Effect of Selective Inhibitors of Nitric Oxide Synthesis on the Course of Experimental Hemorrhagic Shock

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Selective inhibitors of NO synthesis (derivatives of lysine, ornithine, and isothiourea) increased the efficiency of infusion therapy for experimental hemorrhagic shock in rats. These changes were related to improvement of cardiac function (increase in stroke volume, cardiac output, and left ventricular efficiency). Among the three inhibitors, N5-(1-iminoethyl)-L-ornithine dihydrochloride was most potent on this experimental model. This compound improved cardiac function and microcirculation and provided 100% survival of experimental animals.

Key Words: *hemorrhagic shock; blood circulation; nitric oxide; selective inhibitors of nitric oxide synthesis*

Serious changes in the vascular tone serve as a common pathogenetic mechanism for various disorders during shock of different nature. Previous studies showed that overproduction of NO plays an important role in these disturbances and often causes irreversible collapse or death [13].

Hemorrhagic shock is accompanied by increased generation of NO due to activation of inducible NO synthase (iNOS) [7,9].These changes are followed by the development of severe dysfunction [8]. Elimination of NO excess generated by iNOS can improve the course of hemorrhagic shock. Here we studied the effects of selective iNOS inhibitors (infusion therapy of hemorrhagic shock) on systemic hemodynamics, microcirculation, blood gas level, and acid-base balance of the blood in rats.

MATERIALS AND METHODS

Experiments were performed on male albino rats weighing 230-250 g and anesthetized with sodium thiopental (35-40 mg/kg). The carotid artery was catheterized for induction of hemorrhagic shock, measurement of blood pressure (BP), and sampling of the blood. The stroke volume (ml/kg) was estimated by means of tetrapolar rheography [1]. HR was calculated from ECG. The cardiac output (CO, ml/min/100 g body weight) was estimated from the stroke volume as follows: stroke volume×HR. The total peripheral resistance (TPR, dyne×sec×cm^{-5}×10^{-4}/kg) was calculated as follows: BP/CO×1332×60×10^{-4}/kg. The left ventricular efficiency (LVE) was calculated as follows: BP×CO×0.0135. Microcirculation in the serosa of the small intestine was studied by vital contact microscopy. A special scale was developed at the laboratory [2]. Gas level was measured in the arterial blood. The acid-base balance was assayed on an ABL-500 gas analyzer (Radiometer). Hemorrhagic shock was induced by repeated bloodletting from the carotid artery. BP was maintained at 50-40 mm Hg for 20-25 min. The volume of blood loss was 3.1±0.5 ml/kg.

The study was performed in 4 series. In series I (control, n=13), an isotonic solution of NaCl was administered after blood loss. The volume of NaCl 2-fold exceeded the blood loss volume. In the other three series, NaCl solution contained the following selective inhibitors of iNOS (ICN Biomedicals Inc.): N6-(1-iminoethyl)-L-lysine hydrochloride (L-NIL, 50 μg/kg; series II, n=7); N5-(1-iminoethyl)-L-ornithine

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dihydrochloride (L-NIO, 50 μg/kg; series III, *n*=9); and S-methylisothiourea (2 mg/kg; series IV, *n*=10).

The results were analyzed by nonparametric tests (Excel 5.0 software).

RESULTS

Blood loss in rats was accompanied by severe hemodynamic disturbances. They included a drop of BP (by more than 50%); significant decrease in stroke volume, CO, and LVE, and increase in TPR (Table 1). After infusion therapy, BP increased slightly in control rats and animals receiving the inhibitors. However, BP in these animals did not reach the baseline level. Isothiourea was most potent in increasing the level of BP. NO synthase inhibitors (particularly L-NIO and L-NIL) improved cardiac function. The stroke volume and CO increased by 2 times (as compared to the initial value). In series IV these indexes also increased, but did not differ from the control (Table 1). LVE was elevated in all animals receiving NO synthase inhibitors. The increase in LVE was most pronounced in rats receiving L-NIO and minimum pronounced after treatment with S-methylisothiourea.

