Development of an animal model of hepatic fibrosis by excretory-secretory antigen of *Ascaris suum*

AMAL SANTRA, ABHIJIT CHOWDHURY, ALOKE GHOSH, D N GUHA MAZUMDER

Department of Gastroenterology, Institute of Post Graduate Medical Education and Research, 244 Acharya J C Bose Road, Calcutta 700 020

**Objective:** The excretory-secretory (ES) antigens of *Ascaris suum* are known to cause hepatic damage in animals. The present study was aimed at developing an animal model of hepatic fibrosis with these antigens. **Methods:** Three doses of ES antigens of *A. suum* were injected into 24 golden hamsters on days 0, 10 and 20. Batches of 8 animals each were sacrificed at 3 days, 45 days and 90 days after the third injection, after collection of blood. Three groups of 6 control animals each were injected with normal saline and were sacrificed similarly. Liver biochemistry, leukocyte migration inhibition test on cells separated from spleen, and liver histology were carried out. **Results:** Serum ALT levels in experimental animals were significantly higher than those in control animals at days 3, 45 and 90 after the last antigen dose; AST levels were elevated 45 and 90 days after the last dose of ES antigen. Leukocyte migration inhibition in experimental animals was 58.2 (8.5)% 51.6 (11.2)% and 50.5 (12.8)% at days 3, 45 and 90 after the last antigen dose. Marked centrilobular degeneration and necrosis were observed in liver tissue in all the experimental animals sacrificed 72 h after the last antigen dose. Condensation of reticulin around the portal zone with extension into the liver lobule was observed in 4 of 8 and 7 of 8 experimental animals sacrificed 45 and 90 days, respectively, after the last dose. Control animals did not have such lesions. **Conclusion:** An animal model of hepatic fibrosis could be produced by repeated injection of ES antigens of *A. suum*. [Indian J Gastroenterol 2000;19:119-121]

**Key words:** Hepatic degeneration, hepatic necrosis

Various chemical and biological agents initiate hepatic fibrosis, which is characterized by deposition of extracellular matrix components in the hepatic tissue in increased amounts and abnormal distribution. Several animal models and *in vitro* systems of hepatic fibrosis have been developed, e.g., alcohol liver injury model in baboon, CCl₄-induced liver damage in rat, and the murine model of schistosomiasis. Although useful, each of these models has several limitations and fall short of mimicking human disease. However, each model provides information about specific steps in the process of fibrogenesis.

The excretory-secretory (ES) antigens of *Ascaris suum* (pig ascaris) have been shown to be hepatotoxic, and ingestion of this antigen leads to hepatic damage in the hamster, although there is no evidence of fibrosis. However, *A. suum* larvae have been reported to cause mild hepatic fibrosis in pigs and mice. The present study was carried out to determine if ES antigens of the adult *A. suum* can produce hepatic fibrosis in hamsters. If successful, this could help in developing an animal model for the study of the pathophysiology of hepatic fibrosis as well as for screening of antifibrotic drugs.

**Methods**

Live adult *A. suum* worms were collected from the intestine of infected pigs. The worms were washed in sterile normal saline and ES antigens were collected in sterile balanced salt-sugar (BSS) solution. In brief, the worms were kept alive in sterile BSS solution, which was changed every 24 h, for 72 h at 37°C; the ES antigen of the worms thus entered the solution. Aliquots of BSS solution containing ES antigens were taken from each sample and cultured for pathogenic organisms; batches showing contamination were rejected. Bacteria-free BSS containing ES antigens was dialyzed against de-ionized water at 4°C for two days with several changes. The dialyzed ES antigen was centrifuged at 10,000×g for 1 h at 4°C, and filtered through 0.22 μm membrane (Millipore; Bedford, USA). Protein content of the ES antigen so prepared was estimated using BSA as standard. The antigens were stored in sterile containers at -75°C.

Forty-two male Syrian golden hamsters (7-8 weeks old, weighing 35-38 g) were maintained at a constant temperature and humidity environment, in 12 hour light-dark cycles and fed standard laboratory chow. All animal experiments were approved by the institution and were performed in accordance with institutional guidelines for the care and use of laboratory animals. The hamsters were divided into two groups. In the experimental group of 24 hamsters, ES antigens of *A. suum* (equivalent to 100 μg protein in each dose) were injected subcutaneously to each animal on days 0, 10 and 20. In 18 control hamsters, normal saline was injected in the same schedule.

Three days after the last antigen dose, 6 animals from the control group and 8 from the experimental group...
were sacrificed, after collection of blood by cardiac puncture. Liver profile was tested by standard laboratory techniques. Leukocyte migration inhibition (LMI) test, with or without addition of ES antigen, was done on cells isolated from the spleen. Liver histology was studied using hematoxylin and eosin and reticulin stains. Similarly, batches of eight animals from the experimental group and six animals from the control groups each were sacrificed 45 and 90 days after the last antigen dose, respectively. Liver function tests, LMI test and histological studies were carried out in all the animals as described above.

