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ROLE OF HEPATIC MICROSOMAL AND PURIFIED CYTOCHROME P-450 IN ONE-ELECTRON REDUCTION OF TWO QUINONE IMINES AND CONCOMITANT REDUCTION OF MOLECULAR OXYGEN

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Abstract—The possible role of cytochrome P-450 in one-electron reduction of quinoid compounds as well as in the formation of reduced oxygen species was investigated in hepatic microsomal and reconstituted systems of purified cytochrome P-450 and purified NADPH-cytochrome P-450 reductase using electron spin resonance (ESR) methods. Two compounds were selected as model compounds: *N*-acetyl-parabenzoquinone imine (NAPQI) and 3,5-dimethyl-*N*-acetyl-para-benzoquinone imine (3,5-dimethyl-NAPQI). Both compounds could be reduced by oxyhaemoglobin, the semiquinones formed were detectable by ESR and did not reduce molecular oxygen. Both NAPQI and 3,5-dimethyl-NAPQI underwent one-electron reduction in microsomal systems and in fully reconstituted systems of cytochrome P-450 and NADPH-cytochrome P-450 reductase under anaerobic and aerobic conditions. In both incubation systems the semiquinone formation was diminished under aerobic circumstances and concomitant reduction of oxygen occurred, leading to the formation of hydrogen peroxide and hydroxyl free radicals. Both the reduction of the quinone imines and the reduction of oxygen were found to be cytochrome P-450 dependent. Both activities of cytochrome P-450 may also be involved in the bioactivation of other compounds with quinoid structural elements, like many chemotherapeutic agents.

Cytochrome P-450, a terminal acceptor from the electrons transferred by NADPH-cytochrome P-450 reductase and substrate binding site of the microsomal mixed function oxidase system, is a versatile biological catalyst. Firstly, by acting as a monooxygenase cytochrome P-450 is capable of metabolizing a large number of endogenous and exogenous compounds. Although this type of metabolism generally implies bioinactivation, in some cases it has been shown to lead to activation of protoxins to reactive metabolites which initiate toxic events [1, 2]. Among the biotransformation reactions mediated by the cytochrome P-450 system are the oxidation of paracetamol to *N*-acetyl-para-benzoquinone imine (NAPQI†) [3, 4] and one-electron reductions of halogenated alkanes, such as halothane and carbon tetrachloride [5]. Furthermore, by acting as an oxidase the cytochrome P-450 system may generate active oxygen species, such as superoxide anions and hydrogen peroxide ultimately leading to the formation of hydroxyl radicals [6].

The bioactivation of exogenous compounds with quinone structures, like many chemotherapeutic agents, has been demonstrated to proceed via one-electron reduction to semiquinone radicals, which, in a redox-cycle with the quinones under aerobic conditions, may reduce molecular oxygen to superoxide anions. Redox-cycling of quinones and their semiquinones is thought to be responsible for the concomitant oxygen toxicity often observed [7]. The one-electron reduction of, for instance, the quinones adriamycine and daunorubicine was shown to be catalysed by hepatic microsomal fractions [8, 9]. This reductive activity was due to the NADPH-cytochrome P-450 reductase, based on the ability of the purified enzyme to reduce these quinones [10]. However, a role of cytochrome P-450 in reductive biotransformation of quinones has been suggested too [11–13]. This may be of importance when the 15- to 20-fold molar excess of cytochrome P-450 over NADPH-cytochrome P-450 reductase on the endoplasmatic reticulum of the liver cell [14] is taken into account. Furthermore, one could imagine that the oxidase activity of cytochrome P-450, due to the interaction of quinones with cytochrome P-450, might contribute to the oxygen toxicity in cells.

The aim of the present study was to investigate the possible role of cytochrome P-450 in the one-electron reduction of quinoid compounds as well as in the formation of reduced oxygen species. For this purpose, besides rat hepatic microsomes, reconstituted systems of purified cytochrome P-450 and purified NADPH-cytochrome P-450 reductase were used. NAPQI and 3,5-dimethyl-NAPQI were used as model quinones because their one-electron reduced free radicals are rather stable and unreactive towards oxygen [15]. ESR combined with spin trapping was used to detect the free radicals formed.

