Increased Levels of Lipid Oxidation Products in Rheumatically Destructed Bones of Patients Suffering from Rheumatoid Arthritis

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The new indicator for lipid peroxidation (LPO) processes – 9-hydroxy-10,12-octadecadienoic acid (9-HODE) – was used to investigate, whether LPO processes are increased in destructed bone material of patients suffering from rheumatoid arthritis (RA) in comparison to surrounded non destructed bone material. The HODE content in destructed bones exceeded that of non destructed ones of the same patient for a factor of about 3.

In addition similar increases in leukotoxines and epoxy oleic acid in the destructed bone material were observed, indicating an increase of LPO processes in affected bone parts of patients.

Introduction

Rheumatoid arthritis (RA) is the most common chronic inflammatory disease (Wolfe, 1968). The inflammatory process of this disease is connected with thickening of the synovial membrane and an increase in the amount of synovial fluid which loses its high viscosity (Biemond *et al.,* 1988). In later stages of the disease bone erosions are observed, cartilage is lost and finally the joint suffers total destruction.

Involvement of reactive oxygen species (ROS) in the pathogenesis of RA is indicated by the observation that decreased viscosity of synovial fluid from the joints of patients suffering from RA is induced artificially by exposing hyaluronate (the glycosaminoglycane responsible for most of the synovial fluid viscosity) to a superoxide-generating system *in vitro* (McCord, 1974).

Since the synovial membrane allows transfer of molecules to the blood stream it is not astonishing that increased amounts of conjugated dienes – typical for generation of lipid peroxidation (LPO) products – and also of fluorescent LPO-products were detected in blood serum and synovial fluid of patients with inflammatory joint diseases (Lunec *et al.,* 1981). A direct correlation between the content of malondialdehyde (MDA) – a LPO product derived mainly from arachidonic acid hydroperoxides – in human plasma and the activity of the disease was demonstrated (Muus *et al.,* 1979). In addition 4-hydroxy-2-nonenal (4-HNE) – another characteristic LPO product – was detected in human plasma and in the synovial fluid of patients suffering from RA and osteoarthritis (Selley *et al.,* 1992). On the other hand a decrease of vitamin E – which is consumed by LPO processes – was observed in the synovial fluid of rheumatic patients (Fairburn *et al.,* 1992). Finally an oxidative change of IgG (Lunec *et al.,* 1985) was detected. This results in the expression of new antigenic determinants and in the formation of auto-antibodies.

Any tissue injury causes liberation of enzymes which induce generation of distinct LPO products

Abbreviations: 10-HNDE,10-hydroxy-11E,13Z-nonadecadienoic acid; 13-HODE, 13-hydroxy-9,11-octadecadienoic acid; 13-HPODE, 13-hydroperoxy-9,11-octadecadienoic acid; 4-HNE, 4-hydroxy-2-nonenal; 5-HPETE, 5-hydroperoxy-6,8,11,14-eicosa-tetraenoic acid; 9-HODE, 9-hydroxy-10,12-octadecadienoic acid; 9-HPODE, 9-hydroperoxy-10,12-octadecadienoic acid; AMPA, ammoniummolybdato-phosphorus acid; ARDS, adult respiratory distress syndrome; CH, cyclohexane; EE, ethylacetate; IgG, immunoglobulin G; LDL, low density lipoproteins; LOH, hydroxy fatty acid; LOO•, fatty acid peroxyradical; LOOH, hydroperoxy fatty acid; LPO, lipid peroxidation; MDA, malondialdehyde; MSTFA, N-Methyl-N-trimethylsilyltrifluoroacetamide; NP-HPLC, normal phase high performance liquid chromatography; PUFAs, polyunsaturated fatty acids; RA, rheumatoid arthritis; ROS, reactive oxygen species; TLC, thin layer chromatography.

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(Gardner, 1997), e.g. of 5-hydroperoxy-6,8,11,14-eicosatetraenoic acid (5-HPETE) (Herold and Spiteller, 1996). Metalloproteases – also liberated after cell injury (Galis *et al.*, 1994) – induce degradation of iron containing proteins (Gutteridge, 1986). Thus produced free iron ions cleave the enzymatically formed hydroperoxides to radicals (Gardner, 1989). These radicals attack now in non specific reactions any bisallylically activated methylene group of polyunsaturated fatty acids (PUFAs) with about equal probability (Spiteller and Spiteller, 1997). Since linoleic acid is the most abundant polyunsaturated fatty acid in membranes, tissue and blood, the main LPO products are 9-hydroperoxy-10,12-octadecadienoic acid (9-HPODE) and 13-hydroperoxy-9,11-octadecadienoic acid (13-HPODE). These are converted in biological surrounding to corresponding hydroxy fatty acids – 9- and 13-HODE. Since the latter are rather stable to further degredation they accumulate in all LPO processes (Spiteller and Spiteller, 1997). As a consequence determination of 9- and 13-HODE offers an unique possibility to check if a disease is connected with LPO.

