Mechanisms of Drug Action on Recovery Processes of Cardiac Muscle in Myocardial Infarction

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ABSTRACT

The results of histological, histochemical, and electron microscopic studies of the effect of inosine, retabolil, orotic acid with vitamin B₁₂, and preparation P-132, a long-chain fatty acid, on the recovery processes in cardiac muscle after coronary artery ligation are presented. A predominant effect of P-132 has been demonstrated on the process of scar organization and of inosine on the energy supply of the contractile and recovery mechanisms in all sections of the cardiac muscle. The effect of retabolil and potassium orotate are directed toward recovery processes in the necrotic area but are less pronounced than with the preceding preparations. The data presented are evidence of the promising search for pathways of medicinal action on cardiac muscle recovery processes in myocardial infarction.

KEY WORDS inosine orotic acid scar organization perinecrotic zone succinate dehydrogenase

In the development of cardiac insufficiency in myocardial infarction, not only the extent of the necrotic area but also the state of the metabolic processes in the perinecrotic sections and in portions of the myocardium remote from the infarct play an important role. Formation of a focus of necrosis leading to an actual drop to zero of the contractile ability of the myocardium complicates the contraction of the ventricle as a whole.

The state of the metabolism of the perinecrotic area, which is determined to some extent by the dimensions of the postinfarct scar, also plays a large part in determining the degree of reduction in the contractility of the ventricle. Metabolic shifts leading to disturbances of contraction of surviving myofibers become of great importance in this area.

To a certain extent both the infarcted area and the perinecrotic zone determine the probability of development of a postinfarct aneurysm. Correction of metabolic abnormalities in these two areas is necessary to accelerate organization of the scar, to decrease its dimensions, and to improve the contractile function of surviving sections of the myocardium in this area. We should like to emphasize further that in the development of cardiac insufficiency in myocardial infarction, sections of the cardiac muscle which are distant from the focus of necrosis also play a significant part.

It is known that the decrease in myocardial contractility in the necrotic area is compensated for by hyperfunction of the sections of the cardiac muscle outside the infarct, which has been demonstrated by measurement of intramyocardial pressure in experimental myocardial infarction in the work of Lukomskii and colleagues, Shenderov and colleagues, and others.

Hyperfunction of intact sections of the myocardium is provided by activation of energy formation and increase in synthesis of protein containing contractile structures, which is indicated by a sharply increasing incorporation of labeled amino acids, [¹⁴C] glycine¹ and [³⁵S] methionine, into the contractile proteins of the myocardium outside the necrotic area. In addition, as a result of stimuli emanating from the focus of necrosis, a generalized spasm of the coronary arteries outside the area of infarction develops. Therefore, dystrophic processes and hypertrophy, as well as the development of disseminated foci of necrosis, are noted in fibers of the intact area of the heart in myocardial infarction.

Besides generalized spasm of the coronary arteries, increased stress on the hyperfunctioning, surviving myocardium with its insufficient oxygen supply probably plays a definite part in the emergence of these shifts. Obviously, the effect of not one but a combination of pathogenetic factors can be imagined, in which, in addition to those indicated above, the effect of decomposition products of the necrotic tissue entering the blood, elevated circulating catecholamines, autoallergic factors, and general hypoxia plays a definite part.

In recent years increasing attention has been paid to study of the metabolic shifts in the area of myocardium outside the infarct. A reduction in the
activity of various enzyme systems and disruption of mitochondrial structures and function have been noted. Basically, these changes are reversible; however, their duration can vary, depending on the extent of coronary spasm, the degree of hyperfunction of the myocardium, and also the expression of previous infarcts and changes in the cardiac muscle.

Study of the action on recovery processes of the cardiac muscle in myocardial infarction should include not only a search for possible ways to affect the formation of the postinfarct scar, as was carried out earlier in the studies of Gudbjarnason et al., Maroko et al., Bing, Olson, Rona et al., and others, but also on the metabolic shifts in the perinecrotic area and sections of the myocardium distant from the necrotic area.

In this investigation we studied the effect on the course of experimental myocardial infarction of preparations designed to activate synthesis of nucleic acids and proteins in order to increase the activity of a number of enzyme systems and thereby facilitate recovery of the energetic capacities of the myocardium.

