Office de la Propriété Intellectuelle du Canada
Un organisme d'Industrie Canada

Canadian Intellectual Property Office
An agency of Industry Canada

CA 2303343 C 2011/01/04

(11)(21) 2 303 343

(12) BREVET CANADIEN
CANADIAN PATENT

(13) C

(86) Date de dépôt PCT/PCT Filing Date: 1998/09/16
(87) Date publication PCT/PCT Publication Date: 1999/03/25
(45) Date de délivrance/Issue Date: 2011/01/04
(85) Entrée phase nationale/National Entry: 2000/03/15
(86) N° demande PCT/PCT Application No.: JP 1998/004164
(87) N° publication PCT/PCT Publication No.: 1999/013880
(30) Priorité/Priority: 1997/09/16 (JP9/250966)

(51) Cl.Int./Int.Cl. *A61K 31/5585* (2006.01),
C07D 307/93 (2006.01)

(72) Inventeurs/Inventors:
KURUMATANI, HAJIMU, JP;
SASAKI, RIE, JP;
KUMAGAI, HIROKI, JP

(73) Propriétaire/Owner:
TORAY INDUSTRIES, INC., JP

(74) Agent: SMART & BIGGAR

(54) Titre : INHIBITEUR DE LA PRODUCTION DE CHEMOKINE C-C
(54) Title: C-C CHEMOKINE PRODUCTION INHIBITOR

(57) Abrégé/Abstract:
The present invention relates to a C-C chemokine production inhibitor containing a prostanoic acid derivative as an active component, and a method of inhibiting C-C chemokine production by using the inhibitor. Medicines of the present invention are effective to cure circulatory diseases, inflammation, allergic diseases, renal diseases, etc.

ABSTRACT

The present invention relates to a C-C chemokine production inhibitor containing a prostanoic acid derivative as an active component, and a method of inhibiting C-C chemokine production by using the inhibitor. Medicines of the present invention are effective to cure circulatory diseases, inflammation, allergic diseases, renal diseases, etc.

76199-155

DESCRIPTION

C-C CHEMOKINE PRODUCTION INHIBITOR

Technical Field

The present invention relates to a C-C chemokine production inhibitor comprising a prostanoic acid derivative as an active component.

Background Art

In 1987, Matsushima et al. isolated IL-8 as a neutrophilic chemotactic factor from a culture supernatant of human peripheral blood monocytes stimulated by a lipopolysaccharide, and then purified and cloned molecules having a migrating activity for many leukocytes. These molecules have a common structure, and are thus generically named "chemokine". Chemokine mainly has high affinity for heparin, and the common property that it is synthesized as a precursor composed of about 100 amino acids, and then secreted in a mature type comprising about 70 amino acids.

Chemokine generally has four cysteine residues, and is roughly classified into C-X-C chemokine comprising an amino acid held between the first two cysteine residues, and C-C chemokine having no amino acid between the cysteine residues. C-X-C chemokine is also called α chemokine, and C-C chemokine is called β chemokine. The C-C chemokine family

is a generic name of a group of low-molecular-weight proteins having about 30% of homology in the amino acid level, and cysteine at the same four positions.

MCP-1 (Monocyte chemoattractant protein-1) is also named a monocyte chemotactic and activating factor (MCAF) or GDCF (glioma-derived monocyte chemotactic factor), and is a protein comprising 76 amino acids and four cysteine residues. The identification and gene cloning of MCAF, MCP-1 or GDCF have been reported (K. Matsushima et al., J. Exp. Med., 169, 1485-1490, 1989, Y. Furutani et al., Biochem. Biophys. Res. Commun., 159, 249-255, 1989, E. R. Robinson et al., Proc. Natl. Acad. Sci. USA, 86, 1850-1854, 1989, T. Yoshimura et al., FEBS Letters, 244, 487-493, 1989). These documents also disclose methods of producing MCP-1. In the present invention, MCP-1 is an abbreviated name and includes GDCF and MCAF hereinafter.

MCP-1 is produced from hemocytic cells such as monocytes, macrophages, and lymphocytes, as well as various cells such as fibroblasts, endothelial cells, smooth muscle cells, various tumor cells, and the like by stimulation with IL-1, TNF, IFN-γ, LPS, phorbol ester (TPA), or the like, and MCP-1 is known to cause accumulation of very strong monocytes and/or macrophages in a pathogenic region. MCP-1 also has a chemotactic action and activating action on basophils and T cells.

Known other proteins belonging to the C-C chemokine include RANTES, LD78, ACT2, I-309, MCP-2, MCP-3, JE, MIP-1α, MIP-1β, TCA-3, eotaxin, and the like.

Of these proteins, MCP-2, MCP-3 (K. B. M. Reid, Immunol. Today 10, 177-180, 1989), RANTES (P. N. Barlow et al., J. Mol. Biol. 232, 268-284, 1993), and JE (B. J. Rollins et al., Proc. Natl. Acad. Sci. USA, 85, 3738-3742, 1988) are known to have the inductive action to cause chemotaxis of monocytes and/or macrophages to a pathogenic region. RANTES also exhibits the strong chemotactic ability for basophils, eosinophils, and T-cells, and is related to chronic rheumatoid arthritis, endarterial hyperplasia after organ transplantation, rejection after organ transplantation, and allergic diseases.

MIP-1α is known to exhibit the chemotactic action on basophils, eosinophils, T-cells, B-cells, and NK-cells, and eotaxin has the strong chemotactic action on eosinophils.

Pathological progress of migration of eosinophils and basophils is frequently observed in acute serious inflammation, chronic intractable inflammation, bronchial asthma, allergic diseases, parasitic diseases, tumors, eosinophilic gastroenteritis, peptic ulcer, vascular diseases, multiple sclerosis, osteoporosis, and organ re-perfusion disorder. Although migration of monocytes and macrophages to a pathogenic region is also observed in general

inflammation, it is observed particularly in acute serious inflammation, chronic intractable inflammation, and allergic diseases, and also observed in nephritis, pneumonocirrhosis, arteriosclerosis, and malignant tumors.

It is known that diabetes highly frequently causes great vessel diseases such as arteriosclerosis, and microangiopathy causing complications such as diabetic neuropathy, diabetic nephropathy, diabetic retinopathy, and the like. However, it is thought to be important for the angiopathy that macrophages are bonded to the endothelial cells and infiltrated into the vessel walls.

It is also known that in lung diseases, macrophages are increased in the lung, and macrophages play an important role for fibrogenesis in the lung. Accumulation of macrophages is also observed in an affected part of chronic rheumatoid arthritis (RA).

Conventionally, a steroidal agent or non-steroidal anti-inflammatory agent is used for the above-described diseases. However, such medicines are known to suppress leukocyte migration, and at the same time, suppress the functions of many types of cells, thereby causing the problem of causing various serious side effects.

Prostaglandin (PG) includes a group of compounds naturally existing, exhibiting a variety of physiological activities, and having a common prostanoic acid skeleton.

76199-155

The natural PG compounds are classified into PGA, PGB, PGC, PGD, PGE, PGF, PGG, PGH, PGI, and PGJ by the structural characteristics of five-member rings, and further classified into sub-classes 1, 2, 3, etc. by the presence of unsaturation and oxidation. Also, many synthetic compounds analogous to these PG compounds are known. Of these PG compounds, a typical PGI derivative PGI_2 is referred to as "prostacyclin" (refer to Nature, Vol. 268, p688, 1976), and is known as a substance having strong platelet aggregation inhibiting action and peripheral vasodilating action. As compounds in which instability of the PGI_2 is significantly improved, Japanese Examined Patent publication Nos. 2-12226, 2-57548 and 1-53672 disclose PGI_2 derivatives having a skeleton in which the structure of an exoenol ether portion, which is a characteristic structure of PGI_2, is converted into an inter-m-phenylene type. Other known compounds in which stability of prostaglandin is improved include ataprost, iloprost, clinprost, ciprostene, naxaprostene, taprostene, cicaprost, pimilprost, CH-169, and CS570 (refer to Gendai-Iryosha, "Generals of Prostaglandin" No. 1, p. 123, 1994; New Drugs of Tomorrow, p. 15-IV-185, 1996; New Drugs of Tomorrow, p. 15-III-551, 1996). However, it is unknown that these prostanoic acid derivatives have the action to inhibit directly C-C chemokine production.

It is an object of the present invention to provide a

preventive and curative medicine for diseases for which conventional medicines are ineffective, and which causes serious side effects and are characterized by abnormal accumulation or activation of leukocytes such as monocytes and/or macrophages, eosinophils, basophils, and the like.

Disclosure of Invention

The present invention provides a C-C chemokine production inhibitor comprising a prostanoic acid derivative as an active component.

Brief Description of the Drawings

Fig. 1 shows the BPS action on MCP-1 production of THP-1 cells stimulated with LPS.

Fig. 2 shows the BPS action on the amount of MCP-1mRNA expression of THP-1 cells stimulated with LPS.

Fig. 3 shows the BPS action on MCP-1 production of human peripheral blood monocytes stimulated with LPS.

Fig. 4 shows the BPS action on MCP-3 production of THP-1 cells stimulated with LPS.

Fig. 5 shows the effect of BPS administration on the neurotransmission rate of streptozotocin-induced diabetic rats.

Fig. 6 shows the actions of various PG compounds on MCP-1 production of THP-1 cells stimulated with LPS.

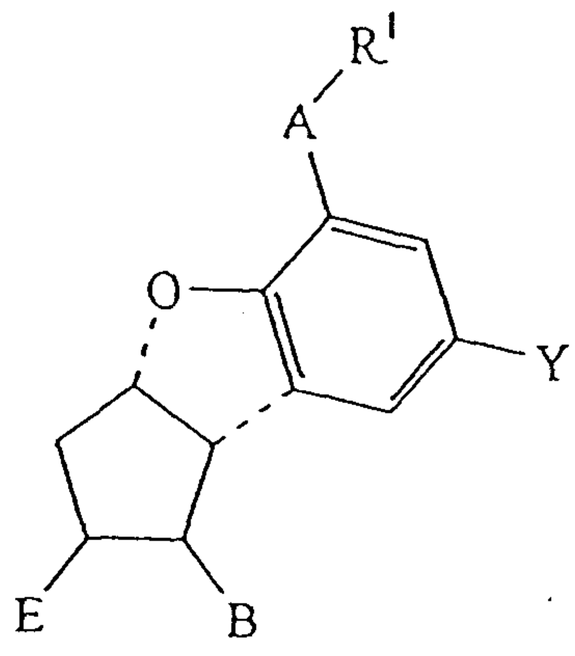
Fig. 7 shows the effect of BPS administration on macrophage infiltration into glomeruli in a glomerulonephritis model (Fig. 7a), and on changes in renal MCP-1 gene expression (Fig. 7b).

Fig. 8 shows the effect of BPS administration on the amount of urinary protein in a glomerulonephritis model

Best Mode for Carrying Out the Invention

As prostanoic acid derivatives of the present invention, derivatives of any type of PGA, PGB, PGC, PGD, PGE, PGF, PGG, PGH, PGI, and PGJ, which have the prostanoic acid skeleton, and derivatives of any of the sub-classes 1, 2, 3, etc., which are classified by the presence of unsaturation and oxidation, may be used. These derivatives include not only natural compounds but also synthetic analogues. Although the basic skeleton of prostanoic acid generally has 20 carbon atoms, the carbon number of prostanoic acid used in the present invention is not limited. PGI derivatives preferably used in the present invention include PGI_1 derivatives, PGI_2 derivatives, PGI_3 derivatives, and salts thereof, but PGI_2 derivatives or salts thereof are preferably used. More preferably, 4,8-inter-m-phenylene prostaglandin I_2 derivatives represented by the following formula (I) or pharmacologically acceptable salts thereof are used.

