Effects of Sulfhydryl Compounds on Interleukin-1-Induced Vascular Endothelial Growth Factor Production in Human Synovial Stromal Cells

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We investigated the effects of various sulfhydryl compounds on interleukin-1 (IL-1)-induced vascular endothelial growth factor (VEGF) production in human synovial stromal cells (HSC). HSC stimulated by IL-1β (100 ng/ml) produced VEGF and interleukin-6 (IL-6) in vitro. Monosulfhydryl compounds, N-acetylcysteine, p-penicillamine, tiopronin and the buctillamine-like disulfhydryl compound, compound A, scarcely affected VEGF or IL-6 production at concentrations of 10⁻⁵ and 10⁻⁴ M. However, the disulfhydryl compound, buctillamine inhibited VEGF production but not IL-6 production at concentrations of 10⁻⁵ and 10⁻⁴ M. These results suggest that buctillamine may be a selective inhibitor of IL-1-induced VEGF production in HSC, and that inhibition of VEGF production may require not only SH groups but also a specific chemical structure.

Key words sulfhydryl compound; vascular endothelial growth factor; synovial stromal cell; buctillamine

Several disease-modifying anti-rheumatic drugs (DMARDs) have been used to control rheumatoid arthritis (RA). While the majority of these DMARDs act as immunomodulatory drugs in RA, some also inhibit the angiogenic process.¹⁻³ A number of angiogenic factors are involved in the neovascularization process in RA joints. These factors, which stimulate vascular endothelial cells in both autocrine and paracrine manners, include acidic fibroblast growth factor, basic fibroblast growth factor, platelet-derived endothelial growth factor and vascular endothelial growth factor (VEGF).⁴⁻⁵ VEGF is expressed by sub synovial macrophages and synovial lining cells in the synovial tissue of RA patients,⁶ and by cultured synovial cells under hypoxic conditions or stimulation by interleukin-1 (IL-1).⁷ In addition, Nagashima et al.⁸ reported that buctillamine, one of the DMARDs, inhibited LPS-induced VEGF production in cultured rheumatoid synovial cells.

As the chemical structure of some DMARDs including p-penicillamine and buctillamine contains SH groups, in the present study, we investigated the effects of sulfhydryl compounds including N-acetylcysteine, p-penicillamine, tiopronin, buctillamine and compound A (Fig. 1) on IL-1-induced VEGF production in human synovial stromal cells (HSC).

MATERIALS AND METHODS

Reagents Recombinant human IL-1β (Genzyme, Cambridge, MA, U.S.A.), N-acetylcysteine (Sigma, St. Louis, MO, U.S.A.), and D-penicillamine (Sigma) were purchased from the sources shown. Tiopronin, buctillamine and compound A were synthesized by the Central Research Laboratories of Santen Pharmaceutical Co., Ltd. Chemical structures of these compounds are shown in Fig. 1. Tiopronin, buctillamine and compound A were identified by their physical data (IR and NMR).

Cell Line and Cell Culture HSC, initiated from normal human synovial tissue, were obtained from the Applied Cell Biology Research Institute (Kirkland, WA, U.S.A.), and were grown in CS-C medium (10% serum: Applied Cell Bi-

Fig. 1. Chemical Structures of Sulfhydryl Compounds
Table 1. Effects of Sulphydryl Compounds on IL-1\-Induced VEGF and IL-6 Production in HSSC (% Inhibition)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration (m)</th>
<th>VEGF</th>
<th>IL-6</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-Acetylcysteine</td>
<td>10^{-3}</td>
<td>2.5</td>
<td>-9.3</td>
</tr>
<tr>
<td>N-Acetylcysteine</td>
<td>10^{-4}</td>
<td>-5.4</td>
<td>3.5</td>
</tr>
<tr>
<td>N-Acetylcysteine</td>
<td>10^{-5}</td>
<td>-0.2</td>
<td>5.1</td>
</tr>
<tr>
<td>N-Acetylcysteine</td>
<td>10^{-6}</td>
<td>-6.4</td>
<td>1.4</td>
</tr>
<tr>
<td>Tiopronin</td>
<td>10^{-3}</td>
<td>14.1</td>
<td>5.7</td>
</tr>
<tr>
<td>Tiopronin</td>
<td>10^{-4}</td>
<td>-4.5</td>
<td>-0.1</td>
</tr>
<tr>
<td>Tiopronin</td>
<td>10^{-5}</td>
<td>46.1**</td>
<td>3.1</td>
</tr>
<tr>
<td>Tiopronin</td>
<td>10^{-6}</td>
<td>66.4**</td>
<td>11.8</td>
</tr>
<tr>
<td>Compound A</td>
<td>10^{-3}</td>
<td>-5.7</td>
<td>0.9</td>
</tr>
<tr>
<td>Compound A</td>
<td>10^{-4}</td>
<td>6.1</td>
<td>7.8</td>
</tr>
</tbody>
</table>