Materials and Methods

The work was carried out on male chinchilla rabbits weighing 2,200 to 2,600 g. Myocardial infarction was produced under pentobarbital sodium anesthesia by ligating the anterior descending branch of the left coronary artery. The animals were killed by decapitation on the first, third, and seventh days after operation. A total of 208 rabbits were operated on; 164 received one of the preparations being studied, and 44 served as controls. Myocardial infarction was diagnosed by means of ECG and by observation at autopsy.

For histochemical study the hearts were frozen in dry ice, and sections were prepared in a cryostat at a temperature of -20°C. Material for electron microscopic examination was fixed in osmium, embedded in Epon, and examined under a UEM-100 microscope. Three areas were distinguished in study of the myocardium: the necrotic area, the boundary or perinecrotic area, and sections of the myocardium distant from the necrotic site. The use of transverse sections through the entire rabbit heart permitted exact determination of the areas mentioned.

Succinate dehydrogenase (according to Nachlas and colleagues), NAD-diaphorase (according to Hess and colleagues), cytochrome oxidase (according to Moog), glucose-6-phosphate dehydrogenase (according to Hess and colleagues), lactic dehydrogenase (according to Hess), phosphorylase (according to Takeuchi), esterase (according to Pearce), aminopeptidase (according to Pearce), acid and alkaline phosphatase (according to Pearce), fat (according to Romeis), and glycogen (according to MacManus) activities were determined histochemically.

The first group of 43 rabbits received inosine (inosine-F), a Japanese preparation of hypoxanthine fiboside (nucleoside), at a dose of 30 mg/kg intravenously once per day, the first time 16 hours after operation and the last 2 hours before sacrifice. The second group of 39 animals received potassium orotate (potassium salt of orotic acid, 2,6-dioxypyrimidine-4-carboxylic acid), which is a pyrimidine base precursor, 30 mg/kg (in combination with vitamin B1, 3 mg/kg), according to a procedure proposed by Meerson and Lukomskii, in the same sequence as the preceding preparation. The third group of rabbits received the long-acting anabolic hormone, retabolil (nandrolone decanoate C33H42O3, at a dose of 5 mg/kg weight one to two times intramuscularly.

The fourth group of animals received preparation P-132, a long-chain fatty acid.

Results

In the first day after experimental myocardial infarction, inosine resulted in a more pronounced cellular infiltrate than in the control, and the enzyme activity was not significantly different from that of the control. On the third day, as a result of the inosine, increased succinate dehydrogenase, NAD-diaphorase, lactic dehydrogenase, and glucose-6-phosphate dehydrogenase, as well as acid and alkaline phosphatase, esterase, and aminopeptidase activities, were noted in the necrotic area (Fig. 1). The histological picture indicates acceleration of lysis of the necrotic mass with a reduction in the area of unorganized necrosis and of the predominance of lymphoid and plasma macrophages in the granular tissue (Fig. 2).

After seven days of experimental myocardial infarction, the activity of all of the above-mentioned enzymes was lower in the necrotic zones on control animals. In addition, a large number of fibrous structures, more intensely stained collagen, were noted, which indicates their more mature state, as well as a reduced number of cellular elements and necrosing muscular fibers than in the control animals (Fig. 3).

On the boundary of the necrotic area, no clear-cut effect of inosine on the activity of the enzymes studied was observed one day after experimental myocardial infarction; however, the number of fibers with deformazan granules was reduced, reflecting lower succinate dehydrogenase activity, and a large number of fibers with linear deformazan appeared, reflecting increased activity of glucose-6-phosphate dehydrogenase. In addition, in the work of Cherpachenko and Sokolova, it was shown that the increase in the number of fibers with deformazan granules noted in experimental myocardial infarction indicates disruption of the ultrastructure and permeability of the mitochondria and a reduction in activity of the corresponding enzymes.
In portions of the myocardium distant from the area of disrupted blood circulation, inosine resulted in a reduction in fibers with diformazan granules one day after production of experimental myocardial infarction at the expense of fibers with linear formazan, as was demonstrated by determination of activity of succinate dehydrogenase, lactic dehydrogenase, and glucose-6-phosphate dehydrogenase. The quantity of glycogen in this area was increased by inosine one day after experimental myocardial infarction. On the third day after the operation, in sections of the myocardium outside the infarcted area (Fig. 4), inosine administration resulted in an increase in succinate dehydrogenase activity in a portion of the fibers, and a more significant and regular increase in glucose-6-phosphate dehydrogenase (Fig. 5) activities was noted. Under the influence of inosine, the glycogen content proved to be increased at this time in a portion of the fibers.

