UP TO DATE

A DOUBLE AND PARADOXICAL ROLE FOR ANGIOGENESIS

Zilton A. Andrade[1]

ABSTRACT

Fibrogenesis and fibrolysis are constant and important features occurring during the pathology of schistosomiasis. New findings have recently indicated that granulation tissue (angiogenesis) is a dominating feature on both occasions. This review article discusses this apparently paradoxical feature displayed by angiogenesis (granulation tissue) during the pathology of schistosomiasis.

KEY WORDS: Schistosomiasis; angiogeneis; granulation tissue; fibrosis and fibrolysis.

RESUMO

Um duplo e paradoxal papel para a angiogênese

Fibrogênese e fibrólise são características constantes e importantes que ocorrem durante a patologia da esquistossomose. Novos achados têm indicado que o tecido de granulação (angiogênese) é uma característica dominante em ambas as ocasiões. Este artigo de revisão discute este papel, aparentemente paradoxal, desempenhado pela angiogênese (tecido de granulação), durante a patologia da esquistossomose.

DESCRITORES: Esquistossomose; angiogênese; tecido de granulação; fibrose e fibrólise.

INTRODUCTION

Recent studies have contributed to the concept that fibrosis, wherever it occurs, represents in reality a process of repair where angiogenesis ("Granulation Tissue"), appears as a dominant feature.

In 1972 Macgree and Patrick stated that any fibrosis was always preceded by a classical repair process, thus contesting the concept of a post-collapse fibrosis, which would follows the events of massive or sub-massive necroses in the liver.

1 Laboratory of Experimental Pathology, Gonçalo Moniz Research Center (Fiocruz), Salvador, BA, Brazil. E-mail: zilton@bahia.fiocruz.br

Received for publication in: 12/8/2013. Accepted in: 16/9/2013.

More recently, Rosmorduc et al. demonstrated that experimental biliary cirrhosis, produced in rats by main bile-duct obstruction, is characterized not only by proliferation of small bile ducts, but also by an intense proliferation of blood vessels due to the liberation of a strong specific factor, vasculo-endothelial growth factor (VEGF), which is related to some degree of local ischemia. Such a relationship between vascular proliferation and fibrosis has also been demonstrated in cases of human biliary cirrhosis (19). A critical review of such findings appeared as an Editorial from The Journal of Hepatology (14), which suggested for the near future, the use of anti-angiogenic drugs as a possible therapy for chronic liver diseases that usually present fibrosis as one of their more serious complications.

The interest of our Laboratory in the relationship between angiogenesis and fibrosis was stimulated since the beginning of our studies with septal hepatic fibrosis, which is invariably present in rats infected with the helminth *Capillaria hepatica* (26). The rats infected with this helminth always develop diffuse septal fibrosis, which eventually involves the entire liver, regardless of the number of parasite-related focal lesions. Sequential morphological studies (6) have also demonstrated the presence of an intense vascular proliferation, especially evident in sections observed under immunofluorescence for laminin, which marks the capillary basement membrane. Such studies also revealed that the vascular proliferation preceded collagen deposition within the newly formed septa (27).

The subject of the relationship between angiogenesis and fibrogenesis disclosed a more complex, although more interesting meaning, when the process of post therapeutic healing of the hepatic lesions caused by *Schistosoma mansoni* were systematically studied (1). What seemed intriguing then was to observe that angiogenesis this time was also outstanding, but did not seem related to fibrogenesis, but rather to fibrosis regression and tissue remodeling, especially observed when the schistosomal obstructive vascular lesions were considered. Such findings were suggestive of a double and paradoxical role played by angiogenesis, a subject to be herein discussed.

