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FORMATION AND METABOLISM OF A GLUTATHIONE-S-CONJUGATE IN ISOLATED RAT LIVER AND KIDNEY CELLS

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Summary: The reaction sequence involved in drug metabolism, which includes initial oxidation by cytochrome P-450, conjugation with GSH, conversion of the glutathione-S-conjugate to the cysteine-S-conjugate, and N-acetylation of the cysteine-S-conjugate to form the mercapturic acid derivative, was reconstituted in vitro using isolated rat liver and kidney cells with paracetamol as substrate. Oxidation and GSH conjugation were catalyzed primarily by the liver cells, while conversion of the GSH conjugate to the mercapturic acid derivative was catalyzed primarily by the kidney cells. With kidney cells, but not with liver cells, added GSH was oxidized to GSSG. Subsequently, GSSG concentration decreased with stoichiometric increase in glutamate concentration. Addition of GSSG to kidney cells inhibited metabolism of the GSH conjugate of paracetamol to the mercapturic acid derivative. These data indicate a relationship between metabolism of GSH conjugates and of GSSG by kidney cells and demonstrate the overall conversion in vitro of a drug to urinary products.

The formation of N-acetylcysteine-S-conjugates (mercapturic acids) of foreign compounds such as drugs and carcinogens involves a sequence of diverse reactions (1,2). Initially the compound is conjugated with reduced glutathione (GSH) either directly or following activation by a cytochrome P-450 dependent oxidation. GSH conjugation is generally catalyzed by glutathione-S-transferase, but may occur spontaneously. The γ-glutamyl group is then removed, presumably by an enzyme termed γ-glutamyltransferase (γ-glutamyltranspeptidase) (1,3,4), and the glycyl residue hydrolyzed by a particulate peptidase (3,5). The remaining cysteine conjugate is then N-acetylated by an N-acetyltransferase located in the endoplasmic reticulum (6). The enzymes catalyzing these reactions are present in several tissues but generally activation and subsequent GSH conjugation are predominant in the liver, and N-acetylcysteine conjugate formation in the kidney (1,2).

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The γ-glutamyltransferase and the particulate peptidase also participate in GSH turnover (3,5). The γ-glutamyltransferase catalyzes transfer of the γ-glutamyl residue from GSH to an amino acid but may also catalyze hydrolysis (3).

We have previously used isolated liver cells and isolated kidney cells for the study of drug metabolism (7,8). In the present investigation, we have combined these two systems to reconstitute the entire reaction sequence described above using paracetamol as substrate.

Materials and Methods

Male Spraque-Dawley rats, 200-250 g, fed ad lib., were used. Hepatocyte isolation was performed by collagenase perfusion as previously described (9). Kidney cells were isolated by a similar collagenase perfusion method (8). Both types of cells excluded both trypan blue and NADH (90-100%). Incubations were performed at 37° in rotating round-bottom flasks under a 93.5% O_2, 6.5% CO_2 atmosphere at a cell concentration of $1\text{-}2 \times 10^6$ cells/ml. The incubation medium for experiments with paracetamol was Krebs-Henseleit buffer, pH 7.4, supplemented with 25 mM Hepes (N-2-hydroxyethylpiperazine-N'-2-ethane sulphonic acid), penicillin (100 IU/ml), heparin (10 IU/ml), horse serum (17.5%) and an amino acid mixture (10). The medium for experiments with GSH or GSSG was Krebs-Henseleit buffer, pH 7.4, containing 25 mM Hepes. The sulfhydryl conjugates of paracetamol were analyzed by high pressure liquid chromatography as previously described (7). GSH and GSSG concentrations were estimated in the deproteinated sample according to Hissin and Hilf (11). Glutamate was measured by the fluorometric assay of Williamson and Corkey (12). Collagenase was obtained from Boehringer/Mannheim GmbH, Mannheim, GFR. Chemicals were at least of reagent grade and purchased locally.