The increase in activity of succinate dehydrogenase and lactic dehydrogenase under the influence of inosine was maintained in this area for seven days after the production of experimental myocardial infarction.
dial infarction, although it was less pronounced. Some increase in DPN activity was noted during this period and the increase in amount of glycogen was maintained.

It was noted in electron microscopic examination of the myocardium remote from the area of disrupted blood circulation that three days after the production of experimental myocardial infarction in the myocardium of rabbits not receiving inosine, mitochondria exhibited swelling, formation of lumina in the matrix, and destruction and considerable decrease in the number of cristae (Fig. 6, upper left); these were associated with sections having obviously normal ultrastructure. In the myocardium of rats receiving inosine, a large number of foci having quite dense, polymorphic mitochondria with a large number of well-preserved cristae (Fig. 6, upper right) were encountered, along with sections characterized by the changes in mitochondria described above. Some groups of mitochondria were small and dense, and others had appreciable constrictions (Fig. 6, lower left). A large number of osmophilic granules of lipoprotein origin and ribonuclein granules were observed in these foci (Fig. 6).

Thus, inosine had profound effects on the myocardium, accelerating scar organization and correcting the metabolic shifts in the perinecrotic area and in sections of the myocardium distant from the necrotic area.

In rabbits receiving potassium orotate with vita-

min B₁₂, a negligible increase in glucose-6-phosphate dehydrogenase activity and a more pronounced increase in acid phosphatase, esterase (Fig. 7), and aminopeptidase activities were noted in the necrotic area three days after ligation of the coronary artery. Seven days after the operation, an increase in the number of fibrous structures was noted in this area. The glucose-6-phosphate dehydrogenase activity was higher in the necrotic area of the “treated” animals than in some zones of the control animals during this period.

At the boundary of the necrotic area, there were no significant differences in activity of the enzymes studied or in the histological picture following the production of experimental myocardial infarction. Seven days after ligation, an increase in the glucose-6-phosphate dehydrogenase activity and a negligible increase in DPN activity were noted in this area.

In the areas of the myocardium distant from the necrosis, no significant differences in enzyme activity were noted three days after production of experimental myocardial infarction, and negligible increases in activities of lactic dehydrogenase, glucose-6-phosphate dehydrogenase, and DPN were noted seven days after operation. Thus, the main direction of the action of this combination of preparations is acceleration of scar organization.

In rabbits receiving retabolil, the histological and histochemical patterns were similar to those noted after the administration of potassium orotate.
with vitamin B₁₂. Again, the most pronounced effect of the preparation was noted in the necrotic area and sections of the myocardium adjacent to it. Collagen staining was more intense after seven days of experimental myocardial infarction in the treated animals, and the number of necrosing muscle fibers was somewhat less.

Neither the enzyme activity nor the histological picture in the necrotic area one day after experimental myocardial infarction was altered by P-132. Three days after the operation, a considerable increase in aminopeptidase, esterase (Fig. 8), acid and alkaline phosphatase, and a large number of connective tissue stroma cells (Fig. 9) were noted. By the seventh day after the operation, there were no significant differences in enzyme activity between the control and treated animals, but the enzymes mentioned were detected, for the most
part, in fibroblasts and not in histocytes, as in the control animals. The number of fibrous structures more intensely stained with collagen was greater, and the number of necrosing muscle fibers was less than in the control.

In the boundary area one day after ligation of the coronary artery under the influence of P-132, some increase in succinate-dehydrogenase, cytochrome oxidase, glucose-6-phosphate dehydrogenase, and lactic dehydrogenase activity was seen in the experimental rabbits. By seven days after the operation in animals receiving P-132, a slight increase in activity of succinate dehydrogenase in comparison with the controls was noted in the boundary area.