. 76199-155



(I)

[wherein R^1 represents the following:

(A) $COOR^2$ wherein R^2 is:

1) hydrogen or a pharmacologically acceptable cation;

2) straight chain alkyl having 1 to 12 carbon atoms, or branched alkyl having 3 to 14 carbon atoms;

3) $-Z-R^3$

wherein Z is a valence bond or straight chain or branched alkylene represented by C_tH_{2t} wherein t represents an integer of 1 to 6, and R^3 represents cycloalkyl having 3 to 12 carbon atoms or substituted cycloalkyl having 3 to 12 carbon atoms and 1 to 3 substituents R^4 each of which is hydrogen or alkyl having 1 to 5 carbon atoms;

4) $-(CH_2CH_2O)_nCH_3$

wherein n is an integer of 1 to 5;

5) $-Z-Ar^1$

wherein Z is defined as the same as the above, and Ar^1 is phenyl, α-naphthyl, β-naphthyl, 2-pyridyl, 3-pyridyl, 4-

pyridyl, α-furyl, β-furyl, α-thienyl, β-thienyl or substituted phenyl (wherein a substituent is at least one of chlorine, bromine, fluorine, iodine, trifluoromethyl, alkyl having 1 to 4 carbon atoms, nitro, cyano, methoxy, phenyl, phenoxy, p-acetoamidobenzamide, $-CH=N-NH-C(=O)-NH_2$, $-NH-C(=O)-Ph$, $-NH-C(=O)-CH_3$ or $-NH-C(=O)-NH_2$);

6) $-C_tH_{2t}COOR^4$

wherein C_tH_{2t} and R^4 are defined as the same as the above;

7) $-C_tH_{2t}N(R^4)_2$

wherein C_tH_{2t} and R^4 are defined as the same as the above;

8) $-CH(R^5)-C(=O)-R^6$

wherein R^5 is hydrogen or benzoyl, and R^6 is phenyl, p-bromophenyl, p-chlorophenyl, p-biphenyl, p-nitrophenyl, p-benzamidophenyl, or 2-naphthyl;

9) $-C_pH_{2p}-W-R^7$

wherein W is $-CH=CH-$, $-CH=CR^7-$ or $-C\equiv C-$, and R^7 is hydrogen or straight chain or branched alkyl or aralkyl having 1 to 30 carbon atoms, and p is an integer of 1 to 5; or

10) $-CH(CH_2OR^8)_2$

wherein R^8 is alkyl or acyl having 1 to 30 carbon atoms;

(B) $-CH_2OH$;

(C) $-C(=O)N(R^9)_2$

76199-155

wherein R^9 is hydrogen, straight chain alkyl having 1 to 12 carbon atoms, branched alkyl having 3 to 12 carbon atoms, cycloalkyl having 3 to 12 carbon atoms, cycloalkylalkylene having 4 to 13 carbon atoms, phenyl, substituted phenyl (wherein the substituent is defined as the same as in (A) 5)), aralkyl having 7 to 12 carbon atoms, or $-SO_2R^{10}$ wherein R^{10} is alkyl having 1 to 10 carbon atoms, cycloalkyl having 3 to 12 carbon atoms, phenyl, substituted phenyl (the substituent is defined as the same as in (A) 5)), or aralkyl having 7 to 12 carbon atoms, two R^9 groups may be the same or different, and when one of the R^9 groups is $-SO_2R^{10}$, the other R^9 is not $-SO_2R^{10}$; or

(D) $-CH_2OTHP$ (THP is a tetrahydropyranyl group);

A is the following:

1) $-(CH_2)_m-$;

2) $-CH=CH-CH_2-$;

3) $-CH_2-CH=CH-$;

4) $-CH_2-O-CH_2-$;

5) $-CH=CH-$;

6) $-O-CH_2-$; or

7) $-C\equiv C-$;

wherein m represents an integer of 1 to 3;

Y is hydrogen, alkyl having 1 to 4 carbon atoms, chlorine, bromine, fluorine, formyl, methoxy or nitro;

B is $-X-C(R^{11})(R^{12})OR^{13}$

76199-155

wherein R^{11} is hydrogen, alkyl having 1 to 4 carbon atoms; R^{13} is hydrogen, acyl having 2 to 14 carbon atoms, aroyl having 7 to 15 carbon atoms, tetrahydropyranyl, tetrahydrofuranyl, 1-ethoxythienyl, or t-butyl; X is the following:

1) $-CH_2-CH_2-$;

2) $-CH=CH-$; or

3) $-C\equiv C-$; and

R^{12} is the following:

1) straight chain alkyl having 1 to 12 carbon atoms, or branched alkyl having 3 to 14 carbon atoms;

2) $-Z-Ar^2$

wherein Z is defined as the same as the above, and Ar^2 represents phenyl, α-naphthyl, β-naphthyl, or phenyl substituted by at least one chlorine, bromine, fluorine, iodine, trifluoromethyl, alkyl having 1 to 4 carbon atoms, nitro, cyano, methoxy, phenyl or phenoxy;

3) $-C_tH_{2t}OR^{14}$

wherein C_tH_{2t} is defined as the same as the above, and R^{14} represents straight chain alkyl having 1 to 6 carbon atoms, branched alkyl having 3 to 6 carbon atoms, phenyl, phenyl substituted by at last one chlorine, bromine, fluorine, iodine, trifluoromethyl, alkyl having 1 to 4 carbon atoms, nitro, cyano, methoxy, phenyl or phenoxy, cyclopentyl, cyclohexyl, or cyclopentyl or cyclohexyl substituted by 1 to

4 straight chain alkyl groups having 1 to 4 carbon atoms;

4) $-Z-R^3$

wherein Z and R^3 are defined as the same as the above;

5) $-C_tH_{2t}-CH=C(R^{15})R^{16}$

wherein C_tH_{2t} is defined as the same as the above, and R^{15} and R^{16} each represent hydrogen, methyl, ethyl, propyl, or butyl; or

6) $-C_uH_{2u}-C\equiv C-R^{17}$

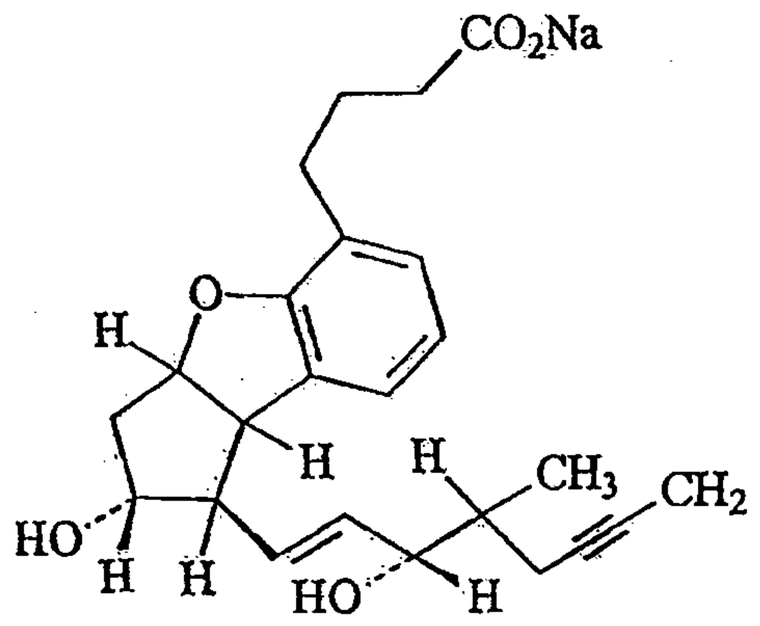
wherein u is an integer of 1 to 7, C_uH_{2u} represents straight chain or branched alkylene, and R^{17} represents straight chain alkyl having 1 to 6 carbon atoms;

E is hydrogen or $-OR^{18}$

wherein R^{18} represents acyl having 1 to 12 carbon atoms, aroyl having 7 to 15 carbon atoms, or R^2 (wherein R^2 is defined as the same as the above); and

the formula represents a d, l or dl form].

Although preferred examples of prostaglandin I derivatives of the present invention include beraprost or salts thereof represented by the following formula (II), ataprost, iroprost, clinprost, ciprostene, naxaprostene, taprostene, cicaprost, pimilprost, CH-169, CS570, and the like, the prostaglandin I derivatives are not limited to these derivatives.



(II)

The prostanoic acid derivatives of the present invention can be produced by known methods. For example, compounds represented by formula (I) or salts thereof can be produced by the method disclosed in Japanese Examined Patent Publication No. 1-53672.

As described above, the prostanoic acid derivatives of the present invention inhibit the production of C-C chemokine, promoters of migration of leukocytes, thus inhibiting chemotaxis to a pathological region.

A typical example of C-C chemokine of the present invention is MCP-1. It has been reported that MCP-1 is produced from hemocytic cells such as monocytes, macrophages, and lymphocytes, as well as various cells such as fibroblasts, endothelial cells, smooth muscle cells, various tumor cells, and the like by stimulation with IL-1, TNF, IFN-γ, LPS, phorbol ester (TPA), or the like. Although

other examples of C-C chemokine include RANTES, LD78, ACT2, I-309, MCP-2, MCP-3, JE, MIP-1α, MIP-1β, TCA-3, eotaxin, and the like. C-C chemokine compounds are not limited to these compounds.

As a result of detailed study of the actions of prostanoic acid derivatives, the inventors found that the compounds have the action to inhibit C-C chemokine production, leading to the achievement of the present invention. In the present invention, curable diseases are not limited as long as the diseases are related to abnormal accumulation of leukocytes, particularly monocytes and/or macrophages, eosinophils, basophils, and lymphocytes, accompanied with abnormal production of C-C chemokine.

Examples of such diseases include circulatory disorders, inflammation, allergic diseases, and renal diseases. More specifically, examples of the diseases include acute serious inflammation, chronic intractable inflammation, nephritis, glomerulonephritis, pyelitis, diabetic nephropathy, pneumonocirrhosis, pneumonia, ARDS, fibroma, ulcerative colitis, chronic rheumatoid arthritis, systemic lupus erythematodes, gout, bronchial asthma, atopic dermatitis, Crohn's disease, osteoarthritis, parasitic disease, eosinophilic gastroenteritis, arteriosclerosis, arterial reocclusion after coronary arterial operation including PTCA, myocardial infarction, malignant carcinoma cutaneum

metastasis, malignant sarcoma metastasis, diabetic microangiophathy, diabetic neuropathy, diabetic retinopathy, diabetic large artery disorder, osteoarthritis.

Any one of the prostanoic acid derivatives of the present invention is administered 1 to 3 times a day in a dose of 0.001 to 1000 mg/adult. Although the C-C chemokine production inhibitor of the present invention may contain at least one prostanoic acid derivative, often it is formulated in a pharmaceutical formulation also containing at least one pharmaceutically acceptable additive. The inhibitor can be orally administered in the form of a solid containing the additives below.

Examples of such additives include an excipient such as starch, lactose, sucrose, glucose, mannitol, calcium carbonate, calcium sulfate, or the like; a binder such as starch, dextrin, gum arabic, tragacanth, methyl cellulose, gelatin, polyvinyl pyrrolidone, polyvinyl alcohol, or the like; a disintegrator such as starch, polyvinyl pyrrolidone, crystalline cellulose, or the like; a lubricant such as magnesium stearate, talc, or the like; a colorant; a flavor; and the like.