Winyard *et al.* (1993) recognized oxLDL in the synovial membrane of RA patients. Comparing the 9- and 13-HODE content in LDL samples obtained from patients suffering from RA with those of healthy volunteers of the same age group we observed increases in 9- and 13-HODE in LDL of RA-patients for a factor of 20–50 (Jira *et al.*, 1997).

In this communication we report on an investigation of the 9-HODE content in destructed and non destructed bone material. Since LPO processes are connected with generation of peroxyl-radicals ($LOO^{\bullet}$) which cause epoxidation of double bonds in unsaturated fatty acids we also investigated the bone materials for the content of leukotoxines and epoxy oleic acid.

Materials and Methods

Materials

N-Methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA) was obtained from Macherey and Nagel (Düren, Germany). All other chemicals were purchased from Fluka (Neu Ulm, Germany). Solvents were distilled before use. Bone material was obtained from the Klinikum Bayreuth. Permisson of the patients to use this material for our investigations was obtained prior to surgery.

Gas chromatography/mass spectrometry

GC was carried out with a Carlo Erba HRGC 5160 Mega Series chromatograph equipped with a flame ionization detector, using a DB-1 fused silica glass capillary column (30m x 0.32 mm i.d., Fison, Mainz-Kastel, Germany), temperature programmed from 80–280 °C at 3 °C min^{-1}. The temperature of injector and detector were kept at 270 and 290 °C, respectively. The carrier gas was hydrogen and the splitting ratio was 1:30 (80%).

Mass spectrometer: MAT-FINNIGAN 95 (Bremen, Germany), ionization by electron impact (70 eV), ion source temperature 220 °C. A Varian 3700 gas chromatograph with a 30 m x 0.3 mm i.d. DB-1 fused silica column (Fison, Mainz-Kastel, Germany) was used for sample seperation.

temperature programme 1: 50–160 °C with 25 °C/min, 160–270 °C with 3 °C/min, 270–300 °C mit 8 °C/min

temperature programme 2: 50–240 °C with 30 °C/min, 240–300 °C with 3 °C/min

Methods

All patients showed typical signs of RA, considering the revised criteria from the American Rheumatism Association (Arnett *et al.*, 1988). After removal of the bone of patients suffering from RA rheumatically destructed and non destructed bone material was separated in respect of characteristic macroscopic (intact or destructed bone structure) and microscopic (histological investigations) marks. Bones were stored until further workup under an atmosphere of argon at –78 °C. About 5 g bone material was homogenized under liquid nitrogen at –196 °C using a mortar. The pulverized material was suspended in 300 ml of a methanol-chloroform-mixture (2:1, v/v) and filtered. The lipid extraction followed the method of Bligh and Dyer (1959). The residue of lipid extract was lyophilized, weighed and divided in two parts for determination of (1) LOOHs and LOHs and (2) epoxy fatty acids.

Investigation of LOOHs and LOHs by determination as hydroxy fatty acid methyl esters

10-Hydroxy-11,13-nonadecadienoic acid (10-HNDE) was added as internal standard to the lyophilized lipid for quantification of monohydroxy fatty acids. The samples were processed in exactly the same manner as described previously (Jira *et al.*, 1996, temperature programme 1).

Investigation of epoxy fatty acids

Qualitative determination as epoxy fatty acid methylesters. About 200 mg of the crude residue obtained from lipid extracts were dissolved in methanol and treated with diazomethane (5% etheral solution) for about 10 min at room temperature. After removal of the solvent under nitrogen epoxy fatty acid methyl esters were enriched by TLC (silicagel 60 PF_{254}, Merck, Darmstadt, Germany, CH/EE 4:1; detection: $UV_{254\,nm}$ and spraying with 10% ethanolic AMPA). The zones containing epoxy fatty acids were detected by cochromatographing 9,10-epoxyoctadecanoic acid methyl ester (R_f=0.63) and 9,10–12,13-diepoxyoctadecanoic acid methyl ester (R_f=0.40) as external TLC standards. These areas were scraped off, eluted with chloroform and solvent was removed under reduced pressure. The samples were trimethylsilylated by treatment with MSTFA for 12 h at room temperature.

