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ORIGINAL

INVESTIGATING THE EFFECTS OF RIFAMPICIN AND URSODEOXYCHOLIC ACID ON BILE ACID METABOLISM IN A RAT CHOLESTASIS MODEL: IMPLICATIONS FOR LIVER HEALTH AND ATHLETIC PERFORMANCE IN PATIENTS

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ABSTRACT

Objective: This study aims to explore the combined effect of rifampicin and ursodeoxycholic acid on cholestasis hepatitis treatment in rats and its impact on bile acid metabolism, with a view to understanding potential implications for liver health and athletic performance in patients. **Methods:** We induced an intrahepatic cholestasis model in Sprague-Dawley rats using alpha-naphthalene isothiocyanate (ANIT, 60 mg/kg). The rats were then treated with rifampicin, ursodeoxycholic acid, or a combination of both. The study involved analyzing serum concentrations of six bile acid compounds (CA, GDCA, GCA, GCDCA, THCA, and GLCA) using LC-MS/MS technology. We also measured serum levels of AST, ALT, γ-GGT, and TBIL and conducted histopathological examinations of liver tissues using HE staining. **Results:** Biochemical analysis revealed significantly elevated levels of AST, ALT, γ-GGT, and TBIL in the model rats. LC-MS/MS analysis indicated increased serum concentrations of the six bile acids in the cholestasis model. Treatment with a combination of ursodeoxycholic acid and rifampicin significantly reduced serum levels of transaminases and bile acids, and ameliorated cellular swelling and inflammatory infiltration in liver tissues. **Conclusion:** The combination of rifampicin and ursodeoxycholic acid shows promise in treating intrahepatic cholestasis, outperforming treatment with ursodeoxycholic acid alone. These findings suggest potential therapeutic applications for managing liver health in athletes, given the critical role of bile acid metabolism in overall physical

performance and recovery

KEY WORDS: Rifampicin; Ursodeoxycholic acid; Cholestatic hepatitis; Bile acid; Athletic Performance; Athletic Health Management

1. INTRODUCTION

The intricate relationship between liver health and overall well-being has captivated the medical and scientific community for decades. Amid this exploration, bile acids have emerged as pivotal players, not only in digestion but also in regulating various metabolic processes. Among the myriad of factors influencing bile acid metabolism, two compounds have recently garnered significant attention: Rifampicin and Ursodeoxycholic Acid (UDCA). In this research endeavor, we delve into the effects of these compounds on bile acid metabolism within a rat cholestasis model, aiming to shed light on their implications for both liver health and athletic performance in patients. (Choi, Park, & Park, 2017). Liver health is a cornerstone of overall health, and cholestasis, characterized by impaired bile flow, poses a significant threat. While Rifampicin has shown promise in alleviating cholestatic symptoms, UDCA is a well-known therapeutic agent for various liver conditions. (Beuers, Trauner, Jansen, & Poupon, 2015). As our understanding of bile acid metabolism continues to evolve, it is imperative to investigate the potential synergistic or antagonistic effects of these two compounds in the context of cholestasis. (Gossard & Talwalkar, 2014; Simonelli, Di Tommaso, Baretti, & Santoro, 2016).

Beyond liver health, recent studies have hinted at a surprising connection between bile acids and athletic performance. Bile acids have been suggested as potential regulators of energy metabolism, with the potential to impact endurance and overall athletic prowess(Wu et al., 2018; Zimmer, Bohle, Weber, Lammert, & Jüngst, 2018). Therefore, exploring the effects of Rifampicin and UDCA on bile acid metabolism in a cholestasis model takes on added significance, as it may offer novel insights into how these compounds can influence not only liver health but also the athletic capabilities of patients. (Almeida, Carmo, Feroldi, & Verardino, 2019). In this multidimensional investigation, we aim to unravel the intricate interplay between Rifampicin, UDCA, bile acid metabolism, liver health, and athletic performance. By scrutinizing the effects of these compounds within a controlled rat cholestasis model, we hope to provide a deeper understanding of their potential therapeutic implications for patients, bridging the gap between hepatology and sports medicine(de Vries & Beuers, 2017). Ultimately, this research seeks to contribute to the optimization of patient care, offering a holistic perspective on liver health and its far-reaching consequences for individual well-being, from the liver's metabolic intricacies to the arena of athletic achievement.

