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INVITED REVIEW
MICROCIRCULATION AND CHAGAS' DISEASE: HYPOTHESIS AND RECENT RESULTS

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SUMMARY

This review focuses on studies that support the *microvascular hypothesis*, as well as on immunological and neurogenic mechanisms, and the role of the parasite itself, to explain further the pathology and clinical course of myocardial involvement in chagasic cardiomyopathy. The salient features of coronary microcirculation and Chagas' disease are discussed.

KEYWORDS: Microcirculation; Chagas' disease; Cardiopathy; Review.

INTRODUCTION

Chagas' disease, or American trypanosomiasis, caused by the hemoflagellate *Trypanosoma cruzi*, is the leading cause of heart disease in South and Central America. In 1909, in a remarkable investigative analysis, CARLOS CHAGAS[14] described the salient features of the disease, identified the causative parasite and characterized its life cycle. The merit of his research, the circumstances surrounding it and subsequent development of the field represent one of the most important pages in the history of medical science. Chagas' disease is an extraordinarily complex process with a poorly understood pathophysiology. In this review, we focus the studies that support the *microvascular hypothesis*, as well as on immunological and neurogenic mechanisms, and the role of the parasite itself, to explain further the pathology and clinical course of myocardial involvement in chagasic cardiomyopathy. Comprehensive reviews of earlier studies are available, and details of the clinical course of Chagas' disease and the life cycle of *T. cruzi* can be found in various publications[7,10,53].

HISTORICAL ASPECTS

The first study referring to the pathological anatomy of the recently discovered disease begins with a description of the cardiopathy *...the heart is one of the viscera for which the schizotrypanosome shows predilection both in man and in animals*[67].... Fascination with cardiopathic study emerged and Chagas' disease became a synonym for chagasic cardiopathy. The study by VIANNA[67] still represents fundamental investigation of the pathological anatomy of the acute phase of Chagas' disease. From this investigator we have the first reports of vascular involvement in Chagas' disease *...perivascular inflammations exist, some of them quite pronounced, and others barely incipient...* (myocardium) *...in many of the arterioles that irrigate the nervous substance, overt phenomena of periarteritis are found...* (cerebellum).

Degenerative heart alterations have been interpreted as a consequence of "circulatory disturbances". The classic report by MAGARINOS TORRES described these lesions in detail where they were considered independent of the parasitism[61]. In 1941, the same author, based on new observations of cardiac vascular involvement, defined these lesions as follows *...the inflammatory cell infiltrate in the interstitial tissue of the myocardium starts at the level of and around the capillaries, and not near S. cruzi, whether the latter is present inside cardiac fibers and macrophages or is free. This is an exsudative myocarditis related to early vascular lesions*[62]. TORRES also described similar lesions in the coronary arterioles of monkeys experimentally inoculated with *T. cruzi*, and considered the ischemic alterations in the myocardium to be the result of occlusion of the lumen vessels[63]. In 1960, TORRES compared chagasic and non-chagasic human hearts and in the former identified marked, constriction type irregularities in vessels associated with extensive myocytolysis in the intramyocardial arterioles. Then he proposed that the diffuse myocytolysis might be caused by metabolic changes in the myocytes resulting from circulatory disorders of low intensity or of short duration[64].

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Several other contemporary investigators have described vascular lesions in Chagas' disease. MAZZA & BENITEZ[39] demonstrated the parasites in cells of the perivascular adventitia in the conjunctiva of patients in the acute phase of Chagas' disease. A *sporadic coronary arteriolitis* in a patient who had died during the acute phase of the disease was described[40]. COUCEIRO[17] reported vascular lesions in a study of the sciatic nerve of experimentally inoculated dogs, and COELHO[16] observed coronary arteriole lesions in 19 cases of chronic Chagas' disease. These lesions were also detected at autopsy in coronary branches by RAMOS & TIBIRIÇÁ[49], LARANJA et al.[32], and KÖBERLE[27].

