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Effect of the Nanoaerosol Form of Medicinal Preparations on Their Basic Activity

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Abstract

The pharmacological effect of the nano aerosol form of diclofenac, ibuprofen, caffeine and barbital *in vivo* was studied. It was established that such a delivery way allowed significantly decreasing the dose not only preserving the base activity, but also elevating it.

Key words: nanoaerosols, diclofenac, ibuprofen, caffeine, barbital

INTRODUCTION

Aerosol therapy is widely used at the present time for the treatment of a row of the upper respiratory tract diseases [1]. Various devices for making aerosols are known, such as nebulisers, inhalers, and powder turbuhalers. Principles of the preparation of aerosol vary, however, the diapason of sizes of particles produced by them is always equal to 0.5–5 μm. Particles of this size cannot reach the alveolar area of the lungs [2], so, their action is limited by the bronchopulmonary tree. In this regard, the development of ways that ensure the aerosol delivery of medicinal remedies affecting other organs and body systems is urgent. The creation of devices capable of producing aerosol particles of the nanometer range is necessary to achieve this goal. This way of delivery has a number of advantages in comparison with traditional peroral and intravenous methods: the noninvasiveness, fast penetration of a medicinal product into blood, absence of the effect of the first passage through the liver and influence from the side of the GIT (gastrointestinal tract) on pharmacokinetics of the delivered substance. Moreover, in case of the nanoaerosol form of delivery, such an important limitation, as the solubility in water, is removed. It is that is a huge obstacle for many modern drugs [2].

In the Institute of Chemical Kinetics and Combustion of the SB RAS (ICKC, Novosibirsk), the installation that allow obtaining the dry aerosol by the method of nucleation with the particle size in the nanometric diapason [3] was

developed. Indomethacin nanoaerosol was obtained using it. At the evaluation of the pharmacological action in experiment on animals, it was found that its effective dose is considerably lower in comparison with the peroral introduction [3].

In order to expand the list of medicinal remedies that can be delivered into the organism by the described method, we conducted analogous experiments with the use other nonsteroidal anti-inflammatory agents (NSAIA) – ibuprofen and diclofenac, as well as studied major pharmacological properties of drugs influencing the Central Nervous System (CNS) – caffeine and barbital**.** All the selected medicinal remedies are included in the list of vitally important preparations of the Russian Federation. Thus, the improvement of their toxicopharmacological characteristics should have a positive impact on the socio-economic aspect of their application.

EXPERIMENTAL

Research was carried on the base of ICKC at the Laboratory of Nanoparticles. Nanoaerosol was obtained by the method of nucleation, according to [3, 4].

All experiments on the study of nano aerosol forms of medicinal preparations were conducted on pubertal mongrel mouse of the mass of 25–30 g received from the vivarium of the Institute of Cytology and Genetics of the SB RAS (Novosibirsk) and contained under standard conditions of the vivarium on the usual food and water ration. Manipulations with animals were implemented in a strict accordance with the legislation of the Russian Federation, provisions of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986).

In each series of experiments, animals were divided into two groups – control and experimental, eight animals each.

The anti-inflammatory activity was evaluated on the model of histaminic inflammation, for the reproduction of which, animals was injected with histamine (0.05 mL) in the concentration of 0.01 % in the aponeurosis of the hind paw [5]. In 1 h after the introduction, animals were subjected to the action of the anti-inflammatory preparation in the form of nanoaerosol. After 5 h thereafter, the animals were killed by method of dislocation of the cervical vertebrae under light anaesthesia.

The reference group was constituted of the animals, which diclofenac in the dose of 20 mg/kg or ibuprofen in the dose of 80 mg/kg was introduced intragastrically once. Animals of the control group were placed for 20 min in a camera, through which a flow of pure air was passed. In the trial group (aerosol), the animals were placed for 20 min in a camera, through which aerosol of diclofenac or ibuprofen was passed in a dose of $8.3 \cdot 10^{-4}$ or $2.4 \cdot 10^{-3}$ mg/kg, respectively.

