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The effect of a synthetic 7-thiaprostaglandin E_1 derivative, TEI-6122, on monocyte chemoattractant protein-1 induced chemotaxis in THP-1 cells

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1 The ability of various prostaglandins (PGs) to inhibit monocyte chemotaxis induced by monocyte chemoattractant protein-1 (MCP-1) was investigated with a human monocytic leukaemia cell line, THP-1. Moreover, to investigate the mechanism of the inhibitory action of PGs the involvement of either intracellular adenosine 3' : 5'-cyclic monosphosphate (cyclic AMP) accumulation or intracellular Ca^{2+} mobilization was studied.

2 TEI-6122, a synthetic 7-thia-PGE_1 derivative, inhibited chemotaxis of THP-1 cells induced by MCP-1 with an IC_{50} of 1.5 pM. Its inhibitory activity was 1000 fold more than that of PGE_1 and PGE_2 (IC_{50} = 2.8 nM and 0.9 nM, respectively), which were more potent than other PGs such as PGA_1, PGA_2, $PGF_{2\alpha}$ and PGI_2 ($IC_{50} \geq 1$ μM).

3 With respect to the effect on intracellular cyclic AMP accumulation in THP-1 cells, TEI-6122 was as potent as PGE_1 and PGE_2, which were approximately 100 to 1000 fold more potent than the other PGs such as PGA_1, PGA_2 and PGI_2. The minimum concentration of TEI-6122 required to increase intracellular cyclic AMP accumulation in THP-1 cells was 1 nM.

4 TEI-6122 and PGE_1 (4 μM) transiently increased intracellular calcium levels in THP-1 cells. When added prior to MCP-1, both PGs partially suppressed the increased in Ca^{2+} caused by this cytokine. There were no significant differences between the activity of TEI-6122 and PGE_1 in either respect.

5 It is concluded that TEI-6122, a synthetic 7-thia-PGE_1 derivative is a much more potent inhibitor of MCP-1-induced THP-1 cell chemotaxis than PGE_1 and PGE_2 which are the best inhibitors among the natural PGs tested, while neither intracellular cyclic AMP accumulation nor effects on Ca^{2+} mobilization account for the extremely potent inhibitory activity of TEI-6122. Thus, either a novel PGE_2 receptor (EP receptor) or a novel intracellular signal transduction system may be involved in the extremely potent chemotaxis inhibitory activity of TEI-6122.

Keywords: Monocyte chemoattractant protein-1; chemotaxis; TEI-6122; prostaglandin E_1; adenosine 3':5'-cyclic monophosphate; Ca^{2+} influx; prostaglandin E_2 receptors

Introduction

Monocyte chemoattractant protein-1 (MCP-1) is a 76-amino acid protein that belongs to the C-C chemokine family and has potent chemoattractant activity that is specific for monocytes (Leonard & Yoshimura, 1990; Oppenheim *et al.*, 1991). It has been shown that MCP-1 is expressed *in vitro* by various cells such as macrophages, endothelial cells, and smooth muscle cells in response to various proinflammatory stimuli (Sica *et al.*, 1991; Wang *et al.*, 1991). MCP-1 expression has been shown also *in vivo* such as within the atherosclerotic lesions of blood vessels from man, rabbits and hypercholesterolaemic primates or within synovial tissues from patients with rheumatoid arthritis (Nelken *et al.*, 1991; Yla-Huttuala *et al.*, 1991; Koch *et al.*, 1992; Villiger *et al.*, 1992; Yu *et al.*, 1992). Thus, MCP-1 has been suggested to play a major role in the accumulation of blood monocytes into inflammatory sites in various diseases such as atherosclerosis, and rheumatoid arthritis. Therefore, it is expected that agents which inhibit monocyte chemotaxis induced by MCP-1 could possess a therapeutic potential for those diseases.

