Affinity for [3H]Iloprost Binding Sites and cAMP Synthesis Activity of a 3-Oxa-methano Prostaglandin I₁ Analog, SM-10906, in Human Platelets and Endothelial Cells

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ABSTRACT—SM-10902 ((+-)methyl [2-{(2R,3aS,4R,5R,6aS)-octahydro-5-hydroxy-4-[(E)-(3S,5S)-3-hydroxy-5-methyl-1-nonenyl]-2-pentalenyl]ethoxyacetate) and its free acid, SM-10906 are new stable 3-oxa-methano prostaglandin (PG) I₁ analogs. Their affinities for [3H]iloprost and [3H]PGE₂ binding sites in human platelets and human umbilical vascular endothelial cells were compared with those of the PGI₂ analog iloprost, PGE₁ and PGE₂ by the radioligand binding assay method. The cyclic AMP (cAMP) synthesis activity of these drugs were also determined in human umbilical vascular endothelial cells. We found that SM-10906 apparently displaced [3H]iloprost binding to the membrane fractions in those cells since the pKᵢ values were 6.30 in platelets, 7.52 in vein endothelial cells and 6.31 in the arterial endothelial cells. The pKᵢ values of SM-10906 for [3H]PGE₂ binding sites were significantly lower than those obtained for [3H]iloprost binding. SM-10902, which is a prodrug of SM-10906, showed low affinity for [3H]iloprost binding sites in those cells. SM-10906 also dose-dependently enhanced the cAMP level in the vascular endothelial cells. Thus, these findings indicate that SM-10906 binds to [3H]iloprost binding to the membrane fractions in those cells since the pKᵢ values were 6.30 in platelets, 7.52 in vein endothelial cells and 6.31 in the arterial endothelial cells. The pKᵢ values of SM-10906 for [3H]PGE₂ binding sites were significantly lower than those obtained for [3H]iloprost binding. SM-10902, which is a prodrug of SM-10906, showed low affinity for [3H]iloprost binding sites in those cells. SM-10906 also dose-dependently enhanced the cAMP level in the vascular endothelial cells. Thus, these findings indicate that SM-10906 binds to [3H]iloprost binding sites and exhibits pharmacological functions such as an anti-platelet action and a cytoprotective action in endothelial cells through the elevation of intracellular cAMP contents.

Keywords: [3H]Iloprost binding site, Cyclic AMP, SM-10906, Endothelial cell, Platelet

Prostaglandin (PG) I₂ and PGE₁ have been shown to be potent peripheral vasodilators (1–3) and inhibitors of platelet aggregation (2–4). Their biological activity is mediated by receptor interaction and expressed through the activation of adenylate cyclase and the subsequent increase in the cyclic AMP (cAMP) level (2). PG₁₂ receptors (IP receptors) have been identified on platelets, neuronal hybrid cells and many other tissues (5–8) by using the PG₁₂ analog [3H]Iloprost. There is evidence that these PGs bind to receptors on platelets and smooth muscles, suggesting that they may inhibit platelet aggregation in the treatment of thrombotic diseases and act as vasodilators in the treatment of vascular occlusive diseases (9–12). In addition, PG₁₂ is also synthesized in monolayer cultures of human umbilical vein endothelial cells, and its release may contribute to the functional modulation of endothelial cells (13). These actions of PG₁₂ may be involved in the modification of the circulatory insufficiency caused by endothelial cell injury such as local ischemia and skin ulcers.

PG₁₂ has the disadvantages of chemical and metabolic instabilities (14). Therefore, we developed a new stable 3-oxa-methano PG₁₂ analog, SM-10902, which has been
shown to be the prodrug whose active form is the free acid SM-10906 (Fig. 1). SM-10906 was shown to exert its anti-platelet and vasodilatory activities through the increase of the cAMP level (15, 16). SM-10906, but not SM-10902, was demonstrated to be an agonist for IP receptors, and SM-10906 stimulated adenylate cyclase activity in mastocytoma P-815 cells (17). Moreover, we (18) suggested that SM-10902 ointment promotes wound healing through the stimulation of angiogenesis and improves blood flow during the neovascularization of a repairing wound and may be useful in the treatment of skin ulcers caused by peripheral circulatory insufficiency.

