Stimulation of Phagocytic Activity of Leukocytes and Macrophages by Traxanox Sodium in Mice and Rats

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Abstract—Phagocytosis of yeast particles by mouse peritoneal macrophages or rat peritoneal polymorphonuclear leukocytes was enhanced by traxanox sodium in vitro. Traxanox sodium (30 and 100 mg/kg, p.o.) enhanced phagocytosis of yeast particles by leukocytes and macrophages under the same conditions. Traxanox sodium (30 mg/kg, p.o.) prevented the suppression of phagocytosis by the above drugs. Combined with theophylline, isoproterenol synergistically inhibited phagocytosis by leukocytes in vivo. Traxanox sodium (100 mg/kg, p.o.) administered alone had no influence on carbon clearance in normal mice. However, traxanox sodium (1–30 mg/kg, p.o.) prevented the suppression of carbon clearance by the treatment with carrageenan, but not by the treatment with ethyl palmitate. These results suggest that traxanox sodium stimulates the phagocytic activity of leukocytes or macrophages and prevents the drug-induced suppression of the phagocytic activity of these cells.

A new synthetic compound, traxanox (9-chloro-5-oxo-7-(1H-tetrazol-5-yl)-5H-[1]benzopyrano-[2,3-b]pyridine) sodium pentahydrate has been shown to possess anti-anaphylactic effects (1, 2). This agent has also been shown to enhance the production of spleen- and thymus-rosette forming cells in C57BL/6 mice (3), a lower responder to sheep red blood cells (SRBC), and to inhibit that in BALB/c mice (4), a higher responder mice to SRBC. Furthermore, the inhibition of phagocytosis of SRBC by spleen adherent cells in BALB/c mice pretreated with dexamethasone was restored by the addition of traxanox sodium in vitro (5). These findings suggest that the immunomodulating effect of this agent is attributable to changes in macrophage functions such as antigen presentation. It was, therefore, supposed that traxanox sodium might regulate the phagocytic activity of leukocytes or macrophages and the functions of the reticuloendothelial system.

In the present paper, we investigated the effect of traxanox sodium on phagocytosis of yeast particles by leukocytes or macrophages in vitro and in vivo. In addition, in mice pretreated with carrageenan, which inhibits the macrophage function (6–8), and ethyl palmitate, which has been shown to be cytotoxic to macrophages (9), the effect of this agent on the suppression of carbon clearance was examined.

Materials and Methods

Animals: Male ICR mice (5–6 weeks of age, Charles River Laboratories, Kanagawa, Japan) and male Wistar rats (250–300 g, Seiwa Institute of Experimental Animals, Fukuoka, Japan) were used. Animals were housed at 23±2°C and 50±5% humidity, and they were allowed free access to food and water.

Compounds: Traxanox sodium pentahydrate (traxanox sodium, Yoshitomi Pharmaceutical Industries), prednisolone (Sigma), isoproterenol hydrochloride (Sigma), theo-
phylline (Sigma), levamisole hydrochloride (Aldrich), carrageenan (Seakem 402), lipo-polysaccharide (E. coli 0111:B4, Difco) and ethyl palmitate (Sigma) were used. Ethyl palmitate was suspended to make a 30% (v/v) suspension by the method of Stuart et al. (10).

For the in vitro experiments, test compounds were dissolved in dimethylsulfoxide (final concentration of 0.1%) and then diluted with Hanks’ solution (pH 7.4). Dimethylsulfoxide alone had no detectable effects on phagocytosis by leukocytes or macrophages. For the in vivo experiments, isoproterenol was dissolved in sterile physiological saline, and the other compounds were dissolved or suspended in 0.5% (w/v) methylcellulose solution prior to use. Doses of traxanox sodium refer to the weight of the non-hydrated sodium salt.

Preparation of peritoneal leukocytes from rats: The procedures were carried out by the previously described method (11).

