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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；Ca^{2+}-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，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 Ca^{2+} superload is the molecular biological basis of myocardial ischemic reperfusion injury. Intracellular Ca^{2+} 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 modulater 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 ischemia and may be concerned with progression of myocardial ischemia[1,2]. 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 vivo*) 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[3]. 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 ℃ 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 % O_2 and 5 % CO_2 for 20 min；(B group) hypoxia group：the test tubes were gassed with 95 % N_2 and 5 % CO_2 for 20 min；(C group) hypoxia-reoxygenation group：having been gassed with 95 % N_2 and 5 % CO_2 for 20 min，the test tubes were gassed with 95 % O_2 and 5 % CO_2 for 20 min；(D group) hypoxia-reoxygenation and serum group (negative control group)：

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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[4].

Statistic analysis All data were shown as $\bar{x} \pm s$ 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).

Tab 1. Effect of anti-digoxin antiserum on endoxin and membrane ATPase activity in hypoxia-reoxygenation induced myocardial injury. $n = 8$. $\bar{x} \pm s$. $^{b}P < 0.05$, $^{c}P < 0.01$ *vs* group A. $^{f}P < 0.01$ *vs* group B, C, D, E, respectively. $^{h}P < 0.05$ *vs* group F.

Groups	Endoxin/ $\mu g \cdot L^{-1}$	Na^+-K^+-ATPase/mmol·g^{-1} protein·h^{-1}	Ca^{2+}-ATPase/mmol·g^{-1} protein·h^{-1}
Group A	0.11 ± 0.04	3.06 ± 0.23	2.86 ± 0.33
Group B	0.16 ± 0.04^{b}	1.40 ± 0.21^{c}	1.66 ± 0.31^{c}
Group C	0.18 ± 0.04^{c}	1.28 ± 0.16^{c}	1.56 ± 0.33^{c}
Group D	0.18 ± 0.03^{c}	1.36 ± 0.20^{c}	1.60 ± 0.33^{c}
Group E	0.18 ± 0.04^{c}	1.49 ± 0.18^{c}	1.68 ± 0.36^{c}
Group F	0.41 ± 0.17^{cf}	3.25 ± 0.74^{f}	2.46 ± 0.40^{f}
Group G	0.56 ± 0.21^{cf}	3.68 ± 0.86^{bf}	3.16 ± 0.42^{fh}

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 tissues, Na^+-K^+-ATPase and Ca^{2+}-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, 0.7846, and 0.7165, respectively, $P < 0.01$ ($n = 24$). The level of endoxin was remarkably positively correlated with Na^+-K^+-ATPase and Ca^{2+}-ATPase activity in cell membrane in hypoxia-reoxygenation induced myocardial injury tissues. By correlation analysis, r was 0.8765, 0.8349, respectively, $P < 0.01$ ($n = 24$).

DISCUSSION

The experiments demonstrated that intracellular Ca^{2+} concentration rise was one of the main causes of irreversible damage during myocardial ischemic-reperfusion injury. Therefore, inhibiting intracellular Ca^{2+} concentration rise has a protective effect on myocardium during ischemic-reperfusion[5,6]. The mechanism of increasing of intracellular Ca^{2+} concentration is related to ATPase activity in cell membrane which is inhibited during myocardial ischemic-reperfusion. When Na^+-K^+-ATPase and Ca^{2+}-ATPase activities in myocardial membrane are inhibited, their ability to transfer and pump out intracellular Ca^{2+} is decreased, therefore intracellular Ca^{2+} 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[7].

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 Ca^{2+} to rise, and consequently induced damage of myocardial cells. ATPase activity in myocardial cell mem-

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 myocardial cell membrane was decreased by anti-digoxin antiserum. 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-antibody compounds can be measured with radioimmunoassay, therefore, the high level of endoxin in myocardial tissues was incorrect, but it reflected content of free endoxin from Na^+-K^+-ATPase bound endoxin in cell membrane.

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 lessened myocardial injury and has a protective effect on hypoxia-reoxygenation myocardium by antagonizing the effect of endoxin.

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地高辛抗血清对缺氧复氧损伤心肌组织内洋地黄素和心肌细胞膜 ATP 酶活性的影响

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关键词 心肌；低氧；内洋地黄素；Na^+-K^+ 交换 ATP 酶；Ca^{2+} 转运 ATP 酶；地高辛；免疫血清

目的：评价地高辛抗血清对缺氧复氧损伤心肌的保护作用及其机制. **方法**：制备心肌组织缺氧复氧损伤模型，观察不同剂量的地高辛抗血清对缺氧复氧损伤心肌组织内洋地黄素水平和心肌细胞膜 ATP 酶活性的影响. **结果**：缺氧复氧损伤可使心肌组织内洋地黄素水平明显升高，心肌细胞膜 ATP 酶活性明显下降；地高辛抗血清能明显拮抗缺氧复氧对心肌细胞膜 ATP 酶活性的抑制作用，使酶活性得到恢复. **结论**：缺氧复氧损伤所致心肌组织内洋地黄素水平升高是缺氧复氧介导心肌损伤的分子生物学基础，地高辛抗血清通过拮抗内洋地黄素的作用，减轻缺氧复氧所致心肌损伤，对缺氧复氧损伤心肌具有保护作用.

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