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COMMENTARY

HEPATOTOXIC METABOLIC ACTIVATION OF PARACETAMOL AND ITS DERIVATIVES PHENACETIN AND BENORILATE: OXYGENATION OR ELECTRON TRANSFER?

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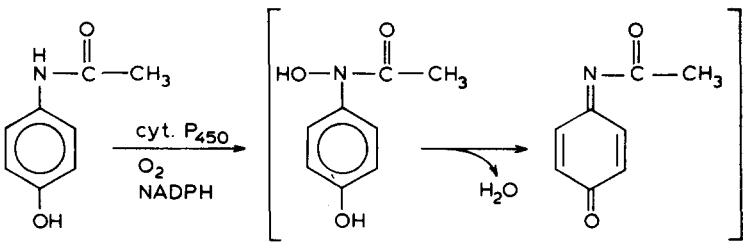
Paracetamol (4-hydroxyacetanilide) is a commonly used mild analgesic which has found increasing acceptance as an aspirin substitute over the past decade [1]. However, at high doses, both in experimental animals and in man, the drug is acutely hepatotoxic and nephrotoxic [2–13]; it can also cause severe hepatic injury when taken over long periods of time [2–4, 6, 12, 14–16]. Poisoning by paracetamol overdosage in suicide attempts is common in Europe and is becoming increasingly prevalent in the United States [17]. Phenacetin (4-ethoxyacetanilide) is another widely used mild analgesic. It has been removed from most analgesic formulations because of the attendant risks of renal damage [18, 19] and methemoglobinemia [20] associated with its use in man. Phenacetin-induced methemoglobinemia has also been observed in experimental animals [21–24]. Recent animal studies have shown that large doses of phenacetin cause hepatic necrosis [23–25]. Benorilate, an ester of acetylsalicylic acid and paracetamol, has been shown to be similar to aspirin in its analgesic [26], antipyretic [27] and anti-inflammatory [28, 29] properties. It has a major advantage over aspirin in that it does not cause gastric bleeding [30, 31]. Some years ago it has been reported that liver necrosis occurred in a 13-year-old girl given benorilate and D-penicillamine together in therapeutic doses for rheumatoid arthritis [32]. Thus, all three compounds have been found to cause hepatic necrosis. Paracetamol hepatotoxicity is dependent upon the mixed function oxydase system [7, 8, 33–35]. It has been demonstrated that paracetamol is converted to an arylating metabolite [9, 36, 37], the identity of which is still uncertain. For the hepatotoxic action of phenacetin a similar mechanism has been proposed. Recent investigations indicate that most of the reactive metabolite arises by oxidative diethylation of phenacetin to paracetamol, followed by further metabolism of paracetamol [23, 38, 39]. In the case of benorilate also, conversion to paracetamol followed by activation of paracetamol has been considered to precede the development of hepatic damage [32]; benorilate undergoes rapid hydrolysis by esterases present in (rat) gut mucosal cells [40] as well as in the blood stream [41].

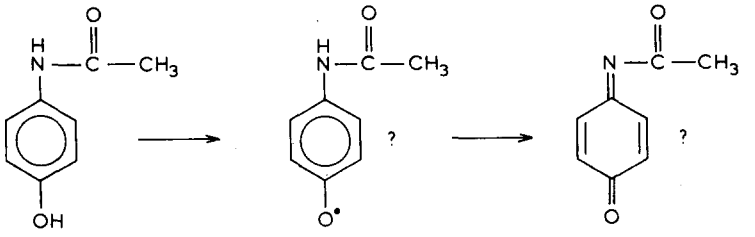
For the possible common mechanism of the hepatotoxic action, the purpose of this commentary is an attempt to integrate some recent findings on the redox behaviour of paracetamol, especially those of relevance to the role of metabolic activation in the hepatotoxicity of the drug.

Metabolic activation of Paracetamol: insertion of oxygen or transfer of electrons?

Up to now the *N*-hydroxyparacetamol/quinoneimine model has been used to explain the hepatic damage by paracetamol [9, 35, 36, 42]:



However, from a recent study it has been concluded that the electrophilic arylating metabolite of paracetamol is not formed from *N*-hydroxyparacetamol [43]. Furthermore, available experimental data suggest that the hepatotoxic action of the drug is dependent upon oxidation to a toxic metabolite by some other pathway. Protection against paracetamol-induced liver damage by radical scavengers such as promethazine [44], glutathione [45–47] and β-dimethylaminoethanol [48] is well-known. Additionally, paracetamol can replace hydroquinone as cofactor for cyclo-oxygenation of arachidonic acid in bovine seminal vesicle microsomes [49]. Moreover, an electrochemical study indicates that the redox behaviour of paracetamol is analogous to that of the *p*-aminophenols: 2 electrons were found for the oxidation [50]:



Oxidation can be insertion of oxygen, but also transfer of electrons. Examples of the second possibility are found in several studies on chemically related compounds. Both *p*-phenylenediamine and *p*-aminophenol have been shown to be suitable substrates for the biological oxidation systems caeruloplasmin and cytochrome *c* oxydase, the expected end products being quinoneimines [51, 52]. Radicals are the initial products of these oxidations. In addition, phenols from ferric hemoglobin by catalytic transfer of electrons from ferrous oxyhemoglobin (a hemeprotein–oxygen complex!) to oxygen [53, 54]. In this respect a recently proposed model for the induction of the cytochrome P-450 linked enzymes is of particular interest [55]. The proposal implies inhibition of the transfer of the second electron from NAD(P)H to the oxy-ferrocytochrome–substrate complex, followed by dissociation of the active oxygen species from the complex. Thus, oxygen dependence does not necessarily imply oxygenation.