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† Abbreviations: NAPQI, *N*-acetyl-para-benzoquinone imine; 3,5-dimethyl-NAPQI, 3,5-dimethyl-*N*-acetyl-para-benzoquinone imine; DMPO, 5,5′-dimethyl-1-pyrroline-*N*-oxide; DTPA, diethylenetriamine-pentaacetic acid; ESR, electron spin resonance; H_2O_2, hydrogen peroxide; DMPO–OH, DMPO-hydroxyl radical spin adduct.

MATERIALS AND METHODS

Materials. Male albino Wistar rats (180–200 g) were used. In order to induce cytochrome P-450 levels animals were pretreated with 3-methylcholanthrene (40 mg/kg, dissolved in arachides oil). Liver microsomes were prepared by differential ultra centrifugation [16]. Cytochrome P-450 from livers of 3-methylcholanthrene-treated rats, and NADPH-cytochrome P-450 reductase from livers of phenobarbital-treated rats were purified to specific contents of 14.8 nmol/mg protein and 45 units/mg protein, respectively, as described elsewhere [17]. Reconstituted systems of these enzyme preparations were active in ethoxyresorufin O-deethylation [17].

NAPQI was synthesized from paracetamol [19], 3,5-dimethyl-NAPQI from 3,5-dimethyl-paracetamol [20]. The latter compound was synthesized as described by Dearden and O'Hara [21]. DMPO, trichloroacetic acid, imidazole and octylamine were purchased from Aldrich-Europe (Belgium), DMPO was purified before use [22]. NADPH, glucose-6-phosphate, glucose-6-phosphate dehydrogenase, catalase and superoxide dismutase were purchased from Boehringer (Mannheim, F.R.G.). l-α-Dilauroylphosphatidylcholine, haemoglobin and DTPA were obtained from Sigma (St. Louis, MO, U.S.A.).

Incubations. Reduction of the quinone imines by oxyhaemoglobin was studied at 20° by addition of either 1 mM NAPQI or 3,5-dimethyl-NAPQI to a solution of 0.1 mg of haemoglobin in 1 ml of 50 mM potassium phosphate buffer (pH 7.4) containing 0.5 mM DTPA.

Reduction of the quinone imines by microsomal or reconstituted systems was assayed at 20° in incubation mixtures (1 ml), consisting of 5 mM glucose-6-phosphate, 0.04 IU glucose-6-phosphate dehydrogenase, 1 mM NAPQI or 3,5-dimethyl-NAPQI and either 1 mg microsomes or reconstituted systems of 10 μg l-α-dilauroyl-phosphatidylcholine, 0.7 units NADPH-cytochrome P-450 reductase and 0.5 nmol cytochrome P-450 in a potassium phosphate buffer (pH = 7.4) also containing 0.5 mM DTPA. Reactions were started by the addition of NADPH (1 mM). H_2O_2 formation was assayed as described by Hildebrandt *et al.* [23] using catalase and methanol. In this case, the reactions were stopped by the addition of 0.5 ml 25% (w/v) cold trichloroacetic acid.

ESR spectroscopy. ESR spectra were recorded on a Varian E-103 spectrometer equipped with an aqueous flat cell. Samples of the incubations were transferred to the ESR cell 1 min after start of the incubation. Anaerobic incubations were performed under nitrogen atmosphere in vials attached to the ESR cell. ESR settings were microwave power 100 mW, microwave frequency 9.460 GHz, field set 3375 gauss, scan range 100 gauss, scan time 8 min, time constant 1 sec, modulation amplitude 1.6 gauss, receiver gain 7×10^5.

RESULTS

Incubations with oxyhaemoglobin

The possibility of reducing NAPQI as well as 3,5-dimethyl-NAPQI by a haem-ferro complex and detecting their semiquinone free radicals by ESR was evaluated by using oxyhaemoglobin, a haemoprotein capable of one-electron reduction of quinones under aerobic conditions [24]. Upon aerobic incubation of NAPQI with oxyhaemoglobin, an ESR spectrum was obtained as shown in Fig. 1A. This spectrum, not detectable in control incubations without oxyhaemoglobin or without NAPQI, is comparable to that earlier report for a NAPQI-derived semiquinone free radical [25]. In the case of 3,5-dimethyl-NAPQI (Fig. 1B), the ESR signal was identical to that reported for the oxygen-centered semiquinone radical of 3,5-dimethyl-NAPQI [26]. Addition of the spin trap DMPO to these reaction mixtures did not affect the ESR spectra. This indicates that neither the NAPQI semiquinone radical, nor the 3,5-dimethyl-NAPQI semiquinone free radical could be spin trapped. It also indicates that no reduction of molecular oxygen to free radicals by the semiquinone radicals of NAPQI or 3,5-dimethyl-NAPQI occurred, since DMPO is capable of spin trapping both superoxide anions and hydroxyl radicals [27].

