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Ursodeoxycholic acid alleviates cholestasis-induced histophysiological alterations in the male reproductive system of bile duct-ligated rats

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ABSTRACT

Ursodeoxycholic acid is the most widely used drug for treating cholestatic liver diseases. However, its effect on the male reproductive system alterations associated with cholestasis has never been studied. Thus, this study aimed to investigate the effect of ursodeoxycholic acid on cholestasis-induced alterations in the male reproductive system. Cholestasis was induced by bile duct ligation. Bile duct-ligated rats had higher cholestasis biomarkers and lower levels of testosterone, LH and FSH than did the Sham rats. They also had lower reproductive organs weights, and lower sperm motility, density and normal morphology than those of Sham rats. Histologically, these animals suffered from testicular tubular atrophy, interstitial edema, thickening of basement membranes, vacuolation, and depletion of germ cells. After ursodeoxycholic acid administration, cholestasis-induced structural and functional alterations were significantly ameliorated. In conclusion, ursodeoxycholic acid can ameliorate the reproductive complications of chronic cholestasis in male patients, which represents an additional benefit to this drug.

1. Introduction

Cholestasis is a liver disorder that arises from imbalanced bile production and excretion. Such impairment leads to intrahepatic accumulation of toxic bile acids, resulting in hepatocytic necrosis, and, eventually, cirrhosis [1]. Cholestasis also adversely affects other organs and causes serious clinical complications. High incidence of hypogonadism has been reported in patients with chronic cholestasis [2].

Ursodeoxycholic acid (UDCA, ursodiol; 3a,7b-dihydroxy-5b-cholanic acid) is a naturally occurring bile acid derived from Chinese black bears [3]. It is the most widely used therapeutic agent for treating cholestatic liver diseases, and is the only FDA-approved drug to treat primary biliary cirrhosis [4]. UDCA is also used to ameliorate other hepatopathies [5–7], and even extrahepatic ones [8–12]. Experimental and clinical data suggests several mechanisms of action for the therapeutic effects of UDCA in cholestatic disorders. Firstly, UDCA modifies the circulating bile acid composition and increases its hydrophilicity, rendering it easily eliminated [3]. It also stimulates the impaired hepatocellular and ductular secretions by increasing the expression of hepatobiliary transporters [3]. Moreover, UDCA exerts cytoprotection against the potentially membranolytic detergent effect of retained endogenous bile acids by stabilizing hepatocytic membranes [13], and inhibiting apoptosis [14]. In addition, immunomodulatory effects of UDCA have been also described [15].

Despite this cumulative evidence of its beneficial effects, the role of UDCA in alleviating the male reproductive system histophysiological alterations associated with long-standing cholestasis remains to be elucidated. Therefore, the present study was undertaken to investigate the potential of UDCA to prevent the development of hypogonadism, and to restore normal reproductive structure and function in rats with chronic cholestasis induced by permanent common bile duct ligation.

2. Materials and methods

2.1. Drugs and chemicals

Ursodeoxycholic acid (Ursofalk®) was purchased from Minapharm (Cairo, Egypt). UDCA was prepared in 0.1 ml of 2.5% sodium bicarbonate on the day of treatment. Diethyl ether was

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supplied by SDFCL (India). All other chemicals and reagents were obtained from Sigma Chemical Company (St. Louis, Mo), unless otherwise specified. All solutions were prepared immediately before use.

2.2. Animal experiments

Adult male Wistar albino rats (*Rattus norvegicus*), weighing 120–140 g, were obtained from the Veterinary Serum and Vaccine Research Institute (Cairo, Egypt). The animals were housed in suitable cages and acclimatized to laboratory conditions for a period of 1 week before the commencement of the experiments. The animals were reared in polypropylene cages and bedded on autoclaved wood shavings. A temperature of 26 ± 2 °C and 12 h light/dark cycle was maintained. The animals were given free access to water and standard rodent food pellets (Agricultural-Industrial Integration Company). All the animals were humanely treated in accordance with the WHO guidelines for animal care, and the experimental protocol was approved by the Ain Shams University Research Ethics Committee.

2.3. Bile duct ligation

Bile duct ligation and sham operation were performed as described previously [16]. In brief, the animals were anaesthetized and maintained throughout surgery using diethyl ether. Under sterile conditions, a midline abdominal incision was made and the common bile duct was exposed. In BDL rats, the bile duct was double-ligated with 4-0 silk, then excised between the ligatures; whereas sham-operated rats had their common bile duct exposed and manipulated but not ligated. Finally, the abdominal incision was closed in layers, and the animals were returned to their cages to recover.

