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Exploring the therapeutic potential of TGF-β in albino mice infected with hydatid cyst using immunohistofluorescence analysis

Afrah A. Sadek
Al-Karkh III, Baghdad Education Directorate, Baghdad, Iraq

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ABSTRACT

This study was designed to rate the efficacy of different antiparasitics against secondary echinococcosis in albino Mus musculus Balb/c borrowed laboratory IHF (immunohistofluorescence) staining method. Mice were experimentally infected with hydatid cysts and then treated with oxfendazole (OFZ), praziquantel (PZQ), and albendazole (ABZ) at 30 mg/kg, 40 mg/kg, and 10 mg/kg body weight, respectively. Treatment was administered once weekly for four months. The combination of OFZ + PZQ treatment presented the highest level of therapeutic efficiency; the other treatments presented descending levels in the sequence: OFZ only, OFZ + ABZ, and ABZ + PZQ. The IHF staining aimed at detection and evaluation of expression for TGF-β3 cytokine within liver and spleen tissues obtained from these animals. Oxfendazole can be considered an encouraging drug under the findings observed for hydatid disease.

KEYWORDS

TGF-β3, Echinococcus granulosus, Oxfendazole, hydatid cysts.

Introduction

Hydatidosis is among the major parasitic zoonoses, which causes considerable morbidity in humans and a wide range of domestic as well as wild animals. The causative agent of Hydatidosis is actually the larval (metacestode) stage of small tapeworms belonging to the genus Echinococcus-most commonly it is Echinococcus granulosus. These parasites have a normal lifecycle involving two obligatory hosts: carnivores (generally dogs or canids) playing the role of definitive hosts, and herbivorous or omnivorous typical intermediate hosts. Humans are accidental intermediate hosts and acquire infection through ingestion of parasite eggs passed in the feces of infected definitive hosts (Wahlers et al., 2012). The resistance of the host to invasion by Echinococcus granulosus is basically mounted through a Th1 immune response, while Th2 dominance makes the host more susceptible to infection (Zhang et al., 2003). These two immune responses are under the control of cytokines, major cellular mediators of innate and adaptive immunity at different stages of immune responses.

Growth factor TGF-β3 belongs to cytokines that regulate growth, differentiation, and apoptosis of cells as well as tissue remodeling and is secreted by immune cells including lymphocytes, phagocytes, and dendritic cells (Vaughn et al. 2000). Beta three also functions mainly as a moderator of immune response by decreasing inflammatory activity but increasing the fibrotic process during a parasitic infection (Harraga et al.,2003).

Because there has not been prior studies in Iraq on the use of oxfendazole—which is a derivative of benzimidazole—in combination with the commonly used antiparasitic agents albendazole and praziquantel, this paper discusses both immunological and pharmacological approaches to the treatment of hydatid disease. We have therefore conducted IHF staining for the evaluation of TGF-β3 expression in liver and spleen tissue from experimentally infected mice.

Materials and Methods

Animal Model

Ninety male Albino mice of 4–5 weeks age with a body weight of about 20±5 grams were obtained from the Drug Control Department, Baghdad. The animals were kept under controlled conditions of housing in the study

facility which ensured acclimatization before the commencement of experiments**.**

Preparation of Antigen and Protoscolices

It is the liver samples, containing hydatid cysts, collected from sheep that were naturally infected with cystic echinococcosis. The infection occurred at the Al-Shula slaughterhouse. Hydatid cyst fluid antigen (HCFAg) extraction was done following Smyth & Barrett's method (1980). Its protein concentration was determined to be 3.36 mg/ml by using Bradford's method from 1976. About 2,000 viable protoscolices were injected into each mouse based on the technique by Wangoo et al. (1989).

Inoculation Procedure

2000±5 protoscolices in 1 ml sterile fluid were injected into each mouse using a 21-gauge needle. The injection sites were sterilized with 70% ethanol. Control group mice received only the protoscolex solution. Negative control group mice were administered 0.2 ml of saline buffer.

