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Effect of Ursodeoxycholic Acid on Ischemia/Reperfusion Injury in Isolated Rat Heart

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In this study, the effects of ursodeoxycholic acid (UDCA) on ischemia/reperfusion injury were investigated on isolated heart perfusion model. Hearts were perfused with oxygenated Krebs-Henseleit solution (pH 7.4, 37°C) on a Langendorff apparatus. After equilibration, isolated hearts were treated with UDCA 20 to 160 μM or vehicle (0.04% DMSO) for 10 min before the onset of ischemia. After global ischemia (30 min), ischemic hearts were reperfused and allowed to recover for 30 min. The physiological (i.e. heart rate, left ventricular developed pressure, coronary flow, double product and time to contracture formation) and biochemical (lactate dehydrogenase; LDH) parameters were evaluated. In vehicle-treated group, time to contracture formation was 21.4 min during ischemia, LVDP was 18.5 mmHg at the endpoint of reperfusion and LDH activity in total reperfusion effluent was 54.0 U/L. Cardioprotective effects of UDCA against ischemia/reperfusion consisted of a reduced TTC (EC_{25}=97.3 μM), reduced LDH release and enhanced recovery of cardiac contractile function during reperfusion. Especially, the treatments of UDCA 80 and 160 μM significantly increased LVDP and reduced LDH release. Our findings suggest that UDCA ameliorates ischemia/reperfusion-induced myocardial damage.

Key words : Ursodeoxycholic acid, Heart ischemia/Reperfusion injury, Cardioprotective effect

INTRODUCTION

The high incidence of atherosclerotic vascular disease and its devastating results has lead to an extensive amount of work directed at gaining a better understanding of ischemia-related pathologies (Braunwald and Kloner, 1985; Chien *et al.*, 1985). Techniques to by pass coronary arterial obstructions including thrombolytic therapy, angioplasty, and surgical grafting have made it possible to reduce the mortality of ischemic tissues and organs. Such techniques have lead to an interest in defining the specific mechanisms leading to irreversible ischemic injury and the seemingly separate phenomenon of reperfusion-induced injury (Gaudel and Duvelleroy, 1984).

Reperfusion of ischemic areas, in particular reoxygenation process, may contribute to further tissue damage (McCord, 1985; Simon and Gregory, 1997). The representative pathophysiological features of reperfusion injury are vascular and microvascular injury (Forman *et al.*, 1989; Maxwell and Gavin, 1991), endothelial dysfunction (Lefer *et al.*, 1990; Lefer *et al.*, 1991; Lum *et al.*, 1992; Mcfalls *et al.*, 1991; Sheridan *et al.*, 1991), accelerated cell necrosis of reperfusion (Hearse *et al.*, 1975; Hearse *et al.*, 1986), and granulocyte activation (Ceriana, 1992). According to Simon and Gregory (1997), there are three main components of reperfusion injury: myocardial stunning, reperfusion arrythmia, and early excess mortality from myocyte injury. The possible interventions of avoiding these ischemia/reperfusion damages can be divided into 1) those that relate to manipulation of the reperfusion setting, 2) antioxidants, and 3) those that are based upon therapeutic agents, with current evidence is mainly from experimental studies.

Ursodeoxycholic acid (UDCA) has been the most representative choleretics since the use of chenodeoxycholic acid (CDCA) was forbidden by its severe hepatotoxicity. Hydrophobic bile salts such as CDCA perturbed the membrane structure, permitting water to permeate deeper into the membrane interior (Drew and Priestly, 1978). Recently, Güldütuna *et al.* (1993) showed that UDCA prevented bile salt damage by stabilizing membrane structure and maintaining

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membrane fluidity. Furthermore, Ono *et al.* (1995) showed that TUDCA, tauine conjuates of UDCA, had a protective effect on liver ischemia/reperfusion injury. They also presented that TUDCA increased bile flow and bile acid secretion, the index of liver cell recovery, by inhibiting the excessive influx of Ca^{2+} into cell and increasing the amount of Ca^{2+} in bile flow, resulting from reduction of the damage in mitochondria and endoplasmic reticulum.