Thus, the present study examined the affinity for \(^{3H}\)iloprost binding sites and cAMP synthesis activity of SM-10906 and SM-10902 in human platelets and human vascular endothelial cells.

MATERIALS AND METHODS

Materials

\(^{3H}\)Iloprost (514 GBq/mmol) and [5,6,8,11,12,14,15-\(^{3H}\)]-PGE\(_2\) (6327.0 GBq/mmol) were purchased from Amersham International plc (Buck, UK) and stored at \(-20^\circ\)C. SM-10902 ((+)-methyl 2-[(2R,3aS,4R,5R,6aS)-octahydro-5-hydroxy-4-[{(E)-(3S,5S)-3-hydroxy-5-methyl-1-nonenyl]-2-pentalenyl]ethoxy]acetate) and SM-10906 (the free acid of SM-10902) were produced by Sumitomo Chemical Industries (Osaka). All compounds were dissolved in ethanol, stored in a freezer and then diluted with distilled water or 15 mM Hepes/Hanks buffer containing 1% bovine serum albumin before use.

Preparation of platelet-rich fraction

Blood was collected from healthy adult volunteers (age range 20–54-years-old) whose informed consent was obtained after they had received a full explanation of the study. The blood samples were put into plastic syringes containing 1 vol. of 3.8% trisodium citrate to 9 vol. of blood. This blood was centrifuged at 180 x g for 15 min at room temperature, and then the supernatant was again centrifuged at 30,000 x g for 10 min at 4°C. The resultant pellet was suspended in the incubation medium (60 mM Tris-HCl, 20 mM MgCl\(_2\), pH 7.4) and homogenized using a Polytron homogenizer (Brinkmann, Switzerland). Aliquots were taken for protein determination according to the method of Lowry et al. (19). The platelet-rich pellet was then frozen in liquid nitrogen, stored at \(-80^\circ\)C and then diluted to appropriate concentrations immediately before use for the binding assay.

Culture of the endothelial cells

Human umbilical vein or arterial endothelial cells were purchased from Dainippon Pharmaceutical Co. (Osaka) and were stabilized overnight at 37°C in a humidified atmosphere of air containing 5% CO\(_2\). After washing with phosphate-buffered saline, the endothelial cells were dispersed in 0.025% trypsin / 0.01% EDTA solution (Kurabo, Osaka). The culture medium (endothelial-SFM supplemented with 10% fetal bovine serum, 5 ng/ml epidermal growth factor and 50 µg/ml bovine pituitary extract (Gibco-BRL, Grand Island, NY, USA) and 1 µg/ml hydrocortisone (Nacalai Tesque, Inc., Kyoto) was added to the solution. The medium containing the endothelial cells was centrifuged at 150 x g for 5 min at 4°C. The resultant pellet was suspended in the culture medium and the endothelial cells were seeded onto a plastic culture flask (150 cm\(^2\); Iwaki Glass, Chiba) coated with 0.1% gelatin. The culture medium was changed every 2–3 days. The endothelial cells were used for the binding assay and for the determination of cAMP content after three to five passages. The confluent cells were harvested using a rubber policeman and suspended in the incubation medium (60 mM Tris-HCl, 20 mM MgCl\(_2\), pH 7.4). The endothelial cells were immediately frozen in liquid nitrogen and stored at \(-80^\circ\)C until used for the binding assay.