In the area distant from the infarction, a clear increase in succinate dehydrogenase, DPN, cytochrome oxidase, lactic dehydrogenase, and glucose-6-phosphate dehydrogenase activities appeared one day after experimental myocardial infarction.

**FIGURE 4**

*Glucose-6-phosphate dehydrogenase outside area of disrupted blood circulation, x 265. (Upper) Three days after operation, low enzyme activity. (Lower) Increase in enzyme activity on third day of experimental myocardial infarction under the influence of inosine.*

Supplement III to Circulation Research, Vols. 34 and 35, September 1974
FIGURE 5

Lactate dehydrogenase outside area of disrupted blood circulation, × 265. (Upper) Three days after operation, reduction in enzyme activity. (Lower) Higher enzyme activity under influence of inosine in three days of experimental myocardial infarction.
under the influence of P-132. Three days after the operation, the increase in lactic dehydrogenase, DPN, glucose-6-phosphate dehydrogenase, and cytochrome oxidase activities was less striking, and succinate dehydrogenase activity as well as glyco-
gen content were comparable to the levels observed in the controls. By seven days after the operation, a considerable increase in activity of only lactic dehydrogenase was noted in the area of myocardium distant from the necrosis.

Discussion

Of the preparations studied, inosine and P-132, a long-chain fatty acid, showed the clearest effect on the process of scar organization. A somewhat different mechanism of action of these preparations on the necrotic area was noted. Preparation P-132 accelerates these processes primarily by means of intensification of lysis of the necrotic masses. The increase in activity in the necrotic area, after three days of experimental myocardial infarction, of acid and alkaline phosphatase, esterase, and aminopeptidase and histological determination of a decrease in the number of necrosing muscle fibers, together with accelerated maturing of connective tissue elements, support this conclusion.

No increase in the activity of enzymes which provide an energy supply for the recovery processes in the necrotic area was noted; however, some increase in activity of succinate and cytochrome oxidases, as well as of glucose-6-phosphate dehydrogenase, was noted in the perinecrotic area in one day and glucose-6-phosphate dehydrogenase and lactic dehydrogenase in three days. This minor activation was preserved until the seventh day of experimental myocardial infarction. The action of
inosine was directed to a greater extent toward activation of energy supply of the recovery processes and the activity of Krebs cycle enzymes, the final electron transport chain, and the pentose-phosphate shunt. The increases were considerable in the necrotic area and in the perinecrotic sections of the myocardium as a result of its action.

P-132 intensified the energy formation processes for only one day in areas of the myocardium distant from the necrosis. The possibility that activation of energy formation processes in this "emergency" stage has great physiological importance is not ruled out. Enzyme activation is considerably decreased in three days, and increase in activity of lactic dehydrogenase alone is preserved for seven days.

Under the influence of inosine, activation of succinate dehydrogenase, glucose-6-phosphate dehydrogenase, and lactic dehydrogenase was manifested in one day, increased in three days, and was preserved to a certain extent for seven days following production of experimental myocardial

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infarction. In this manner the effect of inosine on the energy formation processes was more pronounced, longer, and encompassed the entire area of the cardiac muscle. The effects of retabolil and potassium orotate with vitamin B₁₂ were directed mainly toward the scar organization processes; however, their activity was less striking than that of inosine and P-132.

There is considerable interest in examining the effects of the preparations mentioned on activity of the pentose-phosphate shunt, which can be judged by glucose-6-phosphate dehydrogenase activity. The cardinal importance of the pentose-phosphate shunt in synthesis of nucleic acids and protein and in regenerative and proliferative processes is well known.¹⁴,¹⁵ The increase in the glucose-6-phosphate dehydrogenase activity under the influence of inosine and P-132 and, to a lesser extent, of potassium orotate is especially evident in the perinecrotic area three days after experimental myocardial infarction, i.e., at the time when activation of proliferation processes and formation of

![Image of tissue sections](image-url)

**FIGURE 8**

Esterase in necrosis area, × 265. (Upper) Three days after coronary artery ligation, low enzyme activity. (Lower) Three days of experimental myocardial infarction with administration of P-132: more connective stroma cells containing many enzymes.

