Effect of Anoceptin on Detoxifying Function of the Liver

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The absence of negative effects of anoceptin on the liver tissue and detoxifying function was proven experimentally *in vitro* and *in vivo*. It was proven for the first time that comenic acid (anoceptin active substance) in concentrations of 10^{-4}-10^{-12} does not modulate the growth of liver explants from 10-12-day chicken embryos. The effect of anoceptin on the detoxifying function of the liver was studied on Wistar rats under conditions of hexenal-induced sleep. The drug was injected into the caudal vein for 90 days in a daily dose of 5, 100, or 300 mg/kg. The duration of hexenal-induced sleep was evaluated before and on days 30 and 90 of the study. Anoceptin did not modify the duration of hexenal-induced sleep and the status and detoxifying function of the liver.

Key Words: *liver; comenic acid; anoceptin; hexenal sleep*

Anoceptin is an original Russian analgesic. The analgesic effect of the drug is based on reduction of potential sensitivity of activation gating system of slow $Na_v1.8$ channels of the spinal ganglionar sensory neuron membrane [1]. The dosage form is 1 and 2% solution for intravenous injections, containing 10 or 20 mg active substance, comenic acid (γ-pyrone derivative). The pre-registration clinical trials of the drug are now in progress. Evaluation of drug toxicity and detection of side effects are important aspects in evaluation of the new drug safety. The liver is the main organ of drug metabolism, including analgesic metabolism, in humans and vertebrates [2].

We studied the effect of anoceptin (comenic acid) on detoxifying function of the liver.

MATERIALS AND METHODS

The study was carried out on 300 liver tissue explants from 10-12-day chicken embryos. Liver tissue fragments of about 1 mm^3 were inoculated in Petri dishes with collagen-coated bottom. The explants were cultured for 3 days in a CO_2 incubator (Sanyo) at 37°C and 5% CO_2 [3]. Nutrient medium contained 40% Hanks solution, 40% Eagle medium, 15% FCS, and 5% fetal chicken extract with glucose (0.6%), glutamine (2 mM), and gentamicin (100 U/ml). Comenic acid was added to culture medium in concentrations of 10^{-4}-10^{-12} M. Explants cultured in medium without comenic acid served as controls. The explant area index was expressed in percents; the value in the control was taken for 100%. The cultures were examined through a microscope microteleadapter (series 10, MTN-13 Alpha Telecome). Quantitative evaluation of liver tissue explant was carried out using Photo M 1.2 software.

The significance of differences in the indexes of control and experimental explant areas was evaluated using Student's *t* test.

Anoceptin effect on detoxifying function of the liver was evaluated by the duration of narcotic (hexenal) sleep, reflecting the recovery of microsomal enzymes of liver cells involved in drug metabolism [4]. Experiments were carried out on 160 Wistar rats of both genders in a warm quiet room. The rats were weighed and intraperitoneally injected with hexenal (90 mg/kg) in 0.9% NaCl (solvent). Controls were intraperitoneally injected with 0.9% NaCl. The duration of hexenal

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TABLE 1. Effects of Comenic Acid on the Growth of Liver Tissue Explants from 10-12-Week Chicken Embryos (*M*±*m*)

Comenic acid concentration, M	*n*	Index of area, %
Control	25	100±6
10^{-4}	26	100±4
10^{-5}	23	100±7
10^{-6}	27	100±5
10^{-7}	25	100±8
10^{-8}	24	100±5
10^{-9}	25	100±9
10^{-10}	26	100±6
10^{-11}	27	100±6
10^{-12}	25	100±7

fibroblast-like cells started. After 3 days, two zones formed in the control and experimental explants. The central zone (more dense and dark) consisted of non-migrating cells. The peripheral growth zone formed at the expense of proliferation of hepatocytes and some fibroblast-like cells.

Comenic acid in the studied concentrations did not modify the growth of liver tissue explants from 10-12-day chicken embryos. The index of experimental explant area virtually did not differ from the control (Table 1). The data suggest that comenic acid-based anoceptin is nontoxic for the liver.

Hexenal test showed that anoceptin in doses of 5, 100, and 300 mg/kg (calculated for active substance) administered intravenously daily for 90 days does not impair the detoxifying function of liver microsomal enzymes. Anoceptin in all studied concentrations did not change the duration of hexenal-induced sleep in rats (Table 2).

TABLE 2. Effect of Anoceptin on the Duration Hexenal-Induced Sleep in Albino Rats (*M*±*m*, min; *n*=20)

Day of study	Experimental group, gender							
	control		anoceptin					
			5 mg/kg		100 mg/kg		300 mg/kg	
	males	females	males	females	males	females	males	females
Basal value	28.1±1.6	27.2±2.3	26.2±2.4	25.6±1.8	32.2±2.5	28.6±1.7	31.3±3.2	27.2±2.7
30	30.1±1.7	29.4±2.3	25.2±2.0	27.1±2.3	28.2±1.3	26.5±1.9	27.9±1.5	25.1±1.7
90	26.1±2.4	31.3±3.2	28.8±2.5	29.0±2.1	28.4±1.8	29.0±1.1	29.6±1.3	28.8±1.4

sleep was expressed in minutes from the beginning of narcosis (lateral posture) until its end (turning over). Anoceptin was injected into the caudal vein in doses of 5, 100, and 300 mg/kg (active substance) for 90 days. The duration of hexenal-induced sleep was evaluated before and on days 30 and 90 of the experiment.

RESULTS

Organotypical culturing of tissue fragments is an adequate method for evaluating drug activity. The possibility of strict dosage of the treatments without disturbing the morphofunctional relationships intrinsic of this or that tissue is an advantage of this method. The organotypical culture method suggests working with pure active substances. Hence, experiments were carried out with comenic acid, anoceptin active substance.

On day 1 of culturing, the explants spread on the collagen sublayer and migration of fibroblasts and

Hence, anoceptin (active substance: comenic acid) exhibited no negative effect on liver tissue status and detoxifying function. Presumably, comenic acid is utilized and metabolised not in the liver, but in neurons.

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