Binding assay

The \(^{3H}\)Iloprost and \(^{3H}\)PGE\(_2\) binding sites on platelets and both types of vascular endothelial cells were assessed with \(^{3H}\)Iloprost and \(^{3H}\)PGE\(_2\) as radioligands. Saturation experiments on these cells were performed in the range of 5–100 nM \(^{3H}\)Iloprost and 1–8 nM \(^{3H}\)PGE\(_2\). The displacement experiments were carried out using 12, 16 and 24 nM \(^{3H}\)Iloprost in platelets, vein endothelial cells and arterial endothelial cells, respectively, and 2 nM \(^{3H}\)PGE\(_2\) in all cells. A membrane suspension of 0.4 mg protein/ml in platelets or 0.12 mg protein/ml in the endothelial cells including \(^{3H}\)Iloprost or \(^{3H}\)PGE\(_2\) and various PG reagents in 0.5 ml of 60 mM Tris-HCl and 20 mM MgCl\(_2\), pH 7.4 was incubated for 60 min at 37°C. The incubation was terminated by rapid filtration under vacuum through a glass fiber filter (GF/C; Whatman International Ltd., Maidstone, Kent, UK) using an Automatic Cell Harvester Labomash (LM-101; Labo Science, Tokyo). A 1-ml aliquot of toluene-triton based scintillation fluid was added to the resultant filters. The radioactivity was counted by scintillation spectrometry (2200 Tri-Carb Scintillation Analyzer; Packard Instrument Co., Inc., Downers Grove, IL, USA). The specific bindings of \(^{3H}\)Iloprost and \(^{3H}\)PGE\(_2\) were defined as the difference between the total binding and the non-specific binding in the absence or presence of 0.1
mM iloprost or PGE2. The range of percentages of non-specific bindings to total bindings of [3H]iloprost to the membranes of platelets, vein and arterial endothelial cells were 32–56%, 39–79% and 38–47%, respectively. The values of the dissociation constants (Kd) and the maximal binding sites (Bmax) were calculated by Scatchard analysis. The values of inhibition constants (Ki) were also calculated by displacement experiments and are expressed as pKi values (−log Ki). The values of Hill coefficients of each drug tested here in the platelets and endothelial cells were between 0.91 and 1.16.

**Determination of cAMP in vascular endothelial cells**

Vein and arterial endothelial cells were cultured on 12-well plastic plates coated with 0.1% gelatin. The confluent endothelial cells were washed with 15 mM Hepes/Hanks buffer containing 1% bovine serum albumin. The cells were allowed to preincubate with 15 mM Hepes/Hanks buffer containing 0.5 mM 3-isobutyl-1-methylxanthine (IBMX, Nacalai Tesque) and 1% bovine serum albumin for 15 min at 37°C before the addition of test drugs. The reactions were terminated by the addition of 6% trichloroacetic acid (TCA) after the removal of supernatant. The sample was mixed with water-saturated ether to remove TCA, and the cAMP was extracted. The contents of cAMP in the vascular endothelial cells were determined by radioimmunoassay using a cAMP kit (Yamasa Shoyu Co., Tokyo).

**Statistical analyses**

Values are expressed as the means±S.E.M. The significant difference between the pKi value for [3H]iloprost binding sites and that for [3H]PGE2 binding sites for each drug was analyzed by an unpaired Student’s t-test. Statistical analysis of the pKi value for [3H]iloprost binding sites and the amount of cAMP produced by the test drugs was performed by Dunnett’s multiple comparison after analysis of variance. Differences were considered to be significant when the P value was less than 0.05.

**RESULTS**

**Binding characteristics of [3H]iloprost in human platelets and vascular endothelial cells**

Table 1 shows the values of Kd and Bmax of membranes of platelets, vein endothelial cells and arterial endothelial cells obtained from the Scatchard analysis. The [3H]iloprost binding sites in these cells exhibited a single class of binding sites. The Kd values obtained in the platelets were lower than those of the endothelial cells.

The affinity for [3H]iloprost and [3H]PGE2 binding sites in human platelets and vascular endothelial cells

Table 2 shows the pKi values of each drug in the displacement experiments using [3H]iloprost and [3H]PGE2 binding to human platelets. SM-10906 had a higher pKi values (6.30±0.21) than SM-10902 (5.43±0.02). The order of affinities of the test drugs for [3H]iloprost binding sites was iloprost > SM-10906 > SM-10902 = PGE1 = PGE2. The affinities of iloprost, SM-10906 and SM-10902 for [3H]PGE2 binding sites in platelets were very low.

Tables 3 and 4 show the pKi values of SM-10906 for [3H]iloprost binding sites in vein and arterial endothelial cells, respectively. The pKi values of SM-10906 were higher than those of SM-10902. The affinities of iloprost, SM-10906 and SM-10902 for the [3H]PGE2 binding sites were lower than those obtained for the [3H]iloprost binding sites. The rank order of affinities of these drugs for [3H]iloprost binding sites in the endothelial cells was similar to that in platelets.

