Biochemical Pharmacology, Vol. 16, pp. 2405–2411. Pergamon Press Ltd. 1967. Printed in Great Britain

A COMPARATIVE STUDY OF GRISEOFULVIN-^{14}C METABOLISM IN THE RAT AND RABBIT*

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(*Received* 12 *April* 1967; *accepted* 8 *June* 1967)

Abstract—The excretion and metabolic pathways of griseofulvin-^{14}C were compared in the rat and rabbit with emphasis on biliary excretion and enterohepatic circulation. It has been shown that, in the rat, griseofulvin metabolism is characterized by extensive biliary excretion and appreciable enterohepatic circulation. In the rabbit, biliary excretion represented a minor pathway and the enterohepatic circulation could not be demonstrated. In the biliary cannulated rat during a 24-hr period, about 77 per cent of the dose (i.v.) was found in the bile and 12 per cent in urine, whereas in the rabbit only 11 per cent was observed in bile and 78 per cent in urine. The major metabolite in rat bile was the 4-demethylgriseofulvin with small amounts of the 6-demethyl derivative present. In rabbit bile, however, the major metabolite was the 6-demethylgriseofulvin with very small amounts of the 4-demethyl derivative present. In addition, small percentages of free griseofulvin and unidentified metabolite(s) were observed in the bile of both species. In urine of the intact and cannulated rats, two major metabolites were present, the 4- and the 6-demethyl derivatives of griseofulvin. Under similar conditions, rabbit urine contained 6-demethylgriseofulvin as the predominant metabolite.

GRISEOFULVIN† is an orally effective antifungal agent used widely in the treatment of fungal infections in animals and man. Studies on the urine of treated animals have demonstrated extensive metabolism of this drug, with only trace amounts of free griseofulvin present.[1–6] A single metabolite of griseofulvin, the 6-demethyl derivative, was demonstrated in rabbit and human urine.[2, 3, 7] Tomomatsu and Kitamura[8] have shown a breakdown product of griseofulvin, 3-chloro-4,6-dimethoxysalicylic acid, also to be present in rabbit urine. In the rat, two major demethylated metabolites of griseolfulvin have been demonstrated *in vivo*[1] and *in vitro*.[6] In addition, an extensive biliary excretion was suggested in a previous study.[1]

The purpose of this investigation was to compare the excretion and metabolic pathways of griseofulvin in the rat and rabbit. Special emphasis was placed on the biliary excretion and enterohepatic circulation and their relationship to griseofulvin metabolism in both species.

EXPERIMENTAL

Male albino, Charles River CD rats, weighing approximately 300 g, were

* A preliminary report was presented at the Fiftieth Meeting (1966) of the Federation of American Societies for Experimental Biology.

† This drug is formulated and distributed by Schering Corporation under the trade name of Fulvicin U.F.

anesthetized with sodium pentobarbital* (50 mg/kg, s.c.) and the bile duct was cannulated with polyethylene tubing (Intramedic PE 10). Biosynthetically prepared[6] griseofulvin-^{14}C (sp. act., 95·8 μc/m-mole) was administered in *N,N*-dimethyl formamide (0·2 ml) at a dosage of 7·5 mg/kg via the femoral vein. The rats were then placed in restraining cages and glucose solution was made available *ad libitum*.

Male albino New Zealand rabbits weighing approximately 2 kg were treated in a similar fashion. After anesthesia with sodium pentobarbital (about 40 mg/kg, i.v.), the bile duct was cannulated and the bladder was catheterized through the urethra with polyethylene tubing (Intramedic PE 50). Griseofulvin-^{14}C (7·5 mg/kg in 0·5 ml) was injected through the marginal ear vein. The rabbits were then placed in restraining racks and small amounts of glucose solution were given orally to maintain fluid balance.

Bile and urine samples were collected at specified time intervals and kept frozen until analysis. Total excretion of radioactivity was determined by counting 0·1 ml of urine and bile samples in a Packard liquid scintillation spectrometer. Corrections for quenching were made with an internal standard.

