SHORT COMMUNICATION

Dexamethasone inhibits the production of thromboxane B₂ and leukotriene B₄ by human alveolar and peritoneal macrophages in culture

RICHARD W. FULLER, CHRISTOPHER R. KELSEY*, PETER J. COLE*, COLIN T. DOLLERY AND JOHN MacDERMOT

Department of Clinical Pharmacology, Royal Postgraduate Medical School, London, and *Host Defence Unit, Department of Medicine, Cardiothoracic Institute, Brompton Hospital, London

(Received 21 May 1984; accepted 2 July 1984)

Summary

1. Cultured human alveolar and peritoneal macrophages have been shown to release thromboxane B₂ and leukotriene B₄.
2. The release was facilitated by stimulation of the macrophages with opsonized zymosan A (1.2 mg/ml).
3. The release was inhibited in a concentration-dependent manner by incubation of the cells with dexamethasone (1 nmol/l to 1 μmol/l).

Key words: dexamethasone, human alveolar macrophages, human peritoneal macrophages, leukotriene B₄, thromboxane B₂.

Introduction

Corticosteroids are employed widely in therapeutics, although until recently their mechanism of action was unknown. Corticosteroids facilitate the transcription of macrogrenin, a protein inhibitor of phospholipase A₂ [1]. This enzyme causes the release of arachidonic acid from phospholipids, and is the rate limiting step in the formation of both the cyclo-oxygenase and lipoxygenase products of arachidonic acid metabolism. Inhibition of the synthesis of prostaglandins, leukotrienes and other potent inflammatory mediators, such as platelet activating factor, may account for the major anti-inflammatory action of corticosteroids [2]. This mechanism has been studied extensively in animals [1] and preparations of animal leucocytes [3], but has not been reported previously in preparations of human macrophages that have been purified to near homogeneity.

Macrophages are distributed widely in human lung [4] and the peritoneal cavity [5]. The number of macrophages increases during inflammation [6], and irreversible structural changes due to macrophage activity may underlie the pathology of some pulmonary diseases [7]. Human alveolar macrophages release products of both cyclo-oxygenase [8] and lipoxygenase [9] metabolism, and the most abundant metabolites of these enzymes are thromboxane B₂ and leukotriene B₄.

In the present study, the results show release of thromboxane B₂ and leukotriene B₄ from human alveolar and peritoneal macrophages, with inhibition of release by pretreatment of the cells with dexamethasone.

Materials and methods

Macrophages

Alveolar macrophages were collected from patients with chronic bronchitis during diagnostic bronchoscopy for suspected lung cancer. The procedure was undertaken with informed written consent. Bronchoalveolar lavage was performed with 200 ml of 0.9% (w/v) NaCl [10]. None of the
patients was receiving steroids or non-steroidal anti-inflammatory drugs.

Cells from the bronchoalveolar lavage were filtered through a fine nylon mesh, and washed twice with Dulbecco's phosphate buffered saline (no Ca²⁺ or Mg²⁺ ions) (PBS). The cells were then plated on 25 cm² culture flasks (Falcon Labware) in 7.0 ml of Dulbecco's modification of Eagle's minimum essential medium (DMEM, Gibco Bio-Cult), containing 10% (v/v) foetal calf serum (Gibco Bio-Cult), gentamicin sulphate (50 µg/ml; Sigma London Ltd) with or without dexamethasone (1 µmol/l; Sigma London Ltd). Flasks were maintained for 16 h in a humidified atmosphere at 37°C containing 10% CO₂. Microscopic examination (including electron microscopy) of adherent cells from bronchoalveolar lavage prepared by the same method as described have been shown [8] to contain >98% macrophages.

Initial experiments designed to examine the effect of increasing dexamethasone concentrations on thromboxane B₂ and leukotriene B₄ production by alveolar macrophages were unsuccessful because of the relative paucity of cells. Some experiments were therefore performed with peritoneal macrophages, which could be obtained in much greater numbers. Fluid drained from the peritoneal cavity during peritoneal dialysis usually contains few cells, hence peritoneal fluid samples were only selected when visibly turbid. Samples were washed three times in PBS. The cells were then cultured for 16 h in a humidified atmosphere at 37°C containing 10% CO₂. Microscopic examination (including electron microscopy) of adherent cells from bronchoalveolar lavage prepared by the same method as described have been shown [8] to contain >98% macrophages.