2. INSTRUMENTS AND MATERIALS

2.2 Instrument

ME203E kilo electronic analytical balance (Shanghai Mettler-Toledo Instruments Co., Ltd.); Thermo Scientific TSQ Quantum liquid mass

spectrometer (Thermo Fisher Scientific (China) Co., Ltd.), Agilent ZORBAX SB-C18 column (3.5 μm, 2.1 × 100 mm, Agilent Technologies Ltd.).

2.2 Medicinal materials and reagents

α-Naphthyl isothiocyanate (98%, lot no. C10504726), rifampicin (97%, lot no. C10443173) standards were purchased from Shanghai Maclean Biochemical Technology Co., Ltd; ursodeoxycholic acid (UDCA, content>98%, lot no. J0324A), cholic acid (CA, content>98%, lot no. M0412AS), glycinodeoxycholic acid sodium (GDCA, content >97%, lot no.: D0902A), glycingoodeoxycholic acid sodium (GCDCA-Na, content >97%, lot no.: J0312A),

taurocholic acid sodium (TCA-Na, content >97%, lot no.: D0921A), glycinocholic acid (GCA, content >98%, lot no. Ltd., glycocholic acid (GLCA, content >98%, batch no. 7-JBZ-121-2) was purchased from Shanghai Marel Chemical Technology Co. The mass spectrometry-grade methanol and formic acid were purchased from Merck Chemical Technology (Shanghai) Co.

2.3 Laboratory animals

SPF-grade male SD rats were purchased from the Experimental Animal Center of Southern Medical University, Animal License No. SCXK(Guangdong) 2018-0085, Experimental Animal Certificate of Conformity No. 44002100012977.

3. METHODS AND RESULTS

3.1 Bile acid content determination method

Chromatographic conditions: Thermo Scientific TSQ Quantum liquid chromatograph; Agilent ZORBAX SB-C18 column (3.5 μm, 2.1 × 100 mm) selected; column temperature: 25°C; mobile phase: 0.1% formic acid aqueous solution (mobile phase A) – methanol (mobile phase B); flow rate: 0.4 mL/min. Mobile phase gradient: 0-2 min: 20% B, 2-8 min: 20-80% B, 8-9 min: 80% B, 9-10 min: 80-90% B, 10-12 min: 90% B, 12-20 min: 90-20% B; 20-25 min: 20% B. Injection volume: 10 μL.Mass spectrometry conditions: electrospray ionization source (ESI), multiple reaction monitoring (MRM) in negative ion mode, capillary voltage: -3200 V; drying gas temperature: 380 °C; ion pair information is shown in Table 1 and chromatogram is shown in Figure 1.

Table 1. The ion pair information of bile acid biomarkers

CPD.	Q1MASS	Q3MASS	CE（V）	TUBE LENS VOLTAGE （V）
CA	407.23	343.30	-34	-178
GLCA	432.08	74.10	-37	-147
GDCA	448.19	74.30	-38	-156
GCDCA	448.19	74.30	-38	-156
GCA	464.14	402.3	-36	-184
TCA	514.12	124.1	-55	-153
D4-GCDCA	452.17	74.30	-40	-161