Preparation of peritoneal macrophages in mice: ICR mice were injected intraperitoneally with 2 ml of 5% casein sodium suspension on day 0. In ex vivo experiments, test compounds were administered orally on days 0, 1 and 2. Peritoneal exudate cells were harvested 3 days after the casein injection. About 75–80% of the total cells was macrophages, and the other cells were lymphocytes. These cells were placed on 35 mm plastic dishes and incubated for 1 hr at 37°C in 95% air–5% CO₂. Following this procedure, the nonadherent cells were removed by rinsing the monolayer with Eagle’s MEM. More than 90% of the adherent cells were macrophages as determined by nonspecific esterase staining. The adherent cells were washed three times and resuspended in physiological saline. The carbon particles were washed three times and resuspended in physiological saline to a concentration of 8 mg/ml. The carbon suspension was injected into the tail vein of ICR mice (0.1 ml/10 g body weight) immediately after the administration of test compounds. In some experiments, carrageenan (50 mg/kg, i.p.) or ethyl palmitate (0.1 ml/10 g body weight, i.v.) was administered 24 hr before the carbon injection. The blood samples were obtained 0.5 and 5 min after the carbon injection by puncturing the retro-orbital venous plexus with a fine capillary glass pipette, and 0.03 ml of the blood was hemolysed by the addition of 3 ml of 0.1% Na₂CO₃ solution, followed by the measurement of the optical density at 600 nm of the hemolysed blood. The phagocytic index, [K], was calculated according to the method of Biozzi et al. (13) from the formula: 

\[ K = \frac{\log C_1 - \log C_2}{200 \mu l} \]

containing mouse serum (diluted two-fold) was added to the reaction mixtures, and they were further incubated at 37°C for the incubation time indicated in the results. After the incubation, the number of phagocytizing cells which ingested one or more particles was counted under the microscope (×400) by staining with basic fuchsin (Nakarai Chemicals). The results were expressed as the percent of phagocytizing cells in the total phagocytes (150–200 cells).

Phagocytosis of yeast particles by leukocytes in vivo: One-half ml of 5% (w/v) yeast suspension was injected intraperitoneally into mice immediately after the treatment with each test solution. Two hours later, 4 ml of Hanks’ solution containing 10 U/ml heparin was injected intraperitoneally and the peritoneal fluids were harvested. Leukocytes in the exudates were counted with a Coulter Counter (model ZB). Protein content exuded into the peritoneal cavity was measured using the Biuret reaction.

Phagocytosis of yeast particles by leukocytes or macrophages in vitro: Phagocytosis of yeast particles by leukocytes or macrophages was measured by the previously described method (12). Briefly, the cell suspension (5×10^6 cells/ml, 200 µl; Sigma Chemical Company) containing mouse serum (diluted two-fold) was added to the reaction mixtures, and they were further incubated at 37°C for the incubation time indicated in the results. After the incubation, the number of phagocytizing cells which ingested one or more particles was counted under the microscope (×400) by staining with basic fuchsin (Nakarai Chemicals). The results were expressed as the percent of phagocytizing cells in the total phagocytes (150–200 cells).

Carbon clearance: The carbon clearance test was carried out according to a slight modification of the method of Biozzi et al. (13). Namely, the carbon particles (Pelikan ink, C11/143a, Gunter Wagner, Hanover, Germany) were centrifuged at 2,000 g for 15 min, and the pellets were suspended in physiological saline. The carbon particles were washed three times and resuspended in physiological saline to a concentration of 8 mg/ml. The carbon suspension was injected into the tail vein of ICR mice (0.1 ml/10 g body weight) immediately after the administration of test compounds. In some experiments, carrageenan (50 mg/kg, i.p.) or ethyl palmitate (0.1 ml/10 g body weight, i.v.) was administered 24 hr before the carbon injection. The blood samples were obtained 0.5 and 5 min after the carbon injection by puncturing the retro-orbital venous plexus with a fine capillary glass pipette, and 0.03 ml of the blood was hemolysed by the addition of 3 ml of 0.1% Na₂CO₃ solution, followed by the measurement of the optical density at 600 nm of the hemolysed blood. The phagocytic index, [K], was calculated according to the method of Biozzi et al. (13) from the formula: 

\[ K = \frac{\log C_1 - \log C_2}{200 \mu l} \]
(T₂−T₁), where C₁ and C₂ are the carbon concentrations at time T₁ (0.5 min) and T₂ (5 min), respectively.

