PHARMACOLOGY AND TOXICOLOGY

Effect of the Synthetic Antiheparin Agent Quaternary Ammonium Salt of Oligomer 25-Conidine on Liver Regeneration

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Single injection of quaternary ammonium salt of oligomer-25 conidine to rats with acute CCl_4-induced hepatitis promotes normalization of morphological and biochemical markers of hepatic injury in comparison with untreated animals. On day 3 postinjection the number of mitosis increased 2-3-fold.

Key Words: *quaternary ammonium salt of oligomer 25-conidine; CCl_4; alanine aminotransferase; histidase; bilirubin; mitotic index*

Extensive use of various chemical agents in agriculture and domestic life and of pharmacological preparations increase the risk of toxic damage to the liver. In light of this, studies of new pharmacological modulators of regeneration are of particular importance. CCl_4-induced hepatitis is the most common model of toxic hepatic injury. The toxicity of CCl_4 results from damage to the endoplasmic reticulum, lysosomes, and other membrane structures caused by free radicals, primarily, CCL_3· [3]. These alterations are accompanied by the release of liver-specific enzymes into circulation, disturbances in bile production and secretion, and impairment of protein-synthesizing function of the liver [7].

The effect of quaternary ammonium salt of oligomer-25 conidine (QAS O-25C) on regeneration is of interest, since this highly selective heparin antagonist corrects hypohemocoagulation in patients treated with heparin [5] and, being a polyion, QAS O-25C stimulates the immune system and regulates translation [6]. Previous studies showed that this agent enters the nucleus and mitochondria and activates protein biosynthesis [10,11].

Slow excretion of QAS O-25C from the organism [1] and its predominant accumulation in the liver led us to an assumption that a single injection of this agent produces long-term therapeutic and preventive effects. In the present study we verify this hypothesis.

MATERIALS AND METHODS

Experiments were carried out on male albino rats weighing 180-200 g. Hepatoprotective effect was studied using a model of toxic hepatitis induced by a single subcutaneous injection of 33% CCl_4 oil solution (Aldrich) in a volume of 0.3 ml/100 g body weight. In this experimental series 4 groups with 10 rats in each were used. Two groups were the control: intact and CCl_4-treated. Experimental rats received 2, 4, 6, and 8 mg/kg QAS O-25C (group 1) in physiological saline either 6 h after or 24 h before CCl_4 injection (group 2); QAS O-25C was

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synthesized by A. V. Nekrasov). QAS O-25C (6250 D) is a white powder soluble in physiological saline, LD_{50}=116 mg/kg.

One day after CCl_4 injection, the rats were decapitated, and blood activity of alanine aminotransferase (ALT, EC 2.6.1.2) [4], histidase (EC 4.3.1.3) [9] was measured, the content of total bilirubin and its fraction were determined as described elsewhere [4].

In the second experimental series, toxic hepatitis was induced by 4 subcutaneous injections of 50% CCl_4 oil solution once daily in a volume of 0.4 ml 100 g body weight. Experimental animals were divided into 4 groups with 30 animals each: 2 control groups (as above) and 2 experimental groups receiving QAS O-25C in doses 2 or 4 mg/kg 2 h before the first injection of CCl_4.

On days 3, 6, and 10, the animals (10 rats from each group) were decapitated, and the liver and blood were sampled for biochemical and morphological analyses; ALT and histidase activities and the content of bilirubin were assayed.

The number of mitoses per 1000 hepatocytes (10 fields of view in different hepatic zones) were counted on sections stained with hematoxylin (Serva) and eosin (Sigma), and mitotic index (MI) was calculated as the percentage of cells with mitotic figures. The degree of dystrophic alterations, necrosis, macrophage and fibroblastic reactions were scored using the following gradations: no changes (0), initial (1), weak (2), marked (3), and deep (4) alterations [12].

The data were processed statistically using the Wilcoxon—Mann—Whitney test.

RESULTS

Injection of QAS O-25C 6 h after CCl_4 poisoning produced a marked hepatoprotective effect (Table 1). In particular, in doses 2 and 4 mg/kg this drug reduced the release of liver-specific enzymes (ALT and histidase) into circulation and decreased the content of total bilirubin; the ratio of direct to in-direct bilirubin in animals injected with QAS O-25C 6 h after CCl_4 was 1.5:1 *vs.* 1:1 in untreated controls.

In animals injected with QAS O-25C 1 day before CCl_4 poisoning, the content of direct bilirubin surpassed that of indirect bilirubin by 2-3-times. This probably resulted from a more rapid recovery of glucuronidation in comparison with glucuronide excretion into hepatic ducts. The effect was most pronounced in animals receiving 2 and 4 mg/kg QAS O-25C.

