Histol Histopath (1991) 6: 95-100

Histology and Histopathology

Experimental thioacetamide-induced cirrhosis of the liver

E. Muñoz Torres, J.I. Paz Bouza, A. López Bravo, M.M. Abad Hernández and E. Carrascal Marino
Department of Pathology, Faculty of Medicine, University of Salamanca, Salamanca, Spain

Summary. Hepatic cirrhosis is a complex disease in which several biological, biochemical and chemical alterations are combined, none of these alone being sufficient for diagnosis. The morphological characteristics of the final stages of cirrhosis are well known, but the initial lesions and intermediate stages still have not been fully clarified.

An experimental model of hepatic cirrhosis by chronic administration over 30 weeks of thioacetamide (50 mg/kg twice weekly) to female Wistar rats has been produced. In a macroscopic, microscopic and ultrastructural study. The different lesions that appeared were evaluated according to the dose of the toxic agent administered up, until hepatic cirrhosis was finally installed; this was after 60 doses of the toxic agent (30 weeks).

Discussion is made of the different types of administration and the doses employed to obtain a suitable survival rate for these cases; in our experiments this was 95%.

It has been demonstrated in both human and experimental pathology that once the disease itself has been installed, currently there is no rational or useful treatment for it. A beneficial effect has been demonstrated for certain substances, improving the initial and intermediate lesions, so we conclude by stating that it is necessary to further study the hepatic lesions preceeding cirrhosis. Knowledge of these lesions could form the basis for establishing a useful and rational therapy for such cases.

Key words: Experimental cirrhosis, Hepatotoxic agents, Thioacetamide

Introduction

The study of hepatic dysfunctions is not an easy task. For a better understanding of these it has thus been necessary to establish models of hepatotoxicity in which the metabolism is studied at all levels. With these models it is possible to obtain reproducible lesions by the use of hepatotoxic agents.

Thioacetamide has been used in many studies to explore different aspects of liver cirrhosis and its possible reversability (Bader et al., 1975; Pino and Del Bolt, 1976; Nuber et al., 1980; Lassila and Virtamen, 1984; Willemer et al., 1984). Interest of thioacetamide was first shown in 1943 when this substance, used on oranges as an antifungicide, was found to be a contaminant in orange juice and thus a danger to public health (Cascales Angosto and Ferrandiz García, 1987). Several groups of investigators studied the toxicity of thioacetamide and in 1948 (Fitzhugh and Nelson) it was reported that a single dose of this hepatotoxic agent could produce centrilobular hepatic necrosis and that chronic administration led to cirrhosis and hepatocarcinoma.

As with other hepatotoxic agents, thioacetamide needs to be activated metabolically for its effect to be toxic. The products of its metabolic transformation include acetamide, sulphate and thioacetamide sulphoxide. The toxic effect of thioacetamide is attributed to the latter metabolite.

Thioacetamide is metabolized by the catalytic action of a mixed-action microsomal mono-oxygenase that transforms, it sequentially into sulphoxide and sulphone (Cascales Angosto and Ferrandiz García, 1987) (Table 1).

Several reports by Goldberg et al. (1984), have described that chronic poisoning with TAA by intraperitoneal injections induced changes in the periportal collagen deposit. Zsigmond et al. (1982) have reported modifications in the body weight of animals, accompanied by progressive changes in the liver, such as cellular necrosis and regeneration as well as hepatic fibrosis with the formation of pseudo-lobules. Bodnar et al. (1982) have also communicated that after sufficient time in the organism, TAA leads to hepatic cirrhosis.

Offprint requests to: Dra. Elisa Muñoz Torres, Colegio Universitario de Medicina de Avila, Santo Tomás s/n, 05001 Avila, Spain

In 1986 Zimmermman et al. reported that the addition of TAA to drinking water (300 mg/litre) from the 4h to the 6th month of life led to uniform micronodular cirrhosis of the liver in all the rats tested. With this treatment the survival of the rats was approximately 90%. When the dosage or exposure time were increased the survival rate of the animals decreased and the micronodular chirrhosis became macro-nodular. According to these authors, in order to achieve macro-nodular cirrhosis it is more efficient to increase exposure time rather than dosage since, in the latter case, in the rat, the survival rate is much lower.

