Experimental Parasitology 130 (2012) 58–62

Contents lists available at SciVerse ScienceDirect

Experimental Parasitology

journal homepage: www.elsevier.com/locate/yexpr

Evidence of intra-hepatic vascular proliferation remodeling early after cure in experimental schistosomiasis *mansoni*: An immunohistochemical descriptive study

Nashwa S. Abdel Fattah [a,*], Naglaa S. Ahmed [b]

[a] Parasitology Department, Faculty of Medicine, Ain-Shams University, Egypt
[b] Pathology Department, Faculty of Medicine, Ain-Shams University, Egypt

ARTICLE INFO

Article history:
Received 9 June 2011
Received in revised form 29 September 2011
Accepted 30 September 2011
Available online 12 October 2011

Keywords:
Angiogenesis
Schistosoma
Liver fibrosis

ABSTRACT

Experimental studies have demonstrated the occurrence of angiogenesis, blood vessels formation from pre-existing vessels, in the initial phase of bilharzial granuloma formation and during fibrosis progression in chronic hepatic schistosomiasis. Paradoxically, a recent work demonstrated an occurrence of angiogenesis during fibrosis regression months after curative treatment. Studies regarding the in situ kinetics of blood vessels in the phase of granuloma resolution and liver tissue healing early after treatment are lacking. The current work compared the kinetics of blood vessels by immunohistochemical staining using CD34, vascular endothelial growth factor (VEGF) and actin in the livers of normal control mice, *Schistosoma mansoni* infected mice and mice 2 weeks after curative treatment. The present study demonstrated a process of angiogenesis remodeling in the liver in the curative phase of hepatic schistosomiasis during the stage of granuloma resolution. Such finding raises the evidence of the importance and potential beneficial effect of vascular proliferation in the process of healing and restoration of liver tissue functions. Thus, blocking of angiogenesis may not represent the appropriate therapeutic target for the early treatment of schistosomal liver fibrosis.

1. Introduction

Schistosomiasis *mansoni* is a major helminthic disease in tropical areas characterized by parasite egg-induced granulomatous inflammation and fibrosis (WHO, 1993). Experimental studies have demonstrated angiogenesis, blood vessels formation from pre-existing vessel, in the initial phase of bilharzial granuloma formation (Loeffler et al., 2002). Also, the relationship of angiogenesis and fibrosis has recently been demonstrated to play a role in the pathogenesis of fibrosis during some pathological conditions including hepatic schistosomiasis (Medina et al., 2005; Souza et al., 2006b). Souza et al. (2006a) clearly indicated that angiogenesis preceded the collagen synthesis during early septa formation and fibrosis.

Over the past decade, multiple mechanisms underlying hepatic fibrogenesis have been uncovered. Vascular endothelial growth factor (VEGF) is known to play a role in regulating vasculogenesis, inducing angiogenesis and endothelial cell proliferation (Shi et al., 2003). The process of fibrosis formation from granular tissue in the liver is thought to be dependent on activation of a special cell-type, the parasinusoidal stellate cell (Friedman, 2008a). Stellate cells are well known as the major source of the fibrillar collagens and other components of the liver scar. Also, portal fibroblasts as well as circulating mesenchymal cells derived from the bone marrow are important sources of matrix proteins in fibrosis. Stellate cells, portal myofibroblasts, and bone marrow derived cells converge in a complex interaction with hepatocytes and immune cells to provoke scarring in response to liver injury (Jiao et al., 2009).

Previous studies have revealed that *S. mansoni* soluble egg antigens (Loeffler et al., 2002; Kanse et al., 2005) induce the proliferation of endothelial cells and up-regulate the vascular endothelial growth factor (VEGF) and angiogenesis.

Progressive fibrosis ultimately leads to increased mortality and morbidity from portal hypertension, end-stage liver failure and ultimately cirrhosis, and is associated with an increased risk of hepatic malignancies (Gines et al., 2004).

Abundant evidence indicates that fibrosis is a dynamic process and a prominent feature of liver fibrosis is that of extracellular matrix turnover, including not only its synthesis, but also its degradation (Arthur, 2000). The reversibility of fibrosis in animal models has stimulated the enthusiasm for anti-fibrotic treatment strategies targeting critical regulatory pathways of liver fibrosis (Wynn, 2008; Friedman, 2008b). The role played by angiogenesis in fibrosis development raises the suggestions of the use of

* Corresponding author. Address: 25b Omar Lotfy St., 8th District, Nasr City, Cairo, Egypt.
E-mail address: n_marakby@hotmail.com (N.S. Abdel Fattah).

0014-4894/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.exppara.2011.09.014

anti-angiogenic drugs to become a new treatment of chronic liver diseases (Lai and Adams, 2005). However, no agents are yet approved for this indication (Ghiassi-Nejad and Friedman, 2008).

Since angiogenesis appears to be a key factor for new connective tissue synthesis, an investigation on the behavior of blood vessels during schistosomal granuloma formation and healing seemed to be appropriate. Although this behavior has been investigated in the phase of granuloma formation by some authors, studies regarding the in situ kinetics of blood vessels in the phase of granuloma resolution are lacking.

Andrade et al. (2006) and Andrade and Santana (2010) demonstrated that a process of angiogenesis was occurring during fibrosis regression of hepatic schistosomiasis four months after the curative treatment of *Schistosoma mansoni* infected mice. They assumed that angiogenesis may function along a two-way tract, being important during fibrosis production as well as during fibrosis regression, a finding that stimulated this work to be performed to clarify the role of angiogenesis during early cure from schistosomiasis and whether antagonism of angiogenesis may be of value for the prevention and early treatment of schistosomal liver fibrosis.

