Experimental Parasitology 128 (2011) 44–49

Contents lists available at ScienceDirect

Experimental Parasitology

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

Evaluation of the role of angiogenic factors in the pathogenesis of schistosomiasis

F. Shariati [a], J.L. Pérez-Arellano [b], C. Carranza [b], J. López-Abán [a], B. Vicente [a], M. Arefi [c], A. Muro [a,*]

[a] Laboratorio de Inmunología y Parasitología Molecular, CIETUS, Facultad de Farmacia, Universidad de Salamanca, 37007-Salamanca, Spain
[b] Departamento de Ciencias Médicas y Quirúrgicas, Facultad de Ciencias de la Salud, Universidad de Las Palmas de Gran Canaria, 35080-Las Palmas de Gran Canaria, Spain
[c] Servicio de Hematología, Hospital Clínico Universitario de Salamanca, Spain

ARTICLE INFO

Article history:
Received 29 July 2010
Received in revised form 10 January 2011
Accepted 12 January 2011
Available online 4 February 2011

Keywords:
Schistosoma mansoni
Schistosomiasis
Angiogenesis
VEGF
FGF2
Endostatin

ABSTRACT

Schistosomiasis is one disease produced by helminths, which affect many people in tropical areas. Granuloma formation is the main mechanism involved in the pathogenesis of this disease. Experimental studies have demonstrated angiogenesis (blood vessels formation from pre-existing vessels) in the initial phase of granuloma formation. In the present work, VEGF (vascular endothelial growth factor) levels were analyzed in sera from people diagnosed with different helminthic infections. Patients with schistosomiasis and filariasis had significantly high VEGF levels in compared with healthy people and patients diagnosed with hookworms. In addition, the effects of angiogenesis inhibition using anti-angiogenic factors (endostatin) were evaluated in a schistosomiasis murine model. A lesion decrease was observed in mice infected with *Schistosoma mansoni* and treated with endostatin. Finally, mechanisms of angiogenesis induction were studied and observed that cercariae antigens stimulated the angiogenic factors by host alveolar macrophages.

1. Introduction

Schistosoma mansoni is one of the blood flukes, which inhabit in hepatic portal and mesenteric veins of the host. Female adult worms produce hundreds to thousands of eggs per day, containing ciliated miracidium larva, which secretes proteolytic enzymes to migrate into lumen of the intestine (Gryseels et al., 2006). Early pathological changes arise after mechanical occlusion of the microvasculature by the eggs causing acute vasculitis with endothelial damage and necrosis. Granuloma formation results from a delayed hypersensitivity response generated by the host against antigens secreted by the parasite eggs (Van de Vijver et al., 2006). In initial phases, the inflammatory response is intense with important neovascularization. Moreover, fibrosis was produced in later phases and it was responsible of the pathological disorders of this disease (Silva et al., 2006). However, variations in the magnitude of the disease have been described among different hosts and specifically among different mouse strains (Stavitsky, 2004).

Angiogenesis, the formation of new endothelial vessels from pre-existing post-capillary venule, is a characteristic feature of inflammatory diseases, wound repair and cancer (Carmeliet and Jain, 2000). The angiogenic activity depends on the balance or imbalance between angiogenic and angiostatic mediators. Remodelling and degradation of the surrounding stroma is essential to start an angiogenic phenotype. These stromal changes facilitate recruitment and activation of leucocytes, fibroblast and endothelial cells. While granulomas are traditionally considered to be avascular structures, schistosome granulomas should be seen as an inflammatory condition that initiates a variable degree of wound healing response in which angiogenesis and fibrosis are highly involved (Baptista and Andrade, 2005).

The aim of this work was to evaluate the role of angiogenic factors in the pathogenesis of schistosomiasis. Firstly, vascular endothelial growth factor (VEGF) in sera of patients diagnosed of schistosomiasis and other helminthic diseases was studied. Secondly, the effects of endostatin (angiogenesis inhibitor) in mice infected with *Schistosoma mansoni* were determined. Finally, VEGF and fibroblastic growth factor (FGF2) expression from alveolar macrophages stimulated with different *S. mansoni* antigens were analyzed.

