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CONCENTRATIONS OF SODIUM 3A, 7A--DIHYDROXY-12-OXO 5B CHOLANATE IN BIOLOGICAL MATERIAL AFTER ITS INTRAVENOUS AND INTRANASAL APPLICATION

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SUMMARY

Newly synthetized derivative of bile acid, sodium salt of 3α, 7α-dihydroxy-12-oxo 5β cholanic acid (monoketocholanate) expressed a good characteristic as intranasal transport enhancer of xenobiotics.The aim of our sudy was to explore if it has an influence on bile metabolism and to measure its concentration in blood and bile after intravenous and intranasal administration.

The experiment was performed in vivo on adult male Wistar rats. The determination of monoketocholanate (MKCh) in rats blood and bile, was carried out by high-performance liquid chromatography (HPLC), on an HP ODS2 column, using methanol/acetonitrile/acetate buffer as mobile phase. Absorbances were measured at 210 nm.Blood samples were taken from the prepared right axillary artery in 0, 1, 5, 10, 20, 30, 60, 90, 120 and 180 minutes from the beginning of the experiment. Bile was collected in a half an hour intervals,during the three hour period. The results showed that MKCh changed the amount of excreted bile depending on the way of application. Intranasal application increased the bile volume and the MKCh concentration, both in blood and bile compared to the intravenous application ($p<0.05$).

Distributionm of MKCh through animal organism depends on the way of application of the substance, which probably determines its caracterisation as the transport promotor of applied xenobiotics. HPLC has proved as aa relatively simple, fast and effective method for the determination of synthetic bile acid,MKCh in these biological materials.

key words: bile acids and salts, HPLC method, intranasal and intravenous administration, rats

INTRODUCTION

Bile acids and their salts have found many applications in medicine, agriculture and pharmacy [1, 2, 3]. They are very attractive for substance transport researches, according to their chemical properties and detergent-like action [4]. Some scientists have reported that bile acids can be used in the monitoring of the phylogenetic origin of vertebrates [5]. As far as chemical structure is concerned, keto (oxo) derivatives of natural bile acids have been detected as metabolites, named "tertiary bile acids". As is it known, the hydroxy derivative of cholanoic acid is found only in human bile and some amounts of cholanoic acid metabolite is found only in human feces [6]. The main intermediates in the process of reduction of cholic to chenodeoxycholic acid are 3¤,7¤-dihydroxy-12-keto-5¤ cholanoic acid and its esters [7]. Monoketocholanate, its sodium salt, has been synthesized from 3¤,7¤-trihydroxy-12-keto-5¤ cholanoic acid [8]. This salt appeared to be an effective promoter of intranasal resorption of insulin [9, 10, 11], as well as salicylates or morphine transport through the brain endothelial cells [12, 13].

The aim of this work was to examine the possible influence of MKCh on bile secretion by measuring the amount of excreted bile. Another challenge was to determine, for the first time, the MKCh concentration in blood and bile, after intravenous and intranasal applications of MKCh in experimental rats..

STUDY OBJECTIVE

The aim of this work was to examine the possible influence of MKCh on bile secretion by measuring the amount of excreted bile. Another challenge was to determine, for the first time, the MKCh concentration in

blood and bile, after intravenous and intranasal applications of MKCh in experimental rats.

MATHERIAL AND METHODS

Experiments were carried out in vivo on white Wistar male rats (body weight 200-300 g) in three hour time interval. The animals had free access to water and food(with a 12-hour succession of light and dark periods), and then starved for eight hours prior to the experiment. Experiment described in study, complied with ethical principles according to standards of Good Laboratory Practice.

The doses of administered substances were calculated on the basis of animal's body weight.

MKCh was used in the form of its sodium salt (cholanate), which was synthesized in the Department of Chemistry, Faculty of Sciences Novi Sad. Substance was applied intravenously or intranasally to experimental animals, in the dose of 4 mg/kg b.w. Concentrations of MKCh were measured on a Hewlett-Packard 1090, Series II HPLC instrument, using an HP ODS2 (10 cm·2.1 mm) 5 μm column, whith methanol/acetonitrile/buffer (pH 7.4) as mobile phase, and a flow rate of 1.0 mL/min.