Ro *et al.* (1980) showed that UDCA abolished the spontaneous and ouabain-induced arrythmia in isolated rabbit artia. Moreover, in the heart of anesthetized frog, the epinephrine-induced arrythmia was partially blocked by UDCA. These studies suggested that UDCA could be used as an therapeutic agents to prevent the heart ischemic disease.

In the present study, we evaluated the cardioprotective activity of UDCA in globally ischemic isolated rat hearts.

MATERIALS AND METHODS

Chemicals

Ursodeoxycholic acid, sodium pyruvate and lactate dehydrogenase (LDH) kit were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.), and heparin sodium was supplied from Choongwae Pharmaceutical Co. (Suwon, Korea). All other chemicals were of the reagent grades commercially available locally.

Langendorff heart preparation

Male Sprague-Dawley rats weighing 350-450 g were used for the experiments. The rats were anesthetized with sodium pentobarbital intraperitoneally (100 mg/kg i.p.). The femoral vein was injected with heparin (1,000 U/kg) and the isolation of heart was performed according to the method of Grover *et al.* (1995). While the trachea of rats was intubated and mechanically ventilated, their hearts were perfused in situ with oxygenated Krebs-Henseleit bicarbonate buffer solution (pH 7.4) by retrograde aortic cannulation. The hearts were then excised and quickly moved to a Langendorff apparatus, where they were perfused with oxygenated Krebs-Henseleit solution containing (in mM) NaCl 112, $NaHCO_3$ 25, KCl 5, $MgSO_4$ 1.2, KH_2PO_4 1, $CaCl_2$ 1.25, glucose 11.5, and pyruvate 2 at a constant perfusion pressure (75 mmHg). A water-filled latex balloon attached to a metal cannula was inserted into the left ventricle and connected to a Statham pressure transducer (Gould Inc., Oxnard, CA) for measurement of left ventricular pressure (LVP).

Experimental protocols

The hearts were allowed to equilibrate for 15 min, at which time end-diastolic pressure (EDP) was adjusted to 5 mmHg; this balloon volume was maintained for the duration of the experiment. Preischemia or predrug contractile function, heart rate (HR) and coronary flow (volume of buffer overflowed out of isolated heart chamber during 1 min) were then measured (Grover *et al.*, 1990; Grover *et al.*, 1995). Cardiac contractile function was determined using the double product (DP) of HR × left ventricular developed pressure (LVDP) divided by 1,000. LVDP was calculated from the difference between left ventricular peak systolic pressure (LVSP) and LVEDP. In addition, the first derivative of LVDP, the rates of left ventricular maximal pressure development (+dp/dt) and relaxation (-dp/dt) were monitored by polygraph differentiator (Grass instrument, 7P20). Cardiac temperature was maintained throughout the experiment by submerging the hearts in 37°C buffer which was allowed to accumulate in a stoppered, heated chamber.

For the concentration-response determination, the hearts were treated with 20, 40, 80, and 160 μM UDCA or vehicle (0.04% dimethylsulfoxide) by adding them to perfusate. Drug treatment was started 10 min before onset of ischemia. UDCA was administered directly into the oxygenator of the Langendorff apparatus immediately above the aortic root in a retrograde fashion as solutions in the perfusate. We then rendered the hearts globally ischemic by completely shutting off the perfusate for 30 min. Time to contracture (TTC) during global ischemia was then calculated. TTC was the time (in min) from onset of global ischemia in which the first 5 mmHg increase in LVEDP is observed. The hearts were reperfused and, 30 min later, contractile function (LVDP, DP) and cumulative reperfusion lactate dehydrogenase (LDH) release were measured.

LDH assay

The reperfusion effluent was collected for cumulative LDH release. LDH concentrations were determined using a kit supplied by Sigma Chemical Co. LDH release was expressed as units per liter for the 30 min collection period.

