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Toxic drug effects associated with oxygen metabolism: Redox cycling and lipid peroxidation

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Summary. Various endogenous and exogenous compounds exert cytotoxic effects via oxygen reduction. In general, these are reduced by intracellular enzymes (reductases of various kinds) in one-electron transfer reactions, before they in turn reduce O_2 to O_2^-, the superoxide anion radical. Thus, a cycle is formed of O_2 uptake at the expense of cellular reducing equivalents, notably NADPH, generating further active oxygen species (figs 1, 2). Structures capable of 'redox cycling' include catechols and other quinone compounds, iron chelates, and aromatic nitro compounds. Several anticancer agents, and also some mutagens, operate on this principle, and their toxic effects may be explained by redox cycling. The particular importance of hypoxic conditions for deleterious O_2 effects is given by the concomitant flux through reductive as well as oxidative pathways.

Toxic effects include membrane damage resulting from peroxidative reactions of polyunsaturated fatty acids (lipid peroxidation), as well as the attack of reactive oxygen species on proteins (enzymes) and nucleic acids; thus O_2 metabolism is linked to carcinogenicity and mutagenicity. Lipid peroxidation is also induced by various halogenated compounds such as carbon tetrachloride. Again, hypoxic conditions are particularly critical because, on the one hand, metabolic activation leading to the free radical is enhanced and, on the other hand, oxygen required for the maintenance of lipid peroxidation is still available. - Powerful antioxidant systems of the cell maintain low steady state concentrations of oxygen metabolites, and toxic effects may, in part, also be explained by the constant drain of reducing equivalents resulting from redox cycling.

Most of the oxygen consumed in biological systems is reduced to water by cytochrome oxidase, and the coupling of oxidation to the phosphorylation of ADP by the respiratory chain forms the energetic basis for life, in eukaryotic systems. However, beneficial as this uptake of oxygen may be, there are other aspects of O_2 metabolism, some of which are associated with toxic processes. Consequently, aerobic life encompasses certain dangers which, fortunately, are dealt with by powerful defense systems against oxidative damage.

It is now well-established that there is a formation of oxygen metabolites derived by univalent or bivalent reduction, i.e. the superoxide anion radical, O_2^-, and hydrogen peroxide, H_2O_2, respectively[1]. O_2^- is known to lead to cytotoxic effects[2-4] and to induce, under defined conditions, strand breaks in DNA[5,6], oxidation of thiol groups in proteins[3,4], lipid peroxidation reactions[7-9] etc. It is likely, however, that the effects are not due to O_2^- itself but rather are catalyzed by one of the other reactive oxygen species[2-4]. In other words, O_2^- does not appear to be directly a cytotoxic agent by itself. In contrast, recent evidence points to a direct cytotoxic effect of H_2O_2[10].

In addition to hydrogen peroxide, other peroxides are formed, notably organic hydroperoxides in the form of lipid hydroperoxides (see reviews in Pryor[11]). Chemically feasible, and still under debate with respect to biological significance, are further toxic O_2 derivatives such as singlet oxygen and the hydroxyl radical (see reviews in reference[4]).

Potentially toxic processes associated with O_2 metabolism include a variety of events, both occurring physiologically and pathophysiologically. These range from the generation of mutations and carcinogenesis to aging, inflammation, phagocytosis, immune problems, prostaglandin and leukotriene metabolism, and others.

In this essay we will discuss 2 problems: 1. What is the common mechanism of action of a number of compounds which undergo a redox cycle, i.e. what is the basis of their toxic effects on target cells? This relates to what may be termed oxygen-induced toxicity by redox cycling. And the second problem is: 2. How can

one distinguish these effects from toxic O_2-dependent effects not linked to redox cycling? The major damaging pathway here is that of lipid peroxidation (the cycling involved in the maintenance of radical chain reactions is not discussed here). Other pathways also are of importance in evaluating damaging conditions, and we will briefly consider the particular difficulties encountered in hypoxic states where O_2-dependent damage and reductive pathways of a damaging nature may combine to form particularly critical conditions.

Redox cycling

Various endogenous and exogenous compounds are supposed to exert toxic effects in biological systems via oxygen reduction. In general, these compounds must first be reduced by intracellular enzymes (reductases of various kinds) before they can activate oxygen by electron transfer. Predominantly one-electron oxidation-reduction processes occur. The examples dealt with are shown in figure 1. The reductases are not discussed in detail here; they include NADPH-cytochrome c (cytochrome P-450) reductase[11a], aldehyde reductase [11b] and ketone reductase[11c] activities.

