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Short communication

# Beraprost sodium, an analogue of prostacyclin, induces the expression of mitogen-activated protein kinase phosphatase and inhibits the proliferation of cultured mesangial cells

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**Abstract**

Beraprost sodium, an analogue of prostacyclin, increases intracellular cyclic adenosine monophosphate (cAMP) in cultured glomerular mesangial cells. We examined the effect of beraprost on mesangial cell proliferation. Beraprost was able to inhibit fetal bovine serum-stimulated proliferation of mesangial cells in concentrations enough to increase cellular cAMP. By northern blot analysis, beraprost induced the expression of MKP-1, a mitogen-activated protein kinase phosphatase, in a dose- and time-dependent manner, similarly to dibutyryl cAMP and adrenomedullin. These results indicate that beraprost inhibits the proliferation of mesangial cells and one of the mechanisms might be cAMP-dependent induction of MKP-1. © 1997 Elsevier Science B.V.

*Keywords:* Beraprost sodium; Mitogen-activated protein kinase phosphatase 1 (MKP-1); Mesangial cell; cAMP

## 1. Introduction

The proliferation of glomerular mesangial cells is considered to be one of the major histological characteristics of various glomerular diseases. Thus, the pharmacological intervention of the proliferation of mesangial cells may be important in the treatment of glomerular diseases. The agents which increase cellular cAMP are known to inhibit the proliferation of mesangial cells (Menè and Dunn, 1990). We have shown that beraprost sodium, a stable and orally active prostacyclin (prostaglandin $I_2$) analogue (Hirano et al., 1992), is able to increase cellular cAMP in mesangial cells (Haneda et al., 1996). Therefore, it might be reasonable to hypothesize that beraprost sodium could inhibit the proliferation of mesangial cells.

Mitogen-activated protein kinase (MAPK) was found to play a central role in cell proliferation (Seger and Krebs, 1995) and the phosphorylation of MAPK is necessary for its activation (Anderson et al., 1990). Recently, several laboratories have identified a family of inducible protein phosphatases, MKP-1/CL100/HVH1 (human homologue of vaccinia H1 gene product)/erp (externally regulated phosphatase) and PAC-1 (phosphatase of activated cells-1), with dual protein-tyrosine/threonine specificity and selectivity for MAPK (Sun et al., 1993). The constitutive expression of MKP-1 was found to inhibit cell proliferation (Sun et al., 1993). We have reported that atrial natriuretic peptide (ANP), a cGMP-rising agent, inhibits the proliferation of mesangial cells by inactivating MAPK through the induction of MKP-1 (Sugimoto et al., 1996).

From these observations, we hypothesized that beraprost sodium could induce the expression of MKP-1 and thus inhibit the proliferation of mesangial cells. To prove this hypothesis, we examined the effect of beraprost sodium on the proliferation of mesangial cells and the expression of MKP-1.

## 2. Materials and methods

### 2.1. Mesangial cell culture

Glomerular mesangial cells were obtained from a culture of glomeruli, isolated from male Sprague–Dawley rats

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weighing 100 to 150 g by sieving method, in RPMI 1640 medium containing 20% fetal bovine serum and antibiotics as previously described (Kikkawa et al., 1987). Cells at passage 4 to 8 were used for the following experiments.

### 2.2. Determination of thymidine incorporation

Thymidine incorporation was measured as previously described (Koya et al., 1993). In brief, the cells were incubated in RPMI 1640 with 0.4% fetal bovine serum for 48 h at 37°C in a $CO_2$ incubator. The medium was replaced by RPMI 1640 with 0.4% fatty acid free bovine serum albumin, and the cells were exposed to 1.5% fetal bovine serum with or without various concentrations of beraprost sodium. After 20 h, [$^3$H]thymidine was added to a final concentration of 1.5 μCi/ml and the incubation was continued for an additional 4 h at 37°C in a $CO_2$ incubator. Cells were cooled on ice, washed twice with phosphate-buffered saline (PBS), twice with 10% trichloroacetic acid, and once with 95% ethanol, and solubilized with 1 M NaOH. The radioactivity remaining within the cells was determined in a liquid scintillation counter.

