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OVERVIEW OF ENZYME SYSTEMS INVOLVED IN BIO-REDUCTION OF DRUGS AND IN REDOX CYCLING

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Exactly ten years ago Handa and Sato [1] published a paper which showed that the reduction of several anticancer quinones by microsomal enzymes was associated with increased sulfite oxidation indicating superoxide radical formation ($O_2^{\cdot-}$). Since that time this field has expanded tremendously and numerous compounds of different chemical classes have been examined for their ability to undergo enzyme-catalyzed redox cycles in biological systems (for review see [2–4]). For example, a redox cycling mechanism has been suggested for anticancer activity and cytotoxicity of a number of drugs used in tumor therapy (for review see [2–8]). Similarly various radiosensitizers may be effective via redox cycling. Also therapeutic efficacy and mammalian toxicity of some antimicrobial drugs have been attributed to redox cycling in microorganisms or in humans respectively (for review see [2–4, 9]). The herbicidal as well as the toxic effects of paraquat are most likely due to redox cycling (for review see [10]). Very recently chemical mutagenesis and carcinogenesis have been related to redox cycling too [11].

In general, the respective compound is reduced in a one-electron-step to a reactive intermediate which is able to transfer one electron to molecular oxygen resulting in the formation of superoxide anion radical (Fig. 1). Only if the reductase involved is not inhibited by dioxygen redox cycling can occur. All enzymes catalyzing redox cycling of foreign compounds are flavoproteins with relatively low substrate selectivity. Therefore, the number of enzyme systems being able to catalyze such a reduction step is limited.

For example, with quinonoide compounds semiquinone radicals are formed which depending on their redox potential react with dioxygen (Fig. 1). The superoxide radical formed can undergo a variety of reactions. It spontaneously dismutates to hydrogen peroxide (H_2O_2) and dioxygen (O_2) whereby singlet oxygen ($^1O_2$) is formed. Ground state dioxygen is formed when superoxide dismutase is involved in this step (Fig. 1). Hydrogen peroxide thus formed is either removed by catalase or by glutathione peroxidase (Fig. 1). Hydrogen peroxide is most likely responsible for hydroxyl radical formation which occurs in the presence of reduced metals like ferrous ions (Fig. 1). Superoxide anion is responsible for the reduction of the metal ions. This is the so-called metal-catalyzed Haber–Weiss-cycle (for review see [2, 12]).

The hydroxyl radical is the most reactive oxygen metabolite and is suggested to be responsible for

