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Tauro-β-muricholic acid restricts bile acid-induced hepatocellular apoptosis by preserving the mitochondrial membrane potential

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ABSTRACT

Purpose: β-Muricholic acid (βMCA) is a trihydroxylated bile acid that constitutes the major bile acid in rat and mouse. βMCA is more hydrophilic than ursodeoxycholic acid and has been evaluated for dissolution of cholesterol gallstones. Since it is unknown if βMCA has beneficial effects on hepatocyte cell death we determined the effect of tauro-βMCA (TβMCA) on apoptosis *in vitro*.
Methods: Human Ntcp-transfected HepG2 cells and primary hepatocytes from rat and mouse were incubated with the proapoptotic glycochenodeoxycholic acid (GCDCA) as well as the free fatty acid palmitate in the absence and presence of TβMCA. Apoptosis was quantified using caspase 3/7-assays and after Hoechst 33342 staining. The mitochondrial membrane potential (MMP) was measured fluorometrically using JC-1 (5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethyl-benzimidazol-carbocyaniniodide). Immunoblotting was performed against the proapoptotic Bcl-2-protein Bax.
Results: In Ntcp-HepG2 cells, GCDCA markedly increased apoptosis after 4 h. Co-incubation with TβMCA reduced apoptosis to 49% ($p < 0.01$ vs. GCDCA, each; $n = 6$). While GCDCA (100 μmol/L) reduced the MMP to 34% after 6 h, combination treatment with TβMCA restored the MMP to control levels at all time points ($n = 4$). TβMCA also restored breakdown of the MMP induced by palmitate. GCDCA induced a translocation of Bax from the cytosol to mitochondria that was inhibited by simultaneous treatment with TβMCA in eqimolar concentrations.
Conclusions: TβMCA restricts hepatocellular apoptosis induced by low micromolar concentrations of GCDCA or palmitate via inhibition of Bax translocation to mitochondria and preservation of the MMP. Thus, further studies are warranted to evaluate a potential use of TβMCA in ameliorating liver injury in cholestasis.

1. Introduction

Elevated levels of hydrophobic and toxic bile acids in the liver and in other compartments of the body are found in the most common cholestatic liver diseases primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC) [1]. In the cholestatic liver, bile acid-induced apoptosis of hepatocytes and cholangiocytes is thought to be one of the major pathomechanisms of disease [1]. Especially the glycine-conjugate of chenodeoxycholic acid, glycochenodeoxycholic acid (GCDCA), is a strong inducer of apoptosis [2,3].

At present, ursodeoxycholic acid (UDCA) is the only approved medication for PBC and is often used (off-label) for the treatment of PSC [4]. Whereas UDCA treatment in PBC is able to ameliorate the clinical course of this disease in approximately 50% of patients [5,6], its benefit for patients with PSC is questionable and might even be harmful in high doses [7,8]. Evidently, better suited therapeutic interventions are urgently needed for the treatment of PSC, but also for PBC.

β-Muricholic acid (βMCA) is a natural trihydroxylated bile acid and constitutes the major bile acid in rat and mouse, but does not occur in substantial amounts in man [9]. In addition, βMCA cannot be metabolized in man [10]. βMCA is more hydrophilic than UDCA due to the presence of an additional hydroxyl group in the 6β-position of the steroid ring. In the past, βMCA has been successfully evaluated for the dissolution of cholesterol gallstones *in vitro*

Abbreviations: GCDCA, glycochenodeoxycholic acid; MCA, muricholic acid; TβMCA, tauro-β-muricholic acid; TUDCA, tauroursodeoxycholic acid.

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[11]. In a more recent study, βMCA has been shown to be more effective than UDCA in preventing and dissolving cholesterol gallstones in mice [12]. In addition, TβMCA was also shown to have beneficial effects in both, taurolithocholic acid induced cholestasis in primary rat hepatocyte couplets [13], as well as in taurocholic acid (TCA)-induced cholestasis in colchicine treated perfused rat livers [14].

In rat liver, the toxic bile acid chenodeoxycholic acid (CDCA) is metabolized to βMCA [15] whereas this is not possible in humans. Interestingly, primary rat hepatocytes appear to be less sensible to bile acid-induced apoptosis than human hepatoma cell lines [16]. We therefore hypothesized that βMCA or its conjugates might efficiently prevent or reduce hepatocyte cell death. To address this important issue we tested tauro-βMCA (TβMCA) for its antiapoptotic effect in cell models of GCDCA- and palmitate induced apoptosis.

