PATHOMORPHOLOGICAL CHANGES IN RATS FOLLOWING INFECTION WITH TRYPANOSOMA EVANSI ISOLATED FROM A HETEROLOGOUS HOST

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ABSTRACT

Albino rats infected experimentally with Trypanosoma evansi (isolated from camel) revealed parasitaemia by day 2 post infection. The infected animals became dull and depressed and showed abnormal movements from 2 DPI onwards. Postmortem examination revealed splenomegaly, marked congestion of lungs, petechiae on serous surfaces and the entire surface of liver along with presence of fluid in peritoneal and pericardial cavities from 4-6 DPI (the maximum period of observation). Histopathologically, heart muscles showed hyaline degenerative changes and haemorrhages. Liver parenchyma revealed congestion of central vein and sinusoids. Haemosiderosis was found in liver, spleen, heart and lungs. Renal tubules had some hyaline cast. The organisms could be demonstrated in sections of liver and kidney only.

INTRODUCTION

Acute fulminating trypanosomiasis (surrea) in animals can be detected by microscopic examination of the Giemsa stained blood smears prepared during the febrile stage of the affected animals (Gill, 1991). The diagnosis of trypanosomiasis by peripheral blood smear examination, at any moment of time can detect ≤ 60% infection (Antipin et al., 1964).

The surest method for establishing diagnosis of surra is by way of animal inoculation. Rats and mice are the laboratory animals of choice for this purpose. Rats can receive more inoculum of blood and hence are preferred over mice. The present study was undertaken to know the effects on the pathogenesis of Trypanosoma evansi in a heterologous host, if any, to facilitate diagnosis and to prove Koch’s postulates.

MATERIAL AND METHODS

T. evansi isolated from camel (gifted by Dr. K.M.L. Pathak) was passaged serially in albino rats. The parasites at 10th passage level in rats were used for the present study. Infected rat blood diluted five times in Alsever’s solution was inoculated subcutaneously into eighteen healthy rats @ 1.0 ml each. The infected rats were kept in fly proof cages. Twelve hatchmate rats were inoculated similarly with Alsever’s solution and kept in fly proof cages to serve as uninfected controls. The experimental animals were given water and pelleted ration ad libitum. These animals were observed daily, for the development of clinical symptoms and for monitoring the uptake of the infection. The Giemsa stained blood smear prepared daily from the individual animal from all the experimental rats were examined under light microscope for the development of parasitaemia.

Three rats from the infected and two from the control group were sacrificed each day through day one to six. The parasites in the blood were counted with the help of haemocytometer using WBC counting pipette and diluting the blood twenty times with PBS (pH 7.2), following the method as described for WBC count. The counting was done in WBC counting chamber and the no. of organisms were calculated by employing the following formula:

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Total no. of parasites/ml of blood = Total count of parasites in four chambers x 50 (Dilution factor)

The animals sacrificed each day were examined meticulously at the time of autopsy. Representative pieces from all the major organs irrespective of the presence of gross changes were collected in 10% buffered formalin. The formalin fixed tissues were processed for routine paraffin embedding and sections were cut at 3µ thickness. The tissue sections were stained with haematoxylin and eosin following the standard method for histopathological examination. Perl's method was followed for the demonstration of haemosiderin pigment in the tissue section (Culling, 1963).

RESULTS AND DISCUSSION

The clinical examination revealed dullness, depression and appearance of abnormal movements in a few infected animals from 2 DPI till 6 DPI (the maximum observation period). No abnormality of any kind could be seen in the uninfected control animals. The growth kinetics of the organisms in the inoculated rats has been presented graphically in Fig. 1. It is evident from the graph that the log phase of the organisms started from 3 DPI and the parasites multiplied continuously till 6 DPI. However, examination of Giemsa stained blood smears of the infected animals revealed presence of few T. evansi from 2 DPI and a heavy parasitaemia could be seen around 4 to 6 DPI (Fig. 2). None of the animals in control group revealed T. evansi infection during the period of observation.

The sacrificed animals revealed presence of straw coloured fluid in the pleural and peritoneal cavities invariably from 4 DPI to 6 DPI, splenomegaly, marked congestion of lungs, petechiae on the serous surfaces and liver were also observed. However, changes as observed by Raisinghani et al. (1980) in the natural hosts i.e. camel viz., alopecia, emaciation, facial edema, excessive keratinization of skin could not be observed in rats in experimental studies which may probably be due to occurrence of acute course of disease in rats or may be due to species difference. The observations, however, corroborate the findings of Verma and Gautam (1979) in cattle and buffalo calves experimentally infected with Trypanosomes.

![Graph showing growth kinetics of T. evansi in experimentally infected rats](image-url)
Fig. 2. Photomicrograph of Giemsa stained peripheral blood smear of experimentally infected rat on 5 DPI showing flagellate Trypanosoma organism x 1000.

Fig. 3. Liver parenchyma showing congested central vein and sinusoids on 4 DPI. H&E x 400

Alveoli of the lungs revealed eosinophilic exudate indicative of alveolar oedema. Alveolar blood vessels were congested and massive areas of haemorrhages could be seen from 3 DPI onwards along with focal areas of infiltration with polymorphs. Similar findings have been observed earlier by Patel et al. (1982).

Heart muscles showed hyaline changes and areas of haemorrhages from 2 DPI
onwards. However, Patel et al. (1982) recorded granular degeneration of myocardial fibres and extensive haemorrhages in mice infected experimentally with Trypanosoma organisms.

Liver sections revealed congestion of central vein and the hepatic sinusoids from 2 DPI onwards, followed by massive areas of haemorrhages (Fig. 3). Hepatocytes showed various stages of degeneration and the Von kupper cells were found laden with haemosiderin pigment along with focal areas of mononuclear cell infiltration. Deposition of haemosiderin pigment could be demonstrated with Perl's stain in lungs, heart and spleen as well. There are conflicting reports about the demonstration of the organisms in the tissue sections. However, the organisms could be demonstrated successfully in the liver and kidney sections of the infected rats by Giemsa stain. Similar observations have been reported in rats and mice (Patel et al., 1982), in dogs (Srivastava et al., 1969), in cattle (Verma and Gautam, 1972) and in rabbits, sheep and goats (Losos and Ikede, 1970). Sen et al. (1959) noticed organisms only in kidneys of albino rats where as Srivastava and Ahluwalia (1972) could not demonstrate organisms either intra or extravascularly in any of the organs of the pigs.

Kidneys showed congestion of blood vessels and massive areas of haemorrhages from 3DPI onwards. Some tubules showed the presence of hyaline casts. Poursines et al. (1937) observed degeneration of renal tubular epithelium in rats, guinea pigs and rabbits. Sen et al. (1959) observed congestion and cloudy swelling in glomeruli of rats.

Spleen was haemorrhagic with pronounced haemosiderosis and RE cell proliferation. Srivastava and Ahluwalia (1972) observed similar lesions in dogs and Patel et al. (1982) in mice and rats infected with T. evansi.

Brain revealed congested blood vessels and perivascular cuffing of lymphocytes. Similar findings have been reported in dogs infected with Trypanosomes (Chew, 1968).

All these reports indicate some variations in the pattern of lesions or difference in pathogenesis of the infection in various species of the animals. From the study it could be inferred that infection of heterologous hosts with T. evansi may not induce lesions akin to the natural host. Mechanism of induction of lesions though is not very clear in the affected animals, however, metabolic products/toxins of the trypanosomes besides the causative agent itself have been reported to induce lesions on the various organs in the affected animals. However, there is a need for further exploration for finding out the mechanism/reasons for such changes employing the latest biotechnological tools.

REFERENCES