Administration of Tauroursodeoxycholic Acid (TUDCA) Reduces Apoptosis Following Myocardial Infarction in Rat

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Abstract: Black bear bile has been used in traditional Chinese medicine to treat liver and eye related illnesses for centuries. A major constituent of bile is ursodeoxycholic acid (UDCA). Recent analysis of the cellular effects of UDCA and its taurine conjugate tauroursodeoxycholic acid (TUDCA) have demonstrated their antiapoptotic properties through regulation of Bcl-2 family and survival signaling proteins (Bax, Bad, phosphatidylinositol-3-kinase). In this study, we tested the hypothesis that TUDCA administered to rats prior to a myocardial infarction (MI) would exhibit anti-apoptotic effects and improve cardiac function. Prior to ligation of the left anterior descending (LAD) coronary artery, TUDCA (50 mg/ml, 400 mg/kg, IV) or PBS was administered to rats. Animals were sacrificed 24 hours after ligation for terminal transferase-mediated dUTP-digoxigenin nick end-labeling (TUNEL) and caspase-3 activity to assess apoptosis. Additional TUDCA or PBS treated rats underwent pre-operative, 1 and 4 week transthoracic ultrasounds to assess heart function by quantification of shortening fraction (SF) and infarct area. TUNEL labeling of the cardiac tissue revealed a significant reduction in apoptotic cells in rats given TUDCA prior to ischemic injury (p = 0.05). In support of reducing apoptosis, caspase-3 activity in the TUDCA treated animals also decreased (p = 0.02). By 4 weeks, a significantly smaller infarct area was present in the TUDCA group compared to the PBS group (0.05 vs. 0.13 cm², p = NS) and there was also an improvement in SF. The results provide evidence for TUDCA as a viable treatment for reducing apoptosis in a model of myocardial infarction. Additional studies will distinguish the functional result
of improved cell survival following infarction, suggesting the potential for clinical application of this anti-apoptotic drug in treatment of acute MI.

Keywords: Tauroursodeoxycholic Acid; Apoptosis; Myocardial Infarction.

Introduction

Pharmacological therapy is the primary mode of medical treatment for ischemic heart disease. The directed therapy of oxygen, aspirin, heparin, beta-blockers, and nitrates has been repeatedly proven to limit necrosis caused by the absence of blood flow to the heart. Interestingly, there has been only limited experience with prevention of apoptosis resulting from myocardial infarction. A recent study, however, reported that a hydrophilic bile salt (ursodeoxycholic acid, UDCA) can actually protect the heart against reperfusion injury induced by temporary left coronary artery occlusion, and preserve cellular function (Rajesh et al., 2005). The study, combined with our own experience, is a significant step towards understanding the pharmacology of a traditional Chinese medication that has the potential for widespread adoption as a new drug treatment for myocardial infarction.

History of Bile Acid Therapy

Bile from animals has been used for many centuries to treat certain conditions in China. For example, bear bile is used in the treatment of “heat” related illnesses such as elevated temperature, complaints related to the liver, and eye soreness (Mather, 2005). According to China’s state pharmacopoeia, the action of bear bile is to remove heat from the liver, relieve convulsions and spasms, improve visual acuity, and clear away heat and toxic material (Commission, 2005). Bear bile used in the production of this medicine is also used in tonics and as a food supplement. Whether these formulations are effective for various ailments as purported is not entirely known. This is partly because the high demand for bear bile throughout Asia has led to a recent black market of adulterated bear bile compositions. One controversial study showed that capsules of “bear bile” purchased from a traditional Chinese pharmacy contained primarily bile originating from the pig. Furthermore, the same study reported that only 3% of samples confiscated as criminal evidence claiming to contain bear bile actually did (Lin et al., 1997). The dilution of bear bile with other animal bile likely obfuscates the efficacy of this traditional medicine. Clearly, bear bile has been shown to be an effective medication, but a true understanding of its pharmacology has only been recently elucidated.

