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Chapter 9

HYALURONAN – A HIGH-MOLAR-MASS MESSENGER REPORTING ON THE STATUS OF SYNOVIAL JOINTS: PART II. PATHOPHYSIOLOGICAL STATUS

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ABSTRACT

Reactive oxygen species, which are generated during the oxygen metabolism, participate in physiological catabolism of native high-molar-mass hyaluronan within the joint synovial fluid. It is very plausible that the microenvironment of the oxidative-reductive hyaluronan degradation under physiological conditions is closely to bone cartilages. Yet at the early stage of the acute phase of joint inflammation, the generated oxidants primarily cause damages within the synovial membrane.

Inflammation, a pathophysiological status, can be classified as a full-scale biochemical defensive response of living organism to remove harmful stimuli such as infective pathogenic invaders, toxins, disrupted cells, physicochemical irritants, pollutants, etc. As a powerful generator of the "defender" free radical species, the so-called Weissberger's oxidative system (ascorbate plus cupric ions) may be the organism weapon against above (bio)stimuli. A supportive fact for such a (hypo)thesis is the well known fact of changes in the systemic levels of acute-phase proteins, among others that of ceruloplasmin – a carrier of copper ions.

Keywords: Glycosaminoglycans, Hyaluronan catabolism, Reactive oxygen species, Synovial fluid, Transition metals.

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INTRODUCTION

Synovial joint(s), responsible for one´s ability to move, is a unique complex endobiotic system, function of which is closely related also to oxygen-dependent high-molar-mass hyaluronan turnover. The catabolism of this biopolymer under physiological homeostasis, has been implemented into the article "Hyaluronan – A High-Molar-Mass Messenger Reporting on the Status of Synovial Joints: Part I. Physiological Status" [printed in this book].

This, the second part of the two overviews, is devoted to hyaluronan oxidative degradation under pathophysiological status – mostly at the early stage of the acute phase of joint inflammation, which stage often precedes circumstances classifiable as oxidative and/or nitrosative stress.

Oxidative/Nitrosative Stress

Oxidative and/or nitrosative stress are terms used to describe situations, in which the organism's production of oxidants exceeds the capacity to neutralize them. The excess of oxidative species can cause "fatal" damage to lipids within the cell membranes, cellular proteins and nucleic acids, as well as to the constituents of the extracellular matrix, such as collagens, proteoglycans, etc. [1].

Oxidative and/or nitrosative stress has been implicated in various pathological conditions involving several diseases, which fall into two groups:

(i) diseases characterized by "inflammatory oxidative conditions" and enhanced activity of either NAD(P)H oxidase (leading to atherosclerosis and chronic inflammation) or xanthine oxidase-induced formation of oxidants (implicated in ischemia and reperfusion injury),
(ii) diseases characterized by the implication of pro-oxidants that shift the thiol/disulphide redox equilibrium and cause impairment of glucose tolerance - the so-called "mitochondrial oxidative stress" conditions (leading to cancer and diabetes mellitus) [2].

Oxidants

In a broader sense, oxidation concerns the reaction of any substance with molecules of oxygen, the primary oxidant. In chemistry, however, the term "oxidant" is used for all species able to render one or more (unpaired) electrons.

In a simplified way, oxidants can be classified as free-radical and non-radical species (cf. Table 1; adapted from [3]). They are often classified as reactive oxygen species (ROS) and reactive nitrogen species (RNS). Although the latter, similarly to ROS, contain oxygen atom(s) – e.g. NO+, NO–, NO2Cl – the RNS usually participate at nitrosylation reactions.

Table 1. Main ROS and RNS

Radical		Non-radical	
hydroxyl	$^{\bullet}OH$	peroxynitrite anion	$ONOO^-$
superoxide anion radical	$O_2^{\bullet-}$	hypochloric acid	HOCl
nitric oxide	$^{\bullet}NO$	hydrogene peroxide	H_2O_2
thyil	$-RS^{\bullet}$	singlet oxygen	$^1\Delta_g$ ($^1O_2$)
alkoxyl	$RO^{\bullet}$	ozone	O_3
peroxyl	$ROO^{\bullet}$	nitrosyl cation	NO^+
		nitroxyl anion	NO^-
		nitryl chloride	NO_2Cl