Drug Treatment

Three antiparasitic agents were employed to treat the infected mice:

1. Albendazole (ABZ): 10 mg/kg (0.01 mg/kg HED) per Pérez-Molina et al. (2011)

2. Oxfendazole (OFZ): 30 mg/kg (0.04 mg/ml) per Gavidia et al. (2010)

3. Praziquantel (PZQ): 40 mg/kg (0.06 mg/ml) per Gavidia et al. (2010)

Treatment groups received either a single drug or drug combinations in the following regimens:

- OFZ alone
- OFZ + PZQ
- OFZ + ABZ
- ABZ + PZQ

OFZ was locally sourced (Synanthic®, Fort Dodge, Mexico) and administered orally at 0.25 ml weekly, beginning one week post-infection, for a total duration of four months. The negative control group received only distilled water. After four months, mice were euthanized for tissue collection and analysis.

Histopathology

Tissue samples were processed using an Olympus compound microscope equipped with a Konica camera. Images were captured using 100 ASA sensitivity film to document structural alterations.

Immunohistofluorescence Staining

To evaluate TGF-β3 expression,

immunohistofluorescence was performed using the FITC-labeled antibody method:

1. Tissue Slicing: Liver and spleen samples were sectioned (4–5 µm thick) using a rotary microtome and mounted on charged slides.

2. Dewaxing and Rehydration: Slides were incubated in a 60°C oven, followed by xylene washes and graded alcohol series.

3. Blocking and Staining:

- Tissue sections were encircled with a hydrophobic barrier.
- Peroxidase activity was blocked and non-specific sites were blocked with protein block or BSA.
- Primary antibody (anti-TGF-β3) diluted at 1:50 was applied and incubated at 37°C for 1 hour or overnight.
- FITC-conjugated secondary antibody was added and incubated.

4. Mounting and Observation: Slides were dehydrated, cleared with xylene, mounted with DPX, and examined under an Olympus fluorescence microscope.

Results

TGF-β3 Expression in Liver and Spleen

Immunohistofluorescence results showed visible expression of TGF-β3 in the treated mice. In the spleen sections, (Plates 1-4) there was massive fluorescence from the lymphocytes which means that the expression of this cytokine is at a high level. In a similar manner, hepatocytes also expressed bright green fluorescence within cytoplasm as seen in Plates 5-8. This indicates active immune modulation from both organs after treatment.

Plate 1: Spleen tissue from mice treated with oxfendazole (OFZ), illustrating TGF-β3 expression as indicated by bright green fluorescence in lymphocytes using FITC-based immunostaining.

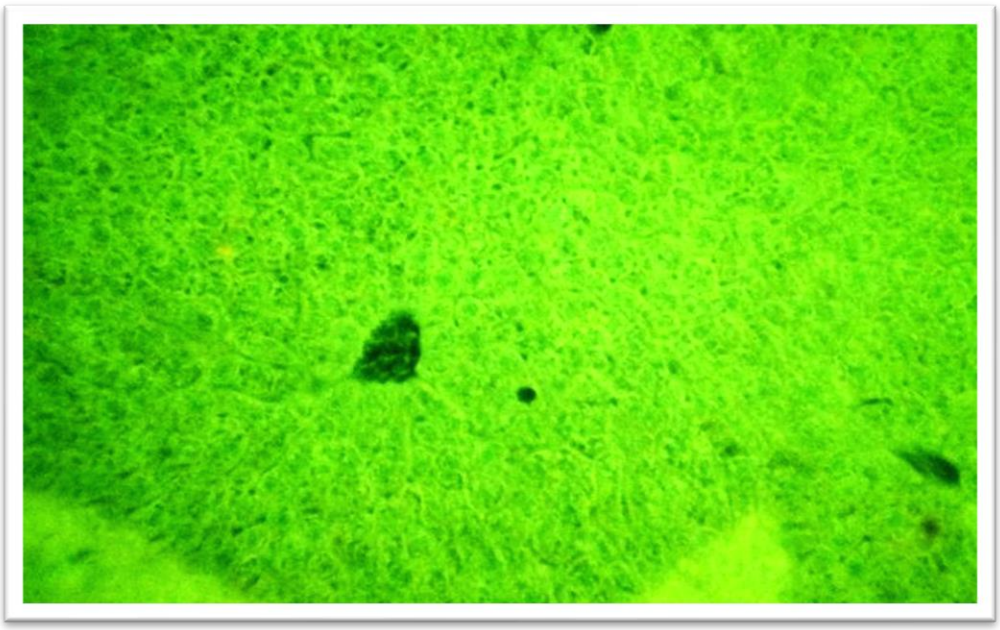

Plate 2: Liver section from OFZ-treated mice displaying TGF-β3 protein expression, evident as bright green fluorescence in both lymphocytes and hepatocyte cytoplasm using the FITC staining technique.