The composition of bear bile varies significantly within the phylogenetic tree of the Ursidae (Bear) family. However, the most common bile acid found in bears is ursodeoxycholic acid (UDCA). Of all bears, North American bears have evolved with substantial amounts of UDCA in their bile. The amount of UDCA in gallbladders ranges from 9% in Asian sun bears (Helarctos malayanus) to 18% in Polar bears (Thalarctos maritimus) and Brown bears (Ursus arctos) as analyzed by high pressure liquid chromatography (HPLC).
TUDCA REDUCES MYOCARDIAL APOPTOSIS

(Hagey et al., 1993). Black bears (Ursus americanus) have a moderate amount of UDCA (15–30%) in their bile (MacDonald and Williams, 1985). As a reference, the total bile acid pool of UDCA in humans is around 3% (Poupon et al., 1997). Black bear bile is notable for its seasonal variation in its composition. For example, UDCA increases during hibernation compared to their active state (Jones and Zollman, 1997; Solá et al., 2006). One hypothesis for this variation is that it has a protective effect during prolonged abstinence from food and water; UDCA protects the bear from gut atrophy, lithogenesis, and bacterial overgrowth during hibernation (MacDonald and Williams, 1985).

The strongest evidence of bear bile’s medicinal effects was demonstrated by a Japanese double blind trial in 1976. The trial showed that UDCA reduced circulating liver enzymes in patients with chronic cholestatic liver diseases (Mijayi, 1976). In 1980, a similar double blind study, reported in the journal Gastroenterology validated the Japanese study (Leuschner et al., 1989). The two studies clarified the clinical efficacy of UDCA in the treatment of primary biliary cirrhosis. Interestingly, there are two meta-analyses of additional randomized placebo-controlled trials showing that UDCA treatment prolongs transplant-free survival in patients with primary biliary cirrhosis (Poupon et al., 1997; Goulis et al., 1999). A thorough review of the literature in 1998 determined that the mechanisms of action were unknown at that time, and UDCA was postulated to protect cell membranes of the biliary tree (Beuers et al., 1998). We now know that UDCA inhibits apoptosis in cholestatic disease as well as other conditions.

**UDCA’s Mechanism of Action**

This important discovery that UDCA prevents apoptosis was made by Rodrigues et al. (1998a). She showed that UDCA reduces hepatocyte apoptosis by a mechanism related to control of mitochondrial membrane permeability. Clarification of the mechanism was done by treating isolated rat liver mitochondria with deoxycholic acid to induce permeability of mitochondria, causing swelling. Incubating mitochondria with UDCA before adding deoxycholic acid protected them from induced damage by almost 50% (Rodrigues et al., 1998a).

Further studies using a taurine conjugate of UDCA, tauroursodeoxycholic acid (TUDCA) to elucidate the mitochondrial associated mechanism provide evidence that protection also occurs via a pathway independent of membrane permeability (Fig. 1). One study used 3-nitropropionic acid* that induced apoptosis in the brain by translocation of the proapoptotic protein Bax from the cytosol to mitochondria. This translocation of Bax and subsequent apoptosis was abrogated by up to 75% by co-incubating rat neuronal cells in TUDCA (Rodrigues et al., 2000). Furthermore, TUDCA inhibits Bax pore formation and cytochrome c efflux from isolated mitochondria, thus preventing caspase activation and poly (ADP-ribose) polymerase (PARP) cleavage and inhibiting apoptosis (Rodrigues et al., 2003). In addition, a recent report has demonstrated beneficial effects of TUDCA on

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*3-Nitropropionic acid is a plant mitotoxin used to interrupt the electron transport chain.
endoplasmic reticulum stress and inflammation in a mouse model of diabetes (Ozcan et al., 2006). In summary, the endogenous bile acids UDCA and its taurine conjugate TUDCA may be potent anti-apoptotic agents that regulate factors in the Bcl-2 family of proteins (Bax, Bad, phosphatidylinositol 3-kinase) thus preventing mitochondrial dysfunction (Castro et al., 2004).

**TUDCA Protection in an Animal Model of Ischemia**

Given that TUDCA is protective against apoptosis in cell cultures, is it effective in treating apoptosis induced by ischemia? In a rodent model of acute stroke, TUDCA decreased infarct size by nearly 50% when compared to the controls. TUDCA was given in a single dose (400 mg/kg, IV) during or 1 hour after temporary ligation and then allowed to reperfuse (Rodrigues et al., 2002). Two days later, the rats were euthanized and their brains were sectioned and stained with 2% 2,3,5-triphenyltetrazolium chloride (TTC). Quantitative analysis of the infarct size showed a significant reduction of infarct volume (49.0% in
TUDCA REDUCES MYOCARDIAL APOPTOSIS

the vehicle group vs. 24.2% in the treatment group, p < 0.05). Furthermore, TUDCA reduced the number of terminal deoxynucleotidyl transferase-mediated 2′-deoxyuridine 5-triphosphate-biotin nick end labeled (TUNEL) cells as compared to controls, indicating less DNA fragmentation.

