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THE FEATURES OF BILE ACIDS EXCHANGE IN RATS UNDER THE INFLUENCE OF CORVITIN

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Corvitin is a soluble form of quercetin (QUE) and its effects are based on the ability to inhibit the activity of 5-lipoxygenase and to block the formation of leukotrienes. Corvitin increases bloodflow in the stomach, pancreas and liver, but its influence on the excretory liver function has not been studied. We investigated the effect of corvitin (2.5, 5, 10 mg/kg intraportally) on bile formation, determined the biliary content of total, free and conjugated bile acids (BAs). Free and conjugated BAs were separated by thin layer chromatography method. It was shown that corvitin increased the content of total BAs in the bile of rats in all tested groups. At a dose of 2.5 mg/kg flavonoid did not change free BAs secretion, but elevated the content of conjugated BAs. Both free and conjugated BAs secretion was increased in rats treated with corvitin at a dose of 5 mg/kg. Increasing of corvitin dose to 10 mg/kg resulted in enhanced secretion of free BAs. Consequently, inhibition of leukotrienes synthesis by corvitin is followed by modulation of total, free and conjugated BAs formation and secretion into the bile.

K e y w o r d s: corvitin, 5-lipoxigenase, leukotrienes, bile secretion, total BAs, free BAs, conjugated BAs.

Enzyme 5-lipoxygenase (5-LO) plays an essential role in the biosynthesis of leukotrienes (LTs). Leukotriene LTB4 is a potent chemotactic and chemokinetic agent for a variety of leukocytes, while cysteinyl leukotrienes C4, D4 and E4 regulate vascular permeability and smooth muscle contraction. These compounds have vasoconstrictor properties and cause prolonged increased pressure in large blood vessels and capillaries. Inhibitors of LT synthesis have been hypothesized to possess therapeutic potential for the treatment of asthma, allergic disorders and other diseases [1]. Physiological effects of LTs on the liver are not studied enough. It was shown that the addition of LTD4 to liver perfusate resulted in a decrease of portal blood flow, bile flow and bile acids (BAs) release [2, 3]. Specific blockers of 5-LO activity do not affect the synthesis of other arachidonic acid metabolites – prostaglandins and thromboxane [4].

Formation of bile by hepatocytes is an important way of removing potentially harmful exogenous lipophilic substances and endogenous compounds such as bilirubin and cholesterol [5]. Bile contains BAs, which are synthesized in the liver from cholesterol. These molecules ensure the stability of bile colloidal system, dispersion and absorption of dietary fats in the intestine, activation of pancreatic lipase, intensification of intestinal motility [6].

Plant polyphenol quercetin (QUE) attracts attention due to a wide range of positive effects on human health including hepatoprotective effect [7]. But its impact on bile formation and secretion has not been studied. It is known that QUE effects depend on applied doses, redox status of target cells and metabolites synthesis [8]. Despite the high effectiveness of QUE its bioavailability is very low. This complicates the investigation of QUE effects *in vivo*. In this study we used a water- soluble form of QUE corvitin at different doses. Corvitin is the inhibitor of 5-LO, it is natural compound without side effects, that has been used to treat cardiac disorders [9]. Previously we found that this flavonoid modulates gastric se-

cretory activity and tissue blood flow in the gastric mucosa, liver and pancreas [10-12].

The objective of the present study was to investigate the dynamics of BAs formation and secretion under the influence of corvitin as inhibitor of 5-LO.

Materials and Methods

This work was carried out in accordance with Declaration of Helsinki (World medical assembly, 1964), Declaration of Principles on Tolerance (28th session of UNESCO, 1995), Universal Declaration on Bioethics and Human Rights related to introduction of new biomedical technologies, accepted in 1997 in the city of Oviedo (Spain) and signed by parliament of Ukraine in 2002, Law of Ukraine No. 3447 IV "About Animals Protection from Brutal Behavior".

Study has been done in acute experiments on 24 linear, male, mature Wistar rats (obtained from the Institute of Pharmacology, Academy of Medical Sciences of Ukraine), weighing 200-240 g, after 18 h of food deprivation. Every rat was anesthetized with thiopentalum natrium (Ukraine, OAO "Kyivmedpreparat", 6 mg/100 g rat b.w.). Common bile duct was then cannulated with polyethylene catheter. The rats were injected intraportally with sodium chloride 0.9% (0.1 ml/100 g rat b.w.) in control group; with corvitin (2.5, 5, 10 mg/kg) (PJSC SIC "Borshchahivskiy CPP", Kyiv, Ukraine) in experimental groups. All treatments were performed after 30 min stabilization of bile flow level. Bile was collected every 30 min during 2.5 h of the experiment by micropipette connected to cannula located in the bile duct. Bile flow was calculated as µl bile per g rat body weight. Euthanasia was performed by dislocation of the cervical vertebrae.

