Effect of Pectin Gel Particles on Endotoxemia Induced by Restraint Stress in Mice

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We studied the effect of pectin gel particles on endotoxemia in mice induced by restraint stress. It was shown that the concentration of LPS in mouse blood increased during restraint stress, which was associated with memory impairment. Pectin gel particles prevented the development of stress-induced endotoxemia and memory impairment in mice.

Key Words: pectin gel particles; restraint stress; endotoxemia

During recent decades, it became evident that inflammation can be triggered by not only infections. It was established that obesity [4], diabetes [6], alcoholism [7], and psychological stress [2] can induce an increase in blood concentration of LPS, which leads to elevation of proinflammatory cytokine level in the blood and development of mild inflammation. High levels of proinflammatory cytokines affect the CNS, which can lead to memory impairment [10]. It can be assumed that prevention of endotoxemia is the best way to avoid inflammatory reactions. It was shown that pectin gels adsorb ions of heavy metals (Cr$^{3+}$, Cu$^{2+}$, and Pb$^{2+}$) [5] and some anions (H$_2$PO$_4^-$, HPO$_4^{2-}$, PO$_4^{3-}$, and F$^-$) [3,8]. However, the interaction of pectin gels with LPS was not studied. We hypothesize that pectin gels due to enterosorbent properties could prevent the increase in blood concentration of LPS and negative consequences related with this shift.

Here we studied the effect of pectin gel particles (PGP) on endotoxemia induced by restraint stress in mice.

MATERIALS AND METHODS

We used PGP obtained by adding calcium chloride solution to water-in-oil emulsion of 1% solutions of pectins CU701, AU701, and CM020 (Herbstreith & Fox KG). The technique for preparation of gel particles by emulsification was previously described for chitosan [1]. PGP were studied under Altami LUM-1 light microscope equipped with Altami Studio 3.2.2 software (Altami) (Fig. 1). The mean diameters of the particles prepared from pectins CU701, AU701, and CM020 were 3.7±0.2, 4.0±0.3, and 3.4±0.2 μ, respectively (averaged for at least 150 particles of each type).

The particles were incubated in sterile saline (pH 6.8) containing LPS (E. coli 0111:B4, 10 µg/ml; Sigma) for 3 h at 37°C to determine the ability of PGP to adsorb LPS in vitro. PGP were incubated in sterile saline with LPS (10 µg/ml) and BSA (10 mg/ml) for 3 h at 37°C to examine the selectivity of endotoxin adsorption. PGP were separated by centrifugation (460 g, 20 min, Beckman Coulter) after the incubation and the supernatants were collected and frozen. The efficiency of endotoxin adsorption was determined by assessment of the amount of residual concentration of LPS in the supernatant. The endotoxin level in the supernatant was measured using Chromo-LAL kits (Cape Cod, Inc.).

The study in vivo was performed on white outbred male mice (n=56) weighing 20-22 g. The animals were kept in standard laboratory conditions with unlimited access to water and food.

The study was conducted in two series. In series I (n=24), the parameters in non-stressed (native) mice and in stressed animals in 3 and 18 h after stress were...
compared. In this series, the stressed mice were administered only 0.2 ml of water per os.

In series II, the effect of PGP was analyzed. Control mice (n=32) received 0.2 ml water per os before stress exposure, mice of other groups received 1 mg PGP in 0.2 ml distilled water. Restraint stress was modeled in 50 ml well-ventilated centrifuge tubes. The animals were placed in the tubes for 3 and 18 h. In both series, the blood was sampled from the portal vein and behavioral responses before the stress, 3 and 18 h after its induction were compared. The concentration of endotoxin in mouse blood plasma was measured as described previously. Plasma concentration of corticosterone was measured using Corticosterone ELISA kit (IBL International).

Novel object recognition test was used to assess memory in mice. Testing was conducted in two sessions: training and testing. During training, the mice were placed on a round arena with two identical objects for 5 min. After 1 day, one object was replaced with a new one similar in size and color, but different in shape. Testing was conducted for 5 min and recorded on a video camera. The data were processed using RealTimer 1.2 (OpenScience) software. The recognition index was the ratio of interaction time with the new object (touching by the forelegs, sniffing and looking over) to the total time of interaction with two objects [11].

Statistical processing of the results was conducted using the Mann—Whitney U test; the data were presented as the arithmetic mean±standard error of the mean (X±m). The differences were significant at \( p \leq 0.05 \).

RESULTS

The effectiveness of endotoxin sorption in vitro after 3-h incubation of LPS with PGP from pectins CU701 and AU701 was 97 and 39%, and for CMO20 — 58%. AU701 and CM020 PGP selectively adsorbed endotoxin (up to 100%), however, selectivity of sorption for CU701 PGP was 64%.

Immobilization for 3 h induced the development of stress reaction. Blood concentration of corticosterone increased by more than 2 times (to 279±22 nmol/liter vs. 129±17 nmol/liter in native controls, \( p \leq 0.05 \)). LPS concentration in the blood of native mice was 1.2±0.4 ng/ml and after 3 h of restraint stress it significantly (\( p \leq 0.05 \)) increased by more than 5 times (Table 1). After 18-h immobilization, blood level of endotoxin did not differ from that in the blood of native animals. Memory impairment in mice was observed after 18-h immobilization. The recognition index in these mice was significantly (\( p \leq 0.05 \)) lower than in native mice. Thus, the effect of PGP on the concentration of LPS was analyzed after 3-h immobilization and on novel object recognition index in 18 h.

PGP did not change the level of stress in mice. Corticosterone concentration in mice receiving PGP did not differ from that in mice receiving water (Table 2). Blood concentration of LPS was significantly reduced after 3-h restraint stress in mice that receiving

<table>
<thead>
<tr>
<th>Group</th>
<th>LPS, ng/ml</th>
<th>Index of recognition</th>
</tr>
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<tbody>
<tr>
<td>Native mice</td>
<td>1.2±0.4</td>
<td>0.62±0.06</td>
</tr>
<tr>
<td>Stress 3 h</td>
<td>6.5±0.8*</td>
<td>0.55±0.13</td>
</tr>
<tr>
<td>Stress 18 h</td>
<td>2.7±1.1</td>
<td>0.41±0.03*</td>
</tr>
</tbody>
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Note. *\( p \leq 0.05 \) in comparison with native mice.