Short Communication

Platonin, a Cyanine Photosensitizing Dye, Inhibits Pyrogen Release and Results in Antipyresis

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Abstract. Intravenous injection of the supernatant fluids from human peripheral blood mononuclear cells (PBMC) incubated with lipopolysaccharide (LPS) caused fever in rabbits. The fever was in parallel with the levels of either interleukin-1β (IL-1β), IL-6, or tumor necrosis factor-α (TNF-α) in supernatant fluids. When incubating the platonin with the LPS-human PBMC, both the levels of IL-1β, IL-6, or TNF-α in supernatant fluids and the pyrogenicity of supernatant fluids were significantly suppressed. The febrile response to supernatant fluids from the LPS-stimulated PBMC was attenuated almost completely by adding anti-IL-1β, but not anti-IL-6 or anti-TNF-α, monoclonal antibody to supernatant fluids. In addition, both the fever and the increased levels of either IL-1β, IL-6, or TNF-α in rabbit serum following an intravenous administration of LPS were significantly attenuated by pretreatment with an intravenous dose of platonin. Furthermore, the fever induced by intravenous injection of IL-1β was reduced by pretreatment of rabbits with intravenous injection of platonin. The data indicate that platonin inhibits production of pyrogenic cytokines (in particular, IL-1β) from PBMC and results in antipyresis.

Keywords: antipyresis, platonin, cytokine

It is generally believed that exogenous pyrogens including lipopolysaccharide (LPS) and other products provoked by the invading organisms induce fever (1, 2). LPS acts on peripheral blood mononuclear cells (PBMC) to stimulate synthesis or release of pyrogenic cytokines including interleukin-1β (IL-1β), IL-6, and tumor necrosis factor-α (TNF-α). These pyrogenic cytokines attack upon targets accessible from blood with generation of stimulatory signals directed to the brain. Prostaglandin (PG) E₂ is thought to be an essential, proximal mediator in the thermoregulator centers in the hypothalamus, and induced by these cytokines (1, 2). This is followed by PGE₂-induced neuronal mechanisms involving cyclic AMP and neurotransmitters.

Platonin, a cyanine photosensitizing dye, is a potent macrophage-activating agent (3, 4) and an immunomodulator (5, 6). It has been prescribed for rheumatoid arthritis (5, 6). Recently, we have provided evidence to demonstrate that platonin has beneficial effects on ameliorating endotoxemia in rat models (7). To our knowledge, it is not known whether platonin has an effect on the synthesis or release of IL-1β, IL-6, and TNF-α from PBMC incubated with LPS as well as an antipyretic effect.

In order to deal with the question, we first carried out experiments to assess the pyrogenic response in rabbits to intravenous (i.v.) injection of supernatant fluids obtained from human PBMC incubated with LPS alone or LPS plus platonin. At the same time, levels of IL-1β, IL-6, and TNF-α in the supernatant fluids were assessed in vitro. Secondly, the effects of pretreatment with an intravenous dose of platonin on the febrile responses and/or increased levels of these cytokines in the serum of rabbits induced by i.v. injection of LPS or IL-1β were assessed. Furthermore, the effects of adding the anti-IL-1β, anti-TNF-α, and anti-IL-6 monoclonal antibody...
(MAb) to the supernatant fluids from human PBMC treated with LPS on the pyrogenic responses to i.v. administration of the supernatant fluids were assessed in rabbits.

Human PBMC was obtained from freshly collecteduffy coat fraction from healthy donors at the Tainan Blood Bank Center (Tainan City, Taiwan). It was isolated by centrifugation over a Ficoll-Paque (Pharmacia, Uppsala, Sweden) density gradient at 400 × g for 30 min at room temperature in a Sorvall RT 6000 B (DuPont, Wilmington, DE, USA) (8). The cells collected at the interface were washed thrice with serum-free RPMI-1640 (Gibco, BRL, Grand Island, NY, USA) and subsequently resuspended in AIM-V medium (Gibco, BRL) containing 100 U/ml of penicillin and 100 μg/ml of streptomycin. The PBMC at a concentration of 1 × 10⁷ cells/ml was incubated with different concentrations of tested agents in a 37°C incubator. After incubation, the PBMC supernatant was harvested by centrifugation (1200 rpm) and stored at −80°C.

Adult male New Zealand white rabbits, weighing between 2.0 and 3.2 kg, were used. The pyrogen assay was carried out using unanesthetized animals restrained in rabbit stocks. Between experiments, the animals were housed individually at the ambient temperature of 22 ± 1.0°C with a 12-h light-dark cycle, with the lights being switched on at 0600 h. Animal chow and water were allowed ad libitum. Experiments were conducted between 0900 and 1700 h, with each animal being used at an interval of no less than 13 days. Throughout the experiment, colonic temperature (Tco) was measured every minute with a copper constantan thermocouple connected to a thermometer (HR 1300; Yokogawa, Tokyo). The colonic temperature of each animal was allowed to stabilize for at least 90 min before any injections.