2. Materials and methods

2.1. Human population

The study group was formed by 53 patients from sub-Saharan areas who mainly were from Mali and Nigeria. They had recently arrived in Canary Island of Spain. They have been living in Spain since 6 months ago and have been diagnosed only by one parasite as shown in Table 1. All patients were diagnosed by direct parasitological tests included: (i) coprology in three stool samples for ova

* Corresponding author. Address: Laboratorio de Inmunología y Parasitología Molecular, CIETUS, Facultad de Farmacia, Universidad de Salamanca, Avda. Campo Charro, s/n. 37007 Salamanca, Spain. Fax: +34 923294515.
E-mail address: ama@usal.es (A. Muro).

0014-4894/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.exppara.2011.01.016

Table 1
Epidemiological characteristics (age and sex) and count of eosinophils in peripheral blood and VEGF measured by ELISA in sera of Sub-Saharan Africa immigrant patients infected with *Schistosoma*, filariae and hookworms. Healthy patients from endemic area are included as control group.

Group of study	Number of patients	Age in years (Mean ± SD)	Sex (Male %)	Eosinophil/μl (Mean ± SD)	VEGF pg/ml (Mean ± SD)
Healthy control group	18	28.9 ± 8.6	94	176 ± 107	123.7 ± 104.0
Infected with *Schisotosoma*	25	21.5 ± 6.3	96	936 ± 542*	274.2 ± 213.0*
S. mansoni	10	24.5 ± 4.7	90	842 ± 669*	213.6 ± 164.0
S. haematobium	15	19.5 ± 7.4	100	999 ± 458*	314.5 ± 237.0
Infected with filariae	13	28.8 ± 10.7	77	1272 ± 638*	250.3 ± 161.0*
Infected with hookworms	15	23.8 ± 4.7	93	880 ± 429*	135.6 ± 109.0

SD, Standard deviation; %, Percentage.
* Statistical differences in comparison with health control group $p < 0.05$.

and parasites by using Kato-Katz and Ritchie techniques, (ii) identification of *Schistosoma haematobium* by examination of urine for eggs with sedimentation, (iii) Knott's test for detection of microfilaremia in blood and (iv) immune chromatographic test (ICT Filariasis, Binax, Portland and Maine) for *Wuchereria bancrofti* detection.

Healthy control group was formed by 18 immigrants from the same geographic area as patients of the study group. For all patients we have done clinical examination including a systemic analytical study, parasitological tests and direct serological examination. There were not detected eosinophilia and presence of parasite in healthy control group. The HUIGC (Hospital Universitario Insular de Gran Canaria)-IRB approved an oral consent process that included a written summary and a short form. The oral presentation and the short form were made in a language understandable to the subject. The interpreter (witness) was fluent in both the language of the participant and the language of the consent. The written summary was signed by the (1) witness and (2) person obtaining consent. The short form was signed by the (1) witness and (2) the participant or participant's legally authorized representative.

2.2. Parasite and antigens

BALB/C mice were maintained under standard conditions in an environment with controlled temperature and humidity with 12 h light/dark cycle and free access to water and food. *S. mansoni* cercariae were obtained from *Biomphalaria glabrata* snails previously infected with *S. mansoni* miracidia (Freire et al., 2003). Mice were sacrificed on the 7 week post-infection (pi) and adult worms were recovered from the hepatic portal and mesenteric veins by perfusion technique (Chatterjee et al., 2007).

Adult worm antigen (AWASm) and cercarial antigen (CSm) from *S. mansoni* were used accordance with Dunne et al. (1997). Briefly, *S. mansoni* worms were recovered by portal perfusion buffer. Recovered worms were washed free of erythrocytes and then snap frozen in liquid nitrogen. Frozen worms were ground into a paste, allowed to thaw and then centrifuged at 10,000*g* at 4 °C for 1 h. After that 1 mM *N*-Tosyl-1 phenylalanine chloromethyl ketone and 1 mM phenyl-methylphonylfluride (Sigma Chemical Co., St Louise, MO) were added. The soluble material was centrifuged, filtered through a 0.22 μm sterile filter and then stored at −80 °C. Cercarial antigen (CSm) was obtained from cercariae harvested within 3 h of being shed from snail, snap frozen in liquid nitrogen and then processed identically to adult worm antigen.

2.3. VEGF detection in human samples

VEGF determination was done in serum from human samples by ELISA technique (Vascular Biotrak Endothelial Growth Factor [(h) VEGF] human ELISA System, GE Healthcare Buckinghamshire, UK) in accordance with manufacture instructions.

