Contents lists available at ScienceDirect IF: 0.926

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading doi: 10.1016/S1995-7645(14)60321-7

Effects of antiarrhythmic peptide 10 on acute ventricular arrhythmia

Bing Sun, Jin-Fa Jiang, Cui-Mei Zhao, Chao-Hui Hu*

Department of Cardiology, Tongji University Affiliated Tongji Hospital, Shanghai 200065, China

ARTICLE INFO

Article history:
Received 15 December 2014
Received in revised form 20 January 2015
Accepted 15 February 2015
Available online 20 March 2015

Keywords:
Cx43
Ventricular arrhythmia
Acute myocardial ischemia

ABSTRACT

Objective: To observe the effects antiarrhythmic peptide 10 (AAP10) aon acute ventricular arrhythmia and the phosphorylation state of ischemic myocardium connexin. **Methods:** Acute total ischemia and partial ischemia models were established by ceasing perfusion and ligating the left anterior descending coronary artery in SD rats. The effects of AAP10 (1 mg/L) on the incidence rate of ischemia-induced ventricular arrhythmia were observed. The ischemic myocardium was sampled to detect total-Cx43 and NP-Cx43 by immunofluorescent staining and western blotting. the total-Cx43 expression was detected through image analysis system by semi-quantitative analysis. **Results:** AAP10 could significantly decrease the incidence of ischemia-induced ventricular tachycardia and ventricular fibrillation. During ischemic stage, total ischemia (TI) and AAP10 total ischemia (ATI) groups were compared with partial ischemia (PI) and AAP10 partial ischemia (API) groups. The rates of incidence for arrhythmia in the ATI and API groups (10% and 0%) were lower than those in the TI and PI groups (60% and 45%). The difference between the two groups was statistically significant (P=0.019, P=0.020). The semi-quantitative analysis results of the ischemic myocardium showed that the total-Cx43 protein expression distribution areas for TI, ATI, PI and API groups were significantly decreased compared with the control group. On the other hand, the NP-Cx43 distribution areas of TI, ATI, PI and API groups were significantly increased compared with the control group (P>0.05). AAP10 could increase the total-Cx43 expression in the ischemic area and decrease the NP-Cx43 expression. Western blot results were consistent with the results of immunofluorescence staining. **Conclusions:** AAP10 can significantly decrease the rate of incidence of acute ischemia-induced ventricular tachycardia and ventricular fibrillation. Acute ischemic ventricular arrhythmias may have a relationship with the decreased phosphorylation of Cx43 induced by ischemia. AAP10 may stimulate the phosphorylation of Cx43 by increasing the total-Cx43 expression and decreasing the NP-Cx43 expression in the ischemic area, so as to decrease ventricular arrhythmia.

1. Introduction

The gap junction (GJ), which is located between myocardial cells, provides a direct communication pathway between ions and small molecular substances, to mediate intercellular communication. As the neighboring cells uncouple, the connecting channel of the gap closes, and subsequently induce slow and heteroplasmic electrical conductions that easily promote arrhythmia[1]. Cx43 is a major ventricular GJ protein, and its functional status is regulated based on the state of phosphorylation. Previous studies showed that Cx43 dephosphorylation may have an active role during the GJ channel closing process[2-5].

Antiarrhythmic peptide (AAP)[3] was named from its potent antiarrhythmic effects. In the AAP family, AAP10 has the same pharmacological effect[4]. Stains *et al*[5] considered the eliciting anti-arrhythmic effects of AAP10 through enhancing electrical conduction between the GJ. However, there was no uniform

*Corresponding author: Chao-Hui Hu, MD, Associate Chief Physician, Department of Cardiology, Tongji University Affiliated Tongji Hospital, No. 389, Xincun Road, Putuo District, Shanghai 200065, China.
Tel: 86+21-66112819
E-mail: huchaohui3486@126.com
Foundation project: This project was supported by the National Natural Science Foundation of China (No: 81300150).

conclusion pertaining to the mechanisms involved in enhancing electrical conduction. Li *et al*[6] speculated that AAP10 may have some protective effect on the Cx43 expression. AAP10 can reduce the rate of incidence of arrhythmia through adjusting the P-Cx43 concentration. However, this viewpoint still falls short of sufficient experimental validation. To further explore the effects of AAP10 on acute ventricular arrhythmia, the author and his team established isolated acute cardiac ischemia SD rat models, and explored the possible mechanisms of the antiarrhythmic effects of AAP10 on acute ventricular arrhythmia from the Cx43 phosphorylation perspective.

