UDC 615.038
https://www.doi.org/10.34907/JPQAI.2021.66.67.007

STUDY OF COMPARATIVE PHARMACOKINETICS AND BIOEQUIVALENCE OF URSODEOXYCHOLIC ACID MEDICATIONS

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One of the most common diseases of mankind is cholelithiasis (GSD). Currently, there is only one existing proven on various links of biliary lithogenesis – ursodeoxycholic acid (UDCA). Its therapeutic area is extensive and includes various diseases of the liver and biliary tract. To confirm the efficacy and safety of the medicine Ursolab, registered by EKOlab CJSC, the main active ingredient of which is ursodeoxycholic acid, the comparative pharmacokinetics and bioequivalence of this medicine and the medicine Ursofalk (Dr. Falk Pharma GmbH, Germany) was studies in 28 healthy volunteers.

Keywords: ursodeoxycholic acid (UDCA), bioequivalence, pharmacokinetics, Ursolab, liver and gall bladder diseases

According to the World Health Organization, more than 2 billion people worldwide suffer from liver diseases. In the CIS countries, from 500 thousand to 1 million medical care encounters related to liver pathology are registered annually. One of the most common diseases of mankind is cholelithiasis (GSD) that is a multifactorial and multi-stage disease of the hepatobiliary system, characterized by a certain clinical picture such as a violation of cholesterol and/or bilirubin metabolism with the formation of gallstones in the gallbladder (GB) and/or bile ducts [1]. Epidemiological data show that 10% of the world's population suffers from GSD and for every decade the number of patients increases by about 2 times [2–5]. At the same time, in developed countries, the number of patients with GSD is 10–40% of the adult population. In Russia, the number of patients with GSD is 5–20% [2,6]. Cholecystectomy, unfortunately, remains to this day the "gold standard" of GSD treatment [3,7,8].

Currently, there is only one substance with a proven effect on various links of biliary lithogenesis – ursodeoxycholic acid (UDCA) [9, 10], respectively, its use for dissolving cholesterol stones today is an alternative to cholecystectomy [11,12].

Ursodeoxycholic acid is a tertiary bile acid formed in hepatocytes and intestines. Unlike its predecessors – primary and secondary bile acids – this acid is hydrophilic and, therefore, non-toxic. The scope of its therapeutic use is very extensive and includes various diseases of the liver and biliary tract such as chronic active hepatitis with cholestatic syndrome, acute hepatitis, toxic liver lesions of various genesis, primary biliary cirrhosis of the liver, primary sclerosing cholangitis, biliary dyskinesia, etc.

Normally, the content of UDCA in human bile is no more than 5% of the total pool of bile acids;

cholic acid – 26–39%, deoxycholic – 16–33%, litocholic – 0.5–5%. When administered at a dose of 13–15 mg/kg per day (orally), the content of UDCA in bile approaches 50%, which makes it the main one among bile acids, and the content of toxic bile acids (cholic, deoxycholic, litocholic, etc.) decreases. The absence of toxicity of UDCA is explained by its higher polarity and, accordingly, hydrophilicity [13,14].

It was found that the cytoprotective effect of UDCA on cholangiocytes and hepatocytes is due to the prevention of the release of cytochrome C from mitochondria, which, in turn, blocks the activation of caspases and apoptosis (programmed cell death). UDCA, embedded in the hepatocyte membrane, improves the fluidity of the phospholipid bilayer, stabilizing the cell structure and protecting them from damage.

In addition, UDCA has an immunomodulatory effect, reducing the production of proinflammatory cytokines (interleukins 1, 2, 6, gamma interferon, etc.), the level of the immune complexes of IdM and autoantibodies, the expression of histocompatibility antigens on hepatocytes (HLA I and II classes), which, in turn, prevents the activation of cytotoxic T-lymphocytes, normalizes the ratio of CD4/CD8 immunocompetent cells and promotes the suppression of immunopathological reactions. Stimulating exocytosis in hepatocytes during cholestasis by activating Ca++-dependent alpha-protein kinase, UDCA reduces the concentration of bile acids (cholic, litocholic, deoxycholic, etc.) which are toxic to the liver cell.

UDCA inhibits the absorption of lipophilic bile acids in the intestine, induces bicarbonates-rich choleresis, which leads to increase in bile passage and stimulates the excretion of toxic bile acids through the intestine. Replacing nonpolar bile acids, UDCA forms nontoxic mixed micelles (liquid crystals with cholesterol molecules). By reducing the synthesis of cholesterol in the liver, its secretion into bile, as well as absorption in the intestine, UDCA reduces the lithogenicity of bile, reduces the cholato-cholesterol index, promotes the dissolution of cholesterol stones (macrolites) and prevents the formation of new crystals (microlites). UDCA is well absorbed in the small intestine and is almost completely bound to serum proteins. In the liver, UDCA is rapidly and actively conjugated with glycine, taurine, N-acetylglucosamine, glucuronic acid and sulfate, which specifies its low level in plasma. In conjugated form, UDCA is released into the bile, where its concentration specifies the effectiveness of the medicine [15].