Microsomal incubations

Upon anaerobic incubation of NAPQI or 3,5-dimethyl-NAPQI with hepatic microsomes and NADPH, the ESR spectra shown in Figs 1C and D were obtained. The respective spectra were similar to those obtained upon incubation of NAPQI or 3,5-dimethyl-NAPQI with oxyhaemoglobin. No ESR signal could be detected in control incubation mixtures without NADPH, NAPQI or 3,5-dimethyl-NAPQI, or when the latter compounds were replaced by paracetamol and 3,5-dimethyl-paracetamol. Under aerobic conditions only the ESR spectrum of a 3,5-dimethyl-NAPQI semiquinone was detectable, although the intensity was approximately 20 times lower than under similar anaerobic incubation conditions.

Aerobic incubation of either NAPQI or 3,5-dimethyl-NAPQI with hepatic microsomes, NADPH and DMPO, in both cases gave rise to an ESR signal of a DMPO–hydroxyl radical spin adduct (DMPO–

Table 1. Formation of DMPO–OH during aerobic incubation of NAPQI and 3,5-dimethyl-NAPQI with hepatic microsomes in the presence of NADPH

System	Percentage DMPO–OH formation NAPQI	3,5-dimethyl-NAPQI
Complete*	90 ± 15	100 ± 10
−microsomes	4 ± 4	3 ± 3
−NADPH	4 ± 3	4 ± 4
−quinone imines	10 ± 5	10 ± 5
+catalase (500 U)	45 ± 10	36 ± 9
+imidazole (1 mM)	54 ± 7	50 ± 8
+octylamine (1 mM)	63 ± 7	55 ± 10

* Contained microsomes, NADPH and quinone imines.

All incubations were performed as described in Materials and Methods. DMPO–OH formation was calculated from ESR signal intensities and expressed as percentage of the maximal signal obtained. Results express means ± SD of three experiments.

