Hypotensive Effect and Tissue Distribution of the Dinitrosyl Iron Complexes, a Nitric Oxide Donor

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Hypotensive effect of the dinitrosyl iron complexes, an NO donor, is compared with distribution of these complexes in organs and tissues after their intravenous administration to wakeful animals. Hypotensive effect of iron complexes depended on dose and post-injection time. There was a strong correlation between hypotensive effect and the content of dinitrosyl iron complex in the studied organs. Effective dose of dinitrosyl iron complexes that did not provoke adverse effects was about 200 mg/kg. This preparation is a prospective source of NO to treat and prevent pathological states related to NO deficiency.

Key Words: *dinitrosyl iron complexes; nitric oxide; tissue distribution; arterial pressure*

Endothelium-derived relaxation factor, the active ingredient of which is nitric oxide (NO), plays a key role in the local regulation of vascular tone and arterial pressure (AP). Disturbances of NO production contribute considerably to the development of hypertension, atherosclerosis, diabetes mellitus, ischemic heart disease, etc. [9]. Nitrovasodilators which have NO as a common active metabolite have been widely used to treat the diseases characterized by endogenous NO deficiency. Hypotensive effect of nitroprusside and nitroglycerin correlate with the amount of NO released by them [2]. However, in addition to NO, nitroprusside releases toxic cyanide [5], while administration of nitroglycerin is associated with a rapidly developing tolerance [6]. Therefore, considerable effort has been focused on the search for new NO donors that could be used in clinics. In this respect, the most perspective among them are dinitrosyl iron complexes (DNIC). These substances produce NO in the cells, and there is a hypothesis that DNIC is the endothelium-derived relaxation factor [10]. It is established that exogenous DNIC has a high hypotensive activity [1,4] and does not differ from the endothelium-derived relaxation factor in respect to vasodilation [13].

The aim of this work was to study the potency of DNIC to act as an NO donor. To this end, the hypotensive effect of DNIC in different doses was compared with its distribution in different organs and tissues of rats. The data obtained propose the optimal dosage of DNIC that on the one hand provides effective tissue level of NO, and on the other hand does not adversely affect the animals.

MATERIALS AND METHODS

Experiments were carried out on male Wistar rats weighing 240-270 g. Arterial pressure was measured by a bloodless method on the tail artery with the help of a Physiograph DMP-4F (Narco Bio-Systems). Then the animals were intravenously injected with the NO donor dimeric diamagnetic DNIC in the doses of 0.54, 1.79, 3.57, and 5.36 mmol/kg, which corresponds to administration of iron in the complex form in the doses of 30, 100, 200, and 300 mg/kg. The following DNIC doses are given in this form.

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After injection, AP was monitored for 3 h. It was measured again after 24 h.

The content of NO, which was incorporated into DNIC, was determined in the blood, liver, kidneys, heart, spleen, lungs, small intestine, and brain 1.5 and 24 h postinjection of DNIC. Injected in the diamagnetic form, DNIC turns into the paramagnetic form in the organism, which makes it possible to determine its content with the help of paramagnetic electron resonance (EPR) [11]. The amount of tissue NO in a complex form was determined according to the amplitude of EPR signal with g-factor values of 2.041 ($g_{\perp}$) and 2.014 ($g_{||}$), which are characteristic of DNIC (Fig. 1). The detailed description of this method is given in [12]. The EPR signals of the samples were recorded in a Radiopan radiospectrometer at 77 K, with a field modulation amplitude of 0.5 mT and UHF power of 10 mW.

The data were statistically analyzed using Student's *t* test.

RESULTS

Figure 2 shows the dynamics of AP after injection of different doses DNIC. Injection of DNIC in a dose of 30 mg/kg (Fig. 2, *1*) did not produce significant changes in AP. When DNIC was injected in a dose of 100 mg/kg, AP gradually decreased (Fig. 2, *2*), but in all the animals is remained above 90 - 95 mm Hg.