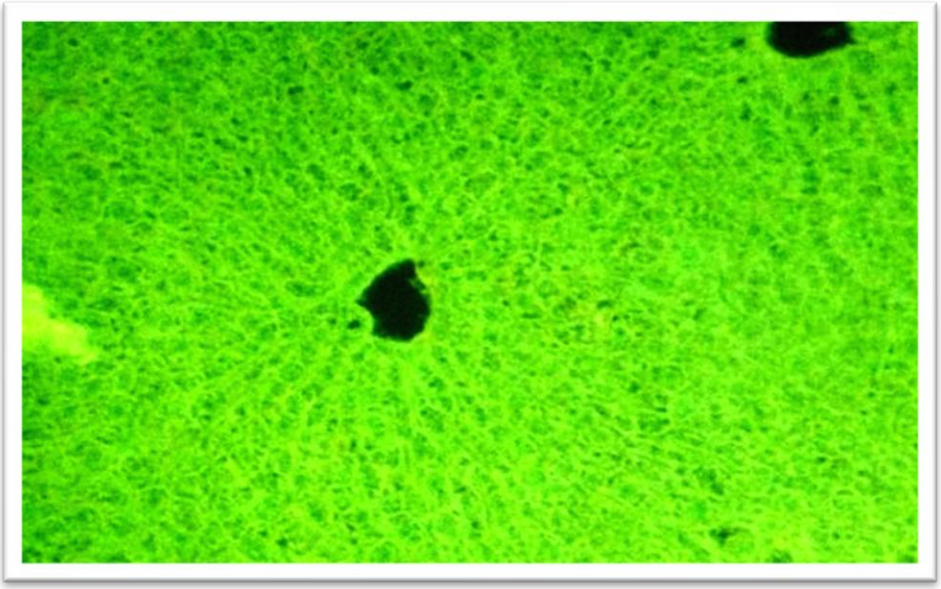

Plate 3: Liver tissue from mice treated with a combination of OFZ and PZQ, showing TGF-β3 expression. Bright green fluorescence is observed in lymphocytes and diffusely within the cytoplasm of hepatocytes using the FITC staining method.

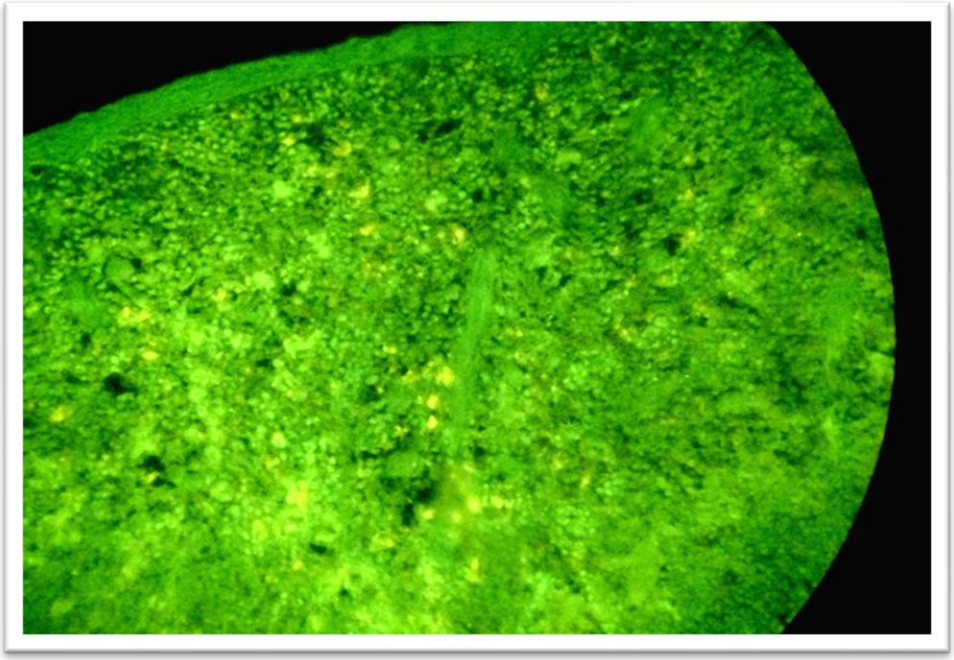

Plate 4: Spleen section from mice treated with OFZ and PZQ, revealing TGF-β3 expression as bright green fluorescence in lymphocytes, visualized using the FITC staining technique.

