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Free Radicals in the Rheumatoid Arthritis

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Abbreviations:

ADAMTS: a disintegrin and a metalloprotease with thrombospondin motifs; AP-1; activator protein 1; COX: cyclooxygenases; CVD: cardiovascular disease; DMARD: disease-modifying anti-rheumatic drug; DPI: diphenyleneiodonium; ECM: extracellular matrix; GM-CSF: granulocyte-macrophage colony stimulating factor; HA: hyaluronic acid; HLA: human leukocyte antigen; IL: Interleukin; IL-1ra: IL-1 receptor antagonist; IKK: I κ B kinase; I κ B: inhibitor κ B; MHC: major histocompatibility complex; MMP: Matrix metalloproteinases; NF- κ B: nuclear Factor κ B; NLS: nuclear localization signals; NSAID: nonsteroidal anti-inflammatory drug; PG: prostaglandin; PGHS: prostaglandin H synthase; RA: Rheumatoid arthritis; ROS: reactive oxygen species; RNS: reactive nitrogen species; SOD: superoxide dismutase; sTNFr: soluble tumour necrosis factor receptors; TCR: T cell receptor; TIMP: tissue inhibitors of metalloproteinase; TGF: transforming growth factor; TNF: tumour necrosis factor.

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Abstract

Rheumatoid arthritis (RA), shown as joint inflammation, pain, swelling, osteoporosis and bone erosion, is a debilitating autoimmune disease. It affects about 2% of the people all over the world. The etiology of RA remains elusive. But free radicals, oxidizing, proteases, cytokines, transcription factors and immune cells are believed to play a role in the onset of arthritis. The enhanced production of ROS and free radicals by all kinds of endogenous sources were observed. ROS and free radicals have multiple effects on joint structure and function. Now many promising methods are under research and may bring great improvements to RA treatment.

1.Introduction of the rheumatoid arthritis

Rheumatoid arthritis (RA) is a chronic inflammatory, autoimmune disease, manifesting joint swelling, pain, functional impairment and muscle wasting and is associated with increased risk of cardiovascular disease (CVD) and osteoporosis. [1-4]. It is characterized by both localized and systemic inflammation with elevated plasma concentrations of pro-inflammatory cytokines, such as interleukin-6 (IL-6), interleukin-1 (IL-1), tumor necrosis factor- α (TNF- α), and acute-phase proteins [1-4,38].

Rheumatoid arthritis has a prevalence of about 2% worldwide and is threefold more common in women than men, which may be attributable to hormonal factors [1-2]. RA is thought to result from an underlying genetic susceptibility, which is manifest in response to an environmental trigger [1-4,38]. The symptoms of RA typically appear during middle age [2]. The associated complications make RA important in public health terms. Rheumatoid cachexia may be an important contributor to the risk of developing these complications and is associated with the elevated production of the inflammatory cytokines IL-1 and TNF- α . [1-2].

2.Pathogenesis of the rheumatoid arthritis

Although RA is generally accepted as an autoimmune disease, the etiology of RA remains elusive [2,38]. The clear-cut genetic contribution in this disease is contained predominantly within the human leukocyte antigen (HLA) class II locus. On one side of the peptide-binding groove of HLA-DR, there is a shared epitope, comprising amino acids 70-74 of the β chain [38]. This epitope is present in > 80% of Caucasian RA patients and is the most important evidence to support the concept that T lymphocyte recognition is important at some stage in the pathogenesis of RA, either in shaping the T cell receptor (TCR) repertoire or in the antigen presentation [38].

The pathology of RA extends throughout the synovial joint (Fig. 1), and in severe cases, involves many other organs. In contrast to the acellular nature of normal synovial fluid, RA synovial fluid is enriched predominantly with neutrophils, but macrophages, T lymphocytes, and dendritic cells are also present. The increase in cellularity, however, is most obvious in the synovial membrane, which becomes infiltrated by cells recruited from the blood [5]. The lining layer of the joint increased greatly, and comprised mostly of activated macrophages with an underlying layer of fibroblast-like cells. Many of the activated macrophages and T lymphocytes express abundant HLA class II and adhesion molecules of relevance in antigen presentation [38]. The deeper layers within the synovium have follicles of lymphoid cells around vessels as well as lymphocytes scattered between them. Neovascularization is prominent in this region in RA [5].

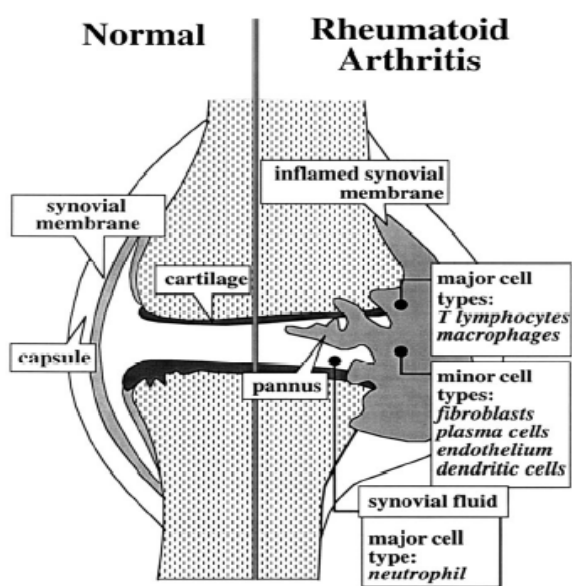


Figure 1. Diagrammatic representation of synovial joint, normal joint (*left*) and rheumatoid arthritis joint (*right*).

