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METABOLISM OF PARACETAMOL TO A GLUTATHIONE CONJUGATE CATALYZED BY PROSTAGLANDIN SYNTHETASE

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SUMMARY: Microsomes isolated from sheep seminal vesicles (SSV) were found to catalyze the metabolic activation of paracetamol as evidenced by rapid formation of paracetamol glutathione conjugate when SSV microsomes were incubated with paracetamol in the presence of arachidonic acid and GSH. The activity was inhibited by indomethacin indicating the involvement of prostaglandin synthetase in the reaction. The initial activity was very rapid, and the affinity for paracetamol in the reaction was high, since formation of the glutathione conjugate was optimal already at 0.2 mM drug concentration.

It is concluded that the activation of paracetamol is due to the peroxidase activity of prostaglandin synthetase in SSV microsomes, since linolenic acid hydroperoxide was also able to support the reaction.

Paracetamol (acetaminophen) is a widely used analgesic and antipyretic drug which is considered to be nontoxic at therapeutic concentration, but produces liver and renal damage when taken in overdoses (1,2). It is currently believed that the microsomal cytochrome P-450-linked monooxygenase system is responsible for activating paracetamol to an electrophilic intermediate that can bind covalently to cellular macromolecules to produce cell damage (3). In the presence of reduced glutathione (GSH), the reactive species is trapped as the corresponding glutathione conjugate (2).

Marnett and coworkers (4,5) have shown that during prostaglandin biosynthesis in sheep seminal vesicle microsomes, several xenobiotics, including benzo(a)pyrene, undergo substantial cooxygenation. In this communication we report that SSV microsomes can catalyze arachidonic acid-dependent metabolism of paracetamol to form a glutathione conjugate in high yield. Prelimi-

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nary evidence indicates that this metabolite is similar, if not identical, to that produced during NADPH-dependent microsomal metabolism of paracetamol.

METHODS AND MATERIALS

Microsomes were prepared as previously described (6) from sheep seminal vesicles that had been stored frozen at -80°.

Incubations were performed at 25° in 0.1 M phosphate buffer, pH 8.0, at a protein concentration of 1 mg per ml of incubate. The final concentration of arachidonic acid was 0.3 mM and that of GSH 2.5 mM. Reactions were started with microsomes and terminated by the addition of 0.2 ml 3 N perchloric acid per ml of incubation mixture.

Protein concentration was measured according yo the method of Lowry et al.

Paracetamol glutathione conjugate formation was determined by high-performance liquid chromatography as described earlier (7).

Arachidonic acid and GSH were purchased from Sigma Chemical Co., St. Louis, Mo. Other chemicals were obtained from local commercial suppliers. Linolenic acid hydroperoxide was produced from linolenic acid in the presence of soybean lipoxygenase as described by Funk et al (8).

RESULTS

In the presence of arachidonic acid, paracetamol and GSH, microsomes isolated from sheep seminal vesicles (SSV) catalyzed the formation of paracetamol glutathione conjugate (Table I). The glutathione conjugate was iden-

Table I

Formation of Paracetamol Glutathione Conjugate Catalyzed by SSV Microsomes

Incubation conditions	Paracetamol Glutathione conjugate nmol/mg protein per min
Complete	22.0
- microsomes	1.3
- paracetamol	0
- glutathione	0
- arachidonic acid	0.9
+ indomethacin, 100 µM	6.5
Complete (boiled microsomes)	0

Incubations were performed as described in Methods.
Paracetamol concentration was 1 mM.

tified by high-performance liquid chromatography and found to cochromatograph with the paracetamol glutathione conjugate formed during aerobic incubation of liver microsomes with paracetamol and GSH in the presence of a NADPH-regenerating system (7). The reaction catalyzed by SSV microsomes was enzymatic in nature, since no activity was detected in the absence of microsomes, or with boiled microsomes. Neither was activity detected if either of GSH, paracetamol or arachidonic acid was absent from the incubation. Indomethacin, a potent inhibitor of prostaglandin synthetase, inhibited the formation of the GSH conjugate of paracetamol by 70% at a concentration of 100 μM (Table I).

