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Free radicals and Dupuytren's contracture

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Abstract

The concentration of substrate expressed as hypoxanthine capable of reacting with xanthine oxidase to release superoxide free radicals (O₂⁻) was measured in control and Dupuytren's contracture palmar fascia. In Dupuytren's contracture palmar fascia the concentration of hypoxanthine was six times that of control and was greatest in "nodular" areas. Xanthine oxidase activity was also detected in Dupuytren's contracture palmar fascia.

These results suggest a greater potential for hypoxanthine-xanthine oxidase generated oxygen free radical formation in Dupuytren's contracture than in control palmar fascia. Production of free radicals may be an important factor in the pathogenesis of Dupuytren's contracture. The benefit of allopurinol in the management of Dupuytren's contracture and other fibrotic conditions may thus be explained, as allopurinol binds to xanthine oxidase and prevents release of free radicals.

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Introduction

Dupuytren's contracture affects the palmar fascia, which becomes thickened and shortened and may lead to disabling fixed flexion deformities of the fingers. The prevalence in white populations is between 4% and 6%, rising to 20% in men over 65,¹ and is higher in diabetics, particularly when retinopathy is present, but lower in patients with rheumatoid arthritis.^{2,3}

The cause is unknown but may include localised ischaemia of the palmar fascia.^{4,5} During ischaemia adenosine triphosphate is broken down, increasing the amount of the purine bases hypoxanthine and xanthine and converting xanthine dehydrogenase to xanthine oxidase^{6,7} (fig 1). Xanthine oxidase, located in the endothelial cells of small vessels,⁸ catalyses the conversion of both hypoxanthine to xanthine and xanthine to uric acid. Both reactions produce superoxide free radicals (O₂⁻) and hydrogen peroxide (H₂O₂). Superoxide free radicals, hydrogen peroxide, and their degradation products may damage various tissues and alter vascular permeability.⁹⁻¹¹ In animal studies allopurinol (a competitive inhibitor of xanthine oxidase) and free radical scavengers such as superoxide dismutase and catalase limited the damage associated with acute ischaemia.^{12,13} Preliminary clinical results suggest that allopurinol may improve Dupuytren's contracture.¹⁴

To see whether free radicals might be important in the pathogenesis of Dupuytren's contracture we have measured the concentration of substrates able to react with exogenous xanthine oxidase to produce superoxide free radicals in Dupuytren's and control palmar fascia. These substrates are most likely to

be hypoxanthine and xanthine; for clarity we express these as hypoxanthine concentrations.

The activity of xanthine oxidase was also measured in Dupuytren's contracture tissue from six patients and compared with values previously found in other human tissues. Samples of normal palmar fascia from control patients were too small for assay of xanthine oxidase activity.

FIG 1—Mechanism of hypoxia induced free radical damage.

Subjects and methods

Palmar fascia was obtained from 10 patients (eight men, two women; age range 45-73) during fasciectomy for Dupuytren's contracture and from 10 patients having carpal tunnel release operations (five men, five women; age range 30-66). Both procedures were performed under tourniquet ischaemia. The effect of tourniquet ischaemia was examined in three patients by measuring the hypoxanthine concentrations in samples of skin subjected to 0-60 minutes of tourniquet ischaemia. All patients gave informed consent.

To assess the relation of cell density and histological appearance to hypoxanthine concentration in Dupuytren's contracture palmar fascia we dissected one large sample of Dupuytren's tissue into 10 pieces. Hypoxanthine concentrations were measured in each piece and representative segments fixed in formaldehyde, embedded in paraffin wax, sectioned, and stained with haematoxylin and eosin. Cell density was determined by using a graticule and 1 mm graduated slide.

