Additional inhibitors of rat serum acute phase \(\alpha_2\)-macroglobulin levels. Effect of 6-mercaptopurine and some lipoxygenase and cyclo-oxygenase inhibitors

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Abstract
With the use of a previously described electro-immunoassay, 4 more compounds were found to depress acute phase \(\alpha_2\)-macroglobulin levels in rat serum following the injection of turpentine. The compounds, meclofenamic acid, dextropropoxyphene, 5,8,11,14-eicosatetraynoic acid and 6-mercaptopurine are thus grouped with a variety of anti-inflammatory agents, cyclo-oxygenase inhibitors, hypolipidaemics and colchicine, all of which share the property of reducing the concentration of \(\alpha_2\)-macroglobulin in rat serum. Compounds with immunosuppressant properties, namely amethopterin, azathioprine, cyclophosphamide and 5-fluouracil, the steroids, betamethasone and dexamethasone, the lipoxygenase inhibitor, nordihydroguaiaretic acid, and MK447, a compound said to stimulate prostaglandin synthesis, did not reduce serum levels of the acute phase protein. 6-Mercaptopurine, a compound known to show anti-inflammatory properties in some systems, proved to be the strongest inhibitor found so far. The rat acute phase protein electroimmunoassay may thus be an effective screen for detecting a new class of anti-inflammatory agents. Knowledge of the mechanisms of action of drugs that depress rat serum \(\alpha_2\)-macroglobulin concentrations may promote understanding of the control of acute phase protein production by the liver.

Introduction
The concentrations of a number of serum proteins elevate during inflammatory reactions in the rat [1–5]. One of these proteins, \(\alpha_2\)-macroglobulin, may be regarded as the prototype acute phase protein in this species [1, 3, 4].

Previous studies [6] with a wide variety of drugs showed that non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin, indomethacin, flufenamic acid, ibuprofen and NW755C, as well as the hypolipidaemics clofibrate and ICI 55,897 (‘Clozic’), reduced elevated rat serum concentrations of \(\alpha_2\)-macroglobulin 48 h after injection of turpentine. The quantitative electro-immunoassay used [6] offers a new and convenient screen for NSAIDs and may be of value for assessing potential anti-rheumatic compounds of the clozic type [7].

Further investigations with the method revealed some additional inhibitors of the elevated concentration of serum \(\alpha_2\)-macroglobulin following oral dosage of turpentine-injected rats. Included amongst these inhibitors were the antimetabolite, 6-mercaptopurine, the NSAID, meclofenamic acid, the mild analgesic, dextropropoxyphene napsylate, and the ‘lipooxygenase inhibitor’, 5,8,11,14-eicosatetraynoic acid.

Materials and methods
Rats
Male Oxford Hooded (PVG/C) rats (approximately 200 g) obtained from the Department of Pathology, University of Sydney, were used in all experiments designed to assess the effect of drugs on \(\alpha_2\)-macroglobulin levels.

Sera
Blood samples from rats were obtained by snipping the end of the pre-warmed tail with scissors or by decapitation.

Rabbit anti-rat \(\alpha_2\)-macroglobulin was prepared as described [6]. The antiserum did not precipitate with normal rat serum and its precipitating properties with acute phase rat serum samples were not altered by absorption with normal rat serum.

Sera were obtained after letting blood samples stand for 1 h at room temperature and then for 4–24 h at 4°C.

Induction of turpentine-induced inflammation
Rats were injected with industrial grade turpentine (Wolsey Castle, Pendle Hill, NSW) (0.5 ml) in the muscle of 1 hind leg.

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Compounds

Allopurinol, l-(+) amethopterin, azathioprine, betamethasone, cyclophosphamide, dexamethasone acetate, gallic acid, 6-mercaptopurine and nordihydroguaiaretic acid were obtained from Sigma Chemical Co., St Louis, Mo., USA. Other compounds used were 2-aminomethyl-4-t-butyl-6-iodophenol hydrochloride (MK447) (Hoffman-La Roche), dextropropoxyphene napsylate (Lilly), 5,8,11,14-eicosatetraynoic acid (Hoffman-La Roche) and 5-fluorouracil (Calbiochem).

All compounds were administered by oral dosage in 0.5-1 ml of water except 5-fluorouracil, which was given intravenously in 0.3 ml sterile physiological saline.

