Protective Effects of Norursodeoxycholic Acid Versus Ursodeoxycholic Acid on Thioacetamide-induced Rat Liver Fibrosis

Vyacheslav U. Buko^{*,†}, Oxana Y. Lukivskaya^{*}, Elena E. Naruta^{*}, Elena B. Belonovskaya^{*}, Horst-Dietmar Tauschel[‡]

^{*}Division of Biochemical Pharmacology, Institute of Biochemistry of Biologically Active Compounds, National Academy of Sciences, BLK-50, Grodno 230030, Belarus and [†]School of Medical Sciences, Krakowska Str. 9, 15-875 Białystok, Poland [‡]Dr. Falk Pharma GmbH, Leinenweberstrasse 5, Freiburg 79041, Germany

Background/objectives: Effects of norursodeoxycholic acid (norUDCA) and ursodeoxycholic acid (UDCA) on liver fibrosis progression and liver fibrosis reversal in thioacetamide (TAA)-treated rats were studied. **Methods:** Advanced liver fibrosis was induced by TAA treatment (200 mg/kg, i.p.) for 12 weeks. In the second experiment resolution of liver fibrosis was assessed after 8 weeks of TAA withdrawal. During 8 last weeks of each trial, fibrotic rats were daily administered with UDCA (80 mg/kg) and norUDCA (equimolar to 80 mg/kg of UDCA) by oral gavage. Liver fibrosis was assessed by Sirius red staining, liver hydroxyproline and serum fibrosis markers determination. **Results:** The TAA treatment resulted in advanced fibrosis and increase in liver hydroxyproline content and serum fibrosis markers. These signs of fibrosis were less pronounced in rats after TAA withdrawal. Treatment with of norUDCA significantly decreased the total and relative liver hydroxyproline contents in rats with fibrosis reversal, whereas UDCA did not change these parameters. Both compounds decreased serum TGF β and type IV collagen contents, whereas other serum markers did not differ from the placebo group. In the fibrosis progression model the square of connective tissue was decreased by norUDCA. Serum type IV collagen and procollagen III-NT contents in these experiments were lowered by both UDCA and norUDCA, whereas rest of serum fibrosis markers were diminished only by norUDCA. **Conclusions:** Both norUDCA and UDCA showed therapeutic and prophylactic antifibrotic effect in rats with TAA-induced liver fibrosis. For most of tested parameters norUDCA was more effective than UDCA, especially in the experiment with liver fibrosis regression. (J CLIN EXP HEPATOL 2014;4:293–301)

Antifibrotic properties of ursodeoxycholic acid (UDCA) have been known for a long time. UDCA is used for treatment of primary biliary cirrhosis in humans which improves clinical indices and helps delay liver transplantation.^{1,2} Application of UDCA in patients with PBC is associated with a considerable decrease in the progress of liver fibrosis.³ Pronounced improving activity of UDCA was found in rodent models of liver fibrosis

induced by bile duct ligation,⁴ carbon tetrachloride administration⁵ and alcoholic liver damage.⁶ Antifibrotic properties of UDCA can be associated inhibition of free oxygen radical-dependent processes in the liver^{7,8} and by reduction in production of cytokines, in particular of tumor necrosis factor- α (TNF α) and tumor growth factor- β (TGF β), inducing apoptosis and coordinating processes of inflammation and fibrogenesis.^{9,10}

24-norursodeoxycholic acid (norUDCA) is a UDCA homolog with a shortened side chain. Recent studies showed that this derivative has unique physiologic and therapeutic properties which are different or superior from those in UDCA. NorUDCA decreases the hydrophilicity and cytotoxicity of circulating bile acid pool and induces their detoxification and elimination. The antifibrotic effect of norUDCA considerably surpassed the similar UDCA action in NEMO-^{Δhepa} mice with specific steatohepatitis¹¹ and in Mdr2-knockout mice with sclerosing cholangitis.¹² There is an experimental evidence for a pronounced therapeutic effect of norUDCA in mice with liver fibrosis provoked by *Schistosoma mansoni* infection.¹³ NorUDCA, in

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Address for correspondence: Vyacheslav U. Buko, Professor, Institute of Biochemistry of Biologically Active Compounds, National Academy of Sciences, BLK-50, Grodno 230030, Belarus. Tel.: +375 152 438411; fax: +375 152 434121

E-mail: buko@bioch.basnet.by

Abbreviations: UDCA: ursodeoxycholic acid; NorUDCA: norursodeoxycholic acid; TAA: thioacetamide; ALT: aminotransferase; AST: aspartate aminotransferase; SEM: standard error of the mean; ANOVA: analysis of variance; TIMP-1: tissue inhibitor of metalloproteinase-1

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contrast to UDCA, significantly ameliorates liver injury in rats with selective bile duct ligation.¹⁴

At present, no clinically effective therapy for liver fibrosis is available. We hypothesize that norUDCA could be a potential therapeutic candidate for fibrotic liver diseases. Therefore, the aim of this study was to compare antifibrotic effects of norUDCA and UDCA on liver fibrosis progression and liver fibrosis reversal in thioacetamide (TAA)-treated rats.