**Statistical analyses:** Results are shown as the mean±S.E. Statistical evaluation was performed by one-way analysis.

**Results**

**Effect of traxanox sodium on phagocytosis of yeast particles by leukocytes or macrophages in vitro:** The time course of phagocytosis of yeast particles by mouse peritoneal macrophages or rat peritoneal leukocytes and phagocytosis at various ratios of phagocytes to yeast particles are shown in Fig. 1. When the ratio of phagocytes to yeast particles was 1:10, the percent of phagocytizing cells to total phagocytes increased in a time-dependent manner during a 30-min incubation. When the ratio of the leukocytes to yeast particles was 1:200, the phagocytosis of yeast particles by leukocytes was time-dependent during a 10-min incubation. When the ratio of phagocytes to yeast particles was 1:10, traxanox sodium (100 μM) more strongly enhanced the phagocytosis of yeast particles by macrophages or leukocytes during a 20-min incubation. Therefore, the effects of test compounds on phagocytosis of yeast particles by phagocytes were assessed under the above experimental condition.

Phagocytosis by leukocytes was significantly enhanced by the treatment with traxanox sodium (30–100 μM) and levamisole (1–10 μM). Prednisolone (100 μM) significantly inhibited phagocytosis by leukocytes (Table 1). Traxanox sodium (30–100 μM) and levamisole (30–100 μM) also enhanced phagocytosis by macrophages as shown in Fig. 1. On the other hand, isoproterenol, theophylline and prednisolone inhibited phagocytosis by macrophages at 30–100 μM, 100 μM and 100 μM, respectively.

**Effect of traxanox sodium on phagocytosis of yeast particles by leukocytes in vivo:** As shown in Table 3, traxanox sodium (100 mg/kg, p.o.) enhanced leukocyte emigration into the peritoneal cavity, and at 30–100 mg/kg, p.o., significantly enhanced phagocytosis by leukocytes. Theophylline, isoproterenol and prednisolone at doses of 100 (p.o.), 1.0 (s.c.) and 30 mg/kg (p.o.), respectively, inhibited or showed a tendency to inhibit the protein

![Fig. 1](image-url)
exudation and phagocytosis by leukocytes.

Traxanox sodium (30 mg/kg, p.o.) prevented or had a tendency to prevent the suppression of the protein exudation and phagocytosis by isoproterenol (1 mg/kg, s.c.) or theophylline (100 mg/kg, p.o.). Combined with theophylline, isoproterenol synergistically inhibited the protein exudation and phagocytosis by leukocytes. As shown in Table 4, traxanox sodium prevented this synergistic suppression of phagocytosis by the leukocytes, but not the suppression of the protein exudation by isoproterenol and theophylline administered simultaneously.

### Table 1. Effect of traxanox sodium on phagocytosis of yeast by rat peritoneal leukocytes in vitro

| Compound       | Conc (μM) | Phagocytosis (%) | % of control |
|----------------|-----------|------------------|-------------|
| Traxanox sodium| 0         | 26.3±1.5         | 100         |
|                | 10        | 32.4±1.2         | 123         |
|                | 0         | 28.6±1.9         | 100         |
|                | 30        | 40.0±2.4**       | 142         |
|                | 100       | 44.2±0.8**       | 158         |
| Levamisole-HCl | 0         | 34.2±1.2         | 100         |
|                | 1         | 46.2±0.6**       | 134         |
|                | 10        | 56.0±0.9**       | 163         |
| Prednisolone   | 0         | 28.6±1.9         | 100         |
|                | 100       | 14.9±2.3**       | 49          |

After the cell suspension (5×10⁶ cells/ml, 200 μl) was preincubated with each test solution (40 μl) at 37°C for 10 min, the incubation mixtures containing mouse serum (100 μl) and yeast suspension (1×10⁶ particles/ml, 100 μl) were further incubated for 20 min. Results are given as the mean±S.E. (N=3–5). **P<0.01, significantly different from the control group.