2.4. Mice experimental design

Three groups of six-weeks-old CD1 mice weighing 16–25 g were used as follows: uninfected group, mice infected with 150 *S. mansoni* cercariae per animal, mice infected with 150 *S. mansoni* cercariae and treated with 2.5 mg/kg of endostatin (Sigma) at 36, 38, 40 and 42 days post-infection (O'Reilly et al., 1997). From these animals sera and blood samples were taken weekly from week 0 to 7 after challenged.

Animals were sacrificed at week 7 post-infection (pi) and the following parasitological parameters were assessed: (i) recovery worms, (ii) eggs per gram (epg) determination from liver and intestine (iii) and granulomas count on liver. In addition, liver and intestine were harvested and adult worms were collected and counted. Blood samples were collected and analyzed on hemocytometer Hemavet 950 (Drew Scientific Group). Mice were housed and handled under standard animal laboratory conditions according to the EU regulations. All animal procedures were approved by the University of Salamanca Animal Care and Committee.

2.5. VEGF and FGF2 expression in mice infected with S. mansoni by RT-PCR

Total RNA isolated from liver or intestine of all mice was used to analyze the expression of VEGF and FGF2 by RT-PCR as described previously (Shariati et al., 2009). Briefly, total cellular was isolated with the Rneasy Mini Kit (Qiagen GmbH, Hilden Germany), according to manufactures instructions. RNA was reverse transcribed with the First Strand cDNA Synthesis kit (Roche), according to manufactures instructions. Primers specific for rat VEGF were sense, 5′-CTGCTCTCTTGGGTGCACTGG-3′ and anti-sense, 5′-CACCGCCTTGGCTTGTCACAT-3′. VEGF-PCR reactions were carried out through reverse transcription incubation at 94 °C for 5 min, 35 cycles of 94 °C for 1 min, 55 °C for 1 min, 72 °C for 1 min and a single cycle at 72 °C for 7 min. Primers specific for detection of FGF2 were sense, 5′-GCCGGCAGCATCACTTCGCT-3′ and anti-sense, 5′-CTGTC CAGGCCCCGTTTTGG-3′. FGF2-PCR reactions were carried out through reverse transcription incubation at 94 °C for 2 min, 50 cycles of 94 °C for 30 s, 60 °C for 30 s, 72 °C for 1 min and a single cycle at 72 °C for 5 min. PCR product were analyzed by electrophoresis in agarose gel stained with ethidium bromide with glyceraldehydes 3-phosphate dehydrogenase (GAPDH) as internal control. Specific primers for GADPH were sense, 5′-GGTCGGT GTGAACGGATTTG-3′ and anti-sense, 5′-GTGAGCCCCAGCCTTCTCC AT-3′ that were used as positive control (Shariati et al., 2009).

2.6. VEGF and FGF2 expression in alveolar macrophage stimulated with different antigens of S. mansoni

Macrophages were obtained from male wistar rats of 250–300 g by bronchoalveolar lavage (BAL) as previously described (Porter

et al., 2006). Rats were euthanized with an intraperitoneal of sodium pentobarbital (100 mg/kg bw). Lungs were washing with aliquots of 5 ml of sterile phosphate-buffered saline (PBS, pH 7.4) using sterile Teflon catheter (VYCON Code 123.06, lot 220,987, Vigon, Spain) for collection of BAL fluid. Fluid was immediately withdrawn by gentle suction and was collected in 50 ml tubes and centrifuged at 1000*g* at 4 °C temperature to pellet the cells.

The cells were suspended in 1 ml fresh complete medium containing 44 ml of Dulbecco's modified eagle medium (DMEM), 2 mM glutamine, 10% heat inactivated fetal bovine serum (FBS) and antibiotic solution[(with 10,000 U penicillin and 10 mg streptomycin per ml) (Sigma Chemical Co., St Louise, MO)]. Viability of cells was estimated by the mitochondrial reduction of MTT (3-[4,5-dimethyl-thiazol-2-yl]-2,5-diphenyl tetrazlium bromide) (Sigma) to formazan which was found to be more than 87.5% accordance with Andrade et al. (2004). Cells were then counted, seeded in a 12-well cell culture cluster (Corning, NY 14831, USA) at the density of 1×10^6 cells/well and incubated at 37 °C in 5% CO_2 incubator (RS Biotech, Galaxy). After 2 h, non-adherent cells were washed away with sterile PBS and adherent cells (macrophages) were incubated in fresh complete medium. Cells were treated with a dose of 10 μg/ml lipopolysaccharide (LPS; Sigma; positive control) and 0.1–50 μg/ml of *S. mansoni* antigens (AWASm and CSm). Non-stimulated macrophages were used as negative control. All experiments were performed in triplicate.