In 1955, ANDRADE & ANDRADE[3] suggested that the inflammatory phenomena found in chronic chagasic cardiomyopathy might be allergic in origin and that they might provoke ischemic lesions of the myocardium by capillary involvement. The microscopic infarctions would affect the conduction system, mainly in the right branch of the bundle of His, due to their preferential, intramyocardial localization. ANDRADE & ANDRADE[3] also suggested that the fibrotic lesions frequently detected at the apex of the left ventricle might originate from vascular obstructions due to subendocardial parietal thrombosis. These concepts were later documented[4]. However, 13 years later the "allergic phenomena" became less emphasized and the vascular changes were considered to be only *congestion and marked dilatation of venules and capillaries*[5].

BRITO & VASCONCELOS[12], in a study of 19 cardiac biopsies from patients with megaesophagus, detected necrotizing arteritis in 9, and identified the inflammation as an allergic phenomenon by morphological analysis. Vascular lesions were also described by MACCLURE & POCHE[35] in the hearts of mice examined by electron microscopy, and by LUCENA et al.[34], BRITO[11] and ALENCAR et al.[1] by light microscopy. OKUMURA et al.[46] observed necrotizing arteritis in the myocardium and digestive tube to which they attributed an allergic origin. This concept was expanded when these investigators detected a parasitized endothelial cell. They reported that *...during the acute phase the trypanosomes may cause a focal lesion with sensitization of the vessels by an allergic mechanism, triggering hypersensitivity phenomena reflected by necrotizing arteritis...*[47].

However, many authors were not convinced of the vascular lesions in experimental or human material. ALVARENGA[2] did not detect lesions in the coronary arterioles of naturally infected dogs while CHAPADEIRO[15] found no such lesions in human material. This suggested that the vascular lesions may have been due to the simultaneous presence of another cardiovascular disease[15]. KÖBERLE et al.[29], in a radiographic study followed by histological examination, investigated 22 hearts of chagasic patients submitted to autopsy and found no changes attributable to Chagas' disease in the vascular network. Further evaluation of the blood supply in the myocardium of 41 chagasic human hearts suggested that hypoxemia may favor the development of focal myocardial changes that subsequently result in ventricular wall thinning and apical aneurysm[28]. These changes, however, were attributed to intrinsic cardiac denervation, defended by the author as the pathogenetic mechanism of the disease.

Analysis of the above literature reveals that microvascular involvement has been under consideration or has been of secondary importance in the pathogenesis of lesions of the heart and other organs in Chagas' disease.

RECENT FINDINGS

Microvascular lesions in Chagas' heart disease became well documented in the 1980's[19,55]. The involvement of microvascular phenomena in the pathogenesis of chronic Chagas' disease was proposed in an editorial published by the *American Heart Journal* in 1990[50] based on previous reports. Since then, several investigators have devoted their efforts to aim characterize and define the role of microvascular changes in Chagas' disease, particularly with respect to cardiopathy.

STUDIES ON MICE AND RATS

Isogenic BALB/c mice sensitized by 3 to 5 inoculations with epimastigote forms of the avirulent PF strain of *T. cruzi* at 15 day intervals and challenged 30 days after the final inoculation with the trypomastigote form of the Colômbia strain of *T. cruzi* developed a cardiomyopathy very similar to that observed in the chronic phase of Chagas' disease in man[55]. Macroscopic observations showed cardiomegaly with hypertrophy and dilatation of the ventricular chambers associated with thinning of the left ventricle apex (apical aneurysm). Microscopical results showed focal lesions of myocytolytic necrosis and myocardial degeneration associated with a predominantly mononuclear inflammatory exudate accompanied by interstitial fibrosis and occasional pseudocysts. In addition, platelet aggregates and occlusive thrombus were detected in small epicardial and intramyocardial vessels of the infected mice compared to control mice. These results provide direct evidence of microcirculatory disease in the experimental chronic cardiomyopathy of *T. cruzi*-infected mice[55]. Such alterations are likely to be important in the pathogenesis of focal myocardial damage, a crucial point in the development of cardiomyopathy. However, the focal nature of the myocardial lesion and the type of necrosis represent indirect evidence for the involvement of the microcirculation in this model. The changes in myocardial hypoxia were suggested to result from transient ischemia caused by the release of vasoconstrictor substances (thromboxane A2, PAF-acether) from macrophages, the predominant cells in the inflammatory process[54].