Oxygen metabolism – source of energy

Several oxidant species are produced at the processes occurring in animal cells, including human ones, during metabolism of oxygen, when these cells generate energy. Although the substrate (O2) is – by a cascade of enzymatically driven reactions – reduced within subcellular organelles, mitochondria, to a completely harmless substance, the waste product – water, a fraction of generated ROS may escape from the enzymatically controlled processes:

$$O_2 + 1e^- \rightarrow O_2^{\bullet-} \quad (1)$$

$$O_2^{\bullet-} + 1e^- + 2H^+ \rightarrow H_2O_2 \quad (2)$$

$$H_2O_2 + 1e^- + H^+ \rightarrow {}^{\bullet}OH + H_2O \quad (3)$$

$$^{\bullet}OH + 1e^- + H^+ \rightarrow H_2O \quad (4)$$

net reaction

$$O_2 + 4e^- + 4H^+ \rightarrow 2H_2O \quad (5)$$

As indicated by the reaction steps (1), (2), and (3), oxidants, namely $O_2^{\bullet-}$, H_2O_2, and $^{\bullet}OH$ are intermediate products of the enzymatically controlled cascade. Their reactivity and presumable site of action can be assessed by physico-chemical parameters, such as standard reduction potential (E^{θ}) and half-life ($t_{1/2}$) of the given species (cf. Table 2).

Table 2. Standard reduction potential (and half-life) for some dioxygen species in water, pH 7, 25 °C[a]

Species	(reaction)	E^{θ} [V]	$t_{1/2}$ [s]
O_2	(1)	-0.33[b]	reactive
$O_2^{\bullet-}$	(2)	+0.89	10^{-6}
H_2O_2	(3)	+0.38	long living
$^{\bullet}OH$	(4)	+2.31	10^{-9}

[a]Adapted from [4].

[b]The greater the positive E^{θ} value, the greater is generally the species reactivity, i.e. the ability to catch an electron [cf. reactions (1)–(4)].

With regard to the high (positive) value of E^{θ} and to the short half-life values, escape of $^{\bullet}OH$ and $O_2^{\bullet-}$ from the sphere immediately surrounding mitochondrion can be virtually excluded. Yet the neutral molecule H_2O_2 is considered to be movable one, which can escape as from the "body" of the mitochondrion as well as from the cell body itself. It is comprehensible that in some tissues the actual H_2O_2 concentrations may reach 100 μM or more as e.g. in human and other animal aqueous and vitreous humors. The hydroperoxide levels at or below 20–50 μM seem, however, to have limited cytotoxicity to many cell types [5].

Oxygen metabolism – a defence mechanism against viral/bacterial invaders

Along with the above four-electron reaction (5), several specialized cells – or more precisely their specific (sub)cellular structures – are able to reduce O_2 molecules producing the superoxide anion radical, which in aqueous (acidic) milieu can form the reactive perhydroxyl radical ($^{\bullet}O_2H$).

Nitric oxide, called also nitrogen monoxide ($^{\bullet}NO$), a (bioactive) free radical, is produced in various cells/tissues by NO-synthase (NOS) enzymes. The three distinct NOS isoforms are P_{450}-related hemoproteins that during L-arginine oxidation to L-citrulline produce $^{\bullet}NO$. Two of the permanently present enzymes that participate in the regulation of the blood vessel tonus are termed constitutive NOS (cNOS), while the third one is called an inducible NOS (iNOS). The level of $^{\bullet}NO$ produced by iNOS increases markedly during inflammation, a process accompanied with abundant production of the superoxide anion radical.

The two radical intermediates – $O_2^{\bullet-}/^{\bullet}O_2H$ and $^{\bullet}NO$ – serve as precursors of various ROS and RNS, including hydrogen peroxide, peroxynitrite/peroxynitrous acid, hypochlorous acid, *etc*. On respiring air, human beings by utilizing one mole of O_2 ingest 6.023×10^{23} molecules of oxygen, of which approximately 1–3 % is assigned to the generation of ROS/RNS that defend the organism against viral/bacterial invaders [6].

It has been noted that certain organ systems are predisposed to greater levels of oxidative stress and/or nitrosative stress. Those organ systems most susceptible to damage are the pulmonary system (exposed to high levels of oxygen), brain (exhibits intense metabolic activity), eye (constantly exposed to damaging UV light), circulatory system (victim to fluctuating oxygen and nitric oxide levels) and the reproductive systems (at risk from the

intense metabolic activity of sperm cells) [3]. In some cases, however, the intermediate and/or the "final" reactive oxidative species may also damage cells/tissues of the human host. Imbalance between the extent of damage and self-repair of the functionally essential structures may result in a broader host tissue injury, eventually leading to a specific disease.