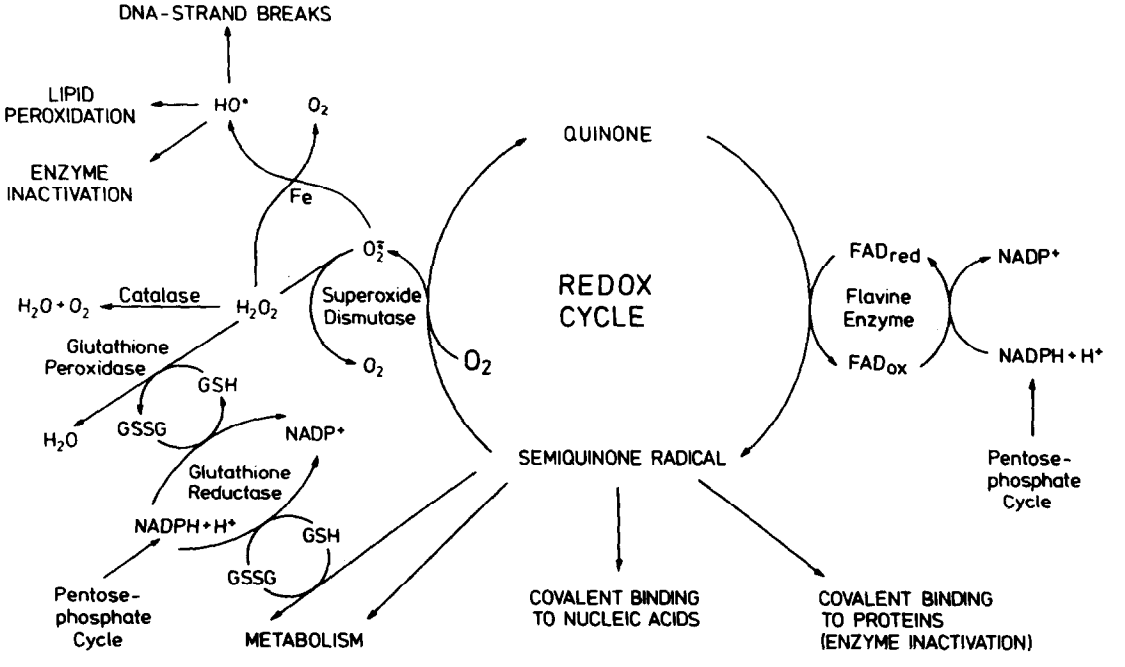

Fig. 1. Redox cycling of quinonoide compounds, oxy radical formation and inactivation (from [12]).

Table 1. Enzymatic systems and compounds involved in redox cycling

Compounds	Reducing enzyme systems	References (original and/or reviews)
Anthracyclines (adriamycin = doxorubicin, daunomycin, aclacinomycin A)	Microsomal and nuclear NADPH-cytochrome P-450 reductase	[1–8, 23–28]
	Mitochondrial NADH dehydrogenase	[8, 29–32]
	Xanthine oxidase	[4, 29]
	Ferredoxin reductase	[33, 34]
Paraquat	Microsomal NADPH-cytochrome P-450 reductase	[10, 35, 36]
	Xanthine oxidase	[37, 38]
	Ferredoxin reductase	[39]
	GSH reductase	[38, 40]
Anthracenediones (e.g. mitoxantrone)	Microsomal NADPH-cytochrome P-450 reductase	[41, 42]
	Mitochondrial NADH dehydrogenase	[42]
Quinones like mitomycin C, AZQ, etoposide (VP-16)	Microsomal and nuclear NADPH-cytochrome P-450 reductase	[43–48]
Quinones like 1,4-naphthoquinone, benzo(*a*)pyrene-3,6-quinone, β-lapachone	Microsomal NADPH-cytochrome P-450 reductase	[2, 3, 9, 49–54]
	Mitochondrial NADH dehydrogenase	[2, 3, 9, 55]
Bleomycin–Fe-complex	Microsomal and nuclear NADPH-cytochrome P-450 reductase	[56–60]
	Xanthine oxidase	[61]
Adriamycin–Fe-complex	Microsomal NADPH-cytochrome P-450 reductase	[17]
Aromatic nitro compounds (nitrofurantoin, nifurtimox, benznidazole, misonidazole, metronidazole, nitrazepam)	Microsomal NADPH-cytochrome P-450 reductase	[2–4, 9, 62–67]
	Mitochondrial NADH dehydrogenase	[9]
	Mitochondrial NAD(P)H nitro reductase (outer membrane)	[68]
	Xanthine oxidase	[69]

some of the serious damage occurring during redox cycling processes, e.g. peroxidation of membranal lipids and protein- and DNA-damage (Fig. 1). Lipid peroxidation changes cellular integrity and releases toxic reaction products (for review see [12]). Protein damage by oxygen radicals leads to amino acid oxidation resulting in conformational changes and enzyme inactivation (for review see [2–4, 10]). DNA damage by hydroxyl radicals leads to cytotoxicity, mutagenicity and carcinogenicity (Fig. 1) (for review see [2–4, 10]).

QUINONES

Quinones occur in biological systems either by the oxidation of hydroquinones or semiquinones or by exogenous addition. A number of benzoquinone, naphthoquinone and anthraquinone derivatives have been studied to find out whether their toxicity or therapeutic efficacy are due to a redox cycling mechanism. These include the anticancer drugs mitomycin C, AZQ, etoposide and the antimicrobial drug β-lapachone which all have been demonstrated to undergo redox cycling by NADPH-cytochrome P-450 reductase, studied either with the isolated enzyme, with microsomes or with isolated nuclei (Table 1).