Total RNA was extracted from alveolar macrophages using an RNeasy Mini Kit (Qiagen GmbH, Hilden Germany.). A total of 1 μg RNA was used as template for the first-strand DNA synthesis. Primers specific and PCR reactions for VEGF, FGF2 and GAPDH were done as above.

2.7. Statistical analysis

Data of VEGF in human sera, number of eosinophils and epidemiological data of human population were reported as mean ± standard deviation (SD). Linear regression was performed with INSTAT programme. The overall differences among groups were compared by non-parametric Kolmogorov–Smirnov analysis. Data of parasite recovery, eggs per gram of liver or intestine in mice, blood cells and serological data were reported as mean ± SD. Differences in mice groups were performed by analysis of variance (ANOVA). When global differences were detected a post-ANOVA test using the Fisher least significant differences (LSD) analysis was applied. Differences between means were considered statistically significant at $p < 0.05$. All statistical analyses were performed using Statworks, Statview 4.5 and INSTAT software packages for an IBM computer.

3. Results

3.1. Evaluation of VEGF detection protein in human population

Result showed significantly differences in VEGF detection protein between patients with schistosomiasis and filariosis and healthy control patients ($p < 0.05$) (Table 1). Also, no difference was found between patients infected with *S. mansoni* and *S. haematobium*. Moreover, it was observed that there was no difference between patients diagnosed with hookworm infections and healthy control patients.

3.2. Evaluation of the effects of endostatin on S. mansoni infection in a murine experimental model

We studied the number of adult recovery and number of eggs per gram in liver and intestine separately counted on day 46 post-infection. The mean number of worm recoveries was significantly higher in mice infected with *S. mansoni* than in mice infected and treated with endostatin ($p < 0.05$). The mean number of eggs per gram in liver in animals infected with *S. mansoni* was significantly higher ($p < 0.05$) than mice infected and treated with endostatin (Table 2). But, no significant differences were detected in the mean number of eggs per gram of intestine. We also studied the number of granulomas from liver in *S. mansoni* mice infected compared with mice infected and treated with endostatin. The results showed that the mean number of granulomas in mice infected group was significantly higher than mice infected and treated with endostatin ($p < 0.05$).

Red blood cells and platelet counts did not show any difference between groups. Only there was an increasing of the number of eosinophil in mice infected with *S. mansoni* (0.112 ± 0.032) in compared with mice infected and treated with endostatin (0.065 ± 0.023) but differences was not significant (data not shown).

RT-PCR in liver showed that VEGF-mRNA expression decreased in mice infected and treated with endostatin in comparison with mice infected with *S. mansoni* (Fig. 1). Similarly, FGF2 expression in liver also decreased in mice infected and treated with endostatin in comparison with mice infected with *S. mansoni* (Fig. 1). In contrary, VEGF and FGF2-mRNA expression in intestine did not show differences between *S. mansoni* infected group and mice infected and treated with endostatin (Fig. 2).

3.3. Evaluation of the effects of S. mansoni antigens on the VEGF and FGF2 production by rat alveolar macrophages

We studied the effects of different concentrations of different antigens of *S. mansoni* (0.1–50 μg/ml) on the VEGF and FGF2 expression in alveolar macrophages (Fig. 3). The result indicated that macrophages stimulated with 50 μg/ml of cercarial antigen of *S. mansoni* (CSm) were able to induce VEGF and FGF2 mRNA expression. On the other hand, macrophages stimulated with adult worm antigens of *S. mansoni* (AWASm) were not able to produce VEGF and FGF2 angiogenic factors.