### 2.3. Northern blot analysis for the determination of MKP-1 expression

Confluent cells were made quiescent by reducing the concentrations of fetal bovine serum to 0.4% for 48 h. The quiescent cells were incubated in an incubation medium (RPMI 1640 medium with 0.4% bovine serum albumin and 20 mM Hepes, pH 7.4) at 37°C for 30 min. The cells were then exposed to various agents in an incubation medium at 37°C for indicated time interval. The reactions were stopped by rapid aspiration of the medium and by washing twice with ice-cold PBS. Northern blot analysis was performed as previously described (Sugimoto et al., 1996). In brief, 10 μg of total RNA extracted using TRIzol Reagent (Gibco BRL, Gaitherburg, MD, USA) was electrophoresed through a 1% agarose formaldehyde gel and transferred on nylon filter (Nytran, Schleicher & Schuell, Dassel, Germany). The filter was prehybridized in Church buffer (500 mM $NaPO_4$, pH 7.0, 7% sodium dodecyl sulfate, 1% bovine serum albumin, 1 mM EDTA) at 65°C for 2 h and hybridized with $1 \times 10^6$ cpm/ml radiolabeled HVH1 cDNA, a human homologue of 3CH134, at 65°C for 24 h in a rotating hybridization oven. The filter was washed twice with wash buffer A (40 mM $NaPO_4$, pH 7.0, 5% sodium dodecyl sulfate, 0.5% bovine serum albumin, 1 mM EDTA) for 10 min at 65°C, twice with wash buffer B (40 mM $NaPO_4$, pH 7.0, 1% sodium dodecyl sulfate, 1 mM EDTA) in the same conditions, and exposed to Kodak XAR film with intensifying screen at −70°C. The filter was then rehybridized with $^{32}$P-labeled glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA.

### 2.4. Materials

Beraprost sodium was kindly donated by Yamanouchi Pharmaceutical (Tokyo, Japan), dibutyryl cAMP and

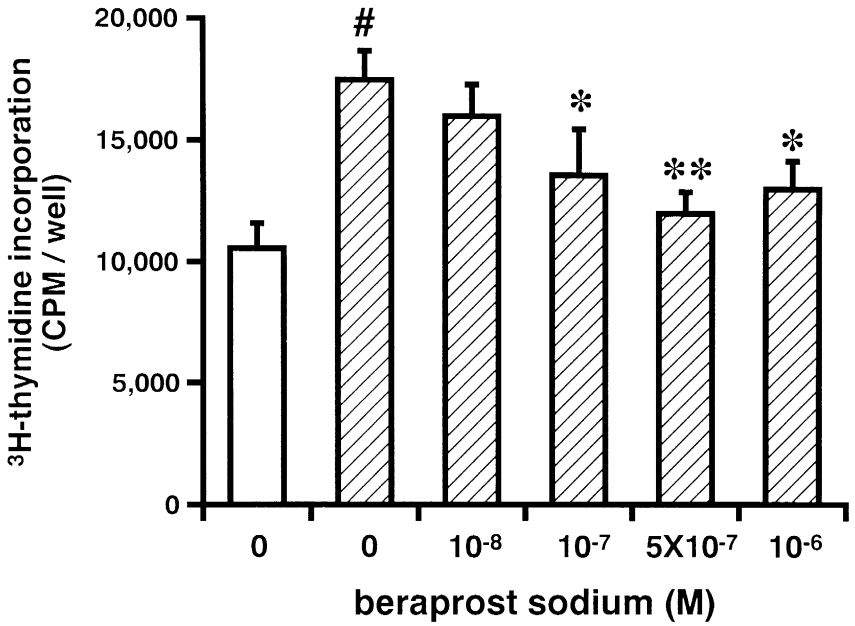


Fig. 1. Effect of beraprost sodium on fetal bovine serum-induced thymidine incorporation into mesangial cells. The quiescent cells were exposed to 1.5% fetal bovine serum with or without various concentrations of beraprost sodium. After 20 h incubation, [$^3$H]thymidine was added to a final concentration of 1.5 μCi/ml and the incubation was continued for an additional 4 h. Basal incorporation of thymidine (without fetal bovine serum) is shown in the left open column and fetal bovine serum-stimulated incorporation is shown in the hatched column. Values are mean ± SD, $n = 3$. $^{\#}$ $P < 0.01$ vs. basal, $^{*}$ $P < 0.05$, $^{**}$ $P < 0.01$ vs. 1.5% fetal bovine serum-stimulated thymidine incorporation without beraprost sodium.