The assessment of the anti-inflammatory activity was carried out according to the index of inflammation that was determined by the formula: $(A - B)/A$, where A is the mass of the healthy paw; B is the mass of the paw

TABLE 1

Anti-inflammatory effect of diclofenac

Groups	Index edema		Anti-inflammatory activity, %
	Average	Relative to control, %	
Control	27.3±1.4*	100	0
Diclofenac perorally, $2 \cdot 10^{-3}$ mg/kg	28.8±1.0**	105.5	−5
The same, 20.0 mg/kg	22.0±1.6*	80.6	20
Control (in the chamber with clean air)	19.0±1.9	100	0
Aerosol diclofenac, $8.3 \cdot 10^{-4}$ mg/kg; size of particles of 15 nm	9.9±1.2***	52.1	47.9

*$p < 0.05$.

**$p < 0.001$.

***$p < 0.0001$ (compared with the control).

TABLE 2

Anti-inflammatory effect of ibuprofen

Groups	Index edema		Anti-inflammatory activity, %
	Average	Relative to control, %	
Control	39.87±2.62	100	0
Ibuprofen, 80 mg/kg perorally	17.45±1.2	44	56*
Control (in the chamber with clean air)	17.9±1.2	100	0
Aerosol ibuprofen, $2.4 \cdot 10^{-3}$ mg/kg; size of particles of 80 nm	8.2±1.2	45.8	54.2**

*$p < 0.05$.

**$p < 0.001$ (compared with the control).

with the induced inflammation. These indicators were compared with the control group (not received anti-inflammatory agents) and calculated the value of the anti-inflammatory effect (in per cent) [6].

To study the analgesic activity of the nanoaerosol form of diclofenac and ibuprofen the standard model was used: the test of the visceral pain "acetic writhing" (AW). It was evoked by the intraperitoneally administration of acetic acid (0.75 %, by 0.1 mL/mouse), the pain response was assessed by the number of cramps with the fifth through eighth minute after introducing acetic acid. The percentage of the repression of the pain response (RPR) was calculated by the formula: $100\ \% \cdot (X - Y)/X$, where X is average number of cramps in the control group; Y is average number of cramps in the test group [6].

The stimulating activity of caffeine in the form of nanoaerosol was studied on the model of chloral hydrate sleep. The soporific preparation chloral hydrate at a dose of 325 mg/kg was intraperitoneally injected with to animals. After the onset of sleep, the mice were placed in a camera with a nanoaerosol of caffeine for 20 min and the wake-up time compared with the control group was registered. The soporific activity of barbital was studied according to the analogous model.

Statistical processing results was conducted using the program package Statistica 7.0, the results are presented as the average (M)± standard error of the mean (SE). The reliability was determined according to the t-criterion of Student. The results were accepted authentic at $p < 0.05$.

RESULTS AND DISCUSSION

It was established that nanoaerosol of diclofenac on the model of the histaminic inflammation in the nanoaerosol form in the dose of $8.3 \cdot 10^{-4}$ mg/kg and size of particles of 60–120 nm exhibited the anti-inflammatory activ-

TABLE 3

Analgesic effect of diclofenac

Groups	AW		Analgesic effect, %
	Number of acts	% Acts	
Control	11.5±1.9	100	0
Diclofenac, 10 mg/kg intragastrically	4.6±1.1**	40	60
Aerosol of diclofenac, $6.4 \cdot 10^{-8}$ mg/kg; size of particles of 23.6 nm	4.7±1.6*	41	59

*$p < 0.05$.

**$p < 0.001$ (compared with the control).

TABLE 4

Stimulatory effect of caffeine on the background of the chloral hydrate sleep

Preparations	Time of sleep onset, min	Sleep duration, min
Control (chloral hydrate)	5.71±0.36	39.7±0.71
Control (chloral hydrate + air)	4.5±0.11	38.5±18.5
Chloral hydrate, caffeine, 15 mg/kg intraperitoneally	6.33±0.24	16.22±1.22*
Chloral hydrate, aerosol of caffeine, $2 \cdot 10^{-3}$ mg/kg; size of particles of 140 nm	4.0±0.11	21.0±1.0**

*$p < 0.05$.

