Protection against superoxide and hydrogen peroxide in synovial fluid from rheumatoid patients

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(Received 26 March 1981; accepted 1 May 1981)

Summary

1. On exposure of synovial fluid to superoxide and hydrogen peroxide, generated enzymically or by activated polymorphonuclear leucocytes, hyaluronic acid is depolymerized and the fluid loses its lubricating properties. The ability of synovial fluid from rheumatoid patients to scavenge superoxide and hydrogen peroxide was therefore examined.

2. Synovial fluid from a range of rheumatoid patients contained no superoxide dismutase activity, insufficient caeruloplasmin to scavenge any superoxide radical and little, if any, catalase activity.

3. Total ascorbate (reduced ascorbate + dehydroascorbate) concentrations in the plasma and synovial fluid of rheumatoid patients were similar in each case. The values are at the low end of the normal range.

4. These results are discussed in relation to the role of oxygen radicals in inflammatory joint disease.

Key words: ascorbic acid, caeruloplasmin, catalase, hyaluronic acid, hydrogen peroxide, rheumatoid arthritis, superoxide, synovial fluid.

Introduction

Oxygen-derived species such as superoxide (O$_2^-$) and hydrogen peroxide (H$_2$O$_2$) appear to be formed in all aerobic cells [1–3]. In the presence of traces of iron salts as a catalyst, O$_2^-$ and H$_2$O$_2$ react together to form the highly reactive hydroxyl radical, OH·, which attacks and destroys most biological molecules [1–6]. Polymorphonuclear leucocytes are known to produce O$_2^-$, H$_2$O$_2$ and OH· during ingestion of bacteria or immune complexes [7]. In inflammatory conditions, such as rheumatoid arthritis, leucocytes accumulate in the joints. It is known that rheumatoid synovial fluid contains much more iron than non-inflammatory fluids [8, 9] and that there is deposition of excess iron in the synovial membranes [10]. Hence if the O$_2^-$ and H$_2$O$_2$ produced by the leucocytes are not scavenged, they can easily react together to form OH·. This radical attacks and destroys hyaluronic acid and so the synovial fluid loses its high viscosity, causing increased friction in the joint [9, 11–15].

In cells O$_2^-$ can be scavenged by the enzyme superoxide dismutase, and H$_2$O$_2$ by catalase [1–3]. Ascorbic acid also reacts with O$_2^-$ [16]. It has been claimed that the extracellular protein caeruloplasmin, which is found in synovial fluid, scavenges the O$_2^-$ radical [17], although this has been disputed [18]. Caeruloplasmin is increased in amount in rheumatoid synovial fluid as compared with normal fluid [19]. McCord [12] found that bovine synovial fluid contained superoxide dismutase activity, but little catalase. There have been no studies of these enzymes in fluids of human origin, however. Ascorbic acid concentrations in the serum of patients with rheumatoid arthritis are about 40% lower than normal [20], but the concentrations in synovial fluid do not appear to have been measured.

In the present paper we report an examination of the ability of synovial fluid from a number of
rheumatoid patients to scavenge \( \text{O}_2^- \) and \( \text{H}_2\text{O}_2 \), including measurements of ascorbic acid concentrations in both serum and synovial fluid.

### Materials and methods

#### Enzyme assays

Superoxide dismutase was assayed by its ability to inhibit the production of formazan from nitro-blue tetrazolium with a mixture of xanthine and xanthine oxidase to generate \( \text{O}_2^- \). Assays were carried out at both pH 10.2 [21] and 7.8 [22].

Catalase was assayed at pH 7 by the fall in \( A_{240} \) from 0.45 to 0.40 as \( \text{H}_2\text{O}_2 \) is destroyed [23]. One unit of enzyme activity destroys 1 \( \mu \)mol of \( \text{H}_2\text{O}_2 \)/min under the assay conditions [23].

#### Determination of ascorbic acid

Ascorbate plus dehydroascorbate was determined with the dinitrophenylhydrazine reagent as described previously [24], except that the colour reagent contained 0.6% \( \text{CuSO}_4 \) to oxidize ascorbate, rather than charcoal. The method was calibrated with freshly prepared ascorbate solutions.

#### Patients

The synovial fluid used in these experiments was a small fraction of samples drawn from patients for authentic medical purposes. All the patients fulfilled the criteria of the American Rheumatism Association for classical or definite rheumatoid arthritis. Bloodstained fluids were not used in these experiments.

#### Results

Samples of synovial fluid from the knee joints of a range of rheumatoid patients were centrifuged to remove cells and then assayed for superoxide dismutase and catalase activities by sensitive spectrophotometric methods (see the Materials and methods section). Fig. 1 shows a typical result for superoxide dismutase. No superoxide dismutase activity could be detected in fluid from the eight patients tested. Addition of authentic superoxide dismutase to the synovial fluid before assay resulted in the expected enzyme activity, which shows that the fluids did not contain any inhibitor of this enzyme (Fig. 1).