The anthracenedione (anthraquinone) mitoxantrone has additionally been studied in mitochondrial incubations in the presence of NADH indicating that besides microsomal NADPH-cytochrome P-450 reductase the mitochondrial electron transporting chain could be involved in redox cycling of anthracenediones (Table 1).

Also with benzo(*a*)pyrene-3,6-quinone a redox cycle occurs with NADPH-cytochrome P-450 reductase (Table 1). This compound and other quinone derivatives of polycyclic aromatic hydrocarbons can be formed by autoxidation of phenolic metabolites [13]. Whether redox cycling of these quinones is of any relevance for the carcinogenicity of polycyclic aromatic hydrocarbons is, however, an open question.

The major problem with quinones is that they often are reactive and bind covalently to GSH, proteins and nucleic acids. This is also true for the semiquinones formed after reduction of the quinones (Fig. 1).

ANTHRACYCLINES

Anthracycline anticancer drugs have been extensively studied in respect to redox cycling, because it has been assumed that the antitumor effects of anthracyclines like adriamycin are due to oxygen radical formation occurring during redox cycling in tumor cells (for review see [2–8]). Also cardiotoxicity, a serious side effect of adriamycin, has been related to oxygen radical formation. In microsomes of various organs adriamycin and other anthracyclines are reduced and in the presence of oxygen superoxide and other oxygen radicals are formed (Table 1). This redox cycle of adriamycin can also be catalyzed by isolated NADPH-cytochrome P-450 reductase indicating that this enzyme is responsible.

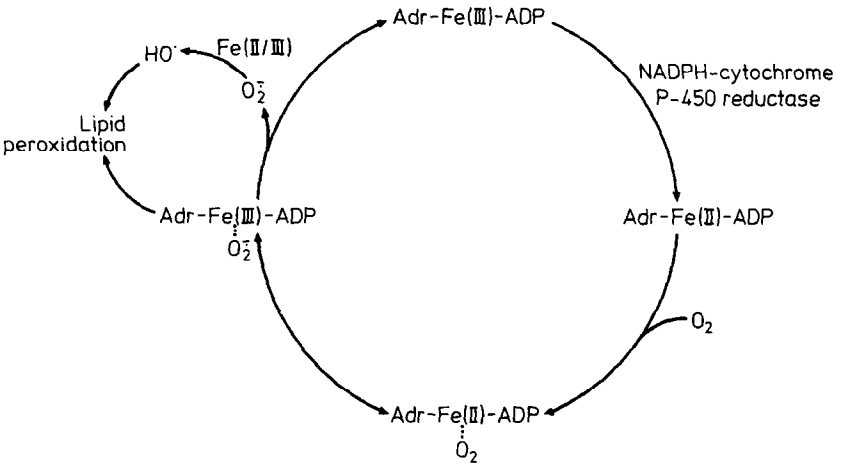

Fig. 2. Scheme demonstrating how microsomal lipid peroxidation could be stimulated by adriamycin (Adr) in the presence of an ADP–Fe-complex. ADP may be replaced by any other endogenous chelator.

On the other hand, in microsomes NADH-enzymes are mostly unable to mediate an anthracycline redox cycle. Therefore, even if NADH-cytochrome b_5 reductase, a single electron-transferring enzyme too, would redox cycle adriamycin, this enzyme might not be involved under physiological conditions. Redox cycling of adriamycin has also been observed in isolated nuclei and is obviously dependent on nuclear NADPH-cytochrome P-450 reductase (Table 1). If the anticancer activity of adriamycin is indeed due to DNA damage caused by oxygen radicals originating from redox cycling of adriamycin, it is important to note that this process occurs near the cellular DNA.

