J Huazhong Univ Sci Technol［Med Sci］
DOI 10.1007/s11596-013-1095-x 33(2):189-194,2013

Efficacy and Safety of Tauroursodeoxycholic Acid in the Treatment of Liver Cirrhosis: A Double-blind Randomized Controlled Trial

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Summary: No direct comparison of tauroursodeoxycholic acid (TUDCA) and ursodeoxycholic acid (UDCA) has yet been carried out in the treatment of liver cirrhosis in China. We designed a double-blind randomized trial to evaluate the potential therapeutic efficacy of TUDCA in liver cirrhosis, using UDCA as parallel control. The enrolled 23 patients with liver cirrhosis were randomly divided into TUDCA group (n=12) and UDCA group (n=11), and given TUDCA and UDCA respectively at the daily dose of 750 mg, in a randomly assigned sequence for a 6-month period. Clinical, biochemical and histological features, and liver ultrasonographic findings were evaluated before and after the study. According to the inclusion criteria, 18 patients were included in the final analysis, including 9 cases in both two groups. Serum ALT, AST and ALP levels in TUDCA group and AST levels in UDCA group were significantly reduced as compared with baseline (P<0.05). Serum albumin levels were significantly increased in both TUDCA and UDCA groups (P<0.05). Serum markers for liver fibrosis were slightly decreased with the difference being not significant in either group. Only one patient in TUDCA group had significantly histological relief. Both treatments were well tolerated and no patient complained of side effects. It is suggested that TUDCA therapy is safe and appears to be more effective than UDCA in the treatment of liver cirrhosis, particularly in the improvement of the biochemical expression. However, both drugs exert no effect on the serum markers for liver fibrosis during 6-month treatment.
Key words: liver cirrhosis; tauroursodeoxycholic acid; ursodeoxycholic acid; serum markers

Liver cirrhosis is a frequent consequence of the long clinical course of all chronic liver diseases. The hepatic lesions may result, at least in part, from the intracellular accumulation of potentially toxic endogenous bile acids. The primary end-point of antifibrotic therapy in cirrhotic patients should be the relief of the serum markers of liver damages and the reduction of fibrosis in the context of cirrhosis. Present studies have demonstrated that the administration of ursodeoxycholic acid (UDCA), a hydrophilic bile acid, leads to improvement in the condition of patients with cirrhosis and gallstones[1-3]. However, the therapeutic effects of long-term UDCA administration may be counteracted by the concomitant increases in the liver of lithocholic acid, a hepatotoxic bile acid resulting from the biotransformation of UDCA, and the limited small bowel absorption and conversion of UDCA by bacteria in the colon[4, 5]. Tauroursodeoxycholic acid (TUDCA), the taurine conjugate of UDCA, is the physical active form of UDCA when secreted into the bile. Compared to UDCA, TUDCA is better absorbed by intestine and liver because of being fully ionized and water soluble at the various pH values. TUDCA undergoes reduced biotransformation to more hydrophobic metabolites[6]. Oral dosing with TUDCA produces similar changes in biliary and circulating bile acid composition and concentrations as UDCA, but with higher proportions and concentrations of UDCA and conjugates, suggesting moderately enhanced bioavailability[7, 8]. Therefore, this compound has shown more favorable metabolic properties in the treatment of liver diseases.

The beneficial effects of TUDCA on clinical and biochemical markers of liver function in patients have been reported extensively[9]. These beneficial effects appear in a variety of human liver diseases including primary biliary cirrhosis (PBC), chronic viral hepatitis and cholesterol gallstones. Thus it is conceivable that TUDCA might also prove to be effective in improving abnormal liver function in liver cirrhosis. Now no direct comparisons between TUDCA and UDCA are available in patients with cirrhosis in China. Therefore, we de-

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signed a double-blind, randomized controlled trial to compare the efficacy and safety of these two hydrophilic bile acids in such patients.