METHODS

Modeling of Liver Fibrosis

Modeling of liver fibrosis desires suitable experimental situation reproducing activation of fibrogenesis and inhibition of fibrolysis causing the following conditions: mirroring of morphological features seen in human diseases; gradual and discrete progression of pathological changes; high reproducibility and low mortality; reversibility and irreversibility of fibrotic changes; development of pathophysiological complications.¹⁵

TAA is a commonly used hepatocarcinogen for induction of experimental liver fibrosis. Based on biochemical and morphological observations, the TAA-induced rat liver cirrhosis resembles to the human liver disease and can be used as a suitable animal model for studying of mechanisms of both liver fibrosis progression and reversibility and testing of antifibrotic and regulating agents.^{16,17}

Rat Liver Fibrosis Progression Model

Ability of tested compounds to slow down progression of fibrosis was tested during induction of fibrosis in delayed treatment protocol. Male Wistar rats weighing 230–250 g (47 animals) handled at temperature-controlled conditions and had free access to water and standard chow. Experimental liver fibrosis was induced by intraperitoneal TAA treatment (200 mg/kg b.w.) 3 times a week for 12 weeks (Figure 1). After the first 4 weeks of the TAA treatment, the rats were administered with the test compounds. The control group and one of the TAA-treated groups, which were used as an experimental control for the tested substances, received a 0.8% aqueous hypromellose (hydroxypropyl methylcellulose) solution by gavage. The test compounds, UDCA (80 mg/kg b.w.), the equimolar amount of norUDCA (77.1 mg/kg b.w.), suspended in 0.8% aqueous hypomellose and placebo, were administered daily, intragastrically by oral gavage during the last 8 weeks of the trial. All drugs were dissolved in 0.8% aqueous hypomellose.

Rat Liver Fibrosis Reversal Model

Reversal of liver fibrosis was studied in male Wistar rats weighing 230–250 g (45 animals) after 12 weeks of intraperitoneal TAA administration (200 mg/kg b.w. 3 times a

Figure 1 Schedule of the experimental procedures. Upper panel—Fibrosis progression model. Rats were treated with TAA (200 mg/kg b.w. intraperitoneally) 3 times a week for 12 weeks. After the 4 weeks of the TAA treatment, the rats were administered with the test compounds. Lower panel—Fibrosis reversal model. Rats were treated with TAA (200 mg/kg b.w. intraperitoneally) 3 times a week for 12 weeks. After this the TAA treatment was stopped and during following 4 weeks rats were treated daily with either UDCA or norUDCA.

week), by discontinuation of the toxin for 8 weeks (Figure 1). Rats were maintained in a temperature-controlled environment and fed *ad libitum* with a standard rat chow. Age-matched control rats were housed and handled in the same manner. One of the TAA-treated groups was sacrificed directly after stopping the TAA treatment and served as a control for a spontaneous regression of fibrosis. At the end of 12 weeks of the treatment, the rest of fibrotic animals were divided into three groups, and during following 4 weeks groups were treated daily with either UDCA (80 mg/kg b.w.) or the equimolar amount of norUDCA (77.1 mg/kg b.w.) suspended in 0.8% aqueous hypromellose administered via oral gavage. One of the groups with liver fibrosis reversal, which was used as an experimental control for the tested substances, as well as the non-fibrotic control group received a 0.8% aqueous hypromellose solution by gavage.

Rats were killed via terminal bleeding under pentobarbital anesthesia (40 mg/kg, i.p.). The care, use, and procedures performed on these rats were approved by the Ethic Committee of the National Academy of Sciences, Belarus, and complied with NIH guidelines.