The cells from peritoneal dialysis fluid were resuspended in 10 ml of PBS, layered on 15 ml of Ficoll-Paque [5.7% (w/v) Ficoll 400, 9% (w/v) sodium diatrizoate; Pharmacia], and centrifuged at 400 g for 40 min at 18°C. Cells at the interface of the PBS and Ficoll-Paque were removed and washed three times in PBS. The cells from peritoneal dialysis fluid were resuspended in 10 ml of PBS, layered on 15 ml of Ficoll-Paque [5.7% (w/v) Ficoll 400, 9% (w/v) sodium diatrizoate; Pharmacia], and centrifuged at 400 g for 40 min at 18°C. Cells at the interface of the PBS and Ficoll-Paque were removed and washed three times in PBS. The cells were then cultured for 16 h in DMEM (with 10% foetal calf serum and 50 µg of gentamicin sulphate/ml) containing selected concentrations of dexamethasone. Microscopic examination of the cells from the gradient revealed them to be >99% mononuclear cells (macrophages and lymphocytes) and after prolonged culture the adherent cells were >95% macrophages (a few fibroblasts were observed).

Flasks containing either the alveolar or peritoneal cells were then washed three times with DMEM to remove non-adherent cells. The release of thromboxane B₂ and leukotriene B₄ from cultured macrophages was measured by incubation of cells for 3 h in 5 ml of DMEM in the presence or absence of 1.2 mg of opsonized zymosan A/ml. A flask treated in a similar manner but with no cells added was used as the medium blank for the radioimmunoassays.

**Opsonized zymosan A**

A suspension of zymosan A (20 mg/ml dry weight; Sigma London Ltd) in PBS was opsonized with pooled human serum by incubation at 37°C for 30 min. The opsonized zymosan was then washed three times in PBS.

**Measurement of thromboxane B₂ and leukotriene B₄**

At the end of the 3 h incubation, 4.5 ml of the incubation medium was removed and added to 0.45 ml of Tris-HCl buffer (0.5 mol/l) at pH 7.4. The samples were centrifuged at 3000 g for 5 min to remove the zymosan A particles, and the supernatants stored at -20°C.

Samples for analysis were thawed, and from each were taken two volumes of 2.2 ml. [³H]-Thromboxane B₂ (approx. 3000 c.p.m.) was added to one of the volumes and [³H]leukotriene B₄ (approx. 3000 c.p.m.) to the other volume. The fluid was acidified to pH 3.5 with 10% (v/v) formic acid and the arachidonic acid metabolites extracted in 8 ml of distilled ethyl acetate. The organic phase was evaporated to dryness under N₂. The residue was resuspended in 0.5 ml of water (leukotriene B₄) or the Tris-HCl buffer employed in the thromboxane B₂ radioimmunoassay [11]. An estimation of the recovery of leukotriene B₄ (mean 66.3%, range 41.2-78.5%) and thromboxane B₂ (mean 83.4%, range 64.0-94.0%) through the extraction procedure was made by counting 100 µl of the resuspended sample.

Thromboxane B₂ and leukotriene B₄ were measured by radioimmunoassay as reported previously [11, 12]. Results were corrected for recovery as described. The sensitivity of the leukotriene B₄ assay varied between 14 and 26 pg/assay tube, and that of the thromboxane B₂ assay was 2.6 to 7.6 pg/assay tube (confidence limit 2 sd). The leukotriene antibody was obtained from Wellcome Diagnostics (U.K.). The thromboxane antibody and the unlabelled eicosanoids were generous gifts from Dr P. V. Halushka and the Upjohn Co. respectively.

**N-Acetyl-β-D-glucosaminidase (EC 2.2.1.30) activity**

The method employed has been described previously [13]. The protein content of the cultured macrophages was determined by a modification of the method of Lowry et al. [14], and
Inhibition of arachidonates from human macrophages

revealed $3.59 \times 10^6$ cells/mg of protein (range 3.44-3.78 $\times 10^6$).

**Statistical analysis**

In the text results are expressed as mean ± 1 SD, and statistical analysis was performed by using paired Student's t-test where appropriate.

**Results**

**Thromboxane B$_2$**

There was a concentration-dependent reduction in the release of thromboxane B$_2$ by dexamethasone (1 nmol/l to 1 $\mu$mol/l) from both unstimulated peritoneal macrophages and those stimulated by opsonized zymosan A. The mean (± SD) IC$_{50}$ for the inhibition of thromboxane B$_2$ release by dexamethasone in three studies was $3.88 \pm 2.89$ nmol/l for the unstimulated cells, and $8.54 \pm 4.30$ nmol/l for those treated with zymosan A. The release (mean ± SD) of thromboxane B$_2$ from cells not treated with dexamethasone was 60.1 ($\pm 21.5$) and 186 ($\pm 45$) ng/mg of cell protein, in the absence or presence respectively of opsonized zymosan A.

Experiments with alveolar macrophages were limited to one concentration of dexamethasone because of the relative scarcity of cells. The results are shown in Table 1. There was a reduction in thromboxane B$_2$ production by both stimulated and unstimulated cells, and this change was significant for the unstimulated cells ($P < 0.025$). The results did not reach statistical significance for stimulated cells ($P < 0.1$), due partly to the wide variation in the amount of thromboxane B$_2$ produced.