1 MATERIALS AND METHODS

1.1 Patients

Between June 2009 and August 2011, consecutive patients with cirrhosis were enrolled into the study. These patients attended the first visit or follow-up at the Department of Gastroenterology of Union Hospital. Diagnosis of liver cirrhosis was based on the standard criteria revised in 2000 by the branch of infectious, parasitic diseases and hepatic association of Chinese Medical Association[10]. Inclusion criteria were as follows: cirrhosis based on clinical, laboratory and ultrasonographic findings; age between 18 and 75 years; written informed consent. Exclusion criteria were as follows: body mass index (BMI) >28 kg/m^2; serum total bilirubin concentration ≥171 μmol/L; hepatic failure that needed hepatic transplantation; malignancies; gallbladder disease; active gastrointestinal bleeding; hepatic encephalopathy; hepatorenal syndrome; intestinal infection; peptic ulcer; severe heart and renal dysfunction; severe endocrine, hematologic and neuropsychiatric diseases; pregnancy or lactation; excessive alcohol consumption (≥40 g per day). Patients were also excluded if they had been treated with interferon, UDCA, colchicines, anti-virus or immunomodulatory drugs in the previous 3 months. Other exclusion criteria were as follows: allergy to the drug; severe adverse reactions or quit for no reason.

The study protocol conformed to the ethical guidelines of the 1975 Helsinki declaration. The study was approved by the ethics committee of Union Hospital. All patients provided written informed consent before enrolment.

1.2 Study Design

This was a randomized, double-blind, parallel-group trial. Patients were randomized in a 1:1 ratio to receive either TUDCA (taurolite, Bruschettini S.R.L., Italy) or UDCA (ursofalk, Losan Pharma GmbH, Germany) of 750 mg/day, which was administered in three divided doses at mealtime for 6-month periods. Before and at 3rd and 6th month after treatment, fasting blood was collected for clinical examination, and compliance was assessed by counting the number of unused capsules. B-ultrasonography was performed before and at 6th month after treatment. At least 1/5 patients undertook percutaneous liver biopsy before and at 6th month after treatment. Patients were instructed to report worsening of symptoms or development of unusual symptoms at any time.

1.3 Methods

The random list was generated by a computer. Unrelated people completed the drug compiling work. Physician released the drug by the observing order and the random number of the drug. Both TUDCA and UDCA capsules were the same in appearance, color and size. The blind was retained at the pharmaceutical clinical research base of Union Hospital and revealed at the end of the trial. Liver function including serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), total bilirubin (TBil), albumin, globulin; serum markers for liver fibrosis including hyaluromic acid (HA), laminin, precollagen Ⅲ and collagen Ⅳ; prothrombin time (PT), international normalized ratio (INR), blood urea nitrogen (BUN), serum creatinine and blood routine were all determined by routine laboratory methods. The morphological changes of the liver were examined using B-ultrasonography. The progression of disease was assessed by histological examination of liver biopsy specimens. Histological stage was determined according to the accepted criteria[11].

1.4 Statistical Analysis

The primary end point was the decline more than 25% from baseline in serum ALT, AST, ALP and GGT. Secondary endpoints included the significant difference in serum markers for liver fibrosis and histologic stage from baseline, and safety. Statistical analysis was conducted using the SPSS 17.0 for Windows. Comparisons between the two groups were performed using Mann-Whitney *U* test for continuous variables and Fisher exact test for qualitative variables. Comparisons of the variables in the same group were performed using the Wilcoxon signed ranks test and Friedman test. All the analyses were two-sided. Descriptive data were given as median with interquartile range, with a *P* value of less than 0.05 considered statistically significant.

2 RESULTS

2.1 Patients and Treatment Compliance

Twenty-three consecutive patients with liver cirrhosis were investigated during the study period, and three of them were not considered eligible for gastrointestinal bleeding (one patient) and using anti-virus drugs (two patients). The remaining 20 patients (Child-Pugh A or B) were enrolled in the study. Two withdrew from the study during follow-up for reasons unrelated to treatment. One of them died of newly diagnosed hepatocellular carcinoma in UDCA group. The other patient refused to continue in TUDCA group. Therefore, the analysis was performed on data from 18 patients, and 9 patients in each group. All 20 randomized patients were analyzed for safety.

At entry, there was no significant difference between the two groups in terms of gender, etiology, age, BMI and Child-Pugh score (table 1). The parameters of liver function, liver fibrosis, coagulation function, blood routine and renal function between the two groups had no significant difference at baseline. Compliance with treatment was good, and no patient took less than 95% of the capsules dispensed.