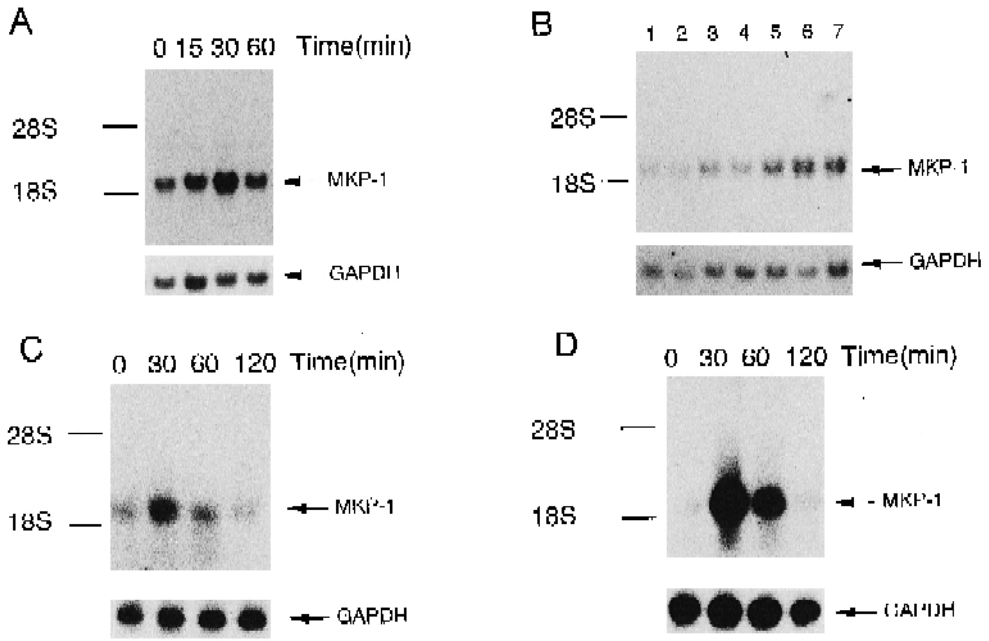


Fig. 2. Beraprost sodium-induced expression of MKP-1 in mesangial cells. The quiescent mesangial cells were treated with $10^{-6}$ M beraprost sodium for the indicated time interval (A), various concentrations of beraprost sodium (lane 1: without beraprost sodium, lane 2: $10^{-8}$ M, lane 3: $10^{-7}$ M, lane 4: $2 \times 10^{-7}$ M, lane 5: $5 \times 10^{-7}$ M, lane 6: $10^{-6}$ M, and lane 7: $10^{-5}$ M) for 30 min (B), 1 mM dibutyryl cAMP for the indicated time interval (C), or 100 nM adrenomedullin for the indicated time interval (D). Total RNA (10 μg) was subjected to northern blot analysis. The filter was hybridized with $^{32}$P-labeled HVH-1 cDNA and then rehybridized with $^{32}$P-labeled GAPDH cDNA as indicated.

adrenomedullin were purchased from Sigma (St. Louis, MO, USA) and [$\alpha$-$^{32}$P]dCTP (3000 Ci/mmol) was purchased from Dupont NEN Research Products (Boston, MA, USA). HVH-1 cDNA was kindly provided by Dr. K.-L. Guan, University of Michigan, and human GAPDH cDNA was a gift from Dr. P. Killen, University of Michigan. All other reagents were chemical grade and purchased from standard suppliers.

### 2.5. Statistical analysis

Results were expressed as mean ± S.D. Analysis of variance followed by Fisher's comparison was used for multiple comparisons.

## 3. Results

In order to evaluate the effect of beraprost sodium on the proliferation of mesangial cells, fetal bovine serum-stimulated incorporation of [$^{3}$H]thymidine into the cells was examined. As shown in Fig. 1, beraprost sodium inhibited fetal bovine serum-stimulated thymidine incorporation into mesangial cells in a dose-dependent manner with a significant inhibition at the concentrations from $10^{-7}$ to $10^{-6}$ M, the concentrations enough to increase cellular cAMP (Haneda et al., 1996).