4. Discussion

The main helminthoses that affect the human population are geohelmintosis, schistosomiasis and filariasis (Watkins, 2003). These worms also are responsible for parasitic infection in immigrant patients asymptomatic with absolute or relative eosinophilia (Pardo et al., 2006; Carranza-Rodriguez et al., 2008). In the present work we evaluated VEGF concentration in the sera of patients with parasitological diagnosis of schistosomiasis, filariasis and hookworms infections. We selected patients from sub-Saharan who did not show co-infection. We found that patients diagnosed of schistosomiasis had a high level of VEGF in sera compared with healthy patients group. These results are similar to those reported by other authors in *S. mansoni* infections (Tawfeek et al., 2003). We observed that there were no significant differences between VEGF levels in patients with schistosomiasis produced by *S. mansoni* and *S. haematobium*. Angiogenesis plays a fundamental role in many physiological and pathological processes, including the development of hepatic fibrosis. In cirrhosis, blood serum levels of VEGF are decreased (Shi et al., 2001). However, high VEGF levels were detected in sera of patients with schistosomiasis, although controversial results were found in the VEGF detection among the different clinical phases of schistosomiasis (Tawfeek et al., 2003; de Toledo et al., 2009).

In addition, we studied the effects of angiogenic inhibitor in an experimental murine model of *S. mansoni*. We use endostatin as a

Table 2
Parasitological data in groups of experimental mice.

Animals (CD1 mice)	Number of animals	Recovery worms (Mean ± SD)	N eggs/liver (Mean ± SD	N eggs/intestine (Mean ± SD)	N granuloma/liver (Mean ± SD)
Healthy control group	6	–	–	–	–
Infected with *S. mansoni*	6	21 ± 6.4	598 ± 726	144 ± 146	78.43 ± 26.11
Infected with *S. mansoni* and treated with endostatin	6	10.2 ± 3.5*	282 ± 413*	115 ± 140	23.80 ± 11.2*

SD, Standard deviation.
* Statistical differences $p < 0.05$.

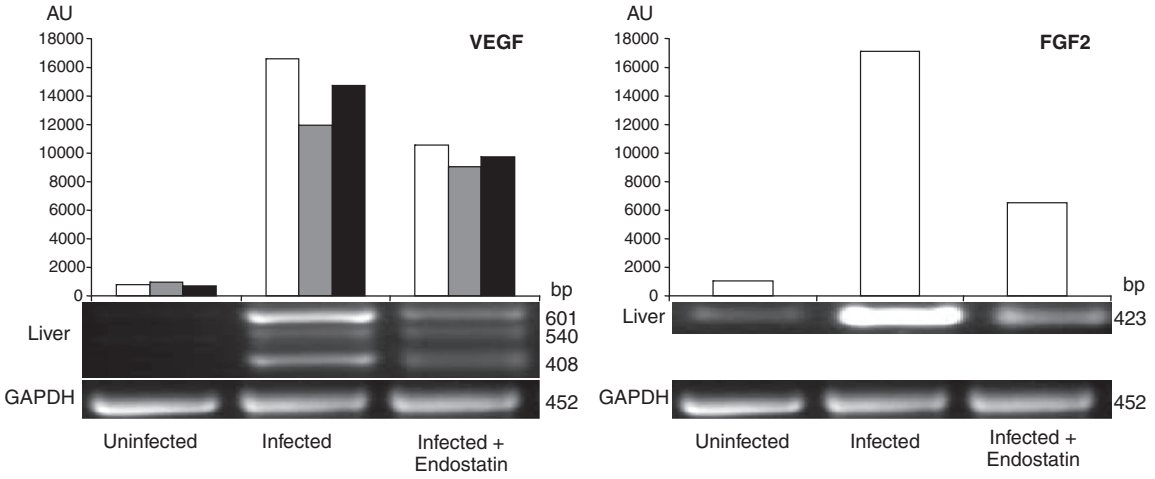

Fig. 1. Detection of VEGF and FGF2 by RT-PCR in liver. Three experimental groups were used: (i) uninfected mice (ii) mice infected with *S. mansoni* (iii) mice infected with *S. mansoni* and treated with endostatin. VEGF was expressed as three bands and arbitrary units (AU) calculated by densitometry of each band 601 bp (white bars), 540 bp (grey bars), and 408 bp (black bars). FGF2 expression showed a band with 423 bp (white bars) and densitometry analysis of it as arbitrary unit (AU). GAPDH expression was used as internal control.