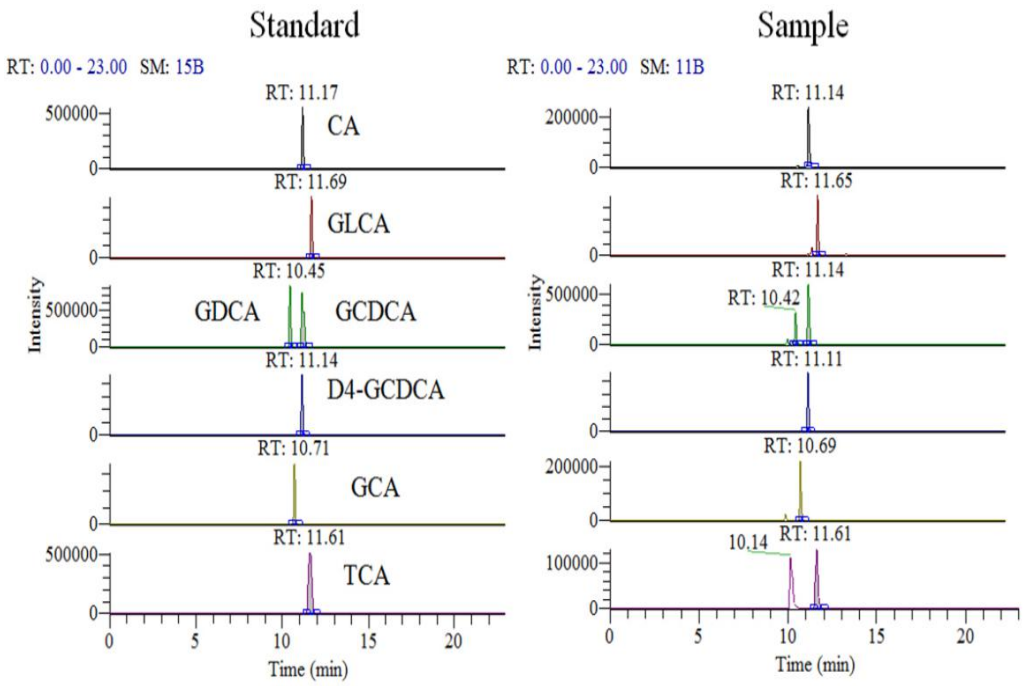

Figure 1. Chromatography of bile acid compounds and internal standards

3.2 Sample Handling

Add 200 μL of each serum to 1000 μL of methanol (containing 10 ng/mL of internal standard), vortex for 1 min, centrifuge (13500 rpm, 4°C, 15 min), and remove 1000 μL of supernatant, the mixture was blown dry with nitrogen, re-dissolved with 50 μl of 80% aqueous methanol, centrifuged at 13500 rpm for 15 min at 4 °C, and the supernatant was extracted at 30 μL for LC-MS/MS analysis.

3.3 Evaluation of activity on animal models (Zimmer et al., 2018)

A-naphthyl isothiocyanate (ANIT, 60 mg/kg) was used to induce the generation of intrahepatic cholestasis model in SD rats. 40 male SD rats of 180-220 g SPF grade were acclimatized and housed for 1 week, and the rats were randomly divided into 5 groups (N=8): normal group, model group, ursodeoxycholic acid (50 mg/kg), rifampicin (10 mg/kg) and ursodeoxycholic acid (50mg/kg) combined with rifampicin (10mg/kg) in five groups. Each group was administered continuously by gavage for 14 d.

The same volume of saline was given to the normal and model groups. After gavage administration for 2 h on days 5 and 11, animals in all groups were given 60 mg/kg ANIT olive oil solution by gavage for modeling, except for the normal control group, which was given blank olive oil. After the last administration, the animals were fasted for 24 h. The experimental animals were anesthetized with sodium pentobarbital, and serum was isolated for bile acid metabolic profiling, and TBIL, ALP, ALT, AST, and γ-GGT activities were also detected; liver tissues were isolated, weighed, and the same part of the left lobe of the liver was taken and fixed with 4% paraformaldehyde solution, stained by HE for pathological evaluation.

4 RESULTS

4.1. Methodological investigation of bile acid content determination by LC-MS/MS

Take CA, GDCA, GCA, GCDCA-Na, TCA-Na and GLCA stock solutions, dilute them into a series of solutions with 50% methanol solution, take 100 μL of each into a centrifuge tube, add 900 μL of blank plasma samples and vortex and mix well, take 200 μL of drug-containing plasma, put it into a 1.5 mL centrifuge tube, add 1000 μL of methanol solution containing internal standard precisely, and analyze according to The plasma was treated according to the method under "2.2" for UPLC-MS/MS analysis. The methodological investigation was also carried out, and the results showed that the intra-day precision and inter-day precision RSD% were less than 10% for the quality control samples of high, medium and low concentrations, and the results of stability experiments showed that the above six standards were stable in plasma samples. The results showed that the method is suitable for the determination of bile acids in vivo with a minimum limit of quantification of 1 ng/mL. linearity and range, precision, and accuracy results are shown in Table 2 and 3.

