Biochemical Mechanism of Oxidative Damage by Redox-Cycling Drugs

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Biochemical mechanisms of production of redox intermediates of redox-cycling drugs include: photochemical events, either photoionization process or electron transfer from photoexcited states; electron exchange of reduced form of a drug with the oxy state of oxygen-binding hemoproteins; oxidation by catalytic metal centers (oxidases, peroxidases, oxygenases) of the reduced forms of drugs; or electron transfer to the oxidized form of a drug from activated intracellular electron transfer chain (mitochondria, microsomes, etc.).

Further reaction of these drug free radicals can lead to oxidative damage by either direct attack of biological macromolecules or via oxygen reduction, giving $O_2^-$, $H_2O_2$, and $OH^-$. The reaction pathway depends on the presence of metal ions, natural scavengers, enzymes that control relative concentrations of reactive species, and availability of oxygen in the environment.

Redox processes have been linked to pharmacological effects of drugs since long time. Many drugs are redox molecules, and in many instances oxidative damage (lipid peroxidation, DNA cleavage, enzyme inactivation, etc.) is evident in tissues undergoing drug action. The recent theory of “active” oxygen species as mediators of oxidation damage is therefore pivotal to current interpretation of the mechanism of action of several drugs (1). The aim of the present article is to review the biochemical factors of relevance to determine the efficacy of a foreign organic molecule (xenobiotic, drug) to act through reversible redox cycling in a certain biological environment. The best characterized redox systems of this type include quinones and bipyridyl cations, which give rise to semiquinoid structures and nitroaromatic compounds, with their reduction products up to the amine level. They give relatively stable free radicals as one-electron redox intermediates according to the one-electron equilibria (1)–(3).

Redox enzymes, like oxidases, peroxidases, and oxygenases, have been shown to produce one-electron intermediates from drugs containing phenol, hydrazine, thiol, polyhalogenalkyl, phenothiazine and other groups (2). Metal ions, which belong to the category of redox-cycling agents, will not be treated here unless strictly associated to the action of organic drugs.

Primary Sources of Oxidative Damage by Drugs

In this respect, four groups of sources can be distinguished which are likely to operate in vivo: physical...
agents, mononuclear oxygen-carriers (e.g., Hb, Mb), metal centers of catalytic oxygen activation, and cellular electron transport systems (e.g., respiratory chains). In general, the primary event is a single-electron donation or abstraction process, giving rise to an organic free radical.

Physical Agents

The light-sensitized action of drugs is a well known occurrence in drug mechanisms. Often photodynamic effects are related to a redox reaction with free radical intermediates. The photochemical event usually involves a photoionization, in which an electron is either added to or ejected from a drug. In biological systems, such reactions can occur under much milder conditions than ordinary photochemistry, owing to the presence of suitable light-sensitive electron donors and/or acceptors. The enhancement by light of the tripanocidal action of crystal violet (a triazylmethyl dye), is related to the increased formation of the carbon-centered radical of the drug (9) by photoreduction in the presence of reduced pyridine nucleotides. Photooxidation of phenothiazines to cation free radicals is responsible, via subsequent electron transfer to melanin, for the skin pigmentation seen in patients undergoing chlorpromazine treatment (4).

On the other hand, visible light can excite anthra-cycline quinone antibiotics, like Adriamycin and daunomycin, to produce a direct electron transfer from the excited state of the drug to oxygen and thus give rise to "active" oxygen species without the intermediate production of free electrons (5).

