**BASIC PHARMACOLOGY**

**Endoxin Antagonist Lessens Myocardial Ischemia Reperfusion Injury**

Yong-Sheng Ke, De-Guo Wang, He-Gui Wang, and Shang-Yin Yang

Department of Cardiology, Yijishan Hospital, Wannan Medical College, Wuhu, 241001 China

Summary. Objective: To elucidate whether endoxin is one of important factors involved in myocardial ischemia reperfusion (MIR) injury, the change of myocardial endoxin level was determined in rats with MIR injury model and the effects of anti-digoxin antiserum (ADA), an endoxin specific antagonist, on MIR injury were studied.

Methods: MIR injury model was obtained by ligating left anterior descending coronary artery 30 min followed by 45 min reperfusion. Sprague Dawley rats were randomly divided into six groups of 10 rats, each. Sham group, MIR group, normal saline group, ADA 9, 18 and 36 mg kg\(^{-1}\) were respectively injected via tail vein after 45 min reperfusion. ECG was continuously recorded. After reperfusion left ventricular myocardium samples of ischemic area were processed immediately. Myocardial endoxin level, Na\(^{+}\)-K\(^{+}\)-ATPase, Ca\(^{2+}\)-ATPase, Mg\(^{2+}\)-ATPase activities, and intramitochondrial Ca\(^{2+}\) content were measured.

Results: Myocardial endoxin level was significantly increased; Na\(^{+}\)-K\(^{+}\)-ATPase, Ca\(^{2+}\)-ATPase, Mg\(^{2+}\)-ATPase activities were remarkably decreased; intramitochondrial Ca\(^{2+}\) content was remarkably increased and occurred scores of ventricular arrhythmias were significantly increased in early stage of reperfusion in rats with MIR. In all groups with ADA, myocardial endoxin level was remarkably decreased; Na\(^{+}\)-K\(^{+}\)-ATPase, Ca\(^{2+}\)-ATPase and Mg\(^{2+}\)-ATPase activities were drastically increased; intramitochondrial Ca\(^{2+}\) content was declined; ST segments and ventricular arrhythmias were significantly improved.

Conclusion: Myocardial endoxin level was increased in MIR, which implies that the elevated endoxin may be one of major factors inducing MIR injury. This postulate is supported by the observation that ADA has protective and therapeutic effects against MIR injury probably by antagonizing the action of endoxin. The underlying mechanism may be ascribed to restoration of energy metabolism, and attenuation of intracellular Ca\(^{2+}\) overload.

Key Words. endoxin, ischemia reperfusion injury/myocardium, Na\(^{+}\)-K\(^{+}\)-ATPase, Ca\(^{2+}\)-ATPase, Mg\(^{2+}\)-ATPase, anti-digoxin antiserum

Introduction

Myocardial ischemia results in an increase in intracellular Na\(^{+}\) concentration ([Na\(^{+}\)]\(_i\)), which secondarily increases intracellular Ca\(^{2+}\) ([Ca\(^{2+}\)]\(_i\)) via Na\(^{+}\)-Ca\(^{2+}\) exchange, resulting in cellular injury. The rise in [Na\(^{+}\)]\(_i\) can be ameliorated by limiting the stimulus for Na\(^{+}\)-H\(^{+}\) exchange or limiting Na\(^{+}\) influx via either the Na\(^{+}\)-H\(^{+}\) exchanger or the Na\(^{+}\)-K\(^{+}\)-2Cl\(^{-}\) cotransporter. Theoretically, the rise in [Na\(^{+}\)]\(_i\) could also be limited by increasing Na\(^{+}\) efflux during ischemia via the Na\(^{+}\)-K\(^{+}\) pump (Na\(^{+}\)-K\(^{+}\)-ATPase) [1–3]. Whereas Na\(^{+}\)-K\(^{+}\)-ATPase activity is primarily driven by ionic gradients and the availability of ATP, there is evidence that activity of the Na\(^{+}\)-K\(^{+}\)-ATPase is regulated by some substances. The studies have shown that endoxin is an endogenous ligand of digitalis receptor that inhibits the Na\(^{+}\)-K\(^{+}\)-ATPase activity in cell membrane, has competitive displacing activity against \([3H]\)-ouabain binding to the enzyme, inhibits \(^{86}\)Rb uptake into intact human erythrocytes, and cross-reacts with anti-digoxin antibody. Therefore, endoxin may be an endogenous inhibitor of Na\(^{+}\)-K\(^{+}\)-ATPase [4,5]. Several papers reported [6–8] that the serum endoxin level was remarkably raised in patients with acute myocardial infarction and in model animals with experimental myocardial ischemia-reperfusion (MIR). But, it is still unclear that the change of endoxin levels is one of causes and/or outcomes of acute myocardial ischemia. In the present study, the effects of the endoxin antagonist, anti-digoxin antiserum (ADA), on myocardial endoxin levels, ATPase activities, and arrhythmias induced by MIR were observed in the rat.

