Griseofulvin-Induced Hepatopathy
Due to Abnormalities in Heme Pathway

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ABSTRACT. 1. The effect of long-term griseofulvin (GRIS) topical administration on some indicators of liver damage was examined.
2. Liver porphyrin accumulation was significant; however, no porphyrin crystals were observed under light microscopy.
3. An earlier onset of hepatopathy was established (3-fold) increase of direct bilirubin values after 7 days of treatment; hepatic injury was confirmed by measuring a 6-fold increase of free bilirubin.
4. Enhanced values of alkaline phosphatase and glutamic oxalacetic transaminase (GOT) confirmed the onset of cholestasis.
5. Topical application of GRIS induced measurable hepatopathy. Nevertheless, we cannot discard the possibility that this hepatopathy could also be attributed in part to a direct reaction to xenobiotics.

KEY WORDS. Griseofulvin, hepatopathy, cholestasis, heme pathway

INTRODUCTION
Griseofulvin (GRIS) is an antifungal widely used for the oral treatment of skin fungal infections; however, it also exhibits a number of undesirable side effects. In laboratory animals, the action of GRIS is manifold, it is well known that it can induce protoporphyrina and hepatocarcinogenesis (De Matteis et al., 1966; Denk et al., 1981). De Matteis and Gibbs (1975) first demonstrated that, after administration of GRIS to mice, experimental protoporphyrina very quickly develops; this reaction was attributed to a rapid loss of ferrochelatase activity, the enzyme responsible for inserting iron into the protoporphyrin ring. This specific effect of heme biosynthesis characterizes GRIS as one of the few compounds capable of inducing porphyria in the normal liver of both humans and animals (De Matteis, 1978).

It also is recognized that protoporphyrina may be associated with significant hepatobiliary disease, including enlargement and darkening of the liver, liver failure, jaundice, cirrhosis, and histological evidence of porphyrinemia and cholestasis (De Matteis et al., 1966; Gschnait et al., 1975; Lefkowitch et al., 1983; MacDonald et al., 1981; Singer et al., 1978; Weston-Hurst and Fagot, 1963).

Experimental induction of protoporphyrina in laboratory animals fed GRIS or 3,5-dietoxycarbonyl-1,4-dihydocollidine has clarified the nature of the liver injury associated with the deposition of hepatic protoporphyrin crystals (Gschnait et al., 1975; Lefkowitch et al., 1983).

We have developed an alternative model to induce murine experimental protoporphyrina by topical application of GRIS; we also have proposed the most likely mechanisms that would explain the porphyrinogenic action of GRIS under these conditions (Navone et al., 1991; Vazquez et al., 1987).

We have investigated the effect of long-term GRIS administration on some indicators of liver damage to establish whether or not some kind of hepatopathy develops by the topical use of the antifungal, as it occurs in feeding models.

With this aim, blood and gallbladder bilirubin levels, as well as plasma activity of alkaline phosphatase and glutamic oxalacetic transaminase (GOT), were determined in both controls and GRIS-treated animals.

MATERIALS AND METHODS

Animals
CF I male mice housed in controlled-climate conditions with free access to normal laboratory animal food (Purina 3, Asociación de Cooperativas Argentinas, San Nicolás, Buenos Aires, Argentina) and water were used. All animals received humane care as outlined in the Guide for the Care and Use of Laboratory Animals.

Experimental design and obetion of samples
Animals received GRIS dissolved in olive oil (500 mg GRIS/10 ml oil) using a cotton sprinkler for topical application on the back skin in a standardized amount, every 48 hr for a whole period of 52 days and were sacrificed at different times during the intoxication. Control animals were treated only with olive oil. Treatments were made at the same time of day. The mice previously heparinized were killed under ether anesthesia by cardiac puncture and bled. Whole blood was centrifuged at 600 g during 15 min and the plasma was separated. The liver and gallbladder were removed after ice-cold saline perfusion and immediately processed. Liver homogenates were prepared, homogenizing the whole organ in 0.25 M ice-cold sucrose for 30 to 45 s in an Ultraturrax Homogenizer (Janke and Kunkel, Stau-
fen, Germany). The gallbladder was homogenized in 0.5 ml saline using a micropotter.

**Assays**

Hepatic endogenous porphyrins were measured fluorometrically in the whole homogenate, according to the method of Polo et al. (1988). Plasma and gallbladder bilirubin, plasma alkaline phosphatase and GOT levels were measured with specific commercial kits (Wiener Lab, Rosario, Argentina).

**Statistical analysis**

The Newman-Keuls test was used to assess the degree of significance. A probability level of 0.01 to 0.05 was used in testing for significant differences between controls and treated animals.

**RESULTS**

**Clinical aspects**

GRIS treatment was well tolerated, and mortality was approximately the same as among untreated mice. Progressive differences between the livers of experimental and control mice became grossly apparent. The livers of the control mice showed reddish-brown pigmentation in contrast to the livers of the GRIS-treated animals, which looked light brown. Liver enlargement was the most prominent feature; liver weight in GRIS-treated animals rose to 10% (P<0.05) of the body weight at day 52, revealing a discrete hepato-megalia, without any variation in the animal’s body weight (Fig. 1).

**Biochemical findings**

No significant variations were detected in control animals in all the parameters analyzed during the whole period of GRIS intoxication.

**HEPATIC ENDOGENOUS PORPHYRINS.** We have previously reported that no significant changes in liver porphyrin content were observed in short-term (up to 24 hours) treatment (Polo et al., 1988). However, we have observed here that porphyrin accumulation was increased by 150% (P<0.01) after day 24 and that porphyrin levels stayed high, reaching 310% (P<0.01) at day 52 (Fig. 2).

