Occurrence of (*E*)-4-hydroxy-2-nonenal in plasma and synovial fluid of patients with rheumatoid arthritis and osteoarthritis

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Abstract

(*E*)-4-Hydroxy-2-nonenal (HNE), a cytotoxic propagation product of lipid peroxidation, is present in the synovial fluid (0.54 (0.19) $\mu\text{mol/l}$; mean (SE), $n=9$) and plasma (0.34 (0.09) $\mu\text{mol/l}$, $n=9$) of patients with rheumatoid arthritis. This compound was also found in the synovial fluid (0.24 (0.19) $\mu\text{mol/l}$, $n=9$) and plasma (0.09 (0.03) $\mu\text{mol/l}$, $n=9$) of patients with osteoarthritis. The concentration of HNE in the plasma of patients with rheumatoid arthritis was significantly greater than in patients with osteoarthritis.

(*E*)-4-hydroxy-2-nonenal (HNE) (figure) was first identified as a major toxic product formed during microsomal lipid peroxidation.¹ It has since been found to occur in a variety of normal tissues and body fluids of rats,²⁻⁴ mice,⁵ dogs⁶ and humans.⁷ Increased concentrations of HNE have been reported in vitamin E deficient rats,² in mice infected with malaria,⁵ and dogs with neuronal retinal ceroidosis.⁶

(*E*)-4-hydroxy-2-nonenal is a highly electrophilic agent with reactivity towards molecules with sulphhydryl (-SH) groups such as cysteine,⁸ glutathione,⁹ and sulphhydryl proteins such as DNA polymerase.¹⁰ It also reacts with amino groups in low density lipoproteins¹¹ and deoxyguanosine.¹²

(*E*)-4-hydroxy-2-nonenal elicits a variety of biological effects including inactivation of glucose-6-phosphatase,¹ adenylate cyclase,¹³ 5'-nucleotidase,¹³ and cytochrome P450,¹ lysis of erythrocytes,¹ the reduction of superoxide anion production by human neutrophils,¹⁴ the potentiation of human platelet aggregation,¹⁵ chemotaxis of rat neutrophils,¹⁶ and the induction of oedema of the foot in rats.¹⁷ (*E*)-4-hydroxy-2-nonenal is cytotoxic against Ehrlich tumour ascites cells¹⁰ and human umbilical cord vein endothelial cells.¹⁸ It causes gastric ulceration in rats,¹⁹ is genotoxic and cytotoxic in rat hepatocytes,²⁰ and toxic to the human malarial parasite.²¹ (*E*)-4-hydroxy-2-nonenal inhibits the proliferative response to phytohaemagglutinin and alloantigens²² and c-myc oncogene expression,²³ and modifies low

density lipoproteins, inducing enhanced uptake by macrophages.¹¹

There is evidence that free radical initiated lipid peroxidation may affect inflammation. Increased formation of malondialdehyde occurs in the liver of mice during acute carrageenin induced inflammation of the foot.²⁴ (*E*)-4-hydroxy-2-nonenal has been detected in the pleural exudate of rats after the intraperitoneal injection of isologous serum²⁵ and in the subcutis of rats after the subcutaneous injection of Sephadex.²⁶ Increased pentane formation occurs during chronic inflammation induced in rats by Freund's adjuvant.²⁷ Increased concentrations of conjugated dienes and fluorescent lipid peroxidation products have been reported in the serum and synovial fluid of patients with inflammatory joint diseases.²⁸ The concentration of malondialdehyde in the plasma of patients with rheumatoid arthritis correlates with disease activity.²⁹ The presence of material reactive with thiobarbituric acid in the synovial fluid of patients with rheumatoid arthritis correlates with bleomycin detectable iron and disease activity.³⁰

In this study, we compared the concentrations of HNE in the plasma and synovial fluid of patients with rheumatoid arthritis with those in patients with osteoarthritis.

Patients and methods

PATIENTS

Nine patients with classical or definite rheumatoid arthritis, defined according to the criteria of the American Rheumatism Association,³¹ and nine patients with osteoarthritis were studied. The degree of joint inflammation was determined by a semiquantitative determination of pain, state of the synovial fluid, and thickness and tenderness of the synovium: 1=mild, 2=moderate, and 3=severe. Two patients with rheumatoid arthritis and one patient with osteoarthritis were receiving non-steroidal anti-inflammatory drugs (NSAIDs). The remaining patients were treated with local corticosteroid injections.

