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Comparative Histopathological Changes in Acute *Toxoplasma gondii* (RH Strain) Infected and Treated Mice

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Abstract

The aim of the present study was to investigate the comparative histopathological changes due to acute *Toxoplasma gondii* (RH strain) infection in infected and treated mice which might be useful observations for future research on toxoplasmosis in veterinary and medical science. Thirty Swiss albino mice were randomly divided into five equal groups (*i.e.* Gr. I, II, III, IV and V). The mice of group II, III, IV and V were intraperitoneally inoculated with 5×10^3 tachyzoites of serially passaged *T. gondii,* respectively). On three days post-infection (DPI) all the mice of group III, IV and V, showing the sign of acute toxoplasmosis (Ascites and respiratory distress) were treated with sulphadoxine with pyrimethamine @21.20 mg kg^{-1} body weight orally, azithromycin @200 mg kg^{-1} body weight orally and clindamycin @6 mg per mice, orally, respectively. Histopathological changes of spleen, liver, lungs and kidney of infected and treated groups were more or less similar. Spleen of Gr II, Gr III, Gr IV and Gr V revealed diffused infiltration of lymphocytes in the red pulp. Fatty degenerative changes in liver were the prominent feature in all the infected groups of mice. Lungs showed pneumonic changes and kidney showed intravascular haemolysis. Intestine of Gr III revealed perivascular cuffing of lymphocytes in submucosa and heart showed focal haemorrhages in myocardium whereas, intestine and heart of other treated groups did not reveal any significant changes.

Key words: Mice, *Toxoplasma gondii* (RH strain), Sulphadoxine-Pyrimethamine, Azithromycin, Clindamycin, Histopathology

Introduction:

Toxoplasma gondii is an obligatory, heterogenous, intracellular protozoan parasite which infects all warm blooded animals (Tenter et al., 2000, Dubey, 2009) including man and birds as intermediate host and cat as the definitive as well as intermediate host worldwide. It poses a great hazard to all immuno compromised hosts. It is usually asymptomatic in immune-competent individuals but may occasionally lead to severe ocular and neurological disorders (Furtado et al., 2013). When it comes to immune-compromised and congenitally infected individuals, toxoplasmosis can result in lethal systemic disease and eventually death (Shen et al., 2016). Currently, the control of *T. gondii* mainly depends on chemotherapy, such as the combination of sulfadiazine and pyrimethamine, but these drugs have serious side effects (Petersen and Schm, 2003). There are no effective drugs available to kill *T. gondii* cysts in tissues. Therefore, new efficient drugs and safe protective therapies are needed. Although spiramycin, clindamycin, azithromycin and sulphonamides in general are antitoxoplasmic.

There are several advantages of azithromycin over the more commonly used macrolides antibiotics, such as erythromycin. It has been reported that azithromycin in a 3-day course was found to be as effective as 5- to 10-day course of other antibiotics, including erythromycin, amoxycillin/calvulanic acid and penicillin V in treating respiratory tract infections (Dunn and Barradell, 1996). Oral treatment of this drug often associated with various adverse effects related to the gastrointestinal tract like cramping, diarrhoea, nausea, abdominal pain and vomiting (Dunn and Barradell, 1996). The reasons for the discrepant findings reported in animal models presumably include different experimental conditions comprising differences in both the infection model and treatment protocol. In this study, we screened three medicines such as Sulphadoxine-Pyrimethamine, Azithromycin and Clindamycin, that have different pharmacological effects and combined them .We evaluated the protective efficacy against the challenge of RH strain of *T. gondii* in a mouse model and to investigate the histopathological changes in different visceral organs.

Materials and Methods:

Mice: Thirty adult healthy Swiss albino mice of either sex aged 6-12 weeks and average weight of 25-30 gm were housed six per cage, and offered drinking water and feed *ad libitum* throughout the study period.