Aliquots of bile and urine containing, whenever possible, 10,000 c/min were acidified to pH 1 with 1 N HCl and extracted with ether. To determine if conjugates of the drug or its metabolites were present, a duplicate aliquot of the urine and bile was adjusted to pH 5 (acetate buffer) and incubated with Glusulase.† After incubation, the samples were acidified to pH 1 and extracted with ether. All ether extracts and the remaining aqueous residues were analyzed in a Packard liquid scintillation spectrometer. The difference in the amount of radioactivity present in ether extracts of Glusulase-treated and untreated samples was assumed to represent the amount present in conjugated form (as glucuronides or sulfates or both).

Ascending paper chromatography[6] was used to study the metabolites of the drug present in the ether extracts of bile and urine samples. The chromatographic strips were scanned for radioactivity with a Nuclear-Chicago (Actigraph III) automatic strip counter equipped with a digital integrator. The positions on the paper strip of cochromatographed authentic griseofulvin and some of its derivatives were located under u.v. light.

RESULTS

Biliary and urinary excretion of radioactivity in the rat and rabbit

Fig. 1 shows the excretion pattern of radioactivity over a 24-hr period after injection of griseofulvin-^{14}C (7·5 mg/kg, i.v.) into cannulated animals. About 90 per cent of the injected dose was recovered during this time interval. The rat excreted 77 per cent of the dose via the bile and 12 per cent in the urine; the rabbit excreted only 11 per cent via the bile and 78 per cent in the urine.

Most of the biliary radioactivity was excreted during the first 2-hr period in both species (Fig. 2). Very little radioactivity was excreted in rabbit bile after 2 hr, but in the rat appreciable levels were still present during the 8 to 24-hr period. It is also evident in Fig. 2 that the radioactivity present in the bile of both species existed to a

* Commercial preparation obtained from Richlyn Laboratories.

† Obtained from Endo Labs; each ml contained 100,000 units of glucuronidase and 50,000 units of sulfatase.

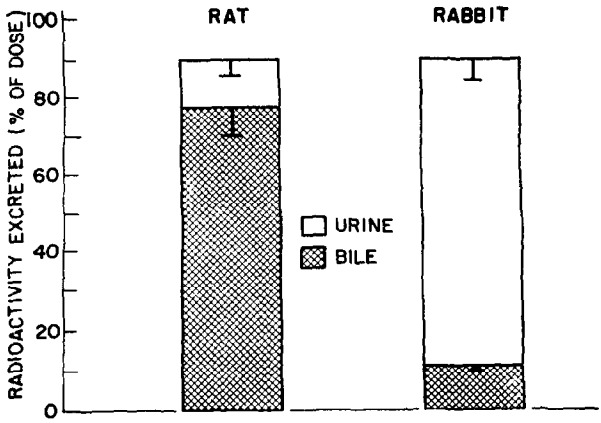

FIG. 1. Excretion of radioactivity in urine and bile over a 24-hr period after i.v. administration of griseofulvin-^{14}C. Results show mean values ± S.E. for three animals.

