缺血，缺血再灌流伤害与 ischemic preconditioning 之机转：药物发展之应用

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Attenuation of Post-Ischemia Reperfusion Injury by LS-NTU-106 (an Aporphine Alkaloid) in rat hearts.

Wei-Luen Chang¹, Shoei-Sheng Lee², Ming-Jai Su¹

¹Institute of Pharmacology and ²Department of pharmacy, College of Medicine, National Taiwan University, Taipei, Taiwan.
Abstract

Previous studies in our lab have indicated that pretreatment of LS-NTU-106 before ischemia could reduce the ischemia and ischemia/reperfusion injury. However, Reperfusion therapy has become a practical and effective strategy in the salvage of ischemic myocardium. The objective of this study was further to evaluate whether LS-NTU-106 administered at the time of coronary reperfusion could have the same cardioprotection as pretreatment before the ischemia/reperfusion period. Anesthetized, open-chest rats were subjected to 60 min of regional ischemia and 120 min of reperfusion. Regional ischemia could induce severe arrhythmia including ventricular tachycardia and ventricular fibrillation. Animal with similar arrhythmia pattern were randomly received vehicle or LS-NTU-106 10 min before the onset of reperfusion only. Infarct size was reduced in the LS-NTU-106 treated groups in a dose-dependent manner compared with control. (LS-NTU-106 $10^{-6}$ mole·kg$^{-1}$ 22.60±2.23%; vs LS-NTU-106 $10^{-7}$ mole·kg$^{-1}$ 31.55±3.26%; vs control 43.57±2.74% P<0.05). It also reduced plasma concentrations of creatine kinase and cardiac MPO activity. After 120 min reperfusion recovery of developed pressure was 78±6 mmHg and 90±5 mmHg in control and LS-NTU-106 ($10^{-6}$ mole/kg$^{-1}$) treatment group. Recovery of rates of pressure development (+dp/dt) and relaxation (-dp/dt) also significantly improved in hearts treated with LS-NTU-106. These protective effects afforded by LS-NTU-106 from post-ischemia reperfusion injury were abrogated by opioid receptor antagonist, naloxone and IKATP blocker 5HD.
Introduction

Myocardial ischemia–reperfusion injury represents a clinically relevant associated with thrombolysis, angioplasty and coronary bypass surgery (1). Injury of myocardium due to ischemia–reperfusion includes cardiac contractile dysfunction, arrhythmias and irreversible myocyte damage (2-7). Efforts to mitigate or prevent ischemia-reperfusion injury have traditionally focused on finding ways to block events associated with ischemic injury. In 1986, Murry et al (8) first introduced the concept of ischemic preconditioning in which repetitive brief episodes of ischemia protected the heart against the deleterious effect of more prolonged ischemia (ischemic preconditioning, IPC). A variety of mediators, including adenosine, opioid, and bradykinin, which act on cardiac cell membrane receptors have been implicated in the mechanism of IPC, and have the potential to mimic the effects of IPC to protect heart from ischemia/reperfusion injury, while pretreatment these agents have been shown to reduce arrhythmia, infarct size, and to improve contractile function in the stunned myocardium (9).

Traditionally, the importance of opioid receptor agonists and antagonists has focused on the treatment of pain. However, it has been recently found that the heart may be modulated by opioids both in physiological and pathophysiological states (10). The first evidence of the importance of the opioid receptor as an integral component of preconditioning-induced cardioprotection was published in 1995. Schultz et al (11) demonstrated that naloxone, a nonspecific opioid receptor antagonist, could blunt the cardioprotective effects of IPC in a rat model. Subsequently, Chien and Van Winkle (1996) (12) have demonstrated those opioid receptors are also involved in IPC in the rabbit heart. Infusion of morphine in the absence of IPC could induce cardioprotection in
the in vivo rat heart (13). Additionally, in a porcine model of myocardial ischemia, Schulz et al. demonstrated the importance of endogenous opioids in myocardial salvage since naloxone could abolish cardioprotection induced by IPC as assessed by infarct size reduction (14). Cardioprotection afforded to the ischemic myocardium via opioid receptor stimulation also appears to be due to KATP channel activation. Bell et al demonstrates a role for the mitochondrial KATP channel in both preconditioning and opioid-induced cardioprotection in cardiac tissue (15).