Parasite: The virulent modified mouse adopted RH strain of *T. gondii*, obtained from Division of Parasitology, Indian Veterinary Research Institute (IVRI), Izatnagar. – 243122, Bareilly, U. P, was maintained in our laboratory by continuous passage at 3-4 days interval in mice to produce (Sreekumar, 2001; Velmurugan, 2006). Peritoneal exudates of mice developed acute toxoplasmosis were cryopreserved for further study.

Grouping and experimental infection of the mice: The mice were randomly divided into five equal groups (*i.e.* I, II, III, IV and V). The mice of Group II, III, IV and V were experimentally inoculated with 5×10^3 *T. gondii* serially passaged tachyzoites intraperitoneally (I/P), and mice of Group-I were maintained as uninfected control.

Chemotherapy: The infected mice of group III, IV and V were treated with the following drugs when infection was noticed at 72-74 hours post infection (3rd DPI). The following drugs were used: On 3 days post-infection (DPI) all the mice of Gr III, IV and V, showing the sign of acute toxoplasmosis were treated with sulphadoxine with pyrimethamine @ 21.20 mg kg^{-1} body weight orally, azithromycin @ 200 mg kg^{-1} body weight orally and clindamycin @ 6 mg per mice, orally, respectively.

Combination of Sulphadoxine and Pyrimethamine: The *Toxoplasma* tachyzoites inoculated mice of Group-III having six mice were treated with sulphadoxine with pyrimethamine (Pyralfin, LupinPharma. Ltd.) @ 21.20 mg per kg body weight orally. Required amount of medicines were administered with PBS (pH-7.2). Ultimately 1ml of PBS (7.2) containing required dose of drug was given to each mice.

Azithromycin: The *Toxoplasma* tachyzoites inoculated mice of Group IV were treated with azithromycin (Azithral, Alembic Pharma Ltd.) @ 200 mg/kg body weight, orally as per Araujo et al. (1988).

Clindamycin: The *Toxoplasma* tachyzoites inoculated mice of Group V were treated with Clindamycin (Clincin, Indi Pharma Pvt. Ltd.) @ 6 mg per mice, orally as per Araujo et al. (1974). Required amount of medicines were administered with PBS (pH-7.2). Ultimately 1ml of PBS (pH 7.2) containing required dose of drug was administered orally to each mice.

Collection and staining of different organs: Mice of infected groups (Gr I and Gr II) were examined carefully and after confirmation of clinical signs, the mice were euthenized with diethyl ether or chloroform and post mortem examination was carried out. Then different organs like liver, lung, spleen, kidney, heart, intestine and brain were collected asceptically for histopathological examination. A piece of these organs were preserved in 10% formalin solution and the sections were cut and stained with Harris Haematoxylin and Eosin stain (H & E) following the standard method (Lillie and Fullmer, 1976).

Results and Discussion:

Histopathological changes:

Infected control group:

(a) Spleen: Diffuse proliferation of follicular lymphocytes obliterating the reticular structure of red pulp was noticed. Intravascular haemolysis was also found (Figure 1). Focal sub- capsular proliferation of reticuloendothelial cells along with lymphocytic infiltration with a few plasma cells was also observed (Figure 2).

(b) Liver: Intra and interlobular blood vessels of liver revealed intravascular haemolysis. Diffuse fatty changes were the prominent feature of liver hepatocytes (Figure 3). Focal precipitation of haemosiderin pigments were also noticed in the liver parenchyma.

(c) Lungs: Lungs showed focal areas of edema, pneumonia and emphysema. The pneumonic lesions comprised of neutrophils, lymphocytes with a few macrophages and plasma cells. Exfoliations of alveolar epithelial cells were also marked. Blood vessels of lungs also showed intravascular haemolysis (Figure 4 and 5).

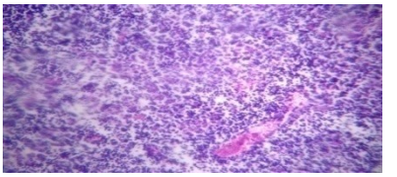

Figure 1: C. S. of spleen of infected mice showing diffuse infiltration of lymphocytes in the red pulp (H & E, ×400).