In the present paper, we focused on prostaglandins (PGs) as possible inhibitors of MCP-1-induced chemotaxis, since there have been reports that PGs of the E series exhibit anti-inflammatory properties (Zurier & Quagliata, 1971; Fantone *et al.*, 1983; Kelly *et al.*, 1987), and also that intracellular adenosine 3' : 5'-cyclic monophosphate (cyclic AMP) accumulation can result in inhibition of neutrophil chemotaxis (Harvath *et al.*, 1991). Anti-atherogenic properties of PGs in a balloon injury model or a cholesterol-fed model (Sinzinger *et al.*, 1992; Braun *et al.*, 1993; Motoyama *et al.*, 1994) also suggested to us that PGs may inhibit MCP-1-induced chemotaxis, although it has not been clarified whether inhibition of monocyte chemotaxis is involved in their anti-atherogenic effects. Thus, we investigated the ability of various PGs to inhibit chemotaxis induced by MCP-1 with a human monocytic leukaemia cell line, THP-1. Furthermore, to investigate the involvement of PGE_2 receptors (EP receptors) in the inhibitory action of PGs, we studied the effects of PGs on intracellular cyclic AMP accumulation and Ca^{2+} influx in THP-1 cells.

Methods

MCP-1-induced chemotaxis assay

MCP-induced chemotaxis was produced with a 96-well microchemotaxis chamber having a polycarbonate filter (5 μm pore size; NeuroProbe Inc.) as previously described (Falk *et al.*, 1980) with a slight modification. Briefly, 4×10^5 THP-1 cells in 200 μl of RPMI-1640 medium supplemented with 10% foetal calf serum (FCS; Bio Wittaker, Walkerssville, Maryland, U.S.A.), 4 mM glutamine (Sigma Chemical Co., St. Louis, MO, U.S.A.) and 80 μg ml^{-1} gentamycin (Schering-Plough) were placed in the upper compartment with 20 ng ml^{-1} MCP-1 in 35 μl of the same medium in the lower

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compartment. The compounds to be tested were dissolved at 0.1 M in dimethyl sulphoxide (DMSO) and then serially diluted in the culture medium. The serially-diluted compounds (10 μl) were added to the upper compartment of each well, where the final concentration of DMSO was not more than 0.1%. DMSO itself had no effect on MCP-1-induced chemotaxis even at 0.1%. The chamber was incubated at 37°C in a humidified atmosphere of 5% CO_2 for 2 h and then the filter was removed, fixed and stained with Diff-Quick (Harleco, Gibbstown, NJ, U.S.A.). After removing the cells on the upper side of the filter, the filter was set to a microplate reader (*E* max; Molecular Devices), and the absorbance at 550 nm was measured. The number of cells migrating in response to MCP-1 was determined by measuring the difference in absorbance between the well in the presence of MCP-1 (± the compound tested) and the well in the absence of MCP-1. The inhibitory effect of the compound was expressed as % of control as follows: % of control = {(the number of cells migrating in response to MCP-1 in the presence of the compound tested) ÷ (the number of cells migrating in response to MCP-1 in the absence of the compound tested)} × 100.

Measurement of intracellular cyclic AMP accumulation

The amount of cyclic AMP accumulated in THP-1 cells was measured with ELISA after extraction according to a previously described method (Severn *et al.*, 1992) with a slight modification. Briefly, 1×10^6 THP-1 cells in a total volume of 250 μl of RPMI-1640 medium supplemented with 10% FCS were aliquoted in Eppendorf tubes in the presence of 2 mM 3-isobutyl-1-methylxanthine (IBMX) (Sigma Chemical Co., St. Louis, MO, U.S.A.), an inhibitor of phosphodiesterase. Various concentrations of PGs to be tested were added and incubated at 37°C in a humidifed atmosphere of 5% CO_2 for 10 min. The reaction was stopped by placing the tubes in a heat block at 98°C for 10 min. The tubes were then clarified by centrifugation at 10,000 *g* for 15 min. The amount of cyclic AMP in the supernatant was determined with a ELISA kit (Amersham International plc, Amersham, UK) according to the manufacturer's instructions. The background amount of cyclic AMP in the absence of PGs (IBMX alone), which was approximately 1 pmol/10^5 cells, was subtracted. ED_{50} was defined as the drug concentration required to induce 50% of the amount of cyclic AMP produced by 10^{-6} M PGE_1 in each experiment.