Although experimental studies demonstrated that ischemic and pharmacological preconditioning attenuates ischemia-reperfusion injury, these interventions have to be applied before the prolonged “index” ischemia. In the long run, clinical as well as preclinical results using various cardioprotective strategies to attenuate reperfusion injury have been rather unsatisfactory (16, 17). The inconsistent results among different species (including humans) and the difficulty in translating these cardioprotective strategies into clinical practice have dampened the enthusiasm for such therapeutic approaches. However, the implementation of cardioprotective therapy at the time of reperfusion is clinically feasible because the onset of reperfusion is more predictable and is under the clinician’s control.

LS-NTU-106 is a phenolic aporphine alkaloid isolated from plants of several families such as Lauracea (18). It was found to be a partial Ca$^{2+}$ channel agonist with strong Na$^+$ and K$^+$ channel-blocking activities to exhibit antiarrhythmic activity (19). Recently, we also indicated that pretreatment of LS-NTU-106 before ischemia could reduce the ischemia and ischemia/reperfusion injury. The cardioprotective effects of LS-NTU-106 in the I-R rats may be correlated with its antioxidant activity and
upregulation of NO production (20). However, application of drugs before reperfusion therapy has become a practical and effective strategy in the salvage of ischemic myocardium. The objective of this study was further to evaluate whether LS-NTU-106 administered at the time of coronary reperfusion could have the same cardioprotection as pretreatment before the ischemia/ reperfusion period, and to investigate its signal pathway of action.
Methods

Animal preparation

Male Sprague–Dawley rats (250–350 g b.wt.) underwent myocardial ischemia by a temporary occlusion of the left main coronary artery as previously described (21). Briefly, rats were anesthetized with urethane (1.25 g/kg i.p.) and placed on an operating table. A Millar catheter with high fidelity pressure sensor (model SPC 320, size 2F, Millar Instruments, Houston, TX, U.S.A.) was inserted via the right carotid artery into the left ventricle. After tracheotomy, the animals were ventilated with room air by a rodent ventilator (model 683, Harvard, USA) with a stroke volume of ~12 ml/kg body weight and at a rate of 60 strokes/min. The chest was opened and the ribs were gently spread. The heart was quickly expressed out of the thoracic cavity, inverted and a 7/0 silk ligature was placed under the left main coronary artery. The heart was repositioned in the chest and the animal was allowed to recover for 15 min. A small plastic snare formed from a Portex P-270 cannula was threaded through the ligature and placed in contact with the heart. Tightening the ligature could then occlude the artery and reperfusion was initiated by withdrawing the polyethylene tubing. Regional myocardial ischemia was verified by the presence of a zone of cyanosis in the area of distribution of the occluded vessel and by changes in the electrocardiogram consistent with the presence of transmural regional myocardial ischemia (ST – segment elevation).

Before and during the ischemia or reperfusion period, heart rate (HR), left ventricular developed pressure (LVDP), the maximum and minimum first derivative of LVSP (+dp/dt_max and –dp/dt) and ECG changes were recorded on a personal computer with a data analysis software (PowerLab data acquisition system, AD Instruments, Castle
Experimental groups

All animals proceeded to coronary artery occluded for 1 hour followed by 2 hours of reperfusion were randomly assigned to one of six groups (Fig 1). Animals were infused with a bolus of LS-NTU-106 (10^{-7}; 10^{-6} mole/kg) or vehicle (0.9% NaCl) from a jugular vein 10 min before coronary reperfusion. To demonstrate the mechanism of the cardioprotective effect induced by LS-NTU-106, two antagonists were used. A non selective opioid antagonist, naloxone (1mg/kg; 3mg/kg), an IKATP blocker, 5HD (5mg/kg) was given 10 min before LAD occlusion. Doses of antagonists selected were those known to block cardiac preconditioning.

