Prostacyclin analogs inhibit fibroblast migration

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Received 5 November 2001; accepted in final form 21 March 2002

Prostacyclin (PGI2) is a potent mediator in the coagulation and inflammatory processes. The current study was designed to evaluate the effect of three stable analogs of prostacyclin, carbaprostacyclin, ciprostene, and dehydro-15-cyclohexyl carbaprostacyclin (DHCC), on fibroblast chemotaxis. Human plasma fibronectin and platelet-derived growth factor (PDGF)-BB were used as chemoattractants. The mechanism by which these analogs might exert their effect also was evaluated.

MATERIALS AND METHODS

The prostacyclin analogs carbaprostacyclin, ciprostene, and DHCC were purchased from Cayman Chemical (Ann Arbor, MI). The protein kinase A (PKA) inhibitor KT-5720 was purchased from Calbiochem (San Diego, CA). KT-5720 (10−5 M) was dissolved in DMSO, and carbaprostacyclin (10−6 M), ciprostene (10−3 M), and DHCC (10−3) were separately dissolved in ethanol. PDGF-BB, purchased from R&D (Minneapolis, MN), was dissolved in 4 mM HCl with 0.1% BSA at 10 μg/ml. Tissue culture supplements and media, except fetal calf serum (FCS), were purchased from Invitrogen (Grand Island, NY). FCS was purchased from Biofluid (Rockville, MD).

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Fibronectin directed HFL-1 chemotaxis concentration dependently. When added to HFL-1, carbaprostacyclin (10⁻⁶ M) inhibited fibronectin-directed migration at all concentrations of fibronectin assessed (Fig. 1). Carbaprostacyclin’s inhibition of migration directed by fibronectin (20 μg/ml) was concentration dependent, reaching significance at 10⁻⁷ M (Fig. 2). Inhibition of chemotaxis was detectable at the highest concentrations after 4 h of incubation. After 6 h, the number of migrating cells did not increase in the presence of carbaprostacyclin but was still increasing in the control (Fig. 3). Ciprostene and DHCC (both 10⁻⁶ M), other prostacyclin analogs, also significantly inhibited fibroblast migration toward human fibronectin (Fig. 4). This inhibitory effect could not be attributed to cytotoxicity, since cell viability was unaffected by up to 10⁻⁵ M of the prostacyclin analogs examined, as detected by the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide assay (data not shown).

To examine whether the carbaprostacyclin effects were specific to fibronectin, we also used PDGF-BB as chemoattractant for HFL-1 migration. PDGF-BB stimulated HFL-1 chemotaxis until the prozone level for the chemoattractant was reached (100 ng/ml). Carba-prostacyclin (10⁻⁶ M) added with HFL-1 inhibited migration toward a range of PDGF-BB concentrations (Fig. 5A). Carbabrostacyclin inhibition of PDGF-BB-induced migration of HFL-1, moreover, was concentration dependent over a range similar to that which inhibited chemotaxis toward fibronectin (Fig. 5B).
To determine whether carbaprostacyclin inhibited chemokinesis, we used checkerboard analysis: varying concentrations of fibronectin were placed both above and below the filter in the blind well chamber. Cells in the chamber were incubated for designated time periods, then removed for staining and counting. Y-axis: fibroblast chemotaxis expressed as a percentage to 12-h time course without analog; x-axis: time in hours. Data are expressed as means ± SE from 3 experiments, each performed in triplicate (*P < 0.05 by Tukey procedure at each time period).

Fig. 3. Inhibition of fibroblast chemotaxis by the PGI$_2$ analog carbaprostacyclin: time course. Fibronectin (20 μg/ml) was used as the chemoattractant. Various concentrations of carbaprostacyclin were added to the fibroblasts in the top wells of the chemotaxis chamber. Cells in the chamber were incubated for designated time periods, then removed for staining and counting. Y-axis: fibroblast chemotaxis expressed as a percentage to 12-h time course without analog; x-axis: time in hours. Data are expressed as means ± SE from 3 experiments, each performed in triplicate (*P < 0.05 by Tukey procedure at each time period).

Fig. 4. Inhibition of fibroblast chemotaxis by 3 PGI$_2$ analogs. Fibronectin (20 μg/ml) was used as chemoattractant. Carparostacyclin (10$^{-6}$ M), ciprostene (10$^{-6}$ M), or dehydro-15-cyclohexyl carbaprostacyclin (DHCC, 10$^{-6}$ M) were added to fibroblasts immediately before cells were placed in the top wells. Y-axis: fibroblast chemotaxis as percentage of control; x-axis: PGI$_2$ analogs in the presence of fibronectin. Data shown are means ± SE from 3 experiments, each performed in triplicate (*P < 0.05 by t-test).