Measurement of Ca^{2+} influx

Intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) was measured as described previously (Yamagami *et al.*, 1994). Briefly, THP-1 cells were incubated with 1 mM Fura-2 AM in HEPES-Tyrode buffer at 37°C for 30 min, and then harvested, washed, and resuspended (2×10^6 cells ml^{-1}) in HEPES-Tyrode buffer containing 0.1 mM $CaCl_2$. The cells were further incubated at 37°C for 15 min, and kept at room temperature until used. Fura-2 fluorescence was measured in a CAF-100 fluorometer (JASCO Corporation, Japan). Samples were excited at 340 nm or 380 nm. Emission at 500 nm was continuously recorded. $[Ca^{2+}]_i$ were calculated as described previously (Sozzani *et al.*, 1993).

Materials

PGE_1, PGE_2, PGA_1, PGA_2, $PGF_{2\alpha}$ and PGI_2 were purchased from Funakoshi Pharmaceutical Co. (Tokyo). The methyl ester of PGE_1 was from Sigma Chemical Co. (St. Louis, MO, U.S.A.). TEI-6122, (17R)-17, 20-dimethyl-7-thia-PGE_1 methyl ester, was synthesized in our laboratories (Tanaka *et al.*, 1985). Isocarbacyclin was also synthesized in our laboratories (Shibasaki *et al.*, 1983). Stock solution of prostanoids (0.1 M) were prepared in dimethyl sulphoxide (DMSO). *E. coli*-derived recombinant human MCP-1 was purchased from Peprotech, Inc. (Rocky Hill, NJ, U.S.A.).

Statistical analysis

Results were analysed for statistical significance by Student's *t* test for unpaired observations.

Results

MCP-1-induced chemotaxis

We studied the inhibitory activities of various PGs including TEI-6122, a synthetic 7-thia-PGE_1 derivative, against chemotaxis induced by MCP-1 in a human monocytic leukaemia cell line, THP-1. The concentration of MCP-1 used was 20 ng ml^{-1}, submaximal for the induction of chemotaxis in THP-1 cells, since the *E. coli*-derived MCP-1 used gave a maximal chemotactic response in THP-1 cells at a concentration of 100 ng ml^{-1} as we have recently reported (Ishii *et al.*, 1995). TEI-6122 inhibited MCP-1-induced THP-1 cell chemotaxis with an IC_{50} of 1.5 ± 0.88 pM, while the IC_{50} of PGE_1 was 1.4 ± 0.25 nM (Figure 1). Thus, TEI-6122 was 1000 fold more potent than PGE_1. We also studied the inhibitory activity of the methyl ester of PGE_1, since TEI-6122 is a methyl ester derivative of 7-thia-PGE_1. The methyl ester was approximately 10 fold less potent than the parent PGE_1 (data not shown). Figure 2 shows the inhibitory activities of authentic PGs against MCP-1-induced THP-1 cell chemotaxis. Thus, PGE_2 was as potent (IC_{50} = 0.9 nM) as PGE_1, while PGI_2 was much less potent (IC_{50} = 30 μM) than PGE_1. A stable PGI_2 analogue, isocarbacyclin, was approximately 10 fold more potent than PGI_2 (data not shown). On the other hand, PGA_1, PGA_2 and $PGF_{2\alpha}$ showed relatively weak inhibitory activities in MCP-1-induced THP-1 cell chemotaxis (IC_{50} = 1.3 μM, 2.0 μM and 6.0 μM, respectively).