The major site of irreversible tissue damage originates at the junction of the synovium with the cartilage and bone, a region termed the *pannus*, an area rich in macrophages [38,42]. The cells of the pannus attach

and migrate into the underlying cartilage and the bone and are related with the erosion of these tissues [38]. At the same time, macrophages and other inflammatory cells are recruited to the joint and produce a number of mediators, including IL-1 and TNF- α . The concentration of IL-1 and TNF- α in

plasma is significantly higher in RA patients than in normal people. And the concentration correlates with RA disease activity [42].

The destruction of the cartilage seen in rheumatic disease is now considered to be mostly due to the activity of metalloproteinases, enzymes produced by activated macrophages and fibroblasts in response to proinflammatory cytokines [44-46]. The activity of metalloproteinases is regulated to some extent by tissue inhibitors of metalloproteinases (TIMPs), which irreversibly bind the active MMP to form a 1:1 complex [53-56]. Much of the connective tissue destruction in RA is due to imbalance between the metalloproteinases and the TIMPs [44-46, 53-56]. The bone damage includes focal erosions, systemic osteoporosis and, as a consequence, increased risk of fracture. The focal erosions of bone are because of that osteoclast mediated resorption overwhelms the new bone formation [42].

3. The effect of free radicals on proteins on cartilage matrix.

Destruction of articular cartilage is an irreversible consequence of arthritis. Cartilage consists of chondrocytes and two major components of matrix, a type II collagen-fibril network with associated small proteoglycans (Fig 2.) [2]. Chondrocyte keeps the homostasis of the extracellular matrix, so apoptosis of chondrocyte is related with the destruction of articular cartilage [42,45]. Cartilaginous proteins are important targets for degradation in arthritis. Chondrocytes and synovial cells synthesize many proteases in response to inflammatory stimuli. Members of each of the four classes of protease – serine/threonine proteases, cysteine proteases, aspartic proteases, and metalloproteases – have been implicated in the degradation of cartilage [44]. However, current data indicate that the metalloproteases is most important for damage of cartilage in RA. This class of enzyme is characterized by the presence of an essential metal ion (usually zinc) within the active site [38,44]. Of

the metalloproteases, the members of two families, the matrix metalloproteases (MMPs) and the ADAMTSs (a disintegrin and a metalloprotease with thrombospondin motifs) family, have been implicated in the breakdown of collagen and aggrecan, respectively [56]. The MMPs can be classified into subfamilies on the basis of structure and substrate selectivity. A convenient grouping is that of collagenases, stromelysins, gelatinases, and membrane-type MMPs (MT-MMPs). Of these enzymes, the collagenases (MMP-1, -8, and -13) are the most specific, since they alone are able to cleave native fibrillar collagens to yield fragments three-quarters and one-quarter the size of the original molecule [38,54-56]. Once the initial cleavage has been made in the collagen fibrils, the triple helix unwinds, rendering the resulting fragments excellent substrates for the gelatinases, MMPs -2 and -9. The stromelysins are characterized by a broad substrate specificity and broad optimum pH range [44]. The MT-MMPs contain a transmembrane C-terminal domain. It has been shown that MT1-MMP is capable of digesting fibrillar type collagens I, II, and III and other extracellular components [44].

All the MMPs are synthesized as inactive proenzymes. Enzyme latency is maintained by ligation of a cysteine residue in the prodomain to the active-site Zn^{2+} ion. Activation occurs via destabilization of the Cys–Zn interaction, followed by a cleavage, resulting in the release of the prodomain [44,55]

Traditionally, the theory regarding MMP activation *in vivo* postulates the attack of susceptible regions in the propeptide MMP domain by soluble or by a membrane-bound proteases. But many research showed that the MMP activation could be achieved *in vitro* by a wide variety of agents that share one common characteristic: thiol reactivity. Based on these observations, a new "cysteine-switch" hypothesis raised [53]. In this model, the zinc atom at the active site is coordinately bound to an unpaired cysteine thiol group located approximately at the 80th residue of the propeptide domain.

Disruption of this interaction is believed as the critical step in initiating the process of MMP

auto-activation. Reactive oxygen species undergo facile reactions with thiol groups and may serve as a common mechanism of activation for several different MMPs [53]

Another metalloproteases family termed ADAMTSs is also very importance in cartilage turnover [56]. It had been known for many years that in cartilage, aggrecan is cleaved by aggrecanases at five unique sites along the core protein after glutamic acid residues [56]. Recently, it was found several aggrecanases belongs to the ADAMTS family [56]. *In vitro* studies suggest that IL-1 can cause cartilage destruction by stimulating the release of matrix metalloproteinases, including aggrecanases and other degradative products [42].