Four subcutaneous injections of 50% CCl_4 oil solution to rats induced acute toxic hepatitis con-

TABLE 1. Effect of QAS O-25C on the Severity of Liver Damage under Conditions of CCl_4-Induced Experimental Hepatitis (*M±m*, *n*=10)

Biochemical parameter, % of control (intact animals)	CCl_4	QAS O-25C (mg/kg) 6 h after injection of CCl_4				QAS O-25C (mg/kg) 1 day before injection of CCl_4			
		2	4	6	8	2	4	6	8
ALT	182.5±5.1*	122.4±1.8*	138.8±3.5*	160.3±2.9*	186.3±9.7	118.3±12.1*	139.1±5.3*	159.2±9.9*	188.3±10.8
Histidase	315.3±3.9*	250.8±8.1*	249.0±10.3	285.7±9.9*	310.9±12.2	242.3±5.9*	245.5±10.1*	289.4±4.1*	319.4±20.4
Total bilirubin	148.3±16.8*	108.2±8.8*	115.7±4.3*	130.1±8.3	150.3±15.9*	110.9±4.8*	114.9±7.6	135.4±5.5	147.8±6.9*
Bilirubin, % of total bilirubin									
direct	50.7±8.2	59.3±4.3	60.3±2.1	61.0±5.7	68.2±6.4*	71.9±0.9*	74.0±5.2*	75.9±3.9	77.8±7.0
indirect	49.3±5.9*	40.7±1.9*	39.7±2.4	39.0±3.4*	31.8±1.8*	28.1±2.2	26.0±1.9	24.1±2.8*	22.2±4.1*

Note. *p<0.05 compared with the control.

TABLE 2. Morphological Characteristics of Liver in Rats Four after Injections of CCl_4 and Treated with QAS O-25C, Carnosine, and Vitamin B_{12} ($M\pm m$, n=5)

Groups	Time after the start of experiment, days	Dystrophic changes	Intensity of necrosis	Macrophagic reaction	Fibroblastic reaction	Granulations	MI, %
		points					
CCl_4	3	2.7±0.3*	2.3±0.5*	1.7±0.3	2.4±0.3*	1.3±0.4	0.93±0.1*
	6	1.7±0.3	0.3±0.3*	1.3±0.2*	1.0±0.1	0	0.07±0.01*
	10	1.0±0.4*	0	1.0±0*	0	0	0.03±0.001*
CCl_4+QAS O-25C, 2 mg/kg	3	2.0±0.1*	1.3±0.15*	2.0±0.1	1.3±0.2*	1.0±0.1	2.67±0.07*
	6	1.7±0.3	0	1.4±0.3	1.2±0.1	0	0.2±0.01*
	10	0.7±0.2	0	1.0±0.1	0.1±0.05	0	0.067±0.01
CCl_4+QAS O-25C, 4 mg/kg	3	2.0±0.3*	1.3±0.3*	1.7±0.3	1.7±0.3	1.0±0.1	1.67±0.1*
	6	1.7±0.2	0	1.6±0.2	0.7±0.2	0.3±0.1	0.1±0.02
	10	1.0±0.1	0	0.6±0.3	0	0	0.08±0.01*
CCl_4+carnosine	3	2.3±0.3	1.3±0.3*	1.7±0.3	2.3±0.3	1.0±0.1	1.86±0.02*
	6	1.7±0.3	0	1.0±0	1.0±0	0	0.20±0.01*
	10	1.0±0.2	0	1.3±0.3*	0.3±0.1	0	0.06±0.01*
CCl_4+vitamin B_{12}	3	2.5±0.35*	1.0±0.2*	2.0±0.3	1.0±0.1*	1.0±0.1	0.7±0.07*
	6	1.7±0.1*	0	1.3±0.2	1.0±0.2	0	0.13±0.01*
	10	1.7±0.2	0	1.0±0.1	0.3±0.1	0	0.067±0.02

Note. *p=0.05 compared with the control.

firmed by morphological examination of the liver (Table 2). We observed considerable dystrophic changes in hepatocytes, focal necroses, enhanced macrophagic reaction, proliferation of fibroblasts, initial fibrosis, and solitary postnecrotic granulation foci. Mitotic figures appeared among unaltered hepatocytes, mitotic index increased to 0.93%.

More serious damage to hepatocyte membranes in the second series was accompanied by a rise of serum ALT and histidase activity (8.3- and 14.7-fold in comparison with intact control, respectively) and bilirubin concentration (4.6-fold, Table 3).

In QAS O-25C-treated rats, the release of liver-specific enzymes and bilirubin was decreased on day 3 postinjection. The drug decreased the number of necrotic foci, prevented dystrophic changes in hepatocytes, and simultaneously stimulated liver regeneration: MI increased 2-2.87-fold attaining 2.6 and 1.67% for groups receiving 2 and 4 mg/kg QAS O-25C, respectively.

On days 6 and 10, serum ALT and histidase activities and bilirubin concentration decreased in all animals.

Thus, our finding suggests that QAS O-25C promotes normalization of biochemical markers of liver damage in comparison with untreated animals.

