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VASOPRESSOR PROPERTIES OF NO SYNTHASE INHIBITOR T1059. PART II. HEMODYNAMIC EFFECTS ON HYPOVOLEMIC DISORDERS

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The NO synthase inhibitor T1059 (1-cyclohexanoyl-2-ethylisothiourea hydrobromide) in experiments modeling acute hemorrhagic shock with a single parenteral injection at nontoxic doses (i.p., 10 mg/kg; i.m., 30 mg/kg) showed pronounced hypertensive activity that was much longer than that of phenylephrine and reduced short-term mortality of experimental animals. The results indicated that further pharmacological development of T1059 was promising to design an original domestic drug with antishock activity that is acceptable at the prehospital treatment stage.

Keywords: isothiourea derivatives, NO synthase inhibitors, hypovolemic disorders, hypertensive action.

Part I of our research found that isothiourea (ITU) derivative T1059 was water soluble; moderately toxic (LD_{16}, LD_{50}, and LD_{84} values i.p. were 274, 380, and 523 mg/kg); capable of competitive inhibition of NO synthase with significant selectivity for eNOS and iNOS (IC_{50} values for nNOS, iNOS, and eNOS were 60.3, 1.8, and 3.2 μmol); and showed prolonged (at least 90 min) vasopressor action associated with moderate bradycardia in normal Wistar rats after a single i.p. injection [1]. These results prompted studies of the vasopressor properties of this compound in experiments modeling acute hypovolemic hypotension.

EXPERIMENTAL PART

The studies used male Wistar rats (3 – 4 months, 230 – 320 g). The source of the laboratory animals and the conditions under which they were housed were the same as in Part I of the research [1].

T1059 drug substance for the present study was synthesized in the Laboratory of Radiation Pharmacology, A. F. Tsyb MRRC using the published method [1]. HPLC and GC found contents of 1-cyclohexanoyl-2-ethylisothiourea hydrobromide in the drug substance of at least 95%; related impurities, <0.5%; other impurities and organic solvents, <0.5%. The sulfate ash content according to pharmacopoeial methods was <0.1%; heavy metals, <0.001%; mass loss on drying, %; bacterial endotoxin level, <0.35 EU/mg. The microbiological purity corresponded to category 1.2B.

T1059 was used in the tests as solutions (0.5 – 3%) prepared before injection using sterile normal saline (0.9%; OAO Dalkhimfarm, RF). T1059 was injected i.p. to experimental animals once at doses of 5, 10, and 20 mg/kg and i.m. at doses of 20 and 30 mg/kg. The T1059 dose was determined using previous results for the NOS-inhibitory activity of this compound *in vivo* and its vasopressor activity in normal rats [1].

The reference drug was phenylephrine, which was chosen to two reasons. First, phenylephrine, in contrast to endogenous adrenomimetics (epinephrine, norepinephrine, dopamine), is a selective α_1-adrenoreceptor agonist. Its specific activity is limited to vasoconstrictive and vasopressor action

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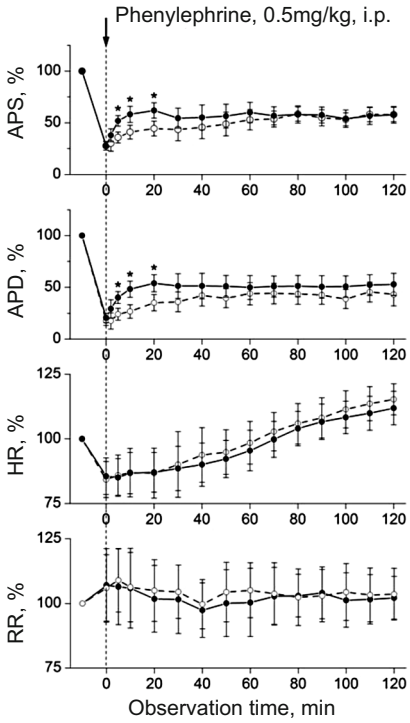

Fig. 1. Effect of phenylephrine (0.5 mg/kg) with a single i.p. injection on AP, HR, and RR of anesthetized Wistar rats with acute hemorrhagic shock. Here and in Figs. 2 and 3: scatter in points corresponds to ±SME; open circles, control points; filled circles, experimental animals. * Statistically significant differences ($p < 0.05$) vs. the control according to the Dunn criterion.

