A Model of Hyperhomocysteine-Induced Endothelial Dysfunction in Rats

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Intragastric methionine (3 g/kg daily for 7 days) elevates homocysteine concentration and increases the endothelial dysfunction coefficient. This protocol of methionine treatment is an adequate model of hyperhomocysteine-induced endothelial dysfunction and can be used for studies of the endothelio- and cardioprotective effects of drugs.

Key Words: *endothelial dysfunction; methionine; homocysteine; hyperhomocysteinemia*

According to the data of the WHO MONICA project, the classical risk factors (tobacco smoking, high systolic BP, excessive body weight, and hypercholesterolemia) can not completely explain for the dynamics of cardiovascular complications, as their prevalence in men and women is 40 and 15%, respectively. Studies of the possible role of homocysteine (HC) in the development of cardiovascular diseases started after detection of liability to atherothrombosis in patients with severe hyperhomocysteinemia (HC>100 μmol/liter) [6]. Experimental data accumulated by the present time are persuasive evidence of the cause-and-effect relationship between moderate hyperhomocysteinemia and cardiovascular disease.

We developed a protocol of intragastric methionine treatment for induction of hyperhomocysteine-induced endothelial dysfunction of rats.

MATERIALS AND METHODS

Experiments were carried out on 40 adult male Wistar rats (180-220 g). Solution for intragastric administration of methionine was prepared *ex tempore* with Twin-80 polysorbate and 1% starch solution.

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Methionine (Polisintez Company) was administered according to the following protocols: 1) two daily intragastric doses of 5 g/kg for 7 days (n=10); 2) one daily intragastric dose of 5 g/kg for 14 days (n=10); 3) daily intragastric dose of 3 g/kg for 7 days (n=10); 4) daily intragastric administration of 1 ml/kg 10% Twin-80 for 7 days (control, n=10).

The status and body weight dynamics were studied during the entire period of methionine treatment.

After methionine treatment (on days 15 and 8 of the experiment) survivors were narcotized with chloral hydrate (300 mg/kg), catheters were inserted in the left carotid arteries for BP recording, and the drugs were bolus injected in the femoral vein. Hemodynamic parameters systolic (sBP) and diastolic (dBP) BP, and heart rate (HR) were measured continuously on a Biopac device.

In addition to BP measurements, functional tests were carried out with subsequent evaluation of changes in sBP and dBP: endothelium-dependent vasodilatation in response to intravenous acetylcholine (40 μg/kg; 0.1 ml/100 g) [2,4] and endothelium-dependent vasodilatation in response to intravenous sodium nitroprusside (30 μg/kg; 0.1 ml/100 g) [3,4].

Endothelial dysfunction was evaluated by endothelial dysfunction coefficient (EDC) calculated by the formula: $EDC=SBP_{NP}/SBP_{AC}$, where SBP_{NP} was

the area of the triangle above BP recovery curve, the points of the lesser leg of the triangle were the point of the maximum drop of BP and the point of BP plateau onset in nitroprusside functional test, and SBP_{AC} was the area of the triangle above BP recovery curve in acetylcholine test, the difference between the point of the end of bradycarditic cardiac component and the point of BP recovery was taken for the lesser leg of the triangle [2-4].

The development of hyperhomocysteinemia was evaluated by serum level of HC in experimental animals. The concentration of HC was measured by immunoturbidimetry using Pliva-Lachema Diagnostica s.r.o. kits.

The significance of differences between the absolute parameters was evaluated by the differential method of variation statistics with estimation of the mean shifts (M), mean arithmetic ($\pm m$), and error probability (p) by Student's tables. The differences were considered significant at $p<0.05$. Statistical calculations were carried out using Microsoft Excel 7.0 software.

RESULTS

Intragastric methionine in two daily doses of 5 g/kg for 7 days caused death of 60% experimental animals. Starting from day 2 of methionine treatment, the animals were inert, moved little, refused from food and water; starting from day 3, the animals started to die with the clinical picture characteristic of acute cerebrovascular disorders. Autopsy showed the morphological picture of ischemic stroke, which was in line with the data on thrombin formation increase and blood rheology disorders under the effect of hyperhomocysteinemia [1,5,7].

Intragastric methionine in a single daily dose of 5 g/kg for 14 days caused death of 40% animals by day 7 of the experiment. The morphological and clinical picture of animal death was similar to that in experimental series 1.