**$p < 0.001$ (compared with the control).

ity that exceeded the effect of the reference group (diclofenac, 20 and 0.002 mg/kg intragastrically) (Table 1).

Thus, the introduction of diclofenac in the nanoaerosol form to animals allowed significantly reducing (by three orders) the dose, in comparison with the reference preparation introduced in the peroral form.

The results of the study of the anti-inflammatory activity of the nanoaerosol form of ibuprofen are represented in Table 2. It can be seen that the introduction of ibuprofen to animals in the nanoaerosol form also decreased the dose, in comparison with the reference preparation introduced intragastrically.

The analgesic effect of diclofenac and ibuprofen was studied on the model of AW (Table 3). Diclofenac in the nanoaerosol form in the dose of $6.4 \cdot 10^{-8}$ mg/kg was found to have displayed the analgesic activity, comparable to the reference group (10 mg/kg perorally). The introduction of diclofenac to animals in the nanoaerosol form provided a significant decrease of a dose, in comparison with the reference preparation that was delivered perorally. We have earlier studied the analgesic activity of nanoaerosol form of ibuprofen on the model of AW. It turned out that in the dose of $6.4 \cdot 10^{-8}$ mg/kg with the size of particles of 68.1 nm the analgesic activity was comparable to the reference group (80 mg/kg of ibuprofen intragastrically) [7, 8].

When studying the effect of the nanoaerosol form of caffeine – a central nervous system stimulator (CNS) – the time decrease of the soporific effect in mice on the background of the preparation chloral hydrate [6] served the assessment criteria. Experiment results are represented in Table 4.

The best result was established for the nanoaerosol form of caffeine in the dose of $2 \cdot 10^{-3}$ mg/kg with the size of particles of 140 nm after its exposition over 20 min. In comparison with control groups, the sleep duration has decreased on average of 47 %. Consequently, caffeine in the nanoaerosol form displays the base activity in a dose that is several orders of magnitude lower than parenteral.

The soporific preparation of the central action, *viz.*, barbital, has become no less inter-

TABLE 5

Stimulatory effect of barbital when awaking from the chloral hydrate sleep

Preparations	Time of sleep onset, min	Sleep duration, min
Control (chloral hydrate)	8±1.81	39.7±1.74
Control (chloral hydrate + air)	5.88±0.83	25.0±0.01**
Chloral hydrate, barbital, intraperitoneally, $2.4 \cdot 10^{-3}$ mg/kg	5.71±0.6	25.86±1.5**
Chloral hydrate, aerosol of barbital, $2.4 \cdot 10^{-3}$ mg/kg	9.0±0.01	<20

*$p < 0.05$.

**$p < 0.001$ (compared with the control).

esting research object. The goal of this series of experiments was the study of the base activity of barbital at the aerosol way of delivery. After 20 min of the exposition in a camera with a nanoaerosol of barbital, the drowsiness and sedation were not observed. To confirm observations an experiment, analogous to the one described above for caffeine (Table 5), was conducted. It was revealed that barbital in the nanoaerosol form in the dose of $2.4 \cdot 10^{-3}$ mg/kg reduced the soporific action duration of the preparation chloral hydrate by 50 % having shown the antagonism to the soporific effect.

CONCLUSION

Based on the carried out research, it can be concluded that nanoaerosol way of delivery allowed: 1) decreasing by several orders of magnitude the effective anti-inflammatory activity and analgesic dose of ibuprofen and diclofenac; 2) lowering the effective dose of the stimulator of CNS, caffeine, in comparison with the parenteral introducing way; 3) changing fundamentally the base activity of the soporific preparation barbital.

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