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A MODEL OF MYOCARDIAL LESIONS OF VARIED SEVERITY

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To evaluate the therapeutic effects of drugs it is necessary to study their mechanism of action on models of pathological processes [2]. Both vascular and metabolic disturbances in the myocardium play very important roles in the development of isochemic heart disease [3, 6, 7]. However, experimental models of myocardial lesions combining disorders of the coronary circulation, hemocoagulation, and metabolism, and leading to pathological changes in the heart muscle, are still in an early stage of development and have not yet become widely used in pharmacological or other research.

The aim of this investigation was to develop experimental heart lesions corresponding to various clinical manifestations and suitable for studying the therapeutic efficacy of new cardiotropic drugs.

EXPERIMENTAL METHOD

Experiments were carried out on eight dogs weighing 6-11 kg, 61 rabbits weighing 2.6-3.2 kg, and 30 albino rats of both sexes weighing 200-240 g. The animals were divided into five groups: group 1 (13 rabbits) – myocardial lesions of micronecrosis type produced by subcutaneous injection of isoproterenol (5 mg/kg) and theophylline (20 mg/kg) once only, or twice with an interval of 24 h between them; group 2 (15 rabbits) – toxic myocarditis, produced by subcutaneous injection of isoproterenol (5 mg/kg) and caffeine (50 mg/kg), once only and twice with an interval of 24 h; group 3 (15 rabbits and 20 rats) – focal myocardial dystrophy, produced by subcutaneous injection of thyroxine (0.02 mg/kg) and isoproterenol into rabbits (5 mg/mg) and rats (75 mg/kg), once only and twice with an interval of 24 h; group 4 (10 rabbits) – microfocal myocardial infarction, produced by intravenous injection of 0.5 U/kg body weight of pituitrin, followed after 15 min by subcutaneous injection of isoproterenol (5 mg/kg), which was repeated 5-6 h later; 24 h after injection of pituitrin a repeated injection was given, and isoproterenol was injected in the same doses and in the same order; group 5 (eight dogs, eight rabbits, and 10 albino rats) – a myocardial infarct of macrofocal type was produced in dogs by intravenous injection of 0.3 U/kg of pituitrin, followed 20 min later by 3 mg/kg of isoproterenol; the latter injection was repeated in the same dose 6 h later, and 24 h after the first injection of pituitrin a second injection of 0.15 U/kg was given intravenously, after which isoproterenol was injected in the same dose and order as previously. Under aseptic conditions and under trimeperidine–thiopental anesthesia with controlled respiration, 24 h after the second injection of pituitrin the anterior interventricular branch of the left coronary artery was ligated below the origin of the first collateral. Pituitrin and isoproterenol were injected into the rabbits by the scheme in group 4, and the coronary artery was ligated without artificial respiration, by a strictly midline incision through the sternum without injury to the pleural cavity. Pituitrin was injected intraperitoneally into the rats

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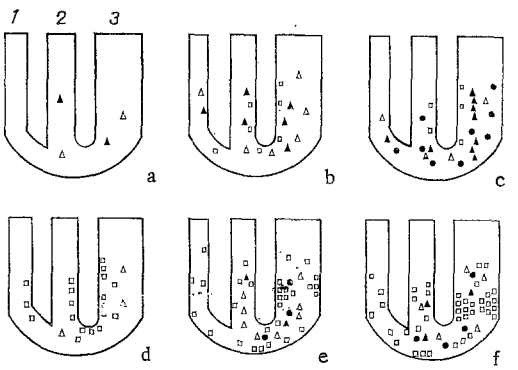

Fig. 1. Schemes showing typical localization of lesions in myocardium. 1) Wall of right ventricle; 2) ventricular septum; 3) wall of left ventricle. a) Control; b) micronecroses; c) myocarditis; d) focal dystrophy; e) microfocal myocardial infarct; f) macrofocal myocardial infarct. Squares denote necrosis, circles vascular lesions and edema; empty triangles – contracture, filled triangles – myocytolysis.

in a dose of 1 U/kg and isoproterenol subcutaneously in a dose of 35 mg/kg in the same order as in the rabbits. The coronary artery was ligated 24 h after the second injection of pituitrin, with artificial respiration.

Parameters of development of the pathological process were ECG changes in standard and chest leads, recorded with a tape winding speed of 100 mm/sec (Élkar-4 electrocardiograph), together with parameters of the blood clotting system [4] and the results of morphological investigation (usually with a polarizing microscope also) of sections through the heart fixed in 10% formalin in 0.1 M phosphate buffer, on the 1st, 3rd, and 7th days after the beginning of the experiment, and subsequently stained with hematoxylin and eosin by Carazzi's method, with picrofuchsins, and by Selya's method. The results were subjected to statistical analysis by Wilcoxon's nonparametric test [1].