Apoptosis versus Necrosis in Cardiac Cell Death

Apoptosis, or programmed cell death, has characteristic morphological changes throughout the process of self-destruction. During apoptosis, the cell attempts to maintain the mitochondria and sarcolemmal membranes, while condensing the nuclear chromatin (pyknosis), shrinking the outer membranes, fragmenting the nucleus (karyorhexis), and cleaving DNA at uniform lengths. In contrast, during oncosis, cells swell edematously; membranes bud early; chromatin clumps irregularly; and the mitochondria are randomly damaged. Both oncosis and apoptosis lead to cell death and irreversible changes afterwards including necrosis.

Similar to a stroke, the lack of blood flow in a myocardial infarct causes cell death. The etiology behind cardiomyocyte death during ischemia is complex and involves a combination of apoptosis and necrosis. Typically, apoptosis is associated with pathophysiological situations of non-ischemic cell death for which cancer, embryonic development and aging are examples. However, an increasing body of evidence exists that apoptosis plays a major role in myocardial ischemia and heart failure (Elsasser et al., 2001). Although the role of apoptosis in ischemic related cardiac cell death is not completely known, several studies have shown that acidosis, re-oxygenation, and reperfusion are necessary for induction of apoptosis and specifically that hydrogen peroxide and free radicals are causative agents. (Elsasser et al., 2001; Yaoita et al., 2000).

Animal Model Selection

Rats have long been used as a model for myocardial infarction as originally described by Selye et al. (1960). Since then, numerous studies have characterized the rat model to determine the qualitative and quantitative changes in myocardial remodeling following surgical occlusion of the coronary vessels (Fishbein et al., 1978). The progression of histological changes that occurs following myocardial infarction in the rat includes cell death, an acute inflammatory response at the edge of the infarct area, vascularization and collagenation, chronic inflammation, resorption of necrotic tissue, and finally scar maturation and contraction (Fishbein et al., 1978). In order to compensate for lost function, the myocardium distant from the infarct adapts to the increased stresses, develops increased vascularity, and hypertrophies (Anversa et al., 1984; 1985; 1986).

†The visual appearance of apoptosis is like the etymological Greek origin of the word meaning the dropping of leaves from a tree. Normally only a few leaves are seen on the ground surrounding a healthy tree, however a diseased tree can shed its leaves suddenly.
One difficulty of using the rodent model is the variation in distribution of the coronary arteries (left anterior descending or LAD, circumflex, and septal branches). This variation of distribution in coronary artery territory can be a confounding factor in the design of experiments that are based upon therapy for myocardial infarction (Anversa et al., 1986). Of five common strains of rats (Lewis, Sprague-Dawley, Wistar-Kyoto, Brown Norway, and Dahl salt sensitive), the Lewis inbred strain has the most uniform branching pattern. In the Lewis rats, the circumflex originates high off the LAD (83%) and its septal branch originates typically from the left coronary artery (83%) (Liu et al., 1997). The regularity of the coronary artery distribution is important in the creation of uniform infarcts that are consistently sized; thus reducing experimental variability.

Methods

The experimental protocol was developed in cooperation with the Departments of Surgery, Neurosurgery, and Medicine. The protocol was then submitted to the Institutional Animal Care and Use Committee and approved under protocol No. 0301A39301. All animals in this study received humane care in compliance with the document entitled Guide for the Care and Use of Laboratory Animals formulated by the National Academy of Sciences (National Academy Press, 2101 Constitution Ave, NW, Washington, DC, 20418, ISBN 0-309-053777-3).

Rodent Intubation and Ventilation

Lewis rats were sedated using 5% enflurane-O₂ filled canister. While sedated, the rat was intubated with a 16 gauge over-the-needle intravenous angiocatheter (Ethicon, Cincinnati, OH) (Stark et al., 1981), and ventilated using a non-rebreathing circular ventilation system (Harvard Apparatus, Harvard Ventilator 683, Holliston, MA) at a respiratory rate of 85, tidal volume of 2.5 ml, and Enflurane at 1.5–2.0% (Rivard et al., 2006).