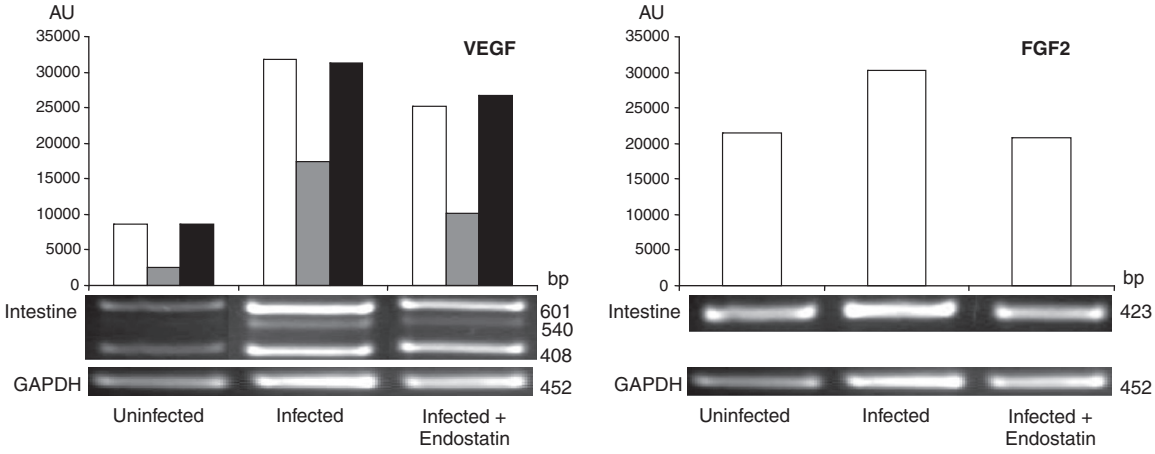

Fig. 2. Detection of VEGF and FGF2 by RT-PCR in intestine. Three experimental groups were used: (i) uninfected mice (ii) mice infected with *S. mansoni* (iii) mice infected with *S. mansoni* and treated with endostatin. VEGF was expressed as three bands and arbitrary units (AU) calculated by densitometry of each band 601 bp (white bars), 540 bp (grey bars), and 408 bp (black bars).FGF2 expression showed a band with 423 bp (white bars) and densitometry analysis of it as arbitrary unit (AU). GAPDH expression was used as internal control.

specific inhibitor of angiogenesis (Abdollahi et al., 2005). The results showed that there was significant reduction in the number of adult worms in mice infected and treated with endostatin. Also we have shown a significant reduction in the number of eggs in liver and number of granulomas in mice treated with endostatin compared with *S. mansoni* infected mice. This indicates that the inhibition of angiogenesis induced a decreasing of injury characteristics trigger hepatic formation of the granuloma and subsequent fibrosis.

Moreover, we studied the mechanisms involved in the reduction of injury. Firstly, we analyzed data of the blood cells count (red, white blood and platelet cells). No significant differences in number of blood cells (specifically in eosinophils) were found between *S. mansoni* infected mice and mice treated with endostatin. The results obtained in this experiment are consistent with observations of eosinophils in human population infected with schistosomiasis, filariasis and hookworm infections. This is opposite to that found by our group when we analyzed the relationship be-

