VIEWPOINT

Drug derived radicals: mediators of the side effects of anti-inflammatory drugs?

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Reactive oxygen species in rheumatoid arthritis

It is now generally accepted that oxygen free radicals and other reactive oxygen species play some part in the pathology of inflammatory joint disease, though whether this is a major or minor part remains to be established.¹ A *radical* is any species containing one or more unpaired electrons (denoted by a superscript dot, $\cdot$). Examples are the superoxide radical ($O_2^{\cdot-}$) and hydroxyl radical ($OH\cdot$). The term *reactive oxygen species* is a broader term that includes not only $O_2^{\cdot-}$ and $OH\cdot$ but also certain non-radical oxygen derived species, such as hydrogen peroxide (H_2O_2) and hypochlorous acid ($HOCl$), which are potentially, damaging.) Increased formation of reactive oxygen species is probably a generalised response to tissue injury, as may be the increased synthesis of other 'injury mediators' such as prostaglandins, leukotrienes, interleukins, and tumour necrosis factors.^{1,2} There is therefore nothing special in showing increased activity of reactive oxygen species in any human disease: it is almost to be expected.^{1,2} What needs to be done (as for any putatively important injury mediator) is to show that reactive oxygen species are important contributors to disease activity (table 1). This has been exceptionally difficult because reactive oxygen species are hard to measure, but fortunately new methods are becoming available.¹

Sources of reactive oxygen species in rheumatoid arthritis

Synovial fluid neutrophils, and macrophages in the pannus, probably generate some $O_2^{\cdot-}$ and H_2O_2 in the inflamed rheumatoid joint.³ In the

presence of 'catalytic' iron ions, which often exist in synovial fluid,⁴ $O_2^{\cdot-}$ and H_2O_2 lead to the formation of highly reactive $OH\cdot$ which can attack and damage lipids, carbohydrates, DNA, and proteins.⁵ Reaction of H_2O_2 with haemoglobin, liberated as a result of traumatic microbleeding in the joint,⁶ can generate oxo-haem species and associated globin radicals that can oxidise many biological materials, including lipids.^{7,8} Eventually, if the H_2O_2 is in excess over the haemoglobin, the haem ring falls apart and 'free' iron is released in a form that can catalyse $OH\cdot$ production.⁹ The pannus overgrowing the cartilage contains many macrophages, presumably secreting $O_2^{\cdot-}$, H_2O_2 , eicosanoids, interleukin 1, and the free radical nitric oxide, $NO\cdot$. Under certain circumstances,¹⁰ $OH\cdot$ might be produced by the reaction of $NO\cdot$ with $O_2^{\cdot-}$

It has also been proposed^{11,12} that the inflamed rheumatoid joint undergoes repeated cycles of hypoxia-reperfusion during movement; this may result in the generation of reactive oxygen species by several mechanisms, including oxidation of hypoxanthine and xanthine by the enzyme xanthine oxidase.¹²

Neutrophils contain myeloperoxidase, an enzyme which uses H_2O_2 to oxidise Cl^- ions into hypochlorous acid¹³

Hypochlorous acid is highly reactive but more selective as an oxidant than is $OH\cdot$: it reacts quickly with methionine and with protein—SH groups, for example.¹³ Myeloperoxidase has many of the properties of a 'typical' peroxidase enzyme: it can oxidise a wide range of substrates in the presence of H_2O_2 . Such peroxidase mediated oxidations almost always¹⁴ proceed by free radical mechanisms in which the substrate (SH_2) is oxidised to a free radical ($SH\cdot$)

The Roman numerals represent the normal oxidation state of the iron in the peroxidase haem ring. Hydrogen peroxide oxidises the resting enzyme ($Fe(III)$) to a nominal $Fe(V)$ state, probably an $Fe(IV)$ state plus an amino

Table 1 Some of the basic criteria that must be satisfied before implicating any agent as an important mediator of tissue injury in human disease

- 1 The agent is always present at the site of injury
- 2 Direct application of the agent to the tissue at concentrations within the range found in vivo reproduces most or all of the injurious effects
- 3 Removing the agent or inhibiting its formation decreases the injury to an extent approximately proportional to the degree of removal of the agent, or inhibition of its formation

The problems with free radicals: (1) and (3) obviously rely on accurate assay methods. Free radicals are difficult to measure: it is rarely clear what percentage of radicals formed are actually trapped when a trapping method is used and 'end products' of free radical damage are rapidly removed by excretion from the body or by metabolism (or both). Molecular targets of free radical damage are numerous and their relative importance can change markedly as a result of changes in radical concentration or cell type. Thus it is hard to know what targets of injury should be measured.

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acid radical. The enzyme returns to Fe(III) in two one-electron steps, in each of which a substrate is oxidised by the removal of one electron, so generating the substrate radical, SH•.

Attempts to measure reactive oxygen species in rheumatoid arthritis

This research group and others have spent considerable time developing ‘markers’ of oxidative damage and ‘traps’ for reactive oxygen species and applying them to patients with rheumatoid arthritis.^{1-11 14 15a} Experience over several years has shown that interpatient variation is enormous. For example, synovial fluid from one patient may show clear evidence of OH• generation (using phenylalanine or salicylate as ‘detector molecules’)^{15a} or of exercise induced increases in oxidative lipid and protein damage (presumably due to hypoxia-reperfusion events¹¹). A patient with an identical degree of disease activity (as measured by clinical or laboratory parameters) may show none of these. One of the factors influencing such discrepancies may be the nature of the treatment regimen. Anti-inflammatory drugs are often proposed to decrease free radical production, but they might often enhance it instead.