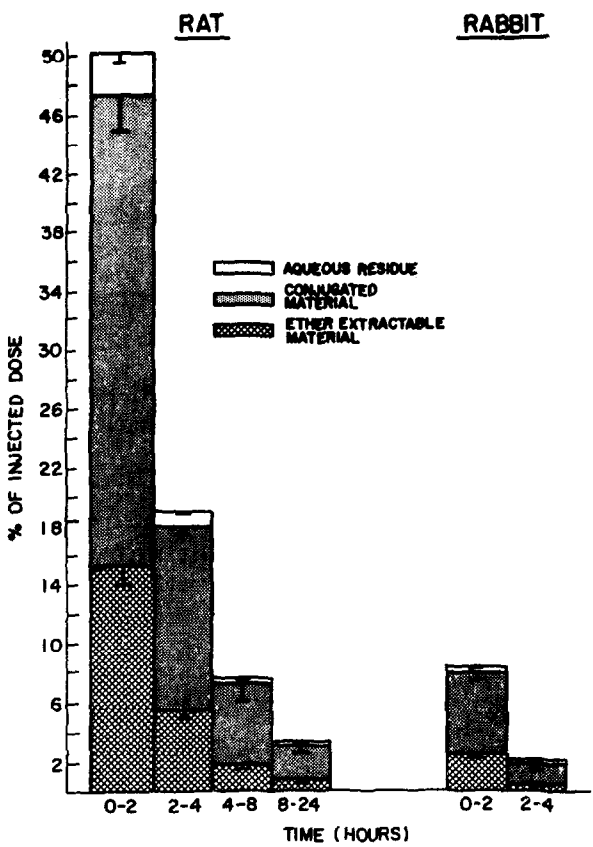

FIG. 2. The excretion and metabolic pattern of radioactivity recovered from rat and rabbit bile after i.v. administration of griseofulvin-^{14}C. Each value represents the mean ± S.E. of three animals.

large degree in conjugated form. Small amounts of radioactivity not extractable with ether (aqueous residue) were present in the bile even after Glusulase hydrolysis.

Metabolic pattern of griseofulvin in bile

A comparison of the chromatographic pattern of ether extractable radioactivity in bile samples (0–2 hr) from the rat and rabbit is presented in Fig. 3. Spots marked 1, 2, and 3 at the bottom, correspond to the position on the chromatographic paper

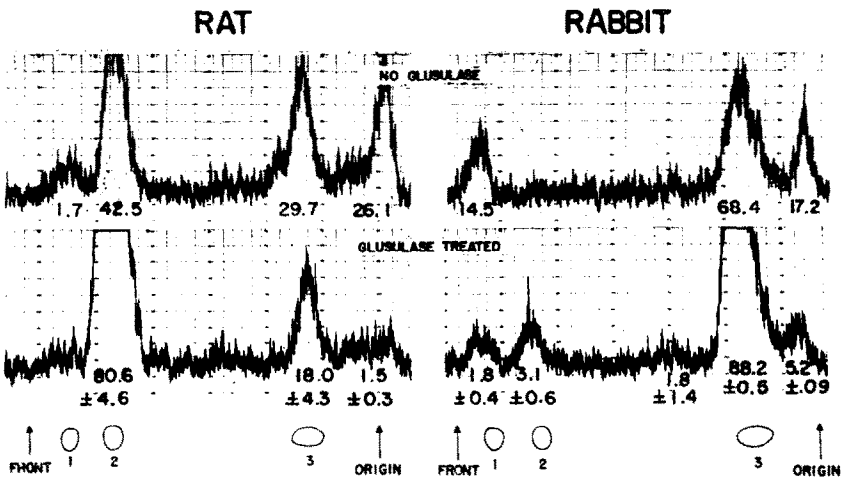

FIG. 3. Metabolic fate of griseofulvin-^{14}C in bile (0–2 hr). Zones marked 1, 2, and 3 on the paper strip correspond to the position of authentic griseofulvin, 4-demethylgriseofulvin, and 6-demethylgriseofulvin respectively. The numbers (± S.E.) show the percentage of total radioactivity on the paper strip associated with each peak.

strip of authentic griseofulvin (R_f 0·89), 4-demethylgriseofulvin (R_f 0·78), and 6-demethylgriseofulvin (R_f 0·21) respectively. The upper portion of this figure (no Glusulase) shows the metabolic pattern of bile samples prior to hydrolysis and represents about 30 per cent of the radioactivity found during 0–2 hr. It serves merely to illustrate that these samples contain appreciable amounts of nonconjugated metabolites of griseofulvin.

The lower portion (Fig. 3) represents the metabolic pattern found in ether extracts of Glusulase-treated samples. These samples represent about 95 per cent of the radioactivity present in the bile at this time period. At least two metabolites of griseofulvin were observed (Fig. 3) in rat bile, with the major radioactive component corresponding chromatographically to 4-demethylgriseofulvin (81 per cent) and a minor component (18 per cent) corresponding to 6-demethylgriseofulvin. In the rabbit, the relative ratio of the metabolites was reversed, with 6-demethylgriseofulvin comprising the largest peak of radioactivity (88 per cent) and small amounts (3·1 per cent) of 4-demethylgriseofulvin visible only after glusulase hydrolysis. Free griseofulvin appeared in rabbit bile to the extent of about 1·8 per cent of total radioactivity and was present in even smaller amounts in rat bile. Several small unidentified peaks (particularly at the origin) were also observed on the radioscan (Fig. 3).

