MECHANISM OF EARLY INJURY TO THE CARDIOVASCULAR SYSTEM BY CARDIOCYTOTOXIC SERUM

M. F. Sirotina

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A combined morphological and functional study of the capillaries and small vessels of the heart and viscera (lungs, liver, kidneys, spleen, skeletal muscles, etc.) of 50 dogs was undertaken during the development of acute cytotoxic injury caused by injection of anticardiac serum (ACS) into the coronary circulation in a dose of 0.5-1.5 ml. The investigation showed that injection of ACS was followed by a widespread reaction of the microcirculation of the whole body with disturbance of capillary permeability for macromolecular compounds and considerable changes in the morphological structure of the microvessels, which were most severe in the myocardium of the left ventricle.

KEY WORDS: anticardiac cytotoxic serum; myocardium; coronary circulation; capillary permeability.

The immunologic aspects of lesions of the myocardium of coronary and noncoronary genesis continue to attract the attention of many investigators. In the past an important role for antiorgan antibodies in the etiology of ischemic heart disease and myocardial infarction has been suggested for antiorgan antibodies [1, 4, 7, 8]. However, the pathogenetic role of antiheart antibodies and the pathophysiology of disturbances caused by them has not yet been fully studied. The question why, as a result of the action of a comparatively small quantity of anticardiac antibodies on the heart, profound hemodynamic disorders forming a symptom-complex resembling cardiogenic shock, should be observed [2, 6], has not yet been explained. Considering information obtained in the last decade on the antigenicity of proteins composing the vascular wall [10, 15], it must be supposed that allergic alteration of the blood vessels, especially microvessels, must play an important role in the general picture of acute immunologic injury to the myocardium.

To shed light on some aspects of this problem, in the present investigation morphological and functional studies were made of the capillaries and small vessels in different parts of the heart and viscera (liver, lungs, spleen, kidneys, etc.) in the course of development of acute cytotoxic injury, caused by intracoronary injection of anticardiac serum (ACS).

EXPERIMENTAL METHOD

Fifty dogs were studied during the first 2 h after intracoronary injection of ACS (12 after 5-20 min, 38 after 1.5-2 h). The immune serum was injected into the descending or circumflex branch of the left coronary artery in a dose of 0.5-1.5 ml without opening the chest [6]. The titer of the serum used varied from 1:640 to 1:800 in the complement fixation test.

To determine the functional characteristics of the microvessels the rate of removal of Evans' blue dye and radioactive homologous albumin from the vascular system and the distribution of labeled proteins in the tissues after their injection into the general circulation were studied [5]. To determine local disturbances of capillary permeability, Young's method [12] was used. The protein composition of the pericardial exudate was investigated by the diffuse salting out method [3]. The morphological techniques used included staining sections with hematoxylin-eosin and by Weigert's method and impregnation with silver by Foot's method. The tissue for electron-microscopic study was fixed with glutaraldehyde and osmic

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TABLE 1. Changes in Rate of Elimination of Evans' Blue Dye from Blood Stream of Dogs after Intracoronary Injection of ACS (M±m)

Time of investigation	Concentration of Evans' blue in blood serum				
	D×1000			%	
	12 min	60 min	120 min	60 min	120 min
Before injection of ACS	207	187	167	90±1,5	83±1,6
After injection of ACS	190	153	133	80±4,2 $P_{1-2}<0,05$	69±5,6 $P_{1-2}<0,05$

acid. The materials were embedded in Araldite and sections stained by Reynolds' method [14]. Coons' immunofluorescence method also was used [9].

EXPERIMENTAL RESULTS

Injection of small doses (0.5-1.5 ml) of ACS into the coronary circulation as a rule led to profound disturbances of activity of the cardiovascular system with a fall of blood pressure to the shock level in the first 15-20 min. Later throughout the period of investigation (2 h) the systemic blood pressure continued to remain much lower than initially, and distinct changes were found in cardiac activity: The ECG revealed zonal injuries to the myocardium [6]. Coons' immunofluorescence method revealed rapid fixation of the injected antibodies in the microvessels of the catheterized region of the left ventricular myocardium. Meanwhile morphological and functional investigations of the capillary network of the heart and other internal organs (liver, spleen, lungs, kidneys, etc.) showed the presence of a widespread reaction of the microvascular system of the body as a whole. The results of function tests of the capillary membranes, carried out during the first 2 h after injection of ACS, showed intensification of transport through the vascular wall. The experiments showed that the experimental animals' own plasma proteins, labeled with Evans' blue, were removed more intensively from the blood into the surrounding tissues (Table 1).

In parallel experiments ^{131}I-labeled homologous albumin from an outside source, injected into the circulation, also passed through the vascular wall much more rapidly (on average by 15%; $P<0.05$). Besides the more rapid removal of radioactive protein from the blood stream into the extravascular spaces, as a rule the more marked accumulation of indicator in the myocardial tissue was observed. As Fig. 1 shows, the calculated value of the coefficient of permeability of the tissue-blood barrier of the heart (left ventricle) was increased fourfold, whereas the corresponding coefficient for a skeletal muscle (biceps femoris) was increased by only 1.5 times. An unequal degree of injury also was found in different parts of the capillary network in the myocardium itself.