BAs panel analysis. Free and conjugated BAs were separated by thin layer chromatography method that has been described by Veselskiy et al. [13]. For this purpose, 0.1 ml of bile was added to 1.9 ml of cold ethanol-acetone (1 : 3) mixture. Samples were kept cool (–10 to 0 °C) in an ice chamber for 25-30 min and then centrifugated for 10-12 min at 3000-4000 r.p.m. The extracts were then poured in conical glass test tubes and dried at 37-40 °C. Dry remainders were dissolved in 50-100 µl of ethanol-water mixture (6 : 4). Samples (5-10 µl) were applied on chromatography plates (15×15 cm silica gel plates (Kavalier, Czech). Free and conjugated BAs were separated in the system containing amyl ester acetic acid, toluole, butanole, acetic acid and water (3 : 1 : 1 : 3 : 1, respectively) in glass chromatography chambers. Chromatograms were dyed after five times sprinkling with the dye stuff (15 ml icy acetic acid, 1 g phosphomolybdic acid, 1 ml sulphuric acid and 5 ml of 50% trichloroacetic acid solution) and dried at 60-70 °C for 5 min. Quantitative determination of BA content was performed with the use of densitometer GP-920 (Shimadzu, Japan) under reflected light (λ 620 nm). This method allowed separating BAs mixture into following fractions: taurocholic acid (TCA), taurochenodeoxycholic acid + taurodeoxycholic acid (TCDCA + TDCA), glycocholic acid (GCA), glycochenodeoxycholic acid + glycodeoxycholic acid (GCDCA + GDCA), cholic acid (CA), chenodeoxycholic acid + deoxycholic acid (CDCA + DCA). BAs content was calculated as 1 mg per 1 g of rat body weight.

Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Newman-Keuls post hoc test. The data were presented as mean ± SEM. *P*-values equivalent to a signifcance level of 0.05 were considered statistically signifcant. Observed power (alpha 0.05) = 0.9. Value of partial eta-squared (η_p^2) was defined as the proportion of the effect + error variance that is attributable to the effect.

Results and Discussion

This is the first report on the effect of corvitin on the total production and secretion of BAs in rats. Earlier the attention of researchers was focused on the study of hepatoprotective properties of QUE at liver damage by various factors. In particular, it was found that this flavonoid protects hepatocytes from chronic ethanol toxicity by modulation of mitochondria function and liver enzymes activity [14]. To the best of our knowledge, there is no sufficient data regarding choleretic effect of purified QUE *in vivo*, however, it has been reported that herbal extracts containing this flavonoid stimulate bile secretion [15]. We identified earlier that corvitin evokes an increase of bile volume in rats: after injection of 2.5, 5 and 10 mg/kg flavonoid total bile volume was increased by 20.9% ($P < 0.01$), 30% ($P < 0.001$) and 20.4% ($P < 0.05$), respectively [16].

Influence of corvitin on total BAs secretion in rats. We found that corvitin at a dose 2.5 mg/kg increased BAs secretion in bile. The content of total bile acids (TBAs) in the 2nd, 3rd, 4th and 5th half-hour samples of bile was increased by 20.4%

($P < 0.05$), 29.1% ($P < 0.05$), 34.1% ($P < 0.001$), 38.2% ($P < 0.001$), respectively.

Corvitin at a dose of 5 mg/kg caused an increase in the content of TBAs in half-hour samples by 38.6% ($P < 0.01$), 46.8% ($P < 0.01$), 50.4% ($P < 0.001$), 56.2% ($P < 0.01$), respectively. At a dose of 10 mg/kg corvitin increased the level of TBAs in the 2nd, 3rd, 4th and 5th half-hour samples by 32.2% ($P < 0.05$), 23.1% ($P < 0.05$), 35.4% ($P < 0.01$) and 39.9% ($P < 0.01$), respectively. It is necessary to note that the content of TBAs secreted from the liver into the bile was higher in a long-term period of the experiment, indicating that corvitin amplified de novo synthesis of TBAs in the liver. The maximum increase in TBAs production was observed after treatment with 5 mg/kg corvitin (Fig. 1).

The data obtained show that under the influence of corvitin the biliary TBAs efflux in rats is increased. These results are consistent with the findings of Zhang M. et al. [17]. They found that after QUE injection the level of TBAs in rats bile was enhanced. Recently another group of researchers reported that QUE, isolated from black beans, activated TBAs secretion in mice liver [18].