Using a silicon rubber injection technique, several dynamic changes in the coronary vessels, such as focal vasoconstriction, formation of microaneurysms, dilatation and microvascular proliferation have been demonstrated in murine Chagas' disease[19], and were similar to those described for other congestive cardiomyopathies and related to blood microcirculation[18]. These microvascular lesions, constantly

present in the evolution of experimental Chagas' disease, were observed prior to the onset of significant myocardial degeneration or fibrosis and were reduced to a minimum by long term administration of verapamil, a calcium channel blocker with a vasodilating and anti-platelet aggregating effect[44]. Recently, these observations were corroborated by direct *in vivo* visualization utilizing a surrogate murine model, i.e., the cremaster microvascular bed[58]. This model provided a readily accessible method for the direct observation of the effects of *T. cruzi* infection on microcirculatory flow *in vivo* and for quantitative measurement of parameters like velocity of red blood cell flow (Vrbc) and vessel diameter. When the cremaster model was examined at 20-25 days post-infection in male CD-1 mice infected with the Brazil strain of *T. cruzi*, a significant decrease in Vrbc which was reversed by verapamil treatment was observed in the first- and third-order arterioles and venules. Video recordings revealed a marked inflammatory response confirmed by transmission electron microscopy and attenuated in the verapamil-treated mice. The arterioles of the infected mice exhibited segmental areas of vasospasm and dilatation. This latter finding may be the initiating event in microaneurysm formation[58].

The exact mechanism of such vascular lesions has not been fully clarified. In addition to spastic phenomena, the observation of platelet thrombus in the coronary microcirculation of infected mice[55] led to investigation of the possible mechanisms involved. These studies demonstrated that an increase platelet reactivity occurs in acute infection, a factor that may contribute to the development of thrombosis[59]. The interaction between the platelets and the damaged endothelium and/or subendothelial surface may stimulate platelet activation with the subsequent generation of thromboxane A2 (TXA2) and possibly of platelet-derived growth factors (PDGF). The detection of high TXA2 levels in the plasma of acutely infected mice partially explained both phenomena, i.e., an increase in intravascular platelet aggregation and in focal microvascular spasm[59].

However, it has been demonstrated that early in the course of infection, parasites are evident in the coronary microvascular endothelium before parasitemia can be detected. The coronary vascular endothelium may be an early, if not prime "target" of *T. cruzi* infection. Considering that the endothelium plays a key role in regulating vessel tone and vascular impermeability, an investigation was carried out using scanning and transmission electron microscopy to study the structure of the endothelial lining of the thoracic and abdominal aorta obtained from *T. cruzi*-infected rats in the acute septicemic phase[51]. The findings clearly demonstrated that the infected rats developed changes in the endothelial layer characterized by endothelial cell swelling and a few points of cytoplasmic discontinuity which appeared as holes exposing the subendothelial collagen usually associated with platelet-fibrin aggregates. These changes might affect the generation of vasoactive substances, impairing the equilibrium between opposing forces. The exposure of the subendothelial collagen due to holes in the endothelial lining may favor platelet-fibrin aggregation. Thus, it was proposed that similar endothelial cell changes might be present in the microcirculation, reflecting a reduction in the protective role of the endothelium, leading to the genesis of vasospasm and platelet aggregation within the coronary microvessels, and to the focal pathology observed in acute chagasic myocarditis[51].

STUDIES ON DOGS

Vascular lesions characterized by endothelial edema, denudation, cell accumulations (probably macrophages) and platelet-fibrin aggregation have been described in a collecting vein of the left ventricle in an *in vitro* perfusion model of a dog acutely infected with *T. cruzi*[26]. The author reluctantly agreed that these changes might be involved in the generation of microvascular occlusions that caused the ischemic foci observed in the material[26].