**Effects of SM-10906, PGI2 and PGE1 on cAMP production in vascular endothelial cells**

Figure 2 shows the time course of the effects of SM-

| Table 1. Binding characteristics of [3H]iloprost in human platelets, human umbilical vein endothelial cells and human umbilical arterial endothelial cells |
|---|---|---|
| | n | Kd (nM) | Bmax (fmol/mg protein) |
| Platelets | 4 | 38.9±5.3 | 2381.2±181.6 |
| Human umbilical vein endothelial cells | 4 | 63.7±9.7 | 2315.3±362.2 |
| Human umbilical arterial endothelial cells | 5 | 74.4±15.8 | 5844.9±666.3 |

Values are each a mean±S.E.M. The significant differences were analyzed by Student’s t-test: *P < 0.05, **P < 0.01.

| Table 2. The pKi values of several drugs for [3H]iloprost and [3H]-PGE2 binding sites in human platelets |
|---|---|---|
| Drugs | [3H]iloprost binding sites | [3H]PGE2 binding sites |
| Iloprost | 7.26±0.04 (4) | 5.11±0.26 (3)<sup>IV</sup> |
| SM-10906 | 6.30±0.21 (4) | 5.10±0.31 (4)<sup>IV</sup> |
| SM-10902 | 5.43±0.02 (4) | 5.48±0.13 (3) |
| PGE1 | 5.49±0.02 (4) | 8.52±0.83 (3)<sup>IV</sup> |
| PGE2 | 4.40±0.07 (4) | 7.64±0.36 (3)<sup>IV</sup> |

Numbers in parentheses indicate the number of experiments. Values are each a mean±S.E.M. The significant differences between the pKi value for [3H]iloprost binding sites and that for [3H]PGE2 binding sites for each drug were analyzed by an unpaired Student’s t-test: *P < 0.02, **P < 0.01.
and cAMP production in human vein and arterial endothelial cells. In these cells, the production of cAMP induced by the test drugs was very slight (data not shown), so the cAMP contents were determined in the presence of 0.5 mM IBMX, a cAMP-phosphodiesterase inhibitor. The production of cAMP began immediately after treatment with SM-10906 at 1 μM and reached a peak level at 10 min. PGE1 and PGI2 also caused the production of cAMP, similar to SM-10906. The cAMP contents were compared 10 min after the addition of these PGs at various concentrations to the vascular endothelial cells treated with IBMX.

Tables 5 and 6 show the effects of the test drugs on the cAMP production in vein and arterial endothelial cells, respectively. In these two types of cells, SM-10906 induced a dose-related elevation of the cAMP level and showed a significant effect above a concentration of 100 nM. The rank order of cAMP production by these drugs in vein and arterial endothelial cells was SM-10906 = PGE1 ≈ PGI2 and PGE1 > SM-10906 > PGI2, respectively.

**DISCUSSION**

IP receptors have been identified on the membranes of platelets (20, 21), human gel-filtered platelets (22), mouse mastocytoma P-815 cells (23) and many other tissues (12). In the present radioligand binding study using platelets, the [3H]iloprost used as a radioligand for the IP receptor was observed to have a Kd value (38.9 ± 5.3 nM, Table 1) similar to those previously observed in platelet membranes (13.4 nM, Ref. 20), human gel-filtered platelets (45.2 nM, Ref. 21) and washed human platelets (18.5 nM, Ref. 21).

We report here that SM-10906 bound to the [3H]iloprost binding sites not only in platelets but also to those 10906, PGI2 and PGE1 on cAMP production in human vein and arterial endothelial cells. In these cells, the production of cAMP induced by the test drugs was very slight (data not shown), so the cAMP contents were determined in the presence of 0.5 mM IBMX, a cAMP-phosphodiesterase inhibitor. The production of cAMP began immediately after treatment with SM-10906 at 1 μM and reached a peak level at 10 min. PGE1 and PGI2 also caused the production of cAMP, similar to SM-10906. The cAMP contents were compared 10 min after the addition of these PGs at various concentrations to the vascular endothelial cells treated with IBMX.