Infusion of isotonic NaCl solution and selective inhibitors of NO synthesis had a modulatory effect on systemic hemodynamics. They caused the same changes in BP, stroke volume, and CO. The test parameters

TABLE 1. Systemic Hemodynamics in Rats with Hemorrhagic Shock after Treatment with Isotonic NaCl Solution and Selective Inhibitors of NO Synthesis (*M*±*m*)

Parameter	Series	Baseline	After blood loss	Time after the end of infusion, min	
				10	60
BP, mm Hg	I	141±3	59±4	100±4^{o}	100±5^{o}
	II	142±4	58±4	97±4^{o}	96±6^{o}
	III	125±4	49±8	101±5^{o}	99±8^{o}
	IV	148±5	60±4	133±4^{ox}	124±9^{ox}
CO, ml/min/100 g	I	15.5±0.2	4.4±0.2	12.8±0.9^{o}	11.5±0.8^{o}
	II	15.3±0.2	4.2±0.4	22.6±1.3^{o+}	18.2±2.2^{o+}
	III	15.5±0.5	5.4±0.9	26.4±1.6^{o}*	25.9±2.8^{o}*
	IV	15.5±0.5	5.2±0.5	15.1±2.0^{o}	11.3±2.0^{o}
Stroke volume, ml/kg	I	0.37±0.01	0.13±0.01	0.35±0.02^{o}	0.32±0.02^{o}
	II	0.38±0.02	0.13±0.01	0.58±0.05^{o+}	0.47±0.06^{o+}
	III	0.42±0.03	0.16±0.03	0.64±0.03^{o}*	0.65±0.05^{o}*
	IV	0.37±0.01	0.14±0.01	0.39±0.02^{o}	0.31±0.04^{o}
TPR, dyne×sec×cm^{-5}×10^{-4}/kg	I	7.3±0.2	11.0±0.7	6.4±0.3^{o}	7.3±0.5^{o}
	II	7.5±0.3	11.6±1.2	3.6±0.3^{o+}	4.5±0.4^{o+}
	III	6.5±0.2	9.2±2.2	3.2±0.1^{o}*	3.2±0.3^{o}*
	IV	7.8±0.3	10.2±0.8	8.5±1.6	9.8±1.6
LVE, kgm/kg/min	I	296±6	36±3	179±18^{o}	162±17^{o}
	II	293±8	33±5	296±22^{o+}	241±26^{o+}
	III	263±14	41±10	376±20^{o}*	355±54^{o}*
	IV	310±7	42±5	272±36^{ox}	189±12^{o}
HR, bpm	I	425±10	342±10	375±21	358±15
	II	413±20	340±7	397±13	376±20
	III	390±38	360±34	394±25	399±25
	IV	413±7	353±13	353±15	350±19

Note. Here and in Table 2: $p<0.05$: ^{o}compared to the end of blood loss; $^{+}$between series I and II; *between series I and III; ^{x}between series I and IV.

increased to a different extent after administration of these agents. TPR decreased in series I-III, but remained practically unchanged in series IV (Table 1).

Administration of NO synthase inhibitors was followed by similar changes in blood flow velocity and number of functioning capillaries. However, the degree of these changes was different after treatment with test agents (Table 2). Ten minutes after the end of infusion, blood flow velocity increased most significantly in series II (NaCl+L-NIL). The increase in blood flow velocity was least pronounced in series IV (NaCl+isothiourea). Similar changes were observed in the number of functioning capillaries.

No between-series differences were revealed in gas content and acid-base balance of the arterial blood. The buffer base deficiency was significantly reduced after administration of L-NIO (-6.2 *vs*. -8.6±1.8 and -9.4±2.1 mmol/liter in series II and IV, respectively).