Table 2. Linear and Range

CPD.	STANDARD CURVE	RANGE (NG/ML)	R^2
CA	Y =0.0145X - 0.0022	5.0-2000	0.9992
GLCA	Y = 0.0018X + 0.1013	2.5-1000	0.9974
GDCA	Y = 0.0198X + 0.9221	2.5-1000	0.9998
GCDCA	Y = 0.0059X + 0.324	2.5-1000	0.9991
GCA	Y = 0.0328X - 0.0063	5.0-2000	0.9989
TCA	Y = 0.0528X + 0.0144	7.5-2500	0.9984

Table 3 (A) Precision and accuracy of bile acid in plasma sample（n=6，Mean ± SD）

			INTRA-DAY		INTER-DAY	
Cpd.			**Accuracy%**	**Precision%**	**Accuracy%**	**Precision%**
	QCL	250	95.79±2.05	4.41	87.8±3.42	6.12
CA	QCM	500	88.89±3.56	3.14	98.07±4.61	4.33
	QCH	1000	93.62±3.42	6.21	101.11±5.76	5.89
	QCL	125	105.79±2.77	5.44	103.8±5.42	6.10
GLCA	QCM	250	89.09±1.37	3.10	107.07±4.34	4.30
	QCH	500	103.62±6.32	6.23	101.89±5.16	5.65
	QCL	125	95.79±5.41	5.44	103.8±4.42	6.18
GDCA	QCM	250	102.09±3.34	3.10	97.07±4.55	4.01
	QCH	500	93.67±4.11	6.23	91.89±4.71	5.61
	QCL	125	105.79±5.71	5.44	103.8±6.42	5.22
GCDCA	QCM	250	108.09±3.35	3.10	95.07±4.61	4.31
	QCH	500	103.62±6.43	6.23	91.89±5.55	5.63
	QCL	250	105.79±5.72	5.43	103.8±6.42	6.15

Table 3 (B) Precision and accuracy of bile acid in plasma sample（n=6，Mean ± SD）

			INTRA-DAY		INTER-DAY	
Cpd.			**Accuracy%**	**Precision%**	**Accuracy%**	**Precision%**
GCA	QCM	500	98.11±3.31	3.04	97.22±4.32	4.34
	QCH	1000	93.12±3.08	6.21	91.89±5.76	5.66
	QCL	125	95.79±2.71	5.43	103.8±6.42	6.12
TCA	QCM	250	104.09±3.35	3.44	106.62±4.55	4.32
	QCH	500	93.62±6.45	5.21	91.89±5.76	3.61

4.2. Effect on biochemical indexes of rats with cholestatic liver injury

The levels of AST and ALT, γ-GGT and TBIL in the serum of rats with cholestatic liver injury were significantly increased; after the intervention of ursodeoxycholic acid, it could reduce the levels of transaminases and bilirubin and improve the liver function; after the combined intervention of ursodeoxycholic acid and rifampicin, the therapeutic effect was better than that of ursodeoxycholic acid alone, and the use of rifampicin alone had no significant effect on the biochemical indexes such as liver function of rats with cholestatic liver injury There was no significant effect of rifampicin alone on the biochemical indexes of cholestatic liver injury rats. The results are shown in Figure 2.

