Glycidipine, a Promising Hypotensive and Cardioprotective Agent

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Toxicological pharmacological study of the molecular complex of nifedipine and glycyrrhizic acid 1:10 (glycidipine) obtained using mechanochemical technique was carried out. High hypotensive and cardioprotective effects of the agent were demonstrated. Chronic administration (45 days) produced no toxic effects in vital organs and systems of Wistar rats and ISIAH rats.

Key Words: *vegetable metabolites; complexes; hypotensive and cardioprotective activity; rats*

According to World Health Organization data, the mortality from cardiovascular diseases occupies the 2nd-3rd place in European Union, USA, CIS, Canada, and Japan. Highest mortality rate is registered for chronic heart insufficiency and essential hypertension [5,9]. Prevalence of arterial hypertension grows from year to year, and elderly patients more frequently suffer from cardiovascular complications (myocardium infarction, stroke, renal pathology). Benefits from antihypertensive therapy and BP control are well established, however majority of patients receive inadequate care. According to recommendations of European Society of Cardiology, renin-angiotensin-aldosterone system modulators (antagonists of angiotensin A2 receptors, angiotensin-converting enzyme inhibitors), calcium channel blockers and β-adrenoblockers appear to be important components of complex heart failure therapy, especially against the background of hypertension [1,6,12]. Calcium channel antagonists are a thoroughly studied class of antihypertensive agents. The most prominent in this group of medicinal agents is derivative of 1,4- dihydropyridine, selective blocker of slow calcium L-type channels, nifedipine (NF), which exerts vasodilatatory, antianginal, and hypotensive actions. However, side effects appear to be substantial obstacle to wide use of NF and other dihydropyridine derivatives (nimotop, isradipine, nicardipine, *etc*.). To minimize toxic effects and improve bioavailability, one can employ technique of complexation with glycyrrhizic acid, glycoside produced by wide-spread *Fabaceae* plants *Glycyrrhiza glabra L., G. uralensis Fisch*, and *G. korshinskyi Grig* (liquorice). As a result this complexation, the so called "effect of pharmacon glycosid clathration" can be observed in virtue of significant reduction of drug dose with preservation of high specific activity and in many cases in amplification of pleiotropic properties [7]. In addition, complexation considerably improves drug solubility in water.

The objective of this study was the toxico-pharmacological investigation of NF molecular complex with glycyrrhizic acid (glycidipine, GD).

MATERIALS AND METHODS

The study was carried out on male and female Wistar rats weighing 180-240 g and ISIAH male rats with stress-induced hypertension weighting 250-300 g. The animals were obtained from the laboratory of experimental animals of Institute of Cytology and Genetics, Siberian Division Russian Academy of Sciences and were kept under standard conditions. All manipula-

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tions on animals were carried out in accordance with "European convention for the protection of vertebrate animals used for experimental and other scientific purposes" (Strasbourg, 1986). In each experiment, 10 Wistar and ISIAH rats were used.

GD was synthetized using mechanochemical approach [2,4] in Institute of Solid State Chemistry and Mechanochemistry, Siberian division of Russian Academy of Sciences in the group of organic compound mechanochemistry (head of the group A. V. Dushkin, Doctor of Chemistry).

For evaluation of the antihypertensive effects, a cannula was inserted into the carotid artery of anesthetized (thiopental sodium 30 mg/kg intraperitoneally) rats (registration on LabLinc V device, Coulbourn instruments) or in virtue of applying tail cuff on awake animals (LE 5007 Automatic blood pressure computer, Letica). ECG was registered in standard lead II using subcutaneous electrodes (LabLinc V, Coulbourn instruments). Arrhythmia was induced by intravenous administration of 250 mg/kg calcium chloride to anesthetized animals.

Blood samples for the analysis were taken from cervical vessels. Clinical blood analysis was carried out on hematological analyzer Medonic CA 530, biochemical analysis was performed using standard diagnostic kits (Olvex-diagnisticum) on photometer 5010 (Boehringer Mannheim). Material for morphological investigations was histologically treated on MICROM (Carl Zeiss). Stained preparations were examined in transmitted light by light microscopy, whereas heart preparations were additionally examined in polarized light on Axioskop 40 microscope with subsequent microphotography using AxioCam MRc camera (software AxioVision 4.5).

Myocardium ischemia (MI) was modeled using pituitrin (Endokrininiai) and isadrine (Sigma Aldrich Cheme GmbH). Pituitrin was administered intraperitoneally 0.5 U/kg, isadrine was administered subcutaneously 100 mg/kg. Isadrine injections were repeated 6 h later, and 24 h later pituitrin and isadrine were again administered in the same doses. GD and reference drug isoptin administered 3 times for 24 h in parallel with MI development (GD intraperitoneally, isoptin according to the same scheme 5 mg/kg). All bioenergetics parameters were estimated in the myocardium. After separation, the hearts were immediately placed in liquid nitrogen and stored in Dewar vessels.