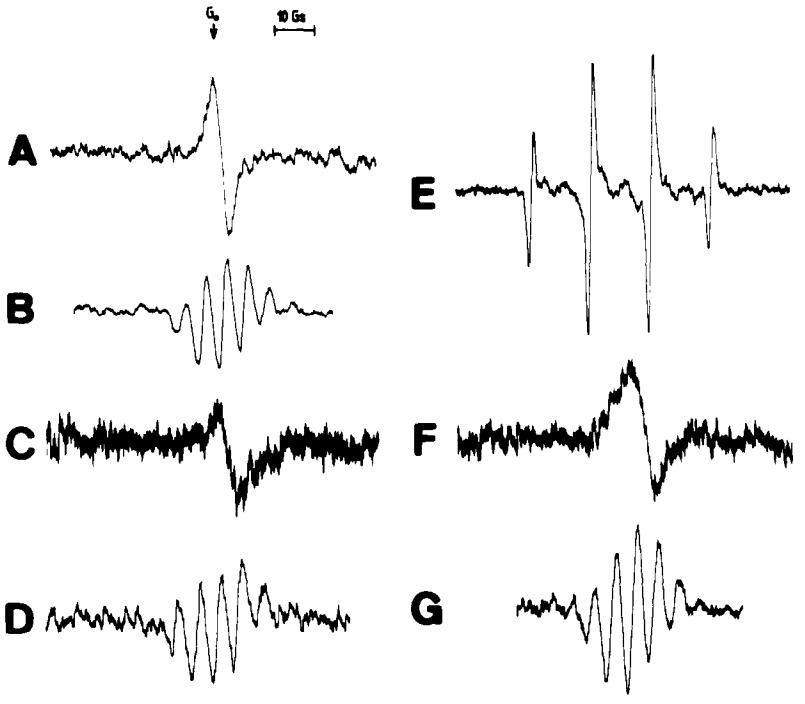

Fig. 1. ESR spectra obtained on incubation of NAPQI or 3,5-dimethyl-NAPQI in different systems. ESR settings as described in Materials and Methods, deviations are mentioned. (A) Aerobic incubation of NAPQI with oxyhaemoglobin; (B) aerobic incubation of 3,5-dimethyl-NAPQI with oxyhaemoglobin (gain 3.2×10^5, time constant 3 sec); (C) anaerobic incubation of NAPQI with hepatic microsomes and NADPH (gain 2.5×10^6); (D) anaerobic incubation of 3,5-dimethyl-NAPQI with hepatic microsomes and NADPH; (E) aerobic incubation of NAPQI, microsomes and NADPH in the presence of DMPO (gain 2.5×10^5); (F) anaerobic incubation of NAPQI with a reconstituted system of cytochrome P-450 and NADPH-cytochrome P-450 reductase in the presence of NADPH (gain 2.5×10^6); (G) anaerobic incubation of 3,5-dimethyl-NAPQI with reconstituted system of cytochrome P-450 and NADPH-cytochrome P-450 reductase in the presence of NADPH. All incubations were performed as described in Materials and Methods.

OH), $\alpha_N = \beta_H = 14.8$ gauss [27, 28] (Fig. 1E). In control incubations without the quinone imines, a small DMPO–OH spectrum was detected, in all other controls ESR signal intensities were negligible. Addition of catalase to microsomal incubation mixtures with NAPQI or 3,5-dimethyl-NAPQI decreased the DMPO–OH formation, suggesting that H_2O_2 was involved in the formation of the hydroxyl radical adduct of DMPO. Since the semiquinones of NAPQI and 3,5-dimethyl-NAPQI have been shown not to reduce molecular oxygen, the observed reduction of oxygen can be expected to be a result of the oxidase activity of cytochrome P-450. This hypothesis could be substantiated by the strong inhibitory effects on DMPO–OH formation of both imidazole and octylamine, cytochrome P-450 inhibitors which bind as ligands to the central haem iron ion of both oxidized and reduced cytochrome P-450 [29]. The results on microsomal DMPO–OH formation are summarized in Table 1.

Incubations with reconstituted systems

During anaerobic incubation of NAPQI or 3,5-dimethyl-NAPQI with a reconstituted system of purified cytochrome P-450, NADPH-cytochrome P-450 reductase and NADPH, relatively strong ESR spectra of the respective semiquinones were observed (Figs 1F and G). These signals could not be detected in any of the control incubations, i.e. in incubation mixtures without cytochrome P-450, NADPH-cytochrome P-450 reductase, NADPH, NAPQI or 3,5-dimethyl-NAPQI or with denaturated cytochrome P-450. As was observed in the case of microsomes, the NAPQI- as well as 3,5-dimethyl-NAPQI-derived semiquinone radical formation was significantly reduced under aerobic incubation with the reconstituted system of purified cytochrome P-450 and purified NADPH-cytochrome P-450 reductase.

The role of cytochrome P-450 in the reduction of oxygen was further studied in aerobic incubations with a reconstituted system of purified cytochrome P-450 and purified NADPH-cytochrome P-450 reductase. Under aerobic conditions and in the presence of DMPO, NADPH and either NAPQI or 3,5-dimethyl-NAPQI, the formation of a DMPO–OH radical was significant. In control incubation mixtures without NAPQI or 3,5-dimethyl-NAPQI, weak DMPO–OH spectra were detectable by ESR, in all other control incubation mixtures, i.e. in the absence of cytochrome P-450, NADPH-cytochrome P-450 reductase or of NADPH, the DMPO–OH formation was negligible. The hydroxyl radical generation in reconstituted systems in the presence of NADPH and NAPQI or 3,5-dimethyl-NAPQI was efficiently

Table 2. Formation of DMPO–OH during aerobic incubation of NAPQI and 3,5-dimethyl-NAPQI in a reconstituted system of purified cytochrome P-450 and purified NADPH-cytochrome P-450 reductase

System	Percentage DMPO–OH formation	
	NAPQI	3,5-dimethyl-NAPQI
Complete*	76 ± 10	100 ± 10
−cytochrome P-450	4 ± 4	4 ± 4
−NADPH-cytochrome P-450 reductase	8 ± 4	9 ± 4
−NADPH	4 ± 4	6 ± 4
−quinone imines	20 ± 5	20 ± 5
+catalase (500 U)	14 ± 5	13 ± 5
+superoxide dismutase (50 μg)	73 ± 5	96 ± 15
+imidazole (1 mM)	26 ± 5	35 ± 5
+octylamine (1 mM)	42 ± 8	50 ± 7

* Contained NADPH, quinone imines and reconstituted systems of purified cytochrome P-450 and purified NADPH-cytochrome P-450 reductase, concentrations and incubation conditions as described in Materials and Methods.