Oxyhemoproteins

Many redox drugs react with oxyhemoglobin according to the reaction (4):

\[ \text{HbO}_2 + \text{RH} + \text{H}^+ \rightarrow \text{MetHb} + \text{H}_2\text{O}_2 + \text{R}^- \]  

(4)

Phenols (6) and phenylhydrazines (7) are typical of this mechanism. The reaction leads to Hb oxidation, but it is actually a reduction of the bound oxygen in which one electron is donated to the [HbFe(II) O] by the drug and another one by the iron; with H_2O_2, HbFe(III) and the drug radical are products. The reactions involving the resulting free radical, O_2, and the other products, HbFe(III) or H_2O_2, are sources of further oxidizing species. In particular, MetHb and H_2O_2 may behave as a peroxidase-like system (see below), which has been invoked by other authors (8) as the real source of phenylhydrazine-derived free radicals, according to a two-electron reduction of a ferryl (FeIV) form of oxy Hb. Furthermore, quinones, like menadione (9) and Adriamycin (10) may actually oxidize [Hb Fe(II)O_2] to HbFe(III) + O_2 with resulting formation of semiquinones. This is a particular case of a more general mechanism, which will be treated below. These reactions are likely to be responsible for the hemolytic effects of many drugs including also antimalarial 8-aminoquinoline (primquine) and sulfonamides. These drugs are potentially hemolytic, especially in individuals with glucose-6-phosphate dehydrogenase deficiency which impairs the H_2O_2 detoxifying power of the cell via GSH-dependent enzymes. Similar reactions with oxy Hb may occur in the presence of dicvine and isouramil, two alygone which are present in broad beans and may be responsible for the hemolytic anemia (favism) affecting some glucose-6-phosphate-deficient subjects (11). These compounds are an aminophenol (divicine) and a hydroquinone (isouramil) of pyrimidine structure and are likely to give rise to semiquinone form upon very facile autoxidation (12), in a process apparently similar to the autoxidation of their structural analog dialuric acid (13). It will be of interest to investigate the influence of Hb on this autoxidation. In any case, oxidation of Hb occurs in red cells treated with dicvine, especially when the reduced forms of the alygone are continuously regenerated by suitable electron donors like reduced glutathione or ascorbate (14).

Catalytic Metal Centers

Metal impurities catalyze so-called "autoxidation" of quinols, like neurotoxic substituted dopamines (13), with formation of drug free radicals and superoxide or hydrogen peroxide. In this context it should be recalled that O_2^- may also react with the oxidized quinonic forms of such compounds, like in the case of α-methyl-dopa, giving rise to reactive semiquinones (15). Metal centers of metalloenzymes are able to carry out reactions with a number of drugs and in many cases a free radical intermediate has been detected. Phenols (16), aromatic amines (17), hydrazines (18), and thiols (19) undergo one-electron oxidation in the presence of peroxidases and H_2O_2. Xanthine oxidase is able to use many drugs as single-electron acceptors from xanthine (20), but the physiological significance of such reactions is doubtful. Ceruloplasmin, a four-electron oxidase, is able to oxidize many of its substrates with free radicals as intermediates (21). Cytochrome P-450 (a mono-oxygenase) has been suggested to form free radicals during microsomal oxidation of polycyclic hydrocarbons and tetrachloroalkanes, but definitive unambiguous evidence for this process is still missing (2).

Electron Transfer Chains

This group encompasses the largest number of cases. Here are the oxidized forms of redox drugs to be reduced, usually to a free-radical intermediate, by biological electron donors, on the basis of suitable redox potentials, enzyme specificity and often membrane permeability, since most electron transfer chains are membrane-bound. Therefore, if processes listed under physical agents can be either reductions or oxidation of a drug and those under oxyhemoproteins and catalytic metal centers are oxidation, reactions of the electron transfer type will be reduction of the drug and require
anaerobic conditions to be studied in detail. Quinones like alloxan (22) and anthracyclines (23), nitroaromatic compounds like nitrofurans (24) and nitroimidazoles (25), bipyridyliums (26) are the best characterized systems which have been shown to produce free radicals in the presence of mitochondrial or microsomal electron transfer chain and even whole cells or tissue homogenates. It is established in nearly all such cases, that the drug “intercalates” within the electron transfer chain at a monoelectronic step, such as flavin or cytochrome, then diverting electrons from physiological multielectron reduction of terminal acceptor to a single-electron intermediate which uncouples the concerted electron flux (28).