The mechanism of single electron transfer from NADPH-cytochrome P-450 reductase to adriamycin has not been worked out. It is generally believed that single electron transfer from cytochrome P-450 reductase to any other electron acceptor is similar to the transfer from the reductase to cytochrome P-450 [4]; this means that the electron is transferred from NADPH via FAD and FMN to the acceptor [14]. On the other hand, NADPH-cytochrome P-450 reductase is a superoxide generating enzyme. Superoxide radicals might also be able to reduce adriamycin. But under strictly anaerobic conditions or when all oxygen has been consumed by redox cycling the glycosidic bond of adriamycin is reductively cleaved, the same adriamycin semiquinone that reacts with dioxygen being the precursor (Table 1).

It has been observed that adriamycin increases lipid peroxidation in microsomes [15]. Because superoxide radicals are unable to elicit lipid peroxidation, it is now generally accepted that iron ions are a prerequisite for adriamycin-stimulated lipid peroxidation [12, 16–19]. Several mechanisms exist which could explain this (Figs 1–3): (a) An iron-catalyzed Haber–Weiss-reaction could result in hydroxyl radical formation which in turn induces lipid peroxidation (Fig. 1). (b) Adriamycin could complex iron ions and the complex could be reduced by NADPH-cytochrome P-450 reductase (Fig. 2). A highly reactive adriamycin–Fe–oxygen-complex

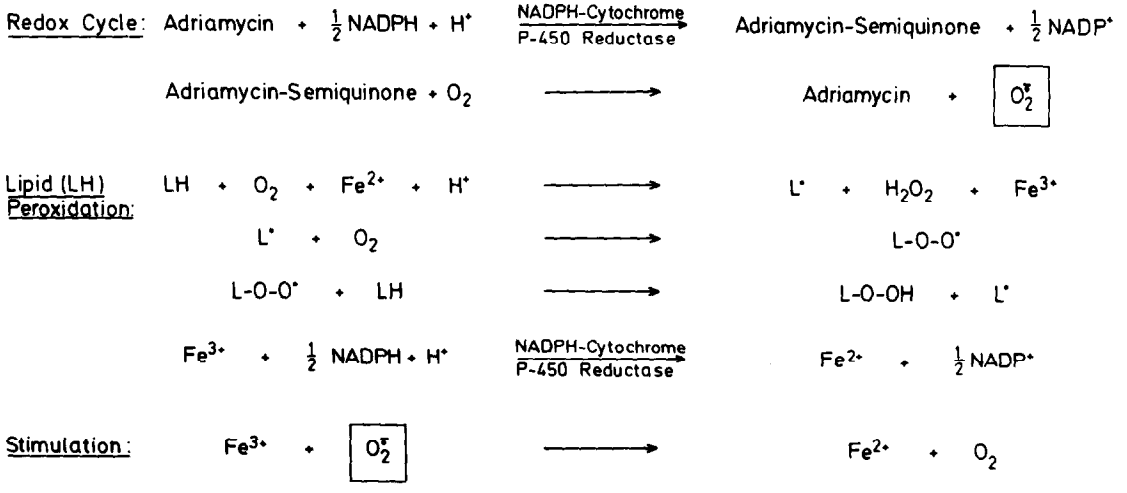

Fig. 3. Scheme demonstrating how microsomal lipid peroxidation could be stimulated by redox cycling of adriamycin (from [20]).

formed under aerobic conditions could directly react with unsaturated fatty acids. (c) We favour a mechanism which is based on the fact that ferric ions can be reduced by superoxide to ferrous ions, a rate-limiting step in lipid peroxidation induced by NADPH-cytochrome P-450 reductase (Fig. 3). The excessive amounts of superoxide formed during redox cycling of adriamycin would accelerate iron ion reduction, thereby stimulating lipid peroxidation (Fig. 3).

In mitochondria adriamycin is reduced by an NADH-dependent enzyme, probably the NADH-dehydrogenase of the mitochondrial respiratory electron transporting chain (Table 1). There is no doubt that in mitochondria adriamycin and other anthracyclines are reduced to semiquinones, although the exact mechanism is still obscure.