Ten minutes after injection of DNIC in a dose of 200 mg/kg, there was a significant decrease in AP, which in 2-2.5 h reached the minimal level of 80-85 mm Hg (Fig. 2, *3*). The dose of 300 mg/kg produced almost immediate drastic fall in AP of 75-80 mm Hg (Fig. 2, *4*). This fall was characterized by a rather large scatter in AP, which in some animals was decreased by 55-60 mm Hg. The decreased level of AP was maintained throughout the entire recording period, which was accompanied by pronounced disturbances of heart rhythm. In 24 h postinjection of DNIC, all the animals restored AP to the initial level independently on the DNIC dose (data are not shown).

The data on DNIC distribution in organs and tissues show that EPR signal of DNIC cannot be detected 1.5 h postinjection of DNIC in a dose of 30 mg/kg (Table 1). For the dose of 100 mg/kg, EPR signal was stable only in blood, kidneys, and brain. In all examined organs and tissues, injection of 200 or 300 mg/kg resulted in a characteristic EPR signal. The DNIC-related EPR signal was absent 24 h postinjection, with the only exception of an animal, in which DNIC was detected in the liver and kidneys after its injection in the dose of 300 mg/kg.

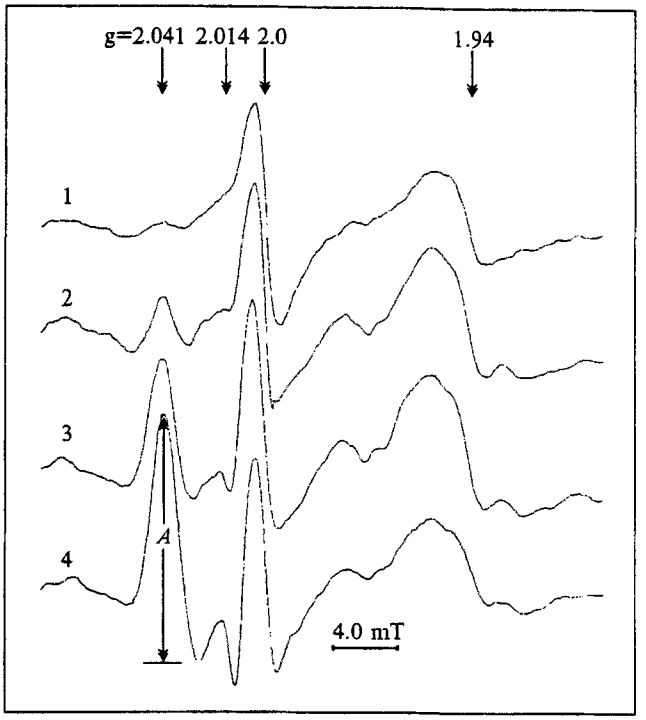

Fig. 1. A characteristic EPR signal in rat kidneys 1.5 h postinjection of DNIC.

Doses of DNIC (in mg/kg): *1*) 30; 2) 100; 3) 200; *4*) 300. A: the component of EPR signal whose amplitude was used to estimate tissue level of DNIC.

Thus, intravenous injection of the potent NO donor DNIC resulted in its dose-response distribution in the organs and tissues. The NO released from DNIC produced hypotensive effect, which depended on both dose of the donor and postinjection time.