Statistical analysis

All values are expressed as means ± S.E.M.. Data were analyzed the unpaired Student's t-test between two groups and one-way analysis of variance (ANOVA) followed by the Dunnett's test for multiple comparisons. All statistical differences were determined at $p<0.05$ level.

RESULTS

Cardiac contractility and coronary flow

We examined the complete concentration-response studies for UDCA in ischemic rat hearts. As shown in Table I, cardiac contractile function and heart rate were similar for all experimental groups before drug administration. UDCA did not affect preischemic cardiac function and heart rate. In the vehicle-treated group, cardiac contractile function and heart rate was significantly depressed after 30 min reperfusion, indicating severe ischemia/reperfusion damage. UDCA significantly improved reperfusion cardiac function in a concentration-dependent manner as shown in reperfusion LVDP and DP, beginning at concentrations of 40, 80 and 160 μM, respectively, indicating marked cardioprotection. During reperfusion, HR was slightly decreased in the vehicle-treated rats. However, UDCA treatment did not change the decrease of HR at reperfusion endpoint. UDCA did not affect preischemic coronary flow. In the vehicle-treated group, coronary flow was significantly decreased after 30 min reperfusion. The UDCA significantly improved coronary flow during reperfusion at concentrations of 160 μM and we also supposed that EC_{25} was 63.1 μM (Fig. 1).

Table I. Effects of UDCA on cardiac conractility before and after 30 min of global ischemia

Parameter	Before ischemia		After ischemia
	Predrug	10 min Postdrug	30 min Reperfusion
LVDP(mmHg)			
Vehicle	86.5±6.0	97.3±4.4	18.5±3.6
UDCA (μM)			
20	88.3±5.9	99.0±2.5	34.5±8.4
40	86.3±5.8	96.9±5.3	33.4±4.3*
80	85.9±5.5	96.7±4.8	45.7±9.5*
160	86.5±4.9	93.8±4.1	82.4±6.1**
HR(beats/min)			
Vehicle	301.5±6.7	300.5±6.5	265.0±4.8
UDCA (μM)			
20	294.0±6.9	298.0±7.2	256.0±9.8
40	304.0±10.7	310.0±12.9	258.0±7.4
80	295.0±4.2	318.0±10.3	241.0±12.3
160	296.0±10.0	311.0±11.1	262.0±3.3
DP(LVDP×HR)/1000			
Vehicle	26.1±1.9	29.2±1.3	4.9±1.0
UDCA (μM)			
20	26.0±2.1	29.5±0.7	8.8±1.1
40	26.1±1.8	30.2±2.5	8.5±0.9*
80	25.4±1.9	30.8±2.2	10.9±2.3*
160	25.6±1.7	29.0±0.7	21.7±1.9**
(-dp/dt)/(+dp/dt)			
Vehicle	0.95±0.03	0.98±0.01	0.65±0.02
UDCA (μM)			
20	0.94±0.03	0.99±0.00	0.70±0.04
40	0.96±0.03	0.97±0.02	0.77±0.05
80	0.95±0.03	0.98±0.02	0.82±0.05*
160	0.94±0.04	0.95±0.04	0.92±0.02**

Values are means ± S.E.M. for 7 hearts per group. *, ** Significantly different ($p<0.05$, $p<0.01$) from vehicle-treated group. LVDP, left ventricular developed pressure; HR, heart rate; DP, double product; (-dp/dt)/(+dp/dt), the ratio of left ventricular maximal pressure development and relaxation.