Results

Paracetamol is activated both by isolated liver and kidney cells as indicated by the formation of paracetamol sulfhydryl conjugates (7,8 and Fig. 1). The major sulfhydryl conjugate detected in the liver cell incubate was glutathione-S-paracetamol; only small amounts of cysteine-S-paracetamol and no N-acetylcysteine-S-paracetamol were formed. However, in the kidney cell incubations, which had a much lower rate of total sulfhydryl conjugate formation, only the cysteine and N-acetylcysteine conjugates were formed, and no glutathione-S-paracetamol could be detected. If one assumes that the cysteine and N-acetylcysteine conjugates are derived from

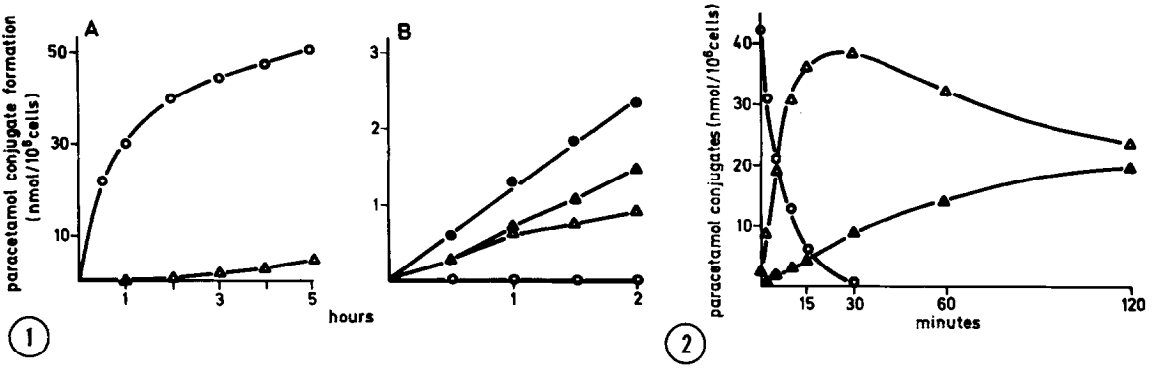


Fig. 1. Formation of sulfhydryl conjugates of paracetamol by cells isolated from rat liver (A) and kidney (B).
Incubations and metabolite analysis were performed as described in Materials and Methods.
o, glutathione-S-paracetamol; Δ, cysteine-S-paracetamol; ▲, N-acetylcysteine-S-paracetamol; ●, total paracetamol conjugates.

Fig. 2. Metabolism of glutathione-S-paracetamol by isolated rat kidney cells.
Isolated kidney cells were added to a medium containing glutathione-S-paracetamol formed by liver cells isolated from phenobarbital-treated rat. The liver cells were removed by centrifugation prior to addition of the kidney cells.
o, glutathione-S-paracetamol; Δ, cysteine-S-paracetamol; ▲, N-acetylcysteine-S-paracetamol.

the GSH conjugate, it would appear that, compared to liver cells, the kidney cells are more active in the further metabolism of the GSH conjugate.

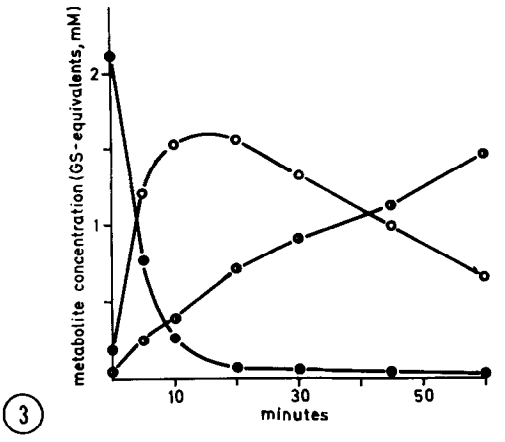
To examine these subsequent reactions, kidney cells were added to an incubation medium containing glutathione-S-paracetamol, preformed by incubation of liver cells with paracetamol (Fig. 2). A rapid disappearance of glutathione-S-paracetamol with a concomitant increase in cysteine-S-paracetamol (≃4 nmol/min per 10^6 cells) was observed. The cysteine conjugate was then acetylated at a lower rate (≃0.3 nmol/min per 10^6 cells) to N-acetylcysteine-S-paracetamol. The total of the three sulfhydryl conjugates was essentially constant during the incubation, indicating stoichiometric conversion of the GSH conjugate to the cysteine and N-acetylcysteine conjugates. Metabolism of paracetamol by kidney cells contributed minimally to the total concentration of sulfhydryl conjugates under these conditions (Fig. 1b).