ANGIOGENESIS AND SCHISTOSOMIASIS

When the discussion about the relationship between angiogenesis and fibrosis began to be explored, it was to be expected that schistosomiasis would need to be included among the pathologies to be especially evaluated, considering the multiple relationship between vascular changes and fibrosis presented by the pathology of this helminthiasis, especially when Manson's schistosomiasis was considered. The fundamental lesion in schistosomiasis is represented by periovular granuloma, which is formed within host tissues around mature eggs retained in the tissues of a susceptible host, such as man. As a matter of fact, the *Schistosoma mansoni* eggs when shed by the female worms are still immature and remain as such for around 6 days, then causing little or no host tissue reaction (23). Soon afterwards,

with egg maturation being completed by differentiation of the embryo (miracidium), the now mature egg starts releasing lytic secretions through the micropores present in the egg shell. Such miracidial secretions present toxic and lytic effects upon the host tissues, which result in inflammatory reactions and focal host tissue destruction, a fundamental change that allows the eggs to pass through the host intestinal wall toward the intestinal lumen, where they may emerge into the feces and eventually reach the exterior where they may complete their life cycle. However, when this mechanism fails, the mature eggs remain trapped within the host tissues causing irritation due to their lytic secretions, which is amplified by the host immune system, when formation of periovular granulomas usually occurs (20). Although these granulomas observed in tissue sections usually appear avascular, serial observations on the dynamics of their formation reveal the important participation of angiogenesis. Periovular granulomas contain numerous endothelial cells, which can be revealed by histochemical methods for factor VIII (16). Furthermore, *in vitro* studies have demonstrated that ovular antigens stimulate endothelial cell proliferation (12) and also induce activation of VEGF, and so angiogenesis (17). Although these alterations of vascular proliferation are evident, the final result is fibrosis, which occurs together with the progressive disappearance of vessels, as classically happens with the repair of wounds. However, it should be stressed that angiogenesis has its particular dynamics during the evolution of schistosomal periovular granuloma (6). At the early stage capillaries appear diffusely distributed inside the granuloma, but gradually come to appear concentrated at the granuloma periphery, then coinciding with the formation of a fibrous capsule. In the liver, in the presence of a high parasite burden, the eggs appear lodged inside the host larger portal spaces, generating the so-called pipe-stem fibrosis, a basic lesion characteristic of the severe form of the disease (hepato-splenic schistosomiasis). Then, the presence of numerous periovular granulomas with their peripheral vascularization, generates an angiomatoid appearance of the fibrous portal tissue, which may be better observed during more recent infections (3). However, with the passing of time, the tendency is towards the formation of a fibrous plaque, dense and poorly vascularized. Then, the technique of vascular injection with plastic of different colors, followed by digestion in strong acid, allows for the simultaneous observation of the vascular system of the liver, when the portal and hepatic veins, plus the hepatic artery can be demonstrated with different colors and at the same time (2). Thus, the portal vein shows a poor presence, with several obstructive and destructive lesions, contrasting with the exuberance of the hepatic artery (hypertrophy and hyperplasia), again contrasting with the near-normal appearance of the hepatic veins.

So, although the presence of angiogenesis during fibrogenesis in schistosomiasis is evident, the final result is a considerable reduction of the hepatic vasculature, in spite of compensatory hypertrophy of the hepatic artery, This latter carries a considerable importance for the clinical evolution of schistosomal

hepatopathy, since the hepatic parenchyma then comes to be much dependent on blood supply from the hepatic artery (3, 4).

RE-MODELING

By the end of 1970 new and effective drugs against schistosomiasis began to be used for the treatment of schistosomiasis and their efficacy stimulated new concepts, not only for epidemiological studies, but also for the understanding of a complex vascular hepatic pathology. A study made in an endemic area of the State of Bahia, over six years, using the new curative drugs against schistosomiasis revealed that the treatment was not only curative, but also preventative against the development of the most severe forms of the disease (8). These results were soon confirmed in different parts of the world (11, 13, 24).

Such results appeared clear cut in younger patients followed over years or months (9). Treated patients presented with disappearance or attenuation of the manifestations of portal hypertension, such as disappearance of signs of collateral circulation and splenomegaly. In the face of such favorable reports, sanitary authorities came to regard chemotherapy as the fundamental measure for schistosomiasis control, while researchers were encouraged to be engaged in new studies on the biology of hepatic fibrosis, its causes and mechanisms. However, the destination of the schistosomal vascular obstructive lesions was apparently not considered in such studies. This may in part be due to the wrong, but resilient notion, that hepatic fibrosis itself is a cause of portal hypertension, both in schistosomiasis as in cirrhosis, in spite of the demonstration that hepatic cirrhosis may appear in asymptomatic individuals (21), while there are records documenting individuals with the classical Symmers´ fibrosis, causing asymptomatic presentation without splenomegaly (22).