The activity of MMP and ADAMTS is modulated by the some endogenous tissue inhibitors of MMPs (TIMPs), includes four molecules (TIMP-1, 2, 3 and 4). These molecules bind tightly to the active site of activated metalloproteases with a 1:1 stoichiometry and have K_i values of less than 10^{-9} M [2,38].

Recent studies showed that the mutated p53 gene is overexpressed in RA synovium, and the mutated p53 abolished the down-regulation of the promoter activity of hMMP-1 by wild-type p53 [54]. The phenomenon perhaps forms a link between the tumor like hyperplasia in RA synovium and the overexpression of MMPs [6,54].

4. The effect of free radicals on hyaluronic acid (HA) structure [2,45-48]

Another major components, proteoglycan aggregates, is composed of a noncovalent association between aggrecan, hyaluronic acid (HA), and link protein [44]. In addition of the involvement in the structure of articular cartilage, HA is also the principle component of synovial fluid, effecting gel formation and subsequent joint lubrication [2].

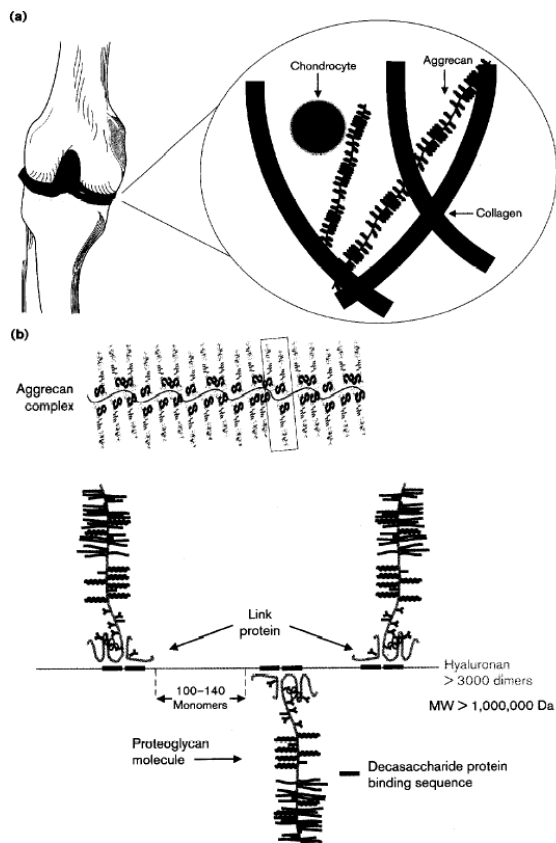


Figure 2. (a) The macroscopic representation of cartilage within a joint. The inset shows the component of cartilage consists of chondrocytes embedded in an extracellular matrix of aggrecan and collagen. (b) A schematic diagram of proteoglycan aggregate (the boxed area is enlarged to show detail). Core protein fragments bearing glycosaminoglycan chains arrange themselves laterally at regular intervals along the hyaluronan backbone via noncovalent interaction with link protein.

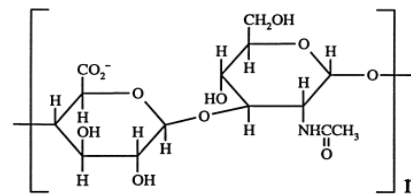


Figure 3. The hyaluronan acid.

Many research has implicate the degradation of HA is essential in arthritis. Current, the degradation of hyaluronic acid is thought mainly caused by reactive oxygen species (ROS), such as HO[·] and HOCl/CIO⁻ [2,46,48]. The ROS are thought to be released from the activated leucocytes and lymphatic cell during acute and chronic inflammation *in vivo* [46-48].

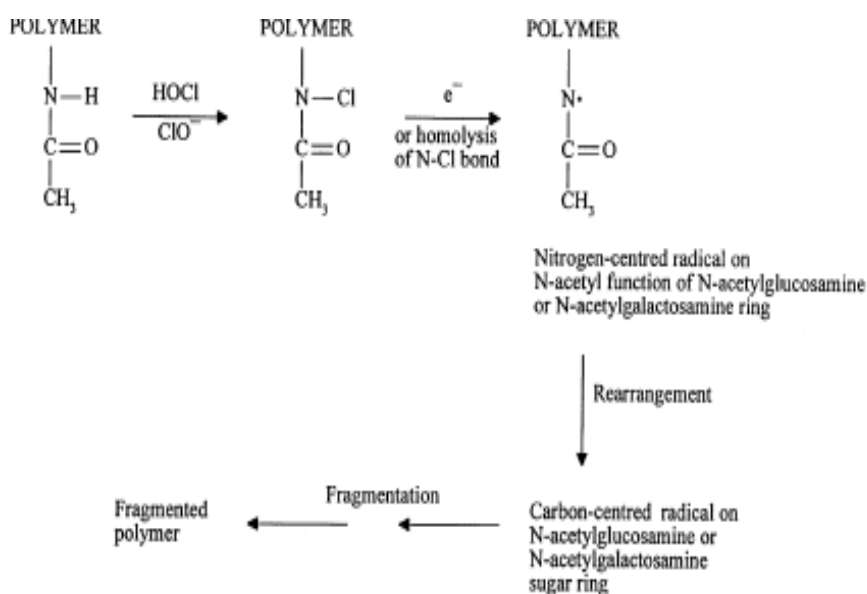
HO[·] attacks on hyaluronic acid, related polymers and monomers has been studied by both direct, rapidflow EPR and EPR spin trapping. Evidence has been obtained, with the monomers, for essentially random hydrogen-atom abstraction at all the ring C-H bonds with glucuronic acid, and at all sites except the N-acetyl side chain and C(2) with N-acetylglucosamine. At both low pH (<4) and high pH (>4), acid- and base-catalysed rearrangement happened, respectively, result in the loss of these species. The rate of loss of these species is dependent on the substrate, with those derived from N-acetylglucosamine undergoing slower acid-catalysed rearrangement than the glucuronic acid-derived species. The base-catalysed process, which is believed to involve a radical-anion