2. Materials and methods

2.1. Transfection and culture of human HepG2 hepatoblastoma cells

Similar as described previously [17], rat Ntcp was inserted in a pcDNA3.1 vector (Invitrogen, Carlsbad, CA, USA) and stably transfected into the human hepatoma cell line HepG2 with Fugene transfection reagent (Roche, Mannheim, Germany). Cells were cultured in minimal essential medium (MEM) from PAA (Pasching, Austria) supplemented with 10% fetal calf serum, 2 mmol/L L-glutamine, 1 mmol/L Na-pyruvate, non-essential amino acids (1% of a 100× stock solution), 100 U/mL penicillin, 0.1 g/L streptomycin, and 1 g/L G418 sulfate. After 24 h of culture Ntcp-HepG2 cells were incubated for another 4 h with the bile acids GCDCA (Sigma, Taufkirchen, Germany), TUDCA (Calbiochem, Nottingham, UK), or TβMCA (Steraloids, Newport, RI, USA) alone, or with combinations of GCDCA + TUDCA or GCDCA + TβMCA, at concentrations of 25 μmol/L each, or with the solvent DMSO (0.1%) as control. In addition, cells were incubated with the free fatty acid palmitate 200 μmol/L) alone or in the combination with TβMCA.

2.2. Culture of primary hepatocytes from rat and mouse

Male Sprague–Dawley rats and male C57BL/6NCrl mice (both from Charles River, Sulzfeld, Germany) were kept according to local regulations with free access to food and water. Similar as described previously [18], the animals were anesthetized by intraperitoneal injection of 50 mg/kg body mass Na-pentobarbital (Merial, Hallbergmoos, Germany). Rat hepatocytes were isolated with a two-step perfusion method using 0.5 mmol/L EGTA and 0.23 g/L collagenase type IV. Mouse hepatocytes were isolated with a modified retrograde perfusion method and filtered through a 80 μm nylon fabric. Cells were further purified by gradient centrifugation with 50% Percoll (GE Healthcare, Freiburg, Germany) in 10 mmol/L Hepes, pH 7.4, and 154 mmol/L NaCl solution. Cell viability was determined by trypan blue exclusion (average viability of more than 90%).

Afterwards, the hepatocytes were cultured at 37 °C in collagen-coated dishes with supplemented Williams E medium (Gibco, Darmstadt, Germany) with 5% fetal calf serum for rat hepatocytes and 10% fetal calf serum for mouse hepatocytes, and with 2 mmol/L L-glutamine, 0.1 μmol/L insulin, 0.1 μmol/L dexamethasone, antibiotics, and antimycotics. After 4 h of culture cells were incubated without serum for another 4 h with the respective bile acids or with the solvent DMSO (0.1%) as control.

2.3. Quantification of hepatocellular apoptosis

As readout of hepatocellular apoptosis, the activation of caspases 3 and 7 was quantified in cell lysates using the Apo-ONE homogeneous caspase-3/7 assay from Promega (Madison, WI, USA) according to the manufacturer's instructions. Nuclear fragmentation in apoptotic hepatocytes was visualized by bisbenzimidazole (Hoechst 33342) staining.

2.4. Mitochondrial membrane potential

Ntcp-HepG2 cells were cultured for 24 h in poly-L-lysine-coated 96-well dishes (4×10^4 cells/well) and incubated for 2, 4, and 6 h with 25, 50, and 100 μmol/L GCDCA, TβMCA, or equimolar combinations of GCDCA and TβMCA. In addition, cells were treated with palmitate in combination with TβMCA. Cells were stained with 2 μmol/L 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolcarbocyaniniodide (JC-1) from Molecular Probes (Eugene, OR, USA) in cell culture media for 30 min and washed twice with HBSS after staining. Fluorescence was determined with a Cyto-Fluor 4000 plate reader (Per-Septive Biosystems, Framingham, MA, USA) at wavelengths of 485 nm (excitation) and 530 and 580 nm (emission). The ratio of green and red fluorescence signals serve as a parameter for the mitochondrial membrane potential $\Delta\Psi_m$ independent of the mitochondrial mass.

2.5. Quantification of intracellular bile acids

Hepatocytes from rats or mice were seeded at a density of 6×10^6 cells in collagen IV coated culture dishes (diameter 10 cm) in appropriate media and incubated for 4 h. They were incubated for another hour with one of the bile acids TCDCA (Sigma), GCDCA, TUDCA, 10 μmol/L each, or without bile acids (control). Afterwards, cells were washed three times with 0.9% NaCl and homogenized in 5 mL 0.9% NaCl by an ultrasonification device (Branson, Danbury, CT, USA). The protein concentration was determined according to the method of Bradford. 4 mL of the homogenate were supplemented with 10 mmol/L sodium acetate, pH 5.6, 5 mmol/L EDTA, 5 mmol/L 2-mercaptoethanol, and 20 U of choloylglycine hydrolase (Sigma) and kept at a temperature of 37 °C to hydrolyze the amidated bile acids. Bile acids were further purified by a solid phase extraction technique, and detected and quantified by capillary gas chromatography, similar as previously described [19].