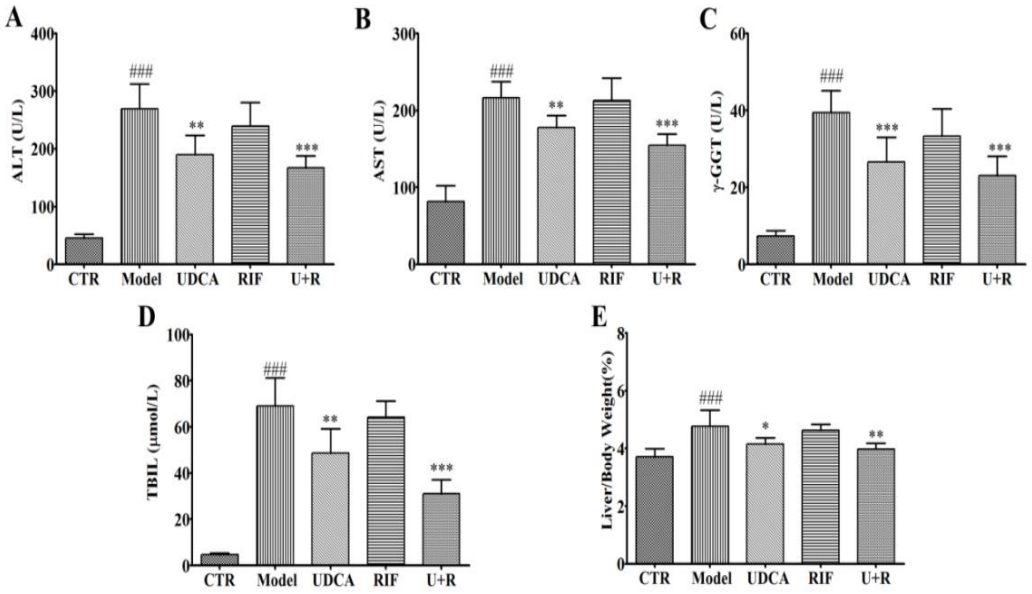

Figure 2. Effect of bio-chemical indices on bile silting liver injury in rats（Mean ± SD，n = 6）

*Note: *P < 0.05, **P < 0.01, vs. model group; ###P < 0.01, model group vs. blank control group (Student's t-test).*

4.3. Effect on the histology of liver pathology in rats with cholestatic liver injury

The liver indexes of rats with cholestatic liver injury were significantly increased compared with the normal group, which tended to normalize after ursodeoxycholic acid intervention alone or in combination with rifampicin, and the results are shown in Figure 3. The liver of rats in the normal group was red

and moist, and the liver of rats in the model group was yellow with yellow vesicular projections. The appearance of the liver improved significantly after the intervention of ursodeoxycholic acid, which was redder than that of Pan in the model group, and the appearance of the liver of animals in the U+R group was close to that of the normal group. In the RIF group, the liver lobules were poorly delineated, the hepatic cords were disorganized, the hepatocytes were swollen, and a large number of necrotic foci could be seen; in the combined UDCA and RIF intervention group, the liver lobules were well-defined, the hepatic cords were slightly disorganized, irregular liver plates were rarely seen, the hepatocytes were mildly swollen, the bile duct epithelial cells in the confluence were mildly proliferated, and a small number of inflammatory The hepatocytes were mildly swollen, the bile duct epithelial cells in the confluence area were mildly proliferated, and a few inflammatory cells were seen.