The prostanoic acid derivatives of the present invention can be used in various forms. Examples of the forms include conventional forms such as a tablet, a sugar-coated tablet, a powder, granules, a troche, a capsule, a pill, a syrup, a spray, and the like. The derivatives may also be parenterally administered in the form of a

sterilized solution, and another solute such as sodium chloride, glucose, or the like can also be used in an amount sufficient for making the solution isotonic. The C-C chemokine production inhibitor of the present invention can be applied to the above-described oral administration as well as parenteral administration of injections, suppositories, etc.

[Examples]

The present invention will be described in detail below with reference to examples.

Example 1

Action on amount of MCP-1 production of human monocyte/macrophage system cells THP-1:

The action of beraprost sodium (BPS) on MCP-1 production was examined by using human monocyte/macrophage system leukemic cells THP-1. Lipopolysaccharide (LPS: Difco Corp.) reactive substrains were isolated from THP-1 cells (obtained from ATCC Corp.), and cultured in a RPMI1640 medium (Gibco Corp.) containing 10% FCS in a flask. The THP-1 cells (1×10^6 cells) were dispensed to a 12-well plate, and activated with 10 μg/ml of LPS. BPS was added 5 minutes before LPS stimulation. A cell supernatant was obtained 24 hours after stimulation, and the amount of MCP-1 production was measured by using a human MCP-1 ELISA kit (R&D Corp.). The amount of production was calculated based on a calibration curve

formed in the range of 31.2 to 2000 pg/ml by using MCP-1 standards contained in the kit. The results indicate that BPS inhibits dose-dependently the production of monocyte chemotactic factor of the THP-1 cells induced by 10 μg/ml of LPS (Fig. 1).

Example 2

Action on expression of MCP-1 mRNA of human monocyte/macrophage system cells THP-1:

The action of BPS on MCP-1 production was examined by using human monocyte/macrophage system leukemic cells THP-1 and the amount of mRNA expression as an index. LPS reactive substrains were isolated from THP-1 cells, and cultured in a RPMI1640 medium containing 10% FCS in a flask. THP-1 cells (1×10^6 cells) were dispensed to a petri dish having a diameter of 10 cm, and activated with 10 μg/ml of LPS. BPS was added 5 minutes before LPS stimulation. The total of RNA was extracted with a LiCl-urea solution (6M urea/3M LiCl/5mM EDTA/0.1M 2ME) 24 hours after stimulation, and dissolved in a TE solution (10 mM Tris-HCl/1mM EDTA, pH 8.0), and then proteins were removed with phenol and chloroform, followed by RNA recovery by ethanol precipitation. RNA was developed by formaldehyde-modified 1% agarose gel, and transferred to a Hybond-N filter (Amersham Corp.), and then MCP-1 mRNA was detected by using a ^{32}P-labeled human MCP-1 probe. The detection was carried out by using a X-ray film or imaging

film, and the amount of expression was digitized by BAS2000. The results indicate that BPS inhibits dose-dependently expression of MCP-1 mRNA of THP-1 cells stimulated by LPS (Fig. 2).

Example 3

Action on MCP-1 production of human peripheral blood-derived monocytes

The action of BPS on MCP-1 production was studied by using human peripheral blood-derived monocytes. The heparinized peripheral blood of a healthy person was superposed on a Histopaque* (Sigma Corp.), and subjected to a centrifugal operation to obtain a monocyte layer. The thus-obtained monocyte layer was reacted with magnetic beads (Miltenyi Biotec Corp.) of anti-CD3 and anti-CD19, and monocytes were purified by a negative selection method using a MACS* column (Miltenyi Biotec Corp.). The thus-obtained monocytes were re-suspended in a RPMI 1640 medium so that 1 x 10^6 cells/ml of cells were obtained. The cells were dispensed to a 48-well plate, and activated with 10 ng/ml of LPS. BPS was added 5 minutes before LPS stimulation. A cell supernatant was obtained 24 hours after stimulation, and the amount of the monocyte chemotactic factor produced was measured by using a human MCP-1 ELISA kit. The amount of production was calculated based on a calibration curve formed in the range of 31.2 to 2000 pg/ml by using MCP-1

*Trade-mark

standards contained in the kit. The results indicate that BPS inhibits dose-dependently the production of MCP-1 of human peripheral blood monocytes induced by 10 ng/ml of LPS (Fig. 3).

Example 4

Action on production of MCP-3 of human monocyte/macrophage system cells THP-1

The action of BPS on MCP-3 production was examined by using human monocyte/macrophage system leukemic cells THP-1. LPS reactive substrains were isolated from THP-1 cells, and cultured in a RPMI1640 medium containing 10% FCS in a flask. THP-1 cells (1×10^6 cells) were dispensed to a 12-well plate, and activated with 10 μg/ml of LPS. BPS was added 5 minutes before LPS stimulation. A cell supernatant was obtained 24 hours after stimulation, and the amount of the MCP-3 production was measured by using a MCP-3 detection method developed by the inventors. The amount of production was calculated based on a calibration curve formed in the range of 0.195 to 12.5 ng/ml by using MCP-3 standards. The results indicate that BPS inhibits dose-dependently not only MCP-1 production of THP-1 cells but also MCP-3 production induced by 10 μg/ml of LPS (Fig. 4).

Example 5

Effect of BPS administration on MCP-1 amount in LPS-induced blood of diabetic rats

76199-155

SD male rats were intravenously administered with 45 mg/kg streptozotocin to induce diabetes. At the eighth week after induction, 2 mg/kg of LPS was administered to the rats to measure the MCP-1 amount in the blood before administration and 3 and 6 hours after administration. BPS was orally administered to the rats 30 minutes before LPS administration. Rats of the same week old as the diabetic rat group were used as a normal group. The results are shown in Table 1. In the diabetic rats, MCP-1 production was significantly increased by administering LPS, as compared with the normal rats. In the rat group administered with BPS, MCP-1 production was significantly inhibited, thereby indicating in in-vivo experiment that abnormal production of MCP-1 due to diabetes is improved by BPS. As a result of measurement of the neurotransmission rate of the ischiatic nerve by using the same diabetic rats, a decrease in the neurotransmission rate due to diabetes was significantly improved by BPS (Fig. 5).

Table 1 Effect of Oral Administration of BPS on MCP-1 amount in LPS-Induced Blood of Rats Administered with Streptozotocin

Treatment	n	MCP-1 amount in blood (ng/ml)		
		Before LPS administration	3 hours after	6 hours after
Normal group	4	0.30±0.11	7.39±1.22‡‡	3.86±1.44‡
Diabetic group				
Untreated	4	0.30±0.04	13.47±4.40#	9.42±1.59
BPS administered	3	0.39±0.13	7.27±2.55‡‡	4.82±4.40‡

Numerals represent average ± standard deviation

‡: $p < 0.05$, ‡‡: $p < 0.01$ in comparison with the untreated diabetic group (Student's test)

#: The value obtained from 3 samples because of defects in a sample.

Example 6

Action on MCP-1 production of human monocyte/macrophage system cells THP-1

The actions of prostaglandin I_2 (PGI_2), prostaglandin E_1

(PGE_1), and prostaglandin E_2 (PGE_2) on MCP-1 production were examined by the same method as Example 1. As a result, MCP-1 production was inhibited by PGI_2, PGE_1, and PGE_2 (Fig. 6).

Example 7

Action on MCP-1 production of human monocyte/macrophage system cells THP-1

The actions of the compounds shown in the table below on MCP-1 production by the same method as Example 1. The action of each of the compounds was shown by an inhibition rate. As a result, MCP-1 production was inhibited by these compounds (Table 2).

Example 8

Action on MCP-1 production of monocytes derived from human peripheral blood

Monocytes derived from the human peripheral blood were isolated by the same method as Example 3, and stimulated with 25 nM 12-o-tetradecanoylphorbol 13-acetate (TPA) to induce MCP-1 production. The action of BPS on MCP-1 production was studied. As a result, 224.7 pg/1 x 10^6 cells of MCP-1 produced by TPA stimulation was decreased to 184 pg/1 x 10^6 cells by 100 nM BPS, and thus it was confirmed that MCP-1 production due to TPA stimulation is inhibited by BPS.

Example 9

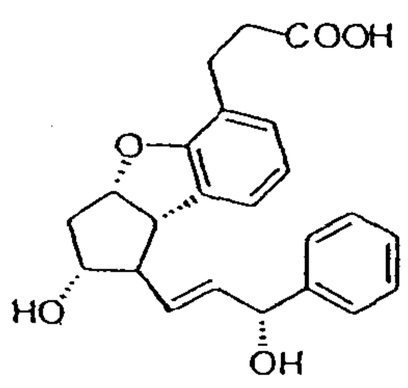
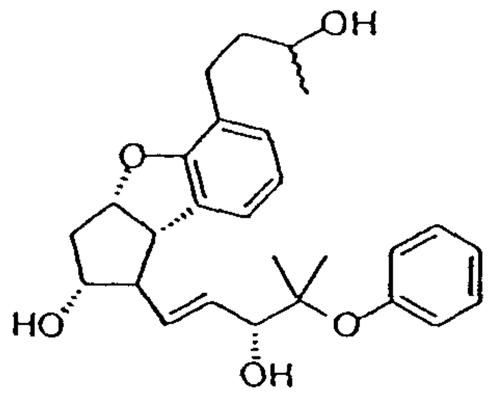
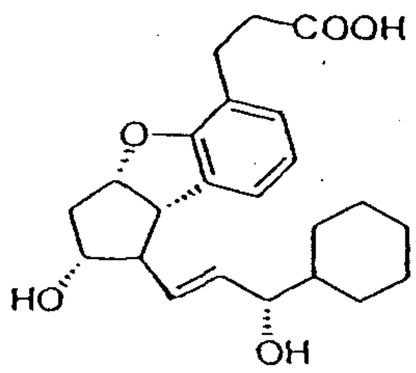
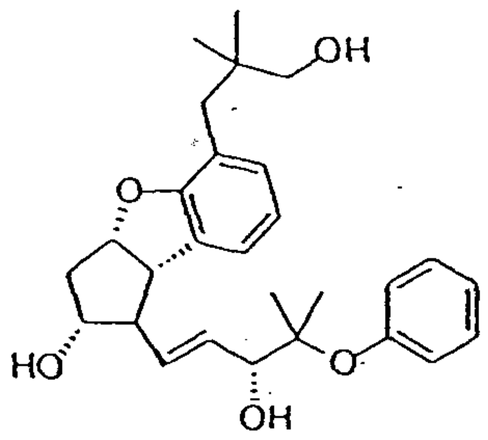
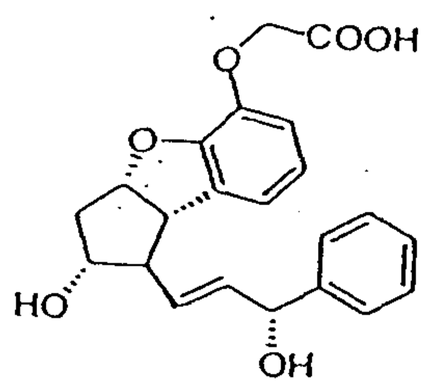
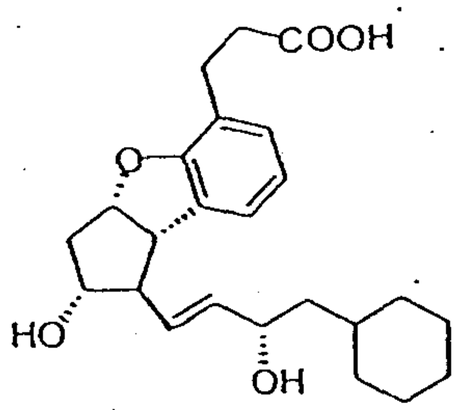
Action on MCP-1 production of monocytes derived from human

peripheral blood

The action of prostaglandin J_2 (PGJ_2) on MCP-1 production due to LPS or TPA simulation was studied by the same method as Examples 3 and 8 using monocytes derived from the human peripheral blood. PGJ_2 was added 1 minute before stimulation. As a result, MCP-1 production due to stimulation by either LPS or TPA was inhibited by PGJ_2 (Table 3).