The main active ingredient of the medicine Ursolab, to which this study is devoted, is ursodeoxycholic acid. Ursolab is a reproduced medicinal product. The equivalence of the reproduced medicine to the reference medicine is usually proved in the framework of a bioequivalence study, which demonstrates that both medicines have the same rate and degree of absorption [16]. Such studies are intended to confirm that the reproduced medicines have the same efficacy and safety as the reference medicine. In this work, bioequivalence was studied for the medicine Ursolab (manufactured by ECOlab CJSC) relative to the medicine Ursofalk (Dr. Falk Pharma GmbH, Germany).

The purpose of this study is to evaluate the pharmacokinetic parameters of bioequivalence of the studied medicine Ursolab, oral suspension 250 mg /5 ml (ECOlab CJSC, Russia), and the medicine Ursofalk, oral suspension 250 mg /5 ml (Dr. Falk Pharma GmbH, Germany), in healthy volunteers after an acute administration under fasting conditions.

MATERIALS AND METHODS

The study was planned as a randomized, open, comparative, cross-sectional, two-period bioequivalence study in healthy volunteers under fasting conditions.

The study determined the concentration of ursodeoxycholic acid in the blood plasma of volunteers after acute administration of 250 mg (1 dose-measuring cup of 5 ml) of each of the medicines under fasting conditions. Based on the data on the concentration of ursodeoxycholic acid in blood plasma, the pharmacokinetic parameters were calculated.

The volunteers, after signing a written form of informed consent, were examined at the clinical center of Clinic "Bessalar" LLC.

The volunteers included in the study were randomized into two groups in a ratio of 1:1. The study plan is presented in Table 1.

The study consisted of screening, two study periods and a "cleaning" period. The duration

Table 1

STUDY PLAN

Study stages/procedures	Screening	Study periods			Final evaluation
		I	"cleaning"	II	
Period duration (days)	up to 10	4	14	4	
Informed consent	X				
Inclusion criteria	X				
Exclusion criteria	X	X		X	
Demographic and anthropometric data	X				
Medical history	X				
Physical examination	X	X		X	X
Laboratory examination (clinical blood analysis, biochemical blood test, clinical urinalysis)	X			X	X
Serology (HIV blood test, syphilis, hepatitis B and C markers)	X				
Urine test for pregnancy	X	X		X	
Breath test for alcohol	X	X		X	
Urine test for narcotic drugs and psychotropic substances, psychoactive drugs	X	X		X	
hospitalization		X		X	
Blood pressure, heart rate, body temperature	X	X		X	X
ECG	X			X	X
Randomization		X			
Administration of the studied medicinal product or the reference medicinal product		X		X	
Blood sampling for PK		X		X	
Registration AEs (adverse events) or SAEs (serious adverse events)		X	X	X	X

of screening was up to 10 days. The duration of each period was 4 days, the "cleaning" period was 14 days. The total duration of the study for one volunteer was no more than 28 days.

The study was planned to include no more than 32 healthy volunteers. As a result, 31 healthy volunteers were included in the study, of which 28 volunteers underwent all screening procedures, were randomized and completed the study in accordance with the protocol

The studied medications were taken by 28 volunteers, 21 of them were men and 7 were women. The average age of the volunteers was 30.82±6.42 years (M±SD). The average height of volunteers was 175.29±6.33 cm. The average weight of volunteers was 72.05±10.13 kg.

Blood sampling to determine the concentration of ursodeoxycholic acid from a cubital catheter or by direct venipuncture was carried out during the periods of administration of the studied medicines according to the schedule: before administration of the medicine a sample of 12.0 h and a sample of 0 (15 minutes before administration of the medicine) will be taken, then in 0,25, 0,5, 0,75, 1,0, 1,5, 2,0, 2,5, 3,0, 3,5, 4,0, 5,0, 6,0, 8,0, 12,0, 24,0, 48,0 and 72.0 h.

Since ursodeoxycholic acid is an endogenous compound, the protocol provides for taking two blood samples 12 hours and immediately before administration of the medicine to assess the endogenous concentration. In this study, the duration of observation of the concentration of the active ingredient was 72 hours, since such a time interval overlaps 4 T1/2 for ursodeoxycholic acid from blood plasma, the value of the parameter T1/2 was about 13–14 hours.