Table 1 Characteristics of study patients

Parameters	TUDCA group (n=9)	UDCA group (n=9)	P values
Male/Female	5/4	4/5	1.0
HBsAg-positive/PBC	7/2	6/3	1.0
Age (years)	53 (40.5—60.5)	50 (37—53.5)	0.376
BMI (kg/m^2)	21.3 (15.4—20.4)	22.8 (18.5—24.3)	0.627
Child-Pugh A/B	7/2	8/1	1.0

PBC: primary biliary cirrhosis; BMI: body mass index

2.2 Efficacy

After treatment for 6 months, significant improvements were achieved in serum ALT, AST, ALP and albumin concentrations in TUDCA group (P<0.05). The mean changes over baseline values of ALT, AST, ALP and GGT were decreased by 42.8%, 45.9%, 38.4% and 47.7%, respectively, and albumin was increased by 16.8% from the baseline. There were significant improvements in serum AST and albumin concentrations in UDCA group (P<0.05). The mean changes over baseline values of ALT, AST, ALP and GGT were decreased by 24.7%, 30.5%, 8.6% and 29.8%, respectively, and albumin was increased by 10.1% from the baseline (table 2, fig. 1). Significant improvements of serum TBil and GGT levels were not found in both TUDCA and UDCA groups. Serum markers for liver fibrosis including HA, laminin, precollagen Ⅲ and collagen Ⅳ did not change significantly during the administration of TUDCA or UDCA (fig. 2). PT, INR, BUN, creatinine and blood routine in both groups remained stable during the treatments.

Table 2 Serum liver enzyme, TBil, albumin and globulin values measured before (baseline) and 3, 6 months after the administration of TUDCA and UDCA

Groups	Baseline	Third month	Sixth month	P values
TUDCA				
ALT (U/L)	116 (32.5—237.5)	34 (14.5—51)	23 (18.5—62.6)	0.045
AST (U/L)	85 (221—55.5)	42 (58.5—27)	39 (29.5—62.5)	0.013
GGT (U/L)	242 (131—356.5)	56 (37.5—87)	50 (33.5—188.9)	0.105
ALP (U/L)	179 (136—296)	132 (98—164)	104 (82—135.6)	0.001
TBil (μmol/L)	24.6 (19.2—41.8)	18.1 (15.2—41.1)	18.4 (13.2—31.1)	0.368
Albumin (g/L)	34.2 (30.5—40)	38.6 (33.9—46.2)	39.2 (34.9—47.4)	0.032
Globulin (g/L)	33.4 (27.1—38.5)	32.7 (27.5—40.9)	31.7 (28—36)	0.459
UDCA				
ALT (U/L)	57 (26—98.5)	32 (26—93)	29 (21—58.5)	0.412
AST (U/L)	74 (52—97)	67 (44.5—100)	43 (38.5—84)	0.003
GGT (U/L)	186 (53—353.5)	88 (40.5—301)	56 (33—378)	0.085
ALP (U/L)	291 (104—402)	196 (126.7—329)	171 (107.5—412.5)	0.641
TBil (μmol/L)	28.1 (23.7—40.9)	21.4 (15.1—37)	26.2 (12.2—40.6)	0.169
Albumin (g/L)	35.5 (32.6—37)	40.2 (35.2—40.9)	36.5 (35.2—42.6)	0.016
Globulin (g/L)	32.9 (30.5—40.4)	39.9 (35.5—42.1)	35.2 (33.9—38.9)	0.097

ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; GGT: gamma-glutamyl transferase; TBil: total bilirubin; TUDCA: tauroursodeoxycholic acid; UDCA: ursodeoxycholic acid

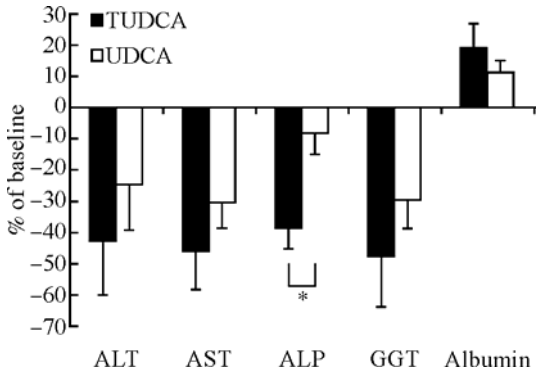

Fig. 1 The percentage of mean changes over baseline values of serum ALT, AST, ALP, GGT and albumin at 6th month after therapy in two groups
*P<0.01

There were five patients subject to percutaneous liver biopsy before and at 6th month after treatment: 3 cases in TUDCA group, and 2 cases in UDCA group. According to inflammatory activity of chronic hepatitis and fibrosis scoring program in China[11], all the patients reached G2-3 and S2-4. Only one patient in the TUDCA group had significant histological relief from G2S3 to G2S1 (fig. 3). The others had no changes in histology.