Statistical treatment of the results was carried out using methods of mathematical statistics and Statistica 7.0 software. Arithmetical mean (M) and standard error of the mean (m) were determined for each parameter. Normalcy of distribution was tested using Kolmogorov–Smirnov test. If the distribution corresponded to normal law, the significance of obtained differences of compared values was assessed using Student's t test. Significance of differences between relative values was assessed using χ^2 test. The differences were significant at $p<0.05$.

TABLE 1. Effects of Test Agents on Parameters of Heart Muscle Lesions in Experimental MI ($M\pm m$; n=5)

Group	LDH activity, mmol/l/h	$\Sigma\ \Delta ST$, μV
Intact	0.36±0.05	0
MI (control)	1.52±0.12	216±39
MI+GD	0.67±0.05*	71±14*
MI+isoptine	0.87±0.06*	107±26*

Note. LDH: lactate dehydrogenase. Here and in Tables 2 and 3: *$p<0.05$ in comparison with the control.

RESULTS

GD was synthetized as stable molecular NF complex with glycyrrhizic acid (1:10 by weight). The complex was characterized by 8.5-fold increased water solubility.

GD investigations were started from assessment of its hypotensive effect. It was established that intravenous administration of GD and NF to Wistar rats in the same dose (3.5 mg/kg) led to similar BP reduction (26%, 37 mm Hg, and 30%, 36 mm Hg). Noteworthy, NF dose in GD is 10 times lower: 0.35 mg/kg. NF in this dose reduces BP only by 9% (11.5 mm Hg). Similar hypotensive effect was observed following intravenous administration of GD to ISIAH rats.

Intraperitoneal GD 3.5 mg/kg administration to Wistar rats resulted in gradual BP reduction to the maximum value of 22 mm Hg (17% of baseline BP) during 30 min and in smooth restitution of BP up to 9 mm Hg (virtually to the baseline level) during 90 min (Fig. 1, *a*). GD pharmacodynamics following administration of 3.5 mg/kg to ISIAH rats under the same conditions of the experiment was similar to that in Wistar rats.

Following intragastrical administration of 3.5 mg/kg GD to Wistar rats, gradual BP reduction to a maximum level of 18 mm Hg over 60 min and gradual BP restitution to 11 mm Hg (virtually to the baseline level) over 180 min were established. At the same time NF administered in a dose of 3.5 mg/kg lowered BP more drastically by 19.5 mm Hg in 30 min, and BP reconstitution occurred over 210 min and was less gradual than following GD administration (Fig. 1, *b*).

Smoothness of BP lowering following GD administration can be similar to second generation of

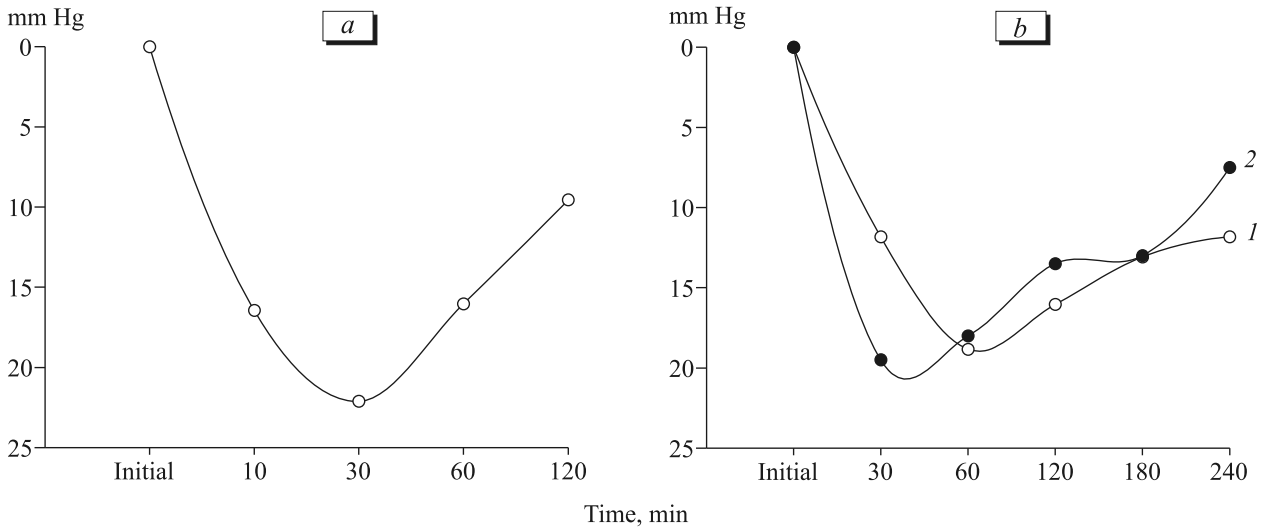

Fig. 1. Hypotensive activity of GD after intraperitoneal (*a*) and intragastrical (*b*) administration to Wistar rats. For *b*: *1*) GD, 3.5 mg/kg; *2*) NF, 3.5 mg/kg.