2.6. Mitochondrial translocation of the proapoptotic protein Bax

Ntcp-HepG2 cells were plated in culture dishes and incubated until 70% confluency in standard culture media. Afterwards, the medium was aspirated and replaced with pure MEM up to 90 min. After stimulation, the cells were washed and harvested with ice-cold homogenization buffer (250 mmol/L sucrose, 20 mmol/L tris-(hydroxymethyl)-aminomethane, 2 mmol/L EGTA, 10 mmol/L leupeptin, 25 mg/L aprotinin, 10 mmol/L 2-mercaptoethanol, 1 mmol/L PMSF, pH 7.4). After 30 min on ice, the cells were homogenized with 30 strokes with a Potter–Elvehjem PTFE pestle. Nuclei were precipitated by centrifugation at 600 RCF for 5 min, and mitochondria and cytosol were separated by centrifugation at 10,000 RCF for 10 min. Mitochondria were resuspended in a volume of homogenization buffer according to the cytosolic volume (1:1). Samples were supplemented with loading buffer, heated to 95 °C, and loaded onto a 12.5% polyacrylamide gel. After electrophoresis and transfer onto PVDF membranes (Millipore, Bedford, MA, USA), the probes were blocked with 5% milk powder and incubated with a polyclonal rabbit-antibody targeting human Bax (Cell Signaling, Danvers, MA, USA). The ratios of mitochondrial and cytosolic Bax were quantified by densitometry.

2.7. Statistics

The results of the different experiments were expressed as means ± SD. Statistical analysis was performed with ANOVA and statistical significance was considered when p was < 0.05.

3. Results

3.1. Glycochenodeoxycholic acid-induced apoptosis is reduced by tauro-β-muricholic acid

In accordance with previous observations [20], GCDCA led to a significant, up to 19-fold increase of hepatocellular apoptosis in comparison with controls as determined by caspase 3/7 activity in human Ntcp-HepG2 cells as well as in primary rat and mouse hepatocytes after 4 h of incubation (Fig. 1A–C). Interestingly, the rodent cells were more resistant against GCDCA-induced apoptosis than their human counterpart, as a fourfold higher GCDCA concentration (100 vs. 25 μmol/L) was necessary to induce a comparable apoptotic effect. Comparable hepatocyte cell quantity and density were used for these experimental settings.

In contrast to GCDCA, the hydrophilic bile acids TUDCA and TβMCA had no apoptosis eliciting effect. Quite the contrary, both, TβMCA and TUDCA, were able to significantly reduce GCDCA-induced apoptosis in Ntcp-HepG2 cells (Fig. 1A) as well as in primary hepatocytes from rat and mouse (Fig. 1 B and C). In this context, the antiapoptotic effects seemed to be even stronger in primary hepatocytes when compared with Ntcp-HepG2 cells (Fig. 1A–C). In addition, nuclear fragmentation of apoptotic cells was determined by staining with bisbenzimidazole (Hoechst 33342). Cells that were incubated with GCDCA showed substantial nuclear fragmentation, which was virtually absent in cells incubated with the hydrophilic bile acids TUDCA and TβMCA. Both, TβMCA as well as TUDCA were able to prevent nuclear fragmentation induced by the apoptotic bile acid GCDCA (Fig. 1D). To the best of our knowledge, this is the first time that an antiapoptotic effect of TβMCA has been described.

3.2. β-Muricholic acid as a protective factor against bile acid-induced hepatocellular apoptosis in rodents

The relative resistance of rodent liver against bile acid-induced apoptosis may be due to the physiological presence of βMCA in these species. In contrast, in human liver βMCA is virtually absent ([9] and data not shown). Quantification of bile acids with capillary gas chromatography detected significant levels of muricholic acid (α- and β-muricholic acid) in lysates of primary hepatocytes of rats and mice under all conditions tested (Table 1). Interestingly, levels of muricholic acid were markedly higher in hepatocytes that were incubated with GCDCA and TCDCA (not significant for mouse) than in controls or in hepatocytes incubated with TUDCA (Table 1), which is most probably due to the conversion of chenodeoxycholic

