Role of Reactive Oxygen and Nitrogen Species in Etiopathogenesis of Rheumatoid Arthritis

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Abstract. Rheumatoid arthritis (RA) is a chronic disease affecting up to 3% of the population in most countries The causes of RA have not been completely elucidated This paper aims to review the role of reactive oxygen and nitrogen species in the etiopathogenesis of RA Reactive oxygen species (ROS), such as superoxide radical, hydrogen peroxide, hydroxyl radical and hypochlorous acid, as well as reactive nitrogen species (RNS), such as nitric oxide and peroxynitrite, contribute significantly to tissue injury in RA Several mechanisms are involved in the generation and action of ROS and RNS Superoxide radical, hydrogen peroxide and nitric oxide do not directly damage the majority of biological molecules They are however converted into the highly reactive hydroxyl radical, which reacts with almost all molecules in living cells The resulting chronic inflammation process can be reduced with antioxidant therapy To date, scavenging, preventive, and enzyme antioxidants are available The most important mode is scavenging of the hydroxyl radical and of hypochlorous acid Another important way is to inhibit production of RNS and ROS by neutrophils, monocytes, and macrophages The control of inflammation in arthritic patients by natural as well as synthetic antioxidants could become a relevant component of antirheumatic prevention and therapy

Key words: Rheumatoid arthritis — Reactive oxygen species — Nitric oxide — Antioxidants

Introduction

Rheumatoid arthritis (RA) is a chronic destructive inflammatory disease targeting synovial membrane and extra-articular tissues Inflammatory infiltrates accumulate and persist in synovial membrane, and the clinical presentation of the syndrome is dominated by destruction of joint architecture (Weyand and Goronzy 1997) The

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most feared consequences are significant levels of pain, functional disability, and rheumatoid organ involvement.

The development of inflammatory reaction in RA is controlled by a number of cellular and molecular components. The adhesion of leukocytes to the endothelium and subsequent migration through the endothelium is a complex process involving many different molecules interacting sequentially. Leukocyte extravasation into the synovial tissue and fluid is an active process mediated by a number of cellular adhesion molecules. Leukocyte-endothelial adhesion is mediated by integrins and selectins on leukocytes, by members of the immunoglobulin superfamily, and by selectins on the endothelium (Halloran et al. 1996; Szekanec et al. 1996). The proinflammatory cytokines tumor necrosis factor α (TNF-α) and interleukin-1 (IL-1) produced in large amounts by inflamed synovial membrane and participating in intercellular signalling play a pivotal role in the acute phase of RA. There is a correlation between the number of mononuclear phagocytes and the level of IL-1 and TNF-α production (Bondeson 1997).

The lack of sufficient knowledge concerning etiology and pathogenesis is obviously the major hampering factor for introducing efficient therapies in most chronic diseases in man, with RA being no exception. Most regimens used today have at best slowed down the progression of the underlying disease process. The presently available therapies fail to be curative. There is an obvious need for a completely new generation of therapies which would selectively target molecular events of pathogenetic importance. There may also be several reasons for reconsidering the use of the currently available drugs that have been introduced on empirical grounds without insight into their mechanism of action. New approaches to therapy have been designed, which include earlier and more aggressive intervention, new drugs, and combination of drugs. They appear to provide adequate control of inflammation, so that long-term damage of RA might be prevented and considerable costs reduced (Klareskog et al. 1995).

The paper aims to review the role of reactive oxygen species and reactive nitrogen species in the etiopathogenesis of rheumatoid arthritis.

Reactive Oxygen Species

The most important reactive oxygen species (ROS) implicated in inflammatory injuries to tissues are the superoxide radical $O_2^{\bullet -}$, hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\bullet}OH$), and hypochlorous acid (HOCl). ROS may arise also in the inflamed joint by several mechanisms. Oxidants can be produced by activated macrophages in the synovial membrane, by chondrocytes, and by activated neutrophils in the synovial cavity. It has also been proposed that movement of an inflamed joint generates sufficient pressure to cause transient ischaemia of the superficial membrane. In health the synovial cavity exhibits negative pressure, and when the joint is exercised, vascular patency is maintained, allowing for nutrition of the avascular cartilage. In rheumatoid synovitis, the cavity pressure is raised and upon

movement this pressure exceeds the capillary perfusion pressure, causing collapse of the blood vessels. This leads to the production of multiple episodes of "hypoxic-reperfusion injury", generating reactive oxygen species (Mapp et al. 1995). ROS oxidize: (a) IgG, inducing rheumatoid factor production, (b) hyaluronan, leading to hyaluronan fragmentation products which may alter immune function, (c) lipids, generating aldehydes which are toxic and may alter T cell/macrophage interactions, (d) lipoproteins, leading to the production of monocyte chemotactic peptides.