Because of the highly reactive nature of ROS/RNS, it is difficult to directly demonstrate their presence *in vivo*. It is considerably more practical to measure the "footprints" of ROS and RNS, such as their effects on various lipids, proteins, and nucleic acids [1].

Indirect ROS/RNS evidence

Most ROS/RNS have very short half-live times thus they cannot be directly detected in the organisms. That is why, as reported also by Valko *et al.* [2], convincing evidence for the association of oxidative/nitrosative stress and acute and chronic diseases lies on validated biomarkers of these stresses. Table 3 summarizes most representative biomarkers of oxidative damage associated with several human diseases.

Table 3. Biomarkers of oxidative damage associated with several chronic human diseases (adapted from [2])

Disease *Biomarker*[a]	Alzheimer's disease	Atherosclerosis	Cancer	Cardiovascular disease	Diabetes mellitus	Parkinson's disease	Rheumatoid arthritis
8-OH-dG			+				
Acrolein		+		+			
AGE	+				+		
Carbonylated proteins						+	
F_2-isoprostanes	+	+		+	+		+
GSH/GSSG	+		+	+	+	+	+
HNE	+	+		+		+	
Iron level						+	
MDA	+	+	+		+		
NO_2-Tyr	+	+	+	+	+		
S-glutathiolated proteins					+		

[a]Abbreviations: 8-OH-dG, 8-hydroxy-20-deoxyguanosine; AGE, advanced glycation end products; GSH/GSSG, ratio of glutathione/oxidized glutathione; HNE, 4-hydroxy-2-nonenal; MDA, malondialdehyde; NO_2-Tyr, 3-nitro-tyrosine

There are numerous further diseases whose pathology involves reactive oxidative/oxygen-derived species, i.e. ROS and/or RNS, at the onset and/or at later stages of the disease [7]. The magnitude and duration of the change in the concentrations of these species appear to belong among the main regulatory events (cf. Fig. 1).

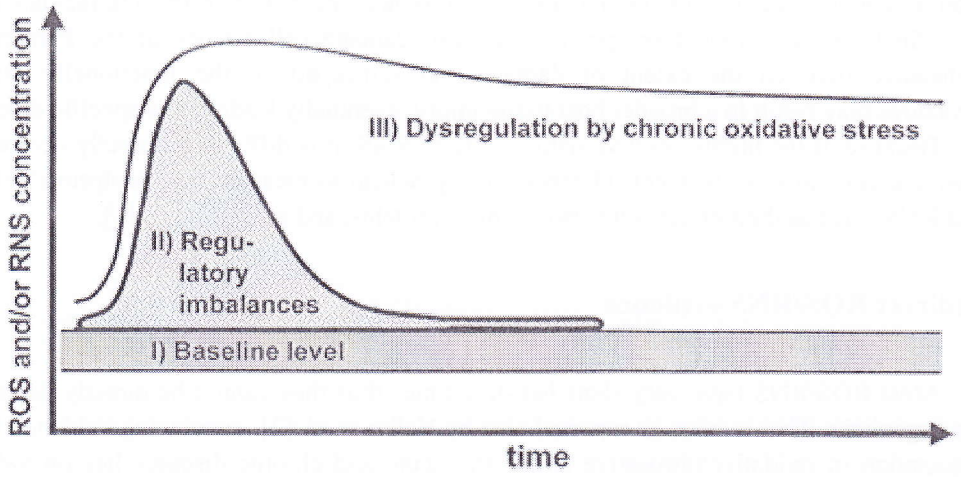


Figure 1. Regulatory events and their dysregulation depend on the magnitude and duration of the change in ROS and/or RNS concentration(s) (adapted from [8]).

Today it is a widely accepted fact that ROS and RNS normally occur in living tissues at relatively low steady-state levels (cf. Fig. 1, stage I "Baseline level"). The regulated increase in the production of superoxide anion radical or nitric oxide leads to a temporary imbalance, which forms the basis of redox regulation (stage II in Fig. 1, "Regulatory imbalances"). The persistent production of abnormally large amounts of ROS or RNS, however, may lead to persistent changes in signal transduction and gene expression, which, in turn, may give rise to pathological conditions (as seen in Fig. 1, stage III "Dysregulation by chronic oxidative stress") [8]. One of the classes of such diseases includes arthritic conditions – inflammatory diseases of joints. A substantial amount of evidence exists for an increased generation of oxidants in patients suffering from acute and chronic inflammatory joint diseases [9,10] – see Table 4.