It can be hypothesized that *in vivo* QAS O-25C enters hepatocyte nuclei and competes with histones for DNA binding sites and, similarly to polyamines, induces conformation changes in DNA molecule, involving additional DNA loci into transcription. This in turn promotes replication and transcription processes [1].

Morphological analysis confirmed our previous findings that QAS O-25C stimulates replication and transcription in animals survived partial hepatectomy [10,11] (Table 2). This polycation inhibits inflammatory and necrotic processes in the liver. High mitotic activity in the liver of QAS O-25C-treated animals indicates enhanced biosynthesis of macromolecules. A decrease in total bilirubin in animals injected with 2 and 4 mg/kg QAS O-25C together with the rise of direct bilirubin suggest that more rapid liver regeneration in treated animals improves bilirubin binding and glucuronidation in hepatocytes (Table 1).

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REFERENCES

1. V. S. Efimov, S. B. Seredenin, O. V. Chernova, *et al.*, *Khim. Pharm Zh.*, **17**, No. 1, 55-59 (1983).
2. V. S. Efimov, O. V. Chernova, T. Z. Berestetskaya, and A. V. Nekrasov, *Farmakol. Toksikol.*, No. 4, 57-60 (1983).
3. V. A. Kostyuk, *Biokhimiya*, **56**, No. 10, 1878-1885 (1991).
4. V. V. Men'shikov, in: *Laboratory Methods in Clinics* [in Russian], Moscow (1987), pp. 186, 225-227.
5. A. B. Nekrasov, T. Z. Berestetskaya, V. S. Efimov, and O. V. Chernova, *Khim. Pharm Zh.*, No. 10, 1211-1215 (1982).

TABLE 3. Biochemical Parameters of Blood Serum from Rats with Experimental Hepatitis Treated with QAS O-25C and Vitamin B_{12} ($M\pm m$, n=10)

Biochemical parameters	Time from the start of experiment	Intact rats	CCl_4	CCl_4+QAS O-25C, 2 mg/kg	CCl_4+QAS O-25C, 4 mg/kg	CCl_4+ vitamin B_{12}
ALT, μmol/h/ml	3	0.60±0.03	5.10±0.24*	4.63±0.18*	4.14±0.21*	4.49±0.16*
			(834.8)	(759.4)	(679.9)	(737.7)
	6	0.65±0.04	3.21±0.21*	1.77±0.11*	2.34±0.19*	2.85±0.23
			(490.1)	(270.3)	(357.1)	(435.4)
	10	0.63±0.04	1.20±0.06*	0.62±0.3*	0.84±0.07	0.95±0.07*
			(191.0)	(97.8)	(132.5)	(151.2)
Histidase, mmol/min/l	3	0.05±0.01	0.795±0.06*	0.46±0.04*	0.53±0.04*	0.69±0.06*
			(1472.6)	(846.3)	(987.0)	(1274.0)
	6	0.05±0.004	0.44±0.02*	0.23±0.17	0.27±0.02	0.38±0.02*
			(876.9)	(462.3)	(533.7)	(748.0)
	10	0.088±0.007	0.35±0.03*	0.088±0.007*	0.19±0.02	0.22±0.02
			(396.8)	(99.8)	(213.2)	(253.9)
Bilirubin, mg/dl	3	0.62±0.03	2.82±0.13	2.04±0.09*	1.63±0.13*	2.40±0.06
			(459.0)	(331.0)	(265.5)	(389.7)
	6	0.74±0.04	1.79±0.11	1.00±0.05	0.99±0.06*	1.06±0.03*
			(241.2)	(134.8)	(133.0)	(142.8)
	10	0.53±0.05	0.88±0.05	0.59±0.05*	0.51±0.01*	0.70±0.04*
			(150.7)	(111.9)	(95.1)	(131.8)

Note. In parentheses: % of control (intact animals); *p<0.05 compared with the control.

6. R. V. Petrov and R. M. Khaitov, *Artificial Antigens and Vacines* [in Russian], Moscow (1988).
7. N. P. Skakun, G. T. Pis'ko, and I. P. Moiseichuk, *Carbon Tetrachloride-Induced Damage to the Liver* [in Russian], Moscow (1989).
8. K. M. Tadzhibaev, *Pat. Fiziol.*, No. 2, 57-60 (1989).
9. S. M. Fedorov, A. Ya. Nikolaev, I. A. Zharskaya, *et al., Vestn. Dermatol.*, No. 3, 12-12 (1988).
10. B. Ya. Khatsernova, S. A. Silaeva, V. A. Golenchenko, *et al., Vopr. Med. Khimii*, No. 3, 83-87 (1989).
11. B. Ya. Khatsernova, S. A. Silaeva, V. A. Golenchenko, *et al., Molecul. Biol.*, **25**, No. 5, 1308-1315 (1991).
12. A. B. Shekhter, A. A. Krel', and Z. G. Rasshchupkina, *Vopr. Revmatol.*, No. 1, 28-34 (1982).
13. H. Vierhapper and W. Waldhaus, *Life Sci.*, **37**, No. 19, 1829-1833 (1985).