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BILIARY EXCRETION OF STERIGMATOCYSTIN BY VERVET MONKEYS

MARTIN STEYN and PIETER G. THIEL*
National Research Institute for Nutritional Diseases, S.A. Medical Research Council, P.O. Box 70, Parowvallei 7503, Republic of South Africa

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Abstract—The major biliary metabolite of the mycotoxin sterigmatocystin was identified as sterigmatocystin glucuronide.

Sterigmatocystin, one of the lesser known mycotoxins, is produced by several fungal species[1]. In addition to being toxic to various experimental animals[2, 3], it also produces hepatocellular tumours after subcutaneous[4] and oral[5] administration. Recently the major urinary metabolite of sterigmatocystin was identified[6] in vervet monkeys (*Cercopithecus aethiops*). This paper reports the results of a parallel study designed to determine the nature and extent of biliary excretion of sterigmatocystin by vervet monkeys.

MATERIALS

Both ^{14}C-labelled and unlabelled sterigmatocystin were produced as described elsewhere[6, 7]. Amberlite XAD-2 and Sephadex stationary phases were obtained from British Drug Houses, London and Pharmacia Chemicals, Uppsala, Sweden, respectively. β-Glucuronidase and aryl sulfatase were purchased from Boehringer, Mannhein and Camag D-5 silica gel for t.l.c. supplied by Camag, Switzerland. Instagel was acquired from Packard Instruments, Switzerland, while [^{14}C]*n*-hexadecane was obtained from the Radiochemical Centre, Amersham. Pentobarbatone (Maybaker) was used for euthenasia. All chemicals used were of analytical reagent grade (Merck).

METHODS AND RESULTS

One male vervet monkey (5·43 kg) received 100 mg ^{14}C-labelled sterigmatocystin *per os* and was sacrificed 40 hr later by i.v. injection of enthatal. For this study the bile was quantitatively drained and washed from the excised gall bladder and stored at −18°. Two male vervet monkeys, (3·60 and 4·00 kg respectively) each equipped with an indwelling bile duct canula connected to a 25-ml latex reservoir, were given a daily oral dose of 10 mg/kg sterigmatocystin for 10 days. Subsequently they were sacrificed as before and their bile was pooled with that obtained from the monkey which received the labelled toxin. The combined bile (8·7 ml) was diluted with distilled water and equilibrated with an equal volume of chloroform (250 ml). Radioactivity was monitored on both phases in a Beckman Liquid Scintillation System. Quenching was corrected for by using an internal quantitative standard of [^{14}C]*n*-hexadecane.

Thirty percent of the radioactivity was found in the chloroform extract and identified as native sterigmatocystin as described under thin layer chromatography. The aqueous phase was lyophylised, dissolved in 10 ml distilled water, and quantitatively transferred to an XAD-2 column (2·6 × 40 cm), which selectively adsorbs conjugates[8]. After washing the column with 5 l. distilled water, the conjugates were eluted with 500 ml methanol. The eluate contained > 95% of the radioactivity applied to the column (Fig. 1). The methanol fraction was evaporated to dryness under reduced pressure in a rotary evaporator at 50° and applied to a DEAE–Sephadex column (1·6 × 90 cm) equilibrated with 0·01 M sodium phosphate buffer (pH 7·0) at 7°. The column was eluted with a linear salt gradient 500 ml 0·01 M sodium phosphate buffer (pH 7·0) +500 ml 0·01 M sodium phosphate buffer (pH 7·0) containing 0·8 M sodium chloride, at a flow rate of 30 ml/hr. The main radioactive peak,

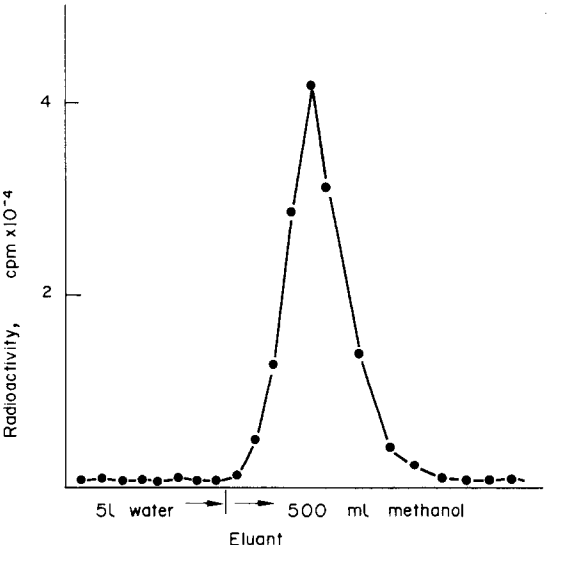

Fig. 1. Elution diagram of radioactive component(s) from XAD-2 column.