Myocardial Infarction

Following successful intubation and antiseptic preparation with Betadine solution, a skin incision was made from the jugular notch to the xiphoid process. Sternotomy was completed and pericardium incised to expose the left coronary artery. In our procedure, we ligated the left anterior descending artery with 6–0 proline. The chest was closed with a three layer closure (sternum, pectoralis muscle, and skin) with 3–0 Vicryl (Ethicon, Cincinnati, OH) and the animal was removed from anesthesia and allowed to convalesce.

TUDCA Preparation and Administration

TUDCA was obtained from TCI America, Portland, Oregon. The 50 mg/ml solution was prepared in 1 × PBS. The pH of the solution was adjusted to 7.4 using NaOH and
then filter sterilized using a 0.22 µm filter. Osmolarity of the TUDCA injection solution was determined twice and averaged using an Advanced™ Micro Osmometer Model 3300 (Advanced Instruments, Norwood, MA). The solution was stored at 4°C, and protected from light until use. Prior to use, the solution was warmed to room temperature. Immediately prior to the myocardial infarction, TUDCA injection was completed using a 3 ml syringe and a thirty-gauge needle inserted in the saphenous vein. This involved establishing vascular access using the following technique: 1) antiseptic preparation of the access site, 2) tourniquet placement around the upper thigh, 3) percutaneous venous puncture. TUDCA (400 mg/kg) was injected over 40 min (0.075 ml/min).

**TUNEL Staining**

Immediately following explantation, hearts were snap frozen using liquid nitrogen. Heart tissue explanted from the animals was serially sectioned to allow for assessment of apoptotic cells by TUNEL staining. Myocardial sections, 10 µm in thickness, were fixed with 1% formaldehyde, post-fixed with acetate-ethanol solution at −20ºC, and washed with phosphate buffer, pH 7.4. An Apoptag in situ apoptosis detection kit (Serological Corp., Norcross, GA) was used for TUNEL staining according to the manufacturer’s recommendations. In brief, the same serial myocardial section for each heart was incubated with equilibration buffer. Terminal deoxynucleotidyltransferase and digoxigenin-dNTP were added to the sections and incubated at 37ºC for 1 hour. Slides were then treated with anti-digoxigenin-peroxidase solution for 30 min, colorized with 3,3′-diaminobenzidine (DAB) substrate, and counterstained with 0.5% methyl green. The number of TUNEL-positive cells was counted on a computer screen grid from at least three random fields at 400× magnification.

**Caspase Activity Assay**

Caspase activation was determined in total protein extracts. For total proteins, cardiac tissue was harvested and homogenized in ice-cold buffer containing 10 mM Tris-HCl buffer, pH 7.6, 5 mM MgCl₂, 1.5 mM KAc, 2 mM DTT, and protease inhibitor cocktail tablets (Complete™; Roche Applied Science, Mannheim, Germany). Equal volumes of 2× Total Protein buffer containing 10 mM Tris-HCl buffer, pH 7.6, 1% Nonidet P-40 were added along with protease inhibitor cocktail tablets and samples remained on ice for 30 min. The lysate was sonicated in 5 sec bursts until homogenized and centrifuged at 3,200 g for 10 min at 4°C; the resultant supernatant containing total proteins was collected. General caspase-3-like activity was determined by enzymatic cleavage of chromophore p-nitroanilide (pNA) from the substrate N-acetyl-Asp-Glu-Val-Asp-pNA (DEVD-pNA; Sigma Chemical Co., St. Louis, MO). The proteolytic reaction was carried out in aqueous solution containing 50 µg total protein and 50 µM DEVD-pNA. The reaction mixtures were incubated at 37°C for 1 hour, and the formation of pNA was measured at 405 nm using a 96-well plate reader.
Pathology

At the time of cardiectomy, the heart was examined in situ. This permitted a subjective evaluation of the infarction. Following cardiectomy, whole heart tissues were fixed in 10% neutral buffered formalin, paraffin-embedded and cut into 3 to 5 µm thick sections from mid-ventricle, and stained with hematoxylin-eosin (H&E) and Masson’s trichrome. Slides were then scanned (HP Scanjet 3970) at a resolution of 600 dpi. Infarct size as a percentage of the total myocardium was then determined using Image J analytical software (open source, National Institutes of Health) using the formula: % Infarct = infarct area/total myocardial area.