### Table 2. Effect of traxanox sodium on phagocytosis of yeast by mouse peritoneal macrophages in vitro

| Compound       | Conc (μM) | Phagocytosis (%) | % of control |
|----------------|-----------|------------------|-------------|
| Traxanox sodium| 0         | 32.0±0.8         | 100         |
|                | 10        | 37.2±2.3         | 116         |
|                | 30        | 52.7±2.7**       | 165         |
|                | 100       | 60.6±3.4**       | 189         |
| Levamisole-HCl | 0         | 32.0±0.8         | 100         |
|                | 10        | 39.2±3.6         | 123         |
|                | 30        | 53.6±3.0**       | 168         |
|                | 100       | 62.6±2.5**       | 196         |
| Isoproterenol-HCl| 0       | 33.1±1.6         | 100         |
|                | 10        | 27.2±1.9         | 82          |
|                | 30        | 17.3±2.9**       | 52          |
|                | 100       | 10.3±0.8**       | 31          |
| Theophylline    | 0         | 33.5±1.6         | 100         |
|                | 30        | 26.9±1.4         | 80          |
|                | 100       | 13.7±1.2**       | 44          |
| Prednisolone   | 0         | 33.9±0.8         | 100         |
|                | 100       | 20.7±2.7**       | 61          |

After the cell suspension (5×10⁶ cells/ml, 200 μl) was preincubated with each test solution (40 μl) at 37°C for 10 min, the incubation mixtures containing mouse serum (100 μl) and yeast suspension (1×10⁶ particles/ml, 100 μl) were incubated for 20 min. Results are given as the mean±S.E. (N=3–5). **P<0.01, significantly different from the control group.
This compound prevented the suppression of phagocytosis by the treatment with prednisolone (30 mg/kg, p.o.).

**Effect of traxanox sodium on phagocytosis**

Table 3. Effect of traxanox sodium, theophylline, isoproterenol and prednisolone on yeast-induced peritonitis of mice in vivo

| Compound          | Dose (mg/kg p.o.) | No. of mice | Leukocyte counts ($\times 10^9$) | Protein contents (mg) | Phagocytosis of yeast (%) |
|-------------------|-------------------|-------------|----------------------------------|-----------------------|----------------------------|
| Traxanox sodium   | 0                 | 20          | 303±25 (100%)                    | 12.8±0.9 (100%)       | 19.8±1.4 (100%)            |
|                   | 10                | 20          | 314±28 (104)                     | 14.4±1.2 (113)        | 20.1±2.1 (102)             |
|                   | 30                | 20          | 249±27 (82)                      | 16.7±1.5 (130)        | 27.3±1.5 (138)**           |
|                   | 100               | 20          | 441±20 (146)**                   | 15.9±1.3 (124)        | 25.9±1.4 (131)**           |
| Theophylline      | 0                 | 10          | 240±25 (100)                     | 12.8±0.6 (100)        | 10.9±0.5 (100)             |
|                   | 30                | 10          | 225±15 (94)                      | 11.0±0.4 (86)         | 10.1±0.7 (93)              |
|                   | 100               | 10          | 230±15 (96)                      | 8.9±0.3 (70)**        | 8.1±0.9 (74)               |
| Isoproterenol-HCl | 0                 | 10          | 240±25 (100)                     | 12.8±0.6 (100)        | 10.9±0.5 (100)             |
|                   | 0.3a              | 10          | 290±25 (121)                     | 10.7±0.3 (84)         | 8.1±0.6 (74)               |
|                   | 1.0a              | 10          | 255±20 (106)                     | 9.3±0.3 (73)**        | 6.7±0.4 (61)**             |
| Prednisolone      | 0                 | 20          | 255±21 (100)                     | 12.4±0.8 (100)        | 17.3±1.6 (100)             |
|                   | 10                | 10          | 370±40 (145)                     | 11.8±0.9 (95)         | 13.8±1.2 (80)              |
|                   | 30                | 19          | 236±14 (93)                      | 9.0±0.5 (73)**        | 10.9±0.7 (63)**            |

*subcutaneous treatment. Yeast was injected intraperitoneally into mice immediately after the treatment with each test solution, and the peritoneal fluids were harvested 2 hr thereafter. Results are given as the mean±S.E. Figures in parentheses show percent of the control. **P<0.01, significantly different from the control group.