76199-155

Table 2 Inhibition rates of various PG derivatives to MCP-1 production of THP-1 cells

Structural formula	Inhibition rate (%)	Structural formula	Inhibition rate (%)
	58		4
	85		28
	61		87

76199-155

Table 3 Action of PGJ_2 on MCP-1 production of human peripheral blood monocytes

Treatment	MCP-1 (pg/10^6 cells)
LPS 10 ng/ml	21872
LPS 10 ng/ml + PGJ_2 10 μM	6
TPA 25 nM	225
TPA 25 nM + PGJ_2 10 μM	2

Example 10

Action on RANTES production of human monocyte/macrophage system cells THP-1

THP-1 cells were prepared by the same method as Example 1, and stimulated with LPS to induce RANTES production. The amount of RANTES production was measured by RANTES ELISA kit (R&D Corp.). The action of PGJ_2 on RANTES production was studied. As a result, 5177 pg/5 x 10^5 cells of RANTES produced by LPS stimulation was decreased to 2403 pg/5 x 10^5 cells by 10 μM PGJ_2, and it was thus confirmed that RANTES production due to LPS stimulation is inhibited by PGJ_2.

Example 11

Action on MCP-1 production and macrophage infiltration in kidney in glomerulonephritis rat model:

The antibody to glomerular basement membrane was administered to form a glomerulonephritis rat model. The rats used were male WKY rats of 9-week old which were purchased

from Japan Charles River. The antibody was obtained by immunizing the rat glomerular basement membrane against rabbits. The amount of urinary protein was increased 4 days after administration of the antibody, reached a plateau 11 days after administration, and then did not change up to the death of the rats. A glomerular lesion such as formation of the crescent was also confirmed by pathological findings, and an irreversible glomerulonephritis model could be formed by administering the antibody to the glomerular basement membrane. MCP-1 production and infiltration of leukocytes such as macrophages and the like in the kidney were increased with the passage of days after administration of the antibody. 1 mg/kg of BPS was orally administered on consecutive days to study the action of BPS on MCP-1 production and infiltration of the macrophages. The MCP-1 production was studied by purifying messenger RNA (mRNA) from the rat kidneys, and determining the amount of MCP-1mRNA expression by quantitative PCR. The macrophage infiltration was studied by immunostaining with an anti-macrophage antibody (ED-1), and then counting the macrophages infiltrated into the glomeruli under a microscope. As a result of comparison between a group administered with distilled water and a group administered with BPS 4 days and 7 days after administration of the antibody, in the group administered

with BPS, infiltration of macrophage per glomerulus was significantly inhibited in parallel with inhibition of MCP-1mRNA expression (Figs. 7a and b). Using the same glomerulonephritis model, the action of BPS on the amount of urinary protein was studied 4 days and 7 days after administration of the antibody. As a result, the amount of urinary protein was significantly decreased by BPS both 4 days and 7 days after the administration (Fig. 8).

Industrial Applicability

Prostaglandin derivatives have the action to inhibit C-C chemokine production, and are effective to cure circulatory diseases, inflammation, allergic diseases, renal diseases, etc.

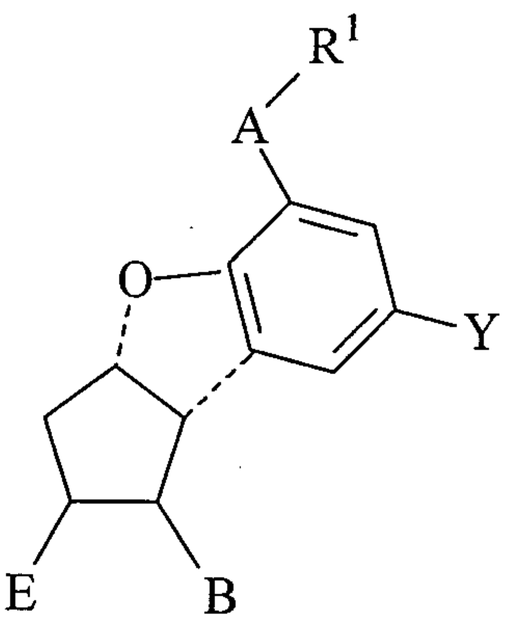
76199-155

CLAIMS:

1. A pharmaceutical formulation for inhibiting C-C chemokine production, comprising:

at least one pharmaceutically acceptable additive; and

a 4,8-inter-m-phenylene prostaglandin I_2 derivative represented by the following formula (I), or a pharmacologically acceptable salt thereof:



(I)

wherein R^1 represents the following:

(A) $COOR^2$ wherein R^2 is:

1) hydrogen or a pharmacologically acceptable cation;

2) straight chain alkyl having 1 to 12 carbon atoms, or branched alkyl having 3 to 14 carbon atoms;

3) $-Z-R^3$

wherein Z is a valence bond or straight chain or branched alkylene represented by C_tH_{2t} wherein t represents an integer of 1 to 6, and R^3 represents cycloalkyl having 3 to 12 carbon atoms or substituted cycloalkyl having 3 to 12 carbon atoms and 1 to 3

substituents R^4 each of which is hydrogen or alkyl having 1 to 5 carbon atoms;

4) $-(CH_2CH_2O)_nCH_3$

wherein n is an integer of 1 to 5;

5) $-Z-Ar^1$

wherein Z is defined as the same as the above, and Ar^1 is phenyl, α-naphthyl, β-naphthyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, α-furyl, β-furyl, α-thienyl, β-thienyl or substituted phenyl (wherein a substituent is at least one of chlorine, fluorine, bromine, iodine, trifluoromethyl, alkyl having 1 to 4 carbon atoms, nitro, cyano, methoxy, phenyl, phenoxy, p-acetoamidobenzamide, $-CH=N-NH-C(=O)-NH_2$, $-NH-C(=O)-Ph$, $-NH-C(=O)-CH_3$ or $-NH-C(=O)-NH_2$);

6) $-C_tH_{2t}COOR^4$

wherein C_tH_{2t} and R^4 are defined as the same as the above;

7) $-C_tH_{2t}N(R^4)_2$

wherein C_tH_{2t} and R^4 are defined as the same as the above;

8) $-CH(R^5)-C(=O)-R^6$

wherein R^5 is hydrogen or benzoyl, and R^6 is phenyl, p-bromophenyl, p-chlorophenyl, p-biphenyl, p-nitrophenyl, p-benzamidophenyl, or 2-naphthyl;

9) $-C_pH_{2p}-W-R^7$

wherein W is $-CH=CH-$, $-CH=CR^7-$ or $-C\equiv C-$, and R^7 is hydrogen or straight chain or branched alkyl or

aralkyl having 1 to 30 carbon atoms, and p is an integer of 1 to 5;

or

10) $-CH(CH_2OR^8)_2$

wherein R^8 is alkyl or acyl having 1 to 30 carbon atoms;

(B) $-CH_2OH$;

(C) $-C(=O)N(R^9)_2$

wherein R^9 is hydrogen, straight chain alkyl having 1 to 12 carbon atoms, branched alkyl having 3 to 12 carbon atoms, cycloalkyl having 3 to 12 carbon atoms, cycloalkylalkylene having 4 to 13 carbon atoms, phenyl, substituted phenyl (wherein the substituent is defined as the same as in (A) 5)), aralkyl having 7 to 12 carbon atoms, or $-SO_2R^{10}$ wherein R^{10} is alkyl having 1 to 10 carbon atoms, cycloalkyl having 3 to 12 carbon atoms, phenyl, substituted phenyl (the substituent is defined as the same as in (A) 5)), or aralkyl having 7 to 12 carbon atoms, two R^9 groups may be the same or different, and when one of the R^9 groups is $-SO_2R^{10}$, the other R^9 is not $-SO_2R^{10}$; or

(D) $-CH_2OTHP$ (THP is a tetrahydropyranyl group);

A is the following:

1) $-(CH_2)m-$;

2) $-CH=CH-CH_2-$;

3) $-CH_2-CH=CH-$;

4) $-CH_2-O-CH_2-$;

5) $-CH=CH-$;

6) $-O-CH_2-$; or

7) $-C\equiv C-$;

wherein m represents an integer of 1 to 3;

Y is hydrogen, alkyl having 1 to 4 carbon atoms, chlorine, bromine, fluorine, formyl, methoxy or nitro;

B is $-X-C(R^{11})(R^{12})OR^{13}$

wherein R^{11} is hydrogen, alkyl having 1 to 4 carbon atoms; R^{13} is hydrogen, acyl having 2 to 14 carbon atoms, aroyl having 7 to 15 carbon atoms, tetrahydropyranyl, tetrahydrofuranyl, 1-ethoxythienyl, or t-butyl; X is the following:

1) $-CH_2-CH_2-$;

2) $-CH=CH-$; or

3) $-C\equiv C-$; and

R^{12} is the following

1) straight chain alkyl having 1 to 12 carbon atoms, or branched alkyl having 3 to 14 carbon atoms;

2) $-Z-Ar^2$

wherein Z is defined as the same as the above, and Ar^2 represents phenyl, α-naphthyl, β-naphthyl, or phenyl substituted by at least one chlorine, bromine,

fluorine, iodine, trifluoromethyl, alkyl having 1 to 4 carbon atoms, nitro, cyano, methoxy, phenyl or phenoxy;

3) $-C_tH_{2t}OR^{14}$

wherein C_tH_{2t} is defined as the same as the above, and R^{14} represents straight chain alkyl having 1 to 6 carbon atoms, branched alkyl having 3 to 6 carbon atoms, phenyl, phenyl substituted by at last one chlorine, bromine, fluorine, iodine, trifluoromethyl, alkyl having 1 to 4 carbon atoms, nitro, cyano, methoxy, phenyl or phenoxy, cyclopentyl, cyclohexyl, or cyclopentyl or cyclohexyl substituted by 1 to 4 straight chain alkyl groups having 1 to 4 carbon atoms;

4) $-Z-R^3$

wherein Z and R^3 are defined as the same as the above;

5) $-C_tH_{2t}-CH=C(R^{15})R^{16}$

wherein C_tH_{2t} is defined as the same as the above, and R^{15} and R^{16} each represent hydrogen, methyl, ethyl, propyl, or butyl; or

6) $-C_uH_{2u}-C\equiv C-R^{17}$

wherein u is an integer of 1 to 7, C_uH_{2u} represents straight chain or branched alkylene, and R^{17} represents straight chain alkyl having 1 to 6 carbon atoms;

E is hydrogen or $-OR^{18}$;

wherein R^{18} represents acyl having 1 to 12 carbon atoms, aroyl having 7 to 15 carbon atoms, or R^2 (wherein R^2 is defined as the same as the above); and

the formula represents d, l or dl form.