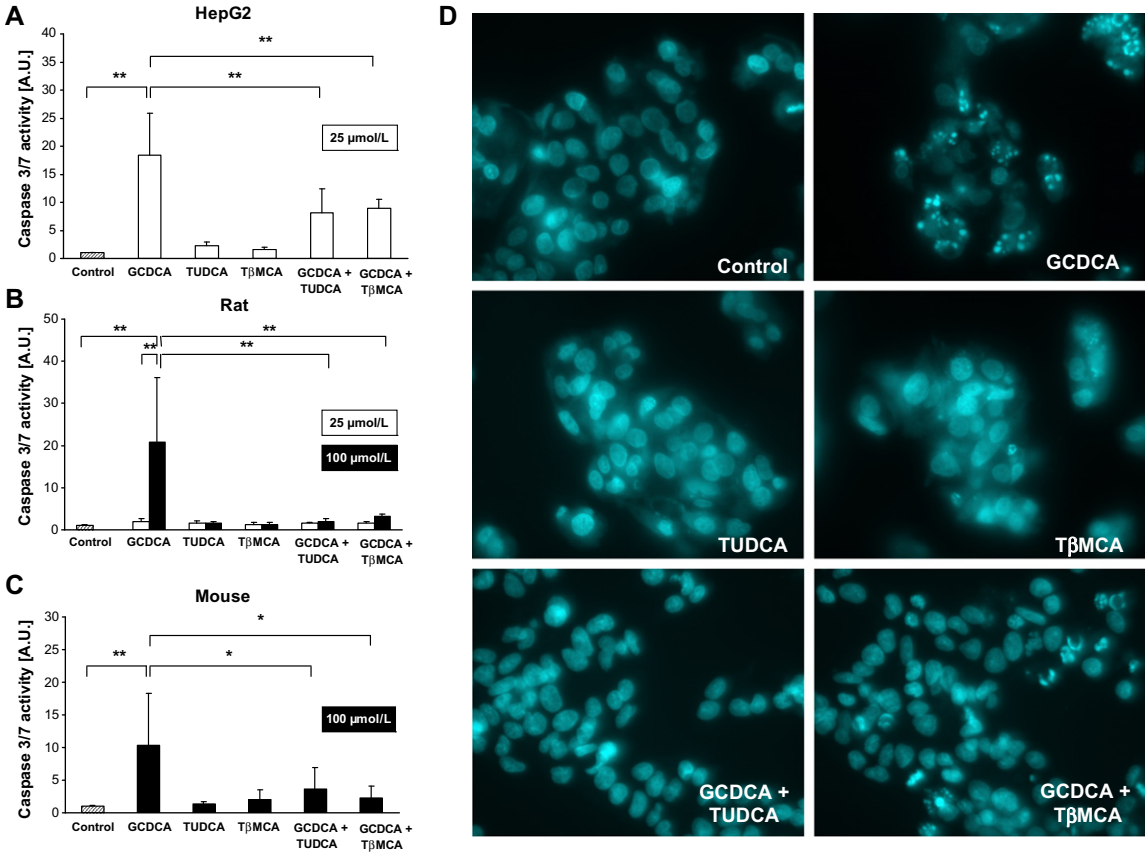

Fig. 1. Glycochenodeoxycholic acid (GCDCA)-induced apoptosis is reduced by tauro-β-muricholic acid. Incubation of human Ntcp-HepG2 cells (A) with the bile acid glycochenodeoxycholic acid (GCDCA; 25 μmol/L) for four hours increased hepatocellular apoptosis as determined by caspase 3/7 activity in comparison with controls. The same occurred in primary hepatocytes from rat (B) and mouse (C), but higher concentrations of GCDCA (100 μmol/L) were required to achieve a comparable effect. GCDCA-induced apoptosis was significantly reduced by the hydrophilic bile acids tauroursodeoxycholic acid (TUDCA) and tauro-β-muricholic acid (TβMCA). Results are expressed as means ± SD of 3–6 experiments, each ($^{*}p < 0.05$, $^{**}p < 0.01$). For illustration of nuclear fragmentation, Ntcp-HepG2 cells were stained with Hoechst 33342 (D). Whereas incubation with GCDCA resulted in substantial nuclear fragmentation, this was not observed for TUDCA and TβMCA. Nuclear fragmentation induced by GCDCA was reduced by both, TUDCA and TβMCA. Concentrations of bile acids were 25 μmol/L, each (40-fold magnification).

Table 1
Quantification of muricholic acid (MCA; i.e., sum of α and βMCA) with capillary gas chromatography. Results are expressed as means ± SD of three experiments, each.

	Muricholic acid (nmol/g protein)
Primary mouse hepatocytes[a]	
Control	165.3 ± 111.2
TCDCA	432.4 ± 250.5
GCDCA	686.4 ± 192.9
TUDCA	161.7 ± 109.5
Primary rat hepatocytes[b]	
Control	72.5 ± 23.4
TCDCA	598.5 ± 239.1
GCDCA	863.9 ± 130.9
TUDCA	304.2 ± 124.8

[a] $p < 0.01$ control vs. GCDCA; $p < 0.01$ TUDCA vs. GCDCA.
[b] $p < 0.01$ control vs. TCDCA, control vs. GCDCA, TUDCA vs. GCDCA; $p < 0.05$ TUDCA vs. TCDCA.

acid to muricholic acid as also described earlier [15]. This conversion of a potentially toxic to a non-toxic and preservative/antiapoptotic bile acid might be one important reason for the observed higher resistance of mouse and rat hepatocytes to GCDCA-induced apoptosis.