Other authors (Cascales Angosto and Ferrándiz García, 1987) have shown that thioacetamide administered by intraperitoneal injections produces acute and chronic hepatopathy according to the length of time administration, with cytotoxic intoxication in the early stages, which coincides with the centrilobular necrosis. More continued treatment gives rise to cholestatic intoxication and the appearance of biliary pigments in plasma and urine (Dashti et al., 1986).

The animal generally used in these studies is the rat. Administration routes are: oral in drinking water, intragastric by stomach cannula, and intraperitoneal injections. The doses vary for each study.

Materials and methods

Seventy female Wistar rats weighing 200 grs. were used in our experiments. Of these 10 were used as controls and the other 60 were administered thioacetamide in intraperitoneal injections, at a dose of 50 mgrs/k of body weight twice a week.

The animals were divided into groups:

1.– Control group (10).

2.– Group 1 (15): receiving thioacetamide for 7 weeks (14 injections).

3.– Group 2 (15): receiving thioacetamide for 15 weeks (30 injections).

4.– Group 3 (15): receiving thioacetamide for 22 weeks (44 injections).

5.– Group 4 (15): receiving thioacetamide for 30 weeks (60 injections).

Five animals, chosen at random in the 7th, 15th and 22nd weeks, were sacrificed from each group. At the same time hepatic biopsies were performed on the other rats, administering general anesthesia by inhalation of ethyl ether. A medial laparatomy was performed, randomly choosing one hepatic lobule, and a biopsy slice was taken with a pointed scalpel. The surface of the cut was coagulated with $AgNO_3$ strips. The remaining rats were sacrificed in the 30th week.

Both the hepatic slices and the livers from necropsy were fixed in 10% formol (aqueous solution of formaldehyde 35-40%) for 24 hours. They were then embedded in paraffin and 5 μm cuts were made and stained with hematoxylin-eosin, Periodic Acid Schiff (PAS), Wilder's reticulin, Mason's and Mallory's trichrome, Perls' method to identify ferric pigment, and Hall's method to identify bilirubin. All these techniques were carried out according to the methods of the Armed Forces Institute of Pathology: (A.F.I.P).

For the ultrastructural study the hepatic slices were fixed in 2% glutaraldehyde and postfixed in 1% osmium tetroxide (isotonic solution according to Zetterguist) according to the standard methods for electron microscope studies.

Results

The survival rate obtained in this experiment was 95%.

The degree of liver hypertrophy was evaluated by calculating liver weight in relation to body weight, a progressive increase in liver weight of the animals treated with thioacetamide was observed with respect to the controls. The livers of the rats treated for 30 weeks reached a weight of 23 grs, whereas those of the control animals with the same body weight (250 grs) weighed 12 grs.

Gross morphology

The livers of the animals treated the longest (Group 4, 60 injections) were observed to be grossly larger, yellowish in color, with a loss of their normal shine. They were hard in texture and had a nodular surface, with nodules of different sizes. Small focal points of haemorrhaging were observed on the surface of the livers (Fig. 1).

Significant gross alterations were not observed in the other animals.

