# Natural Polysaccharide Carrageenan Inhibits Toxic Effect of Gram-Negative Bacterial Endotoxins

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The protective effect of polysaccharide carrageenan on the damaging effect of endotoxins of gram-negative bacteria was studied *in vivo* and *in vitro*. Carrageenan increased mouse resistance to the toxic effect of LPS. The degree of protection depended on polysaccharide concentration and administration time and route. Pretreatment of donor platelets with carrageenan reduced their aggregation activity caused by cooperative effect of LPS and ADP.

**Key Words:** *lipopolysaccharide; carrageenan; toxicity; platelet aggregation*

Endotoxins, or LPS, are among the main components of cell wall in gram-negative bacteria; released into the body, they cause a series of acute physiological reactions: fever, dysmetabolism, disseminated intravascular coagulation; in high doses they cause tissue necrosis, severe intoxication, and death [9]. The search for substances improving body resistance to bacteria and toxins released by them is an important problem. Such cationic compounds as lysozyme [8] and polymyxin [7] neutralize endotoxins by forming macromolecular complexes with them. We previously showed that chitosan, a natural polysaccharide, forms stable complexes with endotoxins, thus appreciably reducing their toxicity [1] and modifying biological properties of LPS [2]. Here we studied the possibility of using polysaccharide carrageenan for this purpose. Carrageenans are *Chondrus armatus* sulfated galactanes containing D-galactose residues and its derivatives; they are widely used as thickening agents and stabilizers in foodstuffs [5]. Intensive studies showed that carrageenans can be regarded not only as foodstuff ingredients, but also as the drugs. For example, the search for anticoagulants, antiviral, antitumorous, and immunomodulating agents among these substances proved to be a promising trend of research [12]. Pharmacological activity of carrageenans is dose-dependent and depends on the type or structure of polysaccharide [5,12].

We studied the protective effect of carrageenan against LPS toxicity *in vivo* and its effect on donor platelet aggregation *in vitro*.

## MATERIALS AND METHODS

Carrageenans (κ- and λ-types) from *Chondrus armatus* (*Gigartinaceae*) were described previously [13]. LPS preparations from *E. coli* 055:B5 and *S. typhimurium* (Sigma) were used in the study.

*In vivo* experiments on evaluation of carrageenan protection from toxic LPS were carried out on outbred and CBA mice (20 g) in 10 groups of animals, 6 per group. Carrageenan was injected intraperitoneally in a dose of 100-200 μg/mouse 7 days or 24 h before or simultaneously with the injection of endotoxin in doses of 0.25-2.00 μg/mouse. The

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animals were observed for 20 days. The protective effect of carrageenan was evaluated by mouse survival and mean life span estimated by the formula [11]. LPS toxicity was evaluated by its intraperitoneal injection with galactosamine [10].

Parameters of hemostasis were determined in the donor citrate plasma before and after 30-min incubation with carrageenan using the Tekhnologiya-standart test systems.

*In vitro* experiments on evaluation of donor platelet aggregation under the effects of LPS and carrageenans were carried out as described previously [3]. Platelet aggregation activity was studied using standard ADP inductor ($2\times10^{-5}$ M) on an AP-2110 aggregometer (SOLAR). The degree of irreversible aggregation of platelets induced by ADP alone served as the control (100% aggregation). In control experiments normal saline was added to platelet-rich plasma instead of the test substances. The results were statistically processed using Student's *t* test.

## RESULTS

We studied the possibility of reducing the toxic effect of LPS with carrageenans in experimental animals. Carrageenans were isolated and characterized as described previously [13]. *E. coli* 055:B5 and *S. typhimurium* LPS served as endotoxins. Carrageenan in a dose of 100-200 μg/animal was injected subcutaneously and intraperitoneally: simultaneously with, 24 h and 7 days before injection of LPS. Carrageenan exhibited a protective effect in all cases: mouse survival and mean life span increased compared to those in the control group. The degree of protection depended on the duration of carrageenan exposure and its concentration. The best protective effect was observed in mice pretreated with κ-carrageenan in a dose of 200 μg/mouse (Fig. 1). The mortality was lower after intraperitoneal injection of carrageenan to random-bred animals 7 days before LPS injection and after simultaneous injection of LPS and carrageenan than after injection of carrageenan in the same doses 24 h before LPS. LPS toxicity estimated from experimental data decreased more than 2-fold after