Table 4. Effect of traxanox sodium on yeast-induced peritonitis of mice treated with theophylline, isoproterenol and prednisolone

| Compound                  | Dose (mg/kg p.o.) | Leukocyte counts ($\times 10^9$) | Protein contents (mg) | Phagocytosis of yeast (%) |
|---------------------------|-------------------|----------------------------------|-----------------------|----------------------------|
| Control                   | 0                 | 250±16 (100%)                    | 13.7±0.6 (100%)       | 15.8±1.4 (100%)            |
| Isoproterenol-HCl         | 1a                | 221±14 (88)                      | 9.2±0.3 (67)**        | 10.0±0.6 (63)**            |
| +Traxanox sodium          | 30                | 280±18 (112)                     | 11.6±0.6 (85)†        | 17.8±1.5 (113)††           |
| Theophylline (TP)         | 100               | 227±21 (91)                      | 9.5±0.4 (69)**        | 8.8±0.6 (56)**             |
| +Traxanox sodium          | 30                | 226±26 (90)                      | 10.3±0.4 (75)         | 20.7±1.2 (131)††           |
| Control                   | 0                 | 260±15 (100)                     | 11.0±0.5 (100)        | 14.0±0.6 (100)             |
| Isoproterenol-HCl         | 1a                | 250±25 (96)                      | 9.4±0.4 (85)          | 9.8±0.7 (70)**             |
| +TP                       | 100               | 275±20 (106)                     | 6.1±0.5 (55)††        | 5.0±0.4 (36)††             |
| +TP+Traxanox sodium       | 3                 | 280±21 (108)                     | 7.1±0.4 (65)**        | 6.6±0.4 (47)**             |
|                           | 30                | 275±30 (106)                     | 5.7±0.3 (52)          | 9.8±0.3 (70)††             |
| Control                   | 0                 | 250±16 (100)                     | 13.7±0.6 (100)        | 15.8±1.4 (100)             |
| Prednisolone              | 30                | 236±14 (94)                      | 8.8±0.4 (64)**        | 8.4±0.5 (61)**             |
| +Traxanox sodium          | 30                | 201±14 (80)                      | 9.3±0.3 (68)**        | 12.4±0.6 (78)††            |

*subcutaneous treatment. ICR mice were injected intraperitoneally with 0.5 ml of 5% yeast suspension immediately after the treatment with each test solution, and the peritoneal fluids were harvested 2 hr thereafter. Results are given as the mean±S.E. Figures in parentheses show percent of the control. **P<0.01, significantly different from the control group. ††P<0.01, significantly different from the isoproterenol, TP or prednisolone group.
of yeast particles by macrophages ex vivo: As shown in Table 5, traxanox sodium (30–100 mg/kg, p.o.) enhanced phagocytosis by macrophages ex vivo. This compound, however, had no effect on macrophage emigration into the peritoneal cavity. Prednisolone (30 mg/kg, p.o.) significantly inhibited both macrophage emigration and phagocytosis by macrophages. Traxanox sodium (3–30 mg/kg, p.o.) significantly prevented the prednisolone-induced suppression of phagocytosis by macrophages, but showed no effect on macrophage emigration.

Effect of traxanox sodium on carbon clearance: Traxanox sodium (100 mg/kg, p.o.) given 24 hr before the carbon injection did not increase the phagocytic index in the mice. Lipopolysaccharide (20 μg/mouse, i.v.), however, increased significantly the phagocytic index. On the other hand, dexamethasone (10 mg/kg, p.o.) significantly decreased the phagocytic index (Table 6). Carrageenan (50 mg/kg, i.p.) given 24 hr before the carbon injection decreased the

