combination of ethoxydol in a large dose with rosuvastatin - 50%.

When compared with the values of the control group during reperfusion with Krebs-Henseleit solution containing ethoxydol (3.8×10^{-4} g/l), IVP parameter is significantly higher by 30 min - by 80%, by 60 min - by 112%, by 90 min - by 70%, and by 120 min - by 60% [Table 2]; the speed parameter + dp/dt is higher by 30 min - by 164%, by 60 min - by 110%, and by 90 min - by 30%; the speed parameter -dp/dt is higher by 30 min - by 164%, by 60 min - by 110%, and by 120 min - by 38% [Table 2].

When compared with the values of the control group during reperfusion with Krebs-Henseleit solution containing a combination of ethoxydol (3.8×10^{-4} g/L) with rosuvastatin 4×10^{-5} g/L, IVP parameter is significantly higher by 30 min - by 54%, on 60 min - by 154%, on 90 min - by 59%, and on 120 min - by 88%; the speed parameter + dp/dt is higher by 30 min - by 132%, by 60 min - by 122%, by 90 min - by 44%, and by 120 min - by 38%; the speed parameter -dp/dt

Table 1: The effect of the reperfusion of ethoxydol and the combination of ethoxydol with rosuvastatin on the resumption of work on isolated Langendorf hearts of rats after simulating total 60-min ischemia (M±m)

Experimental groups	% resumed work hearts
Control, *n*=30	33
Ethoxidol 1×10^{-4} g/l, *n*=21	48*
Ethoxidol 3.8×10^{-4} g/l, *n*=14	71*
Ethoxidol 3.8×10^{-4} g/l+rosuvastatin 4.10^{-5}g/l, *n*=11	80*

Reliability was calculated by Chi-square independence criteria.
*P<0.05 relative to the control group

Table 2: Effect of reperfusion with ethoxydol and combination of ethoxydol with rosuvastatin on the contractility parameters of isolated Langendorf hearts of rats after simulating total 60-min ischemia (M±m; *n*=10)

Experimental group	Parameters			
	HR	IVP	+dp/dt	-dp/dt
Parameters of contractility at 30 min reperfusion				
Control	100±12	8±2	185±24	162±25
Ethoxidol 1×10^{-4} g/l	102±16	12±2	181±35	160±30
Ethoxidol 3.8×10^{-4} g/l	129±10	14±2*	488±63*	284±24*
Ethoxidol 3.8×10^{-4} g/l+rosuvastatin 4×10^{-5} g/l	98±12	12±1	428±26*	381±54*
Parameters of contractility at 60 min reperfusion				
Control	151±10	11±2	268±15	161±20
Ethoxidol 1×10^{-4} g/l	169±13	11±2	294±23	182±12
Ethoxidol 3.8×10^{-4} g/l	161±16	23±3*	564±68*	537±29*
Ethoxidol 3.8×10^{-4} g/l+rosuvastatin 4×10^{-5} g/l	178±12	27±3*	597±41*	510±31*
Parameters of contractility at 90 min reperfusion				
Control	176±18	16±2	293±19	236±28
Ethoxidol 1×10^{-4} g/l	164±14	16±2	302±31	295±31
Ethoxidol 3.8×10^{-4} g/l	180±17	28±2*	403±45*	320±44
Ethoxidol 3.8×10^{-4} g/l+rosuvastatin 4×10^{-5} g/l	212±21	26±4*	423±42*	385±27*
Parameters of contractility at 120 min reperfusion				
Control	154±18	13±1	343±26	250±25
Ethoxidol 1×10^{-4} g/l	152±22	17±1	359±26	257±21
Ethoxidol 3.8×10^{-4} g/l	167±14	21±1*	356±28	335±33
Ethoxidol 3.8×10^{-4} g/l+rosuvastatin 4×10^{-5} g/l	177±14	25±2*	474±39*	467±23*

HR: Heart rate (beats/min), IVP: Intraventricular pressure (mmHg), + dp/dt, - the maximum rate of contraction (mmHg/s), dp/dt is the maximum relaxation rate (mmHg/s), *P<0.05 relative to the control group

is higher by 30 min - by 135%, by 60 min - by 217%, by 90 min - by 63%, and by 120 min - by 86% [Table 2].

Thus, a comparative analysis of the parameters of the effectiveness of ethoxidol, and the combination of ethoxidol with rosuvastatin showed the absence of significant differences in small doses (1×10^{-4} g/l). When comparing with the parameters of the contractility parameters of the control group, it was found that only a large dose (3.8×10^{-4} g/l) of ethoxydol and the combination of ethoxydol with rosuvastatin restored the contractility parameters in the reperfusion period, reaching maximum values by 60 min, and showing a decrease to 120 min. This suggests that the maximum metabolic and subsequent stimulating effect on myocardial contractility manifests itself for the first 60 min, after which exhaustion occurs due to accelerated exhaustion of reserves of energy substrates and the degradation of myocardial proteins.