3.3. Tauro-β-muricholic acid prevents breakdown of the mitochondrial membrane potential induced by glycochenodeoxycholic acid and palmitate

The mitochondrial membrane potential (MMP) is a key indicator of cellular viability, as it reflects the pumping of hydrogen ions across the inner membrane, the driving force behind ATP production. It is known, that GCDCA can affect the MMP in a dose dependent manner [21]. Here, the MMP was quantified in Ntcp-HepG2 cells by determination of JC-1 fluorescence demonstrating a clear-cut MMP breakdown induced by 25 µmol/L GCDCA (Fig. 2A). In contrast, TβMCA (25 µmol/L) did not affect the MMP. Importantly, however, TβMCA was capable to prevent the GCDCA-induced breakdown of the MMP (Fig. 2B and C). This finding was further validated at higher GCDCA concentrations (50 and 100 µmol/L; data not shown). As shown by fluorescence microscopy, the MMP signal diminished after incubation with GCDCA (Fig. 2C), but not in controls and cells that were treated with TβMCA. In fact, TβMCA maintained the MMP despite coincubation with GCDCA.

In order to test whether the preservative/antiapoptotic effect of TβMCA is restricted to GCDCA-induced apoptosis only, we further observed that TβMCA efficiently prevented MMP breakdown induced by the free fatty acid palmitate. While palmitate (200 µmol/L) led to a rapid breakdown of the MMP, coincubation with TβMCA (25 µmol/L) restored it at every time point (Fig. 3A). TβMCA was able to maintain the mitochondrial signal as shown on fluorescence microscopy despite co-incubation with palmitate (Fig. 3B). Thus, the beneficial effect of TβMCA is not restricted to bile acid-induced apoptosis.

3.4. Tauro-β-muricholic acid prevents mitochondrial translocation of the proapoptotic protein Bax

The hydrophobic bile acid GCDCA increased the mitochondrial fraction of the proapoptotic protein Bax in human Ntcp-HepG2

