Enhanced Lipid Peroxidation in Synoviocytes from Patients with Osteoarthritis

BRUNELLA GRIGOLO, LIVIA ROSETI, MAURO FIORINI, and ANDREA FACCHINI

ABSTRACT. Objective. To evaluate the degree of lipid peroxidation of synoviocytes from patients with rheumatoid arthritis (RA), osteoarthritis (OA), and controls and to look at the production of nitric oxide (NO) and its involvement in this process.

Methods. Human synoviocytes were isolated from synovial tissues from patients with RA, OA, and from healthy controls. Cells were maintained in culture for up to 3 culture passages. Lipid peroxidation, verified by the production of malonaldehyde (MDA) and 4-hydroxy-2(E)-nonenal (4-HNE), was determined by colorimetric assay. NO was evaluated by estimating the stable NO metabolite nitrite by the Griess method in the supernatants of unstimulated and interleukin (IL)-1β and tumor necrosis factor (TNF)-α-stimulated cells.

Results. Increased levels of lipid peroxidation were observed for OA-derived synoviocytes compared to RA and controls. The cells in each experimental group produced low amounts of NO both in basal and in stimulated conditions.

Conclusion. In OA, synovial cells underwent a lipid peroxidation process that did not occur in synoviocytes from RA or controls even in the absence of a detectable production of the reactive nitrogen intermediate NO. We can postulate that this peroxidation process might be due to the action of NO secreted by chondrocytes that are known to produce higher levels of this radical in OA compared to RA. (J Rheumatol 2003;30:345–7)

Key Indexing Terms: LIPID PEROXIDATION, RHEUMATOID ARTHRITIS, NITRIC OXIDE, SYNOVIOCYTES, OSTEOARTHRITIS

Lipid peroxidation is a crucial step in the pathogenesis of several diseases including cancer, inflammatory processes, atherosclerosis, ischemic-reperfusion damage, and neurological disorders. Oxidation of lipids is a natural process generated mainly by the effect of several reactive oxygen species that attack the polyunsaturated fatty acids of the fatty acid membrane, initiating a self-propagating chain reaction. Enzymatic and non-enzymatic natural antioxidant defense mechanisms exist; however, these may be overcome, causing lipid peroxidation to take place. Some products of lipid peroxidation are diffusible and can spread the damage far beyond the site of the original free radical attack interfering with the regulation of several metabolic pathways and influencing gene expression.

Rheumatoid arthritis (RA) and osteoarthritis (OA) are common joint diseases that ultimately lead to destruction of cartilage and/or bone remodeling. In both arthritides the loss of the integrity of the extracellular matrix depends on an imbalance between anabolic and catabolic pathways. Inflammatory cytokines and other mediators [enzymes, free oxygen radicals, and nitric oxide (NO)] are mainly involved in matrix cartilage homeostasis. In particular, it is well known that radical oxygen species with oxidative activity, which include reactive nitrogen intermediates, play an important role in the chondrocyte catabolic program, being the mediators and effectors of cartilage damage. Moreover, reactive oxygen species are released during inflammation of the synovial membrane by synoviocytes. Evidence is accumulating about overproduction of NO in OA and RA synovial fluids. It has been shown that this molecule is the primary inducer of apoptosis in human articular chondrocytes and synoviocytes in OA and RA.

We evaluate the degree of lipid peroxidation by synoviocytes from patients with RA and OA and controls, look at the production of NO by means of nitrite products released by these cells, and examine its possible implication in this process.

MATERIALS AND METHODS

Patients. Synovial tissues were obtained from 14 patients with RA (mean age 60 years, range 24–75, disease duration 14 years, range 1–40) and 14 patients with OA (mean age 62 years, range 28–84, disease duration 7 years, range 1–30) undergoing joint replacement surgery. Human synovial tissues were also obtained from 10 controls operated on for trauma with no history of joint...
pathology (mean age 57 years, range 37–70). The diagnosis of RA was made according to American Rheumatism Association revised criteria. The diagnosis of OA was based on clinical, laboratory, and radiologic evaluations. Informed consent from patients and approval by the ethical committee of the hospital were obtained.

**Synovial cells isolation.** Synovial tissues were minced into very small pieces and plated onto 75 cm² cell culture flasks (Costar, Cambridge, MA, USA) in OptiMem 1 with Glutamax-1 medium (Life Technologies, LTD, Paisley, Scotland) supplemented with 50 µg/ml gentamycin (Flow Laboratoires, Biaggio, Switzerland), 2.5 µg/ml amphotericin B (Biological Industries, Kibbutz, Beit Haemek, Israel) and 15% fetal calf serum (Biological Industries) in a humidified 5% CO₂ atmosphere at 37°C. In a few days the synovial tissue fragments adhered to the plastic flasks allowing the cells to come out and begin to divide and multiply. To obtain homogenous populations of fibroblast-like cells, synoviocytes were passaged by brief trypsinization and recultured in medium. Synovial fibroblast cultures were found to be free of contaminating cells (lymphoid and monocytic cells) as assessed by morphology (Wright-Giemsa staining) and negative for the expression of CD4, CD3, CD19, CD14, CD68 by FACS analysis. All experiments described were performed using the cells during the third culture passage.