Quantification of epoxy fatty acids by opening of the epoxid-ring with thiophenol. 10,11-Epoxyheptadecanoic acid methyl ester (5 µg/g lipid) and 10,11-epoxy-13-nonadecenoic acid methyl ester (20 µg/g lipid) were added as internal standards to about 50 mg of the lyophilized crude lipid extract obtained after extraction of the bone (see chapter 2.3). Then the acidic functions were transformed to corresponding methyl esters using diazomethane. After this treatment the evaporation-residue was dissolved in a minimum of methanol (about 100 µl) and stirred under argon with 200 µl thiophenol and 270 µl triethylamine for 24 h at room temperature (Corey *et al.*, 1980). The reaction mixture was treated with about 100 ml of bidestilled water, brought to pH 2 with 2 N hydrochloric acid and extracted three times with $CHCl_3$. The combined organic layers were washed twice with bidest. water, dried over sodium sulfate, filtered and solvent was removed under reduced pressure. The residue was separated by TLC (CH/EE 3:1; detection: $UV_{254\,nm}$) using a mixture of 9-hydroxy-10-thiophenoxyoctadecanoic acid methyl ester and 10-hydroxy-9-thiophenoxyoctadecanoic acid methyl ester (R_f=0.57) as external TLC standards. The corresponding zones were scraped off, eluted with ethylacetate and finally solvent was evaporated applying reduced pressure. The samples were trimethylsilylated with MSTFA (30 min, 40 °C) and analysed by GC and GC/MS (temperature programme 2).

Preparation of the internal standards

Preparation of 10-hydroxy-11E,13Z-nonadecadienoic acid. 100 µl of 10Z,13Z-nonadecadienoic acid (synthesised according to the procedure of Marcel (Marcel and Holman, 1968) were emulgated with 30 ml of a 100 mM NaH_2PO_4-solution (pH 6.5) and 0.02 ml of Tween 20 in an ultrasonic bath. Then about 10 mg of soybean-lipoxygenase (EC 1.13.1.13) were added. The solution was stirred for one hour at room temperature by incubation with oxygen for 1 min in time intervals of 10 min (Teng and Smith, 1985). Fatty acid hydroperoxides were extracted from the aqueous solution with chloroform and the solvent was removed under reduced pressure. Then the hydroperoxy fatty acids containing residue was dissolved in 15 ml methanol and 25 ml 0.1 M borate buffer (pH 9) and reduced by addition of 50 mg $NaBH_4$. The solution was kept for two hours at 0 °C, then brought to pH 2 by adding 2 N HCl. 50 ml of a 0.88% solution of KCl were added and the mixture was extracted three times with chloroform. The combined organic layers were washed with 150 ml of a 0.88% aqueous solution of KCl and the organic solvent was removed under reduced pressure at room temperature. The residue was dissolved in n-hexane/isopropanol 95:5 (v/v), filtered and subjected to a preparative NP-HPLC following the description of Wu *et al.* (1995).

HPLC-conditions:

column:	Ultrasep. FS 100, 6 µm, Bischoff, Leonberg, Germany
eluent:	n-hexane/isopropanol/acetic acid = 98.7/1.2/0.1 (v/v/v)
flow rate:	23 ml/min
UV-detection:	234 nm
elution time:	10.0 min

The product was characterized after methylation and trimethylsilylation by GC/MS:

MS, *m/z* (%): 73(87), 75(88), 93(53), 130(54), 225(44), 306(23), 325(100), 396(26)(M^+)

Preparation of 10,11-epoxy-13-nonadecenoic acid methylester. 50 mg (0.16 mmol) MCPBA (55%) were added to a solution of 50 mg (0.16 mmol) 10*Z*,13*Z*-nonadecadienoic acid in 10 ml methylenechloride. The reaction mixture was stirred for 8 hours at room temperature and the solvent was removed under reduced pressure. The residue was redissolved in ethylacetate and the epoxy fatty acids were enriched by TLC (CH/EE 3:1 v/v) as described above. The corresponding zones (R_f 0.35–0.55) were scraped off, eluted with ethylacetate and the solvent was removed under reduced pressure. The residue was redissolved in n-hexane. 10,11-Epoxy-13-nonadecenoic acid and 13,14-epoxy-10-nonadecenoic acid were separated by analytical NP-HPLC following the method of Balazy (Balazy and Nies, 1989).