When a patient with the hepatosplenic form of schistosomiasis receives specific chemotherapy and, some years later, exhibits regression of hepatic fibrosis, reduction of the spleen size and disappearance of esophageal varices, this means, not only that hepatic fibrosis has been reduced, but that a considerable degree of vascular remodeling has also occurred (5).

In order to observe better how such vascular remodeling occurs, an experimental study was performed in mice (1). Our previous observations had indicated disappearance of the vascular obstructive lesions 4 months following curative treatment in susceptible mice. The excess of elastic fibrils in the sub-endothelium of portal veins then underwent partial removal. New vascular lumens appeared near or within previously obstructed vessels, with proliferation and differentiation of new vascular lumens. However, the most interesting findings were seen with the examination of the plastic casts of the hepatic vasculature. These casts, obtained before and after schistosomiasis treatment, revealed a rich vasculature in the treated animals. New numerous fine vessels then appeared, strongly contrasting

with the picture seen in non-treated controls. At first one would think that the larger obstructed vessels had been opened up as a consequence of treatment, thus allowing for the plastic to permeate through, filling numerous collateral vessels. However, the strong concomitant presence of the vasculo-endothelial growth factor (VEGF revealed that numerous new vessels had now been formed (angiogenesis).

That double and paradoxical role for angiogenesis has much to be considered and well studied. It is well known that fibrogenesis is associated with a strong proliferation of actin-positive cells, not only in the walls of capillaries (pericytes) but also in the interstitial tissues (myofibroblasts).

FINAL COMMENTS

Angiogenesis means the formation of new vessels (capillaries) from pre-existing ones. Two important cellular elements are present in capillaries: endothelial cells and pericytes, there existing an interdependency between these two cellular elements, a capillary cannot be formed in the absence of one of them (7). Pericytes contain actin, so they are contractile cells. They may be detached from the capillary walls and then assume the morphology and function of a myofibroblast, being able to participate in the formation of the extra-cellular matrix (10). A pericyte present in the hepatic sinusoids (Ito cell) has the ability to store lipids. This facilitates its isolation for *in vitro* studies, which have contributed a great deal to our understanding of the relationships between angiogenesis and the formation of fibrous tissue. The recent statement by Lee et al. in this regard is herewith quoted just to give a precise idea of what we are now considering: "*For a long time, the existence and role of pericytes were neglected, but during recent years these cells have gained increasing attention, not only as contractile cells, but also as obligatory regulators of vascular development, stabilization, maturation, and remodeling"*. There is now a possibility that the pathology of schistosomiasis may be used as an adequate model to explore the significance of the double and paradoxical role played by angiogenesis, and it seems that the model of experimental schistosomiasis will allow the means for the investigation of multiple aspects related to the role of pericytes in general, and to the pathology of schistosomal vascular lesions in particular.

REFERENCES

1. Andrade ZA, Baptista AP, Santana TS. Remodeling of hepatic vascular changes after specific chemotherapy of schistosomal periportal fibrosis. *Mem Inst Oswaldo Cruz 101(Supl. 1)*: 267-272, 2006.
2. Andrade ZA, Cheever AW. Alterations of the intrahepatic vasculature in hepatosplenic schistosomiasis mansoni. Amer J Trop Med Hyg 20: 245-232, 1971.
3. Andrade ZA. Hepatic Schistosomiasis. Morphologic aspects. In H. Popper & Schaffner (eds). Progress in Liver Diseases. vol II, Grune & Stratton, New York, 1965.