Venous blood was obtained from each patient and anticoagulated with heparin. Synovial fluid was obtained by aspiration and centrifuged to remove cells and debris.

DETERMINATION OF (*E*)-4-HYDROXY-2-NONENAL

The concentration of HNE in plasma and synovial fluid was determined by high performance liquid chromatography and combined gas chromatography negative ion chemical

Structure of (*E*)-4-hydroxy-2-nonenal.

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ionisation mass spectrometry.⁷ Immediately after collection, plasma or synovial fluid was mixed with 5 µl of butylated hydroxytoluene in methanol (10 mg/ml) and 5 µl of [2,3-²H]HNE in methanol–water (10 mg/ml) and the mixture was vortex mixed. The mixture was added to 5 mg of *O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride dissolved in 200 µl of 1.5 M sodium acetate buffer (pH 5.0) and vortex mixed for one minute. The samples were allowed to stand at room temperature for 15 minutes and then stored at –80°C until analysis. The *O*-pentafluorobenzyl oxime derivative was purified by high performance liquid chromatography and the trimethylsilyl ether of the hydroxyl group of HNE was formed as described previously.⁷ The derivatives were stored at –80°C until analysis.

Gas chromatography negative ion chemical ionisation mass spectrometry was carried out using a Finnigan quadrupole model 3200 gas chromatography mass spectrometry system interfaced to an Incos 2300 data system. The ion source was maintained at 100°C by filament emission (100 mA) and the source pressure was 0.8 Torr. The electron energy and electron multiplier voltage were set at 100–120 eV and 1.1–1.3 kV, respectively. Separations were achieved using a 1.5 m×2 mm (inner diameter) silanised glass column packed with 2% OV-17 on 100–120 mesh Chromosorb Q using methane (flow rate 20 ml/min) as the carrier gas and chemical ionisation reactant. The oven was temperature programmed after sample injection from 150 to 300°C at 10°C min. Selected ion monitoring was accomplished using ions of *m/z* 283, 285, 303, and 305 for the HNE *O*-pentafluorobenzyl oxime trimethyl silyl ether derivative. (*E*)-4-hydroxy-2-nonenal was quantified by comparing the peak areas of the *m/z* 283 ion for the *syn*-isomer of HNE *O*-pentafluorobenzyl oxime trimethyl silyl ether derivative with the *m/z* 285 ion for the *syn*-isomer of [2,3-²H] HNE *O*-pentafluorobenzyl oxime trimethyl silyl ether derivative. Standard curves were prepared by using various amounts of HNE while maintaining a constant amount of internal standard.

Concentration of (*E*)-4-hydroxy-2-nonenal (HNE) in the synovial fluid and plasma of patients with rheumatoid arthritis and osteoarthritis

Patients	Concentration of HNE (µmol/l)		Knee score†
	Plasma	Synovial fluid	
Rheumatoid arthritis (n=9)	0.60	0.53	2
	0.79	1.63	3
	0.52	0.13	2
	0.20	0.26*	2
	0.08	1.36	3
	0.15	0.18	1
	0.54	0.57	2
	0.10	0.11	—
	0.08	0.08*	1
	Mean (SE)	0.34 (0.09)	0.54 (0.19)
Osteoarthritis (n=9)	0.11	0.23	—
	0.07	0.04	2
	0.11	0.07	1
	0.09	0.84	2
	0.11	0.07	—
	0.10	0.04	—
	0.08	0.05	—
	0.12	0.06	2
	0.07	0.80	3
	Mean (SE)	0.09 (0.03)	0.24 (0.19)

*Treated with non-steroidal anti-inflammatory drugs.
†See text for scale used.

STATISTICS
Statistical analysis was performed using the two tailed Student's *t* test. Differences were considered to be significant when *p*<0.05.

Results
The table shows the concentrations of HNE in the plasma and synovial fluid of patients with rheumatoid arthritis and osteoarthritis. There was a statistically significant increase in the concentrations of HNE in the plasma of the patients with rheumatoid arthritis compared with patients with osteoarthritis (*p*<0.05). There was no statistically significant increase in the synovial fluid concentrations of HNE in patients with rheumatoid arthritis compared with patients with osteoarthritis. In rheumatoid patients with high plasma and synovial fluid concentrations of HNE, the knee scores were higher.