Echocardiography

A 15-6L linear ultrasound transducer was interfaced with a Philips Sonos 5500 echocardiography system (Philips Medical Systems, Bothell, WA). The settings were as follows: the mechanical index was set at 0.3–0.6 with compression and gain settings manipulated to enhance visualization of the endocardial-lumen interface. Depth was set at 2 cm with a frequency ranging from 92–103 Hz. Images of the heart were routinely acquired in the short axis window at the level of the mid-papillary muscles for their ease of reproducibility. On occasion, a non-routine view of the short axis window was obtained to achieve the best visualization of the myocardial infarct. Left ventricular measurements were obtained in the short axis window just below the mid-papillary muscles by M-mode echocardiography.

Echocardiographic Data Analysis

Measurements of the heart included the interventricular septum thickness in diastole and systole, posterior wall thickness in diastole and systole, and the ventricular cavity diameter in diastole and systole. In the 2D-echo of the left ventricle in short axis, a tracing was made of the ventricular myocardium to determine the myocardial area in diastole as a surrogate of the left ventricular mass. Both Teicholtz and cubed methods were used to calculate ejection fractions. These measurements were determined from the M-mode measurements of the left ventricular cavity. Shortening fraction (also determined from M-mode measurements of the left ventricular cavity) was calculated using an Excelera workstation (Philips Medical Systems, Bothell, WA).

Results

Dose Infusion Study

In a dose determination study, rats were administered TUDCA (n = 5) at 600 mg/kg using a concentration of 200 mg/ml (Fig. 2). This concentration resulted in significant volume overloading causing right heart failure of the rats. The 200 mg/ml TUDCA concentration
provided a significant sodium load (0.38 M) which was 2.5 times the osmolarity of sodium in normal saline (0.15 M). To reduce the sodium load, the concentration of TUDCA was reduced to 50 mg/ml (0.095 M). The 50 mg/ml concentration was then tested in an osmometer resulting in an average value of 387 mOsm. (ref. 280–300 mOsm). For all subsequent experiments, the 50 mg/ml TUDCA concentration was used.

**Apoptosis Study**

To determine the time course of apoptosis following coronary occlusion, Lewis rats (n = 25) weighing on average 297 ± 29 g were injected with phosphate buffered saline (PBS). The saline injection was completed immediately before commencing surgery. Rats were sacrificed by exsanguination at 12 (n = 4), 24 (n = 15), and 48 hours (n = 6) after coronary artery ligation (Fig. 3). Hearts were then immediately frozen in liquid nitrogen, stored at −80°C, subsequently sectioned, and processed for TUNEL staining. Hearts removed at 48 hours had the greatest number of apoptotic cells per high powered field (5.96 ± 3.8), followed by 12 hours (1.69 ± 0.63), and finally 24 hours (1.55 ± 1.5).

**TUDCA Treatment Study**

To establish whether TUDCA can reduce apoptosis as defined by TUNEL staining, a comparison study was initiated. Lewis rats (n = 11) were treated with 400 mg/kg of TUDCA at the time of coronary artery ligation and survived for 24 hours. The TUDCA

![Figure 2. Tauroursodeoxycholic acid (3α,7β-dihydroxy-5β-cholan-24-oic acid N-(2-sulfoethyl) amide) has a molecular weight of 521.69 and a molecular formula of C_{26}H_{44}NNaO_{6}S. It is the taurine conjugate of ursodeoxycholic acid typically formulated as a sodium salt for increased bioavailability. Available as a powder, it is stable at room temperature (20°C) for three years. The structural formula of ursodeoxycholic acid (3α,7β-dihydroxy-5β-cholan-24-oic acid, or 7β-hydroxyliithocholic acid) is based upon a backbone of cholesterol. It has a molecular weight of 392.57 and formulated: C_{24}H_{40}O_{4}. It is referenced in Merck 13, 9956.](image)
treated group had less TUNEL positive staining when compared to the 24 hour PBS control group (p = 0.05). The number of TUNEL positive cells for the TUDCA treated group was 0.70 ± 0.4 compared to 1.55 ± 1.4 for PBS treated (Fig. 4). There were no significant differences with infarct size as measured by Nissl staining when groups were compared to each other. Using the remaining available tissue, caspase-3 activity assay was performed to validate the results of the TUNEL assay. The results of the caspase-3 activity correlated with TUNEL assay. The 24 hour PBS treated group (n = 6) had more caspase-3 activity (0.125 ± 0.007) when compared to the TUDCA treated group (n = 9, 0.095 ± 0.02, p = 0.02, Fig. 5).