Hypoxanthine assay—All samples were placed immediately in liquid nitrogen, stored at -20°C , and later carefully dissected, weighed, and homogenised with 5 ml ice cold phosphate buffered saline, pH 7.4, in an Ultraturax homogeniser (Sartorius Instruments Ltd) for three 20 second periods and spun at $105\,000\text{ g}$ at 4°C for 30 minutes in a T₁ 40.1 rotor (Beckman). The supernatant was freeze dried, heat denatured to remove any endogenous xanthine oxidase, reconstituted, and filtered before assay. The concentration of substrates able to release superoxide free radicals with the addition of exogenous xanthine oxidase was measured by the superoxide dismutase inhibitable reduction of cytochrome c.¹⁵ One millilitre of sample solution or hypoxanthine (Sigma) standard was mixed with cytochrome c (Sigma; final concentration $50\text{ }\mu\text{mol/l}$) and phosphate buffered saline, pH 7.4, and equilibrated at 37°C for 10 minutes before the addition of 6 mU xanthine oxidase (Sigma) to test samples. Final volume was 2 ml. The rate of cytochrome c reduction at 550 nm was recorded in a Gilford 2600 spectrophotometer, compared with a standard curve constructed with hypoxanthine as substrate, and the result expressed as μmol hypoxanthine/g wet weight of tissue. Each sample was analysed in triplicate.

Xanthine oxidase assay—Sterile operative samples were placed immediately in cold (4°C) Dulbecco's modification of Eagle's medium (Flow Laboratories) containing 10% (vol/vol) fetal calf serum (Flow) with 550×10^6 U benzylpenicillin and 275 mg streptomycin/l. The tissue was chopped into 2 mm cubes and washed in phosphate buffered saline. One cube was placed in each 1.6 cm well of a 24 multiwell culture dish (Flow). One millilitre of phosphate buffered saline, pH 7.4, containing glucose (final concentration 2 mmol/l), hypoxanthine (final concentration $100\text{ }\mu\text{mol/l}$), and cytochrome c (final concentration $50\text{ }\mu\text{mol/l}$), with allopurinol (Wellcome; final concentration $100\text{ }\mu\text{mol/l}$) and superoxide dismutase (Sigma; final concentration 60 mg/l)

where appropriate, was added to each well. Twelve wells were free of allopurinol (six with superoxide dismutase, six without) and 12 contained allopurinol (six with superoxide dismutase, six without). The culture plate was incubated at 37°C for 60 minutes with continuous agitation. All procedures were performed under sterile conditions. The reaction was stopped by adding 1 ml 2mM N-ethylmaleimide (Sigma) and cytochrome c reduction measured. Superoxide release was calculated from the average difference in absorbance of the samples with and without superoxide dismutase. The difference in superoxide release between the samples with and without allopurinol was attributed to xanthine oxidase activity and calculated from a standard curve constructed with known concentrations of xanthine oxidase. Dry weight of each piece of tissue was adjusted to equivalent wet weight and the results expressed as mU xanthine oxidase activity/g wet weight of tissue.

Statistics—Mann-Whitney non-parametric U tests were used for all data.

Results

A sixfold increase in hypoxanthine concentration was found in Dupuytren's palmar fascia compared with control palmar fascia (mean 0.26 (SE 0.04) $\mu\text{mol/g}$ wet weight compared with 0.04 (0.01) $\mu\text{mol/g}$ wet weight); $n=10$ for both groups; $p<0.001$). In the single large piece of Dupuytren's tissue examined and sectioned the hypoxanthine concentration increased with cell density and was double the value in tissue classified as "nodular" compared with "cord" (mean 0.32 (SE 0.03) compared with 0.14 (0.03) $\mu\text{mol/g}$ wet weight; $p<0.005$ (fig 2)). A 1.47% increase in hypoxanthine concentration was found in skin subjected to 60 minutes of tourniquet ischaemia.

The mean xanthine oxidase activity in Dupuytren's contracture tissue from six patients was 13 (SE 6.1) (range 1-41) mU/g wet weight. This is higher than that found for lung and heart but less than that for intestine (table).