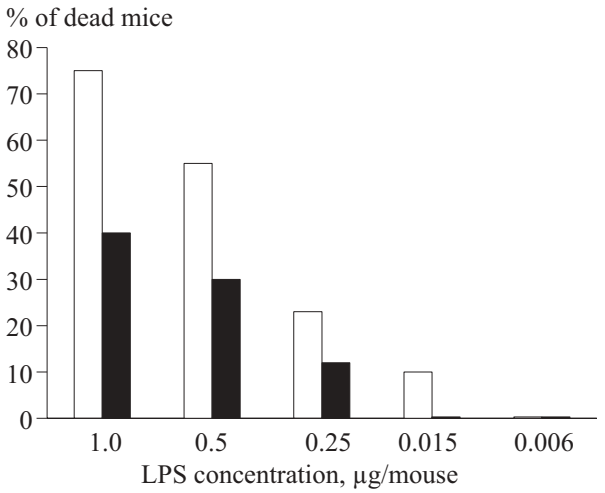


**Fig. 1.** Relationship between the number of survivors and LPS concentration after treatment with LPS (light bars) and LPS+carrageenan (dark bars).

pretreatment with carrageenan (Table 1). A similar protective effect of carrageenan was observed in mice infected by *P. aeruginosa* and *E. coli* [6]. The mechanism of host resistance to endotoxin, induced by carrageenan, can be due to immunomodulating effect of polysaccharide, which was demonstrated for different carrageenans [12], and to endotoxin detoxication at the expense of shielding its toxoform group by carrageenan. The latter hypothesis is supported by $LD_{50}$ values for LPS+carrageenan mixture (Table 1).

Platelets are one of the main cell objects of the primary pathogenic effect of LPS [3]. Evaluation of the effect of polysaccharide on parameters of hemostasis showed that κ-carrageenan in a concentration of 100 μg/ml inhibited (by 3.48 times) spontaneous platelet aggregation, prolonged (by 3.05 times) activated partial thromboplastin time, prothrombin time (by 1.4 times), and thrombin time (by 1.49 times) and significantly stimulated activity of AT-III (by 7.88 times), a potent natural inhibitor of clotting, in comparison with samples without carrageenan. The effects of two carrageenan types (κ and λ) in a dose of 100 μg/ml on aggregation activity of donor platelets (native and under conditions of toxemia) were studied *in vitro*. Platelet aggregation was evaluated in the presence of the test polysac-

**TABLE 1.** LPS Toxicity with and without Carrageenan

| Animals | LPS $LD_{50}$, μg/mouse | LPS $LD_{50}$, μg/mouse (carrageenan injected 7 days before) | LPS $LD_{50}$, μg/mouse (LPS+carrageenan) |
|---|---|---|---|
| CBA mice | 0.4 | 0.8 | |
| Random-bred albino mice | 0.65 | 1.9 | 1.3 |

charides alone and in combination with standard inductor of aggregation (ADP). Preincubation of both carrageenans with platelets for 3 min significantly decreased ADP-induced aggregation: by 37.8 and 33.7% ($p<0.05$) under the effects of κ- and λ-carrageenans, respectively.

In series II we studied platelet protection from the damaging effects of endotoxin; *S. typhimurium* and *E. coli* LPS. Addition of *S. typhimurium* LPS to plasma (200 μg/ml) caused 7.4% platelet aggregation ($p\leq0.05$). Subsequent addition of ADP induced more potent platelet aggregation (109.5%; $p\leq0.05$). Preliminary addition of carrageenan (300 μg/ml) to platelet-rich plasma reduced platelet aggregation to 80.8% ($p\leq0.05$). Preincubation (3 min) of platelets with carrageenan reduced ADP-induced platelet aggregation by 15% in the presence of *E. coli* LPS. Alimentary toxinfection is known to be associated with disseminated intravascular coagulation syndrome with significant damage to platelets. The effect of carrageenan on platelets from patients with alimentary toxinfection was therefore studied. Addition of *S. typhimurium* LPS to patient platelet-rich plasma induced significant cell activation (to 14.2%) compared to that in donor plasma. Subsequent addition of ADP increased platelet aggregation to 133.3%. Pretreatment with carrageenan (before LPS and ADP) inhibited the stimulatory effect of LPS and practically abolished it. Platelet aggregation in this case was 103.4%, which attests to a protective effect of carrageenan on platelets.

Hence, carrageenan can be used for decreasing the toxic effect of LPS. This opens prospects for practical use of carrageenans for reducing toxemia associated with the development of infectious processes caused by gram-negative bacteria, and in emergency, in cases with threatened intoxication.

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