Labeled protein crossed the barrier more rapidly and accumulated mainly in the territory of distribution of the catherized coronary artery (Fig. 2). The considerable and preferential injury to the microvascular system of the myocardium, accompanied by increased outflow of fluid and protein into the intercellular spaces of the heart muscle, and disturbance of pathways of interstitial circulation of these substances led after 1.5-2 h to the accumula- of between 20 and 80 ml of exudate with a high protein concentration (3.4±0.2 g %) in the pericardial cavity. By fractional salting out, all blood serum protein fractions could be detected in the exudate. Light-optical studies of the microvessels of the heart and other organs showed generalized pericapillary edema, depolymerization of the ground substance of the wall of the small vessels, the presence of extravasation, and massive transmural hemorrhages in the myocardium of the left ventricle. Damage to the capillary network of the viscera (liver, lungs, spleen, kidneys, skeletal muscles, etc.) was much less severe. Meanwhile, electron-microscopic investigations revealed signs of intensification of microvesicular transport, widening of interendothelial junctions, and a decrease in the electron density of the basement membranes of the capillaries in the fine structure of the microvessels of all organs. In addition, multiple microthrombi, tears, and holes in the cytoplasm of the endothelial cells, from 0.15 to 0.3 μ in diameter, with partial or total death of the cell organelles and discontinuity of the basement membranes of the small vessels were found in the territory supplied by the catheterized coronary artery. In this zone, as a result of more marked disturbances of transcapillary exchanges and the rapid outflow of fluid (Fig. 3) a greater degree of widening of the precapillary spaces was observed. The extravascular edema was reduced after 2 h, but it was still relatively well marked in the zone of "injury" at this time also.

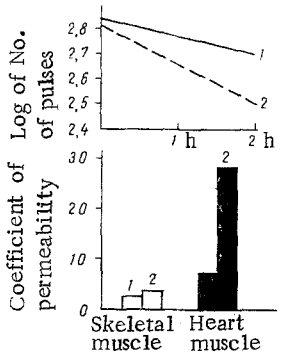

Fig. 1

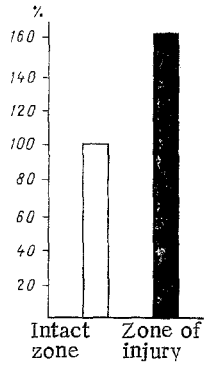

Fig. 2

Fig. 1. Character of removal of ^{131}I-labeled homologous albumin from the circulation and coefficient of permeability of tissue-blood barrier of skeletal muscle and heart muscle (left ventricle) of intact dog (1) and dog receiving injection of 1 ml ACS (2).

Fig. 2. Concentration of Evans' blue dye in different parts of myocardium 2 h after intracoronary injection of ACS (content of dye in "intact" zone taken as 100%).

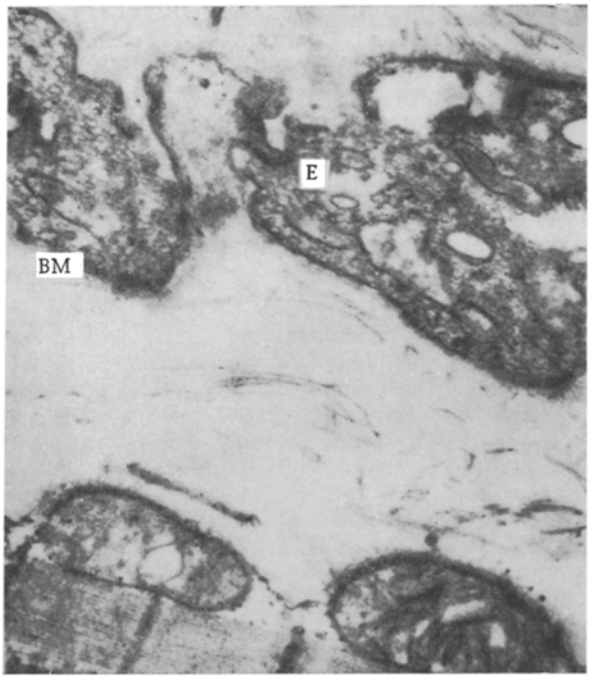

Fig. 3. Myocardial capillary (left ventricle) after intracoronary injection of 1 ml ACS: decrease in clarity of luminal surface of endothelium (E), loosening of basement membrane (BM) of capillary, pericapillary edema (4800×).

The investigation showed that localized areas of the coronary microcirculation are the sites of commencing development of the pathological process. Meanwhile, because of the rapid involvement of the body as a whole in the pathogenetic chain of the immune system and the elimination of many biologically active substances into the general system of the circulation

[1, 11, 13], the microvascular system of the whole body is damaged, but the most severely affected region is the myocardium. In turn, the rapid outflow of components of the circulating blood into the intercellular spaces, on the one hand reduces the venous return to the heart and, on the other hand, disturbs the regular supply of energy-producing material to the functioning cells and, in particular, to the myocytes. The rapidly developing interstitial edema of the myocardium, by modifying the rigidity (compliance) of the heart muscle [6] may evidently disturb its contractility.

The results obtained are evidence that zonal injuries to the myocardium of the left ventricle associated with the antigen–antibody reaction in a particular part of the coronary circulation are accompanied by considerable morphological and functional changes in the microvascular system of the heart and other internal organs. These changes presumably play an important role in the mechanisms of development of the profound hemodynamic disorders in the acute phase of immunologicatrauma to the heart.

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