Analytical procedures were carried out in a specialized analytical laboratory.

All blood plasma samples were subject to analytical study. To determine the concentration of ursodeoxycholic acid in blood plasma, a bioanalytical method was developed and validated using HPLC–MS/MS of the Agilent 1260 Infinity system with a mass-selective detector G6125B.

RESULTS AND DISCUSSION

Ursodeoxycholic acid was absorbed into the blood with a Tmax value of 1.5 [1.0; 2.125] hours (Me [Q25; Q75]) for the tested medicine Ursolab, oral suspension 250 mg / 5 ml (ECOlab CJSC, Russia), and 2.0 [1.0; 3.5] for the reference medicinal product Ursofalk, oral suspension 250 mg /5 ml (Dr. Falk Pharma GmbH, Germany). The average (Mean) maximum concentration of the studied medicines (Cmax) was 4184.59±1974.53 ng/ml (Mean±SD) for the tested medicinal product and 4025.77±1768.49 ng/ml for the reference medicinal product. The average AUC0-t for the tested medicinal product was 16070.7=7220.96 h·ng/ml and 14155.2=4273.44 h·ng/ml for the reference medicinal product. The average pharmacokinetic profiles of the studied medicinal product are shown in Fig. 1.

Safety parameters included physical and systemic examinations, measurements of basic vital signs, clinical laboratory tests and control of adverse events.

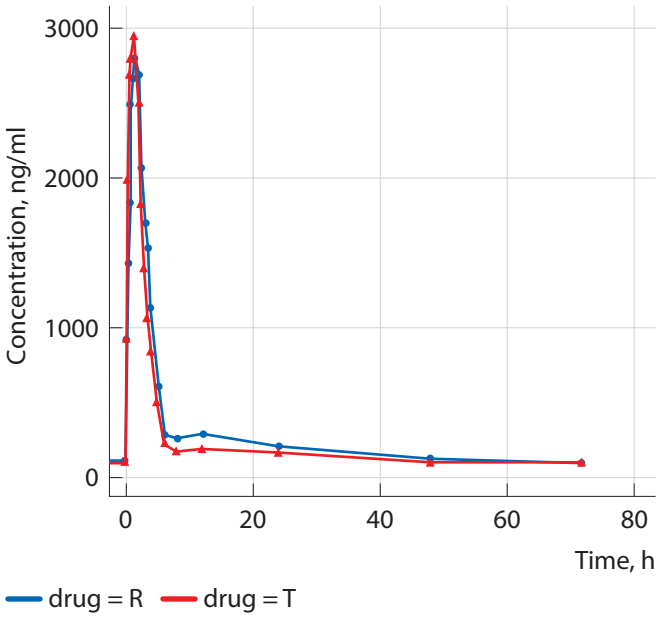

FIG. 1. *Averaged pharmacokinetic profiles: R – Ursofalk, oral suspension, 250 mg / 5 ml (Dr. Falk Pharma GmbH, Germany); T – Ursolab, oral suspension 250 mg /5 ml (ECOlab CJSC, Russia)*

When examined after the study at the end of the clinical part, none of the participants expressed any complaints and they were all physically healthy. The main vital signs of all participants did not change during the study. The vital functions of the body (blood pressure, heart rate) were assessed 12 hours before administration of the studied medicinal product or a reference medicinal product and 1,0, 2,0, 3,0, 4,0, 5,0, 6,0, 7,0, 8,0, 12,0, 24,0, 48,0 and 72.0 hours after administration of the studied medicinal product or a reference medicinal product. Thermometry was performed in the evening when a volunteer was admitted to the hospital at the beginning of the first study period.

During the registration of the main vital signs, each participant was asked about his/her health.

During the entire study, there were no clinically significant changes in the measured parameters, such as blood pressure, heart rate and body temperature.

The bioequivalence of the compared medicinal products was evaluated using an approach based on the assessment of 90% confidence intervals for the ratio of geometric averages for AUC0-t (correct) and Cmax (correct), where AUC0-t (correct) is the area under the pharmacokinetic curve "concentration – time", and Cmax (correct) is the value of the maximum concentration of ursodeoxycholic acid in blood plasma adjusted for the endogenous concentration of ursodeoxycholic acid.

The 90% confidence interval for the geometric mean ratios for the Cmax parameter was 88.49–119.37 (LSM T Geo/R Geo = 102.78), for the AUC0-t parameter 81.35–106.96 (LSM T Geo/R Geo = 93.28). The specified confidence intervals are within the limits of 80.00–125.00%. According to the protocol, the medicinal products are considered bioequivalent if the boundaries of the estimated confidence interval for AUC0-t and Cmax are in the range of 80.00–125.00%. In accordance with this, the tested medicinal product Ursolab, oral suspension 250 mg /5 ml (ECOlab CJSC, Russia), is recognized as bioequivalent to the reference medicinal product Ursofalk, oral suspension 250 mg /5 ml (Dr. Falk Pharma GmbH, Germany).

