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Evaluation of the Hepatotoxic Potential of Minocycline

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Minocycline (25 to 100 μg/g) dose dependently increased serum glutamic oxalacetic transaminase, urea, and bilirubin levels, and the hepatic triglyceride content in mice. In animals pretreated with phenobarbital to enhance minocycline metabolism, the effects on liver triglycerides were attenuated, while the changes in serum glutamic oxalacetic transaminase, urea, and bilirubin were enhanced. It is concluded that part of the toxic effects of minocycline may be produced by a metabolite of minocycline.

Liver damage has frequently been observed after application of excessive doses of the older tetracyclines (for reviews, see references 2 and 3). But unlike tetracycline and doxycycline, until now the hepatotoxicity of minocycline has not been studied systematically. We therefore decided to make a quantitative evaluation of the hepatotoxic potential of minocycline in experimental animals. The study was designed to be similar to earlier studies from this laboratory with tetracycline and doxycycline (2, 3). Thus, a comparison of minocycline with tetracycline and doxycycline should be feasible.

MATERIALS AND METHODS

The experiments were performed on female NMRI mice, weighing 23 to 27 g and kept under standard laboratory conditions (plastic cages with softwood bedding; room temperature, 25°C; 12/12-h dark/light rhythm). They were fed ad libitum with standard diet (Herilan) and tap water.

The animals were treated intravenously with minocycline (25, 50, or 100 μg/g) dissolved in 1.5 mM $MgSO_4$ solution. The control animals received the same volume of the vehicle. Treatment was always performed between 7:30 and 8:30 a.m. Two, 4, 8, or 24 h later, some of the animals were killed by decapitation and exsanguinated. Their blood was collected for the determination of levels of serum glutamic oxaloacetic transaminase (SGOT), urea, and bilirubin. A sample of liver tissue was rapidly excised and frozen in liquid nitrogen. The frozen tissue was weighed, homogenized in saline, and used for the determination of the hepatic triglyceride content. Another sample of liver tissue was obtained for histopathological examination by light microscopy.

In some experiments, the animals were pretreated with phenobarbital (35 μg/g twice daily for 3 days) to induce microsomal enzymes. On day 4, these animals were injected with 50 μg of minocycline per g or saline. They were killed and handled further as described above.

SGOT was measured by the method of Bergmeyer and Bernt (1), serum urea was measured by the method of Fawcett and Scott (6), and bilirubin was measured by the method of Jendrassik and Grof (7). The liver triglycerides were determined enzymatically by the method of Wahlefeld (11).

Minocycline did not interfere with any of the laboratory assays.

Mean values ± standard errors of the mean (SEM) were calculated from 10 single determinations. Differences between mean values of minocycline-treated and control animals were checked by Student's t test and regarded as significant if $P \leq 0.05$.

Because the study covered a period of several weeks and because samples had to be taken at different hours of the day depending on the length of drug action required, some minor variations in the control values (e.g., because of diurnal variations) were unavoidable. Therefore, for clearer presentation of the data in the tables, the deviations of the mean values of the treated animals from their corresponding

TABLE 1. Effects of minocycline on SGOT

Minocycline dose (μg/g)	Change[a] (U/liter) at the following time after administration (h):			
	2	4	8	24
25	+2.1 ± 5.9	−0.7 ± 5.3	+11.5 ± 4.1	+7.3 ± 10.5
50	+39.5 ± 8.1[b]	+12.0 ± 7.9	+5.6 ± 11.1	+6.3 ± 2.1
100	+63.2 ± 14.8[b]	+118.5 ± 10.0[b]	+54.6 ± 16.8[b]	+21.9 ± 5.1[b]
50 (Ph)[c]	+46.3 ± 7.4[b]	+46.0 ± 7.9[b,d]	+23.6 ± 8.8[b,d]	+27.6 ± 3.9[b,d]

[a] Deviations (means values ± SEM) from control value (98.5 ± 8.1 U/liter).
[b] P versus corresponding control, ≥0.05.
[c] Ph, animals pretreated with phenobarbital.
[d] P versus animals not treated with phenobarbital, ≤0.05.