DMPO–OH formation was calculated from ESR signal intensities and expressed as percentage of the maximal signal obtained. Results express means ± SD of three experiments.

inhibited by catalase, but not by addition of superoxide dismutase. Furthermore, the DMPO–OH generation was strongly inhibited by both imidazole and octylamine. The results on DMPO–OH formation in incubations with the reconstituted systems are summarized in Table 2.

To confirm that the DMPO–OH adduct measured was indeed formed from hydroxyl radicals produced during both types of incubations, control experiments were performed to detect possible DMPO–OH production via hydroxyl radical independent routes [27]. Addition of the hydroxyl radical scavengers ethanol and dimethylsulphoxide to both incubation mixtures in the presence of NADPH and NAPQI or 3,5-dimethyl-NAPQI altered the ESR spectra to spectra similar to those of a hydroxyethyl- and a methyl-radical [27], respectively. Furthermore, since addition of superoxide dismutase did not significantly reduce the DMPO–OH signal, a decomposition of a DMPO–superoxide anion spin adduct seemed not to be involved.

H_2O_2 *formation*

Both the experiments with microsomes and the reconstituted systems suggest that H_2O_2 is formed. This hypothesis was further supported by experiments in which the NAPQI- or 3,5-dimethyl-NAPQI-dependent formation of H_2O_2 in reconstituted and microsomal systems was determined. In a completely reconstituted system, the spontaneous H_2O_2 formation was already considerable in the absence of NAPQI or 3,5-dimethyl-NAPQI. H_2O_2 formation was stimulated by a factor of about two, however, upon addition of NAPQI or 3,5-dimethyl-NAPQI. In the microsomal system, NAPQI and 3,5-dimethyl-NAPQI stimulated H_2O_2 formation to a similar extent. In these incubations the cytochrome P-450 ligand binding agents, imidazole and octylamine inhibited the NAPQI- or 3,5-dimethyl-NAPQI-induced H_2O_2 formation in a statistically significant ($P < 0.05$) manner. The results on H_2O_2 formation are summarized in Table 3.

Table 3. H_2O_2 formation during incubation of NAPQI and 3,5-dimethyl-NAPQI in a reconstituted system of purified cytochrome P-450 and purified NADPH-cytochrome P-450 reductase and in a hepatic microsomal system

	H_2O_2 formed (nmol)	
	NAPQI	3,5-dimethyl-NAPQI
Complete reconstituted system*	190 ± 15	210 ± 15
−quinone imines	110 ± 10	110 ± 10
Complete microsomal system†	37 ± 5	38 ± 7
−quinone imines	17 ± 4	17 ± 4
+imidazole (1 mM)	26 ± 3	28 ± 3
+octylamine (1 mM)	29 ± 3	32 ± 3

* Contained quinone imines, NADPH and reconstituted system of cytochrome P-450 and NADPH-cytochrome P-450 reductase.

† Contained quinone imines, NADPH and microsomes.

All incubations were performed as described in Materials and Methods. Results are means ± SD of three experiments, express the amount of H_2O_2 formed during 3 min of incubation in a total volume of 1 ml.

DISCUSSION

NADPH-cytochrome P-450 reductase localized at the endoplasmatic reticulum is generally believed to be the terminal one-electron reductant of quinoid compounds. This type of reduction results in the formation of semiquinone radicals or, upon redox-cycling, in reductive activation of oxygen [8–10]. In view of the molar excess of cytochrome P-450 over NADPH-cytochrome P-450 reductase on the endoplasmatic reticulum of the liver cell [14], it is of interest to determine the possible role of cytochrome P-450 as an alternative in the reduction of quinones and in the concomitant activation of oxygen. For this purpose, the model quinones NAPQI and 3,5-dimethyl-NAPQI were chosen as these compounds form relatively stable semiquinone radicals, which are rather unreactive towards molecular oxygen [15]. These quinone imines are formed in hepatocytes from paracetamol and 3,5-dimethyl-paracetamol, respectively [3, 4, 18].