The assay procedure used would detect any protein that reacted with \( \text{O}_2^- \). Hence the ability of any caeruloplasmin present in the fluid to scavenge \( \text{O}_2^- \) must have been negligible.

Results of the catalase assays are summarized in Table 1. Again, addition of authentic catalase to the samples before assay resulted in the expected enzyme activity, showing that the fluids did not contain any catalase inhibitor.

The values in Table 1 show that the catalase activity of fluids varied from patient to patient, but was generally low or absent. For comparison, pure human catalase has a specific activity of approximately 30 000 units/mg of protein under the same assay conditions.

The samples of synovial fluid were also assayed for their total ascorbate content (i.e. reduced ascorbate plus dehydroascorbate).
TABLE 1. Assay of catalase in synovial fluid from rheumatoid patients

Catalase was assayed by its ability to destroy $H_2O_2$ as described in the Materials and methods section. The drugs below at the concentrations that would have been present in the fluids from patients treated with the drug did not inhibit catalase activity. Addition of 50–500 units of catalase to the fluids resulted in quantitative detection of the enzyme on subsequent assay. "Zero units" means that no destruction of $H_2O_2$ was detected however long the synovial fluid was incubated with this compound.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Drug therapy</th>
<th>Catalase activity (units/ml of synovial fluid)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Indomethacin plus D-penicillamine</td>
<td>35</td>
</tr>
<tr>
<td>2</td>
<td>Aspirin</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>Aspirin</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>Oxyphenbutazone</td>
<td>8</td>
</tr>
<tr>
<td>5</td>
<td>None</td>
<td>14</td>
</tr>
<tr>
<td>6</td>
<td>Indomethacin plus Ibuprofen</td>
<td>17</td>
</tr>
<tr>
<td>7</td>
<td>Naproxen plus D-penicillamine</td>
<td>55</td>
</tr>
</tbody>
</table>

TABLE 2. Ascorbate concentrations in the plasma and synovial fluid of rheumatoid patients

The method used measures both ascorbate and dehydroascorbate. Extra ascorbic acid added to the fluids was detected quantitatively in the subsequent assay, which indicates that the other components of synovial fluid do not interfere with the assay procedure. Ascorbic acid was not lost at a significant rate if fluids were stored frozen at $-20^\circ C$, although most determinations were performed on freshly-drawn samples.

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>Plasma ascorbate ($\mu$mol/l)</th>
<th>Synovial fluid ascorbate ($\mu$mol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>33</td>
<td>35</td>
</tr>
<tr>
<td>2</td>
<td>41</td>
<td>40</td>
</tr>
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<td>3</td>
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<td>5</td>
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<td>6</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>7</td>
<td>31</td>
<td>38</td>
</tr>
<tr>
<td>8</td>
<td>15 (Right knee)</td>
<td>19 (Left knee)</td>
</tr>
<tr>
<td>9</td>
<td>26</td>
<td>23</td>
</tr>
</tbody>
</table>

Plasma samples from the same patients were assayed simultaneously. Results are summarized in Table 2. It may be seen that synovial fluid and plasma had similar total ascorbate concentrations in most cases and that in all cases the concentrations were low (normal serum ascorbate is in the range of 0.040–0.141 mmol/l) [26].

Discussion

The results in this paper have shown that, unlike bovine synovial fluid [12], human rheumatoid synovial fluid has no significant superoxide dismutase activity. Although caeruloplasmin should have been present in these fluids [19], our inability to detect any superoxide scavenging activity strongly suggests that the physiological role of this protein is not that of removing $O_2^-$, in agreement with Bannister et al. [18] and Gutteridge et al. [27].

Human synovial fluid also contains little catalase activity; the small amount present in some cases might perhaps have arisen from the lysis of a small number of erythrocytes. Ascorbic acid concentrations were too low for this molecule to have any significant $O_2^-$ scavenging activity [16]. Since the values in Table 2 represent reduced ascorbate plus dehydroascorbate, and only the former reacts with $O_2^-$ [16], the effective scavenging ability might well be even less than that implied by Table 2.

It follows that synovial fluid from rheumatoid patients has no protection against $O_2^-$ and $H_2O_2$ generated by active leucocytes. Since rheumatoid fluid has a high iron content [8], an interaction of $O_2^-$ and $H_2O_2$ to generate $OH^-$ radicals seems almost certain to occur in vivo. This perhaps accounts for the extensive loss of viscosity of synovial fluid that is seen in inflammatory joint disease [9].

In the rheumatoid patients studied serum and synovial ascorbate concentrations were very similar. It seems likely, therefore, that a similar situation exists in normal humans, in which case synovial fluid ascorbate concentrations will be higher and will exert a greater protection against any $O_2^-$ radical generated.

References

486  D. R. Blake et al.