Experimental groups

Animals were divided into the following groups:

- Control groups - animals received intravenously or intranasally physiological solution and blood was taken after that. Blood samples were taken from the prepared right axillary artery in 0, 1, 5, 10, 20, 30, 60, 90, 120 and in 180 minutes from the beginning of experiment;
- Test groups - animals received 4 mg/kg b.w. of MKCh intravenously (MKCh-iv) or intranasally (MKCh-in) and MKCh concentration was measured in blood at ten time points
- Control groups - animals received physiological solution intravenously or intranasally and bile was collected after that in six time intervals (0-30 min, 31-60 min. 61-90 min, 91-120 min 121-150 min and 151-180 min);
- Test groups received 4 mg/kg b.w. of MKCh intravenously (MKCh-iv) or intranasally (MKCh-in) and bile was measured in six time intervals

Methods

Rats were previously anesthetized by intraperitoneally injected urethane (0.75 mg/kg b.w.)

MKCh solution was applied to rats through the prepared left jugular vein (2.0 mL/kg) or intranasally (0.2 mL/kg). A blood volume of 0.15 mL, was taken with a micropipette, then centrifuged and prepared for the high-performance liquid chromatography (HPLC) experiment, following the procedure developed in the course of this work. The MKCh kinetics in rat's blood after applying it intranasally through the left nostril, was monitored by collecting blood at the determined time intervals.

Bile concentrations of MKCh in the course of time were measured in bile, also after the intravenous (iv) and intranasal (in) application. Cannula for bile collecting was inserted to the immobilized ductus choledohus. Then, MKCh solution was injected intravenously or intranasally during 10 s. The excreted bile was collected in 30-min intervals for 180 min. After measuring a total amount of collected bile, 20 μL of the liquid were taken and prepared for the further biochemical analysis, with 40 μL of acetonitrile.

MKCh absorbances were measured on HPLC at 210 nm. Separation of MKCh in the animal bile lasted 15 min. Quantification was carried out by a computerized procedure of measuring the area under the peak and their comparison with MKCh standards of different concentrations.

Statistical analysis

The data analysis included mean values with standard deviations, Area under the curve (AUC) of MKCh in blood and bile, percentages (%) of excreted MKCh in biological materials. All data were analyzed by the Student's T-test, Analysis of variance (ANOVA) and the probabilities of less than 5% were considered statistically significant.

RESULTS

The very first chromatographic records of monoketocholanate in blood is presented in figure 1 and its presence in bile is given in figure 2.

The results obtained by measuring the volumes of secreted bile in control and threated groups in the course of three hours are presented in the Graf 1.

In the case of intravenous application, the bile volume was reduced by 50% already in the 60th minute ($p<0.01$), and it was significantly lower in all time intervals, according to initial time ($p<0.05$). Compared to controls volume of excreted bile was also much lower, but significantly only in the last measured period ($p<0.05$). On the other hand, the intranasal application of MKCh resulted in an increased bile volume, according to controls and iv application. It was statistically significant beginning from the 60th to 120th minute and in the last measured period ($p<0.05$) compared to i.v. administration, but not compared to controls. Also, the value of AUC bile volume for MKChin was higher than AUC of MKChiv in three hours observing time ($p=0.05$).

The results of measuring time changes of MKCh concentration in blood samples are presented in Graph 2.

As can be seen, there was a statistically very significant difference in MKCh concentration after its intravenous and intranasal application. In the first 10 min, the MKCh-iv concentration was much higher ($p<0.001$), whereas in the 120th min the MKCh-in concentration exceeded the MKCh-iv value ($p<0.01$). The overall amount of excreted MKCh-iv was 248.61 μg and it was significantly higher ($p<0.01$) compared to the excreted concentration of MKCh-in (70.58 μg). However, by comparing the area under the curve (AUC), it can be seen that the difference was not statistically significant (Table 1).

Maximal MKCh concentration in bile was measured in all treated animals in first 30 min, (Graph 3).