Figure 3. The timeline for the apoptosis study involved creation of a myocardial infarction and then survival for 12, 24, or 48 hours. At euthanasia, the heart was removed for sectioning and staining for TUNEL. Additional tissue was processed for caspase measurements for the 24-hour control and experimental groups.

Figure 4. Rats treated with PBS (control) and sacrificed at 12 hours and 24 hours had similar levels of TUNEL staining. In comparison, the PBS treated rats sacrificed at 48-hour showed more TUNEL staining, while rats treated with TUDCA and sacrificed at 24 hours showed significantly less TUNEL staining (p = 0.05).
**Echocardiography Study**

This study was designed to examine whether Lewis rats injected intravenously with TUDCA (400 mg/kg, n = 7) or PBS (n = 6) at the time of infarction could improve cardiac function as determined by echocardiography (Fig. 6). The echocardiography study provided data for myocardial shortening fraction and infarct area. The myocardial shortening fraction as determined by M-mode echocardiography demonstrated a trend toward improvement in the TUDCA treated group vs. the PBS treated group as illustrated in the box plot (Fig. 7). A similar trend toward improvement is also evident in the reduced infarct area following TUDCA treatment (p = 0.111, Fig. 8). Using the Image J analytical software and ANOVA of the hearts stained with Masson’s trichrome, there was no difference between the controls and experimental groups (data not shown).

![Caspase-3 Fluorimetric Assay](image)

*Figure 5.* Treatment with TUDCA reduced caspase-3 activity in rats with 24-hour survival (n = 9, 0.095 ± 0.02) when compared to PBS treated controls (n = 6, 0.125 ± 0.007, p = 0.02).

![Echocardiography Study Diagram](image)

*Figure 6.* For the echocardiography study, serial cardiac ultrasounds were acquired preoperatively, at 1 week, and at 4 weeks. At 4 weeks, cardiac tissue was taken for sectioning, staining with Masson’s trichrome, as well as haematoxylin and eosin.
Figure 7. Rats treated with TUDCA (n = 5) and imaged 4 weeks after myocardial infarction showed a modest improvement in the shortening fraction as compared to PBS treated animals (n = 3, p = NS).

Figure 8. The infarct area is smaller in TUDCA treated animals (n = 6) at 4 weeks as compared to PBS treated controls (n = 4) as measured by echocardiography (p = 0.111).
Discussion

Apoptosis Following Myocardial Infarction

Total occlusion of a coronary artery leads to massive myocardial damage characterized by myocyte death. Myocyte death was originally thought to be principally due to necrosis (Elsasser et al., 2000). However, Anversa et al. (1998) extensively studied the phenomenon and determined that cell death following an acute myocardial infarction is initially and primarily due to apoptosis (86%) rather than necrosis (14%) as shown by TUNEL staining and myosin monoclonal antibody (Kajstura et al., 1996; Anversa et al., 1998).

Treatment strategies involving the use of aurintricarboxylic acid (an endonuclease inhibitor) have been proposed to minimize apoptosis following infarction. Aurintricarboxylic acid reduced infarct size and improved regional contractile dysfunction in a dog model following 1 hour of left coronary occlusion and reperfusion for 24 hours (Zhao et al., 2003). TUNEL staining, DNA laddering, Bcl-2, Bax, caspase-3 assays, and triphenyltetrazolium chloride (TTC) assays revealed a statistically significant improvement following aurintricarboxylic acid treatment. In another myocardial infarction study, 24 hour treatment of rabbits with erythropoietin (EPO) prior to infarction resulted in a reduction in left ventricle infarct size and in TUNEL positive staining. In this setting, EPO acts via the stress-response, Janus-associated kinase-2 (Jak-2), phosphoinositol-3 kinase, and Ras-MAP kinase pathways. Activation of Jak-2 leads to phosphorylation and activation of the downstream transcription factor STAT5. This activation of the Janus Kinase/Signal Transducer and Activator of Transcription (Jak-STAT) pathway prevented apoptosis in cultured myocytes (Stephanou et al., 2000). The most promising evidence for the inhibition of mitochondrial-induced apoptosis is from Rajesh et al. (2005) who examined the effects of UDCA treatment using ischemia reperfusion in the rat. UDCA at 40 mg/kg/IV induced phosphorylation of Akt that resulted in less Bad expression in the mitochondria. Further studies by this group demonstrated that UDCA treatment on isolated mitochondria prevented permeability transition pore opening and subsequent release of cytochrome C.