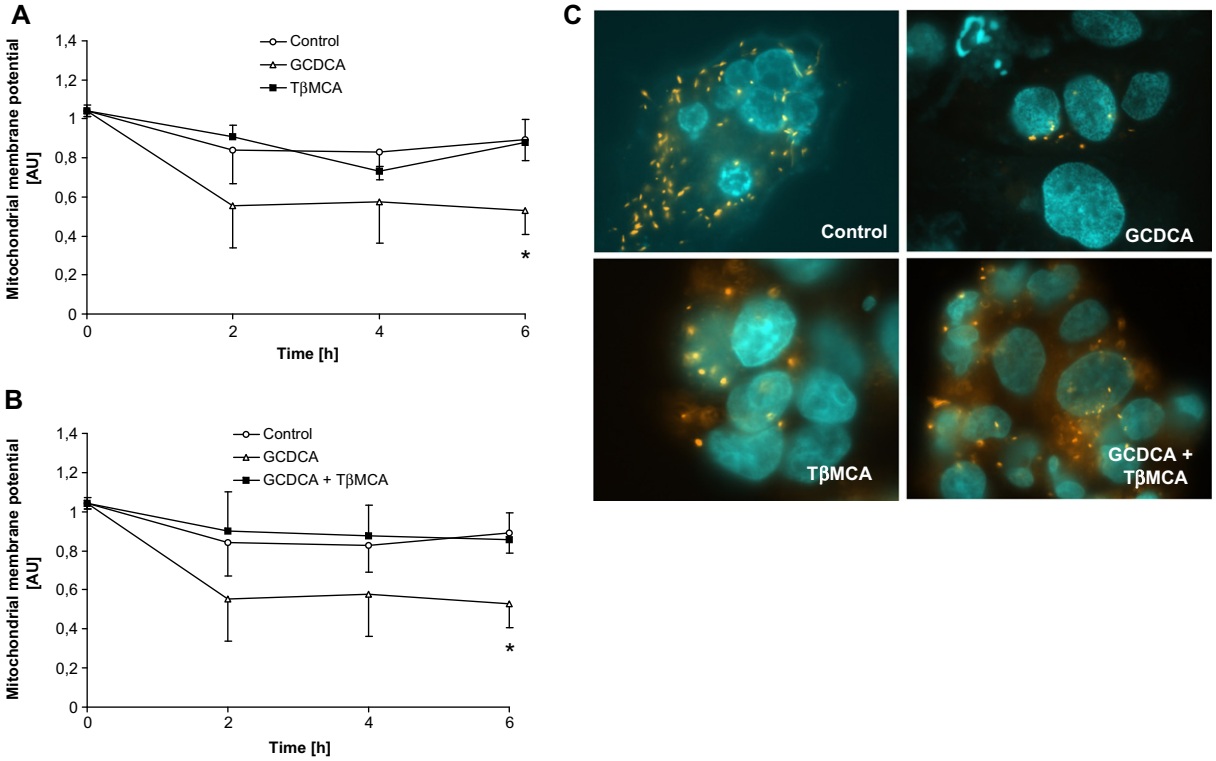

Fig. 2. Tauro-β-muricholic acid (TβMCA) prevents GCDCA-induced breakdown of the mitochondrial membrane potential. The mitochondrial membrane potential was quantified in Ntcp-HepG2 cells by determination of fluorescence with JC-1. Whereas glycochenodeoxycholic acid (GCDCA) induced a breakdown of the mitochondrial membrane potential ($p < 0.05$ vs. control) (A), the hydrophilic bile acid tauro-β-muricholic acid (TβMCA) was able to prevent this breakdown ($p < 0.05$ vs. GCDCA + TβMCA) (B). Concentrations of bile acids were 25 µmol/L, each. Results are expressed as means ± SD of 4 experiments. (C) The mitochondria in Ntcp-HepG2 cells were stained (orange) with JC-1 and images were taken by fluorescence microscopy (100-fold magnification). Whereas the mitochondrial signal diminished after incubation with GCDCA, there was a strong signal in controls and cells that were treated with TβMCA. TβMCA was able to maintain the mitochondrial signal despite coincubation with GCDCA. A representative image of $n = 4$ experiments is shown.