Study of myocardial NADPH activity during staining of sections with triphenyltetrazolium chloride

In the analysis of myocardial survival, only the hearts that resumed work during the reperfusion period while maintaining the coronary perfusion rate of at least 3 ml/min were studied.

Table 3: The effect of a 120-min reperfusion of ethoxydol and a combination of ethoxydol with rosuvastatin on the volume of myocardial necrosis with total 60-minute ischemia (% of total myocardial volume, M±m, *n*=10)

Experimental group	Size of necrosis, %
Control	75±3
Ethoxidol 1×10^{-4} g/l	65±4
Ethoxidol 3.8×10^{-4} g/l+rosuvastatin 4×10^{-5} g/l	51±5*

*P<0.05 compared to the control group

Total hourly myocardial ischemia and subsequent 2-h reperfusion lead to necrosis of 75% of the myocardium [Table 3]. The use of ethoxidol (3.8×10^{-4} g/l) and the combination of ethoxidol (3.8×10^{-4} g/l) with rosuvastatin (4×10^{-5} g/l) limit the zone of necrosis by 14% and 32%, respectively.

CONCLUSION

The study of cardioprotective activity showed that *in vitro* ethoxydol at a dose of 3.8×10^{-4} g/l can significantly improve

the morphofunctional state of cardiomyocytes, which is manifested in an increase in the proportion of postischemic cardiac resumption, reduction of ischemic contracture, and recovery of contractility during the reperfusion period. The use of a combination of rosuvastatin (4×10^{-5} g/l) with ethoxydol (3.8×10^{-4} g/l) resulted in a significant increase in the cardioprotective effect. These data suggest the need for further preclinical study of these *in vivo* pharmacotherapeutic regimens in laboratory animals. Extrapolating the results obtained to the whole organism of a laboratory rat, it can be assumed that the effective concentration of ethoxydol, which allows correction of ischemic myocardial damage, will vary from 50 to 90 mg/kg.

REFERENCES

1. Semenza GL. Hypoxia-inducible factors in physiology and medicine. Cell 2012;148:399-408.
2. Yakushev VI, Filippenko NG, Kizilova IV. Research of dose-dependent endothelioprotective and cardioprotective activity of selective arginase II inhibitor in homocysteine-induced endothelial dysfunction. Res Result Pharm Clin Pharm 2016;2:42-5.
3. Provotorov VY, Korokin MV, Pokrovsky MV. Studying of endothelio -and kardioprotective effects of vitamin в6 and folic acid at modelling methionine-induced hyperhomocysteinemia. Res Result Pharm Clin Pharm 2016;2:16-9.
4. Ivlitskaya IL, Korokin MV, Loktionov AL. Pharmacological efficiency of statins and L-norvalin at an endotoxin-induced endothelial dysfunction. Res Result Pharm Clin Pharm 2016;2:25-35.
5. Denisyuk TA, Lazareva GA, Provotorov VY, Shaposhnikov AA. Endothelium and cardioprotective effects of HMG-Co-areductase in combination with L-arginine in endothelial dysfunction modeling. Res Result Pharm Clin Pharm 2016;2:4-8.
6. Soldatov VO, Shmykova EA, Pershina MA, Ksenofontov AO, Zamitsky YM, Kulikov AL, *et al.* Imidazoline receptors agonists: Possible mechanisms of endothelioprotection. Res Results Pharm 2018;42:11-9.
7. Danilenko LM. Doxorubicin-associated cardiomyopathy: New approaches to pharmacological correction using 3-(2, 2, 2-trimethylhydrazinium) propionate derivatives. Res Results Pharm 2018;4:81-6.
8. Voronkov AV, Pozdnyakov DI. Endothelotropic activity of 4-hydroxy-3,5-di-tret-butylcinnamic acid in the conditions of experimental cerebral ischemia. Res Results Pharm 2018;4:1-10.
9. Danilenko LM, Ya SS, Nadezhdin SV, Timokhina AS, Shcheblykina OV, Kotelnikova AS. Pharmacological screening of substances with cardioprotective effect in the group of 3-oxypyridine derivatives. Res Results Pharm 2018;4:125-31.
10. Sun YY, Yang D, Kuan CY. Mannitol-facilitated perfusion staining with 2, 3, 5-triphenyltetrazolium chloride (TTC) for detection of experimental cerebral infarction and biochemical analysis. J Neurosci Methods 2012;203:122-9.

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