Table 4. Some characteristics registered within SF during inflammatory joint diseases[a]

Diagnosis	SF viscosity	Blood characteristics			H_2O_2 flux [μM/min]
		White ceiis/μL	% of PMNLs	PMNLs/μL	
Healthy	normal	<200	7	<14	<0.003
OA	decreased	600	13	48	0.017
RA	decreased	1900	66	1254	0.276

[a]Adapted from [11].

Abbreviations: PMNL, polymorphonuclear leukocyte; OA, osteoarthritis; RA, rheumatoid arthritis.

Regulatory imbalances within a synovial joint

As schematically reported by Dröge [8] and advocated also in Part I of the chapter "Hyaluronan – A High-Molar-Mass Messenger Reporting on the Status of Synovial Joints" [12], under physiological status, "Baseline level" (cf. Fig. 1) of ROS and/or RNS concentration play an important role as regulatory mediators in signaling processes. In case of the composition of SF of healthy organisms, one may state two border concentrations of ROS (and RNS as well), which are primarily determined by the O_2 level within SF, or more precisely by the H_2O_2 level escaped from mitochondria of chondrocytes and from those of cells of the synovial membrane. A lower one exists at rest regimen of the joint and a higher H_2O_2 level at reoxygenation of the joint tissues during movement of the subject. The high-molar-mass HA however keeps most probably the joint ROS/RNS homeostasis between the two concentration values inside the "Baseline level" (see Fig. 1, stage I).

On accepting the tenet that concentrations of H_2O_2 ranging around 50 μM (sometimes even up to 100 μM) are not toxic to any cells [5], the highest limit (cf. stage I, Fig. 1) of the hydrogen peroxide level in SF, and thus in contact with both chondrocytes and synovial-membrane cells, is close to this concentration (<100 μM). The flux of H_2O_2 in the amount of less than 0.003 μM per minute does not change SF viscosity (cf. Table 4). In light of this observation one can propose that the ROS action, i.e. H_2O_2-degradative action on the high-molar-mass HA, is fully compensated by the *de novo* synthesis of megaDalton hyaluronans by the synoviocytes embedded within the synovial membrane of healthy human beings. Our detailed studies focusing on the H_2O_2-degradative action to HA macromolecules also showed that hydrogen peroxide up to hundreds of micromolar concentrations led to practically no cleavage/decay of high-molar-mass hyaluronan samples when the reaction system was "free" of any transition metal ions, namely those of iron and/or copper [M. Stankovská *et al.*, not published].

Let us now admit the situation of occurrence of temporary "Regulatory imbalances" (stage II in Fig. 1), or more precisely the situation at which an acute inflammation is initiated within the synovial joint. On taking into account the data given in Table 4, the increase in ROS concentration, or more precisely the increase in H_2O_2 flux, appears to be functionally related to the rising number of PMNLs in SF, presenting in the initial phase as Regulatory imbalance. This increase is however associated with the following events: i) infiltration of the increased number of white cells (PMNLS and/or macrophages) from the blood circulation into SF, and ii) activation of these cells in SF. Yet concerning the event given in ii), it has to be emphasized that at the time of infiltration movement of the white blood cells is impeded in the SF, due to its viscosity, which can be characterized as "normal" (cf. Table 4; see Fig. 2) or high caused by the presence of high-molar-mass HA macromolecules. Moreover, it is a well known fact that especially high-molar-mass hyaluronans exert antiimflammatory action or more precisely, the long-sized HA chains quench the PMNLs and macrophages.

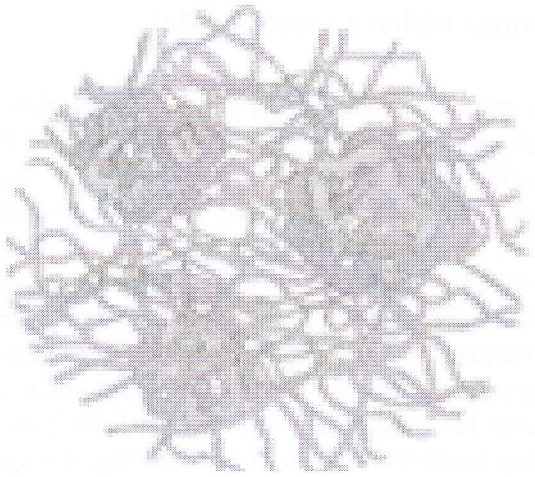


Figure 2. The movement of the white blood cells in the normal/highly viscous SF. The long-sized HA chains are sketched as blue strands.