2. The pharmaceutical formulation according to claim 1, wherein the 4,8-inter-m-phenylene prostaglandin I_2 derivative is beraprost or a pharmacologically acceptable salt thereof.

3. The pharmaceutical formulation according to claim 1 or 2, wherein C-C chemokine is monocyte chemoatractant protein (MCP-1) or monocyte chemoatractant protein 3 (MCP-3).

4. The pharmaceutical formulation according to any one of claims 1 to 3, which is for inhibition of abnormal accumulation or activation of leukocytes.

5. The pharmaceutical formulation according to claim 4, wherein the leukocytes showing abnormal accumulation or activation are monocytes and/or macrophages.

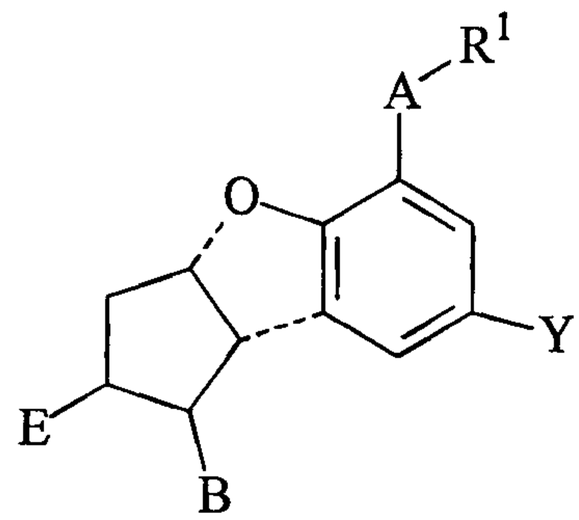
6. The pharmaceutical formulation according to claim 4, wherein the leukocytes showing abnormal accumulation or activation are eosinophils, basophils, or lymphocytes.

7. The pharmaceutical formulation according to claim 1 or 2, which is for treating a disease selected from the group consisting of chronic intractable inflammation, pneumonocirrhosis, pneumonia, ARDS, ulcerative colitis, atopic dermatitis, Crohn's disease, parasitic diseases, rejection after organ transplantation, and endometriosis.

8. Use, for inhibiting C-C chemokine production, of a 4,8-inter-m-phenylene prostaglandin I_2 derivative

76199-155

represented by the following formula (I), or a pharmacologically acceptable salt thereof:



(I)

wherein R^1 represents the following:

(A) $COOR^2$ wherein R^2 is:

1) hydrogen or a pharmacologically acceptable cation;

2) straight chain alkyl having 1 to 12 carbon atoms, or branched alkyl having 3 to 14 carbon atoms;

3) $-Z-R^3$

wherein Z is a valence bond or straight chain or branched alkylene represented by C_tH_{2t} wherein t represents an integer of 1 to 6, and R^3 represents cycloalkyl having 3 to 12 carbon atoms or substituted cycloalkyl having 3 to 12 carbon atoms and 1 to 3 substituents R^4 each of which is hydrogen or alkyl having 1 to 5 carbon atoms;

4) $-(CH_2CH_2O)_nCH_3$

wherein n is an integer of 1 to 5;

5) $-Z-Ar^1$

wherein Z is defined as the same as the above, and Ar^1 is phenyl, α-naphthyl, β-naphthyl, 2-pyridyl,

3-pyridyl, 4-pyridyl, α-furyl, β-furyl, α-thienyl, β-thienyl or substituted phenyl (wherein a substituent is at least one of chlorine, fluorine, bromine, iodine, trifluoromethyl, alkyl having 1 to 4 carbon atoms, nitro, cyano, methoxy, phenyl, phenoxy, p-acetoamidobenzamide, $-CH=N-NH-C(=O)-NH_2$, $-NH-C(=O)-Ph$, $-NH-C(=O)-CH_3$ or $-NH-C(=O)-NH_2$);

6) $-C_tH_{2t}COOR^4$

wherein C_tH_{2t} and R^4 are defined as the same as the above;

7) $-C_tH_{2t}N(R^4)_2$

wherein C_tH_{2t} and R^4 are defined as the same as the above;

8) $-CH(R^5)-C(=O)-R^6$

wherein R^5 is hydrogen or benzoyl, and R^6 is phenyl, p-bromophenyl, p-chlorophenyl, p-biphenyl, p-nitrophenyl, p-benzamidophenyl, or 2-naphthyl;

9) $-C_pH_{2p}-W-R^7$

wherein W is $-CH=CH-$, $-CH=CR^7-$ or $-C\equiv C-$, and R^7 is hydrogen or straight chain or branched alkyl or aralkyl having 1 to 30 carbon atoms, and p is an integer of 1 to 5; or

10) $-CH(CH_2OR^8)_2$

wherein R^8 is alkyl or acyl having 1 to 30 carbon atoms;

(B) $-CH_2OH$;

(C) $-C(=O)N(R^9)_2$

wherein R^9 is hydrogen, straight chain alkyl having 1 to 12 carbon atoms, branched alkyl having 3 to 12 carbon atoms, cycloalkyl having 3 to 12 carbon atoms, cycloalkylalkylene having 4 to 13 carbon atoms, phenyl, substituted phenyl (wherein the substituent is defined as the same as in (A) 5)), aralkyl having 7 to 12 carbon atoms, or $-SO_2R^{10}$ wherein R^{10} is alkyl having 1 to 10 carbon atoms, cycloalkyl having 3 to 12 carbon atoms, phenyl, substituted phenyl (the substituent is defined as the same as in (A) 5)), or aralkyl having 7 to 12 carbon atoms, two R^9 groups may be the same or different, and when one of the R^9 groups is $-SO_2R^{10}$, the other R^9 is not $-SO_2R^{10}$; or

D) $-CH_2OTHP$ (THP is a tetrahydropyranyl group);

A is the following:

1) $-(CH_2)_m-$;

2) $-CH=CH-CH_2-$;

3) $-CH_2-CH=CH-$;

4) $-CH_2-O-CH_2-$;

5) $-CH=CH-$;

6) $-O-CH_2-$; or

7) $-C\equiv C-$;

wherein m represents an integer of 1 to 3;

Y is hydrogen, alkyl having 1 to 4 carbon atoms, chlorine, bromine, fluorine, formyl, methoxy or nitro;

B is $-X-C(R^{11})(R^{12})OR^{13}$

76199-155

wherein R^{11} is hydrogen, alkyl having 1 to 4 carbon atoms; R^{13} is hydrogen, acyl having 2 to 14 carbon atoms, aroyl having 7 to 15 carbon atoms, tetrahydropyranyl, tetrahydrofuranyl, 1-ethoxythienyl, or t-butyl; X is the following:

1) $-CH_2-CH_2-$;

2) $-CH=CH-$; or

3) $-C\equiv C-$; and

R^{12} is the following:

1) straight chain alkyl having 1 to 12 carbon atoms, or branched alkyl having 3 to 14 carbon atoms;

2) $-Z-Ar^2$

wherein Z is defined as the same as the above, and Ar^2 represents phenyl, α-naphthyl, β-naphthyl, or phenyl substituted by at least one chlorine, bromine, fluorine, iodine, trifluoromethyl, alkyl having 1 to 4 carbon atoms, nitro, cyano, methoxy, phenyl or phenoxy;

3) $-C_tH_{2t}OR^{14}$

wherein C_tH_{2t} is defined as the same as the above, and R^{14} represents straight chain alkyl having 1 to 6 carbon atoms, branched alkyl having 3 to 6 carbon atoms, phenyl, phenyl substituted by at least one chlorine, bromine, fluorine, iodine, trifluoromethyl, alkyl having 1 to 4 carbon atoms, nitro, cyano, methoxy, phenyl or phenoxy, cyclopentyl, cyclohexyl, or cyclopentyl or cyclohexyl substituted by 1 to 4 straight chain alkyl groups having 1 to 4 carbon atoms;

4) $-Z-R^3$;

76199-155

wherein Z and R^3 are defined as the same as the above;

5) $-C_tH_{2t}-CH=C(R^{15})R^{16}$;

wherein C_tH_{2t} is defined as the same as the above, and R^{15} and R^{16} each represent hydrogen, methyl, ethyl, propyl, or butyl; or

6) $-C_uH_{2u}-C\equiv C-R^{17}$;

wherein u is an integer of 1 to 7, C_uH_{2u} represents straight chain or branched alkylene, and R^{17} represents straight chain alkyl having 1 to 6 carbon atoms;

E is hydrogen or $-OR^{18}$;

wherein R^{18} represents acyl having 1 to 12 carbon atoms, aroyl having 7 to 15 carbon atoms, or R^2 (wherein R^2 is defined as the same as the above); and

the formula represents d, l or dl form.

9. The use according to claim 8, wherein the 4,8-inter-m-phenylene prostaglandin I_2 derivative is beraprost or a pharmacologically acceptable salt thereof.

10. The use according to claim 8 or 9, wherein C-C chemokine is MCP-1, MCP-3, or RANTES.

11. The use according to claim 8, for the treatment of abnormal accumulation or activation of leukocytes.

76199-155

12. The use according to claim 11, wherein the leukocytes showing abnormal accumulation or activation are monocytes and/or macrophages.

13. The use according to claim 11, wherein the leukocytes showing abnormal accumulation or activation are eosinophils, basophils, or lymphocytes.