2. Materials and methods

2.1. Experimental animals and equipment

A total of 60 SD rats, half male and half female, were provided by the Experimental Animal Center of Shanghai Traditional Chinese Medicine University, weighing (300±50) g. The rats were initially anesthetized with 3% sodium pentobarbital 30 mg/kg (US Biotopped Co.), and treated with 250 μ/kg heparin (Shanghai Fudan Fuhua Pharmaceutical Co., Ltd.), as an anticoagulant. After anesthesia, the costal margin of the abdominal wall below the xiphoid process was explored, and the diaphragm was cut open. The chest wall was cut along the anterior axillary line, turning up to the side of the head. Three branches of the aortic arch were clamped by micro surgery forceps. The aorta (retaining 3-4 mm from the root of heart), pulmonary artery, pulmonary vein, and inferior vena cava were rapidly interrupted. After exposure of the aortic arch, the complete heart was cut along the aortic stump, which was cut below aortic arch. The heart was cut off and was placed into an ice-cold Kreb-Henseleit (K-H) solution (4 ℃); residual blood was gently washed away. The edges of the ascending aorta were clamped with two micro-surgical forceps. The filling canals of the aorta were inserted, fixed by an artery clamp, and the Langendorff perfusion device (AD Instruments Pty Ltd, Australia) was rapidly connected, and fixed by line 0. It was then perfused with a modified K-H buffer, and saturated with 95% O_2 and 5% CO_2. The perfusion fluid temperature was kept at 37 ℃ with a PH value of 7.4. During the experiment, the perfusion pressure was kept at 80-100 cmH_2O and the perfusion flow was 8-10 mL/min. After the heart perfusion model was successfully established, the isolated heart started to re-beat within seconds. When the heart re-started and cardiac functions became stable, a pressure-measuring catheter balloon was inserted into the left ventricle through the mitral valve, and the other end of the pressure-measuring catheter was connected to Multi physiological recorder through pressure sensor.

A 0.3 mm diameter silver wire was used as a pair of electrodes to record the epicardial electrical diagram; the lead wire was connected to the physiological recorder (Shanghai Alcott Biotechnology Co., Ltd.). One of the electrodes was fixed at the right ventricular free wall, the another electrode was fixed at the left ventricular free wall[6].

The ischemia animal model was established by ligating the left anterior descending artery (LAD). Between the left atrial appendage and arterial cone, a wire was used to bypass the LAD; both ends of wire were passed through a hollow plastic tube, which was ligated on another short rubber hose. Myocardial ischemia was induced, and it was observed for 30 minutes.

2.2. Experimental scheme

2.2.1. Grouping and establishment of ischemia-reperfusion model

Rats in control group (Control) and total ischemia group (TI) received isolated heart infusion for 30 minutes, then perfusion was stopped for 30 min. Rats in partial ischemia group (PI) received isolated heart infused for 30 minutes, then regional ischemia was induced by ligating LAD for 30 minutes. After 15 minutes of pretreatment, rats in AAP10 total ischemia (ATI) group were treated with perfusion fluid containing 1 mg/L concentration of AAP10 for another 15 minutes, and perfusion was stopped for 30 minutes. Rats in AAP10 partial ischemia (API) group were treated with perfusion fluid containing 1 mg/L AAP10 concentration for another 15 minutes, then regional ischemia was induced by ligating LAD for 30 minutes.

After pretreatment, the heart was isolated in a K-H solution for 15 minutes; and the stability of the cardiac functions was measured. Time was set to 0 min at the beginning of the experiment. The heart was treated with different perfusion fluids, according to the different groups; then perfusion was stopped to induce total heart ischemia or ligated LAD to induce a partial ischemia (except the control group) for 30 minutes. The experiment was stopped when there was continuous ventricular tachycardia (>1 min) or ventricular fibrillation. The control group did not receive any intervention, and the other processes were the same as above.

2.2.2. Detection of the protein expression

After perfusion in the partial ischemia group, the Evans blue dye test was carried out. The aizen area represented the non-ischemic area, and the undyed area represented the ischemic area. TTC stain was carried out to the cardiac muscle at the undyed area; the red area represented the ischemic area, and the unstained area represented the infarct area. Myocardial ischemia was detected by immunofluorescence staining and analyzed by a laser scanning confocal microscope. The hearts from the control group were directly detected by the above method at the LAD perfusion area.

Western blot detection was also carried out on the myocardial of the groups at the same time. A total of 100 mg of myocardial