To know the mechanism of the growth inhibitory effect of beraprost sodium, we next examined whether MKP-1 mRNA could be induced in mesangial cells by the beraprost sodium. As shown in Fig. 2A, beraprost sodium was able to rapidly induce MKP-1 mRNA when examined by northern blot analysis using HVH-1 cDNA, a human homologue of 3CH134, as a probe and the maximal induction was obtained at 30 min (Fig. 2A). Beraprost sodium-induced MKP-1 mRNA expression was observed in a dose-dependent manner of beraprost sodium with a maximal stimulation at $10^{-6}$ M (Fig. 2B). In order to know whether beraprost sodium-induced expression of mRNA expression would depend on cAMP, we next examined the effects of dibutyryl cAMP, a cell permeable analogue of cAMP, and adrenomedullin, a cAMP-rising agent, on the expression of MKP-1 in cultured mesangial cells. As shown in Fig. 2C and D, both dibutyryl cAMP (1 mM) and adrenomedullin (100 nM) were also able to increase the expression of MKP-1 mRNA in a time-course similar to beraprost sodium.

## 4. Discussion

The present results clearly indicate that beraprost sodium is able to inhibit FBS-induced thymidine incorporation and to induce the expression of MKP-1 mRNA in cultured glomerular mesangial cells. Dibutyryl cAMP, a cell permeable analogue of cAMP, or adrenomedullin, a cAMP-rising agent, was also able to induce the expression of MKP-1 mRNA in a similar time-course as beraprost sodium. Therefore, beraprost sodium might induce the expression of MKP-1 through a cAMP-dependent mechanism.

Beraprost sodium, an analogue of prostacyclin (prostaglandin $I_2$), has been reported to inhibit the proliferation in aortic smooth muscle cells (Koh et al., 1993). The present study confirmed this growth inhibitory effect of beraprost sodium on other types of cells, glomerular mesangial cells, in concentrations enough to stimulate cAMP production (Haneda et al., 1996). Although other agents which increase intercellular cAMP have been reported to inhibit the proliferation of mesangial cells (Menè and Dunn, 1990), the mechanism of the inhibitory effect of cAMP-rising agents on mesangial cell proliferation has not been fully clarified yet.

Since MAPK plays a central role in the proliferation of various types of cells, the inhibition of MAPK may be one of the candidates for the mechanisms of the inhibition of cellular proliferation. The agents which increase cellular cAMP have been reported to inhibit the activation of MAPK induced by various mitogenic stimuli (Seger and Krebs, 1995). We have also reported that cAMP-rising agents including beraprost sodium are able to inhibit endothelin-1 or phorbol ester-induced activation of MAPK in mesangial cells when the cells were treated with cAMP-rising agents for only 10 min (Haneda et al., 1996). In these reports, cAMP was proposed to inhibit the activation of MAPK by preventing Ras-dependent activation of Raf-1 possibly through the phosphorylation of Raf-1.

The activities of MAPK could be regulated not only by the phosphorylation and activation but also by the dephosphorylation. MKP-1, an inducible protein phosphatase, has dual protein-tyrosine/threonine specificity and selectivity for MAPK (Sun et al., 1993). The constitutive expression of MKP-1 was found to attenuate serum- or oncogenic ras-induced MAPK activation and to inhibit cell proliferation, suggesting that the dephosphorylation of MAPK in vivo by MKP-1 could have a negative effect on cell proliferation (Sun et al., 1993). In the present study, beraprost sodium was able to induce the expression of MKP-1 mRNA in mesangial cells. The effect of beraprost sodium was mimicked by dibutyryl cAMP or adrenomedullin. Therefore, in glomerular mesangial cells, cAMP could have a dual inhibitory effect on MAPK: one is the inhibition of the activation of MAPK as a short-term effect (Haneda et al., 1996) and the other is the dephosphorylation of MAPK through the induction of MKP-1 as a relatively long-term effect shown in the present study. We have reported similar dual inhibitory effects of ANP or cGMP on MAPK in mesangial cells (Sugimoto et al., 1996). Thus, cyclic nucleotides might have common dual inhibitory effects on MAPK and inhibit cellular proliferation, although the precise mechanism might be slightly different between cAMP and cGMP (Haneda et al., 1996).

In conclusion, our results suggest that beraprost sodium could inhibit cellular proliferation through both the inhibition of the activation of MAPK and cAMP-dependent induction of the expression of MKP-1 in cultured mesangial cells.

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