Our results indicate that cardiac and vascular components make different contributions to BP recovery after treatment with selective inhibitors. An increase in BP after infusion of isotonic NaCl and S-methylisothiourea was mainly provided by the vascular component. This conclusion was derived from a slight increase in TPR. However, improvement of CO and stroke volume in these rats was less significant than in animals receiving L-NIL and L-NIO. Centralization of blood flow was most pronounced after infusion of S-methylisothiourea. This conclusion was derived from high TPR values. Our previous studies showed that administration of nonselective inhibitors of NO synthesis leads to increased centralization of blood flow [3]. Hence, S-methylisothiourea is less selective than L-NIL and L-NIO. S-methylisothiourea inhibits not only inducible synthase, but also constitutive synthase. These changes are accompanied by impaired perfusion of organs and tissues and, therefore, have an undesirable effect during hemorrhagic shock. It should be emphasized that cardiac component play a role in the consequences of S-methylisothiourea infusion. CO and stroke volume returned to normal after treatment this agent. BP recovery in series II did not differ from the control, but cardiac function was significantly improved after treatment with L-NIL. The cardiac component had an important role in series III with L-NIO. The increase in BP and high values of the stroke volume and CO were revealed in L-NIO-receiving animals. LVE in these rats surpassed the initial values, which attests to improvement of myocardial contractility.

Comparative study of three inhibitors indicates that the vascular component plays less important role than the cardiac component in the restoration of tissue perfusion after infusion therapy of hemorrhagic shock. The survival rate of animals provides support for this conclusion (100, 67, and 44% after administration of L-NIO, L-NIL, and S-methylisothiourea, respectively).

We conclude that infusion therapy with selective inhibitor of NO synthesis L-NIO was most effective. Administration of L-NIO was accompanied by the following changes:

- satisfactory recovery of BP;
- increase in CO, which improves perfusion of organs and tissues under conditions of low TPR;
- increase in CO due to elevation of the stroke volume (improvement of myocardial contractility);
- significant increase in the number of functioning capillaries and blood flow velocity; and
- 100% survival rate.

TABLE 2. Microcirculation in Rats with Hemorrhagic Shock after Treatment with Isotonic NaCl Solution (Series I) and Selective Inhibitors of NO Synthesis: L-NIL (Series II), L-NIO (Series III), and Isothiourea (Series IV; *M*±*m*)

Parameter	Series	Baseline	After blood loss	Time after the end of infusion, min	
				10	60
Blood flow velocity, score	I	0±0	3.54±0.08	1.00±0.25^{o}	1.17±0.18^{o}
	II	0±0	3.67±0.11	0.44±0.11^{o+}	0.78±0.22^{o+}
	III	0±0	3.29±0.14	0.86±0.14^{o*}	0.71±0.14^{o*}
	IV	0.11±0.11	3.33±0.11	1.00±0.28^{o}	1.00±0^{o}
Number of functioning capillaries, % of the baseline	I	100±0	52±4	82±6^{o}	98±8^{o}
	II	100±0	54±6	102±4^{o+}	95±6^{o}
	III	100±0	47±4	95±7^{o}	93±3^{o}
	IV	100±0	45±5	86±7^{o}	83±5^{o}

It should be noted that no absolutely selective synthase inhibitors were synthesized until now. Nearly all selective inhibitors are characterized by non-selectivity of different degree. They can inhibit not only iNOS, but also cNOS. Published data show that L-NIL is more potent than L-NIO in inhibiting iNOS in mice [12]. Our experiments showed that L-NIO is more effective than L-NIL during infusion therapy of hemorrhagic shock in rats. Hence, L-NIO possesses higher selectivity on this model of hemorrhagic shock in rats.

Selective NO synthase inhibitors, L-NIL and L-NIO, decrease the generation of NO during infusion therapy of hemorrhagic shock and improve function of the cardiovascular system. Excessive NO has an adverse effect on coronary blood flow and myocardial contractility [10,11]. Recent studies showed that mitochondrial isoform of synthases serves as an important source of synthases during circulatory shock [4,5]. Particularly, this enzyme is generated in cardiomyocyte mitochondria. Published data show that NO inhibits mitochondrial function [6]. It can be hypothesized that improvement of cardiac function under the influence of selective inhibitors is related to activation of tissue respiration in cardiomyocytes.

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