**Leukotriene B$_4$**

The concentration of leukotriene B$_4$ in the supernatant of unstimulated peritoneal macrophages was below the sensitivity of the radioimmunoassay. In two experiments, leukotriene B$_4$ was detected after stimulation of the cells with zymosan A. In both there was a concentration-dependent reduction in leukotriene B$_4$ release. The IC$_{50}$ values for dexamethasone were 7.06 nmol/l and 2.45 nmol/l. The release (mean) of leukotriene B$_4$ from cells not treated with dexamethasone was $<0.2$ and 6.4 ng/mg of cell protein, in the absence or presence respectively of opsonized zymosan A.

The amount of leukotriene B$_4$ released from alveolar macrophages is shown in Table 1. This shows a reduction in leukotriene B$_4$ release after culture in 1 $\mu$mol of dexamethasone/l. A time course for the effect of dexamethasone on the

<p>| TABLE 1. Thromboxane B$_2$ and leukotriene B$_4$ released from alveolar macrophages |
|-------------------------------------|-----|---------|----------|</p>
<table>
<thead>
<tr>
<th>Addition</th>
<th>Experiment</th>
<th>Control</th>
<th>Dexamethasone</th>
<th>Reduction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thromboxane B$_2$ (ng/mg of protein)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>1</td>
<td>15.3</td>
<td>2.72</td>
<td>82.2</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>35.5</td>
<td>18.8</td>
<td>47.0</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>5.06</td>
<td>2.11</td>
<td>58.3</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>14.9</td>
<td>6.98</td>
<td>53.2</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>17.3</td>
<td>12.6</td>
<td>27.2</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td>17.6 ± 11.1</td>
<td>8.64 ± 7.06*</td>
<td>53.6</td>
</tr>
<tr>
<td>1.2 mg of opsonized zymosan A/ml</td>
<td>1</td>
<td>17.8</td>
<td>2.16</td>
<td>87.9</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>52.1</td>
<td>21.3</td>
<td>59.1</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>6.83</td>
<td>3.08</td>
<td>54.9</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>22.1</td>
<td>10.3</td>
<td>53.4</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>19.0</td>
<td>16.2</td>
<td>14.7</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td>23.6 ± 17.0</td>
<td>10.6 ± 8.27*</td>
<td>54.0</td>
</tr>
<tr>
<td>Leukotriene B$_4$ (ng/mg of protein)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>1</td>
<td>8.5</td>
<td>1.7</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>&lt;0.2</td>
<td>&lt;0.2</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>&lt;0.2</td>
<td>&lt;0.2</td>
<td>–</td>
</tr>
<tr>
<td>1.2 mg of opsonized zymosan A/ml</td>
<td>1</td>
<td>27.5</td>
<td>4.5</td>
<td>83.6</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2.83</td>
<td>1.27</td>
<td>55.1</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>34.1</td>
<td>23.4</td>
<td>31.4</td>
</tr>
</tbody>
</table>

* $P < 0.025$ (two-tailed paired Student's t-test).
release of leukotriene B₄ and thromboxane B₂ revealed no change in under 3 h, a 40% inhibition by 6 h and up to 88% inhibition by 16 h.

N-Acetyl-β-D-glucosaminidase activity and protein estimation

The concentration of N-acetylglucosaminidase was increased in the culture medium after stimulation of the macrophages by opsonized zymosan A. The released enzyme activity of the alveolar and peritoneal macrophages was similar, and dexamethasone caused no change in the release of enzyme.

There was no change in the protein content of the cultured macrophages with increasing dexamethasone concentrations in these experiments.

Discussion

Culture of human peritoneal and alveolar macrophages in the presence of dexamethasone results in a concentration-dependent fall in the release of both thromboxane B₂ and leukotriene B₄. The IC₅₀ for this response was between 1 and 10 nmol/l. These concentrations are within the range achieved in tissue during the clinical use of the drug [15]. The inhibition of arachidonic acid metabolism by corticosteroids has been reported with animal macrophages [3] but never before in human alveolar or peritoneal macrophages.

Human alveolar macrophages have been implicated in the pathogenesis of certain pulmonary diseases such as emphysema and asthma. Some of these conditions respond to treatment with corticosteroids. The demonstration that dexamethasone inhibits the release of two abundant inflammatory mediators may explain some of its therapeutic benefit. The mechanism for the inhibition of release of arachidonic acid metabolites by dexamethasone is probably by production of a protein inhibitor of phospholipase A₂ [1, 16]. The finding that the IC₅₀ values for the inhibition of thromboxane B₂ and leukotriene B₄ were similar is consistent with a single effector pathway for inhibition of the synthesis of both mediators.

In conclusion, human peritoneal and alveolar macrophages synthesize two important inflammatory mediators, namely thromboxane B₂ and leukotriene B₄. The release of both compounds is facilitated by the phagocytosis of opsonized zymosan A. Dexamethasone inhibits the release of thromboxane B₂ and leukotriene B₄, with no accompanying reduction in the release of the lysosomal hydrolase, N-acetyl-β-D-glucosaminidase.

References