| Table 5. Effect of traxanox sodium on phagocytosis of yeast by mouse peritoneal macrophages ex vivo |
|---------------------------------------------------------------|
| Compound             | Dose (mg/kg) | Macrophage counts (×10⁶) | Phagocytosis of yeast (%) |
|----------------------|--------------|--------------------------|----------------------------|
| Control              | 0            | 55±6 (100%)              | 19.4±1.2 (100%)            |
| Traxanox sodium      | 30           | 48±3 (87)                | 30.4±2.8 (157)**           |
|                      | 100          | 59±3 (107)               | 30.0±3.0 (155)**           |
| Control              | 0            | 66±7 (100)               | 27.5±0.8 (100)             |
| Prednisolone         | 30           | 21±2 (32)**              | 15.0±0.4 (55)**            |
| + Traxanox sodium    | 3            | 22±2 (33)**              | 20.3±0.8 (74)††            |
|                      | 30           | 29±5 (44)**              | 21.6±0.8 (79)††            |

ICR mice were injected intraperitoneally with 2 ml of 5% casein suspension (on day 0). Test compounds were administered orally on days 0, 1 and 2. The peritoneal fluids were harvested 3 days after casein injection. Results are given as the mean±S.E. (N=10). Figures in parentheses show percent of the control. **P<0.01, significantly different from the control group. ††P<0.01, significantly different from the prednisolone group.

| Table 6. Effect of traxanox sodium on carbon clearance in mice in vivo |
|---------------------------------------------------------------|
| Time (hr) before carbon injection | Compound                  | No. of mice | Dose (mg/kg) | Phagocytic index (K)×10⁻¹ |
|-----------------------------------|---------------------------|-------------|--------------|--------------------------|
|                                  |                           |             |              |                          |
| 1                                 | Control                   | 6           | 0            | 0.568±0.051              |
|                                  | Traxanox sodium           | 7           | 30           | 0.683±0.041              |
|                                  | Levamisole HCl            | 8           | 10           | 0.622±0.040              |
|                                  | Control                   | 15          | 0            | 0.434±0.033              |
|                                  | Traxanox sodium           | 6           | 10           | 0.433±0.040              |
|                                  |                           | 6           | 100          | 0.498±0.036              |
|                                  | Levamisole HCl            | 5           | 10           | 0.457±0.045              |
|                                  | LPS                       | 6           | 20 (i.v.)    | 1.080±0.105             |
|                                  | Control                   | 13          | 0            | 0.628±0.038              |
|                                  | Dexamethasone             | 20          | 10           | 0.505±0.016**            |
|                                  | Control                   | 9           | 0            | 0.645±0.033              |
|                                  | Traxanox sodium           | 8           | 30           | 0.706±0.042              |
|                                  | Levamisole HCl            | 9           | 10           | 0.713±0.038              |

ICR mice were injected intravenously with carbon particles. Test compounds were administered orally or intravenously at the various time before the carbon injection as shown in the table. Results are given as the mean±S.E. **P<0.01, significantly different from the control group.
phagocytic index. As shown in Fig. 2, traxanox sodium (1–30 mg/kg, p.o.) given simultaneously with carrageenan prevented the carrageenan-induced reduction of the phagocytic index. Levamisole (10 mg/kg, p.o.), however, did not affect the decrease in phagocytic index in this experimental condition. Ethylpalmitate (0.1 ml/10 g body weight, i.v.) given 24 hr before carbon injection decreased the phagocytic index. As shown in Fig. 3, traxanox sodium (3–30 mg/kg, p.o.) and levamisole (10 mg/kg, p.o.) did not have any effect on the phagocytic index.

Discussion

It has been reported that levamisole, which has been used as an immunomodulator (14), enhances phagocytosis by leukocytes (15–17). In the present study, levamisole was confirmed to enhance the phagocytosis of yeast particles by leukocytes and macrophages in vitro. As traxanox sodium has immunomodulating activity (3–5), the effect of this compound on phagocytosis was tested.
In this experimental system, traxanox sodium has a potentiating effect on phagocytosis by leukocytes and macrophages.