It has been suggested that among naturally occurring compounds catecholamines like adrenaline (epinephrine), dopa and dopamine, generate superoxide anion radicals[12–15] after their oxidation by $O_2^{\cdot-}$ formed by flavin enzymes like cytochrome P-450 reductase[13,16,17]. The 1st reaction step probably leads to the semiquinone molecule (fig. 1a) which then reacts with molecular oxygen to form another molecule of $O_2^{\cdot-}$. A reduction of $O_2^{\cdot-}$ to H_2O_2 by the semiquinone is also possible[14]. Both reactions lead to the fully oxidized quinone molecule. Although it is not yet clear whether under physiological conditions the adrenaline quinone molecule thus formed, or the further oxidized adrenochrome, are reduced by direct electron transfer to the semiquinone (which can again react with molecular oxygen), such recycling is very likely (see below). This would lead to reactive oxygen species which can be harmful to the cell. Catechol drugs would behave similarly.

A catecholamine derivate, 5-S-cysteinyl-dopa, a precursor of melanin, has recently been proposed as an antitumor agent[18]. Its efficiency might be due to such a redox cycle in tumor cells where the drug is first oxidized to the semiquinone molecule. This then reacts with oxygen, thereby initiating the cycle. Again, the formation of $O_2^{\cdot-}$ would lead to various reactive oxygen species which are cytotoxic[2–4]. A similar cycle has been proposed even for melanin itself[19]. The neurotoxicity of 6-hydroxydopamine has also been associated with the enzymatic formation of reactive oxygen species which the drug would form after transformation to the semiquinone molecule in presence of oxygen[20,21]. Similarly, the semiquinones of 5-hydroxyindoles (e.g. serotonin) formed in liver microsomes might be able to undergo a redox cycle consuming oxygen and forming reactive oxygen intermediates[22]. The biological or toxicological relevance of the reactions named so far has to be borne out in the future.

Of proven relevance in this sense are a number of clinically used quinone anticancer drugs which probably act on DNA by the formation of reactive oxygen species[23–26]. Anthracyclines like adriamycin (doxorubicin) are reduced by various enzymes or by reducing agents to the corresponding semiquinones[24–29] which readily react with molecular oxygen to form $O_2^{\cdot-}$. This is further reduced to H_2O_2 but might also damage tumor cells directly or via other reactive oxygen species. Because these drugs show cardiotoxicity during chemotherapy it has been assumed that the reactive oxygen species formed during redox cycling induces lipid peroxidation in the heart[25]. However, the evidence for lipid peroxidation is poor, and in recent experiments was not confirmed in vitro[30] as well as in vivo[31]. The direct effects of $O_2^{\cdot-}$, $^{\cdot}OH$ or H_2O_2 formed on the levels of glutathione, various enzymes or other biological molecules might be more important[25,32–34]. As pointed out recently, simply the hypoxic condition induced by the redox cycling of adriamycin could be responsible for the cardiotoxicity observed during chemotherapy[35].

Another anticancer drug which forms reactive oxygen species by a redox cycle is mitomycin C. It has been claimed that hydroxyl radicals are responsible for its cytotoxic activity against tumor cells[36,37]. Similarly, the o-naphthoquinone antibiotic drug β-lapachone is active by means of redox cycling of the quinone molecule, generating $O_2^{\cdot-}$, $^{\cdot}OH$ and H_2O_2 which lead to cytotoxic effects[38], particularly in cells low in defense capacity as is the case in *Trypanosoma cruzi*[38a].

A further quinone drug which presumably catalyzes redox cycles is the diabetogenic compound alloxan[39]. It can be reduced enzymatically to dialuric acid by a two-electron transfer, but it is very likely that as an intermediate a semiquinone moiety is formed. This can react with molecular oxygen to form $O_2^{\cdot-}$. The cytotoxic activity of the drug has been related to the formation of hydroxyl radicals, likely to be formed during this redox cycling[20,39,40].