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![Fig. 2](image-url)  
(a) The vein endothelial cells  
(b) The arterial endothelial cells  

Fig. 2. Time course of the effects of SM-10906, PGI1 and PGE1 on cAMP production in the vascular endothelial cells. In the presence of 0.5 mM IBMX for 15 min, the amount of cAMP induced by 1 μM SM-10906 (●), PGI1 (▲) or PGE1 (■) was determined in the vein endothelial cells (a) and the arterial endothelial cells (b). The cAMP production with or without 0.5 mM IBMX is also shown by ○ (a and b). The results shown are each the mean ± S.E.M. of three wells.
in the cultured vascular endothelial cells and that it had a slightly lower affinity than iloprost in terms of the displacement of \([^{3}H]\)iloprost binding to these cells. The methyl ester of SM-10906, SM-10902, which is the so-called prodrug for SM-10906, was tenfold weaker than SM-10906 in the binding assay. In a previous study using mouse mastocytoma P-815 cells, SM-10906 showed a high affinity for IP receptors, while SM-10902 had little affinity for IP or \([^{3}H]\)PGE_{2} binding (EP) receptors (17). In the present study, the affinities of SM-10906 for \([^{3}H]\)iloprost binding sites obtained from platelets and the cultured vascular endothelial cells coincided with that in mastocytoma P-815 cells. In contrast, both 3-oxa-methano PGI1 analogs and iloprost have very weak affinities for EP receptors.

Thus, the present findings indicate that SM-10906 and iloprost selectively bind to \([^{3}H]\)iloprost binding sites in human platelets and vascular endothelial cells and that there are separate, distinguishable receptors for PGI_{2} and PGE_{2} on these cells.

PGI_{2} and its analogs are known to stimulate cAMP synthesis through IP receptors in platelets and mastocytoma P-815 cells (24, 25). In platelets, SM-10906 increases the cAMP level through the activation of adenylate cyclase in crude membrane fractions, and it inhibits platelet aggregation and the release of adenine nucleotides stimulated by thrombin (15, 16). In our study, SM-10906 induced an elevation of cAMP levels in the vascular endothelial cells. These findings support the existence of IP receptors on these cells as well as platelets.

PGI_{2} is known to prevent damage to endothelial cells (26) and the cells of various other tissues (27-31); that is, it has a so-called cytoprotective action. SM-10906 prevents the endothelial cell injury induced by \(H_{2}O_{2}\) (32). The specific \([^{3}H]\)iloprost binding sites in endothelial cells should be examined because these sites may have an important role in the cytoprotection of these cells. PGI_{2} analogs are also known to prevent damage to endothelial cells (33-35), implying that these analogs show a cytoprotective action through the increase of cAMP production and consequently cause an increase in endothelial antithrombotic activity. Thus, the cell damage
in ischemic disease is closely related to the production of cAMP, thereby causing a decrease in cytosolic Ca\(^{2+}\) contents and inhibiting vascular endothelial cell damage.

In the vascular endothelial cells, the existence and the characteristics of IP receptors are not fully understood. Skidgel and Printz (36) reported that vascular arterial homogenates appear to generate substantial amounts of PGI\(_2\), but venous homogenates do not, generating PGE\(_2\) instead in rats. Schröder and Schror (37) also demonstrated that a continuous basal PGI\(_2\) generation occurs in porcine aortic endothelial cells and that this may be sufficient to completely desensitize PGI\(_2\)-dependent adenylate cyclase activation, presumably at the receptor or GTP-binding protein level. In the present study, higher pK\(_i\) values of iloprost for \([\text{H}]\)iloprost binding sites in vein endothelial cells rather than arterial endothelial cells were observed. Thus, the difference of the pK\(_i\) value of iloprost between vascular endothelial cells may result from the down-regulation of IP receptors caused by endogenous PGI\(_2\) generation and/or the existence of different receptor subtypes in both types of vascular endothelial cells. However, there are not sufficient data at present to explain why iloprost has a similar potency to SM-10906 in the arterial endothelial cells. Although the IP receptor mRNA was expressed only in arteries and not in veins, this expression was limited to smooth muscle cells and was not seen in endothelial cells by in situ hybridization studies in mice (38). Our present radioligand binding assay results suggested that the specific \([\text{H}]\)iloprost binding sites existed in the vein and arterial vascular endothelial cells, the same as in platelets. Further investigation is required to reveal the characteristics of these \([\text{H}]\)iloprost binding sites.

In conclusion, the present findings suggest that the new stable 3-oxa-methano PGI\(_1\) analog SM-10906, which is an active form of SM-10902, selectively binds to \([\text{H}]\)iloprost binding sites and not to \([\text{H}]\)PGE\(_2\) binding sites and induces an elevation of intracellular cAMP contents, with pharmacological functions such as an antiplatelet action and a cytoprotective action in endothelial cells.