2.3 Safety

Both drugs were well tolerated and no side effects were reported. None of the coagulation function, blood routine and renal function results indicated deterioration during therapy, suggesting the safety of both drugs, at least during 6 months of treatment.

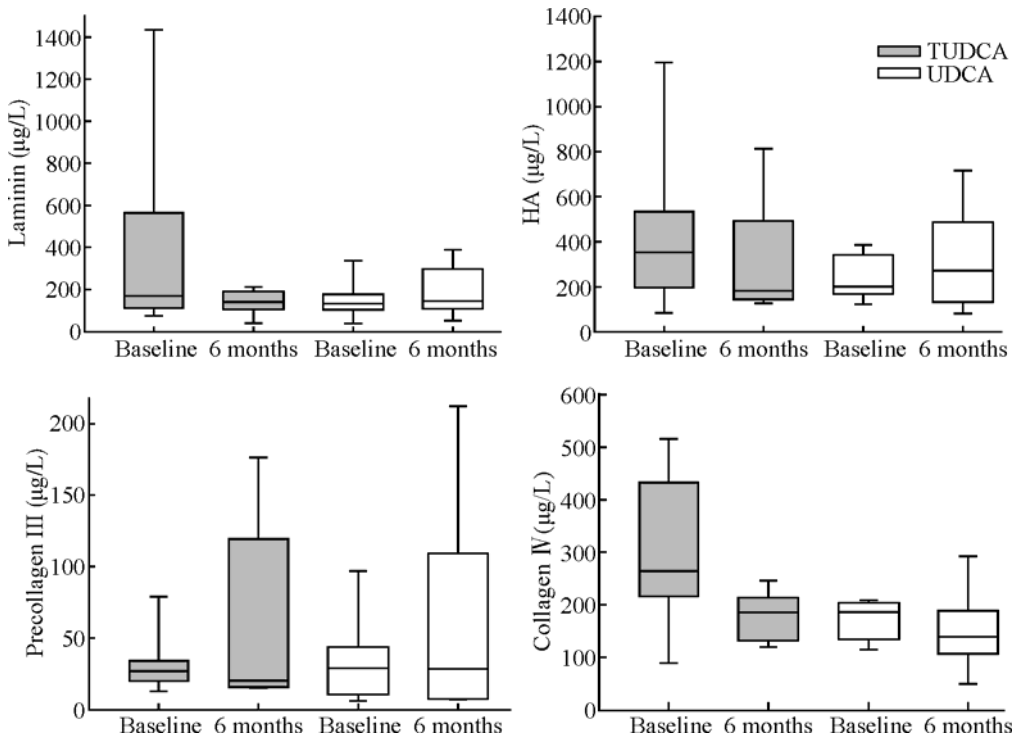

Fig. 2 Changes in serum markers for hepatic fibrosis including laminin, HA, precollagen Ⅲ and collagen Ⅳ before and after the administration of both TUDCA and UDCA
Box plots delineate values as median (bold horizontal line), 75% CI (box), and minimum and maximum values (whiskers). No significant differences were shown in both groups.

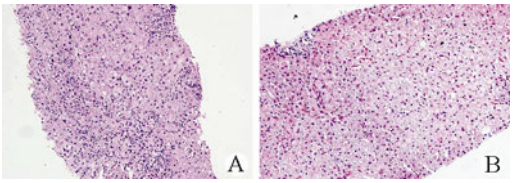

Fig. 3 The significant histological changes before and after the administration of TUDCA in one patient (HE, ×200)
A: before the treatment of TUDCA; B: six months after treatment with TUDCA