Microscopic morphology

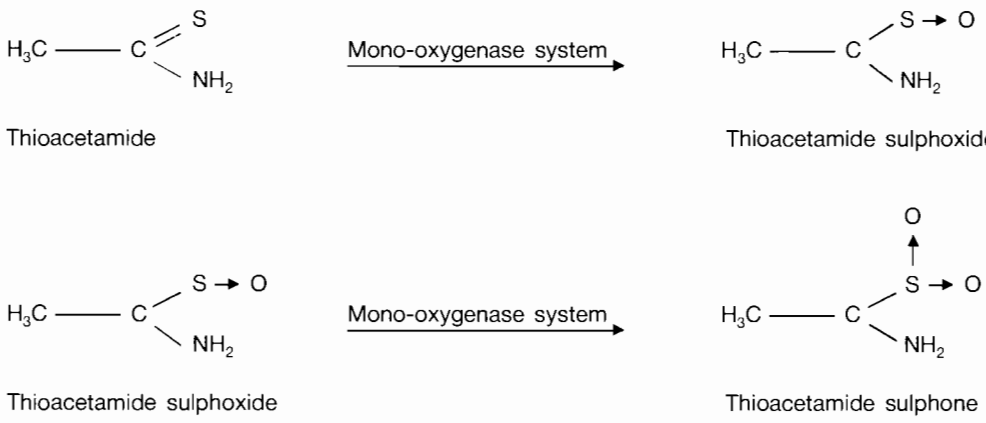
In the 7th week, after 14 injections of thioacetamide, the livers showed a preserved structure; the portal spaces were enlarged with some fine conjuctive projections, although these had not formed bridges. Ansicytosis, binucleate hepatocytes, hypertrophic nuclei and some images of mitosis were also observed. There were also deposits of biliary and ferric pigment, with greater quantities at periportal level.

After 30 injections (15th week), the animals from group 2 showed large portal spaces with connective tissue projections that had not yet formed bridges; hepatocytes with prominent nuclei, or sometimes with multiple nuclei, and small focal points of steatosis. Bile and iron deposits were also observed, mainly in Kupffer cells.

When the animals had received 44 injections (group 3), their portal spaces were enlarged (Fig. 2) with brush-like tracts joining together to form bridges. In some areas incomplete nodules, fine drop steatosis, binucleate hepatocytes, hyperplasia of Kupffer cells, and an irregularly distributed increase in bile deposits could be seen, although the latter were greater in number near the periportal spaces.

The animals that received 60 injections (group 4) showed an altered hepatic structure, with numerous

Table 1



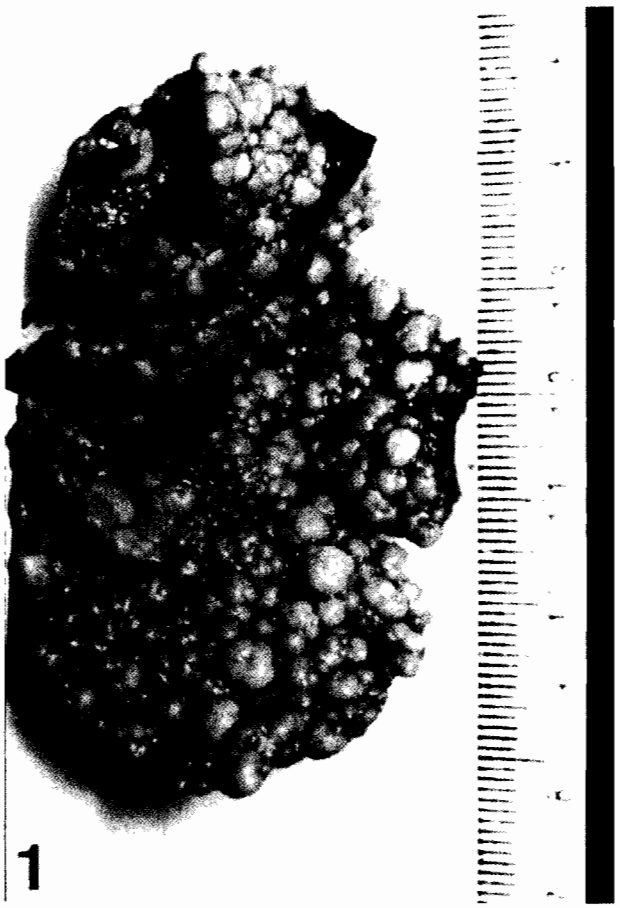


Fig. 1. Gross aspect showing the liver after 60 injections of thioacetamide.