Mechanism of the hepatotoxic action of paracetamol—an alternative proposal

The foregoing may be of special significance as paracetamol can interfere directly with flows of electrons [56]. Therefore, an alternative metabolic activation pathway seems theoretically possible. First, like other substrates, paracetamol must occupy its site on cytochrome P-450 (Fig. 1). In a next step the oxy-ferrocytochrome–paracetamol complex will be produced by electron transfer from NADPH via the cytochrome *c* reductase. This is followed by oxidation of paracetamol to the semiquinone, in a manner analogous to the oxidation of phenols by oxyhemoglobin [53, 54]. As previously described for *p*-benzosemiquinone [57], menadione semiquinone [57] and anthracycline semiquinones [58–62], the semiquinone radical intermediate of paracetamol might undergo a cyclic oxidation–reduction process consisting of the oxidation of the semiquinone to the quinoneimine by molecular oxygen, with generation of superoxide, followed by the reformation of the semiquinone by microsomal NADPH–cytochrome *c* reductase (Fig. 2).

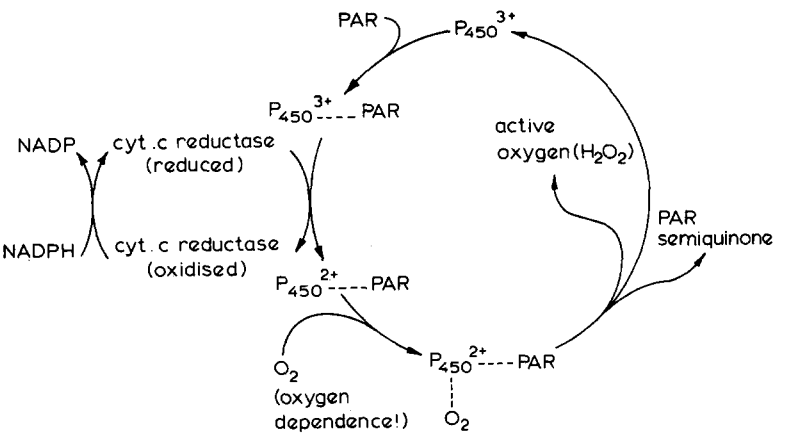


Fig. 1. Proposed model for the hepatotoxic metabolic activation of paracetamol (PAR).

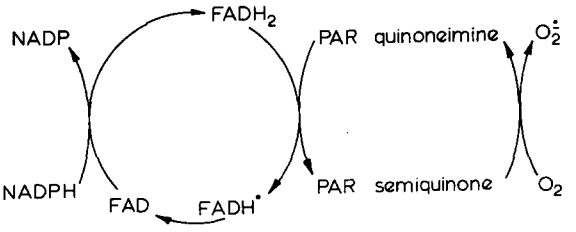
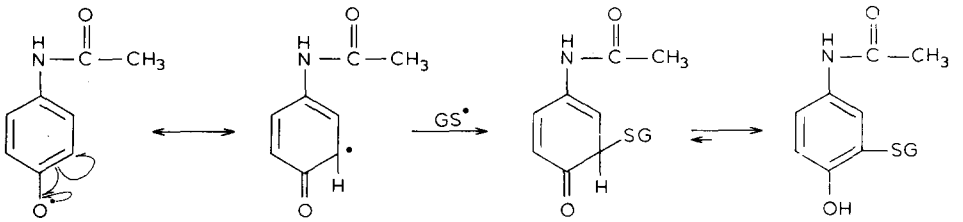


Fig. 2. Proposed oxidation–reduction cycle for PAR semiquinone and associated production of superoxide.

The hepatotoxicity of paracetamol might be due to production of the semiquinone free radical (the semiquinone is far more reactive than the quinoneimine) and/or active oxygen species, such as H_2O_2 and $O_2^{\cdot}$. Both the intermediate and active oxygen can bind and inactivate intracellular proteins. This working hypothesis could also account for the conjugation with glutathione (cf. the formation of 3,5-di-*tert*.butyl-4-hydroxytoluene mercapturic acid [63]):

$R^{\cdot} + GSH \rightarrow RH + GS^{\cdot}$ ($R^{\cdot}$ = semiquinone)
$R^{\cdot} + GS^{\cdot} \rightarrow RSG$
$GS^{\cdot} + {}^{\cdot}SG \rightarrow GSSG$
$R^{\cdot} + GSSG \rightarrow RSG + GS^{\cdot}$



The development of hepatic damage by phenacetin and benorilate will be a pharmacokinetic problem assuming that both compounds undergo initial conversion to paracetamol followed by toxic activation of the paracetamol.

In support of the above concept, preliminary experiments to be published soon have shown that paracetamol is a poor acceptor of active oxygen species. Neither incubation with dihydroxyfumaric acid as a superoxide anion source [64, 65] nor illumination in the presence of methylene blue, a singlet oxygen generator [66–68], resulted in conversion of paracetamol.

Finally, electron transfer may also play a central role in the metabolic activation of other poor acceptors of oxygen.

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