HPLC-conditions:

column: Nucleosil, 3 μm, Bischoff, Leonberg, Germany
eluent: n-hexane/isopropanol/acetic acid = 99.8/0.1/0.1 (v/v/v)
flow rate: 1 ml/min
UV-detection: 200 nm
elution time: 16.4 min

10,11-Epoxy-13-nonadecenoic acid was methylated with diazomethane and characterized by GC/MS:

MS, *m/z* (%): 55(100), 67(87), 81(72), 95(75), 153(13), 169(21), 199(13), 293(3), 306(4), 324(1)(M^+)

Results

The investigated non destructed and rheumatically destructed bones were removed by surgery from patients which showed typical signs of RA, considering the revised criteria from the American Rheumatism Association (Arnett *et al.*, 1988, see Table I).

Rheumatic bones and obviously non-affected bone material as well as the rheumatic synovial membrane were separated after surgical removal according to characteristic macroscopic signs (see chapter 2.3). Bone material was mechanically pulverized at −196 °C and lipids were extracted. The crude lipid was divided in two parts in order to investigate hydroxy fatty acids and epoxy fatty acids.

Hydroxy fatty acids

Quantification of hydroxy fatty acids in non-destructed and rheumatically destructed bones as well as in rheumatic synovial membrane was achieved by adding 10-hydroxy-11,13-nonadecadienoic acid (10-HNDE) as internal standard.

Comparison of the content of 9-HODE in rheumatically destructed and non destructed bones showed significant differences (see Fig. 1): The 9-HODE-content in non destructed bones was found to be 18.3 ± 9.3 μg/g lipid whereas the content of 9-HODE in rheumatic bones was increased to 2–3fold amounts: It was determined to be 43.5 ± 6.6 μg/g lipid. The largest difference between affected and visibly non affected bone material was observed in patient 1: The difference amounted to 5:1. This might be caused by the fact that the bone material originated from different kinds of bones (wrist joint and hip joint).

Remarkably elderly RA-patients showed only low differences in the content of 9-HODE be-

Table I. Age and sex of patients and kind of destructed and non destructed bone-material. From some patients also rheumatic synovial membranes were investigated, indicated by +.

	Age/sex	Kind of rheumatic bone	Kind of non-destructed bone	Rheumatic synovial membrane
1	43, m	wrist joint	hip-joint	–
2	54, m	hip-joint	hip-joint	+
3	56, f	metatarsal bone	metatarsal bone	+
4	65, f	hip-joint	hip-joint	–
5	66, m	knee-joint	knee-joint	–
6	74, f	hip-joint	hip-joint	+
7	80, f	knee-joint	knee-joint	–

tween non destructed and rheumatically destructed bones (patients 6 and 7). This parallels with the observation that the content of 9-HODE in LDL increases dramatically with age (Jira *et al.*, 1996).

The content of 9-HODE in the rheumatic synovial membrane was significant lower than in rheumatically destructed bones and differentiated not very much from that of non destructed bone material of the same person (Fig. 1).

Epoxy fatty acids

Qualitative analysis as epoxy fatty acid methyl ester

Epoxy fatty acid methylesters show uncharacteristic mass spectra. Ions at $[M]^+$, $[M-18]^+$ (loss of water), $[M-31]^+$ (loss of $^{\bullet}OCH_3$) and ions originating from α-cleavages at the epoxy-group are observed with low intensity only. As a consequence their detection requires the knowledge of retentions indices and of comparison spectra. These allowed detection of 9,10-epoxyoctadecanoic acid and 9,10-epoxy-12-octadecanoic acid (leukotoxin A) in form of their methylesters in healthy and rheumatically destructed bones and in the rheumatic synovial membrane. However in contrast to the investigations of *Dudda* (Dudda *et al.*, 1996) on epoxy fatty acids in ischemic porcine heart tissue leukotoxin B and linoleic acid diepoxide were not detected in bones and in the rheumatic synovial membrane.

Quantitative determination of epoxy fatty acids

The lack of intense key ions in the mass spectra of epoxy fatty acid methylester prevented their quantification in bone material by use of the ion tracing method . Localization of epoxy groups by mass spectrometry requires therefore their transformation in derivatives which posess a high tendency to generate α-cleavage products. This is achieved in an already classical procedure for location of double bonds in unsaturated fatty acids in which the double bond is epoxidized and the epoxide reacted with dimethylamine to a mixture of two regioisomeric dimethylaminoalcohols (Audier *et al.*, 1964) or by generation of methoxyalcohols (Minnikin and Patel, 1979). Even better nucleophiles compared to amines or alkoxyradicals are thiols: The unpaired electron-pair of sulphur in the thiol-group in glutathione for instance is able to open an epoxide-ring under physiological conditions. This reaction is catalyzed in the body by glutathione-S-epoxidetransferase . In the course of such a reaction leukotriene A_4 reacts with glutathione by ring opening to generate leukotriene C_4 (Karlson *et al.*, 1983).