In our dosage infusion study, we determined that the concentration of 50 mg/ml (400 mg/kg/IV dose) was appropriate, exhibited no apparent sodium toxicity or volume overload that was associated with the higher concentration of 200 mg/ml (600 mg/kg). The Lewis rat proved to be a good choice in that there was no mortality associated with ligation of the left anterior descending artery. The dosage used in the rat model may have direct relevance to future dose escalation studies. Following myocardial infarction, it is well-established that patients are vulnerable to volume overload and intravenous treatment with a large volume of solution is contraindicated. The dosage of 400 mg/kg given intravenously is a possible starting point for a clinical efficacy safety study.

An Italian 6-month randomized dose response study of TUDCA in the treatment of 24 patients with cholestatic liver diseases determined that a dose of 10 mg/kg/day was appropriate for maximal liver enzyme level reduction. Diarrhea and reduction of total and high density lipoprotein (HDL) cholesterol were the only side-effects noted (Crosignani et al., 1996). Our dose of 400 mg/kg was significantly greater than that of the Italian study.
This difference in effective study dosages opens the door for a pharmacologic investigation of TUDCA. An important component of this study would include plasma concentrations of TUDCA. Recently a sensitive test for determining the amount of TUDCA and its related isomers was developed using an Agilent 1100 series HPLC system (Tessier et al., 2003).

This study demonstrates that treatment with TUDCA at 400 mg/kg results in significant reduction in apoptotic cells when it is given at the time of myocardial infarction as compared to when only PBS saline is administered. Furthermore, we validated the TUNEL staining with a caspase immunoassay. This confirmed that TUDCA directly inhibits the apoptotic cascade induced by myocardial infarction. Additionally, treatment with TUDCA reduces the infarct size and improves shortening fraction as measured by echocardiography.

The independent survival of endothelial cells and myocytes is a plausible explanation of the observed echocardiographic results. Survival of endothelial cells likely improves perfusion via intact microvessels in the infarct penumbra. This in turn could reduce the infarct size and improve the shortening fraction of the TUDCA treated group.

The effect of TUDCA on reducing the number of apoptotic cells in this MI model has a potential therapeutic application in a clinical setting for the treatment of acute MI or in revascularization procedures. This study used an intravenous dosage given at the time of infarct which appears to be within the critical period to protect from apoptosis initiated cell death. The optimal dosage time is likely immediately following myocardial infarction. In fact, Anversa et al. (1998) determined that apoptotic cell loss peaks at 4.5 hours and decreases gradually thereafter. Hypothetically, treatment of patients with coronary events with TUDCA is possible. This could be given in an intravenous solution in the emergency room and continued throughout the critical period into the coronary care unit.

Continuation of additional animal studies using TUDCA is important to determine the extent of myocardial protection. Although the infarct area was smaller in the TUDCA treated animals, the small group sizes limited the power of the study. Similarly, the small group sizes limited the ability of the echocardiography study to determine a statistically significant improvement in myocardial function. There was, however, a trend toward improvement in both the shortening fraction and the infarct area that would benefit from an expanded study with an appropriate power. For example a power calculation (0.80) using one-way ANOVA with two levels of treatment and a SD = 1 with a detectable contrast of 1.01 gives an ‘n’ of 17. Alternatively, a calculation can be done using a paired t-test with a significance of 0.05 and a power of 0.80, a True (µ − µ = 0) of 0.5, and sigma of 0.665 results in an ‘n’ number of 16 (Lenth, 2006). Further improvement in the study design would include an Alzet® osmotic pump (Model 2 ML1) implanted in the femoral or jugular vein. This 2 ml pump would provide a rate of 10.0 µl/hour for 1 week; enough to cover the critical period of increased apoptosis following myocardial infarction.

In conclusion, with its historic origins in Chinese treatment for “heat” related disorders, the bear bile constituent UDCA, and chemically synthesized TUDCA may be an effective drug in the future for preventing apoptotic cell death occurring from myocardial infarction. In consideration of the magnitude of morbidity and mortality from myocardial infarction in the US and worldwide, the potential societal benefits of this traditional medication is exceptional. Our study demonstrated that TUDCA is an effective drug to prevent injury.
mediated apoptosis in the rat myocardium and we plan to address these promising initial findings with future studies.

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