Excretion of Aflatoxin B₁ as a glutathione conjugate

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SUMMARY

Intraperitoneal injection of ³H-GSH (15.4 mg, 0.5 μCi) 15 minutes before a similar i.p. treatment with Aflatoxin B₁ (AFB₁) (2mg/kg) showed less than 40% of the radioactivity in the 24h urine and 30-35% in the bile. Analysis of the urine and bile samples by TLC and separation with XAD-2, showed only traces of the AFB₁-GSH adduct present in the urine and 14% in the bile. AFB₁ metabolites isolated from the urine of the ³H-GSH treated rats were identical with those of rats treated with AFB₁ only. While GSH Conjugate appears therefore to be a major component of biliary excretion of AFB₁, it does not seem to be significant in the urinary excretion of the toxin.

INTRODUCTION

Aflatoxins are potent hepatotoxins and hepatocarcinogenic agents of mould origin (1). They undergo microsome-dependent oxidative metabolism giving rise to reactive intermediates capable of covalent binding to cellular macromolecules (2-4). The formation of these reactive intermediates and their subsequent interaction with target tissue components is believed to be largely responsible for their cytotoxic and carcinogenic effects (5-7). However several reports have shown that the active intermediates could be deactivated by conjugation with tissues glutathione (Fig. 1). This process is significant for the maintenance of tissue integrity. Thus a depletion in the concentration of hepatic glutathione could potentiate covalent binding of the reactive species to target macromolecules with the resultant increase in cytotoxocity or carcinogenicity (8-12). Our earlier report (13) has shown considerable utilization of hepatic reduced glutathione in experimental rats. The present report shows that only a very negligible quantity of AFB₁-GSH conjugate is excreted through the urine during a 24-h period. While a substantial quantity is excreted through the bile.

MATERIALS AND METHOD

Treatment of Animals

Male CD Albino rats (150 - 200g Charles River Mass. U.S.A.) were treated with 15.4 mg (0.5 μCi) each of ³H-GSH (240 Ci/mol New England Nuclear, Boston, Mass, U.S.A.) by intraperitoneal injection. 15 minutes later they were treated with aflatoxin B₁ (2 mg/kg body wt.) intraperitoneally also. A 24h urine sample was collected in a metabolic cage. The urine and washings were pooled and the total volume measured.

Analyses of Urine Sample

0.1ml aliquots of the urine were placed in scintillation vials, mixed with a Triton-Toluene scintillant solution and counted in a Beckman liquid scintillation spectrometer. Total radioactivity in the 24h urine was thus computed. An aliquot of the urine sample was extracted with a chloroform-methanol mixture (3:1 v/v). After centrifugation, the chloroform-methanol layer was separated from the aqueous layer and the radio-activity in each phase determined by scintillation counting. The organic phase was later concentrated and 20 μl aliquots
chromatographed on silica gel G. (Merck Darmstadt, W. Germany) precoated plates and the plates developed with chloroform: methanol (97:3 v/v).

The chromatogram was examined under u.v. for fluorescent metabolites. 20 μl of the concentrated aqueous phase was chromatographed on precoated plates using butanol - acetic acid-water (12:3:5 v/v) as solvent. The plates were air dried, sprayed with ninhydrin reagent and heated in an oven at 150°C for 15 min. Rf values of the ninhydrin sensitive spots were evaluated.

Portions of silica from untreated plates were removed in 0.5 cm divisions. These were suspended in Instagel scintillant solution and the radioactivity was determined. The remaining portions of the aqueous layer were pooled, stirred with XAD-2 for 30 minutes, the resin was washed four times with distilled water (13:14) and then with methanol. The methanol fraction containing the conjugate was dried over anhydrous Na₂SO₄ and concentrated in a rotary film evaporator. The radioactivity of the concentrate was determined and the total concentration computed from this. Similar extracts obtained by incubating aflatoxin B₁ with 10 mM glutathione and rat liver microsomes were used as a reference.

**Collection and Processing of bile**

Male rats (150-200 gm wt) were anaesthetized with Nembutal and the common bile duct was cannulated with 0.5 mm polyethylene tubing. ³H-labelled glutathione (10 mM) was injected into normal animals placed in restraining cages and 15 minutes later, Aflatoxin B₁ (2 mg/kg) was injected and urine and bile were collected for the next 24 h. The bile collected was extracted several times with equal volumes of chloroform and ethylacetate. The aqueous phase was buffered with Tris-buffer (pH 7.4) and incubated with B-glucuronidase / aryl sulfatase for 24 h in a shaking incubator. The chloroform/ethyl acetate extraction was repeated and re-extra-