Anti-inflammatory drugs and oxidative damage

All drugs used in the treatment of rheumatoid arthritis are capable of scavenging OH• in vitro,¹⁶ as do most other molecules.¹⁴ Apart from the special case of salicylate, however, drug concentrations in vivo (e.g. table 2; Baggot *et al*¹⁷) are probably too low to scavenge OH•. As almost everything reacts quickly with OH•, all that matters is concentration—a drug must be present at high concentrations to compete with biological molecules for any OH• generated. Even in simple in vitro radical generating systems, OH• scavengers need to be present at millimolar concentrations to be effective. Similar conclusions apply to HOCl. Although HOCl can react with most drugs in vitro, scavenging of HOCl in vivo is feasible only in a few instances, such as penicillamine, gold thiols

(the thiol is the part active in scavenging HOCl), and phenylbutazone.¹⁸ Some drugs may suppress phagocyte function and so inhibit the generation of reactive oxygen species: this ability is widely claimed on the basis of experiments in vitro (often using inappropriately high drug concentrations) but has rarely been established in vivo.

The reaction of drugs with reactive oxygen species is not necessarily a good thing. For example, oxidation of penicillamine or of phenylbutazone in vitro can generate damaging products; these are often drug derived free radicals. For example, α₁ antiproteinase (the major inhibitor of serine proteases such as elastase in human body fluids) can be inactivated, apparently by reactive oxygen species mediated oxidation, in the inflamed rheumatoid joint.¹⁹ Penicillamine and phenylbutazone could accelerate this inactivation, because free radicals derived from their oxidation can themselves inactivate α₁ antiproteinase.^{20 20a}

Generation of drug derived radicals

In a few instances drug derived radicals might be produced by direct reaction of drugs with reactive oxygen species.^{18 20 20a} More often, however, they might arise because many drugs can be oxidised into radicals by myeloperoxidase, which has (as discussed earlier) a broad substrate specificity (equations 3–5).^{20a 21} Diclofenac²² may be one example. Oxidation could be catalysed by myeloperoxidase within phagocytes, or by enzymes released from activated phagocytes. Mixtures of haemoglobin and H₂O₂ can also oxidise many drugs, including phenylbutazone.^{20a} Hence a scenario can be envisaged in which a drug might suppress certain mediators of inflammation (for example, non-steroidal anti-inflammatory drugs (NSAIDs) inhibiting cyclo-oxygenase) but simultaneously aggravate oxidative damage. Hence it is no surprise that there is, as yet, no completely effective, safe drug for the treatment of rheumatoid arthritis.

If these ideas are correct, then other areas of high peroxidase activity in the human body might be expected to be sensitive to damage by drug induced free radical reactions, provided that H₂O₂ can be generated. Indeed, bone marrow (which has high peroxidase activity due to myeloperoxidase and eosinophil peroxidase enzymes) appears to be especially sensitive to drugs and toxins that can easily generate free radicals.^{21 23 24} A peroxidase has been reported to be present in the stomach of rats and mice.²⁵ If such a peroxidase is also present in the human stomach, and H₂O₂ was generated there in vivo, then drugs might also be oxidised at this site. For example, the gastric toxicity of NSAIDs might include not only the inhibition of cyclo-oxygenase, but also drug derived free radicals generated in the stomach. Several workers have presented evidence consistent with the view that free radicals play a part in indomethacin induced gastric damage in animal model systems.^{26 27}

Can these side effects be decreased?

A large number of damaging free radicals,

Table 2 Actual concentrations of anti-inflammatory drugs found in human body fluids

Drug	Daily dose often used (g)	Peak plasma concentration (mmol/l)
Sulphasalazine	3-4	0.05
Methotrexate	≈0.002	0.0006
Sulindac	0.3-0.4	0.02
Indomethacin	0.15-0.2	0.02
Naproxen	0.5-1.0	0.4
Salicylic acid	5-8	2.0
Ibuprofen	1-3	0.4
Mefenamic acid	1-2	0.08
Aspirin	5-8	2.0 (as salicylate)
Piroxicam	0.02	0.02
Phenylbutazone	0.6	0.5
Penicillamine	1.5	0.2
Hydroxychloroquine	0.4	0.001

The daily doses and peak concentrations are approximate: the ‘free’ drug concentration may be lower because of protein binding. In rheumatoid arthritis there is increased permeability of the synovial membrane, so it is likely that synovial fluid drug concentrations are of the same order of magnitude as those in the plasma. Data from Baggot *et al*.¹⁷

including drug derived radicals,^{20a 23 28 29} can be scavenged by ascorbic acid (vitamin C). The resulting ascorbate radical is poorly reactive and may disappear largely by self reaction, i.e.

Ascorbic acid may also act as a preferred substrate for oxidation by haem protein-H₂O₂ mixtures, preventing them from oxidising other molecules into damaging free radicals.⁸

The concentrations of total ascorbate and the ascorbate to dehydroascorbate concentration ratio are markedly subnormal in patients with active rheumatoid arthritis.³⁰ Giving supplemental ascorbate to such patients does not appear to diminish their disease activity (Blake D R, personal communication). It would be interesting, however, if ascorbic acid were able to minimise the occurrence of drug induced side effects.

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