**PLASMA AND GALLBLADDER BILIRUBIN.** Conjugate plasma bilirubin (Fig. 3A) steadily increased reaching 750% (P<0.01) of the basal values at day 52. Instead, free plasma bilirubin strongly increased up to 900% (P<0.01) at day 45, and then decreased to 240% (P<0.01) at day 52.

Gallbladder bilirubin (Fig. 3B) stayed within basal levels up to day 45 and then decreased at day 52 because of the dramatic diminution observed in the case of free bilirubin (to 90%, P<0.01).

**LIVER INJURY MARKER ENZYMES.** Profiles of alkaline phosphatase and GOT activities were rather similar. In both cases there was an initial increase between days 3 and 5, and from then onward it remained at this level (200%, P<0.01 and 1,000%, P<0.01, respectively), followed by a sharp and sustained enhancement from around day 30 to day 52 (900%, P<0.01 and 2,000%, P<0.01, respectively) (Fig. 4).

**DISCUSSION**

It has become clear that the pathogenesis of liver injury in erythropoietic protoporphyria cannot be only and primarily attributed to crystalline deposits of protoporphyrin within hepatocytes and bile canaliculi, causing cholestasis and secondary hepatocellular damage. Although this mechanism will nevertheless contribute to liver damage, it will occur at later stages of the disease, when ultrastructural hepatocellular abnormalities already are present (Rademakers et al., 1990). Furthermore, it has been shown that soluble protoporphyrin can elicit exactly the same liver injury (Avner, 1982a; Avner et al., 1981).

Protoporphyrin accumulates in all hepatocellular compartments. However, the intracellular mechanisms of its transport have not yet been elucidated (Bloomer, 1988). The rate-limiting step of the over-
all transport from plasma to bile is thought to be the canalicular excretion of protoporphyrin (Avner et al., 1982a).

A ferrochelatase defect may, at first, cause accumulation of protoporphyrin and iron in the mitochondria and later in other organelles and in the hepatocyte cytosol (Koningsberger, 1992; MacDonald et al., 1981; Navone et al., 1991; Rademakers et al., 1990), where it might provoke further oxidative injury, acting in concert with accumulated extrahepatic protoporphyrin.

Heme biodegradation will initially be downregulated by low free heme, which reduces heme oxygenase activity (Schacter, 1988). However, accumulation of heavy metals such as copper and iron, can induce heme oxygenase (Ibraham et al., 1983; Schacter, 1988). Induction of heme oxygenase by iron can even be enhanced by concomitant induction of cytochrome P-450 (Bonkowski, 1991). Cholestasis also can induce heme oxygenase, which will then metabolize heme and thus further deplete the regulatory free heme pool (Schacter and Firneisz, 1983).

The ultrastructural demonstration of the presence of vacuoles containing a lipid-like substance in erythropoietic protoporphyria hepatocytes (MacDonald et al., 1981; Rademakers et al., 1990), is a proof of altered lipid metabolism. Because cholesterol 7α-hydroxylase, the rate-limiting enzyme in bile acid biosynthesis, is a cytochrome P-450-dependent monoxygenase (Vlahcevic et al., 1991), the formation of primary bile acids from cholesterol may be inhibited. This in turn can decrease the bile flow and the solubilizing capacity of bile salts for hydrophobic substances, like protoporphyrin, thereby increasing the lithogenicity of bile (Vlahcevic et al., 1991). Consequently, the bile acid-dependent excretion of protoporphyrin will further be disabled (Avner and Berenson, 1982b). Crystalline protoporphyrin deposits can obstruct bile canaliculi, causing cholestasis (Morton et al., 1988).

The incompetent excretory function of the hepatocyte may therefore be secondary to the metabolic disorder, and not the primary cause of hepatocellular injury (Avner et al., 1983; Koller and Romslo, 1980).

Cholestasis due to biliary obstruction induces heme oxygenase, which suggests bile acid involvement in the regulation of free heme (Schacter and Firneisz, 1983).

Plasma alkaline phosphatase increased activity may be the result of an early manifestation of primary biliary cirrhosis or drug-induced cholestasis. It is well known that high conjugate bilirubin level is a sensitive marker of hidden hepatic injury and that total plasma bilirubin is enhanced in intrahepatic biliary cholestasis, hepatocellular damage, and biliary flow posthepatic obstruction.

Cantoni et al. (1983) reported that administration of 2.5% GRIS in the diet produced protoporphyria and cholestasis. Protoporphyrin became evident as early as after 10 days of treatment, whereas cholestasis developed only 45 days after ingestion of GRIS.

In conclusion, our alternative model to produce murine experimental protoporphyria by topical application of GRIS has been shown to be as effective as the feeding model to trigger a cholestatic status. An earlier onset of the hepatopathy was assessed by the enhancement of direct bilirubin; and hepatic injury was confirmed by free bilirubin increase associated to progressive liver damage. Increased levels of alkaline phosphatase and GOT activities corroborated the onset of cholestasis.

Although endogenous hepatic porphyrin accumulation was significant, no porphyrin crystals were observed under light microscopy (data not shown). Even though porphyrin deposits at levels less than those needed to yield crystals could still have adverse effects on cholestatic mechanisms, the possibility that the hepato- pathy observed after GRIS treatment could be due to a hepatic reac-
tion to xenobiotics, as occurs with other drugs known to provoke a cholestatic syndrome, cannot be discarded.

The support of the Argentine National Research Council (CONICET), the University of Buenos Aires, and of the Spanish Ministry of Education and Science is gratefully acknowledged. We are indebted to Lic. E. Gerez for the preparation of the liver samples used in microscopic studies and to Dr. E. Falzoni for the histological analysis. We are also grateful to Mrs. Beatriz Corvalán for her skilled technical assistance.

References