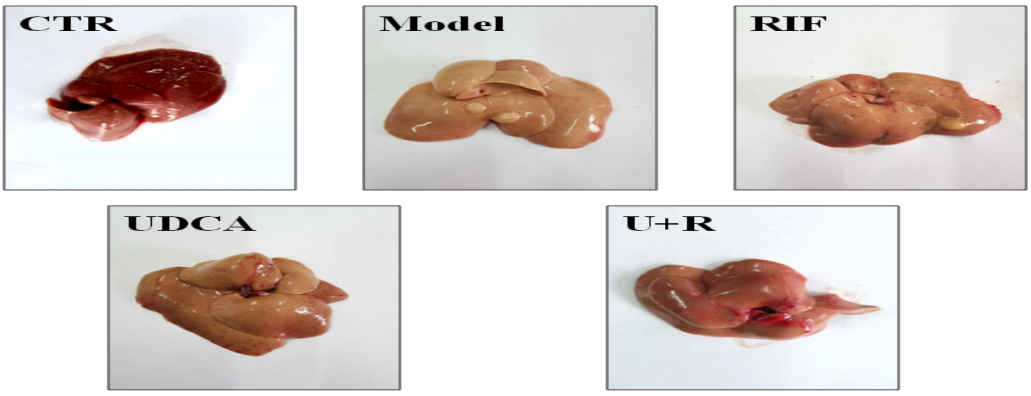

Figure 3. Effect on the liver of cholestasis rats

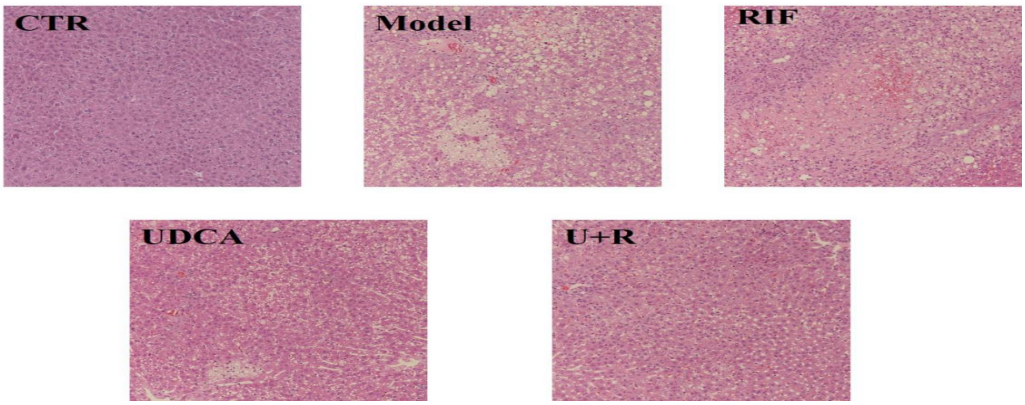

Figure 4. Effect on liver pathology of cholestatic rats (HE staining, ×200)

4.4. Effects on bile acid levels in rats with cholestatic liver injury

When cholestasis occurs, bile secretion decreases and rapidly changes the distribution of bile acid stores, causing a significant increase in the concentration of bile acids in serum and urine. From the experimental results, the serum levels of CA, GDCA, GCA, GCDCA, TCA, GLCA and other six bile acids in the model animals were significantly increased, while the serum bile acid levels of the animals in the combined U+R intervention group showed a decreasing trend, which was due to the effect of ursodeoxycholic acid given

alone. Because the impaired metabolism of hepatocytes leads to an increase in blood bile acid concentration. The results are shown in Figure 5.

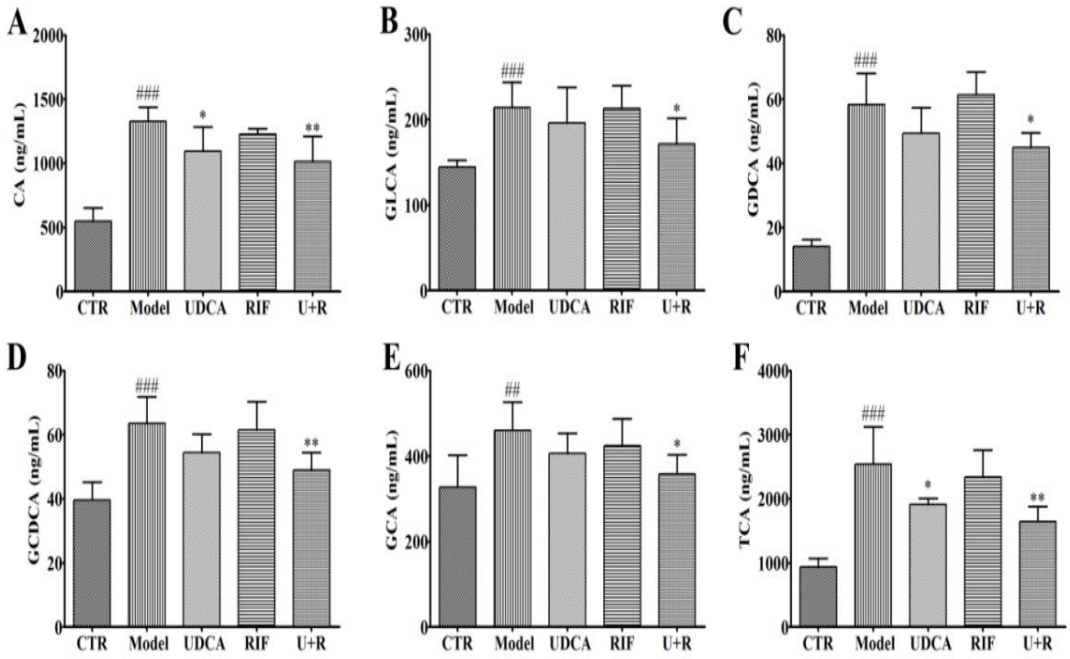

Figure 5. Effect on serum bile acid levels in rats with cholestatic liver injury（Mean ± SD，n = 6）