In analogy to this reaction epoxy fatty acids in bones and in the rheumatic synovial membrane were derivatized after methylation with thiophenol and triethylamine according to a method of *Corey et al.* (1980) (Scheme 1).

The mass spectra of these adducts are characterized by key ions derived by α-cleavage which allowed quantification.

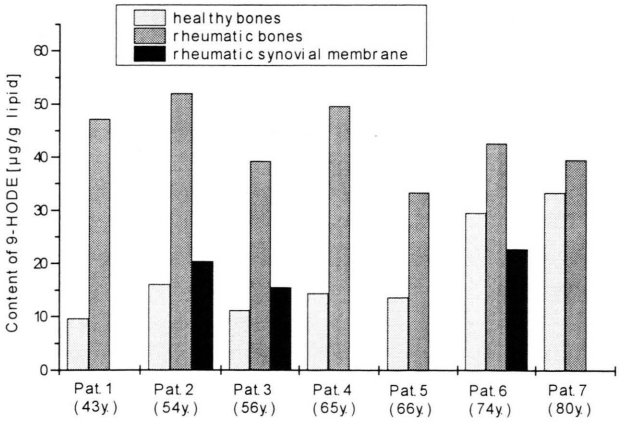
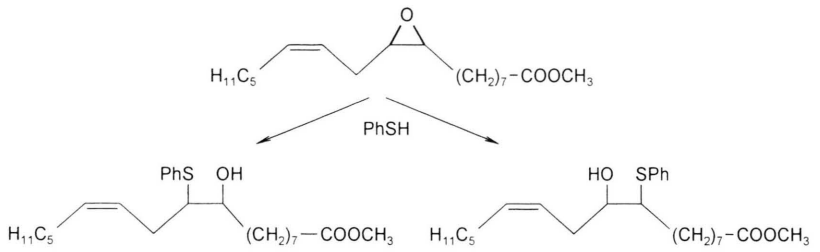


Fig. 1. Comparison of the content of 9-HODE in non-destructed and rheumatically destructed bones and in the rheumatic synovial membrane.



Scheme 1: Conversion of leukotoxin A to corresponding thiophenol adducts

This method offers two advantages in comparison to ring opening with methanol (Minnikin and Patel, 1979) or diethylamine (Audier *et al.*, 1964): On the one hand the reaction conditions are very mild (room temperature), on the other hand peak-overlapping by GC-separation is avoided: Thiophenoxy-trimethylsilyloxy fatty acid methylesters eluate significantly later from the GC-column than usual oxidation products of fatty acids, but earlier than sterols, especially cholesterol, which is present in high amounts in biological tissue.

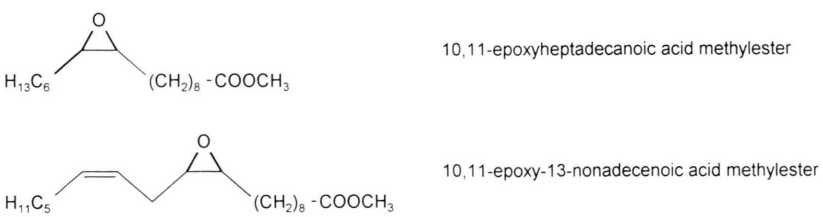
For the derivatization procedure the crude lipid after addition of the internal standards (see Scheme 2) the samples were treated with CH_2N_2 and subsequently with thiophenol and triethylamine for 24 h at room temperature. The resulting thiophenoxy-hydroxy fatty acid methyl esters were separated by TLC and after trimethylsilylation they were analyzed by GC and GC/MS.

The isomeric thiophenoxy-trimethylsilyloxyderivatives of saturated fatty acid methylesters turned out to be inseperable on the used GC columns. Thus the mass spectra contain a mixture of both isomers (Fig. 2A, 2B).