All drug solutions were prepared in pyrogen-free glassware that was heated at 210°C for 5 h before use. All solutions were passed through 0.22-μm-pore-size Millipore bacterial filters. LPS (E. coli serotype 0127:B8) was purchased from Sigma Chem. Co. (St. Louis, MO, USA). Platonin, 4,4',4''-thrimethyl-3,3',3''-triheptyl-7-(2''-thia-zolyl)-2,2'-trimethinethiazolocyanine-3,3''-diiodide was synthesized by Kankohsha Co. (Osaka), and it was obtained from Gwo Chyang Pharmaceutical Co., Ltd. (Tainan, Taiwan). Monoclonal mouse antihuman (anti-h) IL-1β, anti-h TNF-α, and anti-h IL-6 MAb were obtained from R&D Systems (Minneapolis, MN, USA), while an isotope-matched mouse immunoglobulin (IgG1) control MAB was purchased from Chemicon International, Inc. (Teonecula, CA, USA).

PBMC were cultured at a density of 1 × 10⁷ cells/ml in 6-well flat-bottom plates (Nalge Nunc International, Roskilde, Denmark) stimulated with LPS (0.1 – 1.0 μg/ml) alone or cocultured with platonin (0.1 – 1.0 μg/ml) for 24 h. At the end of the experiment, the culture supernatants were collected and stored at −80°C before analysis for secreted cytokines. The amounts of the cytokines TNF-α, IL-1β, and IL-6 in the culture supernatants were determined by using double-antibody sandwich ELISA (R&D Systems) according to the manufacturer’s instructions. Recombinant human IL-1β (16 to 1000 pg/ml), TNF-α (8 to 500 pg/ml), or IL-6 (5 to 300 pg/ml) represented the standards for calibration, and the detection limit of all assays was 20 pg/ml.

Either IL-1β, IL-6, or TNF-α activity in serum samples of rabbits was measured by an in vitro cytotoxicity assay with the IL-1-dependent murine T-cell line D10N4M, IL-6-dependent cell line 7TD1, or TNF-sensitive L.P3 cells, respectively, as previously described (9). One hundred microliters of human PBMC (1 × 10⁷ cells/ml) were stained with 100 μl of trypan blue (0.04%). After treatment with trypan blue, the dead cells will appear blue-purple in color and can be counted in a conventional leucocyte counter. The viability of the PBMC was always equal to or greater than 97%.

Animals were kept at an ambient temperature of 22°C for at least 90 min to obtain thermal balance before drugs were administered. Temperature responses were assessed as changes from the pre-injection value (ΔT°C) and/or the fever index (FI), the area under the curve produced in the 2-h period after the injection of LPS, in terms of degrees centigrade per 2 h were calculated (10). Results were expressed as the means ± S.E.M. for n experiments and compared by one-way analysis of variance (ANOVA) followed by Duncan’s test when appropriate. P<0.05 is considered significant.

To determine the ability of LPS to stimulate the release of cytokines production, the levels of IL-1β, IL-6, and TNF-α in supernatant fluids obtained from human PBMC treated with LPS were assayed at 24 h incubation. As shown in Table 1, the levels of IL-1β, IL-6, and TNF-α in supernatant fluids were increased by incubating PBMC with LPS. Over the dose range of 0.05 – 1.0 μg/ml of LPS, the productions of IL-1β, IL-6, and TNF-α were all increased dose-dependently.

To ascertain whether LPS can act through PBMC to induce a pyrogenic response, supernatant fluids obtained from PBMC (10⁷ cells/ml) treated for 24 h with LPS (0.05 – 1.0 μg/ml) were given i.v. to the rabbits. As shown in Table 1, i.v. administration of LPS produced dose-related fever, as reflected by the FI.

To determine whether the pyrogenic response is mediated by a specific cytokine in supernatant fluids from LPS-stimulated PBMC, we added several cytokine-specific MAb to supernatant fluids at 37°C for
the pyrogenic response to supernatant fluids from the to supernatant fluids. IgG1 control MAb did not affect MAb abrogated almost completely the febrile responses very weak neutralizing action. However, anti-IL-1 a

As shown in Table 2, MAbs to TNF-30 min before they were administered i.v. into rabbits.