Cyclic AMP accumulation

Figure 3 shows the dose-response curves of TEI-6122 and PGE_1 in terms of cyclic AMP accumulation in THP-1 cells. The increase in intracellular cyclic AMP induced by TEI-6122 was very similar to PGE_1. To confirm the similarity of the cyclic AMP-inducing capability between TEI-6122 and PGE_1, we further studied the kinetics of cyclic AMP accumulation induced by either TEI-6122 or PGE_1 in THP-1 cells. At either 10^{-6} M or 10^{-7} M, TEI-6122 and PGE_1 showed similar ki-

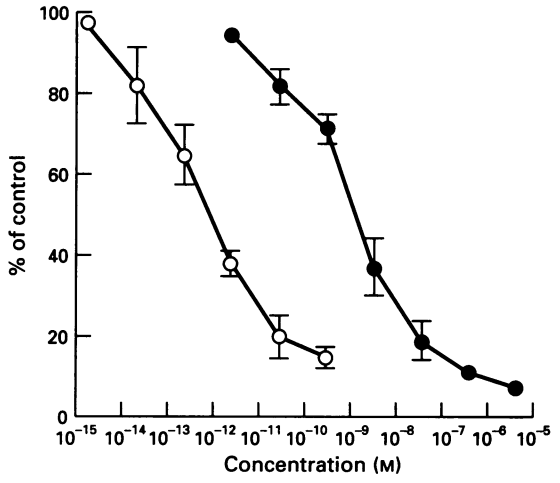

Figure 1 The inhibitory effects of TEI-6122 and PGE_1 on MCP-1-induced chemotaxis. THP-1 cell chemotaxis induced by 20 ng ml^{-1} MCP-1 was produced in the absence or the presence of TEI-6122 (○) and PGE_1 (●). The number of migrated cells in the absence of the compounds was treated as 100% chemotaxis. Values are the means ± s.e.mean from 4 (PGE_1) or 5 (TEI-6122) separate experiments, each performed in triplicate.

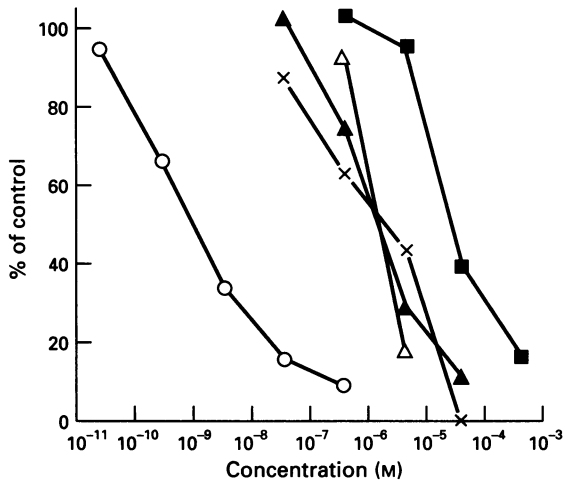

Figure 2 The inhibitory effects of authentic prostaglandins on MCP-1-induced chemotaxis. THP-1 cell chemotaxis induced by 20 ng ml^{-1} MCP-1 was produced in the absence or the presence of PGE_2 (○), $PGF_{2\alpha}$ (X), PGA_1 (▲), PGA_2, (Δ), and PGI_2 (■). The number of migrated cells in the absence of the compounds was treated as 100% chemotaxis. Values are the means from two separate experiments, each performed in triplicate.