As demonstrated in Fig. 1, the formation of paracetamol glutathione conjugate by SSV microsomes was linear with time for only a few seconds and leveled off already after ten seconds of incubation. After two minutes, the reaction had almost terminated. It is of interest to note that the initial reaction velocity was much higher than that usually observed with liver microsomes in presence of NADPH . Also, the affinity for paracetamol was quite high in the reaction catalyzed by SSV microsomes, since measurable

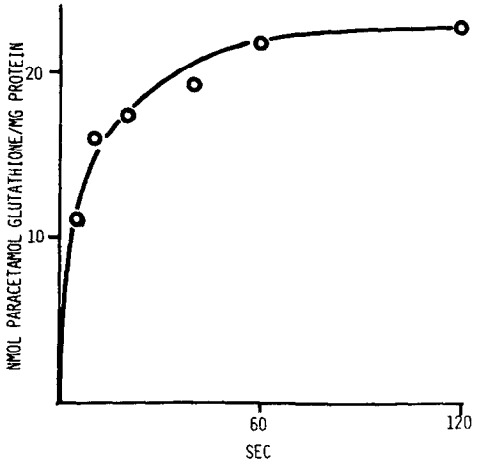

Figure 1. Rate of formation of paracetamol glutathione conjugate in SSV microsomes.
Incubations were performed as described in Methods at a paracetamol concentration of 0.2 mM.

activity was observed already at a drug concentration of 10 μM; the reaction was saturated at about 200 μM paracetamol (Fig. 2).

Finally, the nature of the reaction leading to formation of the paracetamol glutathione conjugate in presence of SSV microsomes and arachidonic acid was further investigated in an experiment where the fatty acid was replaced by a lipid hydroperoxide. As shown in Fig. 3, linolenic acid hydroperoxide was also able to support the metabolism of paracetamol to the glutathione conjugate in presence of SSV microsomes. This activity was somewhat lower than that found with arachidonic acid and terminated after two minutes.

DISCUSSION

The results of the present study clearly demonstrate that sheep seminal vesicle (SSV) microsomes can catalyze the metabolism of paracetamol to a glutathione conjugate which appears to be similar, if not identical, to that formed by liver microsomes in presence of NADPH (7). In both experimental systems, formation of the glutathione conjugate is preceded by the conversion of paracetamol to electrophilic metabolite(s) which spontaneously

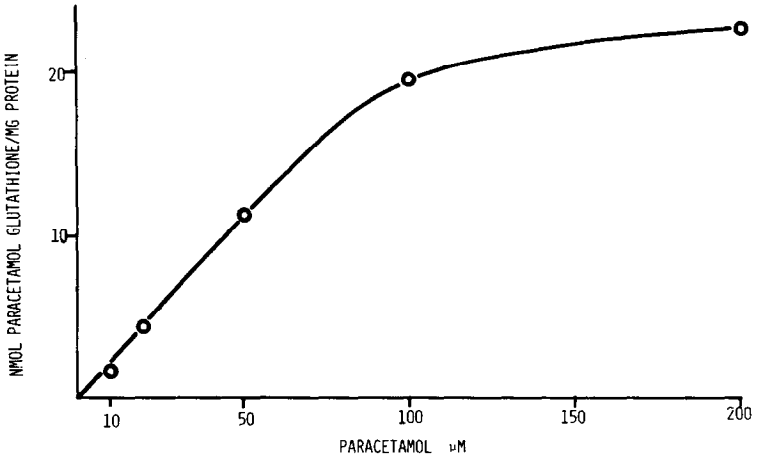

Figure 2. Glutathione conjugate formation in SSV microsomes at different paracetamol concentrations.
Incubations were performed as described in Methods. Time of incubation was 1 min.

