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Effect of Biotin on Biochemical Changes following Isoproterenol-induced Myocardial Infarction in Rats

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Effect of biotin on biochemical changes following isoproterenol-induced myocardial infarction in rats was investigated. Biotin inhibited an increase of myocardial water content, an elevation of plasma lactate dehydrogenase activity and a decrease of myocardial adenosine triphosphate (ATP) level in isoproterenol-treated rats. Prednisolone enhanced the inhibitory effects of biotin on myocardial water content and plasma lactate dehydrogenase activity, but did not influence on myocardial ATP level. A possible mechanism of the inhibition by biotin is discussed.

Introduction

Myocardial necrosis in rats after a single subcutaneous administration of isoproterenol (ISP) was found by Rona, *et al.*[2] The myocardial necrosis was examined macroscopically and histologically, and the general morphological features were found to be similar to those produced by ischemia.[3] Furthermore, biochemical changes produced by the ISP-treatment in rats were investigated.[4–6] Robertson and Peyser[7] reported that an intravenous injection of epinephrine or norepinephrine in cats caused a small but significant increase in myocardial water content based on an increase of the extracellular fluid volume. Shen and Jennings[8] demonstrated that tissue water and sodium contents increased in a transient ischemic model of dog hearts. Our previous paper[6] reported that the relationship between myocardial water content and adenosine triphosphate (ATP) level after the ISP-treatment was inversely proportional. On the other hand, depressed respiration and pyruvate utilization of ventricular heart muscle in biotin-deficient animals were restored by the administration of biotin.[9]

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The present work was undertaken to see the effect of biotin on some biochemical changes induced by ISP.

Materials and Methods

Animals——Male Wistar rats weighing 180 to 220 g were used. Animals were housed in individual cages and allowed free access to food and water. Each experimental group consisted of 5 animals.

Materials——*d*-Biotin was purchased from Iwaki Co., Ltd., Tokyo, Japan. *dl*-Desthiobiotin was purchased from Nakarai Co., Ltd., Kyoto, Japan. *d*-Biotinamide was synthesized by the method of Wolf, *et al.*[10] Prednisolone phosphate was purchased from Nippon Merck-Banyu Co., Ltd., Tokyo, Japan. Isoproterenol hydrochloride was procured from Boehringer Ingelheim GmbH, Germany. Substrates and enzymes used for the assay of metabolites in myocardium and plasma lactate dehydrogenase (LDH) were purchased from Sigma Chemical Company, St. Louis, Mo., USA.

Experimental Model——The necrotic heart was produced by a single subcutaneous injection of ISP (5 mg/kg) 24 hr before the sacrifice for assay. Biotin vitamers and/or prednisolone were given intraperitoneally 30 min before the ISP-injection. In the experiment about the dose response of biotin, the following experimental model was also employed in which ISP (5 mg/kg) was given subcutaneously for 2 consecutive days, and biotin was given intraperitoneally 30 min before each ISP-injection. Rats were sacrificed 24 hr after the 2nd ISP-treatment. The methods preparing the specimens for quantitative determinations of myocardial water content, plasma LDH activity and myocardial adenine nucleotides were described in our previous paper.[6]

Analytical Methods——The ventricular tissue was carefully dissected free of any visible fat, cut in 4 pieces, washed with cold saline and blotted with absorbent paper to remove surface water. Myocardial water content was determined by weighing a wet tissue sample and drying to a constant weight in an oven at 105 to 110°. Plasma LDH activity was determined by the method of Cabaud and Wroblewski.[11] ATP was measured by the method of Lamprecht, *et al.*[12] Adenosine diphosphate(ADP) and adenosine monophosphate (AMP) were simultaneously measured with the method of Adam.[13] Total adenine nucleotides were calculated by summing up ATP, ADP, and AMP values. The experimental result was given as the mean ± standard error.