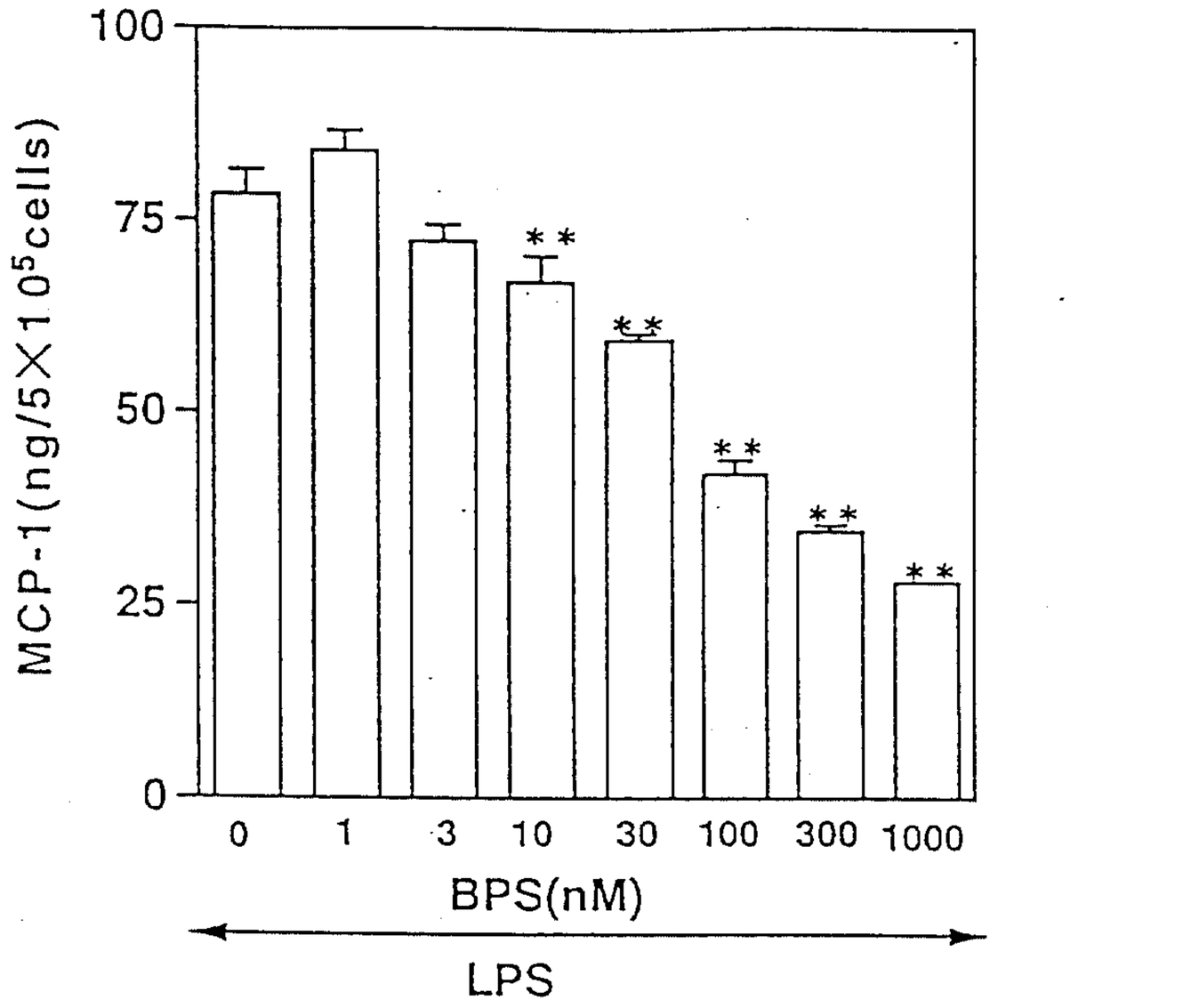
14. The use according to claim 11, which is for treating a disease selected from the group consisting of chronic intractable inflammation, pneumonocirrhosis, pneumonia, ARDS, ulcerative colitis, atopic dermatitis, Crohn's disease, parasitic diseases, rejection after organ transplantation, and endometriosis.

15. A pharmaceutical preparation for treating a disease resulting from abnormal production of monocyte chemoatractant protein 1 (MCP-1), which comprises:

(a) beraprost or a pharmacologically acceptable salt thereof, and

(b) at least one pharmaceutically acceptable additive.

FIG. 1



p<0.01 vs BPS 0

FIG. 2

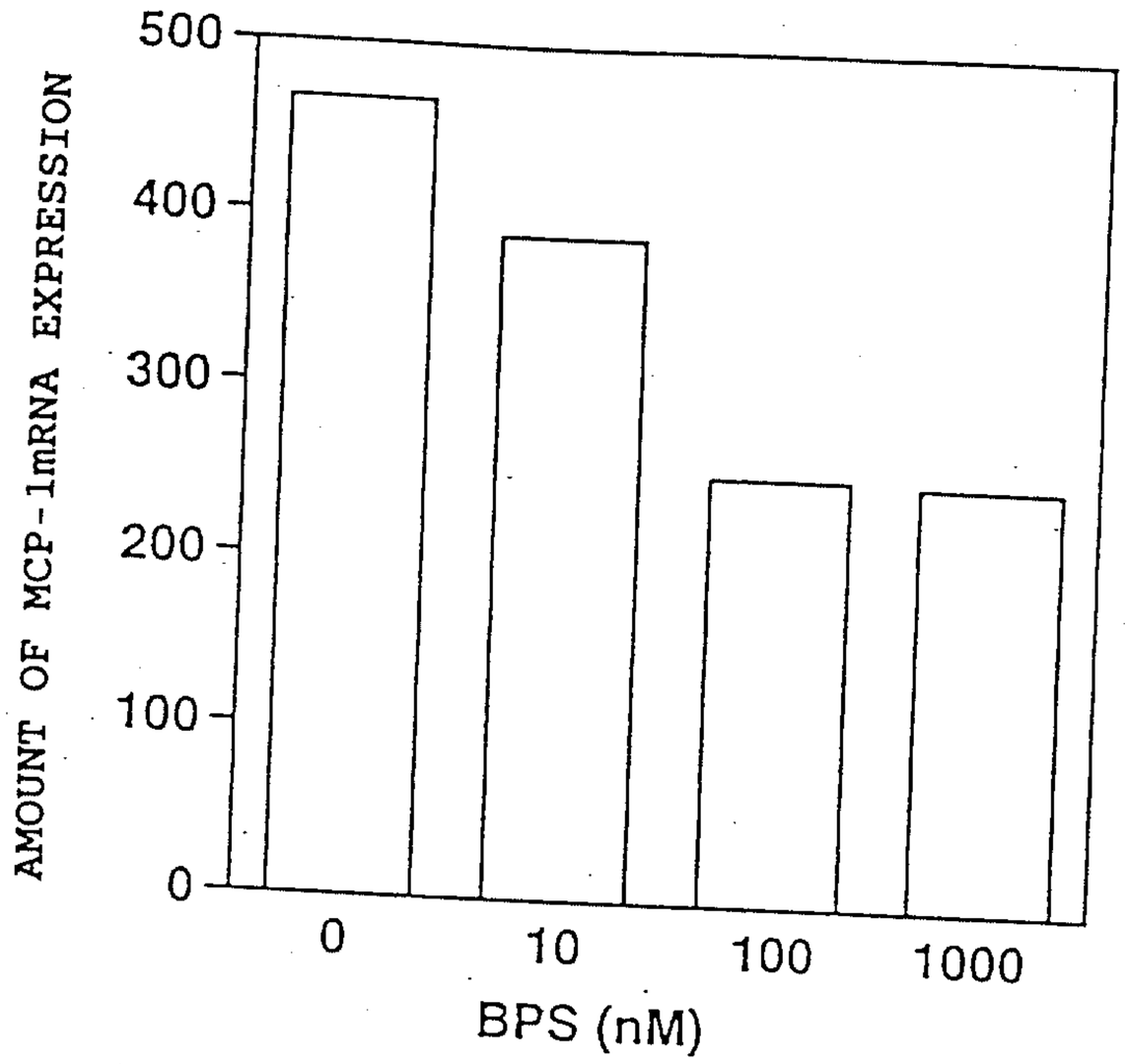

AMOUNT OF MCP-1mRNA EXPRESSION
500
400
300
200
100
0
0
10
100
1000
BPS (nM)

FIG. 3

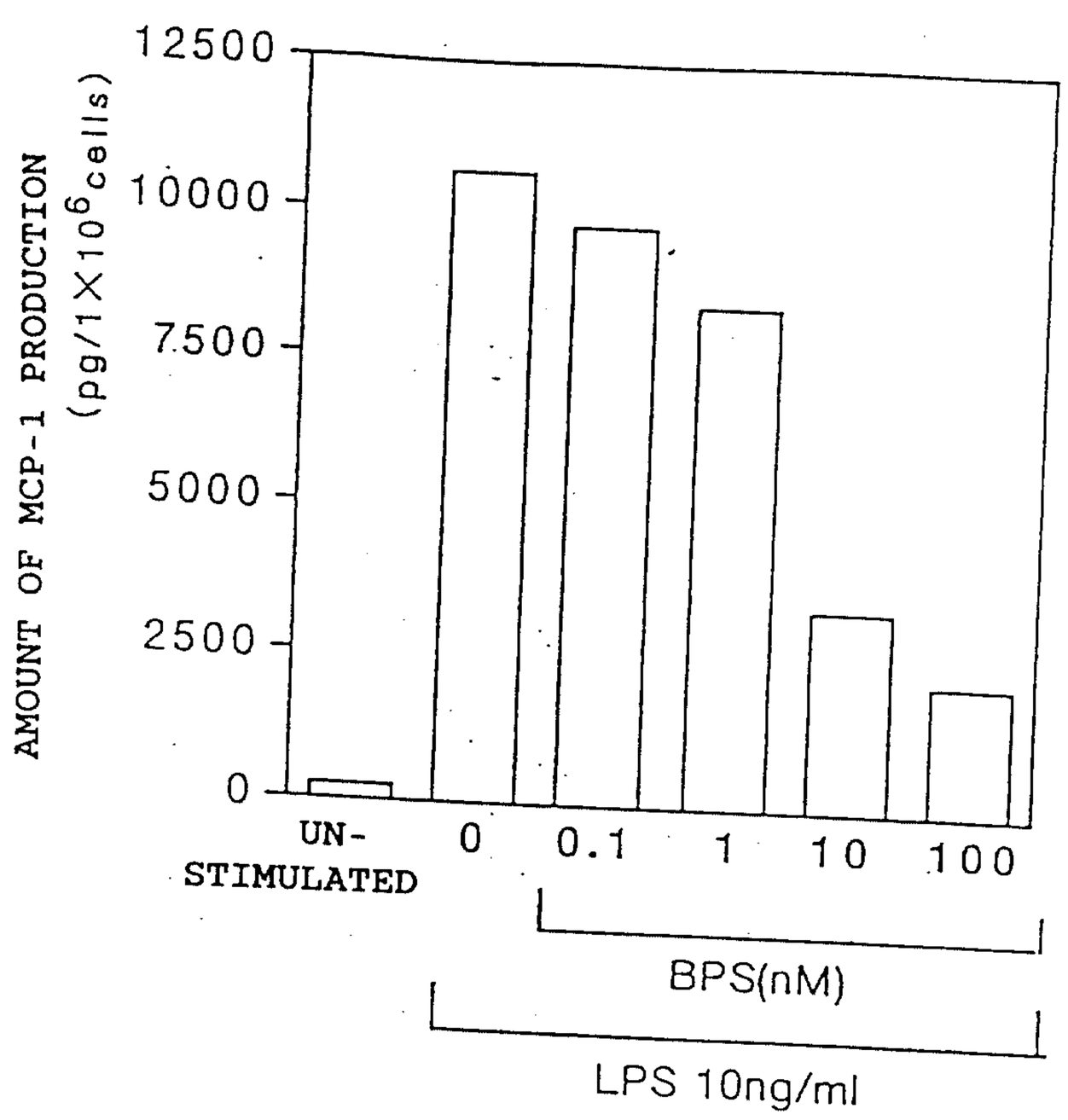

AMOUNT OF MCP-1 PRODUCTION
(pg/1X10^6cells)
12500
10000
7500
5000
2500
0
UN-
STIMULATED
0
0.1
1
10
100
BPS(nM)
LPS 10ng/ml