**Lipid peroxidation assay.** After trypsinization of the flasks 2.5–3 × 10⁶ cells were lysed by repetitive freeze/thawing in distilled water. Malonaldehyde (MDA) and 4-hydroxy-2(E)-nonenal (4-HNE), which are the end products derived from peroxidation of polyunsaturated fatty acids and related esters, were determined in the cell lysates by a colorimetric assay following the kit instruction (Calbiochem, San Diego, CA, USA). Results are expressed as µM nitrite produced. The detection limit for this method is between 0.1 and 1 µM nitrite. The NO production was measured by estimating the stable NO metabolite, nitrite, by a microplate adaptation of the Griess assay. Briefly, 100 µl of each supernatant sample were incubated with 100 µl of Griess reagent (Molecular Probe, Eugene, OR, USA) for 10 min at room temperature and the absorbance at 540 nm was measured with an ELISA plate reader (Multiskan, Labsystem, Helsinki, Finland). Concentrations of nitrite were calculated using sodium nitrite in fresh culture medium as a standard. The results were expressed as µM nitrite produced. The detection limit for this method is between 0.1 and 1 µM nitrite.

**Statistical analysis.** Statistical computations were performed using CSS Statistica-Statistical software (Statsoft Inc. Tulsa, OK, USA). All values are expressed as the mean ± SE. Quantitative variables were compared by means of the Mann-Whitney U-test. Differences were considered significant when p < 0.05.

**RESULTS**

Significantly increased lipid peroxidation, measured as MDA and 4-HNE, was demonstrated in the synovial cells of OA compared to RA patients and controls (p < 0.033 and p < 0.035, respectively) (Figure 1).

Synovial cells from OA, RA, and controls produced very low amounts of nitrites (close to the detection limit of the assay) under the culture conditions tested, i.e., in basal conditions or after stimulation with IL-1β or TNF-α for all the samples evaluated (Figure 2).

**DISCUSSION**

Reactive oxygen species derived from molecular oxygen are highly reactive metabolites. These species are generated by different cell types and are able to react with many biological molecules such as proteins, lipids, and carbohydrates. The reaction of oxygen radicals with lipids is a very well studied process known as lipid peroxidation. NO is a short-lived gaseous free radical formed from L-arginine by a family of enzymes known as NO synthases (NOS). Its role as mediator of tissue damage in inflammatory/autoimmune diseases is well documented. Some authors postulate that NO leads to cartilage matrix degradation inhibiting transforming growth factor-β and activating matrix metalloproteinases; furthermore it has been suggested that during the early phase of OA, NO production may lead to chondrocyte apoptosis. Serum and synovial fluid from OA and RA patients contain significantly higher levels of nitrite compared to those of matched controls, and evidence is accumulating that there is...
an overproduction of these reactive species in OA and RA cartilage.

We evaluated the release of NO by OA, RA, and control-derived synoviocytes both in basal and stimulated conditions and looked at the lipid peroxidation process that can occur in these cells. Synoviocytes from OA, RA, and controls produced low levels of nitrates in both culture conditions tested. On the other hand, they underwent a lipid peroxidation process shown by an increase of MDA and 4-HNE levels that were higher in OA compared to RA and control cells. These molecules are well characterized end products derived from peroxidation of cell membrane polyunsaturated fatty acids and related esters caused by NO and/or other reactive oxygen species. The damaging effect of the process is initiated by a chain-reaction that provides a continuous supply of free radicals, which initiate further peroxidation. This involves the mechanism of oxidative decomposition of n-3 and n-6 polyunsaturated fatty acids, membrane phospholipids, leading to the formation of complex mixtures of lipid hydroperoxide, aldehydic end products such as MDA, and 4-HNE.

Since synoviocytes produced low levels of nitrite in all the conditions tested, one hypothesis we can formulate about the formation of such aldehydes is that NO produced by chondrocytes acts on synovial cells causing lipid peroxidation to occur. It is well known that chondrocytes are the major site of production of mediators of inflammation and in particular of NO and we previously found that cells from patients with OA produced higher levels of NO than those from patients with RA. These findings could explain at least in part the higher peroxidation level found in synoviocytes from OA patients even if other radical species are certainly involved.

Our preliminary data could be useful in understanding the etiopathogenetic mechanisms responsible for different arthritic diseases, and may have implications for the use of specific drugs in their treatment.

ACKNOWLEDGMENTS
The authors wish to thank Mrs. Graziella Salmi and Mrs. Patrizia Rappini for editorial assistance and Mr. Luciano Pizzi for technical assistance. We also thank Mr. Keith Smith for revising the manuscript.

REFERENCES