Analysis of Antioxidant Properties of Chitosan and Its Oligomers

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Oral treatment with chitosan with a molecular weight ~10^5, but not its oligomer, reduced plasma content of free-radical oxidation products in normal rats and animals treated for the bone marrow form of radiation sickness and stimulated the recovery processes in involved bone marrow and peripheral blood.

Key Words: *chitosan; oligochitosan; antioxidant effects; γ-irradiation*

Chitosan (CTS), poly((1,4)-2-amino-2-deoxy-β-D-glucose), is a product of deacetylation of chitin, a polysaccharide second by the prevalence in nature after cellulose. Being a nontoxic, hypoallergenic, and biocompatible substance, it is now intensively studied with the aim of prospective use in medicine and as a component of bioactive additives. Chemical structure of CTS and its capacity to be destroyed under the effect of hydroxyl radicals suggest it as a potential antioxidant.

The data on antioxidant activity of CTS and its derivatives are scanty and contradictory and were primarily obtained on *in vitro* models [10-14]. Antioxidant effects of CTS *in vitro* depend on its molecular weight and deacetylation degree [10,11]. Higher antioxidant activity of oligomers in comparison with high-molecular-weight CTS was reported [12, 14], but these results do not agree with *in vivo* findings [8].

We studied antioxidant activity of CTS of different deacetylation degree and molecular weights (~10^5) and of CTS oligomers *in vivo* in normal animals and in animals exposed to ionizing radiation (as a model of oxidative stress) [2,6].

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MATERIALS AND METHODS

Experiments were carried out on male outbred albino rats (200-230 g). Oligochitosan (OCTS) with a molecular weight of 4×10^3 and 0.82 deacetylation degree and high-molecular-weight CTS with 0.82 and 0.50 deacetylation and mean molecular weight of 1.5×10^5 were used.

The effects of chitosan on LPO intensity in normal animals were studied in experimental series I. The animals were divided into 4 groups, 7 rats per group. Group 1 consisted of intact animals (normal), group 2 rats received distilled water (CTS solvent, placebo control). Animals of two experimental groups received OCTS in a dose of 100 mg/kg (group 3) and CTS (0.82 deacetylation) in a dose of 40 mg/kg (group 4). These doses were selected in accordance with manufacturer's recommendations for bioactive additives based on CTS oligomers and of Institute of Nutrition, Russian Academy of Medical Sciences [4]. Blood for analysis was collected from the sublingual vein on days 3 and 10 after treatment. Plasma concentrations of conjugated dienes and trienes [5] and Schiff's bases [9] were measured.

The therapeutic and antioxidant effects of CTS on the rat blood system under conditions of bone marrow form of medium severe radiation sickness were studied in experimental series II. The animals were exposed to a single total γ-irradiation in a

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dose of 5 Gy (1 Gy/min power; ^{60}Co). The treatment was started 2 h after the exposure. The animals were also divided into 4 groups: irradiation control group (no treatment), placebo control (distilled water), and two experimental groups: oral treatment with OCTS in a single daily dose of 100 mg/kg (group 3) and CTS in a dose of 40 mg/kg (group 4) for 5 days. Plasma concentrations of LPO products and the level of hemoglobin in whole blood [3] were measured on days 7 and 35 after irradiation. The count of bone marrow hemopoietic cells in one femoral bone [1] was evaluated after blood collection.

The results were statistically processed using Biostatistika 4.03 software and nonparametric Kruskal—Wallis and Newman—Keuls tests and Spearman rank correlation coefficients.

RESULTS

CTS and OCTS significantly reduced the content of Schiff's bases 3 days after treatment in comparison with controls and intact animals and did not change the levels of dienes and trienes (Table 1). The level of Schiff's bases in the CTS group was almost 2-fold lower than in rats treated with OCTS.

The intensity of LPO returned to normal 10 days after CTS treatment, while OCTS exhibited a prooxidant effect and significantly increased the content of LPO products in comparison with the control and intact groups (Table 1).

In series II, the content of all studied LPO products decreased significantly 7 days after irradiation, while OCTS showed weak antioxidant activity and reduced only the level of Schiff's bases (Table 2). The level of hemoglobin is very sensitive to radiation injury and reflects body functioning [7]. Seven days after irradiation hemoglobin content in animals receiving CTS and OCTS was significantly higher than in control rats. CTS with 0.82 deacetylation most effectively prevented the drop of hemoglobin (Table 3).