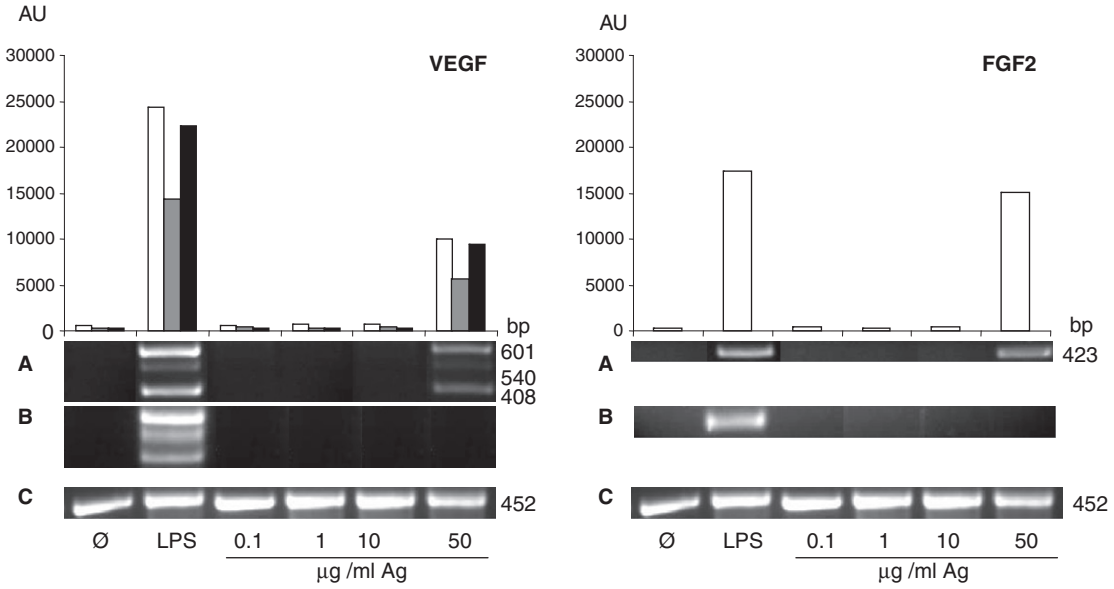

Fig. 3. Effect of *S. mansoni* antigens on VEGF and FGF2 in alveolar macrophages. mRNA expression in rat alveolar macrophages stimulated with cercarial antigen of *S. mansoni* (A) and adult worm *S. mansoni* antigen (B). GAPDH mRNA expression levels from rat macrophages detected by RT-PCR are used as internal positive control (C). Non-stimulated, negative control (Ø), LPS stimulated macrophages, positive control (LPS) and different concentrations of *S. mansoni* antigens, 0.1–50 µg/ml. Arbitrary units are calculated by densitometry of corresponding amplicons. All experiments were performed in triplicate.

tween angiogenesis and strongyloidiasis (Shariati et al., 2010). Secondly we proved the decreasing of the expression of VEGF and FGF2 in liver. This data showed the effective inhibition realised by endostatin.

Finally, the last objective of our study was to identify *S. mansoni* antigens involved in the expression of VEGF and FGF2 angiogenic factors by alveolar macrophages. We used cercarial (CSm) and adult worms (AWASm) *S. mansoni* antigens. Our results showed that cercarial antigens were able to stimulate alveolar macrophages to produce VEGF and FGF2 factors. Our results and the data obtained by Loeffler et al. (2002), where products secreted by *Schistosoma* eggs may promote angiogenesis within hepatic granulomas by up-regulating endothelial cell VEGF, shown the role of angiogenesis in schistosomiasis and its participation in the granulomatous reaction associated to the injury produced.

In summary, this study show the role of angiogenesis in the pathogenesis of schistosomiasis based on three aspects: (i) VEGF detection in patients diagnosed of schistosomiasis, (ii) reduction of worms, eggs recovery and granuloma formation in liver in mice infected with *S. mansoni* and treated with endostatin (iii) cercarial antigen of *S. mansoni* stimulated VEGF and FGF2 production by alveolar macrophages.

Acknowledgment

This work has been supported by Grants Junta de Castilla y León SA116A08 and PI061355. Shariati F, PhD student has been supported by fellowships from the Ministry of Science and Technology of IR of Iran.

References

Abdollahi, A., Hlatky, L., Huber, P.E., 2005. Endostatin: the logic of antiangiogenic therapy. Drug Resistant Update 8, 59–74.

Andrade, M.A., Siles-Lucas, M., Espinoza, E., Pérez Arellano, J.L., Gottstein, B., Muro, A., 2004. *Echinococcus multilocularis* laminated-layer components and the E14t 14-3-3 recombinant protein decrease NO production by activated rat macrophages in vitro. Nitric Oxide: Biology and Chemistry 10, 150–155.

Baptista, A.P., Andrade, Z.A., 2005. Angiogenesis and schistosomal granuloma formation. Memórias do Instituto Oswaldo Cruz 100, 183–185.

Carmeliet, P., Jain, R.K., 2000. Angiogenesis in cancer and other diseases. Nature 407, 249–257.

Carranza-Rodriguez, C., Pardo-Lledias, J., Muro-Alvarez, A., Pérez-Arellano, J.L., 2008. Cryptic parasite infection in recent West African immigrants with relative eosinophilia. Clinical Infectious Diseases 46, e48–50.

Chatterjee, S., Op De Beek, J., Rao, A.V., Desai, D.V., Vrolix, G., Rylant, F., Panis, T., Bernat, A., Van Bergen, J., Peeters, D., Van Mark, E., 2007. Prolonged somatostatin therapy may cause down-regulation of SSTR-like GPCRs on *Schistosoma mansoni*. Journal of Vector Borne Diseases 44, 164–180.

de Toledo, C.F., Carvente, C.T., Shigueoka, D.C., Borges, D.R., 2009. Endothelial markers in schistosomiasis patients with or without portal hypertension. Digestive Diseases and Sciences 54, 1331–1336.