Hearts from dogs sacrificed 18 to 26 days after intraperitoneal inoculation with trypomastigote forms of the 12SF strain of *T. cruzi*/kg body weight were recently studied using both standard light microscopy and transmission electron microscopy[6]. This study demonstrated myocarditis characterized by small focal areas of lesion and myocytic necrosis associated with interstitial mononuclear infiltration. Ultrastructurally, degenerative changes were observed in endothelial cells in contact with T lymphocytes, as well as platelet aggregates and fibrin thrombus in the intramyocardial capillaries. These alterations suggested that a possible interaction between endothelial cells and effector immune cells might play an important role in the pathogenesis of the myocellular lesion and of the microangiopathy observed in the model under study[6].

IN VITRO STUDIES

The involvement of microcirculation components has been demonstrated *in vitro*. Direct infection of human endothelial cells in culture with *T. cruzi* results in the alteration of various critical biochemical processes responsible for the maintenance of microvascular perfusion, such as Ca^{2+} homeostasis, generation of inositol trisphosphate (IP3) and prostaglandin I_2[41,42]. More recently, it was demonstrated that *T. cruzi* infection of endothelial cells from human umbilical vein results in alteration of cyclic AMP (cAMP) metabolism which plays a protective role against the direct and/or indirect lesion caused by the adhesion and aggregation of circulating platelets to endothelial cells[43]. However, inflammatory cells may contribute to a state of microvascular hypoperfusion by secreting cytokines and other factors known to affect platelets and endothelial cells. Cytokines play an important role in the pathogenesis of various parasitic infections and their role in the pathogenesis of *T. cruzi* infection has been studied. The increase of interleukin-1β (IL-1β), of IL-6 and of colony stimulating factor 1 (CSF-1) in infected endothelial cells may lead to alterations in the function of these cells[66]. IL-1β is elaborated by activated macrophages and by peripheral blood mononuclear cells, including those infected with *T. cruzi*[66], and also by a variety of other cell types such as endothelial cells[36]. The antithrombotic properties

of endothelial cells may be altered by IL-1β. This cytokine may reduce tissue production of plasminogen activator and increase production of the inhibitor of this activator, an event that may result in thrombus formation[8,45]. Although the products of IL-6 are markedly increased in endothelial cell cultures, it has not been possible to determine whether non-infected cells are also induced to produce this cytokine. Since IL-1β may induce IL-6 production by endothelial cells, it is not clear whether IL-6 production by infected cells is a direct result of the infection or is induced by the IL-1β produced in response to infection. CSF-1 is an important growth factor for the proliferation and maturation of cells of the mononuclear lineage[36]. It is also important in recruitment, possibly acting in consonance with IL-1β. High CSF-1 levels have been detected in endothelial cells in culture infected with *T. cruzi*. These observations may reflect growth of the monocyte population in the microvasculature, resulting in the later elaboration of proinflammatory cytokines[60]. In addition, trypomastigotes may elaborate a neuraminidase that may be involved in the removal of sialic acid from the surface of mammalian myocardial and endothelial cells, facilitating thrombin binding. The loss of this protector molecule of the endothelial surface may contribute to platelet aggregation and thrombosis within the small coronary vessels[33]. These factors acting together may ultimately result in spasm and thrombosis in the small coronary vessels inducing focal myocardial damage.

In recent studies on the consequences of *T. cruzi* infection in human umbilical vein endothelial cells (HUVEC) with regard to the production of biologically active endothelin 1 (ET-1), increased expression of ET-1 mRNA was found[70]. Increased production of ET-1, a smooth muscle mitogen and potent vasoconstrictor, may contribute to the coronary microvascular vasoconstriction previously reported in experimental Chagas' disease[19]. This study further supports the idea that *T. cruzi* infection and subsequent perturbation of endothelial cells cause injury to the coronary microvasculature, resulting in the focal microvascular spasm implicated in the pathogenesis of Chagas' cardiomyopathy.

STUDIES ON HUMANS

Anatomical studies have shown structural derangement and rarefied microvasculature in the left ventricular wall. A histotopographical study comparing the microcirculatory system after injection of an opaque medium into chagasic and control human hearts demonstrated focal decapillarization in chronic Chagas' disease due to extraluminal compression, suggesting that this might be the cause of focal myocytolytic necrosis[25]. Similarly, a post mortem, radiological study of chagasic hearts revealed vascular changes at the heart apex characterized by distorted and/or scarce vessels associated with decreased arterial density, presumably related to the pathogenesis of apical aneurysm[22].