Quantitation of serum α₂-macroglobulin

This was carried out by rocket electrophoresis [8] as previously described [6] using rabbit anti-α₂-macroglobulin, 100 μl per 12 ml of 0.8% agarose (Sigma) gel.

Standard curves were expressed as an equation of the first degree and the concentrations in the unknown samples (x) were calculated from the height of the rockets (y) by solving the equation y = mx + b [8]. α₂-Macroglobulin levels (mg/ml) from each control and test group of rats were analysed by Hugh's unpaired t-test.

Results

Effect of compounds on α₂-macroglobulin levels in rat serum

To measure the effect of drugs on the α₂-macroglobulin concentration in serum, rats were injected i.m. with 0.5 ml of turpentine and dosed twice daily for 48 h with the compound being investigated. Animals were bled 12 h after the fourth dose and the acute phase protein concentration in serum examined by rocket immunoelectrophoresis. Since the previously reported study [6], which showed that a number of NSAIDs and hypolipidaemic agents inhibited α₂-macroglobulin levels, 4 more inhibitors have been found; meclofenamic acid, dextropropoxyphene napsylate, 5,8,11,14-eicosatetraynoic acid and the most potent inhibitor found so far, 6-mercaptopurine (see table). By contrast, allopurinol, which is structurally related to 6-mercaptopurine, and the immunosuppressants amethopterin, azathioprine, cyclophosphamide and 5-fluorouracil, were inactive. Other compounds devoid of activity were nordihydroguaiaretic acid, gallic acid and MK447. The latter compound was toxic at 20 mg/kg, killing 50% of the rats over the 48-h dosage period. At this dosage the compound elevated the α₂-macroglobulin levels in serum. At 10 mg/kg no toxicity was observed but again the compound proved to be inactive.

No toxicity was observed with meclofenamic acid dextropropoxyphene or interestingly, with nordihydroguaiaretic acid, gallic acid and 5,8,11,14-eicosatetraynoic acid at the doses tested. An occasional death was recorded with 6-mercaptopurine at 50 mg/kg 12-hourly but at 100 mg/kg 12-hourly up to 50% of the animals died at 48 h.

Further testing with the steroids betamethasone and dexamethasone acetate in the dose range 1-5 mg/kg (see [6] for previous studies) showed that the compounds were without activity in lowering the concentration of α₂-macroglobulin in rat serum.

Clofibrate, used as an internal standard in all the experiments [6], inhibited α₂-macroglobulin serum levels by 45-55% at 100 mg/kg (see table).

Effect of compounds on serum α₂-macroglobulin acute phase protein levels in rats.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (mg/kg)</th>
<th>Inhibition (Range %)</th>
<th>Number of rats</th>
<th>Probability (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meclofenamic acid</td>
<td>10</td>
<td>21-25</td>
<td>18</td>
<td>0.005 &gt; p &gt; 0.001</td>
</tr>
<tr>
<td>Dextropropoxyphene napsylate</td>
<td>100</td>
<td>25-35</td>
<td>18</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>6-Mercaptopurine</td>
<td>50</td>
<td>50-85</td>
<td>42</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>5,8,11,14-eicosatetraynoic acid</td>
<td>100</td>
<td>20-30</td>
<td>24</td>
<td>0.005 &gt; p &gt; 0.001</td>
</tr>
<tr>
<td>Clofibrate</td>
<td>100</td>
<td>45-55</td>
<td>126</td>
<td>p &lt; 0.001</td>
</tr>
</tbody>
</table>

* All compounds dosed orally.

* Oxford Hooded rats injected i.m. with 0.5 ml turpentine.

* Rats dosed morning and evening for 48 h following an initial dose given 1 h before turpentine injection.

* Serum α₂-macroglobulin levels assayed by rocket immunoelectrophoresis 48 h after turpentine injection [6].

The following compounds, each tested in 12 rats and with doses in mg/kg in parentheses, were inactive: allopurinol (50), amethopterin (1), azathioprine (40), cyclophosphamide (25), 5-fluorouracil (10 - given i.v.), betamethasone and dexamethasone acetate (1-5), gallic acid (200), nordihydroguaiaretic acid (50 and 100), MK447 (10 and 20). At 20 mg/kg MK447 increased α₂-macroglobulin levels by 26% in the 6 surviving rats.