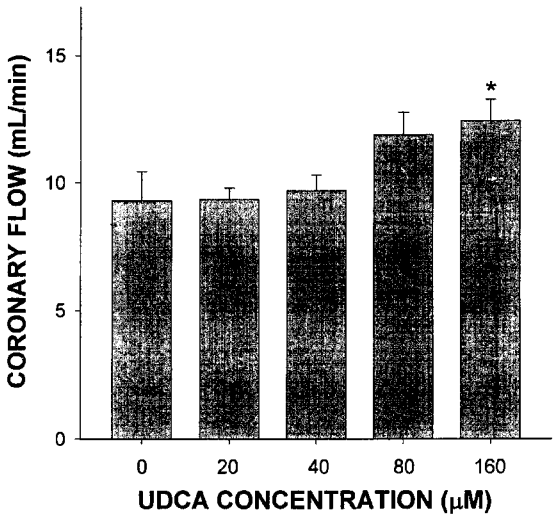

Fig. 1. Concentration-response curve for UDCA with regard to coronary flow during reperfusion in isolated rat heart subjected to 30 min of global ischemia subsequent 30 min reperfusion. Values are means±S.E.M. from 7 hearts per group. * Significantly different from its respective vehicle group value ($p<0.05$)

Time to contracture formation

Effect of UDCA on TTC during global ischemia, the most important parameter that determines the anti-ischemic effect of a substance for cardiac muscles, was presented in Fig. 2. In the vehicle-treated heart, severe contracture was observed and TTC was 21.4 ± 0.6 min. However, the treatment of UDCA significantly increased TTC in a dose-dependent manner. At the highest concentration of UDCA, TTC was increased 28.8% (28.8 ± 0.3 min) compared to the vehicle-treated group and EC_{25}, the concentration that is required for the 25% increase of TTC, was 97.3 μM.

LDH release

Significant LDH release was observed in the reperfusion coronary effluent of vehicle-treated hearts, and this was significantly attenuated by UDCA treatment (Fig. 3). The concentration of released LDH was 54.0 ± 2.8 U/L in vehicle-treated hearts and was 43.9 ± 1.9 U/L (80 μM), 42.8 ± 3.9 U/L (160 μM) in UDCA-treated hearts, respectively.

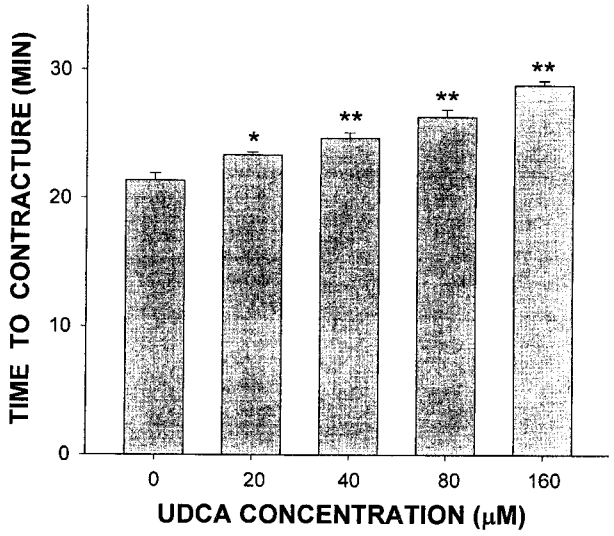

Fig. 2. Concentration-response curve for UDCA dependent on time to contracture during global ischemia. Values are means±S.E.M. from 7 hearts per group. *, ** Significantly different from its respective vehicle group value ($p<0.05$, $p<0.01$)

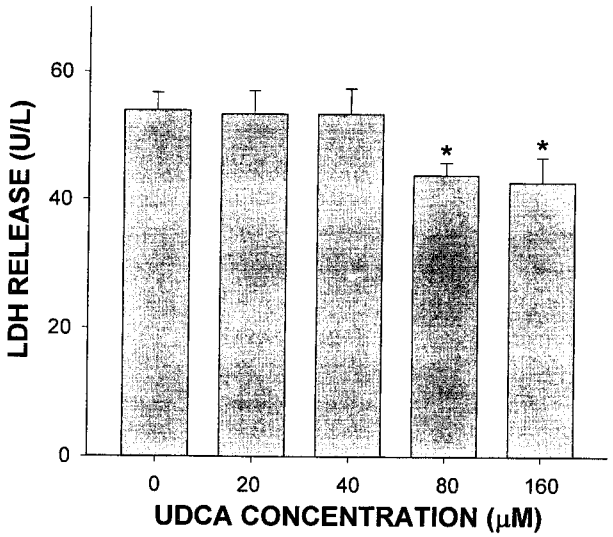

Fig. 3. Effect of UDCA on LDH release during reperfusion in isolated rat heart subjected to 30 min of global ischemia subsequent 30 min reperfusion. Values are means±S.E.M. from 7 hearts per group. * Significantly different from its respective vehicle group value ($p<0.05$)