Even for the hepatotoxicity of the analgesic drug acetaminophen a redox cycle could be responsible[41]. The continuing debate about whether covalent binding of the drug[42] or the observed lipid peroxidation[43] are the underlying mechanisms may be terminated if such a hypothesis is confirmed; there is evidence for the occurrence of the quinoneimine molecule[44,44a] which is reduced in a one-electron step. The metastable semiquinoneimine could easily react with molecular oxygen yielding $O_2^{\cdot-}$ and quinoneimine. The recycling

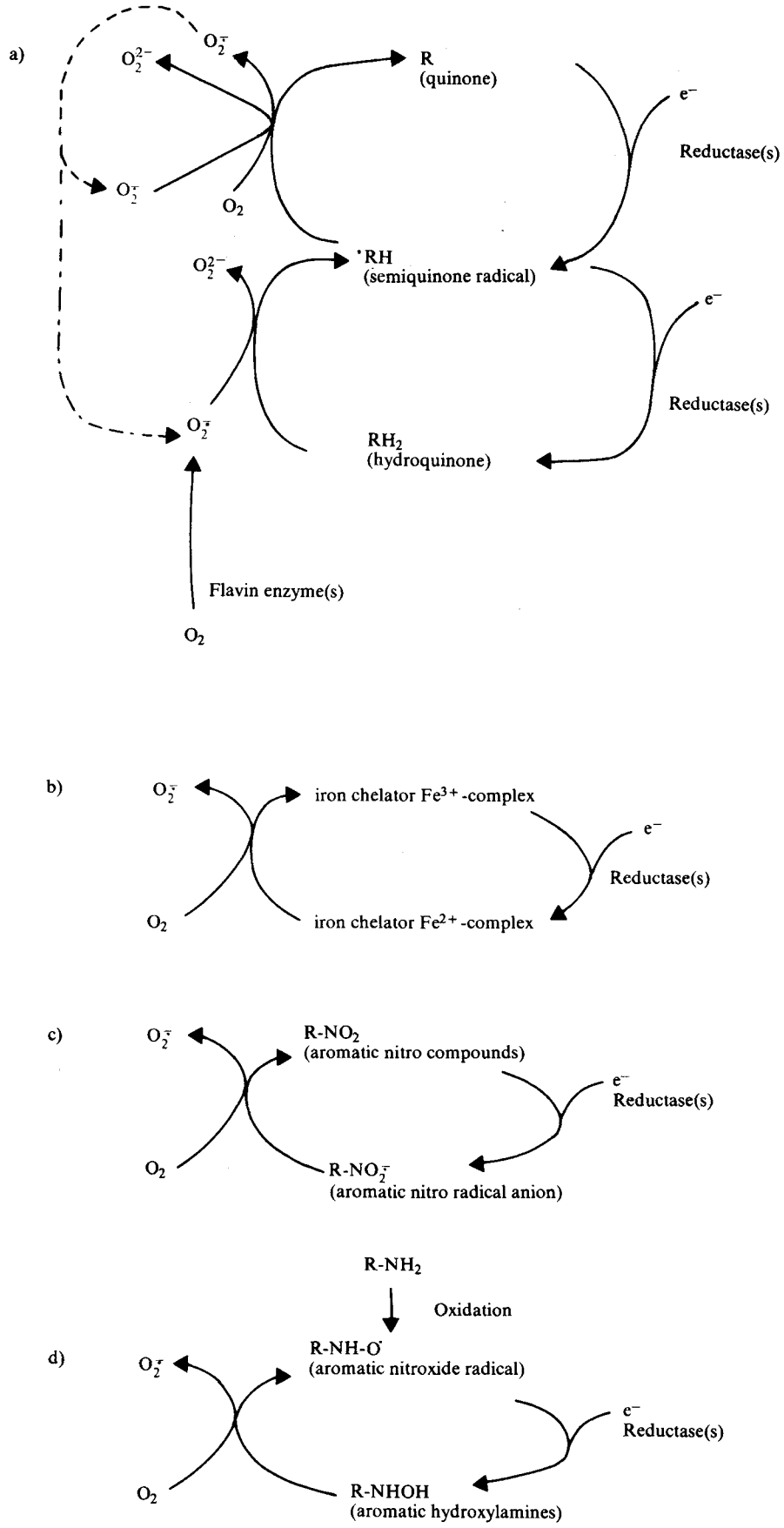

Figure 1. Proposed schemes of redox cycling of different types of drug. Predominant source of reducing equivalents is NADPH.

of the quinoneimine molecule and the formation of excess reactive oxygen species may then result in covalent protein binding of the drug as well as in lipid peroxidation induced by reactive oxygen. The latter probably occurs if protective mechanisms against reactive oxygen species are exhausted. For example, this is the case for glutathione after starvation in acetaminophen-induced lipid peroxidation[43]. An involvement of O_2^- in acetaminophen-induced lipid peroxidation has been suggested[45].