FIG. 4

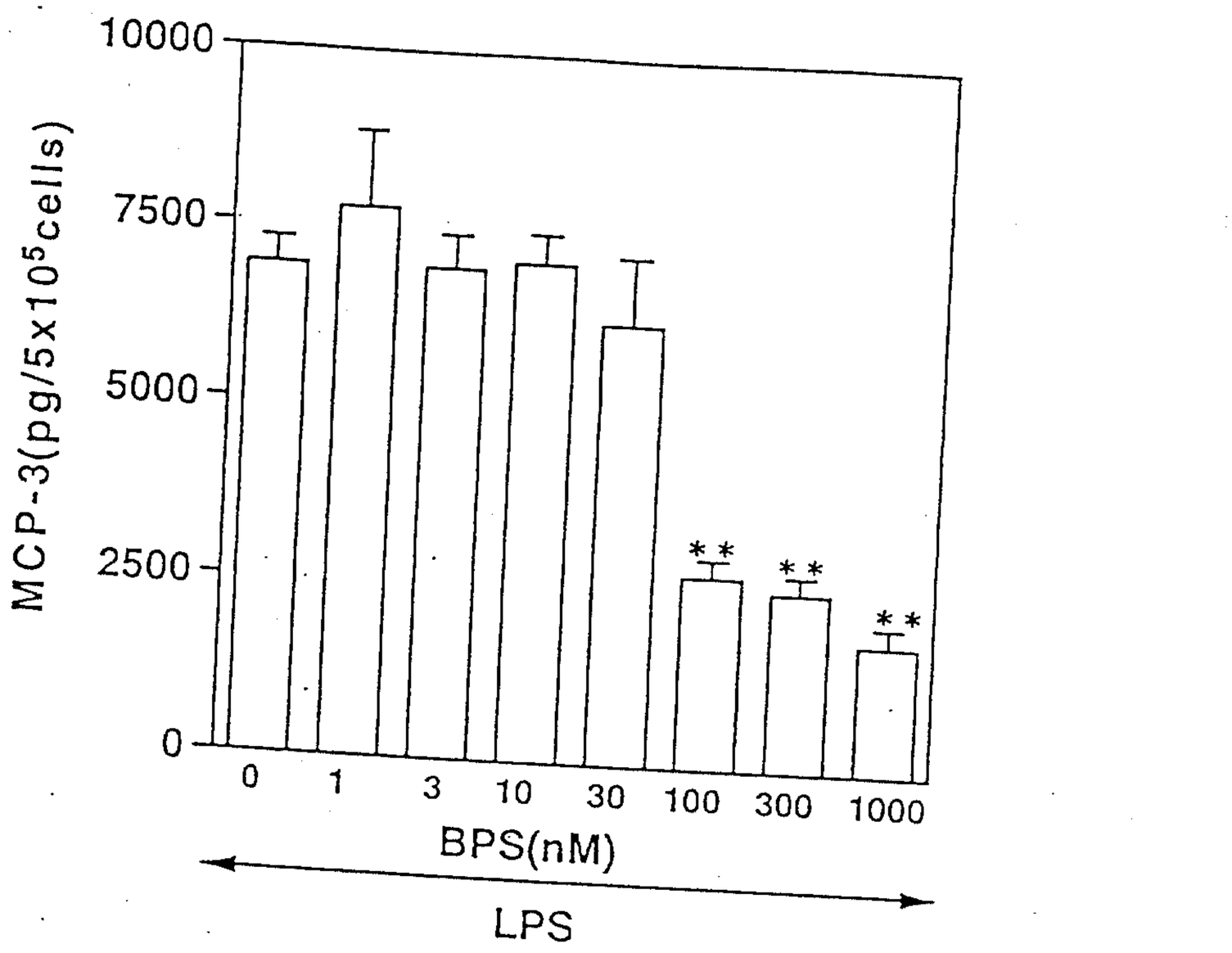

10000
7500
5000
2500
0
MCP-3(pg/5x10⁵cells)
**
**
**
0
1
3
10
30
100
300
1000
BPS(nM)
LPS
p<0.01 vs BPS 0

FIG. 5

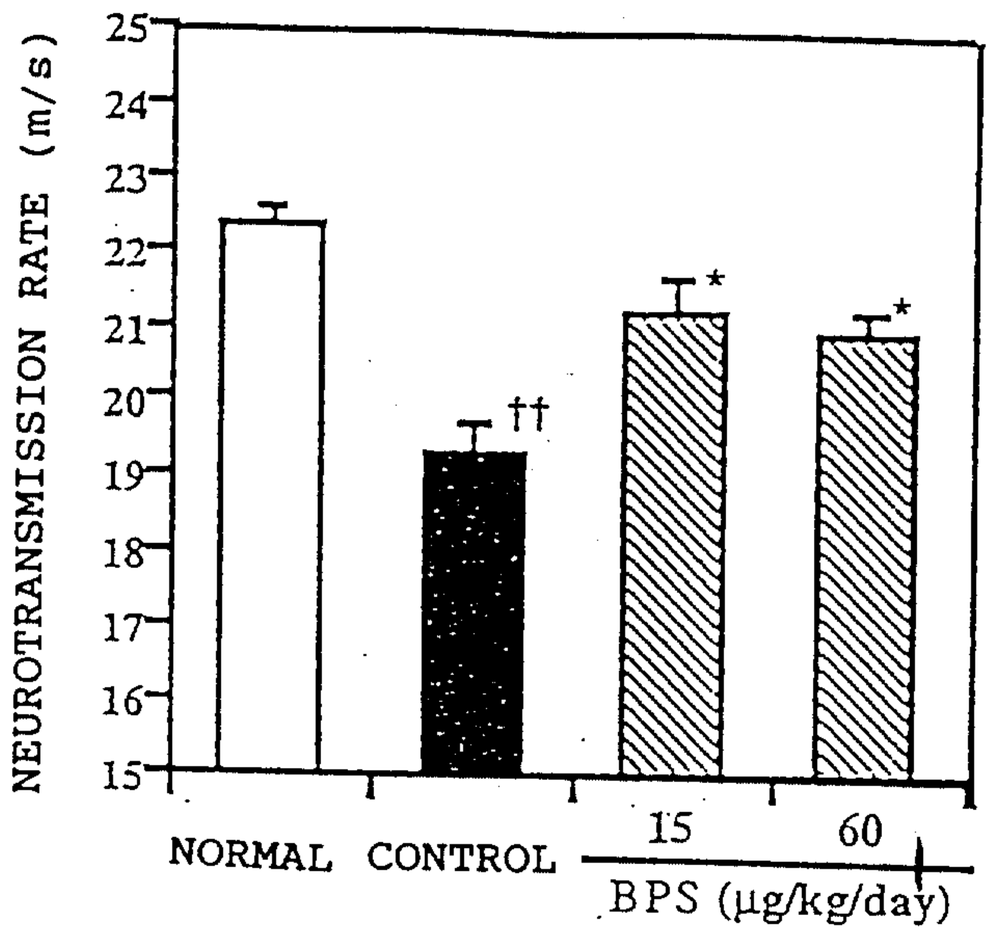

NEUROTRANSMISSION RATE (m/s)
25
24
23
22
21
20
19
18
17
16
15
††
*
*
NORMAL CONTROL
15
60
BPS (μg/kg/day)

FIG. 6

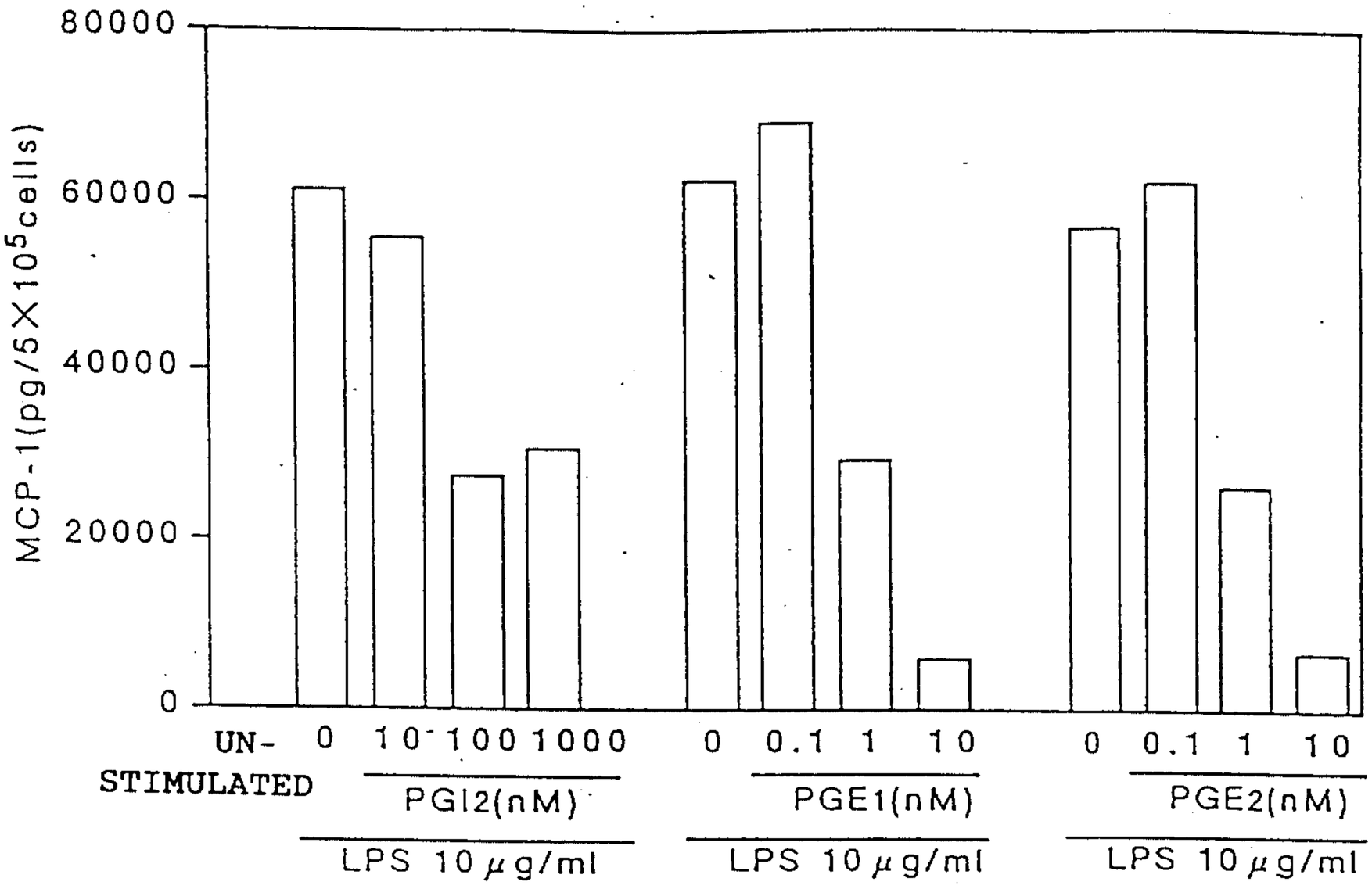

80000
60000
40000
20000
0
MCP-1(pg/5X10^{5}cells)
UN-STIMULATED
0 10 100 1000
PGI2(nM)
LPS 10 μg/ml
0 0.1 1 10
PGE1(nM)
LPS 10 μg/ml
0 0.1 1 10
PGE2(nM)
LPS 10 μg/ml