intermediate, occurs rapidly at pH 7.4, and appears to be less substrate dependent. The base- and acid-catalysed rearrangements lead to strand-breakage and the fragmentation [47].

HOCl/ OCl⁻ is believed to be important in the progression of rheumatoid arthritis [46,48]. The activated neutrophils can generate hypochlorous acid through the action of myeloperoxidase (MPO) [48]. MPO also has been found in the synovial fluid. The cationic enzyme was show binding strongly to the negatively charged HA, it is postulated could lead site-specific cleavage of the polymer [48].



UV-visible experiments have shown that HOCl/OCl⁻ reacts preferentially with *N*-acetyl groups. This reaction is believed to give rise to transient chloramide species, which decompose rapidly to nitrogen-centered radicals via either homolysis (to produce N[•] and Cl[•]) or heterolysis (one-electron reduction, to give N[•] and Cl⁻) of the N—Cl bond. Then the initial nitrogen-centered radicals are suggested to rapidly rearrange to carbon-centered radicals, bringing polymer fragmentation [48]. A



Scheme is shown as follows.

Scheme 1. An outline of the events that may occur on reaction of HOCl/OCl⁻ with the *N*-acetylglucosamine [48].

5. The interactions between free radicals and immune cells

The Rheumatoid arthritis (RA) is an autoimmune disease. There are complex interactions between free radicals and immune cells in RA [12]. Macrophages appear to play a pivotal role in RA [2,12,38]. They are numerous in the inflamed synovial membrane and at the cartilage-pannus junction. They show clear signs of activation, such as overexpression of major histocompatibility complex class II molecules, proinflammatory or regulatory cytokines and growth factors [*eg.* IL-1, IL-6, IL-10, IL-13, IL-15, IL-18, TNF)- α and granulocyte-macrophage colony-stimulating factor (GM-CSF)], chemokines and chemoattractants [*eg.* IL-8, macrophage inflammatory protein (MIP)-1 and monocyte chemoattractant protein (MCP)-1], metalloproteinases and neopterin [38,53]. These molecules may contribute to inflammation and joint destruction both in the acute and chronic phases of RA [38].

Research showed that under some conditions *in vivo*, macrophage foam cells produce superoxide, nitric oxide, and hydrogen peroxide [36, 53]. These reactive oxygens can modulate MMP-2 and -9 activity, either directly or via a derivative radical, ONOO⁻. MMPs are secreted in a latent, zymogen form. This conformation of zymogens is maintained by the thiol interactions between cysteine residues with zinc atom present in the catalytic site. *In vitro*, MMP activation can occur when the prodomain is cleaved by other proteases or when the zinc-cysteine bond is interrupted [53]. ROS, from macrophages and some other sources, are known to react with thiol groups, so they could modulate the activity of MMPs. [53]. ONOO⁻ has the ability to nitrate tyrosine residues of proteins [48]. Research results showed that ONOO⁻ activates pro-MMP-2 by nitration of two tyrosine residues in this protein [48]. It is interesting to speculate that nitration of two tyrosine residues, present within a five amino acid stretch in the hinge region between the propeptide and active domains of pro-MMP-2, could assist in unfolding of the zymogen. Based on many previously published reports, it appears that macrophages

may be able to participate in MMP matrix degradation at several levels. These include: (a) inducing MMP expression in other cells (via secretion of cytokines); (b) producing MMPs; and (c) activating latent forms of secreted MMPs (via production of reactive oxygen species) [53].

The bloodstream neutrophils has enhanced production of superoxide ion and peroxynitrite, and it was suggested that NADPH oxidase together with NO synthase were the major sources of superoxide ion in RA neutrophils [36]. SOD and rutin (vitamin P) were found very efficient suppressors of oxygen radical overproduction by RA neutrophils [36]. In addition, activated neutrophils, can generate hypochlorous acid through the action of myeloperoxidase, seem capable of auto-activating their latent collagenase [48]. The establishment of a chronic synovitis involves the traffic of circulating inflammatory cells into and through the synovial membrane, regulated by cell adhesion molecules, which are in turn regulated by pro-inflammatory cytokines. Manipulation of the expression of cell adhesion molecules can have a profound effect on the inflammatory process in RA [43].