Paraquat has a structure chemically not much different from the quinones mentioned above; it is a herbicide and is toxic to the lung[46,46a], even more so in the presence of 100% oxygen[47]. The damaging effect on plant cells as well as on lung cells has been attributed to its redox properties. This molecule can also form O_2^- by redox cycling[7,39,48a]. Similarly to adriamycin, lipid peroxidation has been suggested as the molecular mechanism for the toxicity of paraquat. However, lipid peroxidation is not evoked by paraquat under normal conditions in vitro and in vivo[49-51]. Only when some protective mechanisms against reactive oxygen species are inactivated can lipid peroxidation be induced by paraquat[50-52a].

Quinoid structures are also involved in the metabolism of benzo(a)pyrene[53,54]. It has been suggested, but not proved, that reactive oxygen species like O_2^-, $^{\cdot}OH$, H_2O_2 etc. are formed which modify DNA, thereby inducing carcinogenesis[53-56].

In this respect a recent hypothesis is of interest, which states that carcinogenesis is mainly related to a low activity of superoxide dismutase in tumor cells[57]. An increase in O_2^- formed by benzo(a)pyrene quinones might be dangerous to the cell[57-59]. It is noteworthy that other benzo(a)pyrene metabolites might also be able to induce the formation of reactive oxygen species[54]. The inhibitory effects of some antioxidants, including vitamin E, on chemical carcinogenesis could be easily explained by this theory[60], because these compounds trap reactive oxygen species. In this connection it may be mentioned that the antitumor antibiotics named above are carcinogenic[26], again possibly due to oxygen reduction during redox cycling of the drugs.

A rather unusual situation exists with the antibiotic bleomycin which is successfully used in cancer chemotherapy[61]. Numerous reports, summarized in a very recent review[62], suggest that DNA breakage caused by bleomycin is due to bleomycin-ferrous ion complexes which activate molecular oxygen to reactive species (fig. 1b). Besides hydroxyl radicals[63,64] the superoxide anion is probably formed by such a complex[63-65]. Whether the bleomycin-ferrous complex is really formed in vivo and whether the ferrous form of the complex is retained by a redox cycle is as yet unknown. However, the fact that reducing agents increase DNA breakage performed by the bleomycin-ferrous oxygen complex[62,66] are indicative of such a redox cycling. Furthermore, it has recently been demonstrated that isolated cytochrome P-450 reductase is able to reduce a bleomycin-ferric ion complex which then causes single-strand breaks of DNA[65]. The reaction required molecular oxygen and NADPH. Other metals also form complexes with bleomycin, but only a few, e.g. cuprous ions, are able to activate oxygen[62].

Bleomycin anticancer chemotherapy is problematic because of the occurrence of a relatively high incidence of lung toxicity. This could be due to increased lipid peroxidation induced by the reactive oxygen species formed. However, after bleomycin treatment in vivo no ethane formation[66a] and only a small increase in malondialdehyde were observed[67a]. Only the Fe^{2+}-induced lipid peroxidation in liver microsomes was found to be increased by bleomycin[66,67]. 1,10-Phenanthroline, another metal chelator which degrades DNA, also produces oxygen radicals in presence of a metal ion such as copper[68].

An increasing number of chemical compounds are thought to activate oxygen by a redox cycle. One group of such chemicals consists of nitro-compounds used as radiosensitizers, e.g. the nitroimidazoles misonidazole and metronidazole, which also have to be reduced to be active in tumor cells[39,69-72]. Oxygen is activated by the nitro radical anion formed during the reduction[39] (fig. 1c). The neurotoxicity of these compounds has been attributed to active oxygen species formed during recycling of the drugs. The lung toxicity of nitrofurantoin has been explained by the same mechanism[73].

Even a number of aromatic amines which are oxidized to nitroxide radicals and reduced to hydroxylamines are thought to catalyze a redox cycle[71,74]. The nitroxide radical would again be formed after the reaction with molecular oxygen (fig. 1d) which is transformed to O_2^-, $^{\cdot}OH$, H_2O_2 etc.