Isolated flavin enzymes like xanthine oxidase or ferredoxin reductase are also able to reduce anthracyclines to semiquinones and to provoke redox cycling (Table 1). These enzymes have often been used to discover the mechanism of redox cycling. However, the mechanism of the reducing process has not been fully elucidated.

PARAQUAT

It has long been known that paraquat and structurally related compounds can easily be reduced forming the paraquat cation radical. The toxic effects of paraquat in plants and in lung have been related to this radical (for review see [10]). It has been shown that paraquat is extensively reduced by microsomal NADPH-cytochrome P-450 reductase also in lung microsomes (Table 1). Oxygen radical formation has been observed. An enzyme-mediated redox cycle of paraquat has been associated with the toxicity of paraquat to the lung.

Redox cycling of paraquat also occurs with isolated xanthine oxidase, ferredoxin reductase and GSH reductase (Table 1), although the latter is probably not a single electron-transferring enzyme.

BLEOMYCIN

It is well known that the antibiotic drug bleomycin which is successfully used in tumor therapy interacts with DNA but splits nucleic acids only in the presence of reduced metals like ferrous ions and dioxygen (for review see [21]). In most studies either a reduced bleomycin–Fe(II)-complex was used or an oxidized bleomycin–Fe(III)-complex was reduced chemically. The reduction of the oxidized complex was also achieved using xanthine oxidase. We found that isolated microsomal NADPH-cytochrome P-450 reductase was very efficient in reducing an oxidized bleomycin–Fe(III)-complex (Table 1) and in the presence of oxygen it catalyzes a redox cycle (Fig. 4). In the presence of DNA strand breaks and release of free bases and malondialdehyde were observed [56, 57]. Recently our studies have been confirmed and extended [58]. We also found good indications for hydroxyl radical formation in this system [59]. The bleomycin–Fe(III)-complex can most likely be reduced by nuclear NADPH-cytochrome P-450 reductase [60], a cell compartment where oxygen radicals would be formed in close proximity to DNA.

NITRO COMPOUNDS

Microsomal NADPH-cytochrome P-450 reductase is also able to catalyze redox cycling of the antimicrobial drugs nitrofurantoin, nifurtimox and metronidazole, the radiosensitizer misonidazole and the hypnotic drug nitrazepam (Table 1). Redox cycling of aromatic nitrocompounds is also catalyzed by xanthine oxidase and mitochondrial NADH dehydrogenase (Table 1). But recent studies suggest that mitochondrial nitro reductase is associated with the outer membrane of mitochondria and therefore not due to the mitochondrial electron transporting respiratory chain (Table 1). The nitro radical anion readily reacts with dioxygen and results in the formation of the superoxide radical and the original nitro compound (Fig. 5). Cytotoxicity of all these drugs has been related to redox cycling (for review see [4, 9]). However, the situation with nitro compounds is very complicated, because they can further be reduced to the hydroxylamines which in turn may undergo redox cycling via the nitroxide radicals (Fig. 5) [22]. But the latter mechanism has not been studied in detail.

CONCLUSION

Figure 5 and Table 1 summarize chemical compounds which undergo redox cycling when reduced by any of the reductases mentioned. There are many more compounds which could not all be named here which undergo redox cycling. It has to be mentioned that although several enzyme systems have been examined with respect to redox cycling it is still unknown which enzyme at what extent takes part in redox cycling. For example, carbonyl reductases

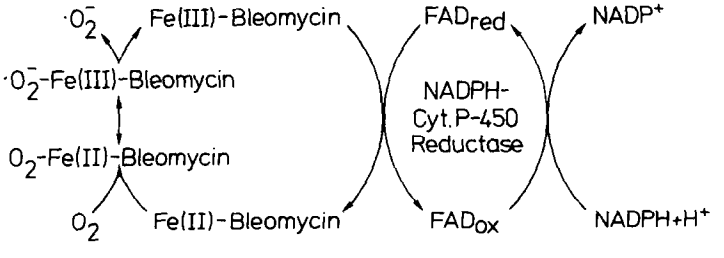

Fig. 4. Redox cycling of the bleomycin–Fe-complex by NADPH-cytochrome P-450 reductase (from [56]).