DISCUSSION

We examined the effect of UDCA on myocardial injury resulting from global ischemia and reperfusion in isolated rat hearts as assessed by mechanical, biochemical evaluation. The principal finding of this study is that UDCA has a potent cardioprotective activity as evidenced by a significant concentration-dependent improvement in TTC and reperfusion cardiac function (LVDP and DP) and a marked reduction of LDH release in reperfusion.

It is well known that oxygen free radicals are generated intracellularly and extracellularly in the myocardium and endothelium during ischemia and reperfusion (Hess and Manson, 1984; Lucchesi, 1990). These reactive oxygen species cause lipid peroxidation of the cell membrane and intracellular Ca^{2+} overload responsible for mechanical and metabolic damage (Josephson *et al.*, 1991; Nakaya *et al.*, 1987). In fact, H_2O_2 induces intracellular Ca^{2+} accumulation in the cardiomyocyte (Josephson *et al.*, 1991) and produces mechanical dysfunction and metabolic changes in isolated perfused heart (Hara *et al.*, 1993; Kokita and Hara, 1996). Woodward and Zakaria (1985) found that addition of either superoxide dismutase or catalase to isolated perfused rat hearts reduced the ventricular fibrillation. Furthermore, it has been reported that UDCA could attenuate the generation of reactive oxygen species and nitric oxide in cultured macrophages (Hattori *et al.*, 1996; Ljubuncic *et al.*, 1996). In our study, protection of hearts from ischemia-reperfusion injury by UDCA may be, at least in part, mediated by the inhibitory effect of UDCA on oxygen free radicals.

The extent of intracellular calcium accumulation is related to the severity and duration of ischemia and the degree of mechanical recovery (Steenbergen *et al.*, 1990). Therefore, inhibition of calcium influx across the membrane of the myocarodial cell may reduce the severity of cell damage. Recently, Ono *et al.* (1995) showed that tauroursodeoxycholic acid (TUDCA) inhibited tissue calcium accumulation and enhanced sinusoidal and biliary calcium output during hepatic ischemia-reperfusion. Moreover, TUDCA stimulates calcium-dependent heaptocellular secretion and modulates cytosolic calcium signal transduction *in vitro* (Beuers *et al.*, 1993). TUDCA may also be cytoprotective (Güldütuna *et al*, 1993). The beneficial action of UDCA on mechanical recovery and myocardial contractility during ischemia and reperfusion may be due to inhibition of intracellular Ca^{2+} accumulation.

In present data, UDCA increased coronary flow. The endothelium releases factors involved in coronary autoregulation and reactive hyperemia. Endothelial dysfunction may occur within 2.5 min of reperfusion after ischemia and may be involved in the pathogenesis of reperfusion injury (Lefer *et al.*, 1991). The vasodilation of coronary arteries by nitric oxide and Ca^{2+} channel blockers protects the myocardium during ischemia and reperfusion (Bernstein *et al.*, 1996; Pabla and Curtis, 1996). It is likely that the cardioprotective activity of UDCA may be in part attributed to its coronary vasorelaxant effects. The increase of coronary flow produced by UDCA may

facilitate the recovery of myocardial contractility and inhibit calcium accumulation during mycocardial ischemia.

We used LDH release as an index of cellular and membrane integrity because this enzyme has been used as surrogate for myocardial necrosis. Our results showed that UDCA partially ameliorated LDH release.

In conclusion, this study demonstrates that UDCA improves the recovery of myocardial contractility and decreases LDH release secondary to ischemia-reperfusion in isolated rat hearts. Therefore, we suggest that UDCA has cardioprotective effects on myocardial ischemia-reperfusion injury.

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