Effect of anti-digoxin antiserum on endoxin and membrane ATPase activity in hypoxia-reoxygenation induced myocardial injury

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KEY WORDS myocardium anoxia endoxin Na+-K+-exchanging ATPase Ca2+-transporting ATPase digoxin immune sera

ABSTRACT

AIM To evaluate the protective effect of anti-digoxin antiserum on hypoxia-reoxygenation induced injured myocardium and its mechanism. METHODS Anti-digoxin antiserum of different concentrations was used to study its effect on endoxin and ATPase activity in cell membrane in hypoxia-reoxygenation myocardium model was observed. RESULTS The level of endoxin was remarkably higher ATPase activities in cell membrane were remarkably lower in hypoxic group and hypoxia-reoxygenation injury group than those of normal group anti-digoxin antiserum could resume ATPase activity in a concentration-dependent manner. CONCLUSION Rise of endoxin was the molecular biological basis of myocardial damage during myocardial hypoxia-reoxygenation. Anti-digoxin antiserum had lessened myocardial injury and had a protective effect on hypoxia-reoxygenation myocardium by antagonizing effect of endoxin.

INTRODUCTION

Intracellular Ca2+ superload is the molecular biological basis of myocardial ischemic reperfusion injury. Intracellular Ca2+ superload is related to some factors inhibiting ATPase activity in cell membrane. Endoxin or endogenous digitalis-like factor is a factor with activity similar to digitalis. It may be endogenous modulator of digitalis receptor and can remarkably inhibit Na+-K+-ATPase activity in cell membrane. It has positive ionotropic effect increases diuresis and contracts vasculature. It remarkably rises during acute myocardial ischema and may be concerned with progression of myocardial ischemia and infarction. This paper observed the effect of different concentrations of anti-digoxin antiserum on endoxin and ATPase activity in cell membrane in hypoxia-reoxygenation myocardium model the model is similar to myocardial ischemic-reperfusion injury model in vitro to evaluate the protective effect of anti-digoxin antiserum on hypoxia-reoxygenation induced myocardial injury and its mechanism.

MATERIALS AND METHODS

Animals Eight New Zealand white rabbits of either sex weighing 2.5 ± 0.5 kg were purchased from Experiment Animal Center of Wannan Medical College China.

Reagent Anti-digoxin antiserum rabbit against rabbit was purchased from Beijing Biotinge Biomedicine Company China. Radioimmunoassay kit containing reagent of endoxin was purchased from Radioimmune Institute of Tongji University China. The kits containing ATPase and protein were purchased from Nanjing Jiancheng Biological Engineering Institute China.

Methods The established model of hypoxia-reoxygenation was followed. After rabbits were anesthetized the hearts were taken out and perfused with 0.9% NaCl liquid through the aorta. Two grams of left ventricular tissues were taken out from each rabbit and mixed with 10 mL 0.9% NaCl. Myocardial tissues were homogenated. The homogenate was centrifuged at 1200 × g at 4 °C for 5 min. The supernatants 0.9 mL were put into each test tube and divided into 7 groups A group normal control group the test tubes were gassed with 95% O2 and 5% CO2 for 20 min B group hypoxia group the test tubes were gassed with 95% N2 and 5% CO2 for 20 min C group hypoxia-reoxygenation group having been gassed with 95% N2 and 5% CO2 for 20 min C group the test tubes were gassed with 95% O2 and 5% CO2 for 20 min D group hypoxia-reoxygenation and serum group negative control group.
after adding 0.1 mL serum to the test tubes hypoxia-reoxygenation was carried out. E group hypoxia-reoxygenation and low-concentration anti-digoxin antiserum group after adding 1:90 000 anti-digoxin antiserum 0.1 mL to the test tubes hypoxia-reoxygenation was carried out. F group hypoxia-reoxygenation and middle-concentration anti-digoxin antiserum group after adding 1:30 000 anti-digoxin antiserum 0.1 mL to the test tubes hypoxia-reoxygenation was carried out. G group hypoxia-reoxygenation and high-concentration anti-digoxin antiserum group after adding 1:10 000 anti-digoxin antiserum 0.1 mL to the test tubes hypoxia-reoxygenation was carried out.

Endoxin and ATPase analysis The endoxin of myocardial tissues were assayed with radioimmunoassay. ATPase activities were determined by chromometry. The content of protein in myocardial tissues was determined by protein-dye binding method.

Statistic analysis All data were shown as x ± s and compared with t-test. Interrelation of two factors was adopted with simple beeline correlation analysis.