In summary, numerous compounds which cannot all be mentioned here are able to maintain a redox cycle in the presence of redox catalyzing enzymes[75]. Because such enzymes are present in all biological systems[39,75] and are relatively unspecific[76], the reactivities towards O_2 of the intermediates formed, and the availability of oxygen itself decide whether reactive oxygen species will occur. In chemotherapy, for example, the drug design is mainly based on the fact that tumor cells are less well supplied with O_2 and are, in such hypoxic states, able to reduce quinoid and other reducible drugs in one-electron steps. They then form metastable intermediates which can reduce oxygen to form O_2^-[77,78].

It is likely that first of all O_2^- is formed (fig. 1a–d); then $^{\cdot}OH$-radicals are produced either by the Haber-Weiss reaction which is catalyzed by traces of iron ions[2-4] or by further reduction to H_2O_2 which reacts with fer-

rous ions in a Fenton-like reaction[2-4] (fig. 2). The resulting effects in cells probably depend very much on the site of formation of the reactive oxygen species (fig. 2). However, O_2^- which is first formed seems to be able to diffuse within the cell, so that it could be formed at a site distant from the site where it is active.

Lipid peroxidation

The attack of reactive oxygen species on polyunsaturated fatty acids, essential constituents of biological membranes, has been shown to result in peroxidative damage of these lipids. A number of recent reviews on lipid peroxidation are available; this subject has received increasing attention during the past few years[7,79-83].

Besides autoxidation of unsaturated fatty acids and radiation-induced lipid peroxidation, a number of enzymes of diverse origin are presumably involved in endogenous lipid peroxidation reactions. As mentioned above, O_2^-, $^{\cdot}OH$ and H_2O_2 may be involved in the initiation step of lipid peroxidation[7,9,79,83]. Furthermore, iron ions probably play a major role in this process. Whether they are merely catalyzing the formation of reactive oxygen species like hydroxyl radicals which would be formed from O_2^- by a modified Haber-Weiss reaction or whether iron ions, either chelated or free, form oxygen complexes, thereby initiating lipid peroxidation, or both, is still controversial[8,84,85].

The biological significance of lipid peroxidation reactions in vivo is far from clear. In addition to the loss of unsaturated fatty acids of lipid membranes, there is the formation of a number of peroxidative lipid breakdown-products; lipid hydroperoxides, fatty aldehydes (e.g. hexanal) and ketones, malondialdehyde and short-chain alkanes like ethane and pentane[7,79-83,85-87]. However, the nature of the precursors and the mechanisms of breakdown of these peroxidized lipids is still unknown, although with some model compounds molecular mechanisms have been described[85,88]. The lipid peroxide decomposition is probably also dependent on the presence of iron ions, either free or heme-bound[8,83-85,89-91]. Furthermore, the increase in conjugated lipid dienes[7,79,83] and the occurrence of low-level chemiluminescence[92,92a] have been taken as indicators of lipid peroxidation. In addition, the formation of fluorescent products and of lipofuscin, representing cross-linked products of aldehydes, probably formed during lipid peroxidation, and amino groups of various cellular macromolecules, seem to be related to lipid peroxidation[83,93,94].

There is increasing evidence that the products formed during lipid peroxidation reactions are cytotoxic[87,95]. For example the cytotoxic lipid fragment 4-hydroxynonenal has recently been isolated and identified from peroxidized liver microsomes[96]. Malondialdehyde, the reaction product of lipid peroxidation usually determined in experiments, exerts several biological effects including cross-linking of proteins[89] and nucleic acids[83,94], and is presumably mutagenic[97,98] and carcinogenic[99]. These reaction steps might be of relevance concerning the toxicity of drugs which induce lipid peroxidation in biological systems.

In addition to reactive oxygen species which may be formed by enzyme reactions, and to the already mentioned redox-active compounds, as well as to some directly lipid-peroxidizing agents like ozone and nitrogen oxides[100], some toxic halogenated aliphatic hydrocarbons are also able to induce lipid peroxidation in vitro and in vivo[79,86,95,101-103]. The classical hepatotoxic agent carbon tetrachloride (CCl_4) has been especially intensively studied in this respect. It has been shown that a reductive pathway involving cytochrome P-450 is responsible for CCl_4-metabolism[81,82,95,101-108]. It has been suggested that trichloromethyl radicals ($^{\cdot}CCl_3$) or dichlorocarbenes ($^{|}CCl_2$) are the ultimate toxic intermediates. The formation of $^{\cdot}CCl_3$ has been demonstrated in vitro and in vivo[109-111]. A controversy still exists as to whether dichlorocarbene is really formed during CCl_4 metabolism[106-108]. Also, the question remains unresolved whether the direct reaction of the $^{\cdot}CCl_3$-radical with macromolecules like proteins, lipids and nucleic acids[104,105,112-116] leads to toxic reactions or whether the concomitant lipid peroxidation process induced by this radical is responsible for the hepatotoxicity observed. It has been suggested that the abstraction of hydrogen by $^{\cdot}CCl_3$ from a methylene group of an unsaturated fatty acid results in the formation of lipid radicals which react with molecular oxygen initiating