Thus one may admit that infiltration of an increased number of white cells into a milieu such as that of SF of healthy human beings need not immediately result in a rise of the ROS concentration or the H_2O_2 level enhancement, respectively. The demand of rapid/acute growth of ROS/RNS level within the joint during the stage II (cf. Fig. 1, "Regulatory imbalances") could not be met in this way. Resulting from our experimental findings, we may hereby offer/recommend our hypothesis/speculation in point of process sequencing which can very quickly, owing to their physiological status, bring about – for a temporary time period – the status possibly be defined as acute inflammation, or – by taking into account the Dröge scheme (cf. Fig. 1 [8]) – the "Regulatory inbalances".

Inflammation

Inflammation generally means a complex biological response of tissues to harmful stimuli, such as infective pathogens, damaged cells, toxins, physical and/or chemical irritants. It is a protective attempt by the organism to remove injurious stimuli and to initiate the healing process for the tissue. Yet inflammation that runs unchecked can lead to various diseases (cf. Table 3), including those connected to synovial joints. Normally, however, inflammation is critically controlled and closely regulated by the body.

Inflammation can be classified as acute or chronic (Table 5). Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of PMNLs from the blood into the injured tissues. Then a cascade of biochemical events propagates and matures the (local) inflammatory response. Chronic inflammation usually leads to a progressive shift in the type of immune cells which are present at the site of inflammation and is characterized by destruction and often by (partial) healing of damaged tissues.

Table 5. Comparison between acute and chronic inflammation (from [13])

Inflammation	Acute	Chronic
Causative agent	Pathogens, injured tissues	Persistent acute inflammation due to non-degradable pathogens, persistent foreign bodies, or autoimmune reactions
Major cells involved	Neutrophils, mononuclear cells (monocytes, macrophages)	Mononuclear cells (monocytes, macrophages, lymphocytes, plasma cells), fibroblasts
Primary mediators	Vasoactive amines, eicosanoids	IFN-γ and other cytokines, growth factors, reactive oxygen species, hydrolytic enzymes
onset	immediate	delayed
duration	few days	up to many months or years
outcomes	resolution, abscess formation, chronic inflammation	tissue destruction, fibrosis

Acute inflammation – a short-term process appearing in a few minutes or hours – is usually characterized by five cardinal signs: rubor, calor, tumor, dolor, and *functio laesa*. However, the acute inflammation of an internal organ may not be manifested by the full set of signs.

Inflammation, and especially the acute one, is associated with elevated systemic levels of acute-phase proteins. These proteins prove beneficial in acute inflammation.

Acute-phase proteins

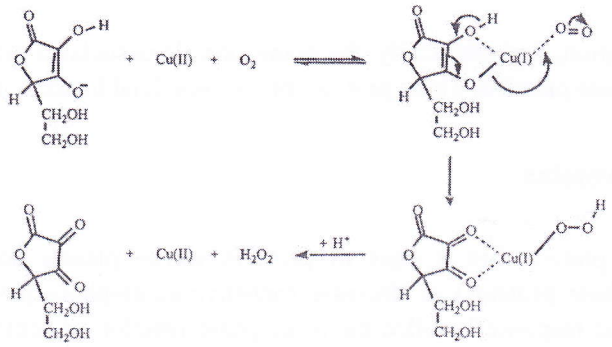
Acute-phase proteins are a class of proteins whose plasma concentrations increase (positive acute-phase proteins) or decrease (negative acute-phase proteins) in response to inflammation. This response is called the acute-phase reaction or acute-phase response. The acute-phase reactants are produced by the liver in response to specific stimulations. The following positive acute-phase proteins belong to the physiologically most prominent ones: C-reactive protein, α_1-antitrypsin and α_1-antichymotrypsin, fibrinogen, prothrombin, complement factors, ferritin, serum amyloid A, α_1-acid glycoprotein, ceruloplasmin, and haptoglobin. Others – negative acute-phase proteins such as albumin, transferrin – give negative feedback on the inflammatory response.