Results

Effect of Biotin Vitamers on Myocardial Water Content and Plasma LDH Activity

Table I shows some biochemical changes after a single injection of ISP in rats and inhibitory effect of biotin vitamers. Both myocardial water content and plasma LDH activity increased 24 hr after the ISP-treatment. Biotin and biotinamide (10 mg/kg respectively) had slight but statistically significant inhibiting effects on an increase of myocardial water content, and more remarkable decreasing effect on an elevation of plasma LDH activity. No effect was, however, observed with desthiobiotin (20 mg/kg). Table II illustrates the dose response of the inhibitory effect of biotin on increased cardiac water content in two experimental models. In experiment I (a single injection of ISP), biotin at 3 and 10 mg/kg showed statistically significant inhibiting effect ($p<0.05$) on the increased cardiac water content, and the effect was highly significant ($p<0.01$) at 30 mg/kg. In experiment II (2 consecutive injections of ISP) also, biotin at 10 and 30 mg/kg showed highly significant effect ($p<0.01$).

Additive Effect of Biotin and Prednisolone on Myocardial Water Content and Plasma LDH Activity

Biotin (10 mg/kg) and prednisolone (20 mg/kg) had almost the same inhibitory effects on an increase of myocardial water content and an elevation of plasma LDH activity by ISP,

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Table I. Effects of Biotin Vitamers on Myocardial Water Content and Plasma LDH Activity in ISP-treated Rats

Treatment	Initial body weight (g)	Myocardial water content (%)	Plasma LDH (IU)
Normal control	190±2.8[a]	76.15±0.06	145±10
ISP (5 mg/kg)	186±2.5	77.74±0.05	235±12
ISP (5 mg/kg)+biotin (10 mg/kg)	186±2.9	77.19±0.13[b]	156±10[b]
ISP (5 mg/kg)+biotinamide (10 mg/kg)	190±2.8	77.23±0.10[b]	161±10[b]
ISP (5 mg/kg)+desthiobiotin (20 mg/kg)	190±2.4	77.59±0.07	223±19

a) All the figures represent the mean ± standard error of five animals.
b) statistically significant from ISP-values with $p < 0.01$

Table II. Dose Response of Effect of Biotin on Myocardial Water Content

Treatment	Exp. I		Exp. II	
	Initial body weight (g)	Myocardial water content (%)	Initial body weight (g)	Myocardial water content (%)
Normal control	212±2.0[a]	76.17±0.06	199±3.1	76.59±0.11
ISP (5 mg/kg)	214±3.3	78.12±0.12	195±3.1	79.02±0.11
ISP (5 mg/kg)+biotin (3 mg/kg)	213±3.1	77.71±0.10[b]	196±3.4	78.80±0.25
ISP (5 mg/kg)+biotin (10 mg/kg)	211±3.2	77.54±0.24[b]	197±2.0	78.61±0.05[c]
ISP (5 mg/kg)+biotin (30 mg/kg)	211±2.5	77.39±0.16[c]	199±2.9	78.40±0.09[c]

a) All the figures represent the mean ± standard error of five animals.
b) statistically significant from ISP-values with $p < 0.05$
c) statistically significant from ISP-values with $p < 0.01$
Exp. I: Biotin was given intraperitoneally 30 min before a single subcutaneous injection of ISP and rats were sacrificed 24 hr after the ISP-treatment.
Exp. II: ISP was given subcutaneously for 2 consecutive days. Biotin was given intraperitoneally 30 min before each ISP-injection and rats were sacrificed 24 hr after the 2nd ISP-treatment.

Table III. Effects of Biotin and Prednisolone on Myocardial Water Content and Plasma LDH Activity in ISP-treated Rats

Treatment	Initial body weight (g)	Myocardial water content (%)	Plasma LDH (IU)
Normal control	189±3.1[a]	76.58±0.06	121± 9
ISP (5 mg/kg)	186±3.1	78.11±0.09	227±12
ISP (5 mg/kg)+prednisolone (20 mg/kg)	187±2.1	77.75±0.04[b]	171±14[c]
ISP (5 mg/kg)+biotin (10 mg/kg)	190±2.3	77.47±0.05[c]	167±11[c]
ISP (5 mg/kg)+prednisolone (20 mg/kg) +biotin (10 mg/kg)	190±2.2	76.79±0.08[c,d,e]	130±13[c]

a) All the figures represent the mean±standard error of five animals.
b) and *c*) statistically significant from ISP-values with $p < 0.05$ and $p < 0.01$, respectively
d) statistically significant from the ISP+prednisolone-value with $p < 0.01$
e) statistically significant from the ISP+biotin-value with $p < 0.01$

as shown in Table III. It should be noted that the simultaneous treatment with biotin (10 mg/kg) and prednisolone (20 mg/kg) enhanced the inhibitory effects of the respective compounds on myocardial water content and plasma LDH activity, though the enhancement was not statistically significant on plasma LDH activity.