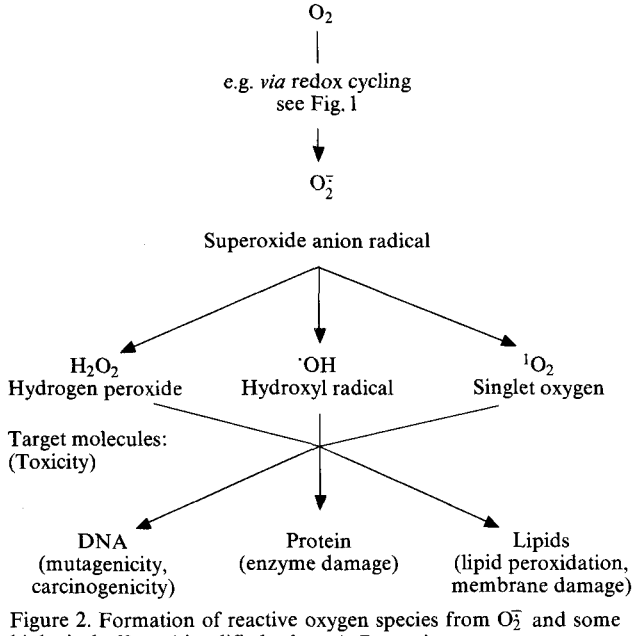

Figure 2. Formation of reactive oxygen species from O_2^- and some biological effects (simplified scheme). Protective systems, enzymatic or via 'antioxidant' reactivity, are not shown here.

lipid peroxidation[79,81,82,95,101]. However, Slater's group[111] has recently demonstrated that in vitro $CCl_3O_2^{\cdot}$ is formed which might be the initiator of lipid peroxidation. Regardless of the nature of the CCl_4-derivative which initiates lipid peroxidation, CCl_4 has first to be reductively metabolized in the cell. It is known that in hypoxia reactive metabolites of CCl_4 are formed much more readily[104,105,113,114,116,117,117a].

Further, it was recently shown that oxygen concentrations play an important role in CCl_4-induced lipid peroxidation[118]: in vivo, the ethane expiration of rats treated with carbon tetrachloride was higher when the animals respired 20% oxygen than with 100% oxygen. Low oxygen concentrations result in higher CCl_4 toxicity[117a,118,119]. This was interpreted as being due to increased metabolic activation of CCl_4. The reactive intermediate formed induces cell damage either by direct binding to cell macromolecules or by initiating lipid peroxidation. It must, however, be mentioned that a recent report is in contrast to the view that lipid peroxidation is catalyzed by a reactive CCl_4 metabolite[120].

Hypoxic conditions prevail in the centrilobular area where the hepatotoxicity after CCl_4 treatment starts. Therefore, a relatively high level of metabolic activation of CCl_4 takes place in this area. On the other hand, oxygen is necessary for the occurrence of lipid peroxidation reactions. Oxygen, however, might not become rate-limiting, because CCl_4-induced lipid peroxidation in vitro was maximal under about 7% oxygen[118]. That products are formed during CCl_4 metabolism in vitro which have similar cytotoxic properties to the reaction products of iron ion-induced lipid peroxidation has already been demonstrated[121,122]. Thus lipid peroxidation may be mainly responsible for the hepatotoxicity observed after CCl_4 treatment, and hypoxic conditions in the liver favor this toxic mechanism.