Table 2. Effects of cytokine-specific monoclonal antibody (MAb) on the pyrogenic response to supernatant fluids from LPS-stimulated PBMC as well as platonin on the pyrogenic response to interleukin-1β (IL-1β) solutions in rabbits

Table 3. Effects of cytokine-specific monoclonal antibody (MAb) on the pyrogenic response to supernatant fluids from LPS-stimulated PBMC as well as platonin on the pyrogenic response to interleukin-1β (IL-1β) solutions in rabbits

30 min before they were administered i.v. into rabbits. As shown in Table 2, MAbs to TNF-α or IL-6 had a very weak neutralizing action. However, anti-IL-1β MAb abrogated almost completely the febrile responses to supernatant fluids. IgG1 control MAb did not affect the pyrogenic response to supernatant fluids from the LPS-stimulated PBMC.

In addition, both the cytokine production by LPS-stimulated PBMC and the pyrogenic response to LPS-stimulated PBMC supernatant fluids in rabbits were significantly reduced by incubating the LPS-stimulated PBMC with various doses of platonin (0.1 – 10 μg/ml),
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As shown in Table 1, i.v. administration of 2 $\mu$g/ml per kg of LPS produced fever in rabbits. The Tco started to rise almost immediately, peaked at 1 – 3 h, and returned to the preinjection level at about 5 h after LPS administration. However, i.v. administration of LPS at a dose of 0.5 $\mu$g/ml per kg had an insignificant effect on Tco (data are not shown here). Again, the fever induced by 2 $\mu$g/ml per kg of LPS was in parallel with serum levels of either IL-1$\beta$, IL-6, or TNF-$\alpha$. Both the fever and the increased levels of IL-1$\beta$, IL-6, and TNF-$\alpha$ in rabbit serum induced by i.v. injection of LPS was significantly attenuated by pretreatment with platonin (5 – 15 $\mu$g/kg, i.v.). Furthermore, the fever induced by IL-1$\beta$ was antagonized by pretreatment with platonin 1 h before IL-1$\beta$ injection (Table 2).

The present results demonstrated that the febrile responses in rabbits to supernatant fluids from LPS-stimulated human PBMC were in parallel with levels of IL-1$\beta$, IL-6, and TNF-$\alpha$ in supernatant fluids. We further showed that adding anti-IL-1$\beta$, but not adding anti-TNF-$\alpha$ or anti-IL-6, MAb into supernatant fluids from the PBMC stimulated with LPS almost completely attenuated the pyrogenic activity exerted by the supernatant fluids in rabbits. In addition, the fever in rabbits in response to i.v. injection of supernatant fluids and the increased levels of IL-1$\beta$, IL-6, and TNF-$\alpha$ in supernatant fluids from the LPS-stimulated PBMC were decreased by incubating the LPS-stimulated human PBMC with platonin. Furthermore, in the present study, both the fever and the elevated levels of IL-1$\beta$, IL-6, and TNF-$\alpha$ in serum induced by systemic administration of LPS in rabbits were attenuated by pretreatment of rabbits with i.v. doses of platonin. Apparently, there is no discrepancy between human and rabbit PBMC in terms of pyrogenic cytokine production responses to LPS. Pretreatment with platonin also antagonized the IL-1$\beta$-induced fever in rabbits. These
findings provide the first evidence showing that platonin acts through the inhibition of pyrogenic cytokines, in particular, the IL-1β release from the LPS-incubated PBMC, to induce its antipyretic effect.

In the present results, i.v. administration (ml/kg) of supernatant fluids from LPS (0.05 µg/ml)-treated human PBMC caused fever in rabbits. However, i.v. administration of LPS (0.5 µg/ml/kg) caused an insignificant change in Tco in rabbits. This indicates that the fever is unlikely to be due to the remaining residue LPS present in supernatant fluids.

Nuclear factor-κB (NF-κB) is thought to play a pivotal role in immune and inflammatory responses through the regulation of genes encoding pro-inflammatory cytokines and inducible enzymes such as cyclooxygenase and inducible nitric oxide synthase (11). NF-κB is likely to be the key transcription factor through which the LPS provokes transduction signal and cytokine gene transcription (11 – 13). Indeed, our unpublished results showed that LPS was able to induce NF-κB activation in human PBMC, which could be abolished by platonin. Systemic administration of the endotoxin LPS also causes a robust expression of the gene encoding IL-1β, TNF-α, and IL-6 in the brain (14, 15). Thus, it is likely that platonin acts through inhibition of the NF-κB mechanism in the PBMC and/or the thermoregulatory centers in the hypothalamus to reduce synthesis or release of pro-inflammatory cytokines and/or PGE2. Platonin may inhibit synthesis or release of pyrogenic cytokines and/or PGE2 in situ and result in antipyresis. Therefore, the current results provide evidence to promote the potential application of platonin as an antipyretic drug in clinical practice.

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References