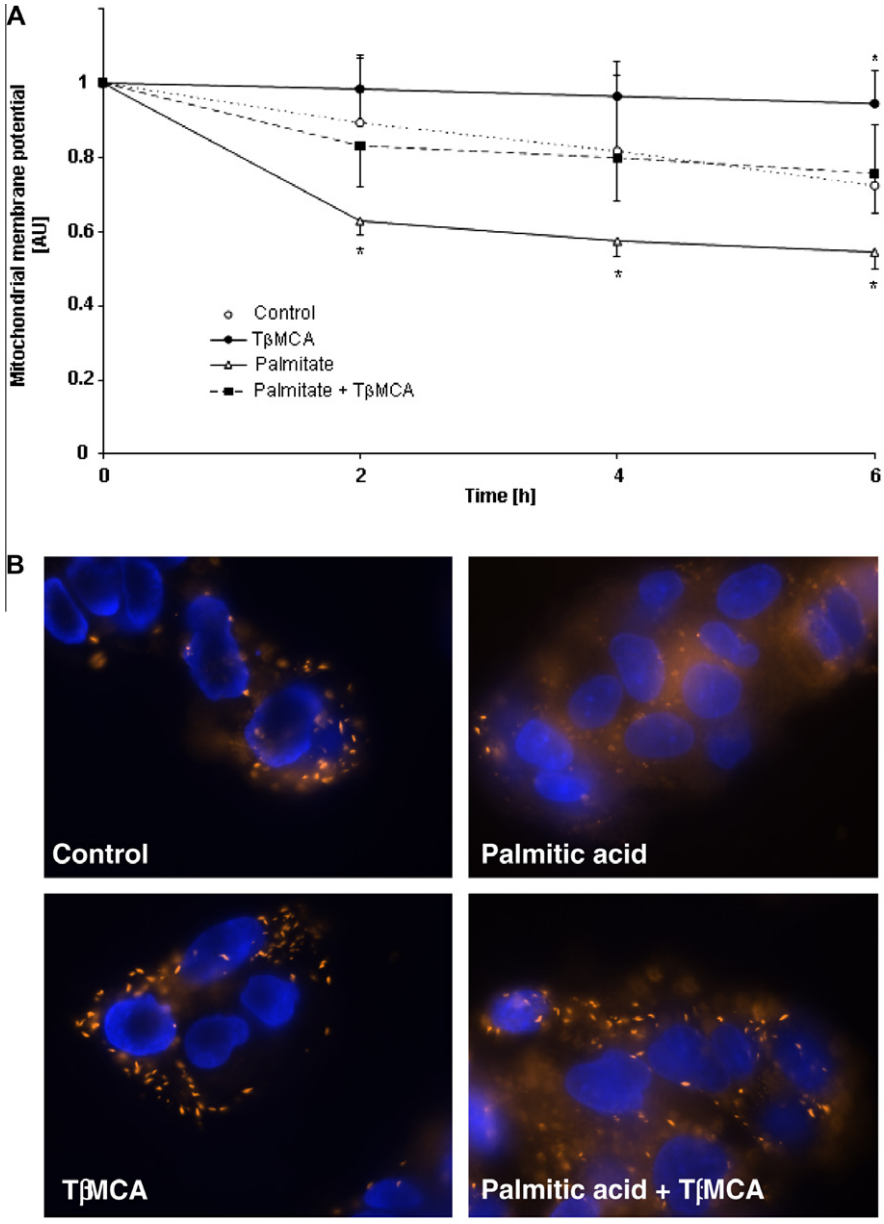

Fig. 3. Tauro-β-muricholic acid (TβMCA) prevents palmitate-induced breakdown of the mitochondrial membrane potential. The mitochondrial membrane potential was quantified in Ntcp-HepG2 cells by determination of fluorescence with JC-1. (A) Palmitate (200 μmol/L) led to a rapid breakdown of the mitochondrial membrane potential which was restored by TβMCA (25 μmol/L). Results are expressed as means ± SD of seven experiments, each. $^{*}p < 0.05$ vs. other variants. (B) After incubation of Ntcp-HepG2 with palmitate, the mitochondrial signal diminished. Again, TβMCA was able to maintain the mitochondrial signal (100-fold magnification). A representative image of $n = 4$ experiments is shown.

cells at a low micromolar concentration of 25 μmol/L, when quantified by immunoblotting and densitometry (Fig. 4A and B). This became statistically significant after 30 min of GCDCA incubation. In contrast, TβMCA (25 μmol/L) had no significant effect on the mitochondrial translocation of Bax (not shown). However, during coincubation TβMCA was capable to prevent the mitochondrial translocation of Bax induced by GCDCA, emphasizing the antiapoptotic potential of TβMCA (Fig. 4C).

4. Discussion

Hepatocellular cell damage induced by toxic bile acids is a pathological key mechanism in chronic cholestatic liver diseases [22]. Although UDCA is the established medical treatment for primary biliary cirrhosis (PBC), up to 50% of patients with early stage disease do not respond sufficiently to this treatment [23]. Even worse, in primary sclerosing cirrhosis (PSC), there is no established medical treatment and the results with UDCA are dissatisfying [7,8]. Therefore, new and better pharmaceutical agents are urgently needed.

In the past, βMCA has been suggested as a promising agent in different models of cholestasis [13,14] and cytoprotective effects have been described [24,25]. Here we demonstrate in cell models of three different species (man, mouse, and rat) that TβMCA has a substantial cell-protective effect avoiding bile acid-induced hepatocellular apoptosis. Interestingly, a fourfold higher concentration of GCDCA was necessary in the rodent hepatocytes to reach a comparable apoptotic effect as in the human Ntcp-HepG2 cells, indicating that the human cell line was more sensitive against