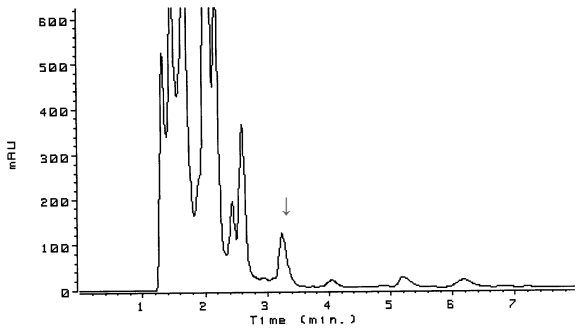


Figure 1. Chromatographic peaks of MKCh in blood after intranasal admistration of 4 mg/kg of MKCh

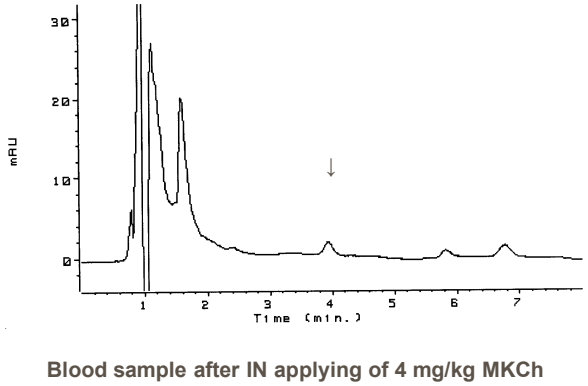
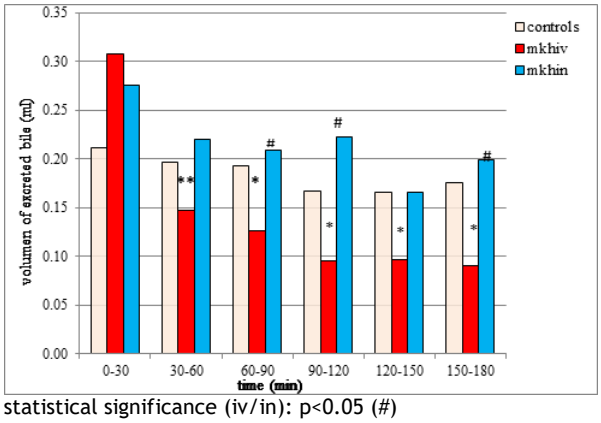
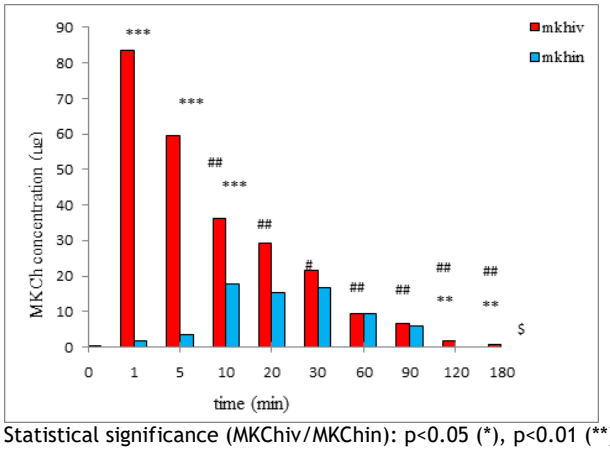


Figure 2. Chromatographic peaks of MKCh in bile after intravenous admistration of 4 mg/kg of MKCh



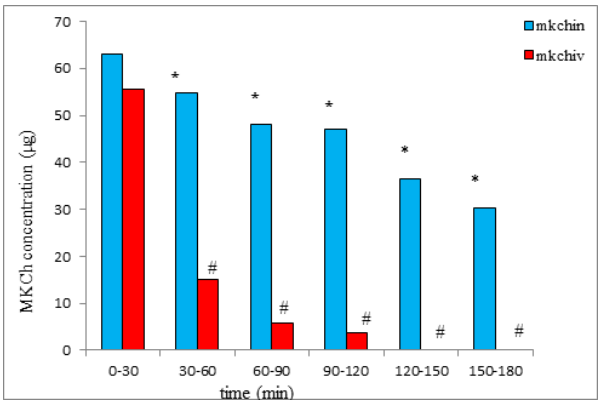
statistical significance (iv/in): p<0.05 (#)
t-test/0-30 min): p<0.05 (*); p<0.01 (**)