**Further Reactions of Redox Intermediates of Drugs: Biochemical Mechanisms of Oxidative Damage**

It is generally agreed that redox intermediates produced by biological activation of drugs (mostly free radicals) can undergo two types of reactions, potentially leading to damage of biomolecules. One type of reaction is direct attack of a macromolecule by an organic free radical, usually leading to irreversible binding of the drug to it. Another pathway is the reaction of the one- or two-electron-reduced drug with oxygen, which leads to production of O$_2^-$ and H$_2$O$_2$. These two “active” oxygen species are capable of numerous reactions, especially in the presence of metal ions with eventual oxidative damage of the target. Free OH$^-$ radicals (27), bound (or crypto) OH$^-$ radicals (28), site-directed (by selective metal coordination) OH$^-$ radicals (29), and liperoxidases (30), have been suggested as suitable candidates for ultimate oxidative step of the damaging process. An interesting case, which is in a way intermediate between the two primary reaction types, is direct reaction of a drug free radical with H$_2$O$_2$ to give OH$^-$ (31). It should be clear in this context that these alternate pathways are somewhat mutually exclusive, as a function of the relative reaction rates under the particular conditions where the radical is originated. A preferential reaction with O$_2$ will prevent the radical from direct attack to a biomolecule, and from this point of view O$_2$ may be considered as a free-radical scavenger. Disproportionation of the radical itself to the fully reduced and fully oxidized forms or electron transfer to a scavenger that gives rise to poorly reactive (toward either oxygen or biomolecules) secondary free radicals may be event of some relevance to eventual protection of biological targets. It is a matter of concentrations and rates, which direction the electron goes, and catalyst availability (metal ions, enzymes) will determine which intermediate will be more dangerous to the biological environment.

**Metal Ions**

Besides being catalysts of autoxidation of reduced drugs, metal ions have a critical role in the production of the ultimate damaging species in many processes of oxidative damage originating from redox activation of drugs. It is established that OH$^-$ radical can be formed from the interaction between O$_2^-$ and H$_2$O$_2$ only in the presence of redox metal ions (32). This process is referred to metal-catalyzed Haber-Weiss reaction and formally is the sum of metal reduction by O$_2^-$ and metal reoxidation by H$_2$O$_2$ (Fenton reaction). A very similar mechanism takes place when drug free radicals reduce chelated Fe(III) directly, without the intervention of O$_2^-$ (33). The actual relevance of such processes to cases where redox cycling of drugs leads to production of O$_2^-$ and H$_2$O$_2$ from the reaction of reduced forms of the drug with oxygen (again, possibly favored by metal ions), depends on the kinetic suitability of the metal coordination for preferential reactions with O$_2^-$, H$_2$O$_2$ and drug free radicals (32,33). Also, the way of generation of drug free radicals, e.g., either radiative or enzymatic (34) seems to affect relative rate of reaction of free radicals with metal ions. It is also well documented that the Fenton reaction is much more effective in damaging macromolecules if OH$^-$ radicals are produced at the site of damage (site-directed Fenton mechanism). This mechanism is favored by site-specific chelation of the metal (29). Mechanisms of this type have been shown to be effective in the case of enzymes (35) DNA (36), viruses (37) and bacteria (38), with ascorbate or paraquat as oxy radical-forming drug.