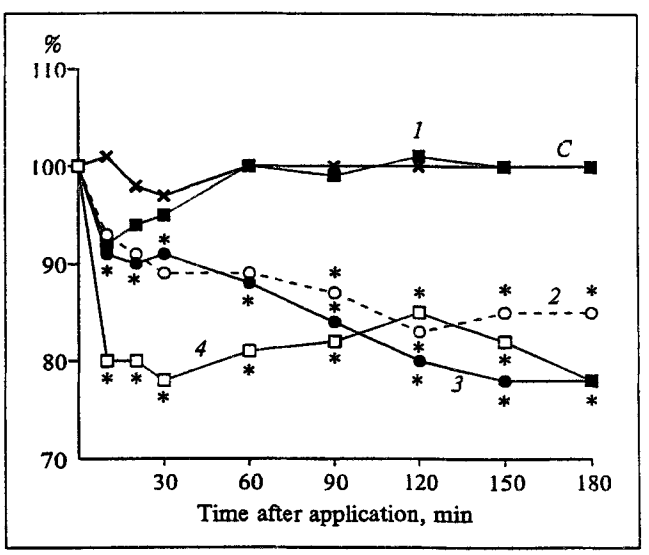

Fig. 2. Changes in arterial pressure in rats after application of DNIC in different doses (in mg/kg): *1*) 30; 2) 100; 3) 200; *4*) 300. *$p<0.05$ in comparison with the control group (C).

TABLE 1. Distribution of DNIC (ng NO/g Wet Weight) in Rat Tissues 1.5 h Postinjection (*M±m*)

Tissue	Dose of DNIC, μg/kg		
	100 (*n*=6)	200 (*n*=5)	300 (*n*=50)
Liver	43±4	80±3*	160±7*+
Kidneys	93±10	136±23	500±30*+
Heart	17±3	50±3*	210±27*+
Spleen	155±6	290±17*	957±67*+
Small intestine	110±3	193±27*	223±27*
Blood	277±6	587±67*	1556±80*+
Lungs	153±10	423±27*	1080±120*+
Brain	23±3	53±3*	90±10*+

Note. $p<0.05$ compared with a dose of *) 100 mg/kg and +) 200 mg/kg. Distribution could not be measured for DNIC given in a dose of 30 mg/kg (*n*=4).

There was a strong correlation between the content of the complex in organs and the degree of AP decrease. Injection of DNIC in a dose of 30 mg/kg induced no fall in AP or accumulation of the complex in tissues. The dose of 100 mg/kg did not provide a stable supply of NO into tissues and produced only a small hypotensive affect. Optimal supply of the tissues with NO was seemingly provided by DNIC in the dose of 200 mg/kg, which produced a decrease in AP without adverse side effects. Further increase of DNIC dose up to 300 mg/kg resulted in profound and persistent hypotension accompanied by cardiac disturbances.

The mechanism of DNIC-induced hypotensive effect are understood rather well. The vasodilator effect of DNIC is based on NO release, which activates soluble guanylate cyclase [8]. In experiments with wakeful [1] and narcotized [4] animals, DNIC was found to produce more efficient and prolong hypotensive effect than nitroglycerin and sodium nitroprusside [2]. The prolonged character of DNIC effect is explained by formation of tissue depot of NO-DNIC with paired thiol protein groups (resulted from $Fe(NO)_2$ transfer from low-molecular-weight DNIC to the proteins), which for a long time provide NO supply to the organism. It is noteworthy that this depot is preserved in tissues even after disappearance of hypotension [1].

DNIC was successfully used in a dose of 200 mg/kg to prevent damage to the cardiovascular system [3,7]. It is important that this protective effect of DNIC was observed 24 h postinjection, when the corresponding EPR signal cannot be recorded in tissues. Therefore, this effect was caused either by accumulation of a small non-detectable amount of NO in the depot or by NO-dependent induction of synthesis of some other protecting factors. The mechanism of this delayed effect of DNIC is yet to be determined.

These data show that intravenous injection of NO donor (DNIC) to wakeful rats resulted in its distribution over the organs and tissues followed by NO release and AP decrease in a dose- and time-dependent manner. The effective dose of DNIC which did not evoked adverse side effects was about 200 mg/kg in respect to iron in the complexes. The hypotensive effect of DNIC correlates with its tissue content. Further study of DNIC as an exogenous source of NO seems to be prospective in view of its possible application for treatment and prevention of pathological states caused by NO deficiency.

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