Quantification of derivatized methylates of epoxy fatty acids was achieved by comparison of the peak areas of the α-cleavage products with analogous peaks obtained from the standard compounds (see Scheme 2). Thus the intensities of the ions *m/z*=215 derived from the methylate of 9-thiophenoxy-10-trimethylsilyloxy octadecanoate (Fig. 2B) and *m/z*=259 derived from the methylate of 10-thiophenoxy-9-trimethylsilyloxy octadecanoate (Fig. 2B) were compared with those derived from the corresponding derivatives of the internal standard 10,11-epoxyheptadecanoic acid methylester (*m/z*=187 and *m/z*=273, Fig. 2A, Scheme 2).

In analogy quantification of 9,10-epoxy-12-octadecenoic acid (leukotoxin A) was achieved by comparing the peak areas of the α-cleavage fragments obtained after derivatisation (*m/z*=213, Fig. 2C and *m/z*=259, Fig. 2D) with those obtained from the internal standard 10,11-epoxy-13-nonadecenoic acid methylester with peaks at *m/z*=213 and *m/z*=273 by determination of mean values.

The content of epoxy oleic acid in rheumatically destructed bones was 4.2 ± 3.2 μg/g lipid. In contrast in non destructed bones of the same patients the amount of this LPO-product was significantly lower (1.9 ± 1.6 μg/g lipid). Nearly no epoxy oleic acid was observed in the bones of patient 1, thus