6. The interaction between free radicals and cytokines in RA

The cytokines are important effectors in immune and inflammation reactions. Many pro-inflammatory cytokines, such as IL-1 α/β , TNF α , GM-CSF and IL-6, are produced by the RA synovial membrane, regardless of therapy [37-38]. There also appears to be a compensatory anti-inflammatory response in RA synovial membranes which includes the IL-1 receptor antagonist protein (IL-1Ra), p55 and p75 soluble TNF receptors (sTNFr), IL-4, IL-10 and transforming growth factor- β (TGF β) [37-38,43].

Now the new concept is that the proinflammatory cytokines were linked in a network with TNF α at its apex. This led to the hypothesis that TNF α was of major importance in rheumatoid arthritis and was a therapeutic target. This hypothesis has been successfully tested in animal models and these studies

have provided the rationale for clinical trials of anti-TNF α therapy in RA patients. Several clinical trials using a chimeric anti-TNF α antibody have shown marked clinical benefit, verifying the hypothesis that TNF α is of major importance in rheumatoid arthritis [38].

Progressive loss of cartilage is the may lead to joint failure in RA patients. IL-1 and TNF- α have been shown implicated in loss of cartilage since they are present in excess in affected joints and are capable of degrading the cartilaginous matrix while inhibiting its synthesis. On the other hand, local growth factors produced by chondrocytes, such as transforming growth factor- β (TGF- β), can counteract the deleterious effects of IL-1 and TNF- α through stimulation of matrix synthesis and inhibition of metalloprotease expression [1-6]. ROS have an important role in the pathogenesis of RA and can be stimulated by proinflammatory cytokines. Hydrogen peroxide, superoxide and NO production is stimulated by cytokines such as IL-1, TNF- α and IFN- β in chondrocytes [38].

Some cytokines generally regarded as possessing immunoregulatory and anti- inflammation. These molecules include TGF- β , IL-4, IL-10, and IL-13 [38]. TGF- β is likely to be a key cytokine involved in repair and fibrosis in the joints. For example, while inhibiting production of metalloproteinases, TGF- β also stimulates the production of type I and type XI collagens. The modulation of different TGF- β isoforms and TGF- β receptors was found to be differentially controlled by NO and ROS [38-40]. In common with TGF- β , IL-4 also displays some immunoregulatory effects such as inhibition of LPS-induced IL-1, TNF α , PGE₂, and 92-kDa gelatinase production in human monocytes [38]. IL-10 also has profound anti-inflammatory and immune-regulatory effects. The inhibition of IL-10 activity using a neutralizing monoclonal antibody can enhance TNF α and IL-1 production. Conversely, addition of recombinant IL-10 to these cultures inhibited TNF α and IL-1 production by approximately 50% [38]. IL-10 induces the production of the endogenous TNF inhibitors, i.e. soluble TNF receptors

from monocyte cultures, while also downregulating surface TNF receptor (TNF-R) expression (94). Thus, many of the properties of IL-10 are compatible with its being a major immunoregulator [38]. IL-4 was more potent in inhibition of IL-1, and additionally IL-4 induced the production of the native inhibitor of IL-1: IL-1ra, which has a role in maintaining a balance with IL-1 in the joint [38,42].

7. The effect of free radicals on transcription factors

Many transcription factors are under ROS or free radicals regulation. Among them, the NF- κ B and AP-1 are especially important in RA. NF- κ B controlled expression of numerous inflammatory molecules in synoviocytes and protected cells against tumor necrosis factor α (TNF α) and Fas ligand (FasL) cytotoxicity. *In vivo* suppression of NF- κ B by either proteasomal inhibitors or intraarticular adenoviral gene transfer of super-repressor I κ B profoundly enhanced apoptosis in the synovium of rats with SCW. This indicated that the activation of NF- κ B protected the cells in the synovium against apoptosis and thus provided the potential link between inflammation and hyperplasia. Intraarticular administration of NF- κ B decoys prevented the recurrence of SCW arthritis in treated joints. Unexpectedly, the severity of arthritis also was inhibited significantly in the untreated joints, indicating beneficial systemic effects of local suppression of NF- κ B [35].

AP-1 is another important transcription factor. IL-1 is implicated in cartilage destruction in RA through promotion of MMPs production [38, 41]. Upregulation of collagenase gene expression by IL-1 is known to require the transactivators Fos and Jun. ROS have been suggested to act as intracellular signaling molecules mediating the biological effects of cytokines. One research demonstrated ROS production by IL-1-stimulated bovine chondrocytes and that neutralizing ROS activity by the potent antioxidant, N-acetylcysteine, or inhibiting endogenous ROS production by diphenyleneiodonium

(DPI), significantly attenuated IL-1 induced c-fos and collagenase gene expression. The inhibitory effect of DPI implicates enzymes such as NADPH oxidase in the endogenous production of ROS [41]. Chondrocytes were also found to produce nitric oxide (NO) upon IL-1 stimulation. That NO may mediate part of the inducing effects of IL-1 [41].