Firstly, oxyhaemoglobin, a known quinone reductant [24], was found to be capable of reduction of both NAPQI and 3,5-dimethyl-NAPQI. The ESR spectra could be assigned to a melanin-like radical of the NAPQI semiquinone [25], and, in the case of 3,5-dimethyl-NAPQI to its one-electron reduced phenoxy semiquinone free radical [26]. Both radicals were found to be stable even under aerobic conditions. By making use of the spin-trap DMPO, it could be shown that, similar to what has been reported recently [15], no reduction of molecular oxygen occurred.

When 3,5-dimethyl-NAPQI was added to a hepatic microsomal system in the presence of oxygen and NADPH, its semiquinone free radical could be detected by ESR. In the case of NAPQI, microsomal one-electron reduction could only be observed under anaerobic conditions. A reconstituted system of cytochrome P-450 and NADPH-cytochrome P-450 reductase was used to determine unequivocally the role of cytochrome P-450 in the one-electron reduction of 3,5-dimethyl-NAPQI and of NAPQI. Both NAPQI and 3,5-dimethyl-NAPQI were only reduced by a complete reconstituted system containing both cytochrome P-450, NADPH-cytochrome P-450 reductase and NADPH. These observations strongly suggest that cytochrome P-450 is the terminal reductant of NAPQI and 3,5-dimethyl-NAPQI.

Previously quinones such as adriamycine and daunorubicine have been shown to be reduced to semiquinones by microsomal fractions and by purified NADPH-cytochrome P-450 reductase [8–10]. In these studies, however, relatively high concentrations of purified NADPH-cytochrome P-450 reductase were used and, in addition, the quinones studied were the sole electron acceptors present. Recently, an NADPH-cytochrome P-450 reductase-dependent one-electron reduction of NAPQI and 3,5-dimethyl-NAPQI has also been shown to occur under such conditions [15]. In contrast, no NADPH-cytochrome P-450 reductase-dependent one-electron reduction of either NAPQI or 3,5-dimethyl-NAPQI was observed in the present study, presumably because the NADPH-cytochrome P-450 reductase concentration in the reconstituted systems was two orders of magnitude lower than in the previous study.

The one-electron reduction of NAPQI and 3,5-dimethyl-NAPQI in the microsomal and reconstituted system was found to be sensitive to the presence of oxygen. Under aerobic conditions the formation of both semiquinone free radicals was diminished and the reduction of oxygen was found to occur. Since neither an NAPQI- nor a 3,5-dimethyl-NAPQI-derived semiquinone radical was able to reduce molecular oxygen, this observation as well as the observed inhibitory effect of imidazole and octylamine (Table 1), well known ligand binding inhibitors of cytochrome P-450 [29], support the hypothesis that the formation of reduced oxygen species is dependent of cytochrome P-450 and NAPQI or 3,5-dimethyl-NAPQI. NAPQI and 3,5-dimethyl-NAPQI thus appeared to stimulate the oxidase activity of cytochrome P-450 under formation of H_2O_2 and hydroxyl free radicals.

As to the identity of the reduced oxygen species, ultimately formed upon aerobic incubation of NAPQI or 3,5-dimethyl-NAPQI in microsomal and reconstituted systems also containing DMPO, ESR clearly identified the presence of a hydroxyl radical adduct to DMPO (Fig. 1E). The efficient scavenging effect of dimethylsulphoxide and ethanol on the formation of the DMPO–OH adduct further substantiates its dependency on hydroxyl radicals. The formation of the hydroxyl free radicals in turn appeared to be the result of dismutation of H_2O_2, in view of the observed inhibitory effect of catalase (Tables 1 and 2). Even though in the experiments DTPA was used as a metal ion chelator, however, a Fenton-like dismutation of H_2O_2 may also have occurred. With ESR, we failed to detect expected superoxide radical anions. This might be caused by a moderate efficiency of DMPO to trap the reactive superoxide anions [27] or, in the case of the microsomal system, the presence of some endogenous superoxide dismutase.