3 DISCUSSION

In the present study, we have compared for the first time the effects of TUDCA and UDCA in patients with liver cirrhosis in China. Our results suggest that TUDCA treatment significantly improves serum ALT, AST and ALP concentrations in patients with cirrhosis. These results confirm and extend the findings of Cagliaris *et al*[12], that administration of TUDCA improves biochemical parameters of cytolysis and cholestasis, and helps to maintain clinical and functional stability during the wait in cirrhotic patients on transplantation waiting lists. The exact mechanisms by which TUDCA improves the serum biochemistries of cirrhosis are not fully elucidated. Recent study showed that it can promote secretin-stimulated hydrocholeresis in rats through Ae2, microtubules, intracellular Ca^{2+}, PKCα, PI3K, PKA and MEK[13]. Besides its protection from disruptive effects of endogenous bile salt, TUDCA has its cytoprotective actions against the intrinsic and extrinsic apoptotic pathways and the endoplasmic reticulum stress responded from direct actions on mitochondrial and endoplasmic reticulum membranes through transcriptional regulation of both death and survival factors[14-16]. Although we didn't measure serum gamma-globulin or IgM in the study, we found a slight but significant increase in the levels of serum albumin in both TUDCA and UDCA groups. The increase in serum albumin may be related in part with the relief of liver function. On the other hand, UDCA is known to decrease HLA antigen expression and interleukin-2 production, and alter natural killer cell activity *in vivo* and *in vitro*[17, 18]. Recent data have also shown a continued decrease in IgM level during UDCA treatment of PBC for more than 10 years[19]. We speculate that the increase in serum albumin might be the result of reduced immune globulin, and TUDCA may have an immunomodulating action as UDCA.

In our patients, serum markers of liver fibrosis were not affected by either TUDCA or UDCA. Both drugs failed to show the beneficial effect on biochemical expression of liver fibrosis after treatment for 6 months. Although no such effect had been adequately studied in human before, both drugs had shown the anti-fibrosis effect in animal studies. TUDCA proved to prevent carbon tetrachloride-induced liver fibrosis in rats by reducing TGF-β1 synthesis, inhibiting hepatic stellate cell activation and decreasing extracellular matrix synthesis[20]. TUDCA also protected hepatocytes against tumor necrosis factor-induced cell death by replenishing mitochondrial glutathione[21]. Moreover, UDCA deters development of immune-mediated liver fibrosis by inhibiting the expression of collagen and other extracellular matrix components in rats[22]. Similar to our study, Voumvouraki *et al* reported recently that UDCA practically had no ef-

fect on the serum markers of hyaluronan, leptin, laminin and collagen Ⅳ in the 6-month treatment of PBC[23]. Considering that 6-month treatment is not sufficient to avoid a possible interference of the natural course of the disease in the results, long-time large scale trials are anticipated to explain the inconsistency between human and animal studies.

In addition, we have found that TUDCA treatment significantly improves serum ALT, AST, ALP and albumin concentrations, and reaches the primary end point more effective than UDCA. Among patients who undertook percutaneous liver biopsy, only one patient in the TUDCA group had significantly histological relief from G2S3 to G2S1. Consequently, our results indicate that TUDCA appears to be more efficient than UDCA in the treatment of liver cirrhosis. Moreover, our results extend the previous pilot crossover study that both treatments have equal efficacy in patients with PBC[24]. However, pathological change in tissue fibrosis is a long process, so long-time observation can give more accurate information.

Furthermore, long-term administration of TUDCA at the dosage used in this study did not cause significant side effects such as diarrhea. None of the patients withdrew because of drug intolerance. All the biochemical parameters of coagulation function, renal function and blood routine were not deteriorated in TUDCA and UDCA groups during the study, indicating the amount of TUDCA taken did not affect these biochemical parameters. Thus, like UDCA, TUDCA appears to have a favorable safety profile.

We are aware that our study has limitations. First, the sample size is clearly small to demonstrate the superiority of the TUDCA treatment to the UDCA treatment. Second, the consistency of fibrosis within the liver of PBC patients is limited, rendering the sampling error more probable for this disease. Despite these limitations, the greater reduction in serum parameters of liver function during TUDCA therapy in the short time suggests its potential efficacy in treatment of various liver diseases.

In conclusion, our study demonstrates that TUDCA therapy is safe and appears more effective than UDCA in the treatment of liver cirrhosis, especially in the improvement of the biochemical expression. However, both drugs show no effect on serum markers for fibrosis during 6-month treatment. Prospective multicentre studies are needed to confirm our preliminary results in order to define the protective role of TUDCA therapy and to avoid possible bias due to small study populations. Future efforts will focus on further elucidation of mechanisms of anti-fibrosis and immunomodulating action of TUDCA at the molecular level, as well as on definition of additional clinical uses of TUDCA.

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(Received May 23, 2012)