Analysis of Liver Histopathology

Liver specimens were fixed in 10% formalin and embedded in paraffin. 5 μ m thick sections were processed routinely for hematoxylin & eosin and Sirius red staining. The slides stained with Sirius red were used for morphometric analysis to determine the percentage of liver tissue affected by

fibrosis using a computer-assisted image analyzer (BIO-SCAN, Minsk, Belarus). Results from 15 random fields per slide (fibrosis index) were calculated as a ratio of the Sirius red positive area to the total area examined and expressed as a relative square of connective tissue (% to the total slide square) as described before.¹⁵

Biochemical Assays

Blood was drawn from all animals at the moment of sacrifice and the contents of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase were determined using standardized test kits (LA-CHEMA, Brno, Czech Republic). Specimens of the liver were immediately snap-frozen for hydroxyproline determination. Hydroxyproline content was determined by a modification of Jamall's method.¹⁸ Liver specimens (250 mg each) were homogenized in buffer and hydrolyzed in 5 ml of 6N HCl at 110°C for 16 h. After the hydrolysis the samples were analyzed for hydroxyproline content biochemically and quantified spectrophotometrically at 558 nm.

Serum Fibrosis Markers

Serum fibrosis markers were analyzed by ELISA technique using commercial kits from R&D Systems GmbH (Wiesbaden, Germany) for TNF α , TGF β , and tissue inhibitor of metalloproteinase-1 (TIMP-1). Assay kits for measurement of hyaluronic acid, procollagen II, procollagen III-NT, as well as type I and IV collagens were from Wuhan USCN Sciences Co, Ltd (Wuhan, China).

Statistical Analysis

Data processing was performed by using Excel and Prism 4 for Windows (GraphPad Software, USA) and are given as means μ standard error of the mean (SEM). Comparison of multiple groups was performed using one-way analysis of variance (ANOVA) followed by Dunnett's post test. A *P* value <0.05 was considered as statistically different.

RESULTS

Liver Histology

Male Wistar rats treated for 3 months with TAA showed severe fibrosis with complete distortion of the lobular architecture characterized by a formation of fibrotic septa throughout the liver parenchyma (porto-portal bridging) (Figure 2A, D). In the group of animals with fibrosis resolution these alterations of liver architecture remain unchanged, but thickness of fibrotic septa was markedly decreased (Figure 2E).

After therapeutic administration of UDCA in the liver fibrosis regression model we did not find visible changes of fibrotic process compared to the TAA-treated group (Figure 2F). The treatment with norUDCA in this experi-

ment had a positive effect on the liver histology and was characterized by decreased fibrotic tissue in the majority of animals of this group (Figure 2G). However, the prophylactic administration of UDCA and norUDCA in the liver fibrosis progression model did not significantly changed histological alterations in gross liver architecture (Figure 2B, C).

The data on the relative square of connective tissue indicated a dramatic increase of the connective tissue in the liver of rats treated with TAA for 3 months as compared to the untreated control group (Figure 3). Cessation of the TAA treatment had already led to a spontaneous decrease of this parameter by almost 33%, when compared to the TAA-treated group. In the liver fibrosis reversal model, neither UDCA nor norUDCA-treated groups affected the fibrosis index as compared to the spontaneous recovery group. However, in the fibrosis progression model the squares of connective tissue significantly reduced due to application of norUDCA whereas UDCA caused a non-significant reduction of the connective tissue deposition.

Biochemical Parameters and Serum Fibrosis Markers in the Liver Fibrosis Reversal Model

TAA treatment of rats for 3 months increased the relative hydroxyproline content very strongly, by almost 280% in comparison to the control group while the total hydroxyproline content increased by 330% (Table 1). A discontinuation of the TAA treatment reversed the relative liver hydroxyproline content in the recovery group by about 14% and the total hydroxyproline content by about 17% with statistical significance. Only norUDCA resulted in an additional therapeutic effect on both parameters, significantly decreasing the relative and total hydroxyproline contents (26.3% and 30.6%, respectively).

The TAA administration during 3 months induced strong increase activities of the serum marker enzymes, ALT, AST and alkaline phosphatase. Withdrawal of TAA led to a significant improvement of the above enzymes. The treatment with either UDCA or norUDCA did not affect activity of these enzymes (Data not shown).

The TAA treatment of rats for 3 months caused a significant elevation of serum TGF β 1 by about 40% in comparison to the control group (Table 1). Withdrawal of TAA and treatment with placebo did not change this parameter after 2 months of recovery. In the groups treated with UDCA and norUDCA, the serum content of TGF β 1 decreased by 41% and 57%, respectively, as compared to the recovery group. The serum values for proinflammatory TNF α did not change in any of the experimental groups. The strongly increased TIMP-1 levels due to TAA treatment reversed already by treatment with placebo or norUDCA and to a greater extent by treatment with UDCA (Figure 3).

