Protective Effects of API\textsubscript{0134} on Myocardial Ischemia and Reperfusion Injury

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Summary: Previous studies have demonstrated that a crude extract from Chinese medicinal herb Andrographis Paniculata Nees (APN) could prevent myocardial ischemia and reperfusion injury. A refined extract API\textsubscript{0134} was studied further. LAD was ligated for 90 min in 20 dogs and then reperfused for 120 min. The animals were randomly divided into 2 groups, API\textsubscript{0134} treated group (n=10), 45 min after ischemia receiving a slow i. v. bolus of 1 mg/kg and then an infusion of 80 μg kg\textsuperscript{-1} min\textsuperscript{-1} for 60 min and control group (n=10) which was given only 5 % glucose in saline. Result showed that the hemodynamics in API\textsubscript{0134} treated group showed better effects of preventing the increase of the LVEDP and maintaining relatively normal CO as compared with control group. Ischemic ECGs were significantly milder. Malignant arrhythmia did not appear in API\textsubscript{0134} treated group. After reperfusion, the infarct size was smaller (5.06±2.67 % vs 10.45±3.11 %, P < 0.01), the damages found in myocardial ultrastructure were significantly milder. It is concluded that API\textsubscript{0134} may protect the myocardium from ischemic reperfusion injury.

Key words: myocardial reperfusion injury; API\textsubscript{0134}

Our laboratory had reported previously that Andrographis Paniculata Nees (APN), a crude extract of Chinese medicinal herb, could attenuate the myocardial injury induced by ischemia and reperfusion\textsuperscript{[1]}. In recent years we have extracted API\textsubscript{0134} from APN. The purpose of the present work is to further observe whether API\textsubscript{0134} possesses protective effect on the myocardial ischemia-reperfusion injury.

1 MATERIALS AND METHODS

1.1 Experimental Procedure

Twenty mongrel dogs of either sex with body weight of 10 to 15 kg, were randomly divided into two groups, with 10 dogs in each group. All dogs were anesthetized with 30 mg/kg of sodium pentobarbital intravenously, and undergone tracheotomy. Artificial respiration was maintained by respirator. The right femoral artery and vein were cannulated for the measurement of arterial pressure (AP) and for fluid and drug administration respectively. A catheter was advanced through the left carotid artery and inserted into the left ventricle for recording left ventricular end-diastolic pressure (LVEDP), and a Swan-Ganz catheter was placed in the pulmonary artery via the right external jugular vein for measurement of cardiac output (CO) by thermodilution technique. After left thoracotomy, the left anterior descending coronary artery (LAD) was dissected free of surrounding tissue at the upper one third, occluded for 90 min and then followed by 120 min reperfusion, initiated by removing the ligature. Epicardial electrocardiograms (E-ECG) were obtained by a monopolar electrode in the area of surgical preparation, 1 point over the ligation and other 9 recording points were located under the site of ligation.

1.2 Drug and Administration Route

API\textsubscript{0134} (API), a brown crystalline powder and an active component extracted from APN, was produced by the Pharmaceutical Factory of Tongji Hospital in Wuhan. A water solution of API 9 mg/kg was given in the treated group. 45 min after occlusion, API 1 mg/kg was administered...
intravenously in bolus for 5 min, and then infusion at a rate of 80 μg · kg⁻¹/min for 100 min until 60 min after reperfusion. In the control group, equivalent amount of 5 % glucose in normal saline was given.

1.3 Parameters Observed

Five min before, 45 and 90 min after occlusion and 60, 120 min after reperfusion, hemodynamics, standard limb lead I and E-ECG were recorded on an eight-channel polygraph system (Nilon, Kohden, Japan). In 9 leads of E-ECG over ischemia area, the sum of ST segment elevation (ST), mean amplitude of Q waves represented myocardial injury. The number of the leads with elevated ST Segment > 2 mv, or depressed ST Segment < 1 mv, and the number of the leads with appearance of Q waves suggested the extent of myocardial ischemia and necrosis. Incidence of arrhythmias was monitored during the experiment.

At the end of each experiment, a 10 % India ink (1.5 ml/kg body weight) was injected into the left atrial appendage for quantification of extent of myocardial ischemia, i.e. risk area. Myocardial samples of the excised heart from regions of central and marginal ischemia, and neighboring non-ischemic area of 4 X 4 X 4 mm were taken respectively for electron microscopic examination. The heart was dissected transversely from base to apex on 0.5 cm thick rings, and stained with 1 % Triphenyltetrazolium chloride (TTC) at 37 °C for 7 min. The unstained regions of myocardium were designated as regions of infarction. The myocardium in risk or infarct area were weighed, and expressed as percentage of the total left ventricular mass. They were sent for light microscopic examination.

1.4 Statistics

All values were expressed as the $\bar{x} \pm \bar{s}$ and compared by means of analysis of variance. Means were considered to be significantly different if $P < 0.05$.

2 RESULTS

2.1 General Condition and Incidence of Arrhythmias

Three dogs which died during surgical preparation were excluded. 9 dogs in the control group, 8 dogs in the treated group with no difference in mean weight, sex and the amount of infusion were used for experiment. 90 min before establishment of ischemia, only isolated ventricular premature beats (VPBS) developed. After reperfusion, in the control group, one dog died of ventricular fibrillation, and the other 8 dogs developed VPBS 5 to 10 min after reperfusion. 5 of them developed multifocal and numerous VPBs and ventricular tachycardia (VT) 20—30 min after reperfusion. Whereas in the treated group, 7 dogs developed isolated VPB, only one dog had isolated VPB 7 min after reperfusion. The rest 6 dogs all developed isolated VPB 30 min after reperfusion.

2.2 Hemodynamics

Before and after reperfusion, there were no significant changes in heart rate (HR) among 2 groups. 45 min after occlusion, both groups showed similar values, i.e. decreased CO, slight increase in mean arterial pressure (MAP) and significant increase in LVEDP. In the treated group, MAP was reduced significantly, which was more conspicuous 90 min after occlusion. MAP gradually recovered after reperfusion. No significant change was observed in LVEDP and CO, whereas in the control group, 90 min after occlusion and during reperfusion LVEDP increased progressively and CO was decreased further and was significantly reduced at the end of experiment.

2.3 E-ECG

In both groups, 45 min after occlusion, similar results were found, i.e. unchanged R wave amplitude, significant ST-segment elevation showing marked increase in $\Sigma$ST and NST, and appearance of Q wave. In the treated group, there was significant decrease in $\Sigma$ST and NST 90 min after occlusion, but with no significance as compared with the control group. After reperfusion, R wave amplitude and $\Sigma$ST were significantly decreased in the control group as compared with the treated group. NST were markedly decreased in both groups, however, there was no significant difference.