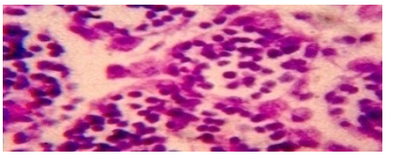

Figure 2: C. S. of spleen of infected mice showing diffuse infiltration of lymphocytes with subcapsular proliferation of endothelial cells (H & E, ×400).

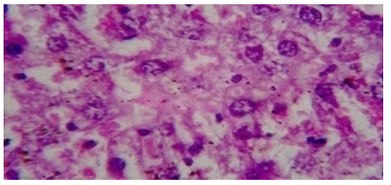

Figure 3: C. S. of liver of infected mice showing diffuse fatty degeneration of hepatocytes (H & E, ×1000)

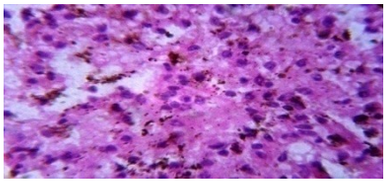

Figure 4: C. S. of lungs of infected mice showing focal area of edematous and pneumonic lesions with haemosiderin like pigments in lungs parenchyma (H & E, ×1000).

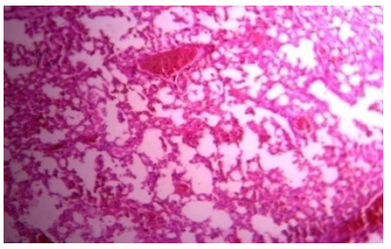

Figure 5: C. S. of lungs of infected mice showing congested blood vessels and emphysema (H & E, ×400).

(d) Kidney: Blood vessels of cortical and medullary regions of kidney were highly congested. Intravascular haemolysis and pigments of haemosiderin was also a common feature (Figure 6). Fatty and hyaline degenerations of the epithelium of proximal and distal tubules were also noticed.

(e) Intestine: Focal perivascular cuffing of lymphocytes in the submucosa was noticed. Diffuse infiltration of leucocytes particularly lymphocytes, neutrophils with a few macrophages and eosinophils in the villi of mucosa were also noticed.

(f) Heart: blood in the ventricle revealed massive haemolysis of red blood cells (RBC) with deposition of haemosiderin pigments. Focal haemorrhages in the myocardium were also evident (Figure 7).

Sulphadoxine and Pyrimithamine treated group:

(a) Spleen: Hyperplasia of follicular lymphocytes infiltrating the red pulp was the remarkable features.

(b) Liver: Diffuse fatty changes of the liver parenchyma were the prominent feature. Blood vessels of liver parenchyma showed intravascular haemolysis with haemosiderin pigments (Figure 8). Fibroblastic proliferation of interlobular septa was also noticed. Focal subcapsular fibroblastic proliferation with infiltration of lymphocytes, neutrophils and a few plasma cells were also observed.

(c) Lungs: Lungs was emphysematous and interalveolar septa were infiltrated by lymphocytes. Congested blood vessels showed partial intravascular haemolysis with sparse haemosiderin pigments.

(d) Kidney: Cortex showed the few atrophic glomeruli. Focal hyaline degeneration of both proximal and distal tubular epithelium with congestion of blood vessels both in cortex and medulla were noticeable features (Figure 9). Focal infiltrations of lymphocytes in the interstitial spaces of proximal and distal tubules were also observed.

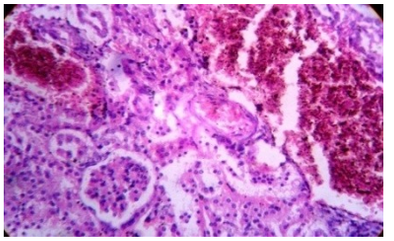

Figure 6: C. S. of kidney of infected mice showing intravascular haemolysis and haemosiderin like pigments in the cortex (H & E, ×400).