Graf 1.Volumes of excreted bile after intravenous (MKChiv) and intranasal (MKChin) application of MKCh



Statistical significance (MKChiv/MKChin): p<0.05 (*), p<0.01 (**), p<0,001(***)
Student T test/1min (MKChiv): p<0.05 (#), p<0.01 (##), p<0,001(###)
Student T test/1min (MKChin): p<0.05 ($)

Graph 2. Time changes of MKCh concentration in rats serum after intravenous ((MKChiv)) and intranasal (MKChin) application



Statistical significance of differences:
Student T-test (0-30 min): p<0,05 (#)
significance (iv:in): p<0,05 (*)

Graph 3. Time changes of MKCh concentration in rat bile after intravenous (MKChiv) and intranasal application

After 60 min, the MKCh-iv value dropped almost quadruple (p<0.05) and continued to fall until the end of the experiment. The presence of MKCh was not registered after 120th min. Compared to the initial period (0-30 min), it was determined a strong decrease of MKCh-iv (p<0.05), which was not in the case of intranasal application of MKCh.The MKCh-in concentration in rats' bile were higher than the MKCh-iv ones, in all measuring intervala and, except in the first and the last half an hour, it was statistically significant (p<0.05). Compared to the first collected period, MKCh-in concentration was, also, significantly higer in all time intervals, (p<0.05). Statistical significance between AUC of MKChiv and MKChin was noted (p<0.05) and presented in table 1.

There was no statistically significant difference between the groups in respect of the overall amount of applied substance(Table 2),nor were differences in the animal body weights (not presented). There is an evident difference (p<0.05) in the amount of excreted bile after intravenous compared to collected bileafter intranasal

Table 1. MKCh concentration after intravenous and intranasal administration of 4 mg / kg MKCh expressed as AUC in blood and bile within the 3 hours interval

	Area under the curve in rat serum		Area under the curve in rat bile	
Area under the curve	AUCiv (μg/mL*h)	AUCin(μg/mL*h)	AUCiv (μg/mL*h)	AUCin(μg/mL*h)
x±SD	34.29±8.53	26.09±19.94	26.19±21.09	116.47±65.94
StudentT test (AUCiv/AUCin)	p=0.47; p>0.05		p=0.017; p<0.05	

Table 2. Total amount of MKCh (%) secreted in bile after intravenous (MKChiv) and intranasal (MKChin) administration within 3 hours

	Total amount of MKCh applied to g)¤animals (	Total amount of MKCh excreted g)¤in bile (	% of MKCh in excreted bile
MKChiv	916±238.95	80.18±56.96	8.30±4.09
MKChin	902±67.4	279.62±150.88*	31.0±15,81*
Student T-test mkchiv:mkchin	p = 0.89 p>0,05	p = 0.022 p<0,05 (*)	p = 0.014 p<0,05 (*)

application of MKCh. Thus, the percent of the excreted MKCh after the intranasal application (30.05%) was more than a 3.5 times higher than the percent of the substance excreted after the intravenous application (8.3%).

Bile acids may increase the solubility of slightly soluble drugs[13].It might be of importance in view of the fact that bile acid derivatives have already beenused for the treatment of various diseaseand enhanced transport through biological barrieres[12,13, 14, 15, 16, 17, 18, 19].Knowing the kinetics of these synthetic derivatives can help in finding simple methods in the application of drugs. This could provide an efficient mode for future bile acid research and, at the same time, the medical praxis in prevention and therapy.

DISCUSSION

Nasal epithelium acts as a barrier for high molecular compounds such as desmopressin, insulin, human growth hormone, etc [14, 15]. On the other way, the intranasal route has been already known as noninvasive way of drug administration for systemic therapy [16]. Earlier studies confirmed the benefits associated with bile salts caracteristics to promote drug transport through intranasal routes [17, 18]. Monoketocholanate (MKCh) was synthetized by selective oxydation of cholic acid in several steps, in aim to obtain the sodium salt of 3α, 7α - dihydroxy-12 - oxo- 5β cholanic acid [8]. MKCh, investigated towards its pharmacokinetic and pharmacodynamic properties, was applied through the intravenous and intranasal route to experimental rats. The bile volume, expressed as area under the curve of total amount of excreted bile in observed period of three hours, had no statistical difference compared to controls for both ways of application (p>0.05). According to that result, we assumed that MKCh bioavailability is good for the intranasal administration.

