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Chitosan and its acyl derivatives included in liposomes protect mice against endotoxin shock

Anti-endotoxin effects of low molecular weight chitosan and its N-monoacylated derivative in solution and in phospholipid liposomes was studied in mice withendotoxin shock. Preventive ten-fold oral and double intraperitoneal injection of liposomal forms of chitosans reduces the toxic effect of endotoxin (lipopolysaccharide, LPS) in the mice and significantly increases the survival rate (83-100%) of animals in the experiment. The interaction of chitosan with LPS was shown to modulate significantly the biological activity of LPS. It was shown that LPS in a complex with N-Ac-Ch-LW reduces induction of pro-inflammatory TNF-α in human blood by 40% in comparison with parent LPS, but does not affect the ability of endotoxin to stimulate blood cells to synthesize the pro-inflammatory cytokine IL-6.

Key words: endotoxin, chitosan, liposomes, anti-endotoxin effects.

Endotoxins of gram-negative bacteria (lipopolysaccharide, LPS), penetrating into the bloodstream interact with immune factors and initiates the biosynthesis of effector molecules complex that cause endotoxic shock in large doses. An important problem of treating patients with endotoxemia is to find drugs to reduce the negative effects of endotoxin on the organism. The strategy of shock therapy is based on the removal of excessive amounts of LPS and/or blocking the binding of endotoxin to receptors on target cells [1]. An important way is the introduction of anti-endotoxin drugs into the human body and animals. Previously, we showed that natural polycations chitosans and its N-acylated derivatives form stable complexes with anionic LPS [3] and modulate significantly the biological activity of LPS [4]. Interactions of (LPS) with the polycation chitosan and its derivatives included in anionic liposomes were studied. The affinity of the interaction of LPS with a liposomal form of the N-acylated chitosan increases in comparison with, that are parent chitosan [2].

In this work we studied protective properties at endotoxemia of low molecular weight chitosan (Ch-LW, 4 -5 kDa) and its N-monoacylated derivative (N-Ac-Ch-LW) in solution and in phospholipid liposomes when administrated intraperitoneally (i/p) and per-oral (p/o) to mice. Endotoxic shock was induced by injection i/p to the mice of LPS from *Escherichia coli* 055:B5 (7.5 mg/kg). Ch-LW was prepared by depolymerization of commercial chitosan with hydrogen peroxide. N-Ac-Ch-LW was obtained by acylated Ch-LW with 3-hydroxytetradecanoic acid hydroxysuccinimide ester. Liposomal forms of chitosans were obtained by entrapped of Ch-LW and N-Ac-Ch-LW on liposomes, preformed from lecithin, cholesterol and dicetyl phosphate [2]. It has been established that N-Ac-X-LM increases the stability of liposomes to the aggressive action of media simulating conditions in some sections of the gastrointestinal tract in 1.4-2 times.

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It was found that the double i/p administration of chitosan or its liposomal form when administered simultaneously with LPS did not significantly affect the survival rate of mice (Table 1).

Table 1.

Effect of X-HM, N-Ac-X-NM and their liposomal forms on the survival of BALB mice in endotoxin shock

Group	Time of introduction	Preparations	Survival, %
I	1-fold i/p injection of LPS 7.5 mg/kg	Liposomes	20
II	2-fold i/p injection of N-Ac-Ch-LW 5 mg/kg	N-Ac-Ch-LW*	100
III	2-fold i/p injection of 5 mg/kg, the administration simultaneously with LPS	Ch-LW	27
		N-Ac-Ch-LW	25
		N-Ac-Ch-LW+liposomes	30
IV	2-fold i/p injection 5 mg/kg, 2 hours before the administration of LPS	Ch-LW	66
		N-Ac- Ch-LW	83
		Ch-LW+liposomes	90
		N-Ac-Ch-LW +liposomes	100
V	10-fold p/o administration 2.5 mg/kg, then LPS	Ch-LW	50
		N-Ac- Ch-LW	66
		Ch-LW +liposomes	76
		N-Ac-Ch-LW + liposomes	83

*Ch-LW – low molecular weight chitosan, N-Ac-Ch-LW– N-monoacylated derivative low molecular weight chitosan. Administrated intraperitoneally (i/p) and per-oral (p/o) to mice.

Intraperitoneal administration of Ac-Ch-LW 2 hours before the LPS injection better protected mice from the toxic effects of LPS, then its liposomal form. The maximum protective activity was shown by liposomes coated with Ac-Ch-LW at their ten-fold per-oral administration before induction of endotoxin shock with LPS. In this case Ac-Ch-LW showed weaker protective properties from the effect of LPS (66% of surviving mice with an average lifetime of 38 h) compared with its liposomal form (83% of surviving mice at 51.4 h).

The interaction of Ch with LPS was shown to modulate significantly the biological activity of LPS. Using ELISA,it was shown that LPS in a complex with N-Ac-Ch-LW reduces induction of pro-inflammatory TNF-α in human blood by 40% in comparison with parent LPS. The formation of the LPS complex with N-Ac-Ch-LW does not affect the ability of endotoxin to stimulate blood cells to produce the pro-inflammatory cytokine IL-6. N-Ac-Ch-LW stimulated cellular immunity reactions. The introduction of hydrophobic substituents into the molecule of chitosan increased its ability to stimulate the bactericidal activity, the adhesive properties and the absorption activity of neutrophils (at a concentration of 1-10 μg/ml).

Thus, preventive ten-fold oral and double intraperitoneal injection of liposomal forms of chitosans reduces the toxic effect of endotoxin in the mice and significantly increases the survival rate (83-100%) of animals in the experiment. With oral administration of liposomal forms of chitosan, its protective effect may increase when bound to the epithelium of the gastrointestinal tract due to mucoadhesive properties of chitosans.

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