The present data demonstrate that cytochrome P-450 is involved both in one-electron reduction of NAPQI and 3,5-dimethyl-NAPQI and in the reduction of molecular oxygen. These two activities appeared to be competing, since under aerobic conditions the quinone reduction was diminished when compared to anaerobic conditions. A proposal for the mechanism of both actions of cytochrome P-450 is given in Fig. 2. Upon binding of NAPQI (or 3,5-dimethyl-NAPQI) to cytochrome P-450, its ferri-complex is reduced by NADPH-cytochrome P-450 reductase to a ferro-complex. This complex may transfer one electron to NAPQI under the formation of a semiquinone radical and a ferri-complex of cytochrome P-450 (pathway a). A similar mechanism of action of cytochrome P-450 has been proposed for the one-electron reduction of compounds like halothane and chloroform [5]. In the presence of oxygen, however, this reaction is competing with the binding of molecular oxygen to the ferro-complex (pathway b), at this stage or later followed by reduction of molecular oxygen to active oxygen species [6]. Whether cytochrome P-450 mediated reduction of a quinone or of molecular oxygen will take place, or reduction of a quinone by NADPH-

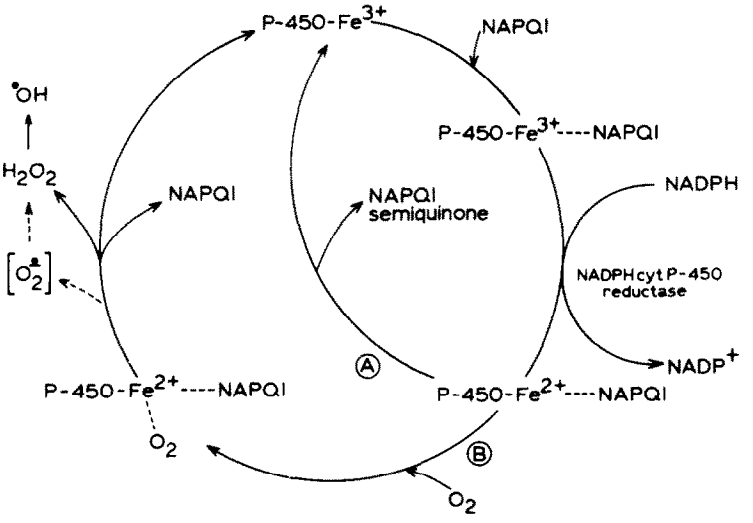

Fig. 2. Proposed mechanism of action of cytochrome P-450 in the reduction of NAPQI and the reduction of molecular oxygen.

cytochrome P-450 reductase, is probably dependent on the actual ratio of cytochrome P-450 to NADPH-cytochrome P-450 reductase present in the system, the affinity of the quinone for NADPH-cytochrome P-450 reductase compared to cytochrome P-450, and as far as cytochrome P-450 is concerned, the electron affinity of the quinone compared to that of molecular oxygen in the presence of the ferro-cytochrome P-450 complex.

Recently, the hepatotoxic action of paracetamol has been related to the formation of active oxygen species [30]. Since during the biotransformation of paracetamol into NAPQI no activation of oxygen was found to occur, it was proposed that redox-cycling of NAPQI once formed would be responsible for the observed oxygen toxicity [31]. Redox-cycling of NAPQI with concomitant reduction of oxygen, however, is rather unlikely since the paracetamol-derived semiquinone radical appears to be unreactive towards oxygen. The data obtained in the present study suggest that the NAPQI-induced activation of oxygen may be the result of uncoupling of cytochrome P-450.

In conclusion, the one-electron reduction of the model quinones NAPQI and 3,5-dimethyl-NAPQI to the corresponding semiquinone free radicals was demonstrated to be cytochrome P-450 dependent under both anaerobic and aerobic conditions in microsomal and reconstituted systems of cytochrome P-450 and NADPH-cytochrome P-450 reductase. Under aerobic conditions this reduction reaction is competing with the transfer of electrons to oxygen giving rise to an uncoupling of the cytochrome P-450 reaction cycle with concomitant formation of active oxygen species, among which are H_2O_2 and hydroxyl free radicals. Both activities of cytochrome P-450 may also be involved in the bioactivation of other compounds with quinoid structural elements.

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