Materials and Methods

Rats. Sixty male Sprague-Dawley rats (Grade II, Certificate: SCXK 2002-0018), weighing (260 ± 50) g, were purchased from Experimental Animal Center of Nanjing Medical University (China). The research protocols were approved by the Animal Care Committee of

*Supported by Anhui Provincial Natural Science Foundation of China (No. 01043902) and Natural Science Foundation of Anhui Education Committee of China (No. 90j0219, 2001kj256).

Address for correspondence: Dr. Ke Yong-Sheng, M.D., Department of Cardiology, Yijishan Hospital, Wannan Medical College, Wuhu, 241001, China. Tel.: 0086-553-573-8856; E-mail: keyongsheng@163.com
Preparation of myocardial mitochondria. A piece of myocardium from area subjected to MIR was minced and digested using a protease enzyme (0.5 mg·mL⁻¹, Nagarse) in cold isolation medium (mmol·L⁻¹; mannitol 225, sucrose 75, Tris-HCl 10, EGTA 2, pH 7.2). Cardiomyocyte mitochondria were isolated by homogenization and differential centrifugation, and mitochondrial protein was measured using a modified Lowry method. All preparative procedures were performed at 4°C. The intramitochondrial Ca²⁺ content was assayed by atomic absorption spectrometry.

Diagnosis and quantification analysis of arrhythmias. Diagnosis standard and quantification analysis of arrhythmias were established by Curtis et al. [10,11] (Table 1).

\[ \text{Arrhythmia score} \times 10 = \text{Type of arrhythmia} \]

<table>
<thead>
<tr>
<th>Arrhythmia score</th>
<th>Type of arrhythmia</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No arrhythmia</td>
</tr>
<tr>
<td>1</td>
<td>Atrial arrhythmia or occasional premature ventricular contraction</td>
</tr>
<tr>
<td>2</td>
<td>Frequent premature ventricular contraction</td>
</tr>
<tr>
<td>3</td>
<td>Ventricular tachycardia (1-2 episodes)</td>
</tr>
<tr>
<td>4</td>
<td>Ventricular tachycardia (&gt;3 episodes)</td>
</tr>
<tr>
<td>5</td>
<td>Ventricular fibrillation (≥5 min)</td>
</tr>
<tr>
<td>6</td>
<td>Ventricular fibrillation (≥5 min) or death</td>
</tr>
</tbody>
</table>

Statistic analysis. All data are expressed as mean ± SD and analyzed using ANOVA followed by t-test. Interrelation of two factors was adopted with simple linear correlation analysis. \( P < 0.05 \) were considered to be statistically significant.

Results

Effects of ADA on the ST segment and arrhythmias. After ligating left anterior descending coronary artery, the ST segment of ECG was quickly elevated. Elevated ST segments and T wave confluence formed monophasic curve at ischemia 5 min, meanwhile, R amplitude and heart rate remarkably decreased. In MIR group, elevated ST segments were gradually reversed after reperfusion 30 min, but ST segments elevation were still larger than 50% R wave amplitude of same lead at reperfusion 45 min. In all ADA groups, elevated ST segments changed into isoelectric level at reperfusion 5 min (Fig. 1). In MIR group, the incidence of reperfusion ventricular arrhythmias (premature ventricular contraction, ventricular tachycardia, and/or reversible ventricular fibrillation) was high during early stage of reperfusion. No episodes of irreversible ventricular fibrillation were observed. Hearts treated with ADA manifested lower incidence of arrhythmias. Arrhythmia scores of treatment groups were significantly lower than that of MIR group (Table 2).

Effects of ADA on myocardial endoxin levels. The myocardial endoxin level in MIR group was significantly higher than that in sham group (Table 3). ADA could significantly decrease the myocardial endoxin level. By correlation analysis, the dose of ADA had a significant negative correlation with myocardial endoxin level: \( r = -0.768, P < 0.01 \).