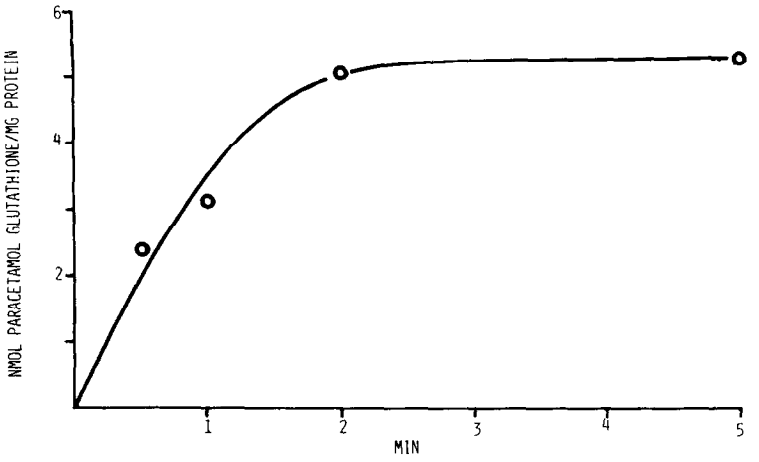

Figure 3. Paracetamol glutathione conjugate formation in SSV microsomes in the presence of linolenic acid hydroperoxide.
Incubations were performed as described in Methods. The final concentrations of paracetamol and linolenic acid hydroperoxide were 0.2 mM and 0.3 mM, respectively.

conjugate with glutathione. The close similarity of the electrophilic intermediates formed from paracetamol in the two systems is supported by similar retention times of the glutathione conjugates on high-performance liquid chromatography (7). It is of special interest to note that both the initial reaction velocity and the affinity for paracetamol are much higher in the SSV microsomal system as compared to the NADPH-dependent monooxygenase reaction in liver microsomes. The apparent K_m-value for paracetamol is only about 60 µM (calculated from data in Fig. 2) in the SSV microsomal system, whereas it is known to be in the millimolar range in liver microsomes (7).

The involvement of prostaglandin synthetase in the metabolic activation of paracetamol by SSV microsomes is suggested by the requirement for arachidonic acid and by inhibition of the reaction by indomethacin. Prostaglandin synthetase, which is present in high concentrations in the seminal vesicles, exhibits both cyclooxygenase activity, catalyzing the oxygenation of arachidonic acid to the hydroperoxyendoperoxide (PGG_2) (9,10), and peroxidase activity, catalyzing the reduction of PGG_2 to the hydroxyendoperoxide (PGH_2) (11,12). It is the latter activity that appears to be responsible for the

metabolic activation of paracetamol. This conclusion is supported by the present observation that linolenic acid hydroperoxide could support formation of the paracetamol glutathione conjugate with SSV microsomes and is also in accordance with previous results by Marnett and Reed (13) demonstrating cooxidation of benzo(a)pyrene to quinones during metabolism of PGG_2 or 15-hydroperoxy-5,8,11,13-eicosatetraenoic acid by SSV microsomes.

The identity of the electrophilic metabolite of paracetamol responsible for formation of the glutathione conjugate has not yet been established. However, since it can be formed as a result of prostaglandin synthetase peroxidase function, its formation appears to involve a one-electron oxidation reaction which could lead to hydrogen abstraction to yield the phenoxy radical of paracetamol. This radical may in turn be further oxidized to the N-acetylimidoquinone prior to reacting with GSH.

The toxicological implications of the metabolic activation of paracetamol as a result of prostaglandin synthetase function are presently unclear. Obviously, this mechanism is of particular interest for the interpretation of the renal toxicity of the drug. The renal medulla is very rich in prostaglandin synthetase, and paracetamol has been reported to interfere with renal prostaglandin production (14). Clearly, the mechanism discussed in this paper may be of importance for the observed inhibitory effect of paracetamol on renal prostaglandin synthesis as well as for the production of reactive paracetamol metabolites in the kidney. These problems are presently under investigation in our laboratory.

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