8. The effect of free radicals on the cyclooxygenase

Prostaglandins (PG) are a family of 20 carbon atom fatty acid compounds, which is synthesized in most tissues in the body [57]. Prostaglandins have important and diverse roles in inflammation. For example prostaglandin E₁ is a potent vasodilator and can increase the intravenous pressure, thus promoting the protein leakage and increase the delivery the pro-inflammatory cells to the site of inflammation [57] Also there are evidences that prostaglandins can sensitize pain receptor in both the central and peripheral neurons [15]. Prostaglandins exert their potential biological function mainly through the binding of specific cell surface receptors [57]. In addition, some kinds of PG, such as PGJ₂

have been found to directly bind to some nuclear receptor and stimulate their function as transcription factors [15].

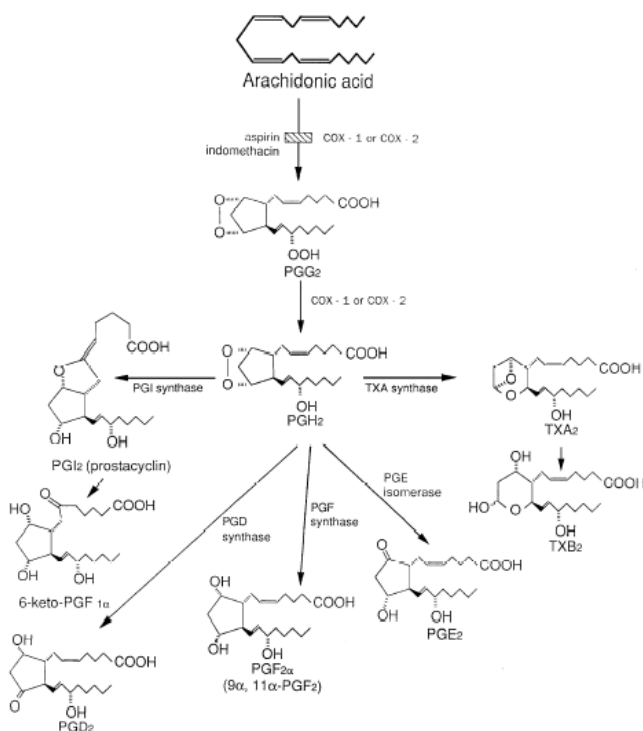


Figure 4. The arachidonic acid cascade [15]. The Cyclooxygenase (COX) is the rate-limiting enzyme in prostaglandin (PG) biosynthesis. There are two isoforms of this enzyme: the constitutive form, COX-1 and inducible form, COX-2 [15].

The synthesis of PG can be divided into three phases as: (i) mobilization of the arachidonic acid from the activation of phospholipase A₂ (PLA₂), (ii) conversion of the arachidonic acid to Prostaglandin endoperoxides, prostaglandin G₂ and then prostaglandin H₂, (iii) then the isomerization or reduction of prostaglandin H₂ to various derivatives, depending on the downstream enzymatic machinery present in a particular cell type [24, 57]. (Fig 4.)

Cyclooxygenase-2 can be induced by various pathogenic stimuli, including proinflammatory cytokines, mitogens, bacteria and ROS [15]. Most of the stimuli are known associate with inflammation and Many anti-inflammation cytokines, such as IL-4, IL-10, will decrease induction of COX-2 [19]. The structure of the COX-1 and COX-2 are very similar, the activity of the both isoforms can be inhibit by aspirin and other nonsteroid anti-inflammtion drugs (NSAIDs). The inhibition by aspirin is due to the irreversible acetylation of the COX active sites [15, 24]. In addition, there is a growing body of evidence to suggest that the nuclear factor-κB is involved in the regulation of COX-2 induction by various stimuli [22-24].

ROS play an important role in inflammation as mediators of injury and potentially in signal transduction leading to gene expression. The ROS scavengers, such as DMSO inhibited IL-1β, TNFα induced COX-2 expression in rat mesangial cells. The NADPH oxidase stimulated COX-2 expression induced by TNFα. But several non-NADPH oxidase-dependent sources of ROS also exist, including mitochondrial electron transport and arachidonate metabolism [22]. So COX-2 is an oxidant stress-inducible gene. An important aspect of COX is its autoaccelerative reaction kinetics; so the ROS produced by inflammatory cells could lead to the deleterious amplification of PGs during inflammation [22]. In addition, it is recognized that there is molecular cross talk between the inflammatory mediators NO and PGs in RA [24].

9. The sources of ROS and free radicals and the oxidative stress

The sources of ROS and free radicals include the neutrophil oxidative burst, Fenton reaction, a series of peroxidases and many other sources. The enhanced production of superoxide ion and peroxynitrite by bloodstream neutrophils and of superoxide ion by monocytes from rheumatoid arthritis (RA) patients was registered. It was suggested that NADPH oxidase together with NO synthase were the major sources of superoxide ion in RA neutrophils, while in RA monocytes superoxide ion was produced by NADPH oxidase and mitochondria. Among the different free radical inhibitors studied (antioxidant enzymes, SOD and catalase; free radical scavengers, bioflavonoid rutin and mannitol; and the iron chelator desferrioxamine), SOD and rutin were the most efficient suppressors of oxygen radical overproduction by RA neutrophils, while mannitol and desferrioxamine were inactive. Iron-catalyzed hydroxyl radical formation was unimportant in RA leukocytes, which mainly produced superoxide ion. This difference between RA neutrophils and monocytes corresponded well to the difference in their NADPH oxidase activities. It has already been shown that NO synthases are able to generate superoxide ion especially under L-arginine-depleted conditions. Thus, NO oxidase apparently participates in the production of superoxide ion by RA neutrophils together with NADPH oxidase. It was also found that NO synthase of RA neutrophils generated the enhanced amount of peroxynitrite. Mitochondrial superoxide production is another source of oxygen radicals in RA monocytes. In addition, the decrease in neutrophil SOD and some other antioxidant enzymes activity and the increase in the levels of "loose" iron in the plasmalemma of neutrophils and monocytes, were also observed. Thus, on the whole, both RA neutrophils and monocytes are under oxidative stress characterized by the overproduction of oxygen radicals and peroxynitrite [1-6, 36-38].