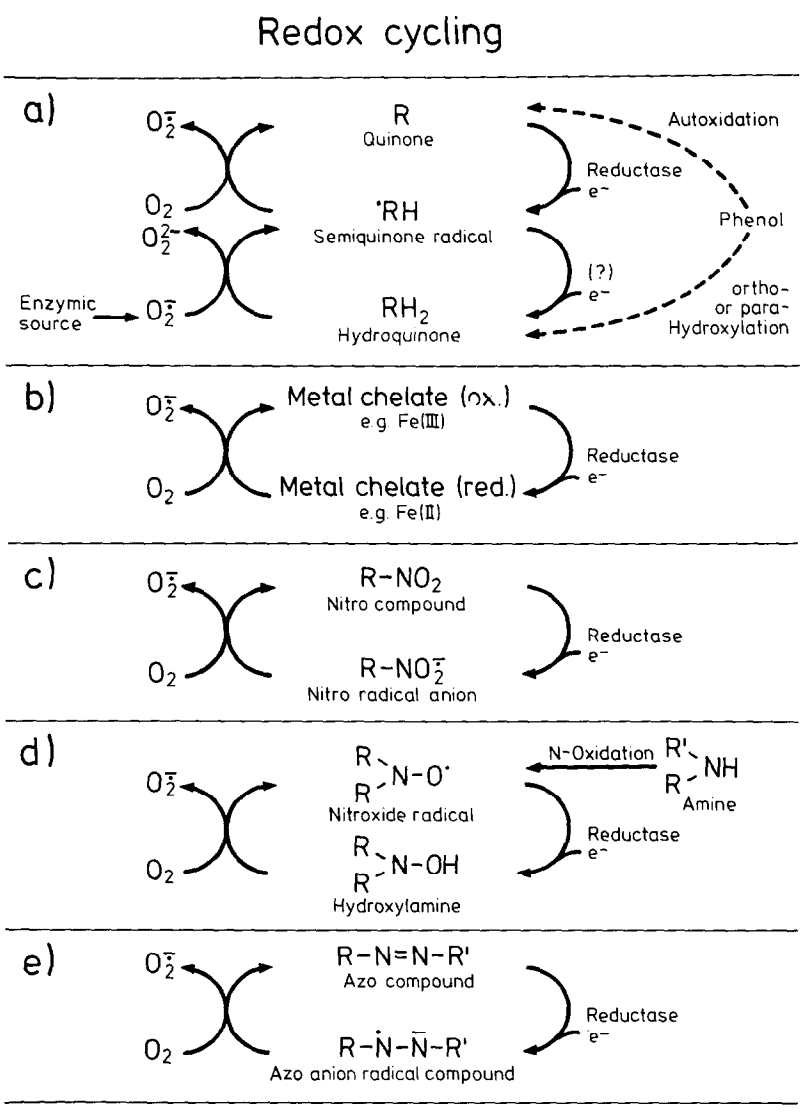

Fig. 5. Mechanism of redox cycling of various compounds via enzymatic reduction and superoxide anion radical formation ($O_2^{\cdot-}$). The azo compound shown is not being dealt within the present paper. Modified from [2].

have been suggested as enzymes involved in redox cycling of drugs. But evidence is missing. Because of its ubiquitous existence in many cells and cell compartments NADPH-cytochrome P-450 reductase is regarded as the major enzyme catalyzing redox cycling of drugs; but other enzymes may be important as well. Furthermore, it is not known what the final toxic event of the redox cycling process is. As mentioned above oxygen radicals which always occur during redox cycling are very dangerous for living organisms. But NADPH depletion could also be a critical event. On the other hand, increased oxygen consumption leads to hypoxic conditions which change the whole intermediary metabolism.

In summary, metabolic activation of drugs via bio-reduction by different single electron-transferring flavine enzymes may be an important mechanism for drug action and drug toxicity.

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