While comparing the biliary metabolic patterns of the rat and rabbit (Fig. 3), one should remember that the total amount of radioactivity excreted in the bile

differs markedly in the two species. Thus, the data presented for the bile (0 to 2-hr period) represent respectively about 50 per cent and 8 per cent of the injected dose in the rat and the rabbit (Fig. 2).

Enterohepatic circulation

The data presented thus far have shown that, although extensive biliary excretion is associated with the metabolic disposition of griseofulvin in the rat, only a small degree of biliary involvement is found in the rabbit. In order to establish the existence of an enterohepatic circulation in both species, urinary excretion and metabolism were compared in intact and biliary cannulated animals after i.v. drug administration.

The results in the rat are presented in Fig. 4. The data to the left (part A) show clearly that the total urinary radioactivity excreted over a 24-hr period was about

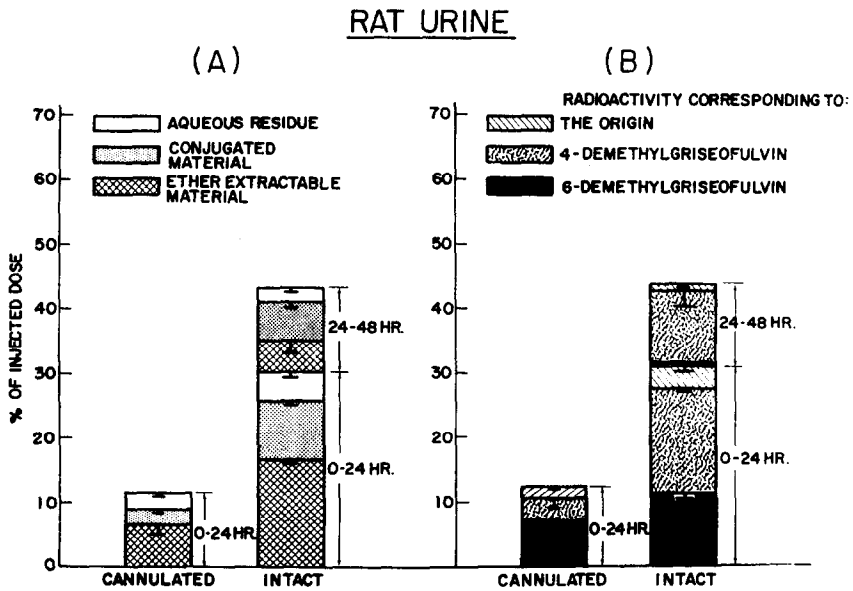

FIG. 4. Comparative studies of urine from intact and cannulated rats after i.v. administration of griseofulvin-^{14}C. Part A represents the excretion and metabolic pattern of radioactivity. Part B shows the metabolic fate of griseofulvin-^{14}C as depicted by chromatography of ether extracts of urine after treatment with Glusulase. Mean values ± S.E. are given for at least three animals.

2·5 times higher in intact than in cannulated rats. An additional 13 per cent of the injected dose was recovered from urine of the intact rat during the 24 to 48-hr period. (The collection period in the cannulated rat was limited to 24 hr, since it resulted in 90 per cent recovery of total radioactivity, Fig. 1.) It is also shown in part A of Fig. 4 that the amount of conjugated material was higher in intact than in cannulated rats.

The chromatographic analysis (Fig. 4, part B) revealed that qualitatively the metabolites in urine were similar in intact and cannulated rats, but that they differed in quantity and relative amounts. In the cannulated rat, the 6-demethyl derivative of griseofulvin exceeded the 4-demethyl derivative by a factor of almost 2. In the intact rat, however, during the same 24-hr period, this factor was 1·5 in favor of the 4-demethyl derivative, which became predominant during the 24 to 48-hr period.

In the rabbit, the total amount of radioactivity present in urine over a 24-hr period was practically identical in intact and cannulated animals, and the distribution of radioactivity into a free, conjugated, and residual form was also quite similar (Fig. 5).