Another type of site-directed, metal-mediated, oxidative damage by drugs occurs when binding of the drug itself directs the damage to specific sites (drug-directed metal coordination). This has been shown in particular in the case of DNA with bleomycin, Adriamycin and their analogs as the best prototypes. The mechanism of action of bleomycin, a glycopeptide-heterocyclic drug that cleaves DNA, depends on two separate binding properties of its structure, one capable to bind DNA through intercalation of its bithiazole moiety, and another one capable to bind Fe(II) and Fe(III) by several of its nitrogenous groups (39). The ligated iron is the site of redox activation of the drug, while the organic moiety has no redox-active residue. First, if Fe(III) is the starting oxidation state, it has to be reduced to Fe(II), by thiols, NAD(P)H, ascorbate, etc. Then the Fe(II) form binds O$_2$. Addition of one more electron will form “activated bleomycin” (B*) which, in the presence of oxygen, is able to cleave DNA [Eq. (5)].

\[
\begin{align*}
B \text{Fe(II)} & \stackrel{\text{O}_2^-}{\longrightarrow} B \text{Fe(II)O}_2^- \\
B \text{Fe(III)} & \stackrel{\text{H}_2\text{O}_2}{\longrightarrow} B \text{Fe(II)O}_2^- \rightarrow \text{DNA cleavage}
\end{align*}
\]

Alternatively, activated bleomycin may be formed from reaction of Fe(III) bleomycin with peroxide. Although no definitive evidence for the ultimate oxidizing species has been presented, the process is analogous with formation of oxidizing complexes of drugs with oxyhemoglobin and peroxidase/H$_2$O$_2$. Also, anthracycline antibiotics are Fe(III)-chelators, and the resulting
chelate binds to DNA, in a way that is apparently different from that of drug intercalation (40). The DNA-bound complex is able to mediate reduction of oxygen by external reductants, like thiols, and to cleave DNA in a process requiring peroxide. Again, a target-directed production of OH· radical by Fenton chemistry is the most likely explanation of the process, although ferryl-ion species, like in peroxidases, can be produced via an iron-peroxo intermediate. Either pathway should account for those cases where OH· radical scavengers prove to be less effective because free OH· is not involved. These cases are analogous to those referred to as “crypto-OH” radicals (28).

It should be noted that quinones like Adriamycin, have a dual potential of redox cycling, i.e. via metal ion redox shuttle or via quinone–hydroquinone redox shuttle. The former case may be operative with either the quinone or the hydroquinone form of the drug as the metal-chelator, the hydroquinone being also capable of reducing it. The latter case requires either the hydroquinone or the semiquinone form to react with oxygen. However, in the semiquinone-mediated cases, the drug has to be free, and therefore the oxidation species will be produced distantly from the DNA target. In fact, in this case OH· scavengers are effective protectors against DNA cleavage (27).

In conclusion, availability of redox metal ions for coordination by target-specific drugs, seems to favor drug toxicity, regardless of intrinsic capability of redox cycling by the drug itself, in addition to the role of free metal impurities in catalyzing OH· radical formation from O2− and H2O2 produced by redox-cycling drugs.

Natural Scavengers of Reactive Redox Intermediates (Natural Noncatalytic Antioxidants)

It is widely accepted that the antioxidant properties of, for example, carotenoids (precursors of vitamin A) tocopherols (vitamin E), and ascorbate (vitamin C) are related to their ability to scavenge free radicals. In any case, the vitamin free radical will be formed, and the protective efficacy of the vitamin actually depends on further reactions of such a radical. In particular, it is established that in aqueous solution containing metal impurities, ascorbic acid produces O2− via its free radical (41) and this makes it rather a pro-oxidant compound. However it has been reported that the vitamin C free radical can be reduced back to vitamin C by a NADH-dependent system (42). In other cases, disproportionation of the scavenger free radicals may be faster than potentially noxious reaction with oxygen or biomolecules.

Among natural antioxidants, the plant pigments of flavonoid nature (e.g., quercetin) appears to be particularly interesting in view of possible protection against redox cycling drug. In our laboratory, evidence has been obtained that such compounds diminish the oxygen consumption and inhibit the formation of the EPR signal of the paraquat and anthracycline free radicals produced by NADPH addition to rat liver microsomes. This reaction appears to prevent OH· radical formation from H2O2 and the drug free radical (43). Inhibition of the anthracycline-augmented oxygen uptake in microsomal incubations has been also found with vitamin E (44). Therefore, a similar mechanism seems to apply for the protective effects of some natural antioxidants against redox cycling drugs, which apparently is lowering the concentration of drug-derived species particularly reactive toward oxygen.