However, it is not clear yet what is the reason of enchansing in bile secretion caused by intranasal application of MKCh. Compared to the first collected period, it was significantly higer in all time intervals. It also remains unclear what could be the possible pathways of distribution and metabolism of MKCh after its intranasal application, which yielded so high MKCh's concentration in the bile. We did not measure the concentration of MKCh in other biological material (feces for example), that could be eventually explaned the route of MKCh kinetics. One of the possible answers could be the interactions of the MKCh with the physiological environment based on its chemical properties (MKCh is the hydrophyllic bile salt). Obviously, the organism reacted to additional amount of bile acid and MkCh distribution took place via penetration to other compartments.

Bile acid derivatives have already been used for the treatment of various disease and enhanced transport through biological barrieres due to their physicochemical properties. They can influence on membrane fluidity, mucus viscosity or on enzyme limitation. They can inhibit the enzymatic activity in the membrane and thus improve the bioavailability of drugs. One of the important mechanism for improving nasal bioavailability lies in their ability to open the thight junction of nasal epithelium [24, 25, 26, 27].

There is an evidence that MKCh can promote the absorbtion of many substances [9-12, 28, 29, 30]. MKCh can affects membrane fluidity and improve passive difusion. Also, MKCh is less toxic than other bile salts [31]. Critical micelle concentrations (CMC) is one of the factors responsible for cytotoxic effects. Essentially, more hydrophobic bile salts cause hemolysis below their CMC [32,33,34]. Hydrophilic ones can cause hemolysis above their CMC. MKCh is supposed to induce hemolysis at a concentration higher than its CMC, due to position of keto group [30, 31]. So, it can be another benefit of using this bile acid derivative in promoting drug penetration through the nasal route.

Chemical transformations of bile acids and determination in biological materials have challenged researchers to pay attention on their biosynthesis or develop various analytical methods [19, 20, 21, 22, 23]. The HPLC method is quite simple and punctilious. It does not require too much time for sample preparation. HPLC is a very convenient for qualitative identification and precise quantitative determination of this originally synthesized bile acid in biological materials. As relatively inexpensive method, it might be commonly used for the determination of other novel synthetic bile acids and contribute to future research of bile acids.

CONCLUSION

Summary, knowledge of the kinetics of this and other synthetic derivatives can help in finding simpler methods in the application of physiologically active de-

rivatives, which may have a medical application. This could provide an effective way of investigating bile acids as well as their medical use in prevention and therapy. In addition, since intranasally given monoketocholate has greatly influenced the volume of the bile, it could be the basis for exploring potentially new cholagogues.

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This paper is the part of dissertation