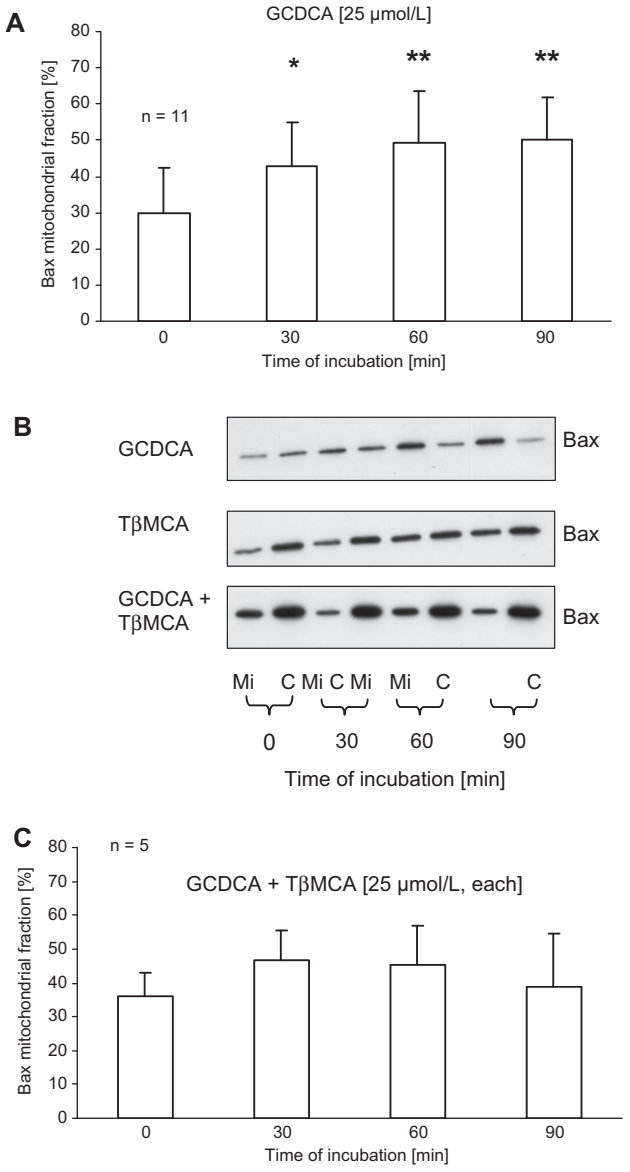

Fig. 4. Tauro-β-muricholic acid (TβMCA) prevents the mitochondrial translocation of the proapoptotic Bcl-2 protein Bax. Ntcp-HepG2 cells were incubated with the two bile acids (GCDCA and TβMCA) at concentrations of 25 µmol/L, each, for 0, 30, 60, and 90 min. Mitochondrial and cytosolic fractions were separated by centrifugation. Whereas GCDCA increased the mitochondrial fraction of the proapoptotic protein Bax (A and B), the hydrophilic bile acid TβMCA had no effect on the mitochondrial translocation of Bax (B and C). Even more, TβMCA was able to prevent GCDCA-induced Bax translocation (B). Results are expressed as means ± SD of 5–11 experiments, each. $^{*}p < 0.05$ vs. untreated controls (time 0), $^{**}p < 0.01$ vs. untreated controls (time 0). A representative Western blot is shown in (B). Mi, mitochondrial fraction; C, cytosolic fraction.

GCDCA-induced apoptosis than their rodent counterparts. One possible explanation is that physiologically βMCA can only be found in rodent, but not in human hepatocytes [9]. In addition, TUDCA can be metabolized to the hydrophobic and toxic bile acid lithocholic acid in the intestine, whereas TβMCA does not undergo such a metabolization in man due to the presence of a 6β-hydroxy group that protects it from 7-dehydroxylation. In humans, βMCA is conjugated to glycine and taurine and undergoes enterohepatic circulation [10]. Thus, βMCA has potential advantages over UDCA, since it is more hydrophilic and not converted into a toxic bile acid. Together with its strong antiapoptotic effects βMCA might therefore be an interesting substance in addition to UDCA for the treatment of cholestatic diseases.

Our findings are an extension of previous studies that already addressed possible beneficial effects of TβMCA in experimental models of cholestasis as well as in necrotic liver cell injury as outlined in the following paragraph.

Indeed, TβMCA has been shown to have beneficial effects in both, taurolithocholic acid induced cholestasis in primary rat hepatocyte couplets via a potential activation of PKC and Ca^{2+} mobilization [13], and in taurocholic acid (TCA)-induced cholestasis in colchicine treated perfused rat livers [14]. While TCA was choleretic in control livers, it promoted cholestasis during colchicine-dependent microtubule dysfunction. TβMCA stimulated bile flow and choleresis, thereby reducing the cholestatic effects of TCA in combination with colchicine [14]. Interestingly, in normal rat livers TβMCA increased the biliary HCO_3^- concentration [14] and a HCO_3^- umbrella has recently been shown to be a protective mechanism against bile acid-induced injury in human cholangiocytes [26]. Furthermore it could be shown that TβMCA reduced LDH-release induced by taurochenodeoxycholic acid (TCDCA) in a 1-h perfusion model [24]. When primary rat hepatocytes were incubated with TCDCA in a (very high) concentration of 1 mmol/L, there was significantly more LDH released into the medium, indicating necrotic cell injury [25]. Again, coincubation with TβMCA reduced LDH release and also decreased intracellular TCDCA content, probably by inducing bile efflux from hepatocytes. These existing together with our current data support the conclusion that βMCA might be a promising substance to ameliorate cholestasis-induced liver cell damage.

We show here, that TβMCA prevents GCDCA-induced breakdown of the mitochondrial membrane potential, a surrogate marker of apoptosis [27] and prevents the mitochondrial translocation of the proapoptotic protein Bax. As inhibition of the permeability transition (PT) pore potently reduces apoptosis, the PT and the mitochondrial potential are central to apoptotic processes [28]. Interestingly, the Bax protein interacts with the PT pore and induces permeability transition with cytochrome *c* release [29,30]. Thus, βMCA might directly target mitochondria as one possible mechanism of action.

It has been shown that elevated serum free fatty acids are features of non-alcoholic fatty liver disease [31]. The saturated C16 free fatty acid palmitate can induce hepatocyte lipoapoptosis in a JNK-dependent manner by activating Bax thereby triggering the mitochondrial apoptotic pathway [32]. TβMCA also prevented palmitate-induced breakdown of the mitochondrial potential. Thus, the beneficial effect of TβMCA appears not to be restricted to bile acid-induced liver damage.

In conclusion, we were able to demonstrate substantial antiapoptotic effects of TβMCA on bile acid-induced apoptosis in cell models from three different species, possibly via inhibition of Bax translocation to mitochondria and preservation of the mitochondrial membrane potential.

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