Values were obtained from 5 to 6 samples. Spontaneous or control VEGF production after 24h incubation was about 160 pg/ml or 470 pg/ml, respectively, and that of IL-6 production was about 40 pg/ml or 1050 pg/ml, respectively. ** Statistically significant when compared with the control group (Scheffe’s test, **p<0.01).

Fig. 2. Effects of Bucillamine on IL-1-Induced VEGF and IL-6 Production in HSSC
Bars represent the means±S.E. of 6 samples.

RESULTS AND DISCUSSION

In a preliminary study, HSSC were characterized as CD14+ CD23+ CD40+ CD54+ CD58+ CD80+ CD86+ CD95+ cells by FACs analysis (data not shown). This shows that cell adhesion molecules in HSSC are similar to RA-derived synovioocytes.7) HSSC stimulated by IL-1β (100 ng/ml) produced VEGF and IL-6 in vitro (Fig. 2). It has recently been reported that IL-1 activates nuclear factor (NF)\-κB and c-Jun NH2-terminal kinase via the MyD88 pathway.10,11) NF-κB and c-Jun activation is, therefore, important for IL-1-induced VEGF and IL-6 production.12,13) p42/p44 mitogen-activated protein kinase (ERK) and p38 mitogen-activated protein kinase (p38) are also important for IL-1-induced VEGF and IL-6 production.9,14)

In the present study, we investigated the effects of various sulfhydryl compounds on IL-1-induced VEGF production in HSSC. Monosulfhydryl compounds, N-acetylcysteine, \-penicillamine, tiopronin and the bucillamine-like disulfhydryl compound, compound A scarcely affected VEGF or IL-6 production at concentrations of 10^{-5} and 10^{-4} M (Table 1). However, the disulfhydryl compound, bucillamine inhibited VEGF production but not IL-6 production at both these concentrations (Table 1 and Fig. 2). The factate dehydrogenase release assay revealed no drug cytotoxicity (data not shown). These results are in contrast to those of a previous study concerning the effect of bucillamine on spontaneous IL-6 production in synovial cells from RA patients in vitro.15) Bucillamine inhibited spontaneous IL-6 production in synovial cells at concentrations under 10^{-5} M. Aono et al.16) also reported that bucillamine inhibited serum-induced IL-6 production in synovial cells from RA patients in vitro at a concentration of 10^{-4} M but not 10^{-5} M. One possible explanation for these controversial results could be differences in the study conditions including cells and stimulators.

We reported that bucillamine and N-acetylcysteine inhibited LPS-induced NF-κB activation and IL-6 production in monocyte cell lines at concentrations greater than 10^{-5} M.17) However, bucillamine inhibited IL-1-induced VEGF production at concentrations of 10^{-5} and 10^{-4} M in this study, while, N-acetylcysteine did not inhibit such production at these concentrations. While study conditions are different, these results suggest that the mechanism by which bucillamine inhibits IL-1-induced VEGF production does not involve the inhibition of NF-κB activation. As inhibition of p38 activation may prevent the production of many cytokines including VEGF and IL-6,9) it is unlikely that the mechanism by which bucillamine inhibits IL-1-induced VEGF production involves inhibition of p38 activation. Although inhibition of c-Jun or ERK activation could be a candidate as the mechanism of bucillamine action, further investigation is necessary to clarify this.

Moreover, bucillamine, but not compound A, inhibited IL-1-induced VEGF production at concentrations of 10^{-5} and 10^{-4} M. The only difference in chemical structure between bucillamine and compound A is the length of the hydrocarbon chain (Fig. 1). These results suggest that bucillamine may be a selective inhibitor of IL-1-induced VEGF production in HSSC, and that inhibition of this production may require not only SH groups but also a specific chemical structure. The inhibition of VEGF production and angiogenesis may be one of the anti-rheumatic mechanisms of bucillamine.

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