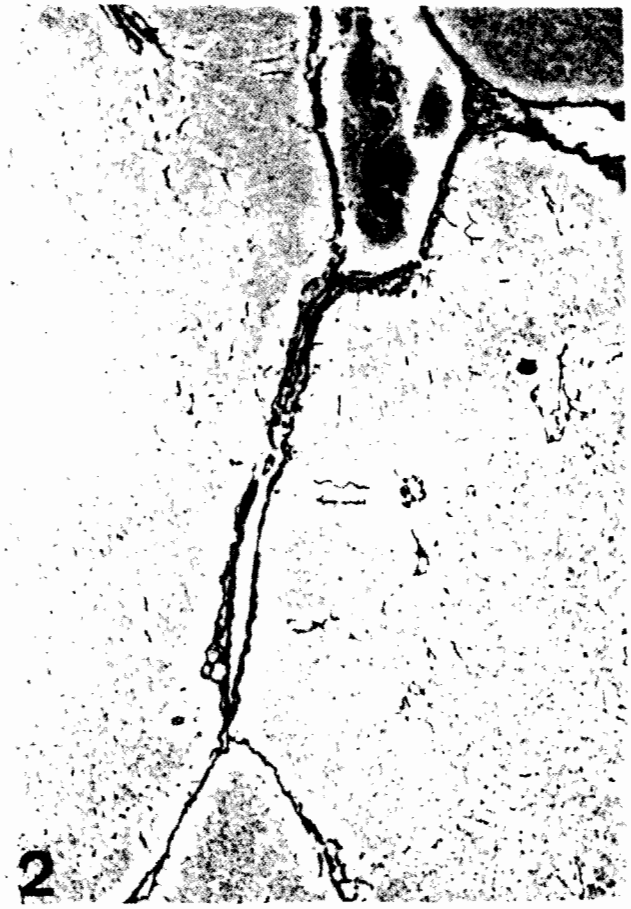


Fig. 2. Enlarged portal spaces joined by fibrous tracts forming bridges. Wilder. × 20

fribrous tracts forming completed nodules of different sizes (Figs. 3, 4); cirrhosis was well established.

Ultrastructural study

The ultrastructural study of the livers of the animals receiving 60 injections of thioacetamide (group 4) revealed parenchymatose destructuralization with numerous hepatocytary remains. The better-preserved hepatocytes showed various nucleoli and considerable breakage of the endomembranes with a loss of cytoplasmic matrix (Fig. 5). Fibroblasts were observed and

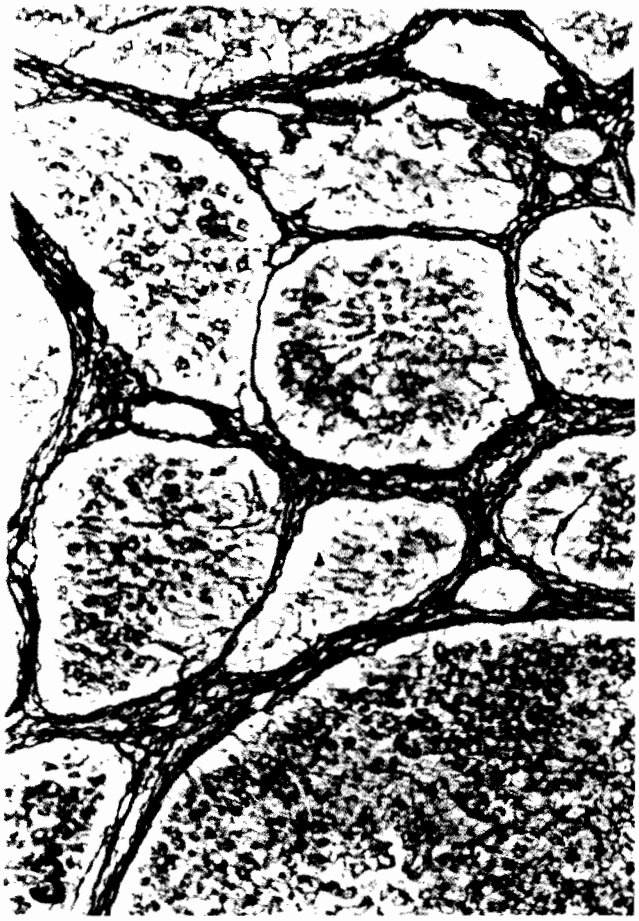


Fig. 3. Altered hepatic structure with thick fibrous tracts forming complete nodules of different sizes. Wilder. × 63