2.4. Experimental groups

Animals were randomly assigned to three groups each of seven rats as follows: Sham Group: rats were subjected to sham operation and served as controls; BDL Group: rats were subjected to bile duct ligation and excision; and UDCA Group: BDL rats were treated with 100 mg/kg of Ursodeoxycholic acid daily via orogastric gavage [17]. UDCA administration started on the second day after BDL and was continued until study completion after four weeks. Sham and BDL groups received the vehicle in a similar manner.

2.5. Sample collection

Four weeks after surgery, the rats were again anesthetized and weighed. Blood samples were collected via retro-orbital puncture and centrifuged, and the supernatent serum was collected. Serum samples were divided into 200 μl aliquotes and frozen at −80 °C until being assayed for bilirubin, liver enzymes, and sex hormone levels. After necropsy, the liver and reproductive organs were immediately excised, cleaned of the adhering connective tissue, weighed, examined for gross lesions, and then processed for histological assessments. The left testis was frozen for later determination of intratesticular testosterone. The left cauda epididymis was excised and placed inside 2 ml of Modified Tyrode's medium (MT6) (125 mM NaCl, 2.7 mM KCl, 0.5 mM $MgCl_2{\cdot}6H_2O$, 0.36 mM $NaH_2PO_4{\cdot}2H_2O$, 5.56 mM glucose, 25 mM $NaHCO_4$, 1.80 mM $CaCl_2$, 100 units penicillin and 4 mg/ml BSA) in 35 °C for later determination of sperm parameters. The penis was amputated after the removal of the skin and subcutaneous tissue for the determination of nitric oxide level.

2.6. Sperm motility

The left cauda epididymis was minced with small scissors in a 35-mm Petri dish containing 2 ml of MT6 medium and left for 15 min at 35 °C for sperm release. An aliquot of sperms in MT6 medium was layered onto clean, grease-free microscopic slides and percentage motility was evaluated using a light microscope at a magnification of 400×. Motility estimates were performed from three different fields in each sample. The mean of the three estimations was used as the final motility score. Samples for motility evaluation were kept at 35 °C [18].

2.7. Sperm count

An aliquot of sperms in MT6 medium was filtered through gauze. Filtered samples were diluted with MT6 medium and infused into a Neubauer-type haemocytometer for microscopic examination. The sperms were counted in the four corner squares and the central square ignoring the cells on the upper and right square boundaries. The data were expressed as the number of cauda sperms/ml [18].

2.8. Sperm morphology

Sperm morphology was determined with the same samples used for sperm motility study. The smears were air-dried then fixed in methanol. After fixation, the samples were stained with 1% aqueous Eosin-Y solution for 1 h, washed with distilled water, passed through neutral resin, and mounted with coverslips [19]. Two hundred spermatozoa from each sample were evaluated, using a light microscope at 1000× magnification with an immersion objective lens. Sperm abnormalities were recorded as percentages of the total number of counted spermatozoa.

2.9. Histopathological examination

After weighing, the median lobe of liver, the right testis, and the right epididymis were fixed in Bouin, dehydrated in ascending series of ethanol, cleared in terpineol, and embedded in paraffin. Paraffin sections, 5 μm thick, were stained with hematoxylin and eosin. Liver sections were assessed for necrosis, bile duct proliferation and fibrosis. The stage of fibrosis was evaluated according to METAVIR scoring system [20]. Fibrosis was staged on a scale of 0–4: F0: no fibrosis, F1: portal fibrosis without septa, F2: few septa, F3: numerous septa without cirrhosis, and F4: cirrhosis.

For each testis sample, 30 round tubular profiles were evaluated for histopathological changes. Testis capsular thickness, tubular diameter, and germinal epithelium height were measured at 400× magnification using an ocular micrometer calibrated with a stage micrometer. Additionally, the state of spermatogenesis was semi-quantitatively evaluated using Johnsen's testicular score system [21]. Each tubular section was given a score from 10 to 1 according to presence or absence of the main cell types arranged in the order of maturity. Presence of spermatozoa scores 10, 9 or 8; spermatids (and no further) 7 or 6; spermatocytes (and no further) 5 or 4; only spermatogonia 3, only Sertoli cells 2, and no cells 1.

2.10. Liver functions

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined as described previously [22] using kits obtained from Biodiagnostics Company (Dokki, Egypt). Serum total and conjugated bilirubin levels were determined according to Walter and Gerade colorimetric method [23] using kits from Biodiagnostics Company (Dokki, Egypt).