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TABLE 2. Effect of minocycline on serum bilirubin

Minocycline dose (μg/g)	Change[a] (mg/100 ml) at the following time after administration (h):			
	2	4	8	24
Total bilirubin				
25	+0.05 ± 0.02	−0.01 ± 0.02	−0.11 ± 0.03[b]	−0.03 ± 0.03
50	+0.14 ± 0.03[b]	+0.04 ± 0.02	−0.05 ± 0.02	−0.03 ± 0.02
100	+0.52 ± 0.03[b]	+0.27 ± 0.05[b]	−0.07 ± 0.02	−0.14 ± 0.02[b]
50 Ph[c]	+0.28 ± 0.07[b]	+0.03 ± 0.03	−0.06 ± 0.05	−0.02 ± 0.03
Unconjugated bilirubin				
25	+0.03 ± 0.02	−0.03 ± 0.02	−0.12 ± 0.02[b]	−0.04 ± 0.03
50	+0.01 ± 0.02	−0.03 ± 0.03	+0.02 ± 0.02	−0.02 ± 0.03
100	+0.48 ± 0.04[b]	+0.16 ± 0.03[b]	−0.07 ± 0.03	−0.18 ± 0.02[b]
50 Ph[c]	+0.20 ± 0.06[b]	+0.01 ± 0.03	−0.05 ± 0.05	−0.06 ± 0.03

[a] Deviations (means values ± SEM) from control values (total bilirubin, 0.38 ± 0.03 mg/100 ml; conjugated bilirubin, 0.35 ± 0.04 mg/100 ml).
[b] *P* versus corresponding control, ≤0.05.
[c] Ph, animals pretreated with phenobarbital.

control values determined at the same time under identical conditions are given.

RESULTS

SGOT. Minocycline caused an increase in the SGOT levels which was only transient after 50 μg/g but was long lasting after 100 μg/g. A dose of 25 μg/g did not raise the SGOT. The increase in the SGOT was greater and lasted much longer in the phenobarbital-pretreated animals (Table 1).

Serum bilirubin. The bilirubin content of the serum was initially increased by minocycline doses of 50 and 100 μg/g. The changes in the total bilirubin content can be ascribed almost entirely to parallel changes in the serum levels of unconjugated bilirubin. The increases in total and unconjugated bilirubin were accentuated by pretreatment with phenobarbital (Table 2).

Serum urea. Minocycline dose dependently raised the serum urea level, with a maximum after 4 h. This effect was not influenced by phenobarbital (Table 3).

Liver triglycerides. The triglyceride content of the liver was increased by minocycline in a dose-dependent manner. Maximum values were found after 8 h. This effect was attenuated by pretreatment with phenobarbital (Table 4).

Histomorphology of the liver. Minocycline caused no gross morphological abnormalities.

DISCUSSION

Liver damage seems to be an uncommon side effect of minocycline therapy in humans (4). Nevertheless, our results show that minocycline at higher doses causes the same kind of changes in the parameters related to liver function as other tetracyclines, i.e., an increase in the SGOT, indicating a reversible change of cell membrane permeability, an increase in unconjugated and total bilirubin, probably caused by interference with glucuronidation and elimination of bilirubin, and an increase in serum urea, which may be caused by reduced renal excretion or more probably may result from an antianabolic effect of the tetracyclines. The latter effect is also supposed to be responsible for the hepatic steatosis resulting from inhibition of lipoprotein biosynthesis (2, 3).

If the order of magnitude of these effects is compared with the order of magnitude of the effects observed by us in earlier experiments with other tetracyclines (2, 3), minocycline more closely resembles tetracycline than doxycycline. That means that on a molar basis, its hepatotoxic potential is high. This is in line with observations by Redin (10) that the 50% lethal doses of minocycline and tetracycline are similar. In regard to the absolute doses required for the initiation of the hepatotoxic effects, minocycline, however, appears to be a safer drug, as significant effects occurred only after doses of 50 and 100 μg/g, which are far above those used in antimicrobial chemotherapy in humans.