Definition of area at risk and area of infarction

Infarct size and ischemic risk area were determined by the triphenyl tetrazolium chloride technique [22]. At the end of reperfusion, the left coronary artery was reoccluded. Methyl blue dye (2 ml; 3% in 0.9% NaCl)) was slowly infused from a jugular vein to delineate the risk area as a perfusion defect. The risk area was cut out, weighed, and expressed as a percentage of total ventricular weight. Thereafter, ventricular tissue was sliced into 1 mm sections for incubation in tetrazolium dye (10 mg 2,3,5-triphenyltetrazolium chloride/ml 0.9% NaCl, pH 7.4) at 37°C for 40 min. Sections were then placed in 10% formaldehyde for 2 days before the infarct (white) tissue was excised. The weight of infarct tissue was expressed as a percentage of occluded zone.
Plasma CK analysis

Cellular damage was evaluated by measuring the plasma CK. The blood samples were drawn from the carotid artery at the end of reperfusion, collected in heparinized tubes. The blood was centrifuged at 1000 g for 5 min. The plasma was kept at 4°C until it was used for determination of CK activity with a commercial kit from Randox.

Myeloperoxidase activity

Myeloperoxidase activity, an index of PMN accumulation, Cardiac tissues collected 120 min after reperfusion, were homogenised in a solution containing 0.5% hexadecyltrimethylammonium bromide dissolved in 10 mM potassium phosphate buffer (pH 7) and centrifuged for 15 min 125000g at 4°C. An aliquot of the supernatant was then allowed to react with a solution of tetra-methyl- benzidine (1.6 mM) and 0.1 mM H₂O₂. The rate of change in absorbance was measured by a spectrophotometer at 405 nm. Myeloperoxidase activity was defined as the quantity of enzyme degrading 1 mmol of peroxide min at 37°C and was expressed in microunits per gram weight of wet tissue.

Drugs

LS-NTU-106 was prepared from isoboldine, isolated from plant Neolitsea konishii K, by selective 9-O-methylation with diazomethane. Urethane, naloxone, 5-hydroxydecanoate (5-HD), 2,3,5-triphenyl- tetrazolium chloride (TTC), were purchase from Sigma Chemical Co. (St. Louis, MO, USA)

Statistical analysis
All values are presented as mean ± standard error. Differences between groups were assessed by one-way ANOVA and Student-Newman-Keuls multiple comparison test where appropriate. p values < 0.05 were considered as significant.
Results

Cardiac function recovery in ischemia-reperfusion

Heart rate (HR), left ventricular developed pressure (LVDP), the maximum and minimum first derivative of LVDP (+dp/dt\text{max} and –dp/dt) at baseline and after 120 min of reperfusion are summarized in Table 1. There were no significant differences between groups at baseline. In all groups, HR increased. However, at 120 min of reperfusion, the recovery of developed pressure was significantly higher in the 10^{-6}\text{mole/kg LS-NTU-106 treated group as compared to control values (90 ± 5 vs 78 ± 6 mmHg, P < 0.05). Recovery of rates of pressure development (+dp/dt) and relaxation (-dp/dt) also significantly improved in hearts treated with LS-NTU-106. The cardiac function recovery of LS-NTU-106 in ischemia-reperfusion were attenuated by opioid receptor antagonist naloxone and IKATP blocker 5HD.}

Ischemia-induced arrhythmias

Ligation of the left coronary artery invariably resulted in ventricular arrhythmias, which commenced within 5-7 min of occlusion, manifesting as ventricular premature contraction, ventricular tachycardia and ventricular fibrillation. Table 2 shows the developed arrhythmias following coronary artery occlusion in the rats. No significant differences in the incidence and duration of ventricular tachycardia were noted among the groups. However, naloxone at 1mg/kg caused a pronounced inhibition of ventricular fibrillation.