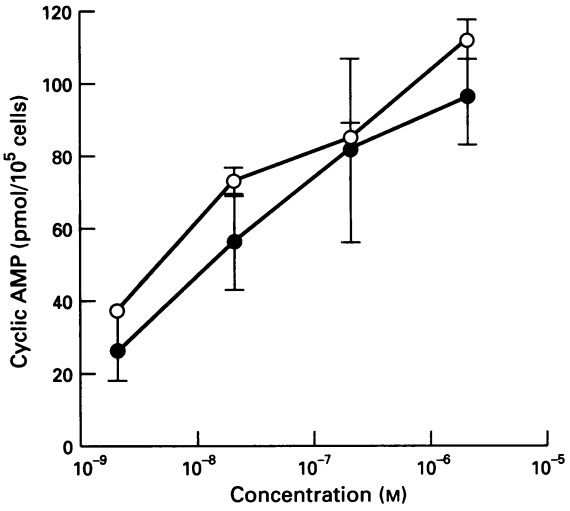

Figure 3 Cyclic AMP accumulation induced by TEI-6122 and PGE_1 in THP-1 cell. The concentration of cyclic AMP was measured after 10 min incubation of THP-1 cells with various concentrations of TEI-6122 (○) and PGE_1 (●). Values are the means ± s.e.mean from 9 (TEI-6122) or 10 (PGE_1) separate experiments, each performed in duplicate.

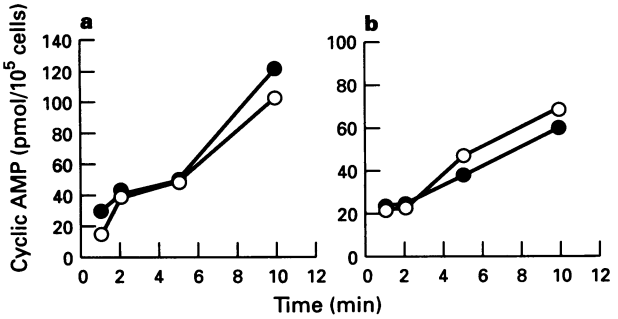

Figure 4 The kinetics of cyclic AMP accumulation induced by TEI-6122 and PGE_1 in THP-1 cell. THP-1 cells were incubated with TEI-6122 (○) and PGE_1 (●) at 10^{-6} (a) and 10^{-7} M (b). The incubation time varied from 1 min to 10 min. Each point is the mean of duplicate samples.

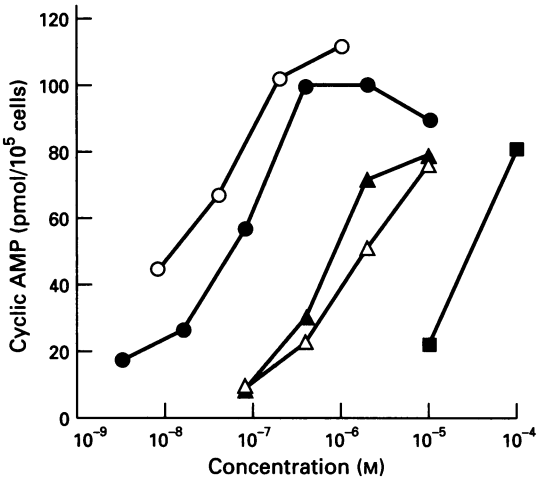

Figure 5 Cyclic AMP accumulation induced by prostaglandins in THP-1 cell. The concentration of cyclic AMP was measured after 10 min incubation of THP-1 cells with various concentrations of PGE_1 (●), PGE_2 (○), PGA_1 (▲), PGA_2, (Δ), and PGI_2 (■). All compounds were examined simultaneously in one experiment. Each point is the mean of duplicate samples.

Table 1 Ca^{2+} influx induced by TEI-6122 and PGE_1 in THP-1 cells

Test agents	$[Ca^{2+}]_i$(nM)
TEI-6122 (4 μM)	36.7 ± 3.4
PGE_1 (4 μM)	37.1 ± 2.8

TEI-6122 or PGE_1 was added to Fura-2-loaded THP-1 cells at 4 μM, and Fura-2 fluorescence was measured. Values are the mean ± s.e. mean (n = 8 from 2 separate experiments). The basal $[Ca^{2+}]_i$ was 19.5 nM (the mean from 2 separate experiments). The difference between both agents was not significant statistically ($P > 0.05$).