Discussion

We have detected higher concentrations of hypoxanthine in Dupuytren's contracture palmar fascia than in control palmar fascia. Longer tourniquet time for operative correction of

FIG 2—Histological appearance (cord or nodule) and cell density versus hypoxanthine concentration in single piece of Dupuytren's contracture palmar fascia.

Comparison of xanthine oxidase activities in different human tissues

Tissue	Xanthine oxidase (mU/g wet weight)	Reference
Lung	0.7	Ramboer, ¹⁹ Krenitsky <i>et al</i> ²⁰
Heart	0	Ramboer, ¹⁹ Krenitsky <i>et al</i> ²⁰
Liver	10-123	Ramboer, ¹⁹ Krenitsky <i>et al</i> ²⁰
Intestine	29-56	Ramboer, ¹⁹ Krenitsky <i>et al</i> ²⁰
Spleen	2.44	Ramboer, ¹⁹ Krenitsky <i>et al</i> ²⁰
Kidney	3.53	Ramboer, ¹⁹ Krenitsky <i>et al</i> ²⁰
Dupuytren's palmar fascia	1-41	Present study

Dupuytren's contracture versus carpal tunnel release may account for less than one tenth of the 600% increase. It is also relevant that hypoxanthine concentrations were higher in the cellular areas of Dupuytren's contracture tissue as it is from these areas that the proliferative process is thought to arise.

Xanthine oxidase activity was also found in Dupuytren's contracture palmar fascia, establishing a potential for oxygen free radical production by the xanthine oxidase-hypoxanthine reaction.

These results support our hypothesis that in Dupuytren's contracture palmar fascia high concentrations of hypoxanthine may react with xanthine oxidase located in the endothelial cells⁸ of narrowed microvessels⁴ to release oxygen free radicals.¹⁴ These free radicals may then damage the perivascular connective tissue and induce a reparative response by surrounding fibroblasts. Alternatively free radicals may directly stimulate proliferation of fibroblasts. We have added free radicals to fibroblasts cultured from Dupuytren's contracture palmar fascia and found, like others,^{10,11} that high concentrations of free radicals are toxic, but that in contrast lower concentrations stimulate fibroblast proliferation.¹⁶ The proliferating fibroblasts may then be responsible for the increase in type III collagen seen in Dupuytren's contracture and the early stages of normal wound healing.^{17,18}

There is increasing evidence to suggest that acute ischaemic damage is mediated by oxygen free radicals.^{12,13} We propose that free radical damage may also occur in chronic ischaemic settings, leading to fibroblast proliferation and localised fibrosis. Our findings also suggest that allopurinol by binding to xanthine oxidase and hence preventing free radical release may be useful in both chronic and acute ischaemia and in Dupuytren's contracture.¹⁴

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Treatment of Niemann-Pick disease type B by allogeneic bone marrow transplantation

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Abstract

Allogeneic bone marrow transplantation was carried out on a 3 year old girl with Niemann-Pick disease type B. Successful engraftment was achieved, and nine months after the procedure there was definite clearing of the sphingomyelin from the liver and pronounced clearing from the bone marrow.

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Any patient with Niemann-Pick disease type B complicated by early or severe hepatic impairment should be considered for bone marrow transplantation.

Introduction

Niemann-Pick disease type B is a lysosomal storage disorder characterised by a deficiency of sphingomyelinase resulting in the accumulation of sphingomyelin in tissues, particularly the bone marrow, liver, spleen, and lungs, but with no discernible neurological abnormality.¹

We report a case of Niemann-Pick disease type B treated by displacement bone marrow transplantation.

Case report

An 8 month old white girl was diagnosed as having Niemann-Pick disease after investigations for an enlarged liver and spleen. The diagnosis was confirmed by finding a very low level of sphingomyelinase activity in her leucocytes. The absence of any obvious neurological impairment placed her