Effect of Biotin and Prednisolone on Myocardial Adenine Nucleotides

Table IV shows the drug effect on myocardial adenine nucleotide levels in ISP-treated rats. An injection of ISP to rats resulted in a significant decrease in the levels of ATP and total adenine nucleotides. Biotin (10 mg/kg) had a significant inhibiting effect on a decrease of ATP level by ISP, but had no effect on total adenine nucleotides. No protection was demonstrated with prednisolone (20 mg/kg) on the decreases of both ATP and total adenine nucleotide levels.

TABLE IV. Effect of Biotin and Prednisolone on Myocardial Adenine Nucleotide Levels in ISP-treated Rats

Treatment	Initial body weight (g)	μmoles/g dry weight	
		ATP	Total adenine nucleotides
Normal control	209±3.2[a]	17.9±0.6	24.8±0.6
ISP (5 mg/kg)	207±3.2	11.7±0.1	18.0±0.7
ISP (5 mg/kg)+prednisolone (20 mg/kg)	211±3.2	11.6±0.4	18.0±0.7
ISP (5 mg/kg)+biotin (10 mg/kg)	210±3.4	13.3±0.4[b]	18.7±0.3
ISP (5 mg/kg)+prednisolone (20 mg/kg) +biotin (10 mg/kg)	211±3.4	12.3±0.2	18.7±0.2

a) All the figures represent the mean±standard error of five animals.
b) statistically significant from ISP-value with $p < 0.05$

Discussion

A potent β-adrenergic stimulating agent, ISP is capable of inducing such increased heart rate and cardiac work that the heart soon falls into hemodynamic coronary insufficiency, myocardial ischemia may be established and the myocardium may undergo necrosis.[14] In the later stage (later than 8 hr after an ISP-injection), myocardial degeneration and necrosis become histologically demonstrable, and the basic and earliest lesions would appear to be hyaline necrosis of the muscle fibers.[15] In our previous report,[6] decreases of ATP and total adenine nucleotides, an increase of myocardial water content and an elevation of plasma LDH activity were observed in this stage. An elevation of plasma LDH activity may reflect the development of degeneration in myocardial cell membranes.

Untreated myocardial cells have a relatively constant water content.[16] In contrast, myocardial cells injured by an acute work load, ischemia or hypoxia have a disturbance of myocardial metabolites, resulting in variations of water content and electrolite compositions.[17~19] Leaf[20] emphasized the importance of sodium pumps in the regulation of the volume of cellular fluid, and suggested that impairment of sodium extrusion lead to a net gain of water. Gains of water and sodium must affect the resulting membrane potential of myocardial cells, their excitability and contractile efficiency.

In ISP-treated rats, biotin had slight but statistically significant inhibitory effects on the changes of myocardial water content and adenine nucleotide level, and rather marked decreasing effect on the elevated plasma LDH activity. The simultaneous administration

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of biotin and prednisolone showed the additive inhibitory effects on an increase of myocardial water content and an elevation of plasma LDH activity by ISP.

The administration of biotin to animals in a single massive dose or in multiple large doses produced essentially no striking pharmacodynamic action.[21,22] Therefore, the mechanism of this inhibition by biotin is probably not *via* pharmacodynamic effect.

Biotin but not prednisolone inhibited a decrease of myocardial ATP level by ISP. Therefore, the inhibitory mechanism of biotin on biochemical changes after ISP may be different from that of prednisolone, though the inhibitory mechanism of prednisolone is obscure.

Pilgrim, *et al.*[23] showed that homogenates of liver from biotin-deficient rats oxidized pyruvate less than did those from normal controls. Summerson, *et al.*[24] reported that the addition of biotin to liver slices from biotin-deficient rats resulted in increased lactate utilization. The tissue from biotin-deficient animals underwent a decrease in phosphoenolpyruvate carboxylase,[25] propionyl-coenzyme A carboxylase[26] and pyruvate carboxylase[27] activities, resulting in a diminished pyruvate oxidation and impaired tissue respiration.[9]

By our experiments, biotin had a significant protective effect on the decrease of myocardial ATP level following ISP. Therefore, it may be assumed that the protective effect of biotin is accomplished through the restoration of depressed pyruvate oxidation.

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