* Present address: National Food Research Institute, Council for Scientific and Industrial Research, P.O. Box 395, Pretoria 0001, Republic of South Africa.

concentrated in fractions 164–193 and containing 89% of the radioactivity applied to the column, was freeze-dried, dissolved in 25 ml distilled water and desalinated on a Sephadex G 10 column (2·6 × 80 cm), equilibrated and eluted with distilled water at a flow rate of 50 ml/hr at 7°. The total amount of radioactivity recovered from fractions 85–110 accounted for >94% of that applied to the column. These fractions were pooled, freeze-dried, dissolved in and diluted with distilled water to 5 ml in a volumetric flask.

THIN LAYER CHROMATOGRAPHY

Aliquots of the purified biliary metabolite were co-chromatographed with the major urinary sterigmatocystin metabolite on 0·5 mm t.l.c. plates coated with Camag D-5 silica gel and developed in chloroform-*i*-propanol–10 *N* ammonium hydroxide–methanol (40:40:8:20). When viewed under 360 nm ultraviolet light, a light blue fluorescent spot was present in the purified bile extract. The spot appeared to be similar to the major urinary metabolite, which ran at an R_f value of *ca* 0·3.

By subjecting 2 ml of the purified bile extract to β-glucuronidase hydrolysis[9], the blue fluorescent spot disappeared and native sterigmatocystin could be detected in the chloroform extract of the hydrolysis mixture (Fig. 2). Incubation of the purified bile extract with aryl sulfatase produced no detectable change in the amount and nature of the radioactive metabolite. By scanning the chromatograms (before and after hydrolysis) on a Bertholdt t.l.c. radioactivity scanner employing a dot printer, the only detectable radioactivity on either chromatogram was localised in the blue fluorescent spot before hydrolysis (sterigmatocystin glucuronide) and in the liberated sterigmatocystin after hydrolysis. The authenticity of the liberated sterigmatocystin was confirmed by t.l.c. in various solvent systems, u.v. spectroscopy and derivative formation[10].

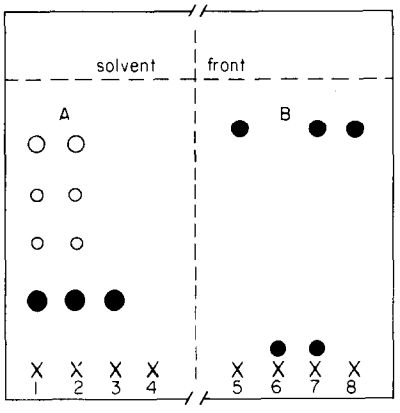

Fig. 2. T.l.c. chromatograms of sterigmatocystin and its biliary metabolite. (a) developed in chloroform–*i*-propanol–10 *N* ammonium hydroxide–methanol (40:40:8:20). (b) developed in carbon tetrachloride–acetone (90:10). ● Radioactive zones; ○ non-radioactive contaminants. × points of sample application. A1: sterigmatocystin glucuronide (ex urine), A2: sterigmatocystin glucuronide plus purified bile extract, A3: purified bile extract before β-glucuronidase treatment, A4: purified bile extract after β-glucuronidase treatment, A5: [^{14}C]sterigmatocystin, A6: purified bile extract before β-glucuronidase treatment, A7: purified bile extract plus [^{14}C]sterigmatocystin, A8: purified bile extract after β-glucuronidase treatment.

DISCUSSION

From the experimental results it is evident that sterigmatocystin is excreted via the bile and urine of vervet monkeys as the same compound, namely sterigmatocystin glucuronide. No evidence was found to suggest that any other conjugates (e.g. sulphates) of sterigmatocystin were present in the bile.

Depending on the vehicle used in administrating sterigmatocystin to vervet monkeys, between 50 and 80% of the sterigmatocystin is recovered in the faeces within 48 hr[11]. In this experiment the toxin was mixed with marshmallow toffee and fed to the animals. This method allows a maximum of 30% absorption[11]. The low absorption of sterigmatocystin is to be expected when viewed against its poor solubility in aqueous buffer solutions[12].

It seems as though the metabolism of sterigmatocystin in vervet monkeys is fairly uncomplicated. The major portion of the dose (*ca* 70%) is excreted unchanged via the faeces. More than 50% of the absorbed toxin appears in the urine as the glucuronide. Approximately 15% of the absorbed dose is excreted via the bile, also as the glucuronide. This leaves *ca* 10% unaccounted for, which may well be the margin of experimental error and losses, especially since three column chromatographic purification steps were employed.

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