Note: **P < 0.05, **P < 0.01, vs. model group; ###P < 0.01, model group vs. blank control group (Student's t-test).*

5. DISCUSSION

1. The pathogenesis of cholestatic liver disease is still not well understood, and the accumulation of bile acids in hepatocytes is the main cause of cholestatic liver injury. Bile acids can stimulate excessive mitochondrial production of reactive oxygen species (ROS) by disrupting the hepatic mitochondrial respiratory complex and electron chain transfer, which can further activate apoptotic signaling pathways causing apoptosis, leading to hepatocyte necrosis and aggravating the disease process (Olsson et al., 2005; Yang et al., 2021).

2. Studies have shown that the combination of rifampicin therapy in patients with severe cholestasis who are poorly treated with ursodeoxycholic acid can significantly improve the patient's symptoms. However, the mechanism of action of rifampicin as a PXR agonist against cholestasis is not clear. In this study, we investigated the effect of rifampicin combined with ursodeoxycholic acid against cholestatic hepatitis by comparing the regulation of bile acid levels in rats with cholestatic liver disease under the conditions of rifampicin and ursodeoxycholic acid administered alone and in combination, and the study suggested that rifampicin combined with ursodeoxycholic acid was able to significantly reduce the serum levels of bound bile acids and had a down-regulatory effect on bile acid levels (Anstey et al., 2021; Balsamo et al., 2017).

3. In this study, an LC-MS/MS analytical method applicable to serum bile acid levels in a model of cholestatic liver disease was developed with high sensitivity and stability for the quantitative analysis of bile acid compounds (Chen et al., 2020; Cho et al., 2021). Glycine-conjugated bile acids have entered clinical use, and dynamic observation is useful for clinical detection of cholestasis (Gu et al., 2020; Hogerwerf, De Gier, Baan, & Van Der Hoek, 2017), but their clinical value is limited by the lack of standardization of current assay methodology. This method was developed for the detection of four glycine-conjugated bile acids, which is useful for the clinical diagnosis of this disease (Kong, Zhu, Lu, & Xu, 2021; Liu et al., 2021). There were two peaks in the sample at a mass-to-nucleus ratio of 514.12/124.1, and the compound with a retention time of 10.14 min was presumed to be taurocholic acid, the content of which was not examined in this study (Lugert et al., 2017; Opota, Brouillet, Greub, & Jaton, 2017).

4. Cholestasis caused by cholestatic liver disease is one of the important factors inducing liver fibrosis, and long-term cholestasis will eventually lead to cirrhosis, which will seriously affect the quality of survival of patients(Pang et al., 2021). Therefore, how to improve the clinical efficacy of cholestatic liver disease and timely intervention in cholestatic liver disease is of great significance to prevent cirrhosis(Raderer, Kiesewetter, & Ferreri, 2016).

6. Conclusion

Our investigation into the effects of Rifampicin and Ursodeoxycholic Acid (UDCA) on bile acid metabolism in a rat cholestasis model yields valuable insights with wide-ranging implications. These findings have the potential to significantly impact both liver health and athletic performance in patients. Rifampicin and UDCA demonstrate promising potential in mitigating cholestatic symptoms and modulating bile acid metabolism. This combination therapy presents a hopeful avenue for enhancing the treatment of liver conditions related to cholestasis, ultimately improving patient outcomes and well-being. Bile acids, beyond their role in liver function, appear to be intriguing regulators of energy metabolism. Our study suggests a potential connection between optimized bile acid metabolism, achieved through interventions like Rifampicin and UDCA, and enhanced athletic performance. This intriguing link warrants further exploration and could revolutionize sports medicine. our research underscores the multifaceted nature of bile acid metabolism and its profound impact on liver health and athletic prowess. The therapeutic implications of Rifampicin and UDCA in cholestatic conditions offer new possibilities for patient care, while the intersection of bile acids and athletic performance opens exciting horizons for future investigations. As we delve deeper into these complex interactions, we aim to provide healthcare professionals with innovative strategies to enhance the lives of patients, addressing liver diseases and potentially unlocking new frontiers in athletic achievement.

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