Ceruloplasmin

The concentration of ceruloplasmin, whose molar mass (≈ 134 kDa) exceeds nearly twice that of albumin, increases markedly under certain circumstances – including those of acute inflammation. Since each ceruloplasmin macromolecule complexes/binds up to eight Cu(II)/Cu(I) ions of which two can liberate relatively easily [14], at the early stage of acute inflammation the actual copper level increases markedly. The consequence of higher ceruloplasmin concentration in blood plasma – accompanied with a rise in the concentration of copper ions – would mean a larger amount of this biogenic trace element that might cross the synovial membrane [15]. Yet, due to the gel-like consistency of SF, the copper ions entering into this specific environment start their redox action in the vicinity of the synovial membrane.

Weissberger's oxidative system

The concentration of ascorbate in SF of healthy subjects reaches the values close to those established in blood serum, i.e. 40–140 μM [16]. Ascorbate, an "actor of physiologic HA catabolism in SF" with copper liberated from ceruloplasmin, creates easily the so-called Weissberger's oxidative system [17,18] – ascorbate-Cu(I)-oxygen – generating H_2O_2 (cf. Scheme 1) [19-21]. Moreover, due to the simultaneous decomposition of hydrogen peroxide by the redox active copper ions, a large flux of hydroxyl radicals may occur [22].



Scheme 1. Generation of H2O2 by Weissberger's system from ascorbate and Cu(II) under aerobic conditions (adapted from Fisher and Naughton [21]).

As evident from the data listed in Table 1 in Part I of this article "Hyaluronan – A High-Molar-Mass Messenger Reporting on the Status of Synovial Joints" [12], iron and copper are the two prevailing redox active transition metals in SF. Although just only a minor fraction of their respective total levels equaling 5.2 μM and 4.3 μM is disposable for Weissberger's and/or Fenton-type reactions, these are the copper ions that better fulfill the requirement of acute (rapid) generation of ROS – particularly of $^{\bullet}OH$ radicals (cf. Fig. 3).

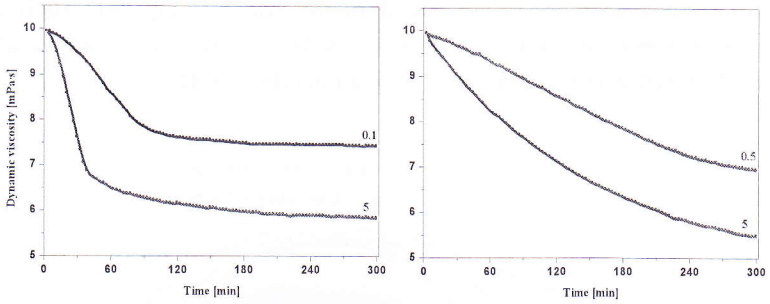


Figure 3. Time dependences of dynamic viscosity of solutions of a high-molar-mass HA sample.

Left panel: Solutions of the HA sample with addition of 100 μM ascorbic acid immediately followed by admixing 0.1 or 5 μM of CuCl2.

Right panel: Solutions of the HA sample with addition of 100 μM ascorbic acid immediately followed by admixing 0.5 or 5 μM of FeCl2.

Figure 3 illustrates the degradative action of ROS by monitoring the viscosity-time profiles of a HA solution into which – along with 100 μM ascorbate – a single transition metal was added [23]. As evident, a significant reduction of the solution dynamic viscosity (η), corresponding to the degradation of the high-molar-mass HA sample, clearly indicates a concentration-dependent manner for each metal (cf. left and right panels in Fig. 3). While the character of the time dependence of η value upon the addition of $FeCl_2$ (5.0 μM) can be described as a gradual monotonous decline, the addition of $CuCl_2$ (5.0 μM) resulted in a literally "dramatic" drop of η value within a short time interval (30 min). A similar drop of η value and two-phase reaction kinetics are identifiable upon the addition of even a minute (0.1 μM) amount of $CuCl_2$ (see Fig. 3, left panel). A possible explanation of this dissimilarity lies most probably in different reaction kinetics of the processes leading to generation of oxygen-derived reactive species in the system ascorbate *plus* $CuCl_2$ and in that comprising ascorbate *plus* $FeCl_2$.