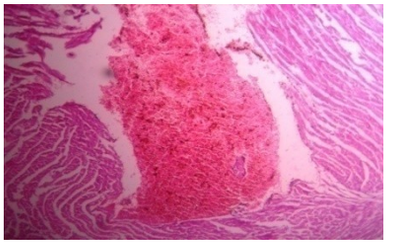

Figure 7: C. S. of heart of infected mice showing haemolysis with haemosiderin like pigments in the ventricle (H & E, ×100).

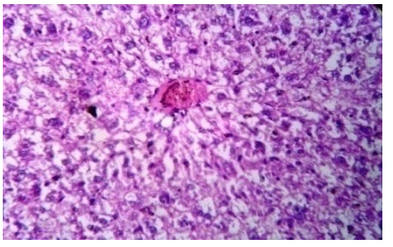

Figure 8: C. S. of liver of sulphadoxine-pyrimethamine treated mice showing diffuse fatty infiltration of the liver hepatocytes with intravascular haemolysis (H & E, ×400).

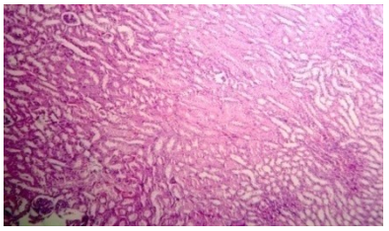

Figure 9: C. S. of kidney of sulphadoxine-pyrimethamine treated mice showing congestion of blood vessels in the cortex and hyaline degeneration of distal tubules (H & E, ×100).

Azithromycin treated group of mice:

(a) Spleen: Diffuse lymphocytic hyperplasia infiltrating the red pulp was the prominent features. Congestion of spleenic vessels with intravascular haemolysis was also noticed.

(b) Liver: Hepatocytes revealed mild fatty degenerative changes. Intravascular haemolysis in both inter and intralobular veins were prominent.

(c) Lungs: Focal edema and pneumonic lesions with lymphocytes, macrophages and a few plasma cells was noticed. Congestion of pulmonary veins with partial intravascular haemolysis with haemosiderin pigments were also revealed (Figure 10).

(d) Kidney: Intravascular haemolysis with pigments of haemosiderin found in the cortical region. Proximal tubular epithelium revealed focal hyaline degeneration (Figure 11).

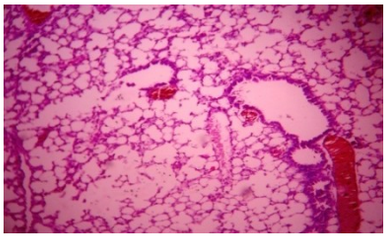

Figure 10: C. S. of lungs of azithromycin treated mice showing severe congestion of blood vessels with emphysema (H & E, ×400).

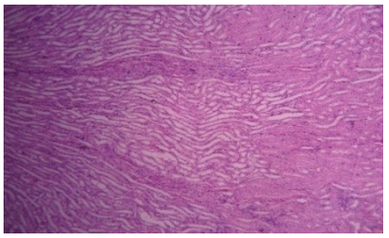

Figure 11: C. S. of Kidney of azithromycin treated mice showing hyaline degeneration of proximal tubules and focal interstitial cell proliferation in the medullary tubules (H & E, ×400).

Clindamycin treated group:

(a) Spleen: Follicular hyperplasia of lymphocytes with congested blood vessels was also noticed. Partial intravascular haemolysiswas the differentiating features.

(b) Liver: Diffuse fatty changes in the hepatocytes were the prominent features.

(c) Lungs: Lungs showed patchy areas of emphysema and pneumonia. Infiltrated cells in the pneumonic lesion were lymphocytes, neutrophils and a few plasma cells. Interalveolar septal edema is also present.

