

## Xanthine oxidoreductase is present in human synovium

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**SUMMARY** It is postulated that the mobile inflamed joint may be subject to cyclical ischaemic reperfusion injury. Xanthine oxidoreductase is an enzyme thought to contribute to oxidative reperfusion injury, and the detection of this activity in human synovium is described. Three normal and five rheumatoid tissues were assayed with a carbon-14 radioassay detecting the conversion of [<sup>14</sup>C]xanthine to [<sup>14</sup>C]uric acid. Rheumatoid synovia contained 0.67–305 μU/g tissue (n=5), while normal synovia contained 1.2–5.0 μU/g tissue (n=3).

**Key words:** reperfusion, ischaemia, ischaemic reperfusion, xanthine oxidase, xanthine, rheumatoid arthritis.

We have previously suggested a mechanism involving ischaemic reperfusion injury to account for the unusual persistence of synovial inflammation.<sup>1</sup> This involved synovial ischaemia induced by exercise and reperfusion injury occurring on resting. In resting inflamed joints with an effusion, intra-articular pressures are raised compared with those of normal joints, but during exercise the pressure in these joints increases further.<sup>2</sup> Lund-Olsen showed that partial pressures of oxygen (P<sub>O<sub>2</sub></sub>) in the synovial fluid are lower in rheumatoid joints than in osteoarthritic or traumatised joints and fall during exercise.<sup>3</sup> It is probable that, as the pressures measured during exercise often exceed the capillary perfusion pressure and indeed are greater than systolic blood pressure,<sup>2,4</sup> occlusion of the vessels supplying the synovium occurs, explaining the drop in synovial fluid P<sub>O<sub>2</sub></sub>. Once exercise has ceased intra-articular pressures drop and P<sub>O<sub>2</sub></sub> levels rise, often above basal levels, consistent, not only with reflow through the synovial vascular bed, but with a reactive hyperaemia.

In many tissues much of the damage resulting from an ischaemic incident has been attributed to the postischaemic reperfusion phase,<sup>5</sup> the ischaemic phase 'priming' the cells for the destructive activity occurring in the reperfusion phase. During temporary ischaemia low oxygen concentrations halt mitochondrial oxidative phosphorylation, and cellu-

lar adenosine triphosphate (ATP) production becomes dependent on anaerobic glycolysis. This is an inefficient means of ATP production from glucose and also results in the production of lactic acid. Increasing levels of lactic acid together with an increasing ratio of NADH to NAD<sup>+</sup> (due to ceased oxidative phosphorylation) eventually lead to the inhibition of glycolysis, and intracellular ATP and ADP levels, already reduced, fall further.<sup>6,7</sup> Among other events the decrease in intracellular ATP and ADP concentration leads to increases in concentrations of adenosine and its breakdown products, including hypoxanthine and xanthine,<sup>7</sup> these last two being substrates for the enzyme xanthine oxidoreductase.

Xanthine oxidoreductase is a cytosolic enzyme normally oxidising hypoxanthine and xanthine to uric acid and reducing NAD<sup>+</sup> to NADH. Della Corte and Stirpe described the conversion of this enzyme from the normal NAD<sup>+</sup> dependent 'D' form to an NAD<sup>+</sup> independent 'O' form, for which oxygen can act as an electron acceptor forming the superoxide anion (O<sub>2</sub><sup>-</sup>) and consequently hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>).<sup>8</sup> (H<sub>2</sub>O<sub>2</sub> is also produced directly by this enzyme.) This conversion has been reported to occur in ischaemia and may be mediated by a calcium dependent mechanism.<sup>9,10</sup> Upon reperfusion, the O form of the enzyme, supplied with oxygen as an electron acceptor, and high levels of hypoxanthine, produces a flux of O<sub>2</sub><sup>-</sup> which may be converted either spontaneously or by the enzyme superoxide dismutase to H<sub>2</sub>O<sub>2</sub>.

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Electron paramagnetic resonance studies have shown the formation of radicals in the reperfusion phase of ischaemic damage in other tissues, particularly the heart,<sup>11</sup> and tissue injury has been reduced by the addition of radical scavengers such as superoxide dismutase, catalase, and mannitol.<sup>10 12</sup> Iron chelators<sup>12</sup> and allopurinol<sup>13</sup> (which on oxidation to oxypurinol by xanthine oxidoreductase is a potent inhibitor of the enzyme) also have beneficial effects in hypoxic reperfusion injury.

The detection of xanthine oxidoreductase in human synovium is therefore of importance as a first step in the investigation of possible mechanisms of radical generation peculiar to a reperfusion phase of synovial injury.