(in particular, not associated directly with inotropic and chronotropic heart functions) [2]. Furthermore, phenylephrine is hydrolyzed by monoamine oxidase (MAO) much more slowly than endogenous catecholamines and is currently considered one of the longest acting α_1-adrenomimetics [2, 3]. Phenylephrine (mezaton; 1% solution for injection, OAO ICN Polifarm, RF) was injected once i.p. at a dose of 0.5 mg/kg to experimental animals, which corresponded to the maximum one-time dose for humans with i.m. or s.c. injection according to interspecies recalculation [3, 4].

The experiments modeled acute hemorrhagic shock induced by acute massive blood loss similar in course to serious clinical hypovolemic disorders [5]. Acute hemorrhagic shock was induced by controlled blood collection of 2.5 mL per 100 g of body mass [47 – 50% of the blood-pool (BP) volume] from the right carotid artery for 8 – 12 min.

Anesthetized animals (sodium thiopental, 60 mg/kg, i.p.; OAO Sintez, RF) underwent tracheotomies followed by catheterization of the left jugular vein and both carotid arteries and were administered heparin (100 U, i.v.; NPO Microgen, RF). The heart rate (HR), respiration rate (RR), systolic (APS) and diastolic arterial pressure (APD), and EKG in standard leads were recorded in the experiments using an RM-6000 polygraph recorder (Nihon Kohden, Japan) or a PowerLab 8/30 system (ADInstruments, Australia). Parameters were recorded for untreated animals and then after blood collection. Control animals were injected with sterile normal saline (0.9%, 0.3 mL, i.p.; OAO Dalkhimfarm, RF); experimental animals, T1059 or phenylephrine. Parameters were recorded for the next 120 min. Animals surviving to the end of the observation period were withdrawn from the experiment by air embolism.

Intergroup differences were processed statistically using nonparametric criteria. Kruskal—Wallis rank analysis of variance using the Dunn criterion was performed for multiple comparisons. Short-term mortalities were compared using the exact Fisher criterion [6]. Differences in all instances were considered statistically significant for $p < 0.05$.

RESULTS AND DISCUSSION

The acute massive blood loss in the used model was 47 – 50% of the BP [4] and caused the animals to develop acute hemorrhagic shock. Moderate bradycardia (HR reduced by 15%) and deep hypotension (APS 28%, APD, 18% of the initial level) were observed (Fig. 1).

The severity of hypotension in control animals ($n = 18$) was partially compensated during the first hour after the blood loss by an increase of vessel tone and acceleration of the HR. The APS was 53%; APD, 44% of the initial value by the 60th minute of observation. However, the low blood flow in general was not adequately compensated. Erratic disturbances of intracardiac conductivity and respiratory arrhythmia followed shortly by cessation of respiration and cardiac contractions with total myocardial ischemia developed during persistent hypotension for 40 – 60 min after blood loss in a significant fraction of these animals. Half (9 of 18) of the control animals perished by the 120th min of the experiment.

Reference drug phenylephrine (0.5 mg/kg, i.p.) quickly raised the AP of rats ($n = 10$) with acute hemorrhagic shock. The AP parameters of experimental animals were statistically significantly greater ($p < 0.05$) than those of controls already 5 min after injection of the adrenomimetic. The hypertensive effect of phenylephrine peaked at 10 – 20 min with the APS greater by 35 – 40% and APD, by 65 – 75% than in the controls and 45 – 55% of the initial level before blood loss. However, the vasopressor action of phenylephrine for this administration mode was brief. A statistically significant hypertensive effect was observed for 15 – 20 min, from 5 to 20 min after injection, which agreed fully with the literature [2, 3]. All recorded parameters in rats that received phenylephrine and control animals were not statistically different starting from 30 min until the end of the observation period. The brief and rather limited amelioration of hypotension by phenylephrine in these experiments did not noticeably affect the course of the shock because half (5 of 10) of the rats in this group perished by the 120th min of the experiment.