Myocardial Infarct Size
The ischemic area (area at risk) and infarct size after 60 min of left main coronary artery occlusion followed by 120 min of reperfusion was evaluated. Figure 2 show the infarct size expressed as a percentage of the area at risk in all experimental groups. The infarct size was 43.57±2.74 % in the control group. Administration of LS-NTU-106 reduced myocardial infarction in a dose-dependent manner to 31.55±3.26% in group given 10⁻⁷ mole/kg and to 22.60±2.23% in the group given 10⁻⁶ mole/kg.

A dose-response effect was observed in the two doses of naloxone plus 10⁻⁶ mole/kg LS-NTU-106 groups. The low dose (1mg/kg, iv) of naloxone partially blocked cardioprotection induced by LS-NTU-106 (29.92±1.60, P<0.05 vs control and 10⁻⁶ mole/kg LS-NTU-106 groups); whereas, the high dose (3mg/kg, iv) of naloxone completely abolished the cardioprotection induced by LS-NTU-106 (37.93 ± 1.46 P<0.05 vs control). Previously, it had shown that high dose of naloxone alone had no effect on infarct size (11).

Plasma CK activity

Plasma CK activity was used to confirm infarct size quantified by TTC staining. A large increase of the enzyme was found in the plasma of I/R rats in control group. (10424.29 ±1180.30U/l). However, administration of LS-NTU-106 resulted in an attenuation of CK release in a dose-dependent manner during ischemia-reperfusion. (7997.78 ±253.43, 10⁻⁷ mole/kg ; 3905.56 ±08.40U/l, 10⁻⁶ mole/kg; p<0.05 vs control) (Fig. 3).

While application of opioid receptor antagonist, naloxone and IKATP blocker, 5HD
in $10^{-6}$ mole/kg LS-NTU-106 treatment group, the effect of decrease in plasma CK by LS-NTU-106 in I/R rat was abolished. Although the difference between groups was not statistic significant, but the trend was not neglect.

Tissue MPO activity in ischemia-reperfusion myocardium

Neutrophil infiltration of the ischemic region during reperfusion is considered to be one of the major mechanisms responsible for reperfusion injury. To determine the effects of LS-NTU-106 on the accumulation of neutrophils into the ischemic myocardium, we examined myocardial myeloperoxidase activity in ischemic regions after 60min ischemia-120min reperfusion. A significant decrease in myeloperoxidase activity was observed in LS-NTU-106($10^{-6}$ mole/kg) treatment group (P<0.05). Pretreatment with opioid receptor antagonist, naloxone or IKATP blocker, 5HD in $10^{-6}$mole/kg LS-NTU-106 treatment group, the effect of decrease in cardiac MPO activity by LS-NTU-106 in I/R rat was abolished.
Discussion

The findings of present study show that (1) treatment of LS-NTU-106 at the time of post-ischemia reduced plasma concentrations of creatine kinase and infarct size in a dose-dependent manner in anesthetized rat subjected to cardiac ischemia-reperfusion. (2) By the end of ischemia-reperfusion, recovery of cardiac function was higher in LS-NTU-106 (10-6mole/kg ) treatment group. (3) LS-NTU-106 also significant reduced MPO activity in ischemia-reperfusion hearts. (4) This protection afforded by LS-NTU-106 from post-ischemia reperfusion injury is abrogated by opioid receptor antagonist, naloxone and IKATP blocker 5HD.

In summary, LS-NTU-106 exerts its cardioprotective effects mainly by stimulated opioid receptor and open the KATP channel to against post-ischemia reperfusion injury.
Reference