1,4-dihydropyridine derivative calcium channel blockers (nifedipine retard, nimodipine, nisoldipine, manidipine, *etc*.) [10].

NF exhibits low antiarrhythmic activity, which however is considered clinically insignificant, and this agent is used in practice only as antianginal and hypotensive drug [3]. As it was already noted, complexation with glycyrrhizic acid often results in strengthening of pleiotropic properties of medicinal compounds. Analyses of GD antiarrhythmic properties following intravenous administration to Wistar rats in the model of calcium chloride-induced arrhythmia showed that GD administered in a dose of 0.12 mg/kg before arrhythmogen injection ensured 80% animal survival, while its administration after arrhythmogen injection resulted in 90% animal survival. NF exhibited no antiarrhythmic activity at the dose of 0.12 mg/kg [14]. In the analogous experiment on ISIAH rats animal survival was 60%, which was apparently determined by higher sensitivity of these rats to calcium chloride.

Comparison of NF and GD effects on slow calcium channels of isolated neurons from mollusc *Lymnaea stagnalis* allow the conclusion that GD blocks slow calcium channels 25 min later than NF [13].

Glycyrrhizic acid [7] and NF [8,11] are known to possess cardioprotective properties, therefore we considered highly interesting to investigate this property. Experiments were carried out on the MI model.

Experimental MI in animals was characterized by *ST* segment elevation from the baseline. Experimental MI therapy with GD resulted in reduction of *ST* deviation and promoted improvement of lactate dehydrogenase hyperenzymemia, which is indicative of antiischemic activity (Table 1).

MI simulation led to typical ischemic disturbances in myocardial energy metabolism: glycolysis activation, Krebs cycle dyscoordination, and energy deficiency.

TABLE 2. Energy Metabolism Parameters in Experimental MI (*M*±*m*; *n*=5).

Parameter	Group			
	intact	control (MI)	MI+isoptine	MI+GD
ATP, μm/g	3.38±0.30	2.17±0.14	3.11±0.31*	3.56±0.19*
CPK-mc, μm/mg/min	19.05±2.20	7.07±0.77	10.87±1.13*	14.93±1.17*
Pyruvate, μm/g	0.08±0.006	0.07±0.007	0.11±0.005*	0.08±0.009
Lactate, μm/g	6.62±0.78	11.07±0.22	8.07±0.32*	7.75±0.23*
Malonic acid, μm/g	0.40±0.06	0.10±0.02	0.21±0.05	0.34±0.06*

Note. CPK-mc: mitochondrial creatine phosphokinase.

TABLE 3. Parameters of Antioxidant System, Oxidative Protein Modification in the Myocardium in Experimental MI ($M \pm m$; n=5)

Parameter	Group			
	intact	control (MI)	MI+GD	MI+isoptine
SOD, arb. unit/mg/min	261.6±11.0	134.0±8.0	212.7±10.0*	157.2±21.0
APH, arb. unit/g protein	7.77±0.4	18.23±0.6	9.12±0.3*	10.89±0.5
KPH, arb. unit/g protein	12.33±2.8	34.81±2.3	16.10±1.8*	21.6±1.4

Note. APH: aldehyde phenylhydrazone, KPH: ketone phenylhydrazone.

GD administration increased ATP level due to intensification of aerobic processes, reduced activity of inefficient anaerobic glycolysis, which was indicated by decreased lactate levels, and normalized oxidation in the Krebs cycle at carboxylic stage (increased malate levels). Under conditions of MI GD exerted beneficial effects not only on energy production, but also on its transportation, which was indicated by increased activity of mitochondrial creatine phosphokinase (Table 2).

In MI simulation, SOD inhibition and increased levels of protein oxidative modification markers aldehyde phenylhydrazone and ketone phenylhydrazone were noted in the myocardium, whereas GD administration produced a significant antioxidant effect manifesting in elevated SOD activity and reduced levels of the markers (Table 3).

Maximum dose of GD successfully administered intraperitoneally was 200 mg/kg, which corresponds to NF 20 mg/kg. It should be noted that NF LD_{50} for intraperitoneal administration to rats is 15 mg/kg and glycyrrhizic acid LD_{50} is 1650 mg/kg. Intraperitoneal administration of GD in the dose range from 20 to 200 mg/kg to rats for 14 days caused no toxic effects.

To evaluate subchronic GD toxicity, investigations on Wistar rats with intraperitoneal administration for 14 days and on ISIAH rats with intragastrical administration for 45 days were conducted.

Administration of GD in a dose of 3.5 mg/kg for 14 and 45 days produced no toxic effects on vital organs of the animals. The conclusion comes from the lack of changes in biochemical parameters and normal heart, liver, kidney, and lung morphology.

Thus, further preclinical and clinical investigations of GD are needed and they may result in creation of an efficient medicinal product for the treatment of cardiovascular (*e.g.* ischemic) diseases possessing cardioprotective action.

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