A situation similar to that with CCl_4 exists with halothane, another halogenated drug which produces, in rare cases, hepatitis in man. An animal model of halothane hepatotoxicity which has recently been introduced includes enzyme induction in the liver as well as hypoxia[123,124]. The hepatotoxicity of halothane has been attributed to a reactive metabolite formed by reductive dehalogenation[103,113,125-127]. Lipid peroxidation, although occurring in vivo after halothane[102,103,128,129] has received much less attention than the hypothesis of reaction of reactive halothane metabolites with cell constituents. However, data on lipid peroxidation using the hypoxic, enzyme-induced animal model are still lacking. It could be possible that, as with CCl_4, halothane metabolites induce more lipid hydroperoxides under hypoxic conditions. Therefore, lipid peroxidation is not exluded as a molecular mechanism of halothane hepatotoxicity.

The correlation between lipid peroxidation and cell injury is not as good as is often expected[130,131]. However, this might depend on the methods used and on the very effective protective mechanisms which are present in most biological systems.

Concluding remarks

The multiple lines of defense against toxic oxygen intermediates consist of enzymatic systems, gluta-

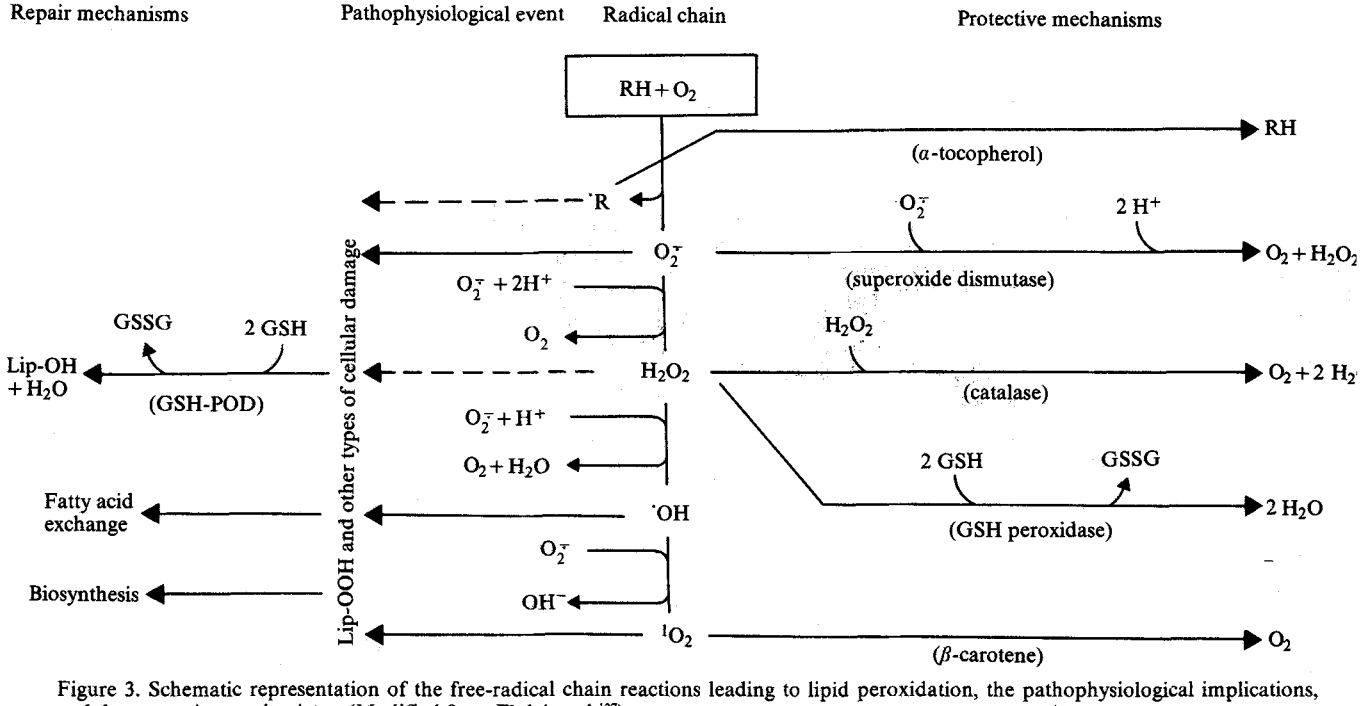

Figure 3. Schematic representation of the free-radical chain reactions leading to lipid peroxidation, the pathophysiological implications, and the protective mechanisms. (Modified from Flohé et al.[137]).