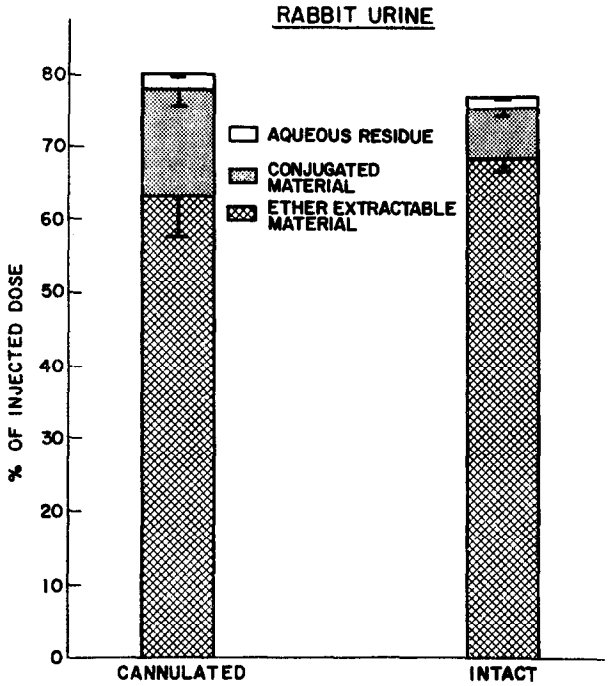

FIG. 5. Comparison of excretion and metabolic pattern of radioactivity in urine of normal and cannulated rabbits over a 24-hr period after i.v. injection of griseofulvin-^{14}C. Mean values ± S.E. for three animals are presented.

Ether extracts of urine, after Glusulase hydrolysis, showed upon chromatography a single radioactive component for the intact and cannulated rabbit (not illustrated). This radioactivity corresponded in R_f to that of authentic 6-demethylgriseofulvin and represented over 95 per cent of total activity present on the paper strip.

DISCUSSION

The data presented in this investigation have demonstrated that the bile represents a major pathway of excretion for griseofulvin metabolites in the rat, but only a minor pathway in the rabbit.

Evidence for an enterohepatic circulation in the rat was provided by comparing the urinary excretion and metabolic patterns of griseofulvin in intact and cannulated animals. Urinary excretion of radioactivity was greater in the intact rat and the composition of metabolites was, to a large extent, a reflection of that found in the bile. Thus, in the urine of the intact rat we found more 4-demethylgriseofulvin (the primary biliary metabolite) and larger amounts of conjugated metabolites.

In the intact rabbit, however, the urinary excretion of radioactivity and its metabolic pattern were very similar to those found in the cannulated animal. This indicates that in the rabbit the enterohepatic circulation, if present, is not extensive.

Closely associated with the route of drug excretion were its metabolites. Thus in the rat, where the biliary excretion was extensive, 4-demethylgriseofulvin was the major metabolite. In the rabbit, where biliary excretion was relatively low, the major metabolite was 6-demethylgriseofulvin.

It should also be mentioned that bile samples from rat and rabbit contained small quantities of unidentified metabolites in addition to small amounts of free griseofulvin.

It has been suggested[9] that unless a compound can form a conjugate it may not appear in large quantities in the bile. Indeed, we have seen that the 4-demethylgriseofulvin, which was present in such large amounts in rat bile, was extensively conjugated. This same metabolite, although present in a small quantity in rabbit bile, also appeared in conjugated form. On the other hand, 6-demethylgriseofulvin, which appeared in bile in relatively small quantities, was excreted in urine largely in free form.

Kaplan *et al.*[7] could not demonstrate a glucuronide conjugate of 6-demethylgriseofulvin under *in vitro* conditions, and postulated its p*Ka* value to be responsible for its inability to form conjugates (sulfates and glucuronides). The results reported in rat[1, 6] and in man,[2] as well as the data presented here (rabbit and rat), would tend to confirm that 6-demethylation of griseofulvin limits, but does not completely prevent, conjugation. This limited ability to conjugate may explain the relatively small amounts of this metabolite appearing in bile.[9]

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