Discussion
The presence of HNE in the synovial fluid of patients with rheumatoid arthritis and osteoarthritis is probably due to the lipid peroxidation which takes place in the inflamed joint in these diseases.^{28 30} Similar concentrations of HNE have been reported in pleural exudate and subcutaneous Sephadex implants in rats.^{17 26} It has been reported that the concentration of lipid peroxides in synovial fluid is higher in inflammatory than in degenerative joint disease.²⁶ We found no difference in the concentration of HNE in the synovial fluid of patients with rheumatoid arthritis compared with those with osteoarthritis. The concentration of HNE in the plasma of patients with osteoarthritis was in the same range as that found in the plasma of normal healthy subjects of similar age (Selley M L, Bourne D J, unpublished data).

The inflammatory reaction in osteoarthritis is believed to be a secondary phenomenon which occurs in other deforming joint diseases in which no primary phlogistic origin is likely.³² This probably explains why the plasma concentrations of HNE in patients with rheumatoid arthritis was significantly higher than in patients with osteoarthritis. There appears to be some correlation between the extent of disease activity as measured by the knee score and the concentration of HNE in the plasma and synovial fluid of patients with rheumatoid arthritis. This correlation is considered to be tentative until studies have been completed on a larger number of patients using other indices of disease activity such as neutrophil flux into the synovial space.

The injection of a mixture of carbonyl compounds containing mainly HNE at a concentration of 0.15 µmol/l produces an inflammatory response in the hind paw of rats.¹⁷ This concentration of HNE is within the range found in the synovial fluid of patients with rheumatoid arthritis and osteoarthritis. In patients with rheumatoid arthritis there was a tendency for the knee score to increase in patients with high plasma and synovial fluid concentrations of HNE. This suggests that HNE may be a mediator of inflammation. (*E*-

4-hydroxy-2-nonenal is chemotactic to rat neutrophils with a median effective dose (ED_{50}) of $0.14 \mu\text{mol/l}$ in the presence of bovine serum albumin.¹⁶ Synovial fluid contains albumin³³ so it is possible that HNE affects the recruitment of neutrophils into the inflamed joint. Synovial fluid from patients with rheumatoid arthritis contains increased numbers of neutrophils³⁴ and the activation of neutrophils by complement fixing immune complexes is believed to play a central part in the immunopathogenesis of rheumatoid arthritis.³⁵ We reported previously that HNE increases the release of arachidonic acid from membrane phospholipids,¹⁵ so HNE could also increase the production of eicosanoids in the inflamed joint.

The source of the HNE found in joint fluids has not been established. Phagocytic cells are a potential source of oxygen free radicals³⁶ which are capable of initiating lipid peroxidation in polyunsaturated fatty acids in membrane phospholipids. We found previously that HNE is produced by human blood monocytes⁵ and HNE is also produced by neutrophils in the Sephadex model of acute inflammation.²⁶

The increased concentration of HNE in the plasma of patients with rheumatoid arthritis suggests that lipid peroxidation in this disease is not confined to the synovial space. There is an increase in lipid peroxidation in the liver during carrageenin induced inflammation in the paws of mice.²⁴ There is an association between rheumatoid arthritis and liver disorders³⁷ and it is possible that the circulating HNE in the plasma of rheumatoid patients originates in the liver. Serum samples from patients with rheumatoid arthritis have a reduced sulphhydryl concentration compared with controls.³⁸ This may be a result of the reaction of HNE with cysteine residues of circulating proteins.⁸

There is evidence of an increased incidence of peptic ulcers associated with rheumatoid arthritis.³⁹ Non-steroidal anti-inflammatory drugs are given to almost all patients with rheumatoid arthritis and their use is associated with peptic ulcers.³⁹ The increase in plasma HNE in patients with rheumatoid arthritis is of particular interest in view of the ulcerogenic properties of this compound. (E)-4-hydroxy-2-nonenal induces peptic ulcers in rats when given by mouth at a dose of only $0.26 \mu\text{mol/l}$.¹⁹ This concentration is within the range of concentrations observed in the plasma of rheumatoid patients. In patients with rheumatoid arthritis with high concentrations of circulating HNE, there may be an increased risk of NSAID induced gastropathy.

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