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Effect of taurine administration on liver lipids in guinea pig

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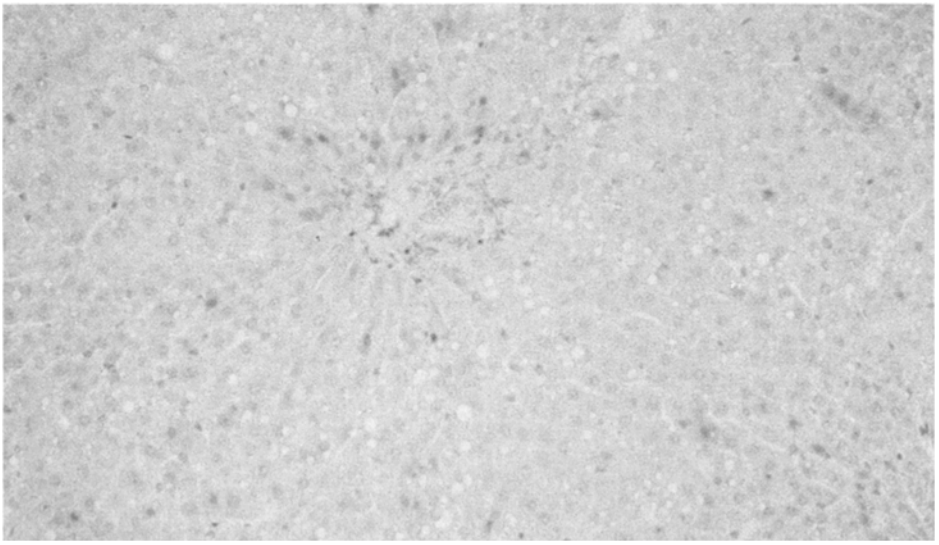
Summary. The oral administration of 0.4% taurine in drinking water for 14 consecutive days showed the following hepatic effects in male guinea pig. The percentage of tauro-conjugated biliary bile acids was increased from 17.2–54.2%; the ratio liver weight/body weight was increased, and fatty change was induced. Liver triglyceride concentration was accordingly increased; diglyceride and phosphatidylcholine concentrations were reduced by the treatment, while phosphatidylethanolamine level was not affected. These changes suggest an adverse effect of taurine administration on phosphatidylcholine hepatic synthesis.
Key words. Taurine; fatty liver; bile acids; lipid metabolism.

Taurine is a sulfur containing amino acid, whose only known physiological role in the liver is the conjugation of bile acids[1]. Its administration to man or to animals with a low biosynthetic capacity for taurine in the liver, e.g., guinea pig or rabbit, can reverse the conjugation pattern of biliary bile acids from glyco-conjugation to tauro-conjugation[2,3]. Beneficial effects have been attributed to taurine administration in the clinical course of acute and chronic hepatitis[4,5], in drug-induced liver diseases[6] and in cirrhosis[7]. More recently, taurine has been administered, at relatively high doses, to children less than two years old to prevent retinal disfunction occurring in the course of long-term parenteral nutrition without taurine[8]. Experimentally taurine feeding alleviated the effects of hepato-toxic agents like naphthylisocyanate[9], carbon tetrachloride[10], and sulfolithocholate[11]. In this last study, however, a 5-day treatment with 0.5% taurine in drinking water induced a mild lipid accumulation and dilatation of endoplasmic reticulum membranes in guinea pig liver as shown by electron microscopy. The growing interest in the clinical use of taurine in adults and children led us to extend this finding by administering a similar dose of taurine for a longer period to guinea pigs.

Materials and methods. Taurine (2-aminoethansulfonic acid) was obtained from E. Merck, Darmstadt, FRG. All chemicals used were of analytical grade. Male Hartley guinea pigs, about 380 g each (purchased from Charles River, Calco, Italy), were housed in metabolic cages. Food and water were available ad libitum. Taurine was administered with drinking water (0.4% solution) which was freely available for 14 consecutive days. On the fifteenth day the animals were sacrificed by decapitation. Liver and bile were collected from each animal at the end of the trial.

Liver lipid analysis. About 2 g of liver were homogenized and their lipids were quantitatively extracted with chloroform-methanol following Folch et al.[8]. The lipid extract was fractionated by thin-layer chromatography and analyzed by gas chromatography following a method derived by Christie et al.[12]. Briefly the extracts were fractionated by thin-layer chromatography on silica gel precoated plates with the following solvent systems; n-hexane-diethyl ether-acetic acid (70:30:1, by vol.) for neutral lipids, and chloroform-methanol-acetic acid-water (65:25:15:4 by vol.) for phospholipids. Bands corresponding to triglycerides (TG), diglycerides (DG), phosphatidylethanolamine (PE) and phosphatidylcholine (PC) were revealed by iodine vapor, scraped off the plate and analyzed by gas-liquid chromatography as previously described[13].

Biliary bile acid analysis. Bile obtained by gallbladder puncture



Fatty infiltration in the liver of guinea pig treated for 14 days with taurine. H and E stain, × 100.

was diluted with isopropanol (1:9, by vol.) and centrifuged at 5000 rpm for 10 min. The clear supernatant was analyzed by high performance liquid chromatography (HPLC) as previously described[14].