A different pattern was observed for experimental animals that received T1059 (Fig. 2). A hypertensive effect de-

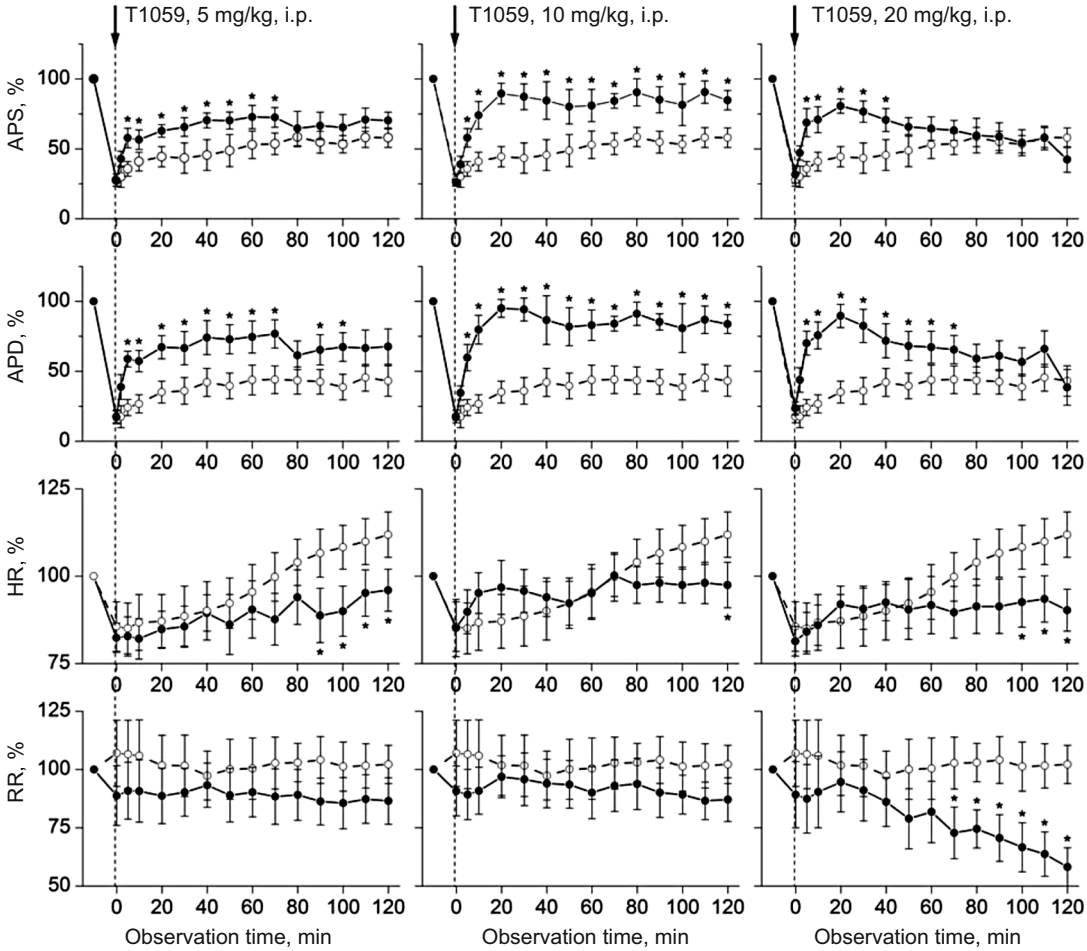

Fig. 2. Effect of T1059 at doses of 5, 10, and 20 mg/kg with a single i.p. injection on AP, HR, and RR of anesthetized Wistar rats with acute hemorrhagic shock.

veloped in rats after a single i.p. injection of T1059 at a dose of 5 mg/kg ($n = 8$) in the same timeframe as for phenylephrine. A statistically significant ($p < 0.05$) increase of AP was also noted already 5 min after injection although the degree of AP rise was 1.2 – 1.4 times greater than with phenylephrine. The hypertensive effect peaked at 10 – 60 min with the APS greater by 40 – 55%; APD, by 70 – 100% than in the controls and 60 – 75% of the initial level before blood loss. The vasopressor action of T1059 lasted 2 – 3 times longer than for phenylephrine. A statistically significant hypertensive effect was observed for 70 min. Increased vessel tone was manifested for up to 100 min of observation.