Table 2 The inhibitory effects of TEI-6122 and PGE_1 against MCP-1-induced Ca^{2+} influx in THP-1 cells

Pretreatment	$[Ca^{2+}]_i$(nM)	*% of control*
None	40.1 ± 5.5	100
TEI-6122	28.9 ± 4.8	72.1
PGE_1 (4 μM)	26.2 ± 4.0*	65.3

Fura-2-loaded THP-1 cells were pretreated with 4μM TEI-6122 or PGE_1 for approximately 5 min, and then stimulated with 20 ng ml^{-1} MCP-1. Values are the mean ± s.e. mean ($n = 13$ from 4 separate experiments). The basal $[Ca^{2+}]_i$ was 13.9 ± 3.5 nM (the mean ± s.e. mean from 4 separate experiments). $[Ca^{2+}]_i$ after stimulation by MCP-1 without pretreatment of TEI-6122 or PGE_1 was treated as 100% (control). *Significantly ($P < 0.05$) different from control. The difference between TEI-6122 and PGE_1 was not significant statistically ($P > 0.05$).

netics of cyclic AMP accumulation during the first 10 min after stimulation (Figure 4). These results indicate that the 1000 fold difference in potency as inhibitors of MCP-1-induced THP-1 cell chemotaxis between TEI-6122 and PGE_1 cannot be ascribed to differences in the ability to increase cyclic AMP levels.

We also compared the potency of the other PGs studied in the chemotaxis assays with their potency in raising cyclic AMP. Figure 5 shows the intracellular cyclic AMP accumulation in THP-1 cells induced by the authentic PGs. PGE_1 and PGE_2 were more potent cyclic AMP-inducers ($ED_{50} = 50$ nM and 22 nM, respectively) than the other PGs. PGE_1 and PGE_2 were approximately 50 fold more potent than PGA_1 and PGA_2 ($ED_{50} = 0.8$ μM and 1.5 μM, respectively), while PGI_2 was ap-

proximately 50 fold less potent ($ED_{50}=40\ \mu M$) than PGA_1 and PGA_2. $PGF_{2\alpha}$ had only a marginal ability to induce intracellular cyclic AMP accumulation even at 100 μM (data not shown). Thus, the ED_{50} values of PGE_1 and PGE_2 in the cyclic AMP assay were approximately 30 fold higher than the IC_{50} values in the chemotaxis assay, while the potency of PGA_1, PGA_2, and PGI_2 in the cyclic AMP assay were very similar to their IC_{50} values in the chemotaxis assay.

Intracellular Ca^{2+} mobilization

We next studied the effects of TEI-6122 and PGE_1 on intracellular Ca^{2+} mobilization in THP-1 cells. TEI-6122 induced a similar increase in Ca^{2+} influx as PGE_1 did at a concentration of 4 μM (Table 1), while neither TEI-6122 nor PGE_1 affected Ca^{2+} mobilization at 0.2 μM. These Ca^{2+} responses occurred rapidly and then declined to the basal level within 5 min. Thus, we also studied the effect of pretreatment of THP-1 cells by either TEI-6122 or PGE_1 on the Ca^{2+} influx induced by MCP-1 (Table 2). TEI-6122 and PGE_1 inhibited Ca^{2+} influx induced by MCP-1 but the difference between the two was not significant statistically ($P>0.05$). Thus, there was no difference between TEI-6122 and PGE_1 in their ability either to induce Ca^{2+} influx in itself or to inhibit the Ca^{2+} influx induced by MCP-1. Moreover, the drug concentration needed to affect the intracellular Ca^{2+} mobilization was as high as 4 μM. Therefore, the ability of TEI-6122 to affect intracellular Ca^{2+} mobilization in THP-1 cells cannot account for the 1000 fold difference in potency between TEI-6122 and PGE_1 as inhibitors of MCP-1-induced THP-1 cell chemotaxis.