As seen in Figure 3, the transition metal – either iron or copper – can play an active role in oxidative HA catabolism. However, the increase in Cu(II) concentration within the joint (and particularly in SF) could lead to an extremely rapid degradation of the native HA macromolecules. How efficiently the chemically generated $^{\bullet}OH$ radicals are "scavenged" within this microenvironment by the locally disposable albumin as well as by the HA polymer fragments of lower molecular size, remains questionable. The oxidative process may escape the control mechanisms and damage/disrupt the synovial membrane. Moreover, the intermediate-sized HA-polymer fragments generated within this microenvironment could participate in the activation of "defender" cells. They may further intensify the inflammation state of the injured tissue(s) as the HA-polymer fragments can in turn augment the inflammatory responses. As reported by Jiang *et al.*, the HA fragments in the e.g. 2×10^5 Da range induce the expression of a number of inflammatory mediators in macrophages, including chemokines, cytokines, growth factors, proteases, and nitric oxide [24]. In this way, the oxidants generated by activated defender cells may enlarge the damage within the

involved joint tissues such as the synovial membrane (cf. Fig. 4). Such an increase in unmediated reactive radicals, generally termed oxidative stress, is an active area of research in a variety of diseases where copper may play an insidious role.

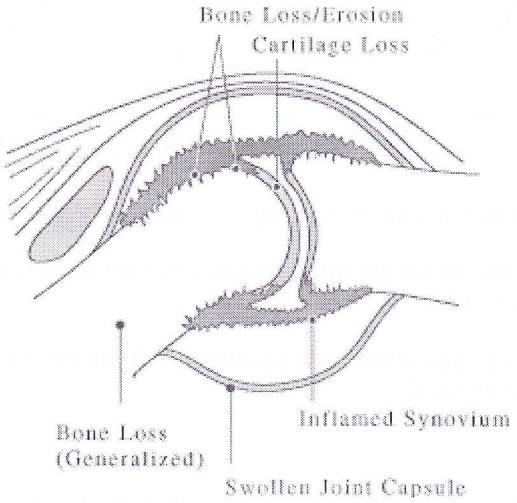


Figure 4. Damages within the inflamed joint tissues.

Moreover, reactive oxygen species appear to disrupt copper binding to ceruloplasmin, thereby releasing "free" copper ions, which in turn may promote oxidative pathology [14]. The damage can be manifested by visually localizable cardinal signs of inflammation – i.e. rubor, calor, tumor, dolor, and *functio laesa*, yet less distinct, repeated (micro-acute) inflammatory injures may lead to a disastrous outcome, e.g. an autoimmune disease such as rheumatoid arthritis.

RELEVANCY AND FUNCTION OF WEISSBERGER'S OXIDATIVE SYSTEM AT ACUTE INFLAMMATION OF THE JOINT

As demonstrated by the results depicted in Figure 3 (left panel) Weissberger's oxidative system is really a prompt/ultimate generator of hydrogen peroxide leading immediately to dramatic flux of $^{\bullet}OH$ radicals. Subsequently these radicals initiate a significant degradation of long-chain HA macromolecules, the process which diminishes markedly the dynamic viscosity of the hyaluronan solution. A similar HA degradative process can be anticipated in SF at the early stage of acute (synovial) joint inflammation. The lower SF viscosity may markedly promote the transition of defender cells from blood through the synovial membrane and further enhance the movement of these cells to the target synovial and periarticular tissues. These cells may simultaneously undergo activation in contact with/binding to biopolymer fragments resulted from ($^{\bullet}OH$) radical degradation of native high-molar-mass

hyaluronans present in SF. The infiltrated defender cells thus may start their more or less specific action inside the intraarticular space.

Chronic inflammation

In acute inflammation, if the injurious agent persists, chronic inflammation will ensue. This process marked by inflammation lasting many days, months or even years, may lead to the formation of a chronic wound. Chronic inflammation is characterized by the dominating presence of macrophages in the injured tissue. These cells are powerful defensive agents of the body, but the "toxins" they release – including ROS and/or RNS – are injurious to the organism's own tissues. Consequently, chronic inflammation is almost always accompanied by tissue destruction. Destructed tissues are recognized by the immunity system and, when "classified" by the body as foreign ones, a cascade of autoimmune reactions could start. Such reactions are well established in diseases such as rheumatoid arthritis, where – along with the (synovial) joints – several further tissues/organs, e.g. lungs, heart, and blood vessels, are permanently attacked, i.e. miss-recognized as foreign ones.