2. Materials and methods

2.1. Experimental animals

Thirty female CD-1 Swiss albino mice (weight, 20 ± 2 g), bred and maintained under conventional conditions at the experimental animal research unit of the Biological Supply Unit at Theodor Bilharz Research Institute (Giza, Egypt), were used. They were fed a standard commercial pelleted diet and were kept in an air-conditioned room at 21 °C. Ethical considerations: All animal experiments were conducted in accordance with valid international guidelines for animal experimentation.

2.2. Schistosome infection

S. mansoni cercariae were provided by the Malacology Lab of the Schistosomiasis Unit (SBSP), Theodor Bilharz Research Institute (TBRI), where laboratory-bred *Biomphalaria alexandrina* are maintained. Infection was subcutaneously performed using freshly shed 80 *S. mansoni* cercariae to each mouse.

2.3. Drugs

Praziquantel (Biltricide, 600 mg tablets) was provided by Alexandria Co. for Pharmaceuticals (Alexandria, Egypt). It was suspended in 7% Tween-80 and 3% alcohol at a concentration of 40 g/l. Praziquantel was administered to mice at a single dose of 500 mg/kg (El-Hoseiny, 1999) and was given to mice intragastrically.

2.4. Experimental groups

Control group: Ten mice were non-infected and non-treated served as the control.

Infected group: Ten mice were infected and non-treated.

Treated group: Ten mice treated with praziquantel 6 weeks after their infection.

All mice were sacrificed 8 weeks post-infection.

2.5. Histopathology and immunohistochemical staining

Biopsies were fixed into formalin (10%) for 2 h followed by paraffin inclusion for histological analysis. Paraffin sections were cut at 4 μm thick and subjected to routine hematoxylin and eosin staining and immunohistochemical staining for CD34 and VEGF to stain endothelial cells and actin to stain myofibroblasts and pericapillary pericytes.

Paraffin sections were fixed on poly-L-lysine coated slides, dried overnight in a 60 °C oven. Then they were deparaffinized and dehydrated. The slides were treated in microwave oven in ready to use antigen retrieval citrate buffer for 10 min, then sections were left to cool at room temperature for 20 min. Slides were stuck to cover plates using PBS pH 7.6 and placed in sequenza center for immunostaining. Endogenous peroxidase activity was blocked by adding 2–3 drops of hydrogen peroxide blocking serum for 5 min at room temperature. Then sections were rinsed well with PBS for 5 min. Two drops of protein blocking serum were added for 10 min and not followed by rinsing. The primary antibody was applied by adding three drops to each section [CD34: a monoclonal mouse antibody (Labvision, catalog No. MA1-37329) supplied as 1 m1 of ready to use antibody], [VEGF: a monoclonal mouse antibody (Labvision catalog No. MS-146-R7) supplied as 1 ml of ready to use antibody] and [Actin: a monoclonal mouse antibody (Labvision, catalog No. MA1-37021) supplied as 1 ml of ready to use antibody] incubated for 2 h at room temperature, followed by rinsing in PBS pH 7.6. The secondary antibody was applied by adding two drops of biotinylated secondary antibody to each section, a supersensitive immunodetection system (Bigenex, catalog No. AD 000-SL), for 30 min at room temperature. Slides were then rinsed in PBS pH 7.6. Two drops of peroxidase labeled streptavidin were added for 20 min at room temperature, then rinsing with PBS pH 7.6. Slides were incubated for 10 min with substrate chromogen (DAB) mixture. Slides were then rinsed with distilled water and immersed in Harris hematoxylin for 3 s, rinsed in tap water, dehydrated in absolute alcohol. Lastly, slides were cleared in xylene, mounted by Canada balsam and covered by glass cover. Negative control slides were processed as by the previous immunostaining procedure, but the primary antibody was omitted from the steps, and PBS was used instead.

3. Results

In the non-infected non-treated control group, vascularity of the livers showed its normal architecture. CD34 and VEGF were expressed in the vascular endothelial cells and sinusoidal endothelial cells; smooth muscle actin was expressed exclusively in vessel walls (portal vessels and centrilobular veins).

In the infected group, we found that endothelial cells, immunohistochemically identified by CD34 and VEGF, are present in great numbers within schistosomal periovular granulomas (Fig. 1a, b). Vascular proliferation forms vascular collars around some granulomas (Fig. 1c). In some areas, when there is fusion of several granulomas, the proliferating small blood vessels appear prominent in the inter-granulomatous tissue and the fibrous tissue assumes an angiomatoid appearance (Fig. 1d). The newly formed granulation tissue shows many actin-staining proliferating spindle-shaped cells most probably myofibroblasts and pericapillary pericytes (Fig. 1e). The schistosomal granulomatous reaction causes partial destruction of the vascular wall of some branches of portal veins (Fig. 1f).

In the treated group, several focal areas of vascular proliferation were seen associated with the presence of a positive staining for CD34 and many actin-stained proliferating cells (Fig. 2a–d). Endothelial proliferation, identified by positive immunostaining for VEGF, within the involuting granulomas and the vascularized areas of fibrosis are quite evident (Fig. 2e, f).

4. Discussion

By means of immunohistochemical staining, the present study demonstrated the participation of angiogenesis in hepatic