In addition, traxanox sodium has anti-asthmatic activity (1, 2). In adult chronic asthmatics, prednisolone can decrease the airway resistance and theophylline and isoproterenol can improve the arterial oxygen tension (18, 19). Furthermore, theophylline and isoproterenol were confirmed to inhibit the phagocytic activity of leukocytes and macrophages at concentrations that were almost the same as those used in the increment of intracellular cyclic adenosine 3',5'-monophosphate (cyclic AMP) content (20, 21). Cyclic AMP and its analogs have been reported to inhibit phagocytosis (20, 21), and anti-allergic agents elevated the intracellular cyclic AMP contents of human neutrophils (22). However, the suppression of phagocytic activity of neutrophils in bronchial asthma is not evident. Recently, the increment of intracellular cyclic AMP contents by amlexanox, which is the anti-allergic agent, can be observed in mast cells, but not in macrophages (22). This finding suggests that the increment of intracellular cyclic nucleotides by drugs does not always occur in all types of cells. Furthermore, since it has been known that theophylline and isoproterenol reduce the blood pressure in high doses (23), the suppression of protein exudation may be mainly attributable to the depression of blood pressure.

In the present study, traxanox sodium prevented the suppression of the phagocytic activity of leukocytes and macrophages treated with isoproterenol, theophylline and prednisolone. Recently, methylxanthines have been shown to competitively antagonize adenosine, a biological modulator (24). This antagonism, unlike that of phosphodiesterase, occurs at the clinically effective doses (24). It is not known if adenosine antagonism relates to the blocking of phagocytosis or mediator release. In addition, glucocorticoids have been reported to inhibit neutrophil functions such as phagocytosis and superoxide production (25, 26) and have been reported to prevent the accumulation of neutrophils at sites of inflammation (19). In this report, prednisolone was confirmed to inhibit protein exudation and macrophage accumulation; and at the same dose, prednisolone inhibited phagocytosis by leukocytes and macrophages. The inhibitory effect of prednisolone may be explained as a direct action on cell membranes (26, 27). The mode of action by which traxanox sodium prevents drug-induced suppression of phagocytosis is unclear, but it is supposed that this compound influences phagocyte functions via many cell membrane receptors.

The results obtained in this study suggest the possibility that traxanox sodium enhances the phagocytic activity not only of peritoneal leukocytes and macrophages but also of Kupffer cells or spleen macrophages. As the carbon clearance test is useful for evaluating the functions of Kupffer cells and spleen macrophages (13), the effect of traxanox sodium on carbon clearance was tested.

Carrageenan has been shown to impair the activity of mononuclear phagocytes in vivo (7-9). In this experiment, carrageenan showed inhibitory effects on carbon clearance. In addition, dexamethasone was also effective in inhibiting carbon clearance. On the other hand, lipopolysaccharide, which is one of many stimulators of the reticuloendothelial system (28, 29), has a potentiating effect on carbon clearance. Therefore this experimental method was confirmed to be useful for estimating the inhibitory or potentiating effect of drugs on the reticuloendothelial system.

Traxanox sodium has no effect on carbon clearance in normal mice but can prevent the carrageenan-induced suppression of carbon clearance. This suggests that traxanox sodium directly affects the phagocytic activity of Kupffer cells and spleen macrophages and has a mode of action different from that of lipopolysaccharide on the reticuloendothelial system. Levamisole showed no preventive effects in the above systems. Okubo et al. (30) have reported that repeated treatments of levamisole could restore the phagocytic activity in tumor-bearing mice. This discrepancy in the effect of levamisole, observed in different laboratories, may depend on the differences in the administration schedule and other experimental conditions.

Di Luzio et al. (31) have shown that ethyl
Palmitate can decrease the phagocytic activity of the reticuloendothelial system because of the death of macrophages. Traxanox sodium and levamisole showed no effect on the suppression of carbon clearance by the treatment with ethyl palmitate. These findings suggest that traxanox sodium has no effect on the viability of macrophages, but it has a preventive effect on macrophage functions. On the other hand, the impaired granulocyte superoxide production in chronic granulomatous disease has been often induced by opportunist infections (32–34). This preventing effect of traxanox sodium on the inhibition of the phagocytic activity of leukocytes and macrophages, one aspect of phagocyte functions, may be of value in the clinical application of this drug.

In conclusion, this study shows that traxanox sodium can potentiate the phagocytic activity of leukocytes and macrophages and prevent the drug-induced suppression of the phagocytic functions.

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