The serum contents of hyaluronic acid (Figure 3), the collagens type I, type IV (Figure 4), procollagen II,

Fibrosis progression model

Fibrosis reversal model

Figure 2 Histological images of liver slides stained with Sirius red from rats with liver fibrosis treated with UDCA or norUDCA. Upper panel—Fibrosis progression model. A. The TAA-treated group (12 weeks); B. Rats treated with UDCA; C. The group treated with norUDCA. Lower panel—Fibrosis reversal model. A. The TAA-treated group (12 weeks); B. Spontaneous recovery of liver fibrosis (8 weeks); C. Rats treated with UDCA; D. The group treated with norUDCA. Representative sections are shown at original magnification 4 $\times$.

Figure 3 Serum hyaluronic acid (ng/ml) and TIMP-1 (ng/ml) contents in rats with liver fibrosis treated with UDCA or norUDCA. Upper panel—Fibrosis progression model. Lower panel—Fibrosis reversal model. Mean $\pm$ S.D. Fibrosis progression model: * $P < 0.05$ compared to the TAA-treated group; ** $P < 0.05$ compared to the group treated with UDCA. Fibrosis progression model: * $P < 0.05$ compared to the fibrosis recovery group; ** $P < 0.05$ compared to the UDCA-treated group.

procollagen III-NT (Figure 5) strongly increased during the administration of TAA, but thereafter they were completely reversed to the levels of the untreated control group by stopping the TAA treatment and by treating the rats with placebo for 8 weeks. Only type IV collagen concentration in the serum was significantly decreased in the groups treated by both UDCA and norUDCA, whereas procollagen II and procollagen III-NT contents decreased only in the group treated with norUDCA as compared to the spontaneous recovery group.

Biochemical Parameters and Serum Fibrosis Markers in the Liver Fibrosis Progression Model

Rats treated with TAA for 3 months showed a dramatic increase in the contents of liver relative hydroxyproline (by about 200%) and total hydroxyproline (by almost 300%). Overall, the treatment of these rats with UDCA or nor-

UDCA for 2 months virtually did not exert any effects on these parameters (Table 1).

The TAA treatment caused a strong increase of serum ALT, AST and alkaline phosphatase activities. Neither treatment with UDCA nor treatment with norUDCA affected these parameters (Data not shown).

The values for serum TGF β and TNF α were significantly increased in TAA-treated animals. Neither UDCA nor norUDCA could significantly prevent the increase of TGF β , but norUDCA almost reversed the increase of TNF α , while UDCA caused only non-significant reduction (Table 1).

The TAA treatment of rats markedly increased the contents of serum type I and IV collagens (Figure 4) and procollagen III-NT (Figure 5). The other serum markers of liver fibrosis, hyaluronic acid and TIMP-1 (Figure 3), were also increased in TAA-treated animals. NorUDCA significantly decreased the serum TIMP and hyaluronate

Table 1 Liver fibrosis area and hydroxyproline contents in fibrotic rats treated with UDCA or norUDCA.

Parameters groups	Liver fibrosis area (% to slide square)	Relative liver hydroxyproline ($\mu\text{g/g}$ wet liver)	Total liver hydroxyproline ($\text{mg}/\text{total liver weight}$)	TGF β 1, (ng/ml)	TNF α , (pg/ml)
Fibrosis progression model					
Control	1.7 $\pm$ 0.18	398 $\pm$ 8.5	4.4 $\pm$ 0.14	54.9 $\pm$ 12.44	3.6 $\pm$ 0.25
TAA, 12 w	14.8 $\pm$ 1.26	1245 $\pm$ 29.7	19.3 $\pm$ 0.38	117.6 $\pm$ 9.56	8.9 $\pm$ 1.75
TAA + UDCA	12.7 $\pm$ 2.23	1287 $\pm$ 40.1	19.4 $\pm$ 0.90	102.0 $\pm$ 6.41	6.7 $\pm$ 0.81
TAA + norUDCA	11.8 $\pm$ 1.57 ^a	1228 $\pm$ 35.9	18.9 $\pm$ 0.67	81.8 $\pm$ 14.01 ^a	4.5 $\pm$ 0.68 ^a
Fibrosis reversal model					
Control	1.5 $\pm$ 0.16	371 $\pm$ 10.3	4.1 $\pm$ 0.23	52.1 $\pm$ 9.29	5.1 $\pm$ 0.16
TAA, 12 w (Peak of fibrosis)	12.7 $\pm$ 1.34	1407 $\pm$ 70.8	17.4 $\pm$ 1.38	74.4 $\pm$ 5.55	5.3 $\pm$ 0.13
Recovery, 8 w	8.5 $\pm$ 0.90	1236 $\pm$ 62.1	14.7 $\pm$ 0.68	63.1 $\pm$ 6.70	5.3 $\pm$ 0.25
Recovery, 8 w + UDCA	8.5 $\pm$ 1.48	1178 $\pm$ 80.6	13.0 $\pm$ 0.93	32.1 $\pm$ 4.53 ^a	5.2 $\pm$ 0.07
Recovery, 8 w + norUDCA	10.6 $\pm$ 1.63	1019 $\pm$ 17.9 ^a	12.0 $\pm$ 0.49 ^a	35.3 $\pm$ 5.83 ^a	5.2 $\pm$ 0.07