REFERENCES

1. Weiner R and Kaley G: Influence of prostaglandin E\(_1\) on the terminal vascular bed. Am J Physiol 217, 563–566 (1969)
2. Moncada S, Gryglewski R, Bunting S and Vane JR: An enzyme isolated from arteries transforms prostaglandin endoperoxides to an unstable substance that inhibits platelet aggregation. Nature 263, 663–665 (1976)
3. Bunting S, Gryglewski R, Moncada S and Vane JR: Arterial walls generate from prostaglandin endoperoxides a substance (prostaglandin X) which relaxes strips of mesenteric and coeliac arteries and inhibits platelet aggregation. Prostaglandins 12, 897–913 (1976)
4. Emmons PR, Hampton JR, Harrison MJG, Honour AJ and Mitchell JRA: Effect of prostaglandin E\(_2\) on platelet behavior in vitro and in vivo. Br Med J 2, 468–472 (1967)
5. Siegl AM, Smith JB, Silver MJ, Nicolaou KC and Ahern D: Selective binding site for \([\text{H}]\)prostacyclin on platelets. J Clin Invest 63, 215–220 (1979)
6. MacDermot J, Blair IA and Cresp TM: Prostacyclin receptors of a neuronal hybrid cell line: divalent cations and ligand-receptor coupling. Biochem Pharmacol 30, 2041–2044 (1981)
7. Shepherd GL, MacDermot J, Blair IA and Lewis PJ: Prostacyclin receptors on human platelets. Clin Sci (Medical Research Society) 61, 29p (1981)
8. Rucker W and Schror K: Evidence for high affinity prostacyclin binding sites in vascular tissue: Radioligand studies with a chemistry stable analogue. Biochem Pharmacol 32, 2405–2410 (1983)
9. Carlson LA and Eriksson I: Femoral-artery infusion of prostaglandin E\(_1\) in severe peripheral vascular disease. Lancet 1, 155–156 (1973)
10. Carlson LA and Olsson AG: Intravenous prostaglandin E\(_1\) in severe peripheral vascular disease. Lancet 2, 810 (1976)
11. Kahan A, Weber S, Amor B and Menkes J: Epoprostenol (prostacyclin) infusion in patients with Raynaud’s syndrome. Lancet 1, 538 (1983)
12. Coleman RA, Smith WL and Narumiya S: Classification of prostanoid receptors: properties, distribution, and structure of the receptors and their subtypes. Pharmacol Rev 46, 205–229 (1994)
13. Weksler BB, Marcus AJ and Jaffe EA: Synthesis of prostaglandin I\(_2\) (prostacyclin) by cultured human and bovine endothelial cells. Proc Natl Acad Sci USA 74, 3922–3926 (1977)
14. Cho MJ and Allen MA: Chemical stability of prostacyclin (PGI\(_2\)) in aqueous solutions. Prostaglandins 15, 943–954 (1978)
15. Yamamoto T, Satoh K, Nisimura T, Horikawa N, Mine T, Hirohashi T and Hara Y: Pharmacological properties of the new stable prostacyclin analogue 3-oxa-methano-prostaglandin I\(_1\), Arzneimittelforschung 44, 483–490 (1994)
16. Nishimura T, Yamamoto T, Komuro Y and Hara Y: Antiplatelet functions of a stable prostacyclin analog, SM-10906 are exerted by its inhibitory effect on inositol 1,4,5-trisphosphate production and cytosolic Ca\(^{2+}\) increase in rat platelets stimulated by thrombin. Thromb Res 79, 307–317 (1995)
17. Oka M, Nogishi M, Yamamoto T, Satoh K, Hirohashi T and Ichikawa A: Prostacyclin (PGI\(_2\)) receptor binding and cyclic AMP synthesis activities of PGI\(_2\) analogues, SM-10906 and its methyl ester, SM-10902, in mastocytoma P-815 cells. Biol Pharm Bull 17, 74–77 (1994)
18. Yamamoto T, Horikawa N, Komuro Y and Hara Y: Effect of topical application of a stable prostacyclin analogue, SM-10902 on wound healing in diabetic mice. Eur J Pharmacol 302, 53–60 (1996)
19. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ: Protein measurement with the Folin phenol reagent. J Biol Chem 193, 265–275 (1951)
20. Tsai A-L, Vijjeswarapu H and Wu KK: Interaction between platelet receptor and iloprost isomers. Biochim Biophys Acta 942, 220–226 (1988)
21. Tsai A-L, Hsu M-J, Vijjeswarapu H and Wu KK: Solubilization of prostacyclin membrane receptors from human platelets. J Biol Chem 264, 61–67 (1989)
22 Eggerman TL, Anderson NH and Robertson RP: Separate receptors for prostacyclin and prostaglandin E2 on human gel-filtered platelets. J Pharmacol Exp Ther 236, 568–573 (1986)