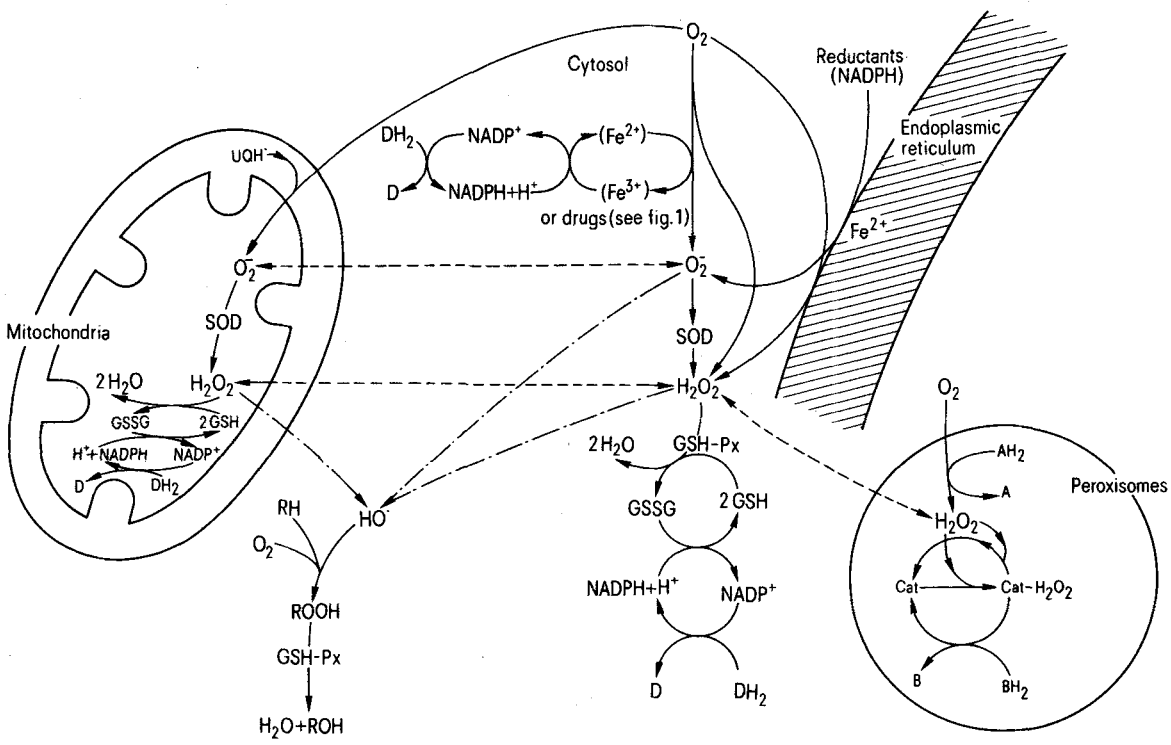

Figure 4. Scheme to illustrate, in a simplified manner, the linkage of Fe^{2+} or drug-mediated redox cycling to cellular reductant systems, notably to the NADPH/$NADP^+$ system, and to cellular oxidant systems as well as defense reactions (compare fig. 3). NADPH is involved (a) in O_2^- radical production and (b) in hydroperoxide reduction by GSH peroxidase reactions. For reasons of space, redox cycling is represented in detail only in the cytosol but is known to occur at membranes of endoplasmic reticulum and mitochondria possibly at higher rates.

thione peroxidase, catalase, and superoxide dismutase, and further of antioxidant capacities such as those of β-carotene and α-tocopherol[1,4,11,132-135](see fig. 3). As stated above, these defense systems counteract the toxic effects, and the balance between prooxidant and antioxidant reactivities is presently under intense study in interdisciplinary groups in areas ranging from biochemistry and biochemical pharmacology into clinical medicine.

This brief discussion of the present state of knowledge on oxygen-linked toxic drug effects has centered on the production of O_2^- radicals by redox cycling and on lipid peroxidation. It was further emphasized that the level of oxygen available at the reaction site may become critical in directing the pathway of generation of potentially toxic intermediates. In addition, the simple fact of an inception of extra O_2 consumption due to redox cycling according to figure 1, and also by lipid peroxidation, may have dangerous consequences in cells on the verge of being hypoxic, a point of importance in pathophysiological conditions. A final point in this respect is to mention that the drain of electrons to maintain redox cycling could also, by itself, lead to metabolic consequences, e.g. via the decrease of the NADPH/$NADP^+$ redox potential (compare fig. 4). Such a problem might lead to critical conditions in cells low in substrate supply for NADPH regeneration, e.g. in the pentosephosphate pathway.

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