^a $P < 0.05$ compared with the TAA, 12 w group (Fibrosis progression model) and with the recovery, 8 w group (Fibrosis reversal model).

Fibrosis progression model

Fibrosis reversal model

Figure 4 Serum type I and type IV collagen (ng/ml) contents in rats with liver fibrosis treated with UDCA or norUDCA. Upper panel—Fibrosis progression model. Lower panel—Fibrosis reversal model. Mean $\pm$ S.D. Fibrosis progression model: * $P < 0.05$ compared to the TAA-treated group; ** $P < 0.05$ compared to the group treated with UDCA. Fibrosis progression model: * $P < 0.05$ compared to the fibrosis recovery group; ** $P < 0.05$ compared to the UDCA-treated group.

Figure 5 Serum procollagen II and procollagen III-NT (ng/ml) contents in rats with liver fibrosis treated with UDCA or norUDCA. Upper panel—Fibrosis progression model. Lower panel—Fibrosis reversal model. Mean $\pm$ S.D. Fibrosis progression model: * $P < 0.05$ compared to the TAA-treated group; ** $P < 0.05$ compared to the group treated with UDCA. Fibrosis reversal model: * $P < 0.05$ compared to the fibrosis recovery group; ** $P < 0.05$ compared to the UDCA-treated group.

values, while UDCA did not change these parameters with statistical significance.

Both UDCA and norUDCA decreased in serum type I and type III collagens as well as procollagen II contents with statistical significance whereas only norUDCA lowered the serum procollagen III-NT value (Figures 4 and 5).

DISCUSSION

Treatment of rodents with TAA is known to lead to liver fibrosis and eventually cirrhosis, with the morphological and biochemical changes resembling that of the human disease.¹⁹ Both of our models of experimental liver fibrosis reproduced a pronounced fibrotic liver disease, as was indicated by serum fibrosis markers, histopathological changes, and liver hydroxyproline content. The treatments with both UDCA and norUDCA demonstrated a clear antifibrotic effect in comparison with

spontaneous regression as well as in the fibrosis progression model.

Reversibility of liver TAA-induced fibrosis induced by TAA has been shown in different laboratories.^{20,21} Earlier we found that TAA withdrawal in the same experimental model led to partial spontaneous reversibility of liver fibrosis in four weeks and was characterized by decreased fibrosis rate, total and relative hydroxyproline contents and the liver/body weight ratio.¹⁷

While demonstrating antifibrotic properties in experimental liver fibrosis, UDCA is the mainstay therapy in primary biliary cirrhosis. UDCA significantly decreased liver hydroxyproline content in CCl₄-induced fibrosis reversal as compared to the spontaneous recovery group²² and improved the ultrastructural changes in hepatocytes in some experimental models.⁵ UDCA treatment alleviated development of immunomediated liver fibrosis induced by porcine serum injections lowering the hepatic mRNA

expression of collagens.²³ Multiple studies suggest a beneficial effect of UDCA, decreasing fibrosis scores and liver hydroxyproline content, in cholestatic liver fibrosis induced by bile duct ligation.^{4,24,25} In the model of TAA-induced liver fibrosis reversal presented here, UDCA developed a moderate antifibrotic effect, decreasing the relative square of connective tissue in the liver, but leaving unchanged the liver hydroxyproline content: a “gold standard” for evaluation of liver fibrosis severity. In contrast, in our experiment, norUDCA decreased the hydroxyproline content, as quantified from the amount of collagens in the liver, and did not exert any effects on the square of connective tissue that consists of not only collagens, but also of other compounds such as glycoproteins, proteoglycans and glycosaminoglycans. In the liver fibrosis progression model, the treatment with UDCA affected neither the liver hydroxyproline contents nor the square of the connective tissue. However, in both models, UDCA decreased most of the serum fibrosis markers as TGF β 1 and collagens being among them.