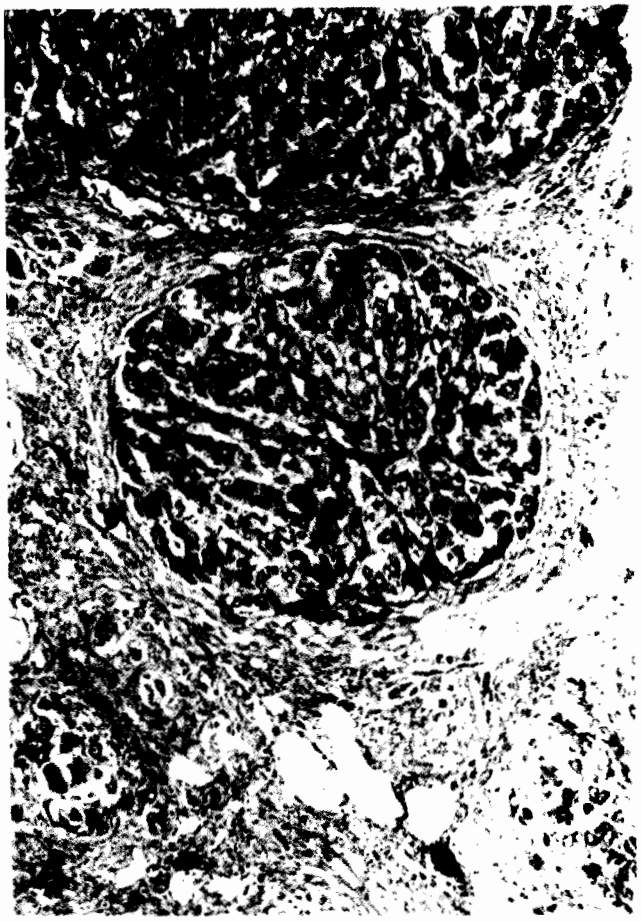


Fig. 4. Nodules of different sizes surrounding fibrous septa. Wilder. × 25

Fig. 5. Nucleus of a hepatocyte with three nucleoli, breakage of endomembranes and loss of cytoplasmic matrix. × 3,600

Fig. 6. Electron micrograph showing numerous fibroblasts (cells of fibroblastic lineage) with bundles of newly-formed fibres (arrows) among hepatocytary debris (*). × 2,800

Fig. 7. Electron micrograph showing two fibroblasts among hepatocytary debris and numerous irregularly-disposed collagen fibres. × 2,800

there were many collagen fibres. The cholangioles remained well preserved.

Under our experimental conditions, at ultrastructural level, thioacetamide led to destruction and necrosis of the hepatocytes with activation of Kupffer cells. However, it did not affect the cholangioles whose cells appeared well-preserved. In addition, there was intense fibroblastic activity with the formation of numerous clusters of collagen (Figs. 6, 7).

Discussion

Most authors have found that chronic thioacetamide intoxication leads to cirrhosis of the liver. Under our experimental conditions we found that by giving the toxic agent enough time to act, cirrhosis is produced. It has also been reported (Cascales Angosto and Ferrándiz García, 1987), that thioacetamide, produces cytotoxic poisoning in the early stages of exposure, and that in continued treatment it gives rise to cholestatic intoxications. These types of intoxication were observed in our animals although the cholestatic effect of the toxic agent appeared in the first seven weeks, bile deposits generally being periportal. This means that under our experimental conditions both effects of the toxin were concurrent practically from the start of the experimental intoxication.

At gross level our results coincide with those described by some authors (Cascales Angosto and Ferrándiz García, 1987), such as the nodular hypertrophic cirrhosis after 60 injections of thioacetamide.