REFERENCES

1. Zimber A, Gespach C. Bile acids and derivatives, their nuclear receptors fxr, pxr and ligands: role in health and disease and their therapeutic potential. Anti-Cancer Agent Med Chem 2008; 8: 540–63.
2. Cabezas G R. Effect of ursodeoxycholic acid combined with statins in hypercholesterolemia treatment: a prospective clinical trial. Rev Clin Esp 2004;204:632-5.
3. Hirai S, Yashiki T, Mima H. Mechanisms for the enhancement of the nasal absorption of insulin by surfactants. Int J Pharm 1981; 9:173-184
4. Cravotto G, Boffa L, Turello M, Parenti M, Barge A. Chemical modifications of bile acids under high-intensity ultrasound or microwave irradiation. Steroids 2005; 70: 77–83.
5. Russel D W.The enzymes, regulation and genetics of bile acid biosynthesis. Ann Rev Biochem 2003;72:137-74
6. Marion J. Sheltawy; M. S. Losowsky. Determination of faecal bile acids by an enzymic method. Clinica Chimica Acta, 1973; 64(2): 127-132
7. H Sobotka, and E Bloch. The Steroids. Annual Review of Biochemistry 1943 12:1, 45-80
8. Kuhajda K, Kantrač J, Mikov M, ur. Biosinteza žučnih kiselina u jetri sisara. Žučne kiseline, određivanje, hemijske transformacije, farmakološko dejstvo. Novi Sad; Ortomedics 1999;
9. Stević S, Stajić D, Stanojević Z, Bukumirić Z, Mikov M, Kuhajda K, Kevrešan S. Intranasal application of bile acids and insulin can affect blood glucose levels in rats. Medicina danas 2009; 8(1-3): 87-91
10. Mikov M, Boni NS, Al-Salami H, Kuhajda K, Kevresan S, Golocorbin-Kon S, Fawcwtt JP. Bioavailability and hypoglycemic activity of the semisynthetic bile acid salt, sodium 3alpha, 7alpha-dihydroxy-12-oxo-5beta-cholanate, in healthy and diabetic rats. Eur J Drug Metab Pharmacokinet 2007; 32(1): 7–12
11. Mikov M, Facwcett JP, Kuhajda K, et al. Pharmacology of bile acids and their derivates: Absorption promoters and therapeutic agents. Eur J drug Metabol Pharmacokinet 2006;31:237-251
12. Baščarević S, Vasović V, Mikov M, Kuhajda K, Kevrešan S.Salicylates transport into the rat brain under the influence of monoketocholic acid. Exp. Toxicol. Pathol. 1998;590(2):91
13. Chein YW, Su KS, Chang SF. Nasal systemic drug delivery. 2nd ed. New York: Dekker; 1989. p. 26.
14. Kissel T, Werner U. Nasal delivery of peptides: an in vitro cell culture model for the investigation of transport and metabolism in human nasal epithelium. J Control Rel 1998;53:195-203.
15. Ken-Ishi H, Hiroyuki K, Hideshi N, Kenji S, Yasunori M. Evaluation of enhancers to increase nasal absoption using chamber technique. Biol Pharm Bull 1994; 17(2): 316-22
16. Costantino HR, Illum L, Brandt G, Johnson PH, Quay SC. Intranasal delivery: Physicochemical and therapeutic aspects. Int. J. Pharm. 2007: 337:1-24.
17. Y.W. Chien. Transnasal Systemic Medications. 1st ed. New York: Elsevier Science Publishing Co. Inc; 1985. p. 100-6
18. Talegonkar S, Mishra PR. Intranasal delivery: An approach to by pass the blood brain barrier. Ind J Pharmacol 2004;36:140-7.
19. Meaney CM, O'Driscoll. CMA Comparison of the permeation enhancement potential of simple bile salt and mixed bile salt: fatty acid micellar systems using the CaCo-2 cell culture model. Int J Pharm 2000;207:21–30
20. Ma¨lkia¨ A, Murtoma¨ki L, Urtti A, et al. Drug permeation in biomembranes: in vitro and in silico prediction and influence of physicochemical properties. Eur J Pharm Sci 2004;23:13–47.
21. Coca E, Ribas B, Trigueros G. A method for quick determination of bile acids in bile of patients with biliary lithiasis. J Liquid Chromatogr 1994; 17: 1349-63