Thirty-five days after irradiation LPO intensity in animals treated with CTS was higher than in in-

TABLE 1. Plasma Levels of LPO Products at Various Terms after Oral Treatment with CTS under Normal Conditions ($\bar{X}\pm m$)

Group	Day after treatment					
	3 days			10 days		
	dienes, arb opt. dens. units	trienes, arb. opt. dens. units	Schiff's bases, arb. units	dienes, arb opt. dens. units	trienes, arb. opt. dens. units	Schiff's bases, arb. units
Intact	0.252±0.005	0.059±0.007	9.10±1.00	0.143±0.005	0.073±0.013	12.20±1.52
Control	0.205±0.019	0.066±0.010	14.10±1.91*	0.138±0.011	0.066±0.010	7.45±1.28*
OCTS	0.248±0.003	0.059±0.001	6.67±0.46*+	0.263±0.006*+	0.178±0.041*+	29.93±5.61*+
CTS	0.230±0.011	0.060±0.008	3.92±0.43*+x	0.143±0.005x	0.087±0.004x	12.15±0.55x

Note. $p<0.05$ compared to *intact, +control, xOCTS groups.

TABLE 2. Plasma Levels of LPO Products in Rats in Different Periods after Irradiation during CTS Therapy ($\bar{X}\pm m$)

Group	Day after treatment					
	7 days			35 days		
	dienes, arb opt. dens. units	trienes, arb. opt. dens. units	Schiff's bases, arb. units	dienes, arb opt. dens. units	trienes, arb. opt. dens. units	Schiff's bases, arb. units
Intact	0.269±0.006	0.048±0.001	0.85±0.06	0.220±0.006	0.035±0.002	0.57±0.05
Irradiation control	0.350±0.035*	0.079±0.012*	0.90±0.08	0.216±0.010	0.030±0.003	0.54±0.06
Control	0.331±0.005*	0.064±0.002*	1.30±0.09*+	0.228±0.009	0.038±0.004	0.58±0.02
OCTS	0.337±0.014*	0.083±0.008*	0.88±0.05o	—	—	—
CTS, 40 mg/kg						
0.82 deacetylation	0.273±0.003+ox	0.044±0.002+ox	0.84±0.05+o	0.298±0.013*+o	0.057±0.002*+o	0.79±0.05*+o
0.50 deacetylation	0.269±0.003+ox	0.048±0.001+ox	1.06±0.06+o	0.258±0.004*+o	0.034±0.002	0.76±0.02*+o

Note. Here and in Table 3: $p<0.05$ compared to *intact, +irradiation control, ocontrol, and xOCTS groups.

TABLE 3. Some Blood System Parameters in Rats During Different Periods after Irradiation (5 Gy) and CTS Therapy ($\bar{X}\pm m$)

Group		Hemoglobin, g/liter		Number of bone marrow cells per femur 35 days after exposure ($\times 10^6$)
		7 days after irradiation	35 days after irradiation	
Intact		137.50±8.10	177.75±3.33	5.39±0.01
Irradiation control		126.00±7.80	142.25±2.96*	3.94±0.34*
Control		112.60±10.20*	124.75±12.34*	3.24±0.41*
OCTS, 100 mg/kg		138.5±3.4^{o}	—	—
CTS, 40 mg/kg	0.82 deacetylation	148.00±5.50^{ox}	158.00±2.27^{*+o}	7.60±0.75^{*+o}
	0.50 deacetylation	121.40±7.83^{o}	160.60±3.38^{*+o}	8.50±0.88^{*+o}

tact and control rats (Table 3). This seems to be due to stimulation of the metabolic and reparative processes and proliferative activity of red bone marrow cells, which was seen from significantly higher hemoglobin content and count of bone marrow cells in animals treated with CTS (Table 3) and from the positive correlation between the latter parameter and levels of dienes (r=0.77; p=0.001), trienes (r=0.63; p=0.030), and Schiff's bases (r=0.80; p=0.001).

By day 35 after irradiation more than 50% animals treated with OCTS died. Hence, this preparation augmented the course of radiation sickness (parameters for this group are not presented).

Thus, OCTS exhibits a short-term antioxidant effect, after which stimulates LPO, which seems to underlie its negative effect on the course of radiation sickness. CTS with 0.82 and 0.50 deacetylation degree is similarly effective antioxidants stimulating reparative processes in the bone marrow and peripheral blood after radiation injury; it can serve as the basis for the creation of new effective antioxidants.

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