No mortality was recorded in animals receiving methionine in a dose of 3 g/kg and 10% Twin-80 throughout the entire period of observation.

Methionin treatment according to the above protocols caused no appreciable changes in BP (Table 1). Functional tests showed that intragastric Twin-80 (10% solution; 1 ml/kg) caused no changes in the endothelial dysfunction coefficient. Intragastric methionine (5 g/kg) for 7 days (twice daily) caused an increase of the endothelial dysfunction coefficient which reached 3.7±0.5 (Table 1). The highest coefficient was found in the group of animals treated with methionine in a single daily dose of 5 g/kg for 14 days: 3.9±0.2. The coefficient also increased significantly in the group receiving methionine in a dose of 3 g/kg for 7 days: 3.3±0.3 (Table 1).

TABLE 1. Effects of Methionine on Hemodynamic Values and Endothelial Dysfunction Coefficient in Hyperhomocysteine-Induced Endothelial Dysfunction ($M\pm m$)

Group		Functional test		S of vascular reaction in AC EDVD and NP EDVD, arb. units	EDC, arb. units
		sBP, mm Hg	dBP, mm Hg		
10% Twin-80, 1 ml/kg (control)	Initial	129.2±4.3	82.4±5.9		0.9±0.2
	AC	74.1±2.9	39.4±3.1	1124.2±63.7	
	NP	67.2±5.1	42.9±5.4	1011.8±94.6	
Methionine, 5 g/kg 2 times per day, 7 days	Initial	117.2±4.1	78.4±5.6		3.7±0.5*
	AC	77.6±6.1	45.4±4.1	794.3±54.0*	
	NP	65.4±7.1	38.3±3.4	2938.1±184.0*	
Methionine, 5 g/kg/day, 14 days	Initial	121.0±4.2	79.6±2.4		3.9±0.2*
	AC	81.4±4.8	42.4±2.5	658.1±71.2*	
	NP	56.1±8.6	34.2±8.3	2566.6±181.4**	
Methionine, 3 g/kg/day, 7 days	Initial	118.9±10.1	76.6±7.2		3.3±0.3*
	AC	80.1±2.9	41.4±2.3	854.6±61.4*	
	HP	72.3±6.7	45.9±4.3	2820.2±210.4*	

Note. S: area above BP recovery curve in drug tests; EDC: endothelial dysfunction coefficient; EDVD: endothelium-dependent vasodilatation; AC: acetylcholine; NP: sodium nitroprusside. *$p<0.05$ in comparison with the control group.

TABLE 2. Serum HC Concentrations in Experimental Animals Receiving Methionine by Different Schemes ($M \pm m$, n=10)

Concentration, μmol/liter	10% Twin-80, 1 ml/kg (control)	Methionine, 5 g/kg 2 times per day, 7 days	Methionine, 5 g/kg/day, 14 days	Methionine, 3 g/kg/day, 7 days
HC	8.6±1.4	92.3±8.8*	104.7±9.4*	53.5±8.1*

Note. *p<0.05 in comparison with the control group.

Measurements of serum HC concentration showed its significant elevation, differing significantly from the control, in all groups of experimental animals (Table 2).

The greatest increment of HC level was found in the sera of animals treated with methionine in a dose of 5 g/kg for 14 days.

These experimental findings showed that daily single and double intragastric methionine dose of 5 g/kg for 14 and 7 days, respectively, caused the death of 40-60% animals. Functional tests in the survivors showed a significant (more than 3-fold) elevation of the endothelial dysfunction coefficient, while biochemical studies showed a more than 9-fold increase of serum concentration of HC in comparison with the control group. However, these protocols of methionine treatment were unfit for simulation of endothelial dysfunction and evaluation of its drug correction because of high mortality in those experimental series.

Intragastric single daily methionine dose of 3 g/kg for 7 days did not lead to animal death. The endothelial dysfunction coefficient increased significantly (3.3±0.3 *vs.* 0.9±0.2 in the control). This was paralleled by an increase of HC concentration to 53.5±8.1 μmol/liter (*vs.* 8.6±1.4 μmol/liter in the control).

Hence, intragastric methionine in a single daily dose of 3 g/kg for 7 days is an adequate experimental model of hyperhomocysteine-induced endothelial dysfunction, which can be used for studies of endothelio- and cardioprotective effects of drugs of various pharmacological groups on this model.

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