22. Jones ML, Chen H, Ouyang W, Metz T, Prakash S. Method for bile acid determination by high performance liquid chromatography. J Med Sci 2003; 23 (5): 277-80
23. Kuhajda K, Kandrac J, Kevresan S, Mikov M, Fawcett JP. Structure and origin of bile acids: An overview. In: Mikov M, Fawcett JP, editors. Chemistry,Biosynthesis, Analysis, Chemical and Metabolic Transformations and Pharmacology. Geneva:Mediset–Publishers; 2007. p. 17–31
24. Gordon GS, Moses AC, Silver RD, Flier JS Carey MC. Nasal absorption of insulin: Enhancement by hydrophobic bile salts. Proc Natl Acad Sci USA 1985; 82: 7419-23
25. Mithani SD, Bakatselou V, TenHoor CN, et al. Estimation of the increase in solubility of drugs as a function of bile salt concentration. Pharm Res 1996;13:163–7.
26. Kushwaha KS, Keshari RK, Rai AK, Advances in nasal trans-mucosal drug delivery, JAPS 2011;01 (07): 21-28.
27. Liu X, Testa B, Fahr A. Lipophilicity and its relationship with passive drug permeation. Pharm Res 2011;28:962–77.
28. Yang, L, Zhang H, Fawcett JP, Mikov M, Tucker I G. Effect of bile salts on the transport of morphine-6-glucuronide in rat brain endothelial cells. J Pharm Sci 2011; 100: 1516–24. doi: 10.1002/jps.22390
29. Al-Salami H, Butt G, Tucker I, et al. Influence of the semisynthetic bile acid MKC on the ileal permeation of gliclazide in healthy anddiabetic rats. Pharmacol Rep 2008;60:532–41.
30. Yang L, Zhang H, Mikov M, and Tucker IG. Physicochemical and biological characterization of monoketocholic acid, a novel permeability enhancer. Molecular Pharmaceutics 2009;6 (2): 448-456 DOI: 10.1021/mp800143w
31. Kuhajda KN, Cvjetićanin SM, Djurendić EA, Sakač MN, Penov Gaši KM, Kojić VV, Bogdanović GM. Synthesis and cytotoxic activity of a series of bile acid derivatives. Hem Ind 2009; 63 (4): 313–18
32. Rodriquez CM, Fan G, Wong PY, Kren BT, Steer CJ. A novel role for ursodeoxycholic acid in inhibiting apoptosis by modulating mitochondrial membrane perturbation. J Clin Invest 1998; 101(12): 2790-9.
33. Kim ND, Im EO, Choi YH, Yoo YH. Synthetic bile acids: novel mediators of apoptosis. J Biochem Mol Biol 2002; 35: 134–41
34. Carubbi, F.; Guicciardi, M. E.; Concari, M.; Loria, P.; Bertolotti, M.; Carulli, N. Comparative cytotoxic and cytoprotective effects of taurohyodeoxycholic acid (THDCA) and tauroursodeoxycholic acid (TUDCA) in HepG2 cell line. Biochim. Biophys. Acta 2002, 1580, 31–9

SRPSKI

KONCENTRACIJA NA 3 A, 7A--DIHIDROKSI-12-KETO 5B HOLANATA U BIOLOŠKOM MATERIJALU POSLE INTRAVENSKE I INTRANAZALNE APLIKACIJE

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SAŽETAK
Novosintetisani derivat žučne kiseline, natrijumova so 3α, 7α-dihidroksi 12 - keto 5ß holanske kiseline (monoketoholanat) ispoljio je osobinu da može da poveća transport intranazalno aplikoovanih ksenobiotika.
Cilj naše studije bio je da ispitamo da li ova supstanca utiče na metabolizam žuči, kao i da izmerimo njenu koncentraciju u krvi i u žuči posle intravenske i intranazalne aplikacije.
Eksperiment je urađen u uslovima in vivo, na odraslim pacovima soja Wistar, muškog pola. Koncentracije monoketoholanata (MKH) u krvi i u žuči eksperimentalnih životinja, određene su metodom tečne hromatografije viske rezolucije (HPLC) na koloni HP ODS2 i pokretnom fazom metanol/acetonitril/acetatni pufer. Ekstinkcije su merene na dužini od 210 nm. Uzorci krvi uzimani su iz preparisane desne pazušne arterije u 0, 1, 5, 10, 20, 30, 60, 90, 120 i u 180 minutu od početka eksperimenta. Žuč je sakupljana u polusatnim intervalima u trajanu od tri sata. Rezultati su pokazali da je MKH uticala na količinu izlučene žuči u zavisnosti od načina aplikacije. Intravenska aplikacija smanjila je volumen žuči, kao i koncentraciju MKH i u krvi i u žuči u odnosu na kontrolu. Kod intranazalnog davanja MKH, povećana je količina izlučene žuči i izmerena statistički značajno viša koncentracija monoketoholanata ($p<0,05$).
MKH se različito distribuira u organizmu u zavisnoti od načina na koji se aplikuje, što verovatno i određuje njene osobine kao promotora transporta aplikovanih ksenobiotika. HPLC metod se pokazao kao relativno jednostavan, brz i efikasan metod za određivanje MKH u ovom biološkom materijalu.
ključne reči: žučne kiseline i soli, HPLC metod, intravenska i intranazalna aplikacija, pacovi