CONCLUSIONS

As part of the registration of the medicine "Ursolab", its bioequivalence relative to the reference medicine "Ursofalk" was studied with an acute administration by healthy volunteers under fasting conditions. Based on the data obtained, it can be affirmed that the studied medicines are characterized by a high degree of similarity in pharmacokinetics. The individual and averaged profiles of the pharmacokinetic curves of the studied and reference medicines have the same forms. The confidence intervals for the ratios of the geometric mean values of the estimated parameters of the studied and reference medicines fully correspond to the established limits.

Thus, the performed study allows us to state the bioequivalence of the medicine Ursolab, oral suspension 250 mg /5 ml (ECOlab CJSC, Russia), relative to the medicine Ursofalk, oral suspension 250 mg /5 ml (Dr. Falk Pharma GmbH, Germany).

REFERENCES

1. *Selezneva E.Ya., Bystrovskaya E.V., Orlova Yu.N. et al. Algorithm of diagnosis and treatment of gallstone disease // Russian Medical Journal. – 2015. – No. 13. – P. 730–737.*
2. *Ivashkin V.T. Diseases of the liver and biliary tract: guidelines for Physicians. – M.: M-Vesti, 2002. – 416 p.*
3. *Ilchenko A.A. Cholelithiasis. – M.: Anacharsis, 2004. – 200 p.*
4. *Simanenkov V.I., Sablin O.A., Lutaenko E.A., Ilchishina T.A. The possibilities of using ursodeoxycholic acid (medicine Urdoxa) for biliary*

dyskinesia // Gastroenterology of St. Petersburg. – 2010. – No. 2–3. – P. 23–26.

5. *Simanenkov V.I., Lutaenko E.A. Methodological recommendations for the use of ursodeoxycholic acid (Urdoxa) in patients with biliary dyskinesia. Available by: http://medznate.ru/docs/index-35443.html.*
6. *Drapkina O.M. Diseases of the biliary tract: new methods of prevention and treatment // Effective pharmacotherapy. – 2011. – No. 9. – P. 44–49.*
7. *Petukhov V.A. Cholelithiasis and the syndrome of impaired digestion. – M.: Vedi, 2003. – 128 p.*
8. *8. Skvortsova T.E. Litolytic therapy with Ursofalk in patients with cholelithiasis and its effect on the state of the hepatobiliary system and intestinal microbiocenosis // Gastroenterology of St. Petersburg. – 2009. – No. 4. – P. 11–13.*
9. *Minushkin O.N. Ursodeoxycholic acid in gastroenterology // Effective pharmacotherapy in gastroenterology. – 2008. – No. 2. – P. 18–24.*
10. *Minushkin O.N., Elizavetina G.A., Ivanova O.I., Shaposhnikova O.F. Ursodeoxycholic acid in the treatment of patients with biliary sludge // Effective pharmacotherapy. Gastroenterology. – 2012. – No. 3. – P. 10–12.*
11. *Roma M.G., Toledo F.D., Boaglio A.C., et al. Ursodeoxycholic acid in cholestasis: linking action mechanisms to therapeutic applications // Clin. Sci. (Lond). – 2011. – No. 21. – P. 523–544.*
12. *Portincasa P., Ciaula A.D., Bonfrate L., Wang D.Q. Therapy of gallstone disease: What it was, what it is, what it will be // World J. Gastrointest. Pharmacol. Ther. – 2012. – № 3. – P. 7–20.*
13. *Bouscarel B., Nussbaum R., Dubner H., Fromm H. The role of sodium in the uptake of ursodeoxycholic acid in isolated hamster hepatocytes // Hepatology. – 1995. – № 21(1). – P. 145–154.*
14. *Day D., Meyer D., Johnson S., Weisbrode S., Thudium D., Rhodes D. Evaluation of total serum bile acids concentration and bile acid profiles in healthy cats after oral administration of ursodeoxycholic acid // Am.J. Vet. Res. – 1994. – № 55(10). – P. 1474–8.*
15. *Raicht R., Cohen B., Sarwal A., Takahasln M. Ursodeoxycholic acid. Effects on sterol metabolism in rats // Biochim. Biophys. Acta. – 1978. – № 531(1). – P. 1–8.*
16. *Ushkalova E.A., Zyryanov S.K., Ushkalova A.V. Reproduced medicinal products and features of their regulation // Neurology, neuropsychiatry, psychosomatics. – 2016. – № 8(3). – P. 82–87.*

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