Dunne, D.W., Webster, M., Smith, P., Langley, J.G., Richardson, B.A., Fulford, A.J., Butterworth, A.E., Sturrock, R.F., Kariuki, H.C., Ouma, J.H., 1997. The isolation of a 22 kDa band after SDS-PAGE of *Schistosoma mansoni* adult worms and its use to demonstrate that IgE responses against the antigen(s) it contains are associated with human resistance to reinfection. Parasite Immunology 19, 79–89.

Freire, N., Rodrigues-Silva, R., Machado-Silva, J.R., Rey, L., 2003. A comparative parasitologic study on *Biomphalaria glabrata* snail and C3H/He mice infected with human and murine isolates of *Schistosoma mansoni* derived from Sumidouro, Rio de Janeiro, Brazil. Memórias do Instituto Oswaldo Cruz 98, 783–787.

Gryseels, B., Polman, K., Clerinx, J., Kestens, L., 2006. Human schistosomiasis. The Lancet 368, 1106–1118.

Loeffler, D.A., Lundy, S.K., Singh, K.P., Gerard, H.C., Hudson, A.P., Boros, D.L., 2002. Soluble egg antigens from *Schistosoma mansoni* induce angiogenesis-related processes by up-regulating vascular endothelial growth factor in human endothelial cells. The Journal of Infectious Diseases 185, 1650–1656.

O'Reilly, M.S., Boehm, T., Shing, Y., Fukai, N., Vasios, G., Lane, W.S., Flynn, E., Birkhead, J.R., Olsen, B.R., Folkman, J., 1997. Endostatin: an endogenous inhibitor of angiogenesis and tumour growth. Cell 88, 277–285.

Pardo, J., Carranza, C., Muro, A., Angel-Moreno, A., Martín, A.M., Martín, T., Hernández-Cabrera, M., Pérez-Arellano, J.L., 2006. Helminth-related eosinophilia in African immigrants, Gran Canaria. Emerging Infectious Diseases 12, 1587–1589.

Porter, D.W., Millecchia, L.L., Willard, P., Robinson, V.A., Ramsey, D., McLaurin, J., Khan, A., Brumbaugh, K., Beighley, C.M., Teass, A., Castranova, V., 2006. Nitric oxide and reactive oxygen species production causes progressive damage in rats after cessation of silica inhalation. The Journal of Toxicological Sciences 90, 188–197.

Shariati, F., Pérez-Arellano, J.L., López-Abán, J., El Behairy, A.M., Muro, A., 2010. Role of angiogenic factors in acute experimental *Strongyloides venezuelensis* infection. Parasite Immunology 32, 430–439.

Shariati, F., Pérez-Arellano, J.L., López-Abán, J., Arefi, M., Martínez-Fernández, A.R., Muro, A., 2009. *Trichinella*: differential expression of angiogenic factors in macrophages stimulated with antigens from encapsulated and non-encapsulated species. Experimental Parasitology 123, 347–353.

Shi, B., Wang, X., Yang, Z., 2001. Vascular endothelial growth factors and liver diseases. Hepato-Gastroenterology 48, 1145–1148.

Silva, L.M., Ribeiro-Dos-Santos, R., Soares, M.B., Andrade, Z.A., 2006. Characterization of the vascular changes in schistosomal portal (pipestem) fibrosis of mice. Acta Tropica 98, 34–42.

Stavitsky, A.B., 2004. Regulation of granulomatous inflammation in experimental models of schistosomiasis. Infection and Immunity 72, 1–12.

Tawfeek, G.M., Alafifi, A.M., Azmy, M.F., 2003. Immunological indicators of morbidity in human *Schistosomiasis mansoni*: role of vascular endothelial growth factor and anti-soluble egg antigen IgG4 in disease progression. Journal of the Egyptian Society of Parasitology 33, 597–614.

Van de Vijver, K.K., Colpaert, C.G., Jacobs, W., Kuypers, K., Hokke, C.H., Deelder, A.M., Van Mark, E.A., 2006. The host's genetic background determines the extent of angiogenesis induced by schistosome egg antigens. Acta Tropica 99, 243–251.

Watkins, B.M., 2003. Drugs for the control of parasitic diseases: current status and development. Trends of Parasitolology 19, 477–478.