Histological examination. Portions of the liver were removed immediately after death, fixed in 10% buffered formalin and embedded in paraffin. Sections were cut at 5 µm intervals and stained with hematoxylin and eosin and Masson's trichrome stain methods. Small pieces of fresh liver were immediately frozen at 20 °C and histochemical investigations on cryostat sections were performed using periodic acid-Schiff (PAS), oil red O and Sudan III.

Results. Table 1 shows the taurine dose, body and liver weight in control and taurine-treated groups. Growth rate and liver weights were not significantly affected by treatment, but the liver weight/body weight ratio appeared significantly higher in taurine fed animals than in controls. The biliary bile acid analysis by HPLC indicated a marked increase of tauro-conjugation (54.7% vs 17.2% in controls) and a decrease of total bile acid concentration (7.6 vs 9.9 mg of bile acids/ml of bile in controls). Figure 1 shows a typical paraffin-embedded liver section stained with hematoxylin and eosin from a taurine treated animal. The cytoplasmic vacuoles observed in most of the parenchymal cells were shown to be lipid droplets by histochemical stain with oil-red O and Sudan III from frozen liver sections. Many cells contained one large fat globule which displaced the nucleus and altered the cell contour. There was no evidence of other relevant cell damage. Table 2 shows the results of the quantitative analysis of the principal lipid classes of liver extracts. Taurine feeding has contrasting effects on glycerolipid metabolism, increasing TG and decreasing DG and PC concentrations, without affecting PE levels.

Discussion. The present results indicate that oral administration of 0.4% taurine in drinking water for 14 consecutive days led to fatty infiltration of the liver in guinea pig. This finding contrasts with the above-mentioned beneficial effects of taurine in clinical and experimental trials but confirms the potential adverse effect described by Dorvil, et al.[11] in the guinea pig. The primary stages of fatty change in that study were evident only by electron microscopy, probably because of the short duration of the treatment (5 days) and not of the doses administered, which were higher than in the present study (0.82 vs 0.47 mg/kg b.wt/day). These doses, corresponding to more than 30 g/day in man, are much higher than current therapeutic administration. We chose to perform the experiments on the guinea pig, as in previous studies[3,11], in order to have a reference for the effects and because doses as high as 15 g/day and 2.25 g/day have been used respectively for human adults[15] and children[8]. In addition to the specific effects on the liver, the treatment induced an evident, but not significant, reduction in growth rate. It cannot be ruled out that this was due to a general toxic effect derived from the high doses administered; but the fact that the major pathological examinations did not show an effect on any organs other than the liver led us to believe during the trial that the liver is the preferential target. The treatment affected the glycine/taurine (G:T) ratio of conjugation of bile acids, increasing about threefold the percentage of tauro-conjugates. This increase was however lower than the almost total tauro-conjugation previously shown by the use of analytical techniques not rigorously quantitative[3,11].

The fatty change indicated by light microscopic examination was confirmed by the fourfold increase of liver TG concentration, which probably accounts for the largest part of the relative increase in liver weight in treated animals (average differences in liver weight and TG quantity: 80 and 67 mg respectively). In addition, the decreased PC and DG concentrations (in spite of the unchanged levels of PE) suggest that a possible explanation for the fatty change may be an increased conversion of DG into TG and a reduced conversion of DG or PE into PC. Thus the effect of taurine administration on guinea pig liver lipids is similar to the fatty change induced by the lack of lipotropic factors such as choline[16]. This effect, probably due to the high doses administered, has not been observed in animal species other than the guinea pig. The wide variations of plasma TG concentrations (28–1393 mg/dl) described in guinea pig[17] support the possibility that the effect of drugs on glycerolipid metabolism in this animal could be stronger than, or different from, effects in other animals.

Table 1. Dose of taurine administered and selected effects 14-day treatment in guinea pig

Treatment	Control	Taurine
n	9	9
Dose (mg/kg b.wt/day)	–	462 ± 40
Initial b.wt (g)	386 ± 52	374 ± 47
Final b.wt (g)	454 ± 67	414 ± 44
Growth rate (g%/day)	1.35 ± 0.48	0.85 ± 0.42
Liver weight (g)	14.1 ± 2.24	14.9 ± 1.53
Liver weight/body weight	0.031 ± 0.003	0.036 ± 0.002*

Values are mean ± SD. *$p < 0.001$.

Table 2. Effect of taurine administration on glycerolipid concentrations in guinea pig liver

Treatment	n	Triglycerides	Diglycerides	Phosphatidyl-ethanolamine	Phosphatidyl-choline
Control	9	1.85 ± 0.52	0.10 ± 0.03	5.88 ± 0.53	13.85 ± 1.14
Taurine	9	**7.15 ± 1.23	*0.07 ± 0.02	6.15 ± 0.62	**9.74 ± 1.86

Values are mean ± SD and are expressed in µmol/g wet tissue. *$p < 0.05$ and **$p < 0.001$.

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