The more pronounced and longer amelioration of hypotension after i.p. injection of T1059 at a dose of 5 mg/kg was also associated with a trend ($p = 0.225$) toward reduced severity of the hemorrhagic shock in this model. Two of eight rats in this group (25%) perished during 120 min of observation.

The hypertensive effect after a single i.p. injection of T1059 at a dose of 10 mg/kg ($n = 11$) was much stronger and longer. A statistically significant increase of AP ($p < 0.05$) was also noted 5 min after the injection although the AP rise was more protracted and peaked by 15 – 20 min after T1059 injection. The AP rose to a level 1.5 – 2 times greater than with phenylephrine ($p < 0.05$). Thus, the hypertensive action of T1059 was 5 – 7 times longer than that of phenylephrine, i.e., the AP parameters of rats that lost half of the BP persisted at the 80 – 95% level of the starting values from 20 min after T1059 injection to the end of the observation period. The vasopressor action of T1059 in various specimens of this group started to weaken at 100 – 140 min after the injection.

The significant and prolonged weakening of hypotension after i.p. administration of T1059 (10 mg/kg) was associated with a pronounced reduction ($p = 0.029$) in the severity of the shock. Only 1 of 11 rats in this group (9%) perished during the experiment. The EKGs of these animals in the late stages showed moderate signs of myocardial ischemia although erratic rhythm and conductivity disturbances were not observed.