Discussion

In this study we have shown that TEI-6122, a synthetic 7-thia-PGE_1 derivative, is 1000 fold more potent as an inhibitor of MCP-1-induced THP-1 cell chemotaxis than either PGE_1 or PGE_2. The extremely potent inhibitory activity of TEI-6122 in the MCP-1-induced THP-1 cell chemotaxis assay prompted us to investigate the mechanism for such an effect. It seems likely that TEI-6122 exhibits its inhibitory activity on MCP-1-induced chemotaxis by interrupting the intracellular signal transduction pathway induced by MCP-1, since it is known that PGs of the E series affect Ca^{2+} influx and cyclic AMP accumulation in the cell via EP receptors (Sugimoto *et al.*, 1992; Honda *et al.*, 1993; Watabe *et al.*, 1993), and intracellular Ca^{2+} mobilization and cyclic AMP-dependent kinases are also involved in the mechanism of MCP-1-induced chemotaxis (Sozzani *et al.*, 1991; 1993; Yamagami *et al.*, 1994). Thus, we have investigated the involvement of intracellular cyclic AMP accumulation, presumably via an EP_2/EP_4 receptor and intracellular Ca^{2+} mobilization presumably via an EP_1 receptor. However, it is very unlikely that either mechanism can account for the extremely potent inhibition of THP-1 cell chemotaxis by TEI-6122, since the potency of TEI-6122 was very similar to PGE_1 with regard to both mechanisms. These results may suggest that an alternative mechanism distinct from that mediated via the EP_1 receptor or via the EP_2/EP_4 receptor is involved in the extremely potent inhibitory activity of THP-1 cell chemotaxis by TEI-6122. An alternative mechanism could also be involved in the inhibition of chemotaxis by PGE_1 and PGE_2, since PGE_1 and PGE_2 inhibited MCP-1-induced THP-1 cell chemotaxis at approximately 30 fold lower concentration than their ED_{50}s in the induction of intracellular cyclic AMP accumulation.

PGE_2 receptors have been classified pharmacologically into at least three types: EP_1, EP_2 and EP_3 receptors (Coleman *et al.*, 1987; Halushka *et al.*, 1989) and the EP_4 receptor has also been described very recently (Coleman *et al.*, 1994). The molecular cloning of the genes encoding the EP_1, EP_2 and EP_3 receptors has confirmed the pharmacological basis of EP receptor heterogeneity (Sugimoto *et al.*, 1992; Honda *et al.*, 1993; Watabe *et al.*, 1993), although two different genes corresponding to the human EP_2 receptor have been reported (An *et al.*, 1993; Regan *et al.*, 1994). To investigate further the mechanism by which TEI-6122 exhibits an extremely high potency in inhibiting MCP-1-induced THP-1 cell chemotaxis, it may be useful to examine the affinity of TEI-6122 for the EP receptor subtypes in detail.

It has been shown that PGs of the E series have a number of anit-inflammatory effects (Zurier & Quagliata, 1971; Fantone *et al.*, 1983; Kelly *et al.*, 1987), although the mechanisms of these anti-inflammatory effects are not clear. It has been recently reported that the administration of PGE_1 or a synthetic PGE_2 derivative decreases the content of macrophages in the kidney in either a rat renal allograft model or a rat glomerulonephritis model (Cattell *et al.*, 1990; Schreiner *et al.*, 1993). It has also been reported that mRNA expression for MCP-1 increases in rat cardiac allografts (Russell *et al.*, 1993) and in glomerulonephritis (Rovin *et al.*, 1994). Taken together, those reports support the idea that the *in vitro* inhibitory property of PGs of the E series against MCP-1-induced chemotaxis, as we presented in this paper, may account for the decrease of macrophage content in the kidney in the above models.

In conclusion, our results show that TEI-6122, a synthetic 7-thia-PGE_1 derivative, is an extremely potent inhibitor of MCP-1 induced chemotaxis of THP-1 cells, and there is a 1000 fold difference in potency between TEI-6122 and PGE_1. With respect to the mechanism, however, neither intracellular cyclic AMP accumulation nor changes in intracellular Ca^{2+} mobilization can account for this difference. Thus, either a novel EP receptor or a novel intracellular signal transduction system may be involved in mediating the effects of TEI-6122. The extremely potent activity of TEI-6122 as an inhibitor of MCP-1-induced chemotaxis could suggest a new therapeutic potential for TEI-6122 and PGs of the E series.

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