The metabolism of GSH conjugates to cysteine conjugates has been suggested to be catalyzed by the same enzyme system involved in glutathione metabolism, i.e. γ-glutamyltransferase and particulate peptidase (1,3,5). In agreement with studies on perfused kidneys (13), GSH rapidly disappeared from the medium in the presence of isolated kidney cells (Fig. 3). However, GSH was initially rapidly oxidized (≃125 nmol/min per 10^6 cells). The GSSG formed then decreased with a concomitant increase in glutamate (≃10 nmol/min per 10^6 cells). Only extracellular GSH was affected by these reactions since the intracellular GSH concentration remained constant during this time of incubation (8). No oxidation or metabolism of added GSH was observed with either isolated liver cells or boiled kidney cells.

Since GSSG appeared to be the form of glutathione metabolized by isolated kidney cells, its effect on glutathione-S-paracetamol metabolism was examined (Fig. 4). With initial concentration of glutathione-S-paracetamol about 50 μM, GSSG at 200 μM inhibited the formation of cysteine-S-paracetamol by isolated kidney cells more than 60%. At 1 mM GSSG, total inhibition was observed.

Discussion

Using paracetamol as substrate and a system of intact isolated liver and kidney cells we have demonstrated the formation and further metabolism of glutathione-S-paracetamol. The liver cells were very active in formation of the GSH conjugate but did not catalyze its further metabolism; the reverse was true for the kidney cells. Hydrolysis of the glycylcysteine-S-paracetamol was rapid since we did not detect any of this conjugate but found a stoichiometric relationship between the formation of the cysteine conjugate and disappearance of the GSH conjugate (cf Fig. 2). The slowest step in the metabolism of glutathione-S-paracetamol was the N-acetylation of the cysteine conjugate. This observation agrees well with the finding that the cysteine conjugate of paracetamol is a urinary product (14).



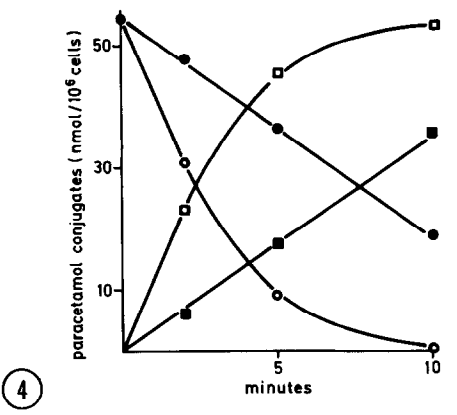


Fig. 3. Glutathione metabolism by isolated rat kidney cells. Incubation and metabolite analysis were performed as described in Materials and Methods. The initial GSH concentration was 2.1 mM.
●, GSH; o, GSSG; ●, Glutamate.

Fig. 4. Inhibition of glutathione-S-paracetamol metabolism by GSSG. Isolated kidney cells were added to medium containing glutathione-S-paracetamol as described in Fig. 2, either without or with GSSG (0.2 mM).
o, glutathione-S-paracetamol (-GSSG); ●, glutathione-S-paracetamol (+GSSG); □, cysteine-S-paracetamol (-GSSG); ■, cysteine-S-paracetamol (+GSSG).

Extracellular glutathione was also metabolized by the isolated kidney cells. Apparently GSSG and not GSH is the substrate for the hydrolytic reactions since added GSH was rapidly oxidized prior to removal of the γ-glutamyl residue. The nature of the GSH oxidase is not yet known. The breakdown of GSSG was accompanied by an increase in free glutamate and appeared to be catalyzed by the same enzymes catalyzing glutathione-S-paracetamol breakdown as indicated by the inhibitory effect of GSSG on this reaction.

The γ-glutamyltransferase catalyzed removal of the γ-glutamyl group may be either a hydrolysis or a transpeptidase reaction. Using purified γ-glutamyltransferase and methionine as acceptor, Tate and Meister (15) found very low transpeptidase activity with GSSG as donor compared to GSH or GSH conjugates. However, the rate of breakdown of GSSG by the kidney cells was faster than that of glutathione-S-paracetamol. Since no amino

acids were added to the incubate, this indicates that the removal of γ-glutamyl residues from GSSG may be a simple hydrolysis.

Acknowledgement

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