4. Andrade ZA. Schistosomal hepatopathy. Mem Inst Oswaldo Cruz 99 (Supl. 1): 51-57, 2004.
5. Andrade ZA. Schistosomiasis and Hepatic Fibrosis Regression. *Acta Tropica 108*: 79-82, 2008.
6. Baptista AP, Andrade ZA. Angiogenesis and schistosomal granuloma formation. Mem Inst Oswaldo Cruz 100: 183-185, 2005.
7. Bergers G, Song S. The role of pericytes in blood-vessel formation and maintenance. *Neuro-Oncology 7*: 452-464, 2005.
8. Bina JC, Prata A. Regressão da hepatoesplenomegalia pelo tratamento específico da esquistossomose. Rev Soc Bras Med Trop 16: 213-218, 1983.
9. Dietze RS, Prata A. Rate of reversion of hepatosplenic schistosomiasis after specific chemotherapy. *Rev Soc Bras Med Trop 19*: 69-73, 1986.
10. Eyden B The myofibroblast: a study of normal, reactive and neroplastic tissues, with an emphasis on ultrastructure. Part 1 – Normal and reactive cells. *J Submicrosc Cytol Pathol 37*: 109-204, 2005.
11. Franke D, Kaiser C, Elsheikh M, Abdalla S, Schafer P, Ehrich JHH, Ultrasonographic investigation of periportal fibrosis in children with Schistosoma mansoni infection: reversibility of morbidity seven months after treatment with praziquantel. Am J Trop Med Hyg 44: 444-451, 1991.
12. Freedman DO, Ottesen EA. Eggs of Schistosoma mansoni stimulate endothelial cell proliferation in vitro. J Infect Dis 158: 556-562, 1988.
13. Homeida MA, Ahmed S, Dafalla A, Sulliman S, Eltom I, Nash T, Bennett JL. Morbidity associated with *Schistosoma mansoni* infection as determined by ultrasound: a study in Gezira, Sudan. *Am J Trop Med Hyg 39*: 196-201, 1988.
14. Lai WK, Adams DH. Angiogenesis and chronic inflammation; the potential for novel therapeutic approaches in chronic liver disease. Editorial. *J Hepatol 42*: 7-11, 2005.
15. Lee JS, Semela D, Iredale J, Shah VH. Sinusoidal remodeling and angiogenesis: a new function for the liver –specific pericyte?. *J Hepatol 45*: 817-823, 2007.
16. Lenzi HL, Sobral ACL, Lenzi JA. Participation of endothelial cells in murine schistosomiasis. Braz J Med Biol Res 21: 999-1003, 1988.
17. Loeffler DA, Lundy SK, Singh KP, Gerard HC, Hudson AP, Boros DI. Soluble egg antigens from Schistosoma mansoni induce angiogenesis-related processes by up-regulating vascular endothelial growth factor in human endothelial cells. J Infect Dis 185: 1650-1656, 2002.
18. Mcgree JOD, Patrick RS. The role of perisinusoidal cells in hepatic fibrogenesis. An electron microscopic study of acute carbon tetrachloride liver injury. *Inter Acad Pathol 26*: 429-440, 1972.
19. Medina J, Sanz-Cameno P, García-Buey L, Martín-Vílchez S, López-Cabrera M, Moreno-Otero R, Evidence of angiogenesis in primary biliary cirrhosis: an immunohistochemical descriptive study. *J Hepatol 42*: 124-131, 2005.
20. Pearce EJ. Priming of the immune response by schistosome eggs. *Parasite Immunol 27*: 265-270, 2005.
21. Popper H. Pathologic Aspects of Cirrhosis. A Review. *Am J Pathol 87*: 228-264, 1977.
22. Prata A, Andrade, ZA. Fibrose hepatica de Symmers sem esplenomegalia. *O Hospital 63*: 177-183, 1963.
23. Prata A. Biopsia retal na esquistossomose mansonica: bases e aplicações no diagnóstico e tratamento. Tese. Serviço de Educação Sanitária, Rio de Janeiro, 1957.
24. Richter J. The impact of chemotherapy on morbidity due to schistosomiasis. *Acta Tropica 86*: 161-183, 2003.
25. Rosmorduc O, Wendum D, Corpechot C, Galy B, Sebbagh N, Raleigh J, Housser C. Hepatocellular hypoxia-induced vascular endothelial growth factor expression and angiogenesis in experimental biliary cirrhosis. *Am J Pathol 155*: 1065-1073, 1999.
26. Souza MM, Junior MT, Assis BCA, Gonzales ACO, Silva TMC, Andrade ZA. Pathogenesis of septal fibrosis of the liver. An experimental study with a new model. *Pathol Res Prac 202*: 883-889, 2006.
27. Souza MM, Tolentino Jr M, Assis BCA, Gonzalez ACO, Silva TMC, Andrade ZA. Significance and fate of septal fibrosis of the liver. *Hepatol Res 35*: 31-36, 2006.