10. Future directions of treatment of RA based on experiments.

For patients of rheumatoid arthritis, the primary therapeutic objectives are the relief of joint pain and improvement in functional status. These goals take on urgency, because the potential for immobility places them at high risk for permanent loss of their independence. Once these patients become bed/chair-bound, medical complications will make recovery improbable [4]. Conventional treatments for RA, including nonsteroidal anti-inflammatory drugs (NSAIDs), disease-modifying antirheumatic drugs (DMARDs) and corticosteroids. However, such treatments are rarely, totally effective and some pharmacological therapies have the potential to cause side effects, such as gastro-intestinal bleeding and bone loss. [1-2]. As a result new therapies are on the way of research, including the inactivation of cytokines, Antioxidant, selective transduction inhibitor, selective COX-2 inhibitor, and TIMPs.

10.1 Selective inhibition of COX-2

The use of nonsteroidal anti-inflammatory drugs (NSAIDs) is achieved only with high dosages of these drugs. At the dosages, the toxicity associated with the chronic use is considerable, such as gastrointestinal (GI) bleeding and renal failure. This is mainly because of the inhibition of the COX-1 [2,4]. So the design of selective inhibitors for COX-2 has been paid great attention.

The first generation of selective COX-2 inhibitors came from animal models in which compounds were sought that were potent anti-inflammatory agents with minimal side effects on the stomach [15]. Nimesulide, etodolac, and meloxicam were discovered in this way. The selectivity ratios of inhibition for these COX-2 inhibitors ranged from 10- to 100-fold selectivity for COX-2. Large-scale clinical trial results of one of this group (meloxicam) clearly show that severe gastric damage is significantly less than that caused by nonselective NSAIDs [15].

The newer compounds specifically designed by medicinal chemists as COX-2 inhibitors, such as **SC58125** and L-745, are more selective, with several 100-fold selectivity for COX-2. Now the binding sites for the selective inhibitors in COX-2 have been described in detail and the three-dimensional structure of the enzyme protein clearly established[15].

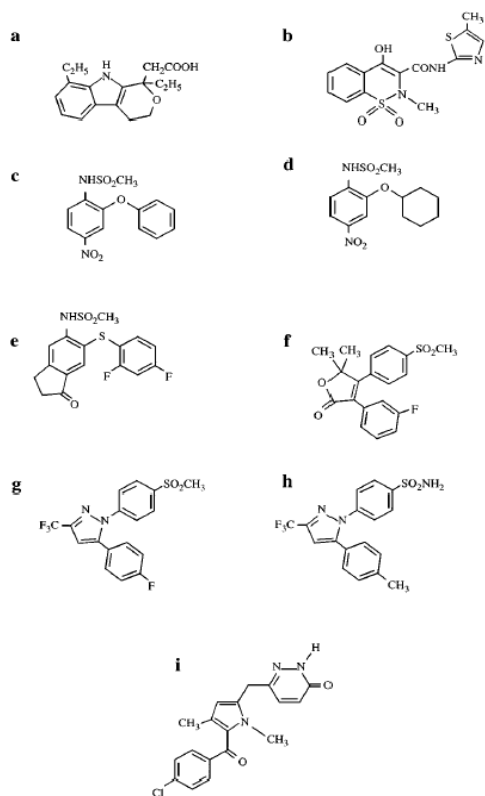


Figure 5. Chemical structures of some selective COX-2 inhibitors. (a) Etodolac; (b) Meloxicam; (c) Nimesulide; (d) **NS398**; (e) L-745,337; (f) DFU; (g) **SC58125**; (h) Celecoxib; (i) RS57067000 [15]

10.2 Newly biologic agents blocking some cytokines

Newly developed biologic agents such as TNF inhibitors and IL-1 receptor antagonists are very promising. But the long-term safety and efficacy of these new biologic agents has yet to be determined [2,38,42]. Monoclonal antibodies bind to their targets with high specificity and therefore have excellent potential as therapeutic agents [8]. In RA, IL-1

stimulates the release of degradative enzymes by synovial fibroblasts at the cartilage–pannus interface. IL-1 also activates chondrocytes to release these enzymes, which probably contributes to the cartilage destruction seen in RA at sites distant to the pannus [42]. Anti-IL-1 monoclonal antibodies significantly reduced cartilage destruction and bone erosion. In addition, anti-TNF α monoclonal antibody suppressed inflammation and produced rapid symptomatic improvement [8].