Fig. 5. Inhibition of fibroblast chemotaxis toward platelet-derived growth factor (PDGF) by the PGI$_2$ analog carbaprostacyclin. A: various concentrations of PDGF-BB were used as chemoattractant either with or without the addition of carbaprostacyclin (10$^{-6}$ M). Y-axis: fibroblast chemotaxis expressed as number of cells migrated/5 high-power fields; x-axis: concentration of PDGF-BB chemoattractant. B: PDGF-BB (10 ng/ml) was used as chemoattractant. Various concentrations of carbaprostacyclin were added to the fibroblasts immediately before cells were placed in the top wells of the chemotaxis chamber. Y-axis: fibroblast chemotaxis expressed as percentage of control; x-axis: carbaprostacyclin concentration. Data shown are means ± SE from 3 experiments, each performed in triplicate (*P < 0.05 by t-test at each paired concentration for A; by Tukey procedure for B).
Table 1. Checkerboard analysis of cell migration stimulated by fibronectin: effect of the prostacyclin analogue carbaprostacyclin

<table>
<thead>
<tr>
<th>Fibronectin Concentration Below Membrane, µg/ml</th>
<th>Fibronectin Concentration Above Membrane, µg/ml</th>
<th>Control</th>
<th>Carprofostacyclin, 10⁻⁷ M</th>
<th>Carprofostacyclin, 10⁻⁷ M</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1 ± 1</td>
<td>7 ± 4</td>
</tr>
<tr>
<td>0.8</td>
<td>0.8</td>
<td>1 ± 1</td>
<td>3 ± 2</td>
<td>19 ± 7</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>2 ± 1</td>
<td>26 ± 7</td>
<td>40 ± 10</td>
</tr>
<tr>
<td>20</td>
<td>20</td>
<td>17 ± 2</td>
<td>119 ± 9</td>
<td>136 ± 12</td>
</tr>
</tbody>
</table>

Values are means ± SE.

cells with the PKA inhibitor KT-5720 (10⁻⁷ M) for 1 h before harvesting for chemotactic evaluation. KT-5720 alone had no effect on chemotaxis toward fibronectin. In contrast, the PKA inhibitor attenuated the carbaprostacyclin inhibition of fibroblast chemotaxis to fibronectin (Fig. 6).

DISCUSSION

The current study demonstrates that the prostacyclin analog carbaprostacyclin is capable of inhibiting fibroblast chemotaxis to both human fibronectin and PDGF-BB in a concentration-dependent manner. The inhibitory effect on fibroblast chemotaxis was further confirmed with other prostacyclin analogs, ciprostene and DHCC. Checkerboard analysis verified that both chemotaxis and chemokinesis were affected. Moreover, carbaprostacyclin’s effect was blocked by KT-5720, an inhibitor of PKA, suggesting that inhibition is mediated by the PKA pathway.

The accumulation of fibroblasts is an important aspect of tissue repair after injury. This accumulation can occur through both chemotactic recruitment and proliferation within the wound. Several mediators, functioning as either stimulators or as inhibitors, can regulate these processes (24–26). In this regard, fibronectin, a multifunctional glycoprotein, and PDGF-BB are both potent fibroblast chemoattractants (24, 25), as well as contributors to fibroblast proliferation (4, 13). Both have been suggested to play important roles in normal wound healing and in the development of fibrotic scars. Inhibitors of fibroblast recruitment and proliferation have also been described, including cigarette smoke (22) and prostaglandin E₂ (15). It is likely that whether repair processes result in restoration of normal tissue function or in excessive accumulation of fibroblasts with resulting scar depends on the balance between inhibitory and stimulatory signals. The current study demonstrates that prostacyclin can function as an inhibitor of fibroblast chemotaxis directed by either fibronectin or PDGF-BB.

Prostacyclin is an arachidonic acid metabolite released from a variety of cell types including mast cells, endothelial cells, and fibroblasts (28, 30, 31). Prostacyclin is a potent regulator of vascular functions. Its vascular effects are generally antagonized by thromboxane A₂ (TxA₂). The balance between prostacyclin and thromboxane, therefore, has been suggested to be an important determinant of coagulability (5, 17). This balance may also contribute to fibroblast recruitment to the site of injury. Although without chemotactic activity on its own, the TxA₂ agonist U-46619 has been shown to potentiate fibroblast chemotaxis toward fibronectin (16).

The production of prostacyclin by endothelial cells is believed to play an important role in regulating acute vascular events. Physiological roles played by prostacyclin production from fibroblasts is less clearly defined. The current study suggests that prostacyclin may also play a role in the balance of mediators that regulate mesenchymal cell participation in repair responses.

Prostacyclin is capable of interacting with IP receptors causing activation of adenylate cyclase and increased levels of cAMP (6, 23). Carbaprostacyclin, ciprostene, and DHCC are stable analogs of prostacyclin and capable of inhibiting ADP-induced human platelet aggregation (1, 2, 14). In the current study, the effect of carbaprostacyclin on fibroblast migration was blocked by a PKA inhibitor, suggesting that the inhib-
and dibutyryl-cAMP (10, 15). This is consistent with a pro-inflammatory effect of carboprostacyclin is mediated through a stable prostacyclin analog. Prostaglandins Med 5: 307–320, 1980.


The current study, in summary, demonstrates that prostacyclin analogs, particularly carboxyprostacyclin, can inhibit fibroblast chemotaxis and chemokinesis. Through such a mechanism, prostacyclin could contribute to the modulation of profibrotic stimuli and, therefore, play an important role in controlling fibrotic responses. The authors acknowledge the excellent secretarial support of Lillian Richards and the editorial assistance of Mary C. Tourak.