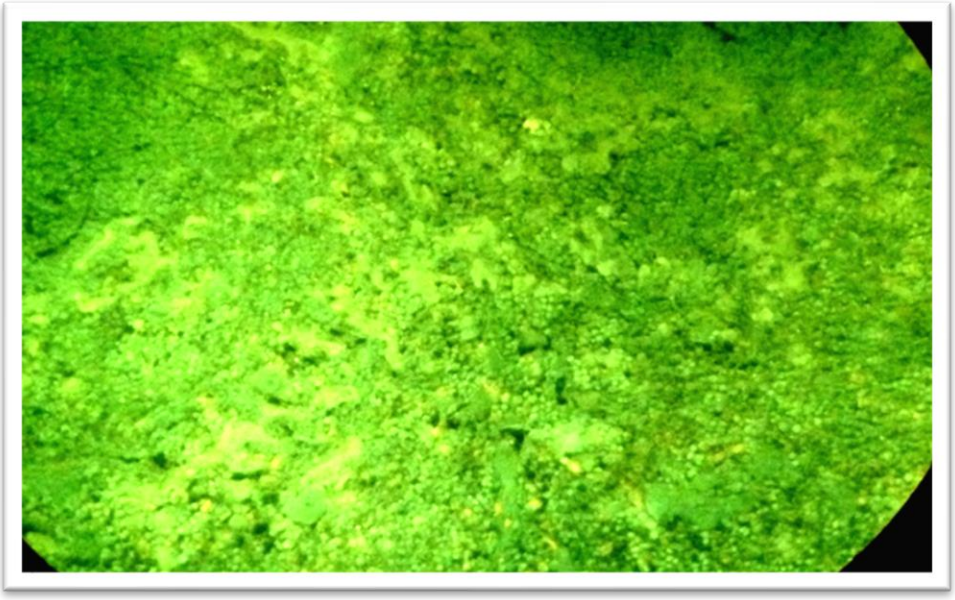

Plate 5: Spleen tissue from mice treated with a combination of OFZ and ABZ, showing TGF-β3 expression as bright green fluorescence in lymphocytes using the FITC staining method.

Plate 6: Liver section from mice treated with OFZ and ABZ, displaying TGF-β3 expression as bright green fluorescence in lymphocytes and within the cytoplasm of hepatocytes, as detected by the FITC-based immunohistochemical method.

Plate 7: Liver tissue from mice treated with ABZ and PZQ, showing TGF-β3 expression indicated by bright green fluorescence in lymphocytes and hepatocyte cytoplasm, visualized using the FITC staining technique.

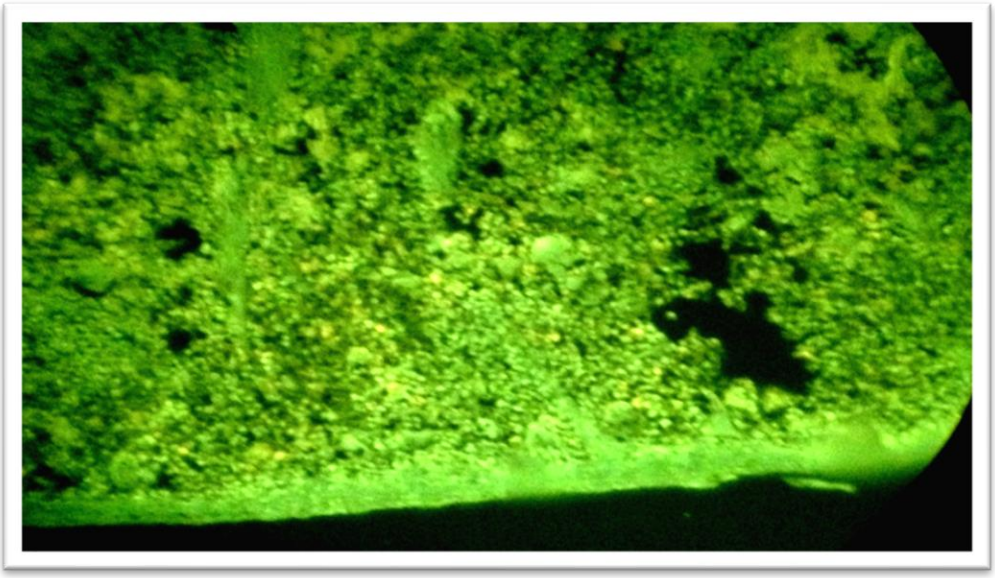

Plate 8: Spleen section from mice treated with ABZ and PZQ, showing expression of the TGF-β3 protein in lymphocytes, marked by green fluorescence using the FITC staining method.