Scheme 2: Internal standards for the quantification of epoxy fatty acids

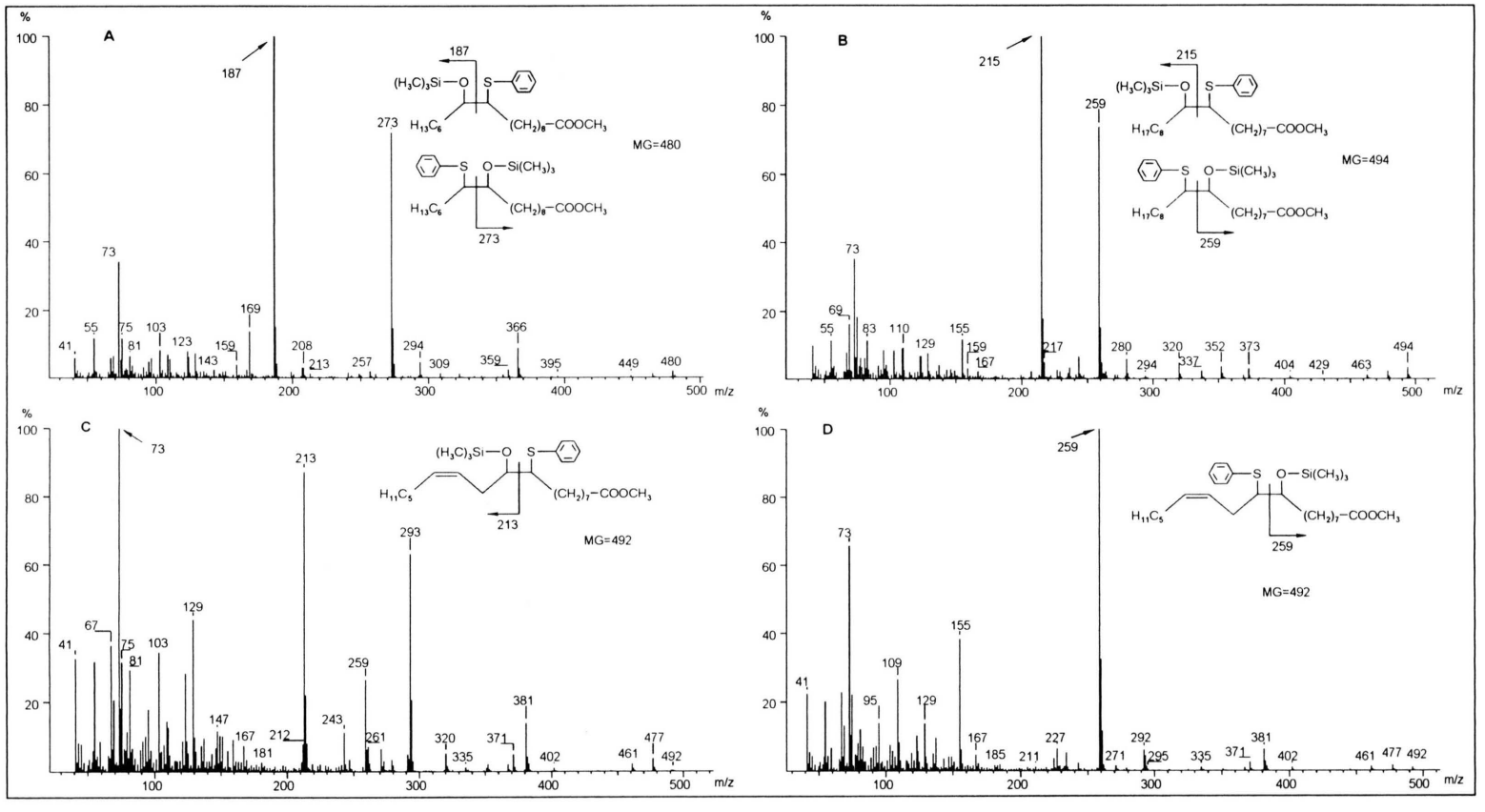


Fig. 2. EI mass spectra of a mixture of regioisomeric trimethylsilylated methyl esters of thiophenoxyhydroxyheptadecanoic acid (A) and thiophenoxyhydroxyoctadecanoic acid (B) and mass spectra of 9-thiophenoxy-10-trimethylsilyloxy-12-octadecenoic acid methyl ester (C) and 9-trimethylsilyloxy-10-thiophenoxy-12-octadecenoic acid methyl ester (D).

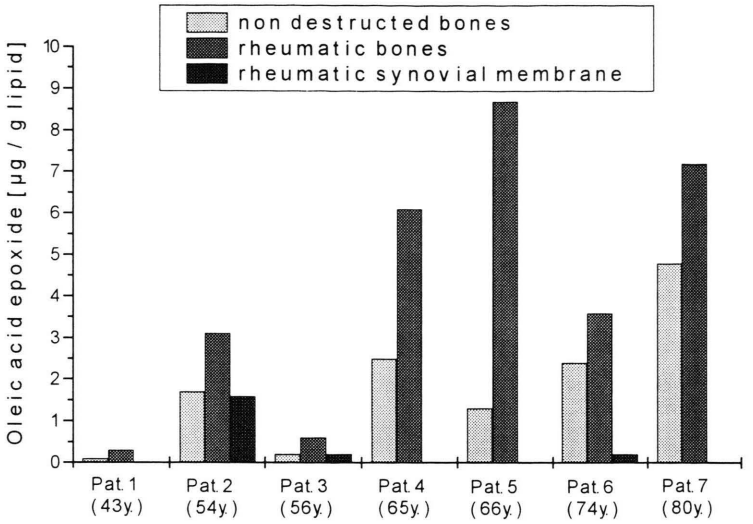


Fig. 3. Comparison ot the content of epoxy oleic acid in healthy and rheumatically destructed bones and in the rheumatic synovial membrane.

indicating that epoxidation may be related to aging. In the rheumatic synovial membrane only small amounts of epoxy oleic acid were detectable (Fig. 3).

In rheumatically destructed bones significantly increased amounts of leukotoxin A (see Fig. 4) were observed in comparison to the content in non destructed bones. In comparison to epoxy oleic acid the content of this monoepoxy linoleic acid was about three times higher.

The mean value of the content of leukotoxin A in non destructed bones was 5.6 ± 2.7 µg/g lipid whereas in rheumatically destructed bones 12.7 ± 4.4 µg/g lipid were detectable (Fig. 4). However these differences in the content of this epoxy fatty acid showed strong individual fluctuations, ranging between 25% and 400%. Again the lowest values were observed in patient 1 while highest values were found in the oldest patients. The content of leukotoxin A in the rheumatic synovial membrane was significantly lower than in rheumatically destructed bones.

Discussion

The involvement of LPO processes in chronical diseases and aging is deduced by increases of a

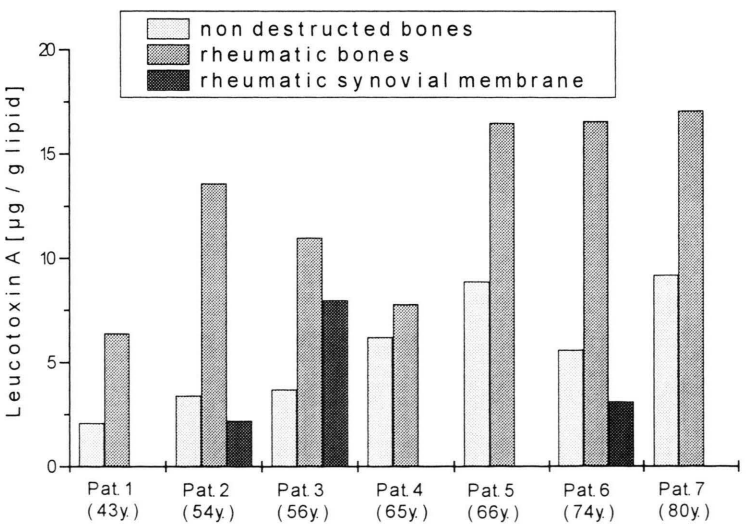


Fig. 4. Comparison of the content of leukotoxin A in healthy and rheumatically destructed bones and in the rheumatic synovial membrane.