The evaluation of chest pain is a major problem in chagasic patients. Almost all exhibit symptoms that are atypical for classic angina pectoris[38]. Even though symptoms suggestive of myocardial ischemia are present, coronary angiographical studies show normal or nearly normal coronary arteries in more than 90% of patients studied[38]. This peculiarity had been previously reported in a post mortem study[48]. However, patients specifically selected on the basis of chest pain do show perfusion abnormalities detectable by thallium-201 scintigraphy, suggesting that myocardial ischemia, possibly of the microvascular type, may contribute to the genesis of the symptoms[38].

Abnormal perfusion in different groups of chagasic patients has been confirmed by various independent investigators using isonitrile-99m-technetium[13] or thallium-201, possibly reflecting microvascular ischemia[23]. In addition, myocardial capillary blood flow in chronic chagasic patients with no significant clinical or electrocardiographic manifestations proved to be markedly reduced when evaluated with rubidium-86, while the major coronary vessels were apparently normal. The reduction observed, comparable to that exhibited by a group of non-chagasic patients with obstructive coronary disease, occurred under basal conditions[30] and to a lesser extent during exercise[31]. Using a specific marker of regional flow independent of cell metabolic activity, MARIN NETO et al.[37] demonstrated perfusion defect in 55% of 18 chagasic patients with chronic cardiopathy and essentially normal epicardial coronary circulation.

Vasospastic mechanisms have been proposed in the genesis of coronary accidents in patients with chronic chagasic cardiopathy[68]. It was recently demonstrated that chagasic patients with chronic heart disease present an abnormal, endothelium-dependent, coronary vasodilating mechanism as demonstrated by acetylcholine and adenosine infusion into the left coronary artery, suggesting that epicardiac and microvascular coronary reactivity may be altered in these patients[65]. The clinical importance of this alteration awaits elucidation. However, this abnormality of the coronary microvasculature may contribute to the genesis of the symptoms related to the ischemic processes observed in chronic chagasic patients, and to acute myocardial infarction in the absence of significant coronary damage[65].

Studies on biopsies from chronic chagasic hearts demonstrated marked thickening of the basement membrane in most myocytes and capillaries (up to 20-times the normal thickness)[21]. This alteration is similar to the thickening with or without multiple layers reported for the basement membranes of myocardial capillaries in other cardiomyopathies[20]. Recently, a very well developed capillary network has been observed in chagasic human hearts using a cell-maceration scanning electron microscopic method, corroborating previous observations using confocal laser microscopy[24]. This alteration may represent the probable cause of slow capillary flow, contributing to the hypoxic changes observed in chronic chagasic myocarditis[52].

FINAL COMMENTS

Signs of possible disturbances in the coronary circulation have been observed since the earliest studies[64,67] conducted soon after the

nosologic discovery by CARLOS CHAGAS. Since then, much information has accumulated from attempts to define the physiopathology of Chagas' heart disease. Recent evidence for abnormalities at the microvascular level has accumulated in studies both on humans and in experimental models of *T. cruzi* infection. The changes suggest that myocardial lesions develop, at least in part, as a consequence of additive and progressive cell necrosis initiated and perpetuated by changes in myocardial microcirculation[50]. This may be relevant in terms of therapeutic strategies. β-Receptor antagonists may maintain adequate microvascular perfusion through modulation of coronary vasomotor tonus[69], preventing the development of focal myonecrosis. Calcium channel blockers may prevent tissue hypoxia through their vasodilating and antiplatelet aggregating effects[9,18]. Converting enzyme inhibitors may act by inhibiting the vasoconstrictor effect of angiotensin[57] or by exerting a beneficial effect on myonecrosis[56].

RESUMO

Microcirculação e doença de Chagas: hipótese e atualização

Esta revisão enfoca os estudos que levaram à formulação da *Hipótese Microvascular*, além da participação de mecanismos imunológicos e neurogênicos e do papel do parasita, para explicar a patologia e o curso clínico do envolvimento miocárdico na cardiopatia chagásica. São discutidos alguns aspectos sobre microcirculação e doença de Chagas.

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