The antifibrotic effects of norUDCA were more pronounced than those of UDCA mainly in liver fibrosis reversal. The treatment of rats with norUDCA in this model led to a significant reduction of both the total hydroxyproline and relative hydroxyproline contents, whereas no changes were observed in the relative square of connective tissue in the liver. However, the treatment of these rats with UDCA decreased the accumulation of fibrotic tissue in the liver evaluated histologically, but did not affect that of liver hydroxyproline.

After application of the compounds in the liver fibrosis progression model decrease of fibrosis rate was noted for norUDCA and UDCA but both compounds did not change the liver hydroxyproline contents in this experimental scenario. However, other important parameters characterizing liver fibrosis decreased significantly, especially quantitative morphometry of connective tissue square in liver tissue, and well established serum markers of fibrogenesis collectively suggest superior antifibrotic properties of norUDCA compared to UDCA in our experimental system.

NorUDCA, a C₂₃ homolog of UDCA that lacks a methylen group in its side chain is not accumulated in the enterohepatic circulation and does not cause hepatotoxicity after its chronic administration.²⁶ NorUDCA has a clear advantage over its parent compound, UDCA, in correction of liver pathology. In experiments with Mdr2-knockout mice having advanced cholestatic liver disease including sclerosing cholangitis and biliary fibrosis²⁷ the administration of norUDCA improved liver damage more effective than UDCA.^{12,28} The authors explained the prevalence of norUDCA over UDCA by increased hydrophilicity of this compound that led to a decrease in its cytotoxicity and induction of detoxification pathways and elimination routes for bile acids. NorUDCA rather than UDCA

attenuated the progression of non-alcoholic steatohepatitis in NEMO- Δ^{hepa} mice with the absence of NF- κ B.¹¹ It inhibited the gene expression of the main regulators of lipid synthesis, FasN, SREBP and PPAR γ , whereas UDCA did not exert any significant effects on these parameters. Simultaneously, norUDCA showed a clear anti-fibrogenic effect in NEMO- Δ^{hepa} mice, significantly improving periductal fibrosis, reducing liver hydroxyproline content, procollagens I and III, mRNA expression and α SMA expression in periductal myofibroblastes, whereas UDCA elevated collagen IAI mRNA expression.

The effect of norUDCA on biliary liver fibrosis was explained by its influence on the ductular reaction.²⁸ Liver fibrosis induced by TAA is also accompanied by ductular disturbances, such as multifocal bile ductular proliferation and biliary dysplasia.²⁹ Therefore, one of the antifibrotic mechanisms of norUDCA could be its effect on the ductular reaction, which was proposed to be a driver of fibrogenesis essentially across all chronic liver diseases.³⁰ Effects on hydroxyproline, TIMP-1, type IV collagen and hyaluronic acid in response to norUDCA indicate direct effects on fibrogenesis pathways, as opposed to indirect (e.g. hepatoprotective or anti-inflammatory). However, norUDCA decreased serum TNF α in liver progressive model that suggest possible anti-inflammatory effect of this compound.

Based on results of our study, norUDCA was superior to UDCA in terms of its efficacy to both slow down fibrosis and induce fibrosis reversal, with direct clinical implications. NorUDCA may offer significant advantage in people with chronic liver disease and progressive fibrosis, and it appears justified to test its clinical efficacy in future clinical trials especially in those with primary biliary cirrhosis currently treated with UDCA, but also other biliary diseases such as primary sclerosing cholangitis and non-biliary chronic liver diseases associated with progressive fibrosis.³¹

In conclusion, norUDCA and UDCA been used with therapeutic and prophylactic intervention alleviated the morphological and biochemical changes in the rat models of TAA-induced liver fibrosis. For most of tested parameters in both experiments norUDCA was more effective than UDCA. Especially, norUDCA had more pronounced antifibrotic effect than its parent compound, UDCA, in fibrotic rats with liver fibrosis reversal where both compounds were used as therapeutic agents. However, the exact mechanisms explaining the advantage of norUDCA over UDCA are to be established.

CONFLICTS OF INTEREST

All authors have none to declare.

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they have no conflict of interest with respect to this manuscript. All institutional and national guidelines for the care and use of laboratory animals were followed.

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