great number of marker compounds (Janero, 1990; Kneepkens *et al.,* 1994; Miyazawa *et al.,* 1990). Very often the observed increases were rather low and overlapped partly with physiological individual variations. The new markers for LPO, 9- and 13-HODE, are not generated – in contrast to MDA and isoprostanes – as final products after a branching cascade of events but as secondary products and they are generated from the most abundant PUFA in biological media – linoleic acid. Thus HODEs are much more enriched than any other LPO product and therefore much larger differences between diseased and physiological processes become evident, as shown by investigation of LDL derived from healthy volunteers and patients suffering from RA (Jira *et al.,* 1997), atherosclerosis (Jira *et al.,* 1998) or also in aged individuals (Jira *et al.,* 1996). In addition remarkable differences were detected in the content of 9- and 13-HODE in LDL samples obtained after a shock (Kreil *et al.,* 1998) or surgery (Kreil *et al.,* 1998) and also in porcine heart tissue after myocardial infarction (Dudda *et al.,* 1996). All these diseases are connected with cell damage.

We used this method to investigate wheather LPO is expressed in bone material of patients suffering from RA. Since during surgery destructed bone material is removed together with intact one we had the unique possibility to exclude individual changes in metabolism by investigation of destructed and obviously non destructed bones of the same individual. And indeed we observed individual differences in the content of 9-HODE for about 300% and even larger (in the case in which a different kind of bone from a not yet affected area was available, patient 1).

The increase in hydroxy fatty acids in bones of patients suffering from RA indicates that LPO processes are not restricted to LDL. The increase of 9-HODE has physiological consequences: 9-HODE was found to be an equal strong proinflammatory mediator as leucotrienes (Moch *et al.,* 1990). It also stimulates the release of interleukin 1β from macrophages and this is connected with bone degradation (Gowen *et al.,* 1983). Thus treatment of RA should try to provide reduction of lipid peroxides by application of reducing reagents but also to avoid LOOH formation at all.

In previous research on RA the possibility of epoxidation had not been considered. Since during LPO peroxylradicals are generated (Gardner, 1989) and since these attack any available double bond by epoxidation . Epoxides of unsaturated fatty acids are produced. Thus for instance lipid hyroperoxides of linoleic acid were detected after burn injury (Ozawa *et al.,* 1988[a]), in lungs of patients suffering from adult respiratory distress syndrome (ARDS) (Ozawa *et al.,* 1988[b]) and in ischemic porcine heart tissue (Dudda *et al.,* 1996). These epoxides caused in animal experiments with dogs acute heart failure (Sugiyama *et al.,* 1987). Due to their cardiotoxic activity they were designed to be „leukotoxines" (Ozawa *et al.,* 1989; Fukushima *et al.,* 1988).

According to these observations and considering that LPO involves bone material as shown above we expected leukotoxine formation also in bones. Leukotoxines do not show key ions in their mass spectra, thus quantification was not possible by using the ion tracing method, but required analysis of thiophenol adducts which generated satisfying mass spectra allowing quantification: And indeed remarkable high amounts of leukotoxines were found. In addition epoxidation products of oleic acid were detected. In contrast to leukotoxines physiological properties of 9,10-epoxyoctadecanoic acid were studied only occasionally: Kinae *et al.* (1981) reported a mutagenic activity of this compound. In animal experiments with rats only a low toxicity of epoxy oleic acid was observed (Chu *et al.,* 1980).

Although generation of epoxides *via* a cytochrome P 450 catalyzed reaction can not be excluded, it seems more reasonable that most of the epoxidation products are the consequence of LPO processes: Such processes had been observed in model oxidations of linoleic acid and in absence of enzymes too.

The observed raised amounts of LPO products of linoleic acid in rheumatically destructed bone materials in comparison to healthy ones seems to be an important evidence that LPO is involved in the joint-destruction of patients suffering from RA.

Acknowledgement

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