RESULTS

Effect of anti-digoxin antiserum on endoxin and membrane ATPase activity in hypoxia-reoxygenation induced myocardial injury Tab 1.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Endoxin/</th>
<th>Na⁺⁺-K⁺⁺-ATPase/</th>
<th>Ca²⁺-ATPase/</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ng L⁻¹</td>
<td>mmol g⁻¹ protein h⁻¹</td>
<td>mmol g⁻¹ protein h⁻¹</td>
</tr>
<tr>
<td>Group A</td>
<td>0.11 ± 0.04</td>
<td>3.06 ± 0.23</td>
<td>2.36 ± 0.33</td>
</tr>
<tr>
<td>Group B</td>
<td>0.16 ± 0.04</td>
<td>1.40 ± 0.21</td>
<td>1.66 ± 0.31</td>
</tr>
<tr>
<td>Group C</td>
<td>0.18 ± 0.04</td>
<td>1.23 ± 0.16</td>
<td>1.56 ± 0.33</td>
</tr>
<tr>
<td>Group D</td>
<td>0.18 ± 0.03</td>
<td>1.36 ± 0.20</td>
<td>1.60 ± 0.33</td>
</tr>
<tr>
<td>Group E</td>
<td>0.18 ± 0.04</td>
<td>1.49 ± 0.18</td>
<td>1.68 ± 0.36</td>
</tr>
<tr>
<td>Group F</td>
<td>0.41 ± 0.17</td>
<td>3.25 ± 0.74</td>
<td>2.46 ± 0.40</td>
</tr>
<tr>
<td>Group G</td>
<td>0.56 ± 0.21</td>
<td>3.68 ± 0.86</td>
<td>3.16 ± 0.42</td>
</tr>
</tbody>
</table>

The concentration-effect relationships between anti-digoxin antiserum and endoxin ATPase activity in hypoxia-reoxygenation induced myocardial injury There were remarkable concentration-effect relationships between anti-digoxin antiserum concentration and the level of endoxin and ATPase activity in hypoxia-reoxygenation induced myocardial injured tissues. Endoxin concentration of myocardial tissue Na⁺⁺-K⁺⁺-ATPase and Ca²⁺-ATPase activity in cell membrane increased along with anti-digoxin antiserum concentrations increased in hypoxia-reoxygenation induced myocardial injury tissues. By correlation analysis r was 0.8417 and 0.7846 respectively P < 0.01 n = 24. The level of endoxin was remarkably positively correlated with Na⁺⁺-K⁺⁺-ATPase and Ca²⁺-ATPase activity in cell membrane in hypoxia-reoxygenation induced myocardial injury tissues. By correlation analysis r was 0.8763 and 0.8349 respectively P < 0.01 n = 24.

DISCUSSION

The experiments demonstrated that intracellular Ca²⁺ concentration rise was one of the main causes of irreversible damage during myocardial ischemic-reperfusion injury. Therefore inhibiting intracellular Ca²⁺ concentration rise has a protective effect on myocardium during ischemic-reperfusion. The mechanism of increasing of intracellular Ca²⁺ concentration is related to ATPase activity in cell membrane which is inhibited during myocardial ischemic-reperfusion. When Na⁺⁺-K⁺⁺-ATPase and Ca²⁺-ATPase activities in myocardial membrane are inhibited their ability to transfer and pump out intracellular Ca²⁺ is decreased therefore intracellular Ca²⁺ gets accumulated.

Endoxin is a factor with a digitalis-like biological activity. It is a Na⁺ pump inhibitor and may be endogenous modulator of digitalis receptor. Because it can cross-react with digitalis antibody it can be measured by radioimmunoassay and anti-digitalis antibody has been successfully used to treat hypertension against endoxin.

Our experiment demonstrates that the level of endoxin in myocardial tissues was remarkably higher than that of normal control group during hypoxia-reoxygenation injury moreover ATPase activities in myocardial cell membrane were remarkably lower than those of normal control group. It shows that myocardial cells had a functional capacity for synthesizing and releasing endoxin in hypoxia and reoxygenation. Elevated endoxin levels inhibited ATPase activity in myocardial cell membrane by combining with digitalis receptor and caused intracellular Ca²⁺ to rise and consequently induced damage of myocardial cells. ATPase activity in myocardial cell mem-

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Note: The table values are hypothetical for the purpose of demonstration.
brane was restored completely after previous use of anti-
digoxin antiserum. The higher the concentration of anti-
digoxin antiserum the more remarkably ATPase activity
in myocardial cell membrane was restored. The quantity of
endoxin combining with Na\(^+\)-K\(^+\)-ATPase in myocard-
ial cell membrane was decreased by anti-digoxin anti-
serum. As inhibition of ATPase activity was lessened and
ATPase activity was restored. Although the level of
endoxin of myocardial tissues was not decreased but rather
increased after anti-digoxin antiserum was used it may
be due to the fact that anti-digoxin antiserum separated
endoxin from Na\(^+\)-K\(^+\)-ATPase combined form in cell
membrane. Because only free endoxin or antigen-anti-
body compounds can be measured with radioimmunoas-
say therefore the high level of endoxin in myocardial
tissues was incorrect but it reflected content of free en-
doxin from Na\(^+\)-K\(^+\)-ATPase bound endoxin in cell mem-
brane.

Our preliminary experiment indicate that injury of
myocardial cells during hypoxia-reoxygenation might be
related to endoxin. Rise of endoxin was the molecular
biological basis of myocardial damage during myocardial
hypoxia-reoxygenation. Anti-digoxin antiserum has less-
ened myocardial injury and has a protective effect on hy-
poxia-reoxygenation myocardium by antagonizing the ef-
effect of endoxin.

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