Elucidation of the precise role of ROS in rheumatoid arthritis is rather complex since it is difficult to measure them in vivo. $O_2^{\bullet -}$, $NO^{\bullet}$, H_2O_2 cannot damage most biological macromolecules directly, and it is widely thought that they exert deleterious effects by becoming converted into the highly reactive hydroxyl radical ($^{\bullet}OH$). $^{\bullet}OH$ could be formed by at least three mechanisms (Kaur et al. 1996): (1) In the presence of "catalytic" iron ions, $O_2^{\bullet -}$ and H_2O_2 may be converted to $^{\bullet}OH$ by the Haber-Weiss reaction. (2) The interaction of $NO^{\bullet}$ and $O_2^{\bullet -}$ forms the cytotoxic product peroxynitrite ($ONOO^-$). At physiological pH, $ONOO^-$ converts to its protonated form – peroxynitrous acid, which decays to generate multiple toxic products, believed to include nitronium ion (NO_2^+), nitrogen dioxide radical ($NO_2^{\bullet}$) and a species resembling $^{\bullet}OH$. (3) Activated neutrophils secrete the enzyme myeloperoxidase, which uses H_2O_2 produced from $O_2^{\bullet -}$ to generate the powerful antibacterial agent hypochlorous acid, HOCl. In a pathway apparently independent of metal ions, HOCl reacts with $O_2^{\bullet -}$ to generate $^{\bullet}OH$.

$^{\bullet}OH$ may react at a diffusion controlled rate with almost all molecules in living cells. Oxidation of proteins, lipids, DNA, uric acid, polysaccharides has been shown to be increased in rheumatoid arthritis patients. In addition, H_2O_2 dependent inactivation of enzymes in cartilage may lead to inhibition of proteoglycan synthesis, so contributing to cartilage destruction in rheumatoid arthritis by interfering with the repair of proteolytic and oxidative damage (Kaur et al. 1996).

Reactive Nitrogen Species

Nitric oxide ($NO^{\bullet}$) represents a key molecule in the inflammatory and degradative cascade of arthritis. $NO^{\bullet}$ production is increased in inflammatory arthritis both in rodent models and humans. Enhanced $NO^{\bullet}$ production was observed in various compartments in vivo but inflammatory synovium and cartilage are the major source of nitric oxide (Miyasaka and Hirata 1997). $NO^{\bullet}$ is a very small molecule synthesised from the amino acid L-arginine by a family of enzymes, the nitric oxide synthases. The activity of constitutive or inducible $NO^{\bullet}$ synthase in the model of peritoneal macrophages cannot be regarded as a determinant of genetically controlled disease inducibility and severity (Zidek et al. 1995). The small size of $NO^{\bullet}$ and its unpaired electron make it a highly reactive and diffusible free radical. $NO^{\bullet}$ can directly interact with metals (predominantly heme complexes) and radicals. Most of the damage to macromolecules is mediated through the reactive nitrogen dioxide $NO_2^{\bullet}$ and peroxynitrite ($ONOO^-$) (Wink et al. 1996). The inducible form of nitric oxide synthase is overproduced in osteoarthritis and rheumatoid arthritis.

The primary producer of NO$^{\bullet}$ amongst the cells normally found in articular joint are chondrocytes (Manfield et al. 1996). NO$^{\bullet}$ released from chondrocytes mediates several catabolic events associated with cartilage degradation, in particular activation of metalloprotease enzymes and suppression of proteoglycan synthesis. NO$^{\bullet}$ plays an important role in the cascade of arthritis because it regulates the activity of the central enzyme in prostaglandin synthetic pathways, cyclooxygenase (COX). Once induced, nitric oxide synthase activity correlated with increased cyclooxygenase activity in chondrocyte cultures and in conditioned media from osteoarthritic human cartilage. When nitric oxide synthase activity was inhibited, inhibition of cyclooxygenase activity was also observed. Exogenous NO$^{\bullet}$ induced cyclooxygenase activity, while NO$^{\bullet}$ scavengers inhibited its activity (Manfield et al. 1996).