23 Mizuno Y, Ichikawa A and Tomita K: Effect of 7-fluoro prostacyclin, a stable prostacyclin analogue, on cAMP accumulation and prostaglandin binding in mastocytoma P-815 cells. Prostaglandins 26, 785–795 (1983)

24 Dutta-Roy AK and Sinha AK: Purification and properties of prostaglandin E1/prostacyclin receptor of human blood platelets. J Biol Chem 262, 12685–12691 (1987)

25 Negishi M, Hashimoto H, Yatsunami K, Kurozumi S and Ichikawa A: TEI-9063, a stable and highly specific prostacyclin analogue for the prostacyclin receptor in mastocytoma P-815 cells. Prostaglandins 42, 225–237 (1991)

26 Johnson AR, Cambell W, Davis L and Schulz W: Influence of prostacyclin on endothelial cell injury (abstract). Fed Proc 42, 772 (1983)

27 Lefter AM, Ogletree ML, Smith JB, Silver MJ, Nicolau KC, Barnette WE and Gasic GP: Prostacyclin: A potentially valuable agent for preserving myocardial tissue in acute myocardial ischemia. Science 200, 52–54 (1978)

28 Konturek SJ and Robert A: Cytoprotection of canine gastric mucosa by prostacyclin: Possible mediation by increased mucosal blood flow. Digestion 25, 155–163 (1982)

29 Blackwell GJ, Radomski M, Vargas JR and Moncada S: Prostacyclin prolongs viability of washed human platelets. Biochim Biophys Acta 718, 60–65 (1982)

30 Fantone JC and Kinnis DA: Prostaglandin E1 and prostaglandin I2 modulation of superoxide production by human neutrophils. Biochim Biophys Res Commun 113, 506–512 (1983)

31 Renkawek K, Herbacznyska CK and Mossakowski MJ: The effect of prostacyclin on morphological and enzymatic properties of CNS cultures exposed to anoxia. Acta Neurol Scand 73, 111–118 (1986)

32 Yamamoto T and Hara Y: The protective action of a stable prostacyclin analogue, SM-10906 on cultured human umbilical endothelial cells. Jpn Pharmacol Ther 24, 789–794 (1996) (Abstr in English)

33 Sakai A, Yajima M and Nishio S: Cytoprotective effect of TRK-100, a prostacyclin analogue, against chemical injuries in cultured human vascular endothelial cells. Life Sci 47, 711–719 (1990)

34 Kainoh M, Maruyama I, Nishio S and Nakadate T: Enhancement by beraprost sodium, a stable analogue of prostacyclin, in thrombomodulin expression on membrane surface of cultured vascular endothelial cells via increase in cAMP level. Biochem Pharmacol 41, 1135–1140 (1991)

35 Kainoh M, Nishio S and Nakadate T: Cytoprotective action of beraprost sodium against peroxide-induced damage in vascular endothelial cells. Pharmacology 45, 61–70 (1992)

36 Skidgel RA and Printz MP: PGI2 production by rat blood vessels: diminished prostacyclin formation in veins compared to arteries. Prostaglandins 16, 1–16 (1978)

37 Schröder H and Schrö K: Prostacyclin-dependent cyclic AMP formation in endothelial cells. Naunyn Schmiedebergs Arch Pharmacol 347, 101–104 (1993)

38 Oida H, Namba T, Sugimoto Y, Ushikubi F, Ohishi H, Ichikawa A and Narumiya S: In situ hybridization studies of prostacyclin receptor mRNA expression in various mouse organs. Br J Pharmacol 116, 2828–2837 (1995)