Enzymes That Control Noxious Reactions of Redox Intermediates of Drug Action

A potentially defensive role may coexist with potential source of damage in the mechanism of various classes of enzymes. Thus oxidases, oxygenases and peroxidases may well produce reactive intermediates when acting on drugs, but they will reduce dioxygen to water, thus decreasing the steady state level of intermediate reduction products of oxygen. An enzyme capable of reducing the vitamin C radical to vitamin C has been already mentioned (42). Production of free-radical intermediates from microsomal or mitochondrial activation of quinones or nitroaromatics can be prevented by the two-electron reduction, forming hydroquinones or nitroso compounds directly, which is catalyzed by DT-diaphorase (45). This reaction may serve a protective function, since hydroquinones and nitroso compounds are less reactive than semiquinones and nitro anion radicals, and hydroquinones are more easily excreted by the cell than semiquinones. The enzymes that are active on O2− and H2O2 are considered to be of primary importance in this respect. It should be kept in mind that their functions appear to be complementary to each other. In fact, a proper balance between superoxide dismutase and the H2O2-removing enzymes (primarily glutathione peroxidase, catalase being localized inside peroxisomes in most cells) seems to be essential with regard to both modality and intensity of “active oxygen” production. Since redox-cycling drugs mostly work through the univalent oxygen reduction pathway, leading to H2O2 via superoxide dismutation, enzyme catalysis of this reaction has to be matched with adequate enzymic control of H2O2. Many natural situations point to actual relevance of such an aspect, in particular to efficacy of redox drugs on targets with a selectively unbalanced [superoxide dismutase]/[GSH-peroxidase (± catalase)] ratio. Antimalarial drugs activated through the oxyhemoglobin mechanism may destroy plasmodial cells because of their deficiency in H2O2-removing enzymes (46). The same argument seems to apply to trypanocidal drugs versus trypanosomes (47), alloxan injury to pancreatic islets (48), tumor killing by anthracyclines (49), drug-induced hemolysis (50), brain sensitivity to oxygen-mediated damage (51), and heart sensitivity to Adriamycin therapy (52). In this respect, a suitable experimental model has been obtained by treating rats with hypolipidemic drugs of the clofibrate
family (53). This treatment leads to decrease of enzymes acting on oxy radicals in liver. This effect is associated with an increased susceptibility of the tissue to enhanced peroxidative risk only in those samples where GSH peroxidase is much more decreased than superoxide dismutase with respect to normal controls.

**Hypoxia and Redox-Cycling Drugs**

It has been already mentioned that there are two generally accepted theories of cell sensitivity to redox-cycling drugs, focusing on either direct attack of cell components by reactive redox intermediates of the drug itself (organic free radicals), or formation of oxygen reactive species like $O_2^-$, $H_2O_2$, and OH. It has also been said that the two hypotheses can not coexist, because the latter requires oxygen, while the former requires lack of oxygen. Hypoxic “oxidative” damage by redox-cycling drugs may therefore be conveniently explained in terms of direct reactions of organic free radicals, although many authors emphasize the role of the reperfusion–reoxygenation step in determining a “burst” of oxygen reduction by organic free radicals accumulated during the hypoxic phase. This is not a trivial difference, since prevention and management of side effects of drug therapy in hypoxic conditions is critically dependent on which mechanism is operating. In fact, antioxygenic enzymes will not be effective in protection against direct damage by organic free radical, while they may be taken in consideration if the reperfusion mechanism is the major cause of injury to ischemic tissues. On the other hand “scavengers” like vitamins A, E, and C have such reaction properties as to be effective in protecting ischemic tissues against purely anoxic free-radical pathways of drug metabolism.

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