EXPERIMENTAL RESULTS

After receiving injections of isoproterenol and theophylline (group 1), all the animals developed tachycardia, cardiac arrythmias, reduction of the R wave on the ECG, flattening of the T wave, and depression of the S–T interval below the isoelectric line. Histological examination revealed small foci of necrosis of cell groups, replaced in the course of time by connective-tissue cells, in the endo- and subendocardial parts of the wall of the left ventricle and ventricular septum. Examination in polarized light revealed lysis of myofibrils in most cardiomyocytes, and sometimes hypercontracture of the outermost sarcomeres was observed [5]. More extensive lesions were found in animals receiving two injections of the drugs with an interval of 24 h (Fig. 1).

The topography and size of the necrotic foci in animals receiving isoproterenol and caffeine (group 2), and likewise the ECG changes were the same as those in the previous group. However, in this series of experiments and, in particular, on the 1st day, edema was observed in the interstitial tissue of the left ventricle, with marked dilatation of capillaries in the subendocardial zone and with signs of stasis. Under the polarizing microscope multiple areas of myocytolysis were observed in the wall of the left ventricle – "the tissue appeared motheaten" [5].

These two types of model, namely micronecroses and diffuse myocarditis, will probably be suitable for the study of drugs stimulating regeneration and affecting inflammatory processes in the myocardium.

Injection of isoproterenol preceded by thyroxine (group 3) caused significant disturbances of the electrical activity of the heart and morphological changes in both rabbits and rats. Lengthening of the Q–S interval, deepening of the Q wave, and changes in the shape and amplitude of the T wave were observed on the ECG. Destruction of cells was observed over wide areas in the subendocardial and subepicardial zones of both the left and the right ventricles, where polymorphs, lymphocytes, and histiocytes were concentrated. Examination of sections in polarized light showed that the myocardium outside the zone of necrosis was virtually unchanged,

and that only rarely after repeated injection of the drugs was increased anisotropy of the A disks visible. It will evidently be possible to use this form of myocardial lesion as a model of severe myocardiodystrophy in order to study the therapeutic effects of appropriate drugs.

Successive injections of pituitrin and isoproterenol (group 4) led to the appearance of characteristic ECG changes: depression of the P wave and simultaneous lengthening of the Q–S interval. Polycardiography showed an increase of 30-60%($P < 0.05$) in the duration of the contraction period and a decrease by 9-12% ($P < 0.05$) in the ejection period. Congestion of arterioles and venules, irregularity of staining of the sarcoplasm of the cardiomyocytes with eosin, and the presence of large foci (large numbers of cells) of necrosis in the subendocardial and subepicardial layers of the myocardium of the left ventricle, ventricular septum, and apex of the heart, and of small foci (small groups of cells) in the middle layers of these same parts of the heart were discovered. In polarized light, especially on the 1st day, areas of myocytolysis were observed, and like areas of segmental increase in anisotropy of the A disks [5], these were topographically more characteristic of the middle layer of myocardium of the left ventricle and ventricular septum. The blood clotting time was reduced by 30-36% ($p < 0.05$) and the plasma recalcification time by 38-60% ($p < 0.05$), whereas the fibrinogen concentration was increased by 35-140% ($P < 0.05$). It can be postulated that this model is similar in its basic pathological manifestations to microfocal myocardial infarction and can be used to study antianginal, cardiotonic, and anticoagulant drugs.

Injection of pituitrin and isoproterenol into dogs before ligation of the coronary artery (group 5) led to the development of a typical behavioral response characteristic of the anginal form of ischemic heart disease: the animal howls, moves restlessly, and its pupils dilate. In the first minutes after injection of pituitrin the respiration rate quickened by 40-50 cycles/min whereas the heart rate fell by 30-100 beat/min. After injection of isoproterenol paroxysmal tachycardia was recorded, with the heart rate rising to 370 beats/min, and the ECG showed displacement of the S–T interval, a change in voltage of the R and T waves, and enlargement of the S wave. The blood fibrinogen concentration was increased by 70-160% ($P < 0.05$), the plasma recalcification time was reduced by 40-50% ($P < 0.05$), and the degree of the thrombotest was increased by more than 1.5 times. Ligation of the coronary artery under these conditions led to a fall in blood pressure in the femoral artery by 30% ($P < 0.05$), to a marked shift of the S–T interval in all ECG leads, and to even greater hypercoagulation of the blood. Histological investigation revealed extensive transmural necroses of the anterior apical region of the heart measuring up to 45 cm^2, together with small (small groups and large masses of cells) foci of necrotic tissue in other parts of the heart. White thrombi, intimately connected with the endocardium, were found in the chambers of the heart and in the mouth of the pulmonary artery. Similar changes also were found in rabbits and rats. It can be concluded from a comparison of the changes in all these parameters that a major myocardial infarct had formed in these animals, with high mortality (50-80%) and accompanied by complications such as fibrillation, asystole, cardiogenic shock, thromboembolism, and the formation of an aneurysm in animals which survived until the 3rd-6th month. This model may be suitable for the study of resuscitation measures after major myocardial infarction.

All five types of experimental pathological lesions of the myocardium (micronecroses, myocarditis, focal dystrophy, micro- and macrofocal myocardial infarction) may thus find different applications for the study of the therapeutic properties of many drugs.

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