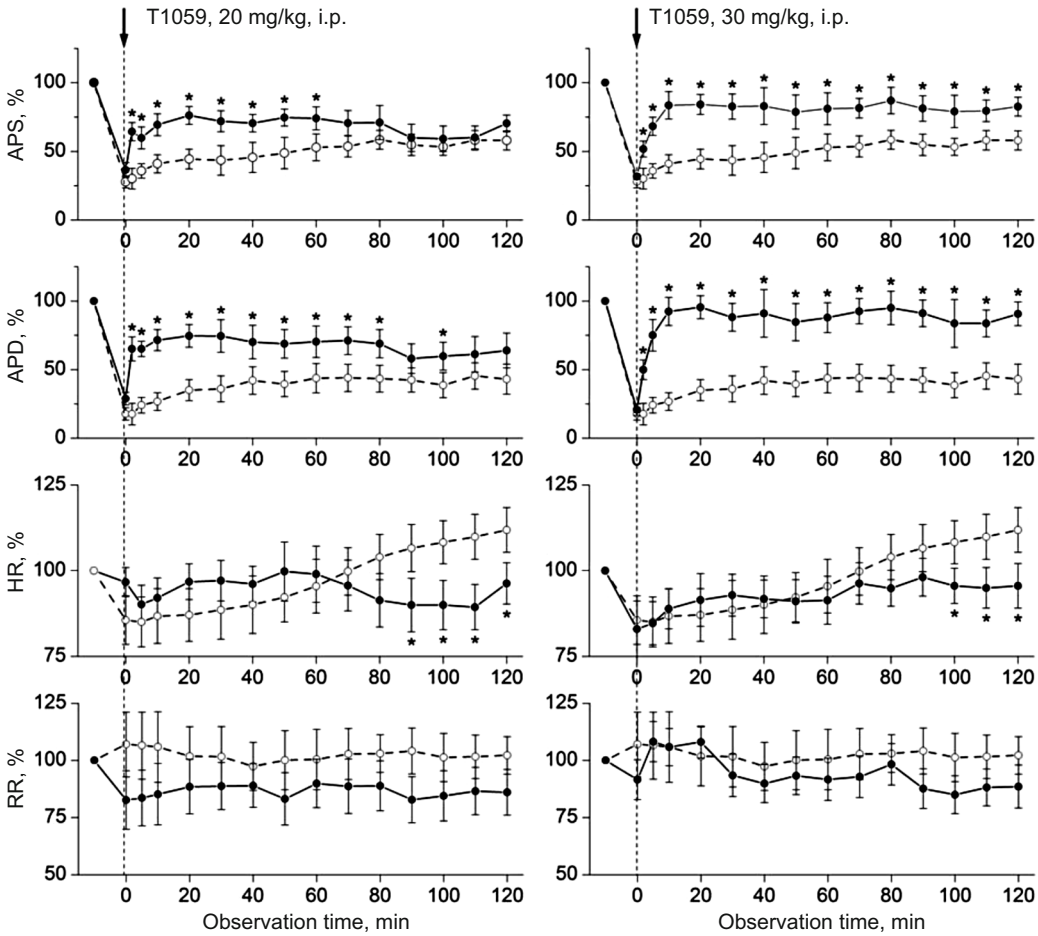

Fig. 3. Effect of T1059 at doses of 20 and 30 mg/kg with a single i.m. injection on AP, HR, and RR of anesthetized Wistar rats with acute hemorrhagic shock.

The vasopressor action after i.p. injection of T1059 (20 mg/kg, $n = 9$) was in general excessive for this model and caused hemorrhagic shock overload, probably as a result of excessive centralized blood flow. The initial development of a hypertensive effect was similar to that of T1059 at a dose of 10 mg/kg. However, pronounced EKG signs of total myocardial ischemia appeared for all rats of this group 15 – 20 min after T1059 injection. Then, cardiac and respiratory failure began and manifested as erratic disturbances of HR and intracardiac conductivity, respiratory arrhythmia, and progressive reduction of HR and AP. The deaths at 2 h in this group (56%) were slightly greater than those for control animals.

In general, vasopressor effects of T1059 after a single i.m. injection at doses of 20 and 30 mg/kg reproduced those of this compound after i.p. injection at doses of 5 and 10 mg/kg (Fig. 3).

The AP rise level after a single i.m. injection of T1059 at a dose of 20 mg/kg ($n = 8$) was 1.3 – 1.6 times greater than for phenylephrine. The vasopressor action of T1059 lasted three times longer than that of the adrenomimetic. A statistically significant hypertensive effect was observed for 60 min. Vessels manifested increased tone for up to 100 min of observation. The action of T1059 was also associated with a tendency (p 0.226) to ameliorate the severity of the shock and to reduce short-term lethality in this animal group (25%).

The hypertensive effect was considerably stronger and longer after a single i.m. injection of T1059 at a dose of 30 mg/kg ($n = 10$). In this instance, the hypertensive effect peaked by the 10th min after T1059 injection. The AP rise level was 1.5 – 2 times greater than for phenylephrine ($p < 0.05$). The hypertensive action of T1059 was 6 – 8 times longer than that of phenylephrine, i.e., the AP parameters stabilized at 75 – 95% of the initial values from 10 min after T1059 injection to the end of the observation period. The vasopressor action of T1059 began to weaken in various specimens of this group at 110 – 160 min post-injection. The lengthy weakening of hypotension was also associated with a pronounced reduction ($p = 0.040$) of short-term lethality of experimental animals. Only 1 of 10 (10%) rats in this group perished during the experiment.

Thus, the results showed that NOS inhibitor T1059 after a single i.p. injection at a dose of 10 mg/kg (~1/27 of LD_{16}) and a single i.m. injection at a dose of 30 mg/kg (~1/9 of

LD_{16}) to Wistar rats with acute hemorrhagic shock had pronounced vasopressor action associated with a prolonged (at least 2 h) hypertensive effect and a statistically significant reduction of short-term (2-h) deaths of animals.

It is noteworthy that the experiments showed that short-term survival in the early stages of uncompensated hemorrhagic shock could be increased also for several other NOS inhibitors. This effect was observed for not only NOS inhibitors with high vasopressor activity [7] but also selective iNOS inhibitors [8, 9] that weakly affect vessel tone during hypovolemia but limit destruction caused by oxidative stress with acute ischemia. In this respect, T1059, which is a selective eNOS and iNOS inhibitor could potentially be effective for short-term prevention of multiple organ failure with acute hypotension. In our opinion, this issue requires further detailed research.

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