10.3 Treatment with anti-inflammation or regulatory cytokines

In normal joints, the effects of many proinflammatory cytokines are balanced by a variety of anti-inflammatory cytokines and regulatory factors. For example IL-1 is counteracted by IL-1 receptor

antagonist (IL-1Ra), which is a natural receptor antagonist of IL-1 for receptors and, as a result, blocks the effects of IL-1. So treatment with anti-inflammation or regulatory cytokines is a useful direction in RA [38]. Addition of IL-1Ra reduced the synovial fibroblast-mediated destruction by up to 45%. In rat long bones, IL-1Ra has been shown to reduce IL-1-induced bone resorption and block formation of osteoclast-like cells in murine marrow cultures in response to IL-1 or ovariectomy. These studies demonstrate that blocking the effect of IL-1 by regulatory cytokines leads to a reduction in RA [42]. Treatment with IL-1Ra was associated with significant reductions in clinical score and in circulating levels of the cartilage turnover marker, cartilage oligomeric matrix protein. Notably, radiographic assessment showed that anti-IL-1 treatment abolished bone erosions of knee and ankle joints. Moreover, histopathologic assessment showed that anti-IL-1 almost completely prevented cellular infiltration, matrix proteoglycan depletion, and bone erosions. Finally, a marker of MMP-mediated aggrecan cleavage was almost absent in the cartilage of animals treated with anti-IL-1 [42].

Anakinra is a recombinant human IL-1ra [38-46]. Anakinra is identical to the naturally occurring non-glycosylated form of IL-1Ra, with the exception of one N-terminal methionine. Monotherapy with anakinra was evaluated in a randomized, controlled, European multicentre study [42]. A total of 472, well-balanced patients with symptoms for 0.5–8 yr and typical features of active disease, were randomly assigned to receive anakinra 30, 75 or 150 mg or placebo once daily by injection for 24 weeks. Usage of NSAIDs (82–89%) and corticosteroids (41–49%) was comparable among the treatment arms. After 24 weeks of treatment, anakinra provided significantly greater clinical improvement than placebo. A significantly higher percentage of patients in the anakinra 150 mg group than in the placebo group responded to treatment. In addition, statistically significant improvements

relative to placebo were observed in each anakinra group for Health Assessment Questionnaire score ($P=0.05$), C-reactive protein ($P=0.004$) and ESR ($P=0.0005$) [42].

10.4 The treatment based on certain kinds of cells.

Based on several line of evidences, macrophage may be a good candidate target for treatment. The first is the correlation between radiological progression of joint destruction and degree of synovial macrophage infiltration. Second, the coincidence of therapeutic efficacy of conventional antirheumatic therapy with downregulation of functions of the mononuclear phagocyte system is in accord with the increasing knowledge of macrophage-specific effects of such drugs. Third, the efficacy of biological therapies directed at cytokines produced predominantly by macrophages has been demonstrated. Fourth, conventional or experimental drugs can be targeted at macrophages, including their subcellular compartments. The final factor in support of targeting monocytes/macrophages for treatment of RA is the differential activation of intracellular signal transduction pathways that underlie different macrophage effector functions, in conjunction with the availability of more specific inhibitors of key metabolic enzymes and/or particular signal transduction pathways.

10.5 Control the ROS in RA patients

Many research have indicated the enhanced ROS in many kinds of cell of RA [31,36,46]. At least in the articular cartilage, the antioxidants can protect the matrix from degradation. Natural non-toxic bioflavonoid rutin (vitamin P) inhibited oxygen radical overproduction in RA and therefore may be considered as a useful supporting pharmaceutical agent for the treatment of "free radical" pathologies [36,46]. The findings reported here suggest the potential efficacy of antioxidants in ameliorating inflammatory diseases not only to inhibit their direct effects, but also to aid in the suppression of

prostanoids when desirable [22], Another direction of control the ROS in the RA patients is that the use of antioxidant mimics, as the work of Miesel *et al.* the analogue of SOD can bring anti-arthritis and anti-inflammatory effect. And more importantly, this method may avoid the problem of short half-life and immunogenicity of SOD enzyme [31].

10.6 Other new methods

Some (E-selectin and ICAM-1) but not all (P-selectin, VCAM-1, PECAM-1) cell adhesion molecules are modulated in patients who respond clinically to drug treatment. E-selectin and ICAM-1 may be important targets for the development of future drug treatments for rheumatoid arthritis, in addition, [43,54]. The activity of MMP and ADAMTS is modulated by the some endogenous tissue inhibitors of MMPs (TIMPs), These molecules bind tightly to the active site of activated metalloproteases with a 1:1 stoichiometry and have K_i values of less than 10^{-9} M, so new products about TIMPs is very promising [2,38].

11. Summary

Rheumatoid arthritis is a debilitating autoimmune disease, shown as joint inflammation, pain, swelling and lead to permanent joint damage. More than 40 million people suffer from the Rheumatoid arthritis. The etiology of RA is not fully understood. Free radicals, proteases cytokines and immune cells are all believed to play a role in the pathogenesis of arthritis. Currently, Several possible directions in treatment of RA are on the way. Among them the selective COX-2 inhibitor is most practical; but the anti-proinflammatory cytokines may be most promising.

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