Results

Biochemical changes

Biochemical evidence of hepatocellular damage was observed in all the three groups (sacrificed on days 3, 45 and 90) of experimental animals. Serum ALT level at the time of sacrifice was significantly elevated in all the three experimental groups compared to respective control animals, but elevation of serum AST was observed only on days 45 and 90, but not on day 3, after the third dose of ES antigen (Table). There was no difference in serum protein concentration between experimental and control animals.

Histological changes

Histological abnormalities were observed in the liver tissues of all the 8 experimental animals sacrificed 3 days after the last dose of ES antigen of A. suum. Marked degenerative changes were observed in the centrilobular hepatocytes (Fig 1). Occasional focal hepatic necrosis characterized by collection of a few mononuclear cells was also observed.

Abnormal histology was observed in the livers of 7 of 8 experimental animals sacrificed 45 days after the last antigen dose. Centrivenular degeneration was ob-

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* \( p < 0.05; \) \# \( p < 0.01; \) compared to respective control groups.

served in 4 animals and focal cell collection and granuloma formation in two animals each. Granulomas were characterized by the presence of epithelioid cells, mononuclear cells and histiocytes. Expansion of portal zone with collection of large numbers of inflammatory cells was seen in one animal. Further, condensation of reticulin fibrils traversing from one portal zone to another for variable distance within the lobule (Fig 2) was observed in 4 of 8 experimental animals.

Ninety days after the last antigen dose all the experimental animals had abnormal liver histology. Focal collection of inflammatory cells and perportal deposition of amyloid were observed in one animal. Granuloma formation was seen in 2 animals. Reticulin condensation similar to those described in the previous group was observed in the liver of 7 animals.

Leukocyte migration inhibition test

Splenocytes of experimental animals collected 3 days, 45 days and 90 days after the last ES antigen dose showed 58.2 (8.5)%, 51.6 (11.2)%, and 50.5 (12.8)% inhibition, respectively, when those cells were cultured with ES

Fig 1: Liver of experimental animal showing centrilobular degeneration; Occasional areas of focal necrosis characterized by liver cell dropout and collection of lymphocytes (arrow) were also seen (H&E, 150X)

Fig 2: Liver of experimental animal showing condensed reticulin fibrils traversing from the portal zone to the liver lobules producing pseudolobule-like appearance (reticulin stain, 150X)

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antigen compared to the migration observed when leukocytes were cultured without ES antigen. No difference in the migration was observed when splenocytes of control animals were cultured with or without ES antigen.

Discussion

The histological lesions observed in the liver of animals challenged with ES antigens by the parenteral routes are interesting. Marked degenerative changes with occasional focal necrosis in the liver were observed 3 days and 45 days after 3 doses of antigen administered subcutaneously. These features appear to be immune-mediated as suggested by the observation of significant LMI in the presence of ES antigen. Similar changes were also reported in pigs after primary and secondary inoculation of larvae of *A. suum*. Granulomatous lesions were observed in some animals at 45 and 90 days. These are believed to result from cell-mediated immune reaction as they are dependent on recognition of antigen by immunocytes. Further, large portal collections of chronic inflammatory cells were seen in the portal zone in one animal. We used the subcutaneous route for antigenic challenge to obtain a more severe immunologic response as compared to that obtained by oral administration.

Hepatic fibrosis was manifested at 45 days after the last antigen dose of ES antigen in half of the experimental animals; it was more frequent after 90 days. The results of our study simulate the hepatic fibrosis developed in mice model by the eggs of schistosoma. In that model, the products secreted by these eggs were found to be antigenic and caused a severe cell-mediated inflammatory reaction after the initial weeks of inflammatory activity, collagen deposition takes place over 8-10 weeks and continues at slower pace.

Liver fibrosis can be induced in rats by using antigenic substances that include swine and horse serum, egg and swine albumin, human gamma globulin and swine serum globulin. Repetitive (twice weekly) intraperitoneal injection of swine serum for five weeks was found to be the most effective in producing hepatic fibrosis in rats. Histologically, the changes are characterized by mononuclear cell infiltration, ductular proliferation and fibrotic response in the portal areas. Compared to this model, our hamster model required fewer doses of antigen; only three doses of ES antigen of *A. suum* were sufficient to produce significant hepatic fibrosis.

Similar to other models of liver fibrosis, the response of individual animals to the ES antigen of *A. suum* is quite variable. Despite this and the small number of animals we studied, it appears that an experimental animal model of hepatic fibrosis could be developed by repeated challenge by ES antigen of *A. suum*. This model could be used for the study of pathophysiology of hepatic fibrosis as well as for screening of potential antifibrotic drugs.

References


Correspondence to: Prof Guha Mazumder, Fax: (33) 475 1799. E-mail: dngm@apexmail.com

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