

a standard solution for investigating the inhibitory action of the extract of the present invention to the nitrogen monoxide production and an example thereof is shown in FIG. 2.

As shown in FIG. 2, production of nitrogen monoxide significantly increased in the presence of LPS at doses higher than 0.1  $\mu\text{g/ml}$  and the extract of the present invention showed substantial inhibitory action to said nitrogen monoxide production induced by LPS.

Further, the extract of the present invention (which was administered simultaneously with the administration of LPS) showed a suppressing action to an increase of NADPH-diaphorase-positive substance and an inducible nitrogen monoxide synthetase in the lung, liver and kidney of LPS-treated mice. Accordingly, it was hereby concluded that the inhibitory action of the extract of the present invention to nitrogen monoxide production was due to inhibition of an expression of the inducible nitrogen monoxide synthetase.

### (3) SUPPRESSING ACTION TO LPS-INDUCED LETHAL TOXICITY

A solution of an extract of the present invention or a physiological saline solution buffered with phosphate was injected intraperitoneally to male Std;ddy mice (25–30 g) together with LPS (0.75 mg/mouse). The extract was a commercially available drug preparation sold, under the trade name Neurotropin, in Japan by Nippon Zoki Pharmaceutical Co., Osaka, Japan. The drug preparation contains non-protein active substances extracted and isolated from inflammatory rabbit skin inoculated with vaccinia virus. As discussed above, it is described at page 1,434 of “Drugs in Japan, Ethical Drugs,” published in August of 1994; edited by Japan Pharmaceutical Information Center; published by Yakugyo Jiho Co., Ltd., and by Y. Takeoka et al, *Int. J Immunotherapy*, XI(2), pp. 49–56 (1995), said descriptions being incorporated herein by reference. After the initial injection, a solution of the extract of the present invention (40 units/kg) or a phosphate-buffered physiological saline solution (control) was injected every 12 hours for five days. The mortality of mice were monitored twice a day for five days. An example of the result is shown in FIG. 3.

As shown in FIG. 3, survival rate of the mice injected with LPS decreased with a lapse of time. However, in the group to which the extract from inflammatory rabbit skin inoculated with vaccinia virus (the drug, Neurotropin) was administered in accordance with the present invention, a substantial suppressing action to LPS-induced lethal toxicity was observed. Thus, as in the test upon the incubated endothelial cell line shown in FIG. 1, the substantial suppressing action of the extract of the present invention to LPS-induced lethal toxicity was confirmed.

### (4) IMPROVING ACTION TO LPS-INDUCED HYPOTENSION

Male Fischer rats (230–250 g) were anesthetized by an intraperitoneal injection of pentobarbital (40 mg/kg) and catheters were inserted into an artery for measurement of blood pressure and heart rate, and also for administration of the extract of the present invention. After blood pressure and heart rate stabilized, the extract of the present invention (60 mg/kg) or a physiological saline solution (control) was injected intravenously. After 30 minutes, LPS (15 mg/kg) was injected and blood pressure was measured every ten minutes for three hours. Average arterial blood pressure of the rats exposed to LPS decreased after about 40 minutes from the administration of LPS and, after 1.5–2.5 hours, a decrease of about 20% was noted. On the other hand, however, in the group to which a drug preparation of an extract from inflammatory rabbit skin inoculated with vac-

cinia virus was injected (Neurotropin, described above), no hypotension took place, but a slight (around 5%) hypertension was observed.

It is clear from the above-mentioned results of the pharmacological tests that an extract from inflammatory tissue inoculated with vaccinia virus which is an effective component of the pharmaceutical composition of the present invention shows an excellent preventive action to the death of cells or animals induced by endotoxin (lipopolysaccharide: LPS). The results also show a substantial inhibitory action toward excessive production of nitrogen monoxide induced by endotoxin. A sudden hypotension upon endotoxin shock is due to an excessive production of nitrogen monoxide of the vascular endothelial cells induced by endotoxin. In the above-described pharmacological test (test 4) on LPS-induced hypotension, the extract of the present invention having an inhibitory action to nitrogen monoxide production showed a preferred action of maintaining normal blood pressure against hypotension after administration of LPS.

The above-described pharmacological test results showing a suppressing action to endotoxin-induced death (test 3), an inhibitory action to nitrogen monoxide production (test 2), and an improving action to LPS-induced hypotension (test 4), are exemplary of test results obtained with various extracts from inflammatory tissues inoculated with vaccinia virus. Thus, such actions were also noted not only for the commercially available extract from inflammatory rabbit skin inoculated with vaccinia virus (Neurotropin) but also for: 1) the extracts described in the examples of Japanese Examined Patent Publication (JP) Sho-63/039,572 B, 2) the extracts described in the examples of JP Sho-63/025,600 B, and 3) the extracts described in the examples of JP Hei-03/043,279 B as well as by various other extracts from inflammatory tissue inoculated with vaccinia virus. The examples set forth in the examples of said Japanese Patent publications are herein incorporated by reference.

In sepsis and other serious bacterial infectious diseases, endotoxin (an intracellular toxin) is produced and a shock symptom is induced by its action. Accordingly, the extracts of the present invention having an excellent inhibitory action toward endotoxin-induced toxicity as mentioned above, is quite useful for the treatment or the prevention of endotoxin-induced shock symptoms, sepsis and various symptoms accompanied thereby. In addition, the extracts of the present invention have an inhibitory action toward abnormal nitrogen monoxide production during the diseased state and, therefore, are also useful as a therapeutic and preventive agent to diseases wherein an excessive nitrogen monoxide production occurs, such as acute hypotension.

We claim:

1. A method for the treatment of sepsis comprising administering to a patient in need of such treatment a pharmaceutically effective amount of an extract from inflammatory tissue inoculated with vaccinia virus.

2. A method for inhibiting endotoxin production in a patient afflicted with an endotoxin-producing disease comprising administering to said patient a pharmaceutically effective amount of an extract from inflammatory tissue inoculated with vaccinia virus.

3. A method for treating endotoxin shock comprising administering to a patient in need of such treatment a pharmaceutically effective amount of an extract from inflammatory tissue inoculated with vaccinia virus.

4. A method for the treatment of sepsis as claimed in claim 1 wherein the inflammatory tissue is a skin tissue.

5. A method for the treatment of sepsis as claimed in claim 4 wherein the inflammatory tissue is a skin tissue of a mammal.