Medications used to treat inflammatory joint diseases

There are many medications available to decrease joint pain, swelling, inflammation and to prevent or minimize the progression of the inflammatory disease. These medications include:

- Non-steroidal anti-inflammatory drugs (NSAIDs – such as acetylsalicylic acid/aspirin, ibuprofen or naproxen).
- Corticosteroids (such as prednisone).
- Anti-malarial medications (such as hydroxychloroquine).
- Other medications, including methotrexate, sulfasalazine, leflunomide, anti-TNF medications, cyclophosphamide, and mycophenolate.

As reported in the Section "Relevancy and function of Weissberger's oxidative system at acute inflammation of the joint", the early acute-phase of (synovial) joint inflammation should, most plausibly, be accompanied with generation of ROS (and RNS) – particularly with $^{\bullet}OH$ radicals. These, however, due to their extremely high electronegativity (-2.31 V) should – in contact with any hydrogen atom containing compounds – entrap a proton ($^{\bullet}H$). By that process the $^{\bullet}OH$ radicals are partially or fully scavenged (cf. Fig. 5). If the resulting radical generated from the given compound/medication is not able to initiate HA degradation, we speak of drug-scavenging, which could moderate the free radical process within the inflamed joint.

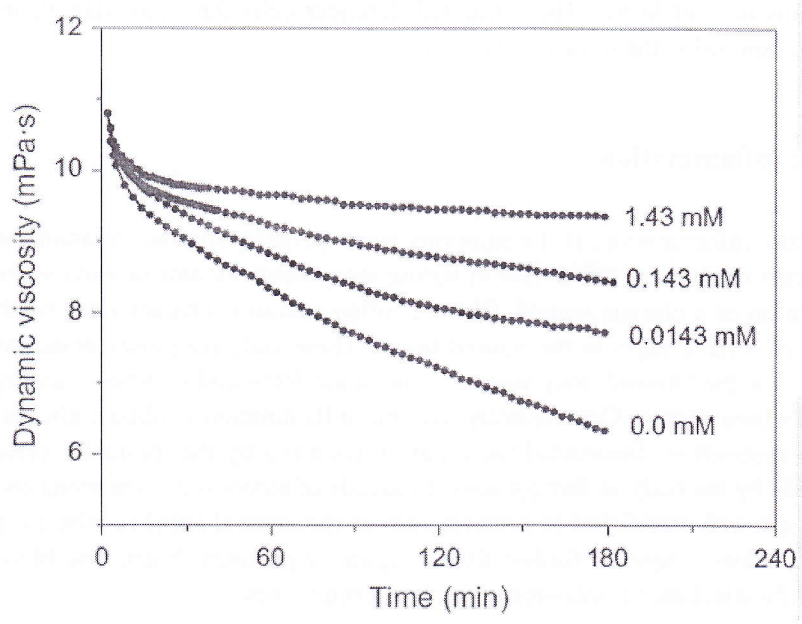


Figure 5. Effect of acetylsalicylic acid on HA degradation in the system 0.1 μM CuCl2 + 100 μM ascorbic acid + 2 mM NaOCl.
Concentration of acetylsalicylic acid added into the system before initiation of HA degradation in mM: 0.0, 0.0143, 0.143, and 1.43.

Figure 5 illustrates such an *in vitro* testing of the scavenging efficiency of acetylsalicylic acid/aspirin. As evident, this drug – based on its activity under aerobic conditions within the system HA-ascorbate-Cu^{2+}-NaOCl – can be classified as a potent scavenger of $^{\bullet}OH$ radicals [25].

Concluding Remarks

With the current understanding that free radicals can act as cell signaling or "messenger" agents it is likely that they also play a role in normal cellular function as well as various disease etiologies. Researchers are now making rapid progress in understanding the role of oxidative stress and nitrosative stress in cardiovascular diseases such as atherosclerosis, ischemia/reperfusion injury, restenosis and hypertension; cancer; inflammatory diseases such as acute respiratory distress syndrome (ARDS), asthma, inflammatory bowel disease (IBD), dermal and ocular inflammation and arthritis; metabolic diseases such as diabetes; and diseases of the central nervous system (CNS) such as amyotrophic lateral sclerosis (ALS), Alzheimer's, Parkinson's, and stroke. The increased awareness of oxidative stress related to disease and the need to measure the delicate balance that exists between free radicals and the given systems in regulating them has given rise to a demand for new research tools.

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