### Materials and methods

Human knee synovium was obtained postoperatively, loosely wrapped with aluminium foil, and frozen in liquid nitrogen. Rheumatoid synovium was obtained from patients requiring joint replacement or synovectomy and non-arthritic samples from patients requiring amputation for malignancy. Samples were stored at  $-70^{\circ}\text{C}$ . The tissue was powdered by grinding with a pestle and mortar cooled with liquid nitrogen. The powdered tissue was then mixed with buffer (0.1 M TRIS/HCl, pH 8.3,  $4^{\circ}\text{C}$ , 1 g tissue + 4 ml buffer) containing the protease inhibitors phenylmethylsulphonyl fluoride (PMSF; 0.1 mmol/l) and *N-p*-tosyl-L-arginine methyl ester (TAME; 1 g/l) and sonicated two 30 s bursts with 30 s cooling) at  $4^{\circ}\text{C}$  in an MSE 150 W sonicator using an MSE titanium microprobe. The preparation was then centrifuged (100 000 g, one hour,  $4^{\circ}\text{C}$ ) and the clear supernatant beneath the fatty upper layer retained. The xanthine oxidoreductase activity of the supernatant was then measured according to the method of Dougherty<sup>14</sup> for serum as follows: supernatant was passed down a pd10 column (Sephadex G25 prepacked; Pharmacia) eluting with bovine serum albumin (1%) in NaCl (0.9%) with PMSF and TAME as above. This removed low molecular weight substrates and inhibitors. The supernatant (0.2 ml) was incubated at  $37^{\circ}\text{C}$  with purified [ $^{14}\text{C}$ ]xanthine (27  $\mu\text{M}$  xanthine, [6- $^{14}\text{C}$ ]xanthine activity 1.9 MBq/ $\mu\text{mol}$ , Amersham International) for three hours. In some incubations allopurinol (0.1 mmol/l) and  $\text{NAD}^+$  (0.7 mmol/l) were included in the incubation solution. The reaction was stopped by the addition of trichloroacetic acid (40  $\mu\text{l}$ , 40%), and after brief boiling to release bound uric acid, and centrifugation, the sample supernatant (0.1 ml) was passed down a Dowex 50 ion exchange column (5 cm Pasteur pipette column, eluant 0.1 M HCl) and the uric acid

Table 1 Xanthine oxidoreductase activity in human synovium\*

Sample		+NAD <sup>+</sup> D+O form	-NAD <sup>+</sup> O form	+Allo- purinol
Rheumatoid synovia	A	305	291	—
	B	30.8	25.7	0.34
	C	21.5	23.5	8.22
	D	3.0	3.1	0
	E	0.67	0	0
Non-arthritic synovia	A	5.0	4.3	0
	B	3.48	3.36	0
	C	1.2	1.16	0

\*Figures are expressed as  $\mu\text{U/g}$  tissue.

traction collected and  $^{14}\text{C}$  measured. Xanthine oxidase standards (0–25  $\mu\text{U}$ ) were included in each experiment. Protease inhibitors were included at all stages of the assay to prevent loss of total activity and conversion of D to O forms of the enzyme, which in some instances is achieved with proteases.

### Results

Non-arthritic synovia contained xanthine oxidoreductase (5.0–1.2  $\mu\text{U/g}$  tissue) which was largely independent of  $\text{NAD}^+$  (4.3–1.16  $\mu\text{U/g}$  tissue without  $\text{NAD}^+$ ) and showed no detectable activity with allopurinol. Rheumatoid synovia contained more xanthine oxidoreductase (305–0.67  $\mu\text{U/g}$  tissue), which was also independent of  $\text{NAD}^+$  (291–0  $\mu\text{U/g}$  tissue without  $\text{NAD}^+$ ) and showed little activity with allopurinol (8.22–0  $\mu\text{U/g}$  tissue) (Table 1).

### Discussion

We have shown that xanthine oxidoreductase activity is present in human synovium in small but detectable quantities. The enzyme was assayed chiefly in its O form, but as it is converted very readily from its D to its O form during preparation and can be independent of proteolytic conversion this indicates simply that conversion to the O form is possible.

The differences obtained between rheumatoid and non-arthritic synovial preparations may not be related to increases in intracellular enzyme levels but rather to the greater cellularity of the rheumatoid synovial preparations. It is for this reason that we have not compared the groups statistically. Attempts to overcome this problem by localising the enzyme using a histological technique on frozen synovial sections have so far failed, possibly owing to lack of sensitivity of the technique used.<sup>15</sup> As

most of the tissue obtained was from patients undergoing joint replacement the levels may be higher in active rheumatoid patients with less advanced disease. It has been reported that xanthine oxidoreductase is present chiefly in capillary endothelial cells, the enzyme being localised by immunological techniques.<sup>16</sup> This would also fit in with our data in view of the increased vascularity of rheumatoid synovia.

Xanthine oxidoreductase has previously been detected in considerable quantities in rat air pouch carrageenan induced granuloma,<sup>17</sup> an interesting observation in view of the structural similarities of the air pouch and the synovial lining. This difference reflects the difference found generally between rat and human xanthine oxidoreductase levels, humans having little of the enzyme in any tissue except liver (30–150 mU/g tissue) and intestine (29–56 mU/g tissue). Xanthine oxidoreductase levels in heart are of the same order as synovial levels (0.16–0.38 mU/g protein).<sup>18</sup>

In addition to this potentially radical producing enzyme, rheumatoid synoviocytes contain high levels of iron saturated ferritin, and the O form of xanthine oxidoreductase is capable of mobilising iron from ferritin by a mechanism largely dependent on superoxide ion.<sup>19</sup> This release of iron together with the production of  $O_2^-$  and  $H_2O_2$  may lead to an iron catalysed Fenton type reaction with the formation of the highly reactive hydroxyl radical. The synoviocyte with high ferritin levels may be particularly susceptible to damage.<sup>20–22</sup>

In conclusion, xanthine oxidoreductase is present in the synovial membrane and may contribute to synovial reperfusion injury directly by production of  $O_2^-$  and  $H_2O_2$  and by iron mobilisation. We are at present examining synovial fluids for changes in substrate and product levels resulting from exercise and subsequent rest.

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