TABLE 3. Effect of minocycline on serum urea

Minocycline dose (μg/g)	Change[a] (mg/100 ml) at the following time after administration (h):			
	2	4	8	24
25	+6.9 ± 2.0	+6.6 ± 1.3[b]	+6.3 ± 1.8	+2.8 ± 2.2
50	+4.7 ± 2.0	+12.5 ± 2.2[b]	+8.4 ± 2.1[b]	+0.6 ± 3.3
100	+7.0 ± 2.5	+39.8 ± 9.1[b]	+8.7 ± 2.2[b]	−0.7 ± 1.6
50 Ph[c]	+14.2 ± 1.8[b,d]	+9.7 ± 1.9[b]	+4.2 ± 2.2	−2.8 ± 1.8

[a] Deviations (means ± SEM) from control value (41.3 ± 2.0 mg/100 ml).
[b] *P* versus corresponding control, ≤0.05.
[c] Ph, animals pretreated with phenobarbital.
[d] *P* versus animals not treated with phenobarbital, ≤0.05.

TABLE 4. Effect of minocycline on liver triglycerides

Minocyclin dose (μg/g)	Change[a] (μg/g) at the following time after administration (h):			
	2	4	8	24
25	+11.0 ± 2.8[b]	+4.5 ± 1.9	+15.4 ± 2.0[b]	+1.6 ± 1.0
50	+9.7 ± 2.9[b]	+26.9 ± 6.4[b]	+34.8 ± 3.6[b]	+4.8 ± 2.0
100	+11.2 ± 5.2[b]	+30.1 ± 7.0[b]	+69.0 ± 9.3[b]	+51.9 ± 7.1[b]
50 Ph[c]	+5.7 ± 2.8	+13.7 ± 3.2[b,d]	+7.2 ± 3.4[d]	+5.3 ± 1.0

[a] Deviations (means ± SEM) from control value (20.4 ± 1.5 μg/g).
[b] *P* versus corresponding control, ≤0.05.
[c] Ph, animals pretreated with phenobarbital.
[d] *P* versus animals not treated with phenobarbital, ≤0.05.

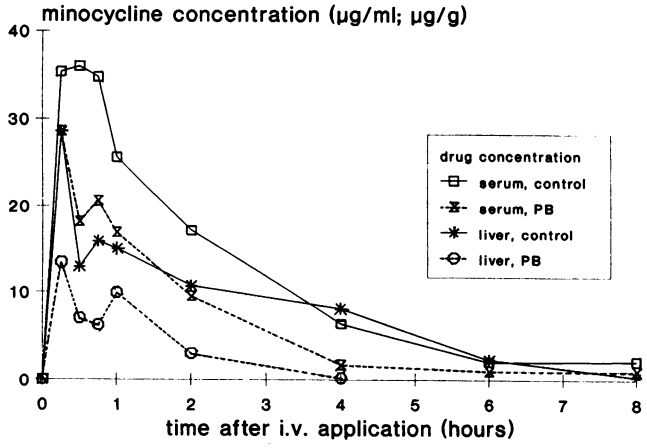

FIG. 1. Concentration of minocycline in blood and liver of mice after intravenous (i.v.) injection of 50 µg of minocycline per g. PB, animals pretreated with phenobarbital (see text). Data adopted from Ludewig-Sandig (8).

Pretreatment with phenobarbital at doses that proved to induce mixed function monooxigenases in our laboratory animals reduced the area under the curve values for minocycline in serum and liver, probably by enhancing its metabolization (Fig. 1) (8). In these animals, the accumulation of hepatic triglycerides was smaller, indicating that minocycline itself might be responsible for this effect. On the other hand, the changes in SGOT, bilirubin, and to some extent serum urea were more pronounced. This suggests that these effects are mediated by a metabolite of minocycline. The nature of this metabolite is presently unknown. DeLeenheer and Nelis (5) and Nelis and DeLeenheer (9) have detected hydroxylated and demethylated minocycline derivatives in urine samples from volunteers. After pretreatment with phenobarbital, the amounts of demethylated derivatives of minocycline were markedly increased in the serum and liver of mice (8). But since these metabolites could be isolated only in extremely small quantities (8), toxicity testing was not possible.

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