(d) Kidney: The epithelial cells of the both proximal and distal tubules were undergone hyaline degeneration. Blood vessels of the nephron around congested with intravascular haemolysis. Focal perivascular cuffing of lymphocytes with a few plasma cells were also noticed (Figure 12).

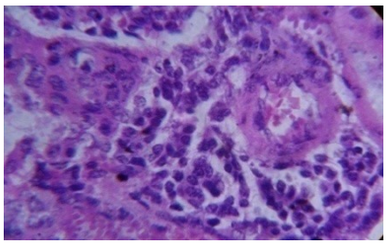

Figure 12: C. S. of Kidney of clindamycin treated mice showing perivascular cuffing of lymphocytes, mononucleate cells and few plasma cells (H & E, ×1000)

Diffuse proliferation of lymphocytes disrupting the follicular nature of white pulp, with intravascular haemolysis of RBC in the spleen of Gr II and Gr IV were the prominent feature (Figure 1 and 2), which indicated the infection was of acute in nature. Whereas, mice of Gr III and Gr IV showed follicular lymphoid hyperplasia of white pulp of the spleen, which was indicative of subacute type of infection. This was in agreement of findings of Suzuki et al. (1973) and Rifaat et al. (1981). A supra-capsular cyst (Figure 12) was found in one mice of Gr-II. The cyst was full of caseated mass. Subscapular proliferation of reticuloendothelial cells along with lymphocytic infiltration with a few plasma cells was also observed the same focal area. Such type of cyst might be produced due to trauma of needle ordue to liberated toxinsby the attached tachyzoites to form such caseated cyst in supracapsular region of spleen.

Diffuse fatty changes in the liver of the infected group (Gr II) and treated groups (Gr III, Gr IV and Gr V), might be due to toxins produced by the tachyzoites. Grimwood et al. (1983) also mentioned the same and named Toxofactor, a glycoprotein molecule (Mw 50-100 kDa)

respectively for such type of changes. All the blood vessels of spleen, liver, kidney and lungs showed intravascular haemolysis of Gr II, Gr III and Gr IV, except Gr V which showed partial intravascular haemolysis.

The same toxic factor might be responsible for intravascular haemolysis in the organs, which reflected erythrocytopenia and leucocytopenia (Sthal et al*.,* 1998). The Gr V mice treated with clindamycin, shown partial intravascular haemolysis. Clindamycin was moderately effective with its static effect on both extra and intracellular tachyzoites in macrophages, thus hindered the release of toxins from tachyzoites phagocytosed (Filice et al*.,* 1991).

Excretion of toxic metabolites through kidney inflicted hyaline degeneration of proximal and distal tubules and congestion of blood vessels both in cortex and medulla (Figure 19). The similar results also reported by Shalaby et al. (1993) that was revealed in Gr II, Gr III, Gr IV and Gr V mice in the experiment. Lungs of infected and group (Gr II) and treated groups (Gr III, Gr IV and Gr V) of mice had showed focal pneumonia and oedema. Emphysema developed as a sequele of same. Davidson et al. (1996) also focused that generalized toxoplasmosis as increased morbidity and mortality occurred from hepatitis and pneumonia. Mice treated with clindamycin (Gr V) had shown focal leucocytic infiltration in the villi and hyper activity of goblet cells in the intestine, which might be due to local infiltration of the drug during absorption through intestine.

Conclusion:

Since the mice are the most studied models for experimental toxoplasmosis the findings of the present study should be useful observation for future research on either acute toxoplasmosis in animal or human, or disseminated toxoplasmosis in AIDS patients.

Conflict of interest:

Authors have no conflict of interests in this study.

Data availability:

All the data in relation to the present study are available.

Author's contribution:

All authors equally participated in designing this article, data analysis and interpreting the results, drafting, editing the manuscript and approved the final version of the manuscript.

Ethical approval:

Obtained from the Institutional Animal Ethical Committee of WBUAFS vide reference no. E. Com. 55 dated 15.06.2010.

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