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Myocardial infarction three days old. (Upper) Control: residues of necrosing muscle fibers are seen among newly formed connective tissue. Hematoxylin-eosin stain, × 170. (Lower) With P-132 administration, necrosing portion of myocardium completely replaced by young connective tissue, rich in cellular elements. Hematoxylin-eosin stain, × 170.

Connective tissue are noted under the influence of the preparations mentioned. This finding indicates the importance of the hexose-phosphate shunt for these processes and its role in supplying pentose, which is necessary for synthesis of nucleic coenzymes, nucleic acids, and NADPH$_2$.

The increase in glucose-6-phosphate dehydrogenase activity under the influence of inosine and P-132 in sections of the myocardium distant from the necrotic area one to three days after experimental myocardial infarction appears to serve the purpose of renewal of the contractile structure proteins and enzymes during the period of compensatory hyperfunction and hypertrophy, which is especially important under the conditions of damage to these substances in myocardial infarction.

In considering the mechanism of action of these preparations on the contractile ability of the myocardium, the assumption must be made that it relates to activation of nucleic acid and protein synthesis. An increase in the numbers of enzyme systems occurs because of this activity, and the
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energy supply for active contraction is intensified, as are the processes of renewal of the contractile, mitochondrial, and membrane proteins.

An increase in lactic dehydrogenase activity occurred to varying extents with administration of the preparations mentioned, predominantly in the area of the myocardium distant from the necrosis and partially in the perinecrotic area. This finding is taken as evidence of the action of these preparations in the process of correction of metabolic shifts, perhaps not participating directly in the contractile mechanism, but showing a negative effect on its efficiency. In this case the increase in activity of lactic dehydrogenase leads to activation of oxidation of the lactic acid accumulated under these conditions, which assists in reduction of acidosis. In addition, it is well known\(^1\) that under conditions of acidosis, the affinity of the sarcoplasmic reticulum for calcium increases sharply, which leads to a decrease in the quantity of ionized calcium. At the same time the affinity of troponin for calcium at low pH is reduced. The affinity of troponin for tropomyosin is increased during systole with formation of a troponin-tropomyosin complex which inhibits the reaction of actin and myosin. In this manner a decrease in acidosis by means of oxidation of lactic acid assists in improving conditions for operation of the contractile mechanisms.

It follows from what has been said that the preparations which we studied exert a stimulating effect, to a greater or lesser extent, on the recovery processes in the cardiac muscle which can assist in prevention of development of cardiac activity insufficiency and postinfarct aneurysm.

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Discussion

Dr. Robert M. Berne, Charlottesville, Virginia: I am very interested in your observations on the beneficial effects of inosine. These findings coincide with those made some years ago by Dr. Lorber and his colleagues and also by Dr. Buckley. They showed that inosine had a positive inotropic (and, I believe, chronotropic) effect on the heart. In contrast, adenosine has a negative inotropic and
chronotropic effect. The mechanism involved has not been clarified, but your observations of improvement with inosine are compatible with these earlier observations on the effect of inosine on the myocardium.

Dr. Nikolaeva: Our clinical studies of the use of inosine in patients with myocardial infarction have shown positive hemodynamic responses.

Dr. J. Gergely. Boston, Massachusetts: I should like to ask you if you have considered use of the so-called sandwich technique, which has been so fruitful in comparing changes in histochemical patterns of skeletal muscles with changes in the activity pattern or innervation. With this technique specimens from control muscle and the muscle to be studied are included in the same preparation to be examined. One can thereby make a direct comparison of enzymatic reactions under identical conditions. I should also like to ask what it means when one finds increased enzyme activity. Does increased activity signify increased production of the specific protein, or are substances present which increase the activity in the histochemical reaction?

Dr. Nikolaeva: In histochemistry we mean by the term “an increase of activity of enzymes” the increased rate of a reaction no matter how it is achieved. At the moment we cannot differentiate between actual increase of enzymatic activity and stimulation of a reaction by a substrate, etc.
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doi: 10.1161/01.RES.35.3_suppl.III-202

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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