Another important radical reaction is that between superoxide and NO$^{\bullet}$, yielding peroxynitrite radical, which plays a role in the oxidation of biological macromolecules contributing to potential deleterious processes. Yet on the other hand, it is involved in the detoxication of superoxide, thus preventing damage normally associated with ROS (Wink et al. 1996). Peroxynitrite is a hydroperoxide, which is also an efficient peroxidase substrate and cyclooxygenase activator. Increased levels of the marker molecule for peroxynitrite, nitrotyrosine, were found in serum and synovial fluid samples from rheumatoid patients (Kaur and Halliwell 1994).

Although there is general agreement that NO$^{\bullet}$ is involved in inflammation, the literature is confusing, as both pro-inflammatory and anti-inflammatory properties have been ascribed to NO$^{\bullet}$ (Stefanovic-Racic et al. 1993). Its anti-inflammatory effect may be accounted for by its ability to inhibit the synthesis of prostaglandin E_2, thromboxane, and IL-6 (Stadler et al. 1993; Amin et al. 1997), and to suppress the production of superoxide by neutrophils through the direct action upon NADPH oxidase (Clancy et al. 1992). However some data indicate that enhanced NO$^{\bullet}$ production is a consequence (and may thus be considered to be a disease-modifying factor) rather than an etiologic principle of the disease (Tanaka et al. 1996; Gilkeson et al. 1997).

Antioxidants in the Management of Rheumatoid Arthritis

In inflammation such as that in joints affected by rheumatoid arthritis or osteoarthritis, ROS are directly involved in tissue injuries and they indirectly facilitate tissue destruction by inactivating α-1-protease inhibitors that form a complex with elastase, a serine proteinase. ROS have been implicated in the damage of hyaluronic acid (HA), which is depolymerised, and synovial fluid loses its lubricating properties, causing friction in the joint (Kataoka et al. 1997). Hyaluronan attacked by ROS, and especially by $^{\bullet}$OH, yields several intermediates and endproducts found in increased concentrations in the synovial fluid and serum of rheumatic patients (Orvisky et al. 1997). In terms of suppressing inflammation, it is therefore desirable to eliminate the ROS that are formed excessively at inflammatory sites (Kataoka et al. 1997).

Different types of antioxidants could be used to advantage in the management of rheumatoid arthritis. By a broad definition, an antioxidant is any substance that, when present at low concentrations compared with those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate (Halliwell 1991). To date, scavenging, preventive, and enzyme antioxidants are classified according to their mode of action (Chapple 1997). The most important mode of affecting the oxidative damage is scavenging of the hydroxyl radical and of hypochlorous acid. On considering the biological importance of scavenging of $^{\bullet}OH$ or HOCl by an anti-inflammatory drug at a site of inflammation, one must also consider whether any radicals produced on oxidation of the drug might not themselves induce biological damage. Another important way in which antioxidants influence the oxidant damage is by inhibiting production of RNS and ROS by neutrophils, monocytes, and macrophages (Halliwell et al. 1988).

Endothelial cells, neutrophils, and macrophages have been shown to be capable of producing both $NO^{\bullet}$ and $O_2^{\bullet -}$. These findings are consistent with the hypothesis that peroxynitrite ($ONOO^-$), which is produced by the combination of $NO^{\bullet}$ and $O_2^{\bullet -}$, may be ultimately responsible for the pathophysiology previously attributed to $NO^{\bullet}$ alone. Thus, the ability to intercept and decompose $ONOO^-$ may represent a novel and critical point of therapeutic intervention (e.g. with Fe porphyrin catalysts) in diseases associated with the overproduction of $NO^{\bullet}$ and $O_2^{\bullet -}$ (Salvemini et al. 1998).

Endogenous $NO^{\bullet}$ formation by inducible NO synthase is enhanced in adjuvant arthritis. Further investigations in animal models and in clinical studies are needed to establish whether inhibition of $NO^{\bullet}$ synthesis may prove a new therapeutic approach in the treatment of inflammatory joint disease (Stichtenoth et al 1994). The arginine-nitric oxide synthase pathways may be a novel therapeutic target in the prevention and management of arthritis (Manfield et al. 1996). Understanding the extent of $NO^{\bullet}$ involvement in chronic inflammation versus cartilage metabolism may help resolve the problem of whether NO synthase inhibition is appropriate (Jang and Murell 1997). There is a need to introduce selective inhibitors of the inducible form of NO synthase, whose activity is preferentially expressed in rheumatoid arthritis (Stefanovic-Racic et al. 1993).

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