Discussion

TGF-β was mainly expressed in the red pulp of the spleen. Macrophages and lymphocytes expressed the strongest staining reaffirming other previous findings where it played a role in immune regulation. In the liver, the cells around the central vein mostly expressed TGF-β signifying its homeostatic function while relating it to inflammation control. IFAT results indicated different antigen concentrations between treatment groups with bright green signals indicating high antigen concentration and tissue specific response differentiating fluorescence intensity between treated groups. It validates TGF-β as a primary immune modulation marker that controls both innate and adaptive immune responses in one organ and is involved in tissue repair among other pathological processes, for instance, fibrosis in another organ such as the liver. Looking at cell movement, it can be said that these cytokines likely make both leukocytes and endothelial cells active to release more cytokines. Also, the use of Freund's complete and incomplete adjuvant during immunization most probably enhanced the stimulation of bone marrow lymphocytes, macrophages, and T cells, hence an increased production of cytokines. These results were found to agree with earlier work done by Reuben et al. (1979), in which it was noted that the

application of a tuberculosis vaccine for the stimulation of macrophages increases lymphocyte numbers and colony-stimulating factor secretion.

TGF-βis a multifunctional cytokine, the major normal growth inhibitor of most cells in vitro or in vivo. In every state, its effects depend not only on cell type but also on the differentiation state and microenvironment of the cell. It has complex and rather elusive roles in liver regeneration. TGF-β effectively participates in regeneration as reported by (Fausto et al., 1991). The liver contains many cell types of hepatic, biliary, and visceral origin which coordinate with this factor during the restoration of mass; it may be facilitating division through matrix remodeling while restraining proliferation. Some researches have mentioned that TGF-β inhibits DNA synthesis in hepatocytes, and among them are Martinez-Hernandez and Amenta (1995) and Uyama et al. (2002). Diseases for TGFβ3 mutations include respiratory disease (Rienhoff et al., 2013) and osteoporosis (Matyas et al., 2014; Bertoli-Avella et al., 2015). Normal hepatocytes do not have the ability to activate latent TGFT however, regenerating hepatocytes acquire this ability (Jakowlew et al., 1991). TGFβ is a negative regulator of hepatocyte proliferation. It controls liver size and immunosuppressive cytokines (Huang et al., 2013).

Granulomatous lesions and inflammatory foci of the liver and other visceral tissues usually express TGF-β at the immunosuppressive level. These are most often in the vicinity of damaged cysts by Echinococcus granulosus. Enhanced TGF-β expression has been noted in sinusoidal epithelial cells of the liver as well as fibroblasts, showing that visceral cells, particularly epithelial cells of the liver, are the main source of TGF-β production. They found this fact in research conducted recently and confirmed that it acts with a paracrine inhibitory effect on hepatocyte growth (Nakatsukasa et al., 1990; Jakowlew et al., 1991; Fausto et al., 1995).

Ueda et al. (2003) stated that TGF-β1 is synthesized in the spleen and released into portal circulation. Expression levels in the spleen have been seen to correlate with plasma concentrations, meanwhile other organs, such as the pancreas and stomach, also contribute TGF-β1 in portal blood exerting similar anti-proliferative effects on hepatocytes (Kahn et al., 1990). The exact mechanism of increased accumulation of TGF-β by the spleen during liver regeneration has not yet been explained (Lin et al., 2011). These findings bring researchers a step closer to understanding how Echinococcus multilocularis invades homeostasis in the liver through changes in pathways of metabolism and gene expression prompted by tissue damage leading to cytokine production (Falk et al., 2005). In mice, immunization with Protozoa elicits both Th1 and Th2 responses (Al-Qaoud & Abdel-Hafez, 2005).

As the parasite grows within the host, patients with hydatid cysts begin to mount a serum accumulation of Th2 cytokines. Zhang et al.. (2003) found that tissue damage and apoptosis seem to provoke the formation of cytokines that may actually mediate the effects of drugs and relief their side effects.

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