FIG. 7a

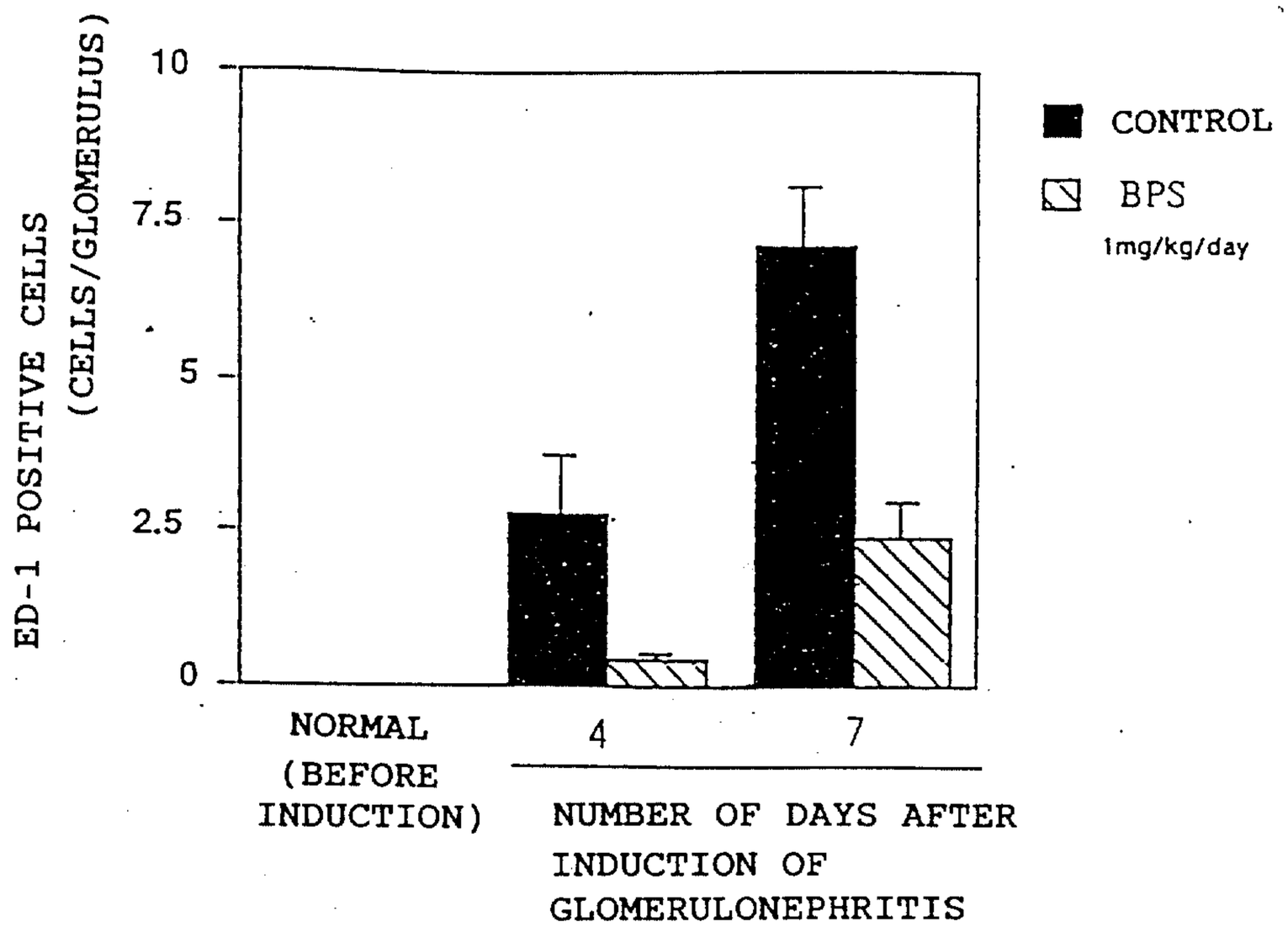

ED-1 POSITIVE CELLS
(CELLS/GLOMERULUS)
10
7.5
5
2.5
0
NORMAL
(BEFORE
INDUCTION)
4
7
NUMBER OF DAYS AFTER
INDUCTION OF
GLOMERULONEPHRITIS
CONTROL
BPS
1mg/kg/day

FIG. 7b

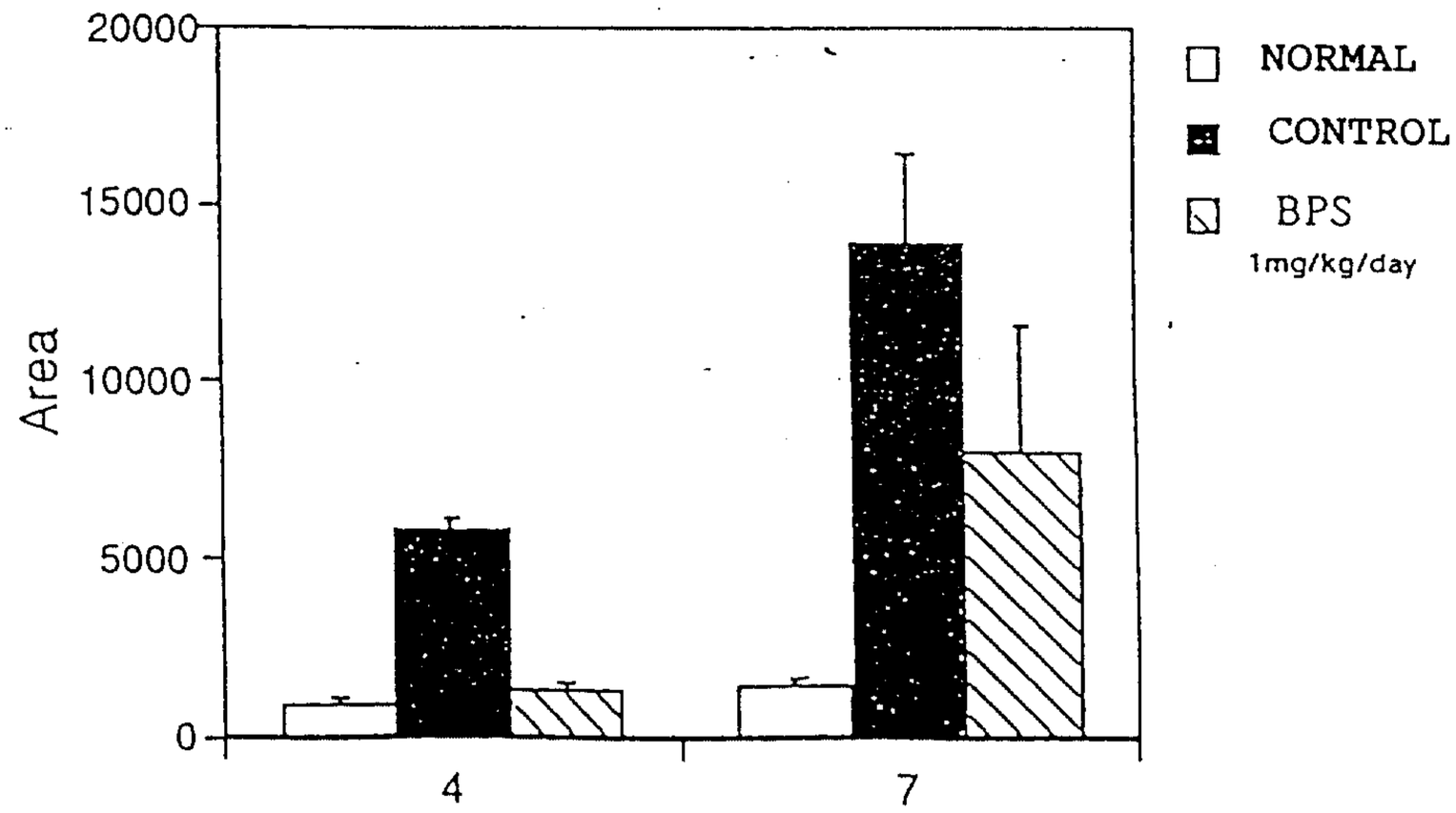
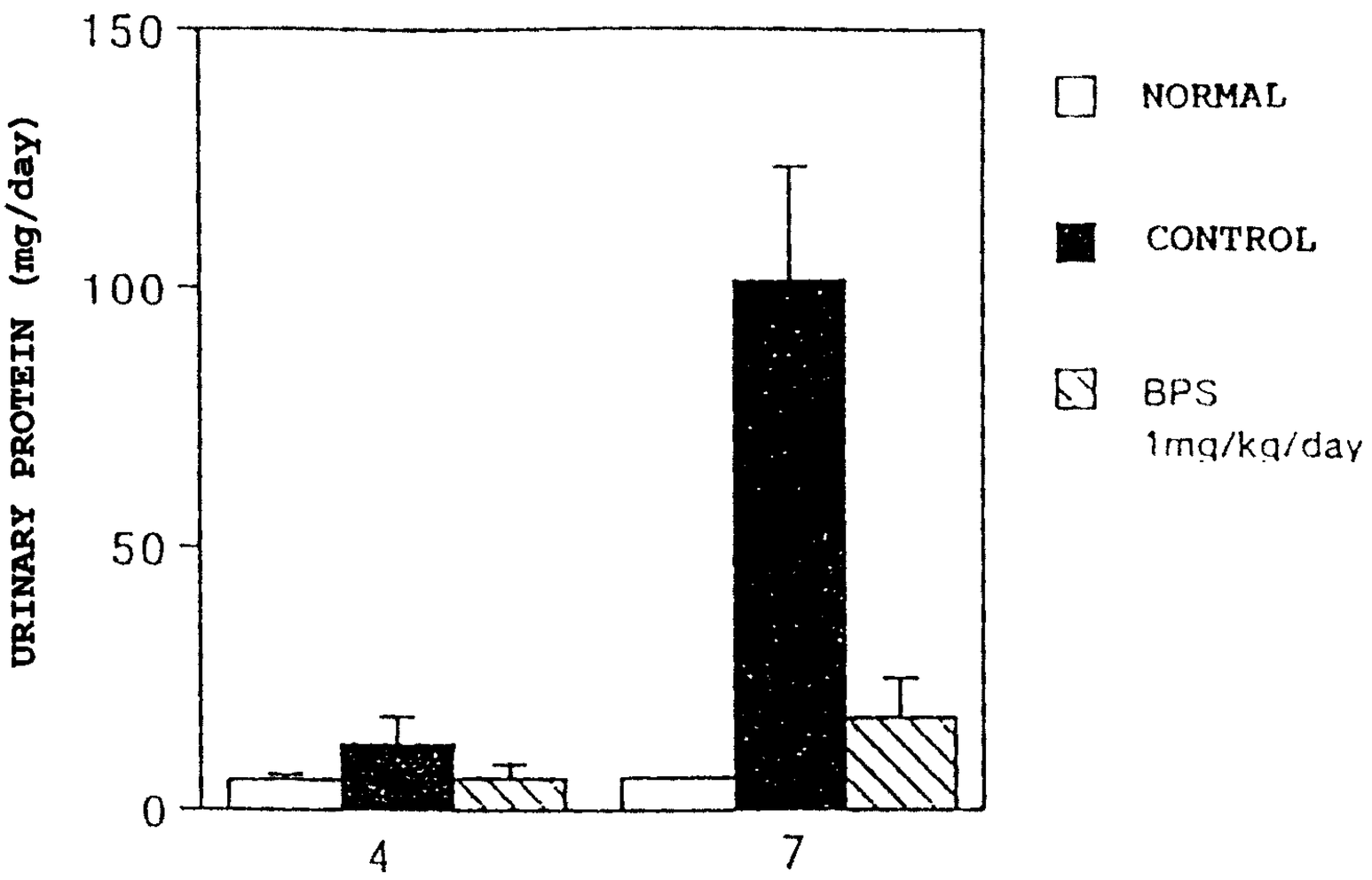

Area
20000
15000
10000
5000
0
4
7
NUMBER OF DAYS AFTER INDUCTION
OF GLOMERULONEPHRITIS
NORMAL
CONTROL
BPS
1mg/kg/day

FIG. 8


150
100
50
0
URINARY PROTEIN (mg/day)
4
7
NORMAL
CONTROL
BPS
1mg/kg/day
NUMBER OF DAYS AFTER INDUCTION
OF GLOMERULONEPHRITIS