We also observed a loss in body weight in the animals as reported by some authors (Zsigmond et al., 1982).

In other studies (Zimmermann et al., 1986), thioacetamide was used in the drinking water, achieving micro-nodular cirrhosis in two months and with a survival rate of 90%. We preferred to use the intraperitoneal route, which perhaps mean that it took longer to reach micro-macronodular cirrhosis but which also led to a higher survival rate (95%). We therefore feel that, in view of the survival rate of the animals, our method is more efficient in comparison with that of the above authors.

Ultrastructurally, thioacetamide has been reported to produce a dilatation of the biliary canaliculi (Zsigmond et al., 1982), an effect which was not observed in any of our animals. Other authors (Cascales Angosto and Ferrándiz García, 1987), obtained ultrastructural data similar to ours, but no other report describing the appearance of a large number of fibroblasts and bundles of newly-formed fibres, has been found, which nevertheless, we observed in greater quantity in the animals treated for a longer period of time. We consider these data to be very important for the evaluation of cirrhosis of the liver. On the other hand, it was surprising to find that an agent as toxic as thioacetamide -which produces a high level of parenchymatose destruction, with breakage of endomembranes and loss of cytoplasmic matrix causing destruction and cellular death (as reflected by the large quantity of hepatocyte debris observed ultrastructurally)- did not harm the cholangioles; ultrastructurally these remained normal at all times.

References

Bader G., Bader N.G., Busch B. and Stiller K.H. (1975). Comparative karyo metric studies on small pseudolobuli and hepatomas in thioacetamide-induced liver cirrhosis. Exp. Pathol. 10, 241-244.

Bodnar A., Kasracsonyi S. and Zsigmond G. (1982). The effect of portacaval shunt on bili flow and maximal biliary excretion of bromsulfophthalein sodium (BSP) in rats with thioacetamide-induded liver cirrhosis. Z. Exp. Chir. 14, 35-39.

Cascales Angosto M. and Ferrándiz García F. (1987). Aspectos bioquímicos y farmacológicos en disfunciones hepáticas. CSIC (eds). Madrid.

Dashti H., Jepsson B., Abdulla M., Srinivas U., Hagestrand I. and Bengmark S. (1986). Changes in the plasma levels of copper, zinc, calcium magnesium and selenium in thioacetamide-induced liver cirrhosis. Acta Pharmacol. Toxicol. 56, 219-222.

Fitzhugh O.G. and Nelson A.A. (1948). Liver tumors in rats fed thiourea or thioacetamide. Science 108, 626-627.

Goldberg H.I., Moss A.A., Stark D.D., McKerrow J., Engelstad B. and Brito A. (1984). Hepatic cirrhosis: magnetic resonance imaging. Radiology 153, 737-739.

Lassila V. and Virtamen P. (1984). Influence of experimental liver injury on rat blood and alveolar bone under stress. Acta Anat. 118, 116-121.

Nuber R., Teush H.F. and Sasse D. (1980). Metabolic zonation in thioacetamide-induced liver cirrhosis. Histochemistry 69, 277-288.

Pino V.L. and del Bolt H.M. (1976). Time course of thioacetamide-induced changes in estrogen metabolism in the liver. Endokrinologie. 68, 137-142.

Willemer S., Sames K., Lidner J. and Schimiegelow P. (1984). Age dependence of early reaction in glycosaminoglycan metabolism of the rat liver on damage following a single dose of thioacetamide. Z. Gerontol. 17, 150-156.

Zimmerman T., Franke H. and Dargel R. (1986). Studies on lipid and lipoprotein metabolism in rat liver cirrhosis induced by different regimens of thioacetamide administration. Exp. Pathol. 30, 109-117.

Zsigmond G., Bodnar A., Schaff Z. and Karacsonyi S. (1982). Bile flow and biliary sulfobromaphtalein sodium excretion in rats with liver cirrhosis and protocaval shunt. Eur. Surg. Res. 14, 344-357.

Accepted July 30, 1990