Synthesis and excretion of BAs provide a direct means of converting cholesterol, which is both hydrophobic and insoluble, into a water-soluble and readily excreted molecule. This process is energy-dependent and requires energy in the form of ATP [19, 20]. A key enzyme of cholesterol conversion into BAs is cholesterol-7-α-hydroxylase (Cyp7a1) [6]. Zhang M. et al. [17] found that the activity of this hepatic enzyme was significantly increased by QUE. The authors demonstrated that QUE significantly increased the expression ATP binding cassette transporter G1 mRNA in the liver and could increase hepatic cholesterol efflux. On the other hand, there is evidence that QUE reduces the level of cholesterol and triacylglycerols in the blood [21]. In another study activated synthesis and altered composition of the BAs pool simultaneously with increased expression of hepatic Cyp7α1 were observed after inhibition of the ileal BAs transport [22].

The present results indicate that corvitin may increase cholesterol delivery from the bloodstream to the liver and its subsequent conversion into BAs by hepatocytes. We believe that this might be a pos-

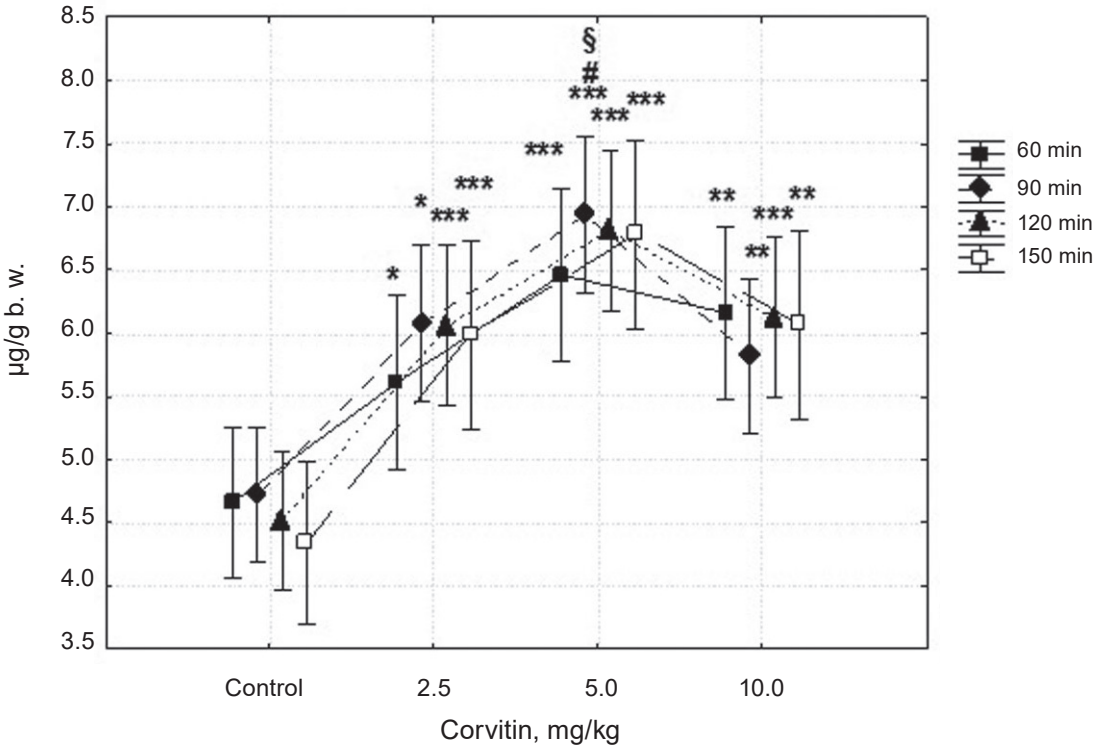

*Fig. 1. Effect of corvitin (2.5, 5 and 10 mg/kg) on TBAs content in bile. Secreted bile was collected each half-hour during 2.5 h of the experiment. Mean ± SEM; n = 6; *P < 0.05, **P < 0.01, ***P < 0.001 versus control group; #P < 0.05 corvitin 2.5 mg/kg versus corvitin 5 mg/kg; §P < 0.05 corvitin 5 mg/kg versus corvitin 10 mg/kg; observed power (at alpha 0.05) = 0.97; partial eta = 0.44*

sible mechanism for QUE induced correction of cholesterol level in the blood and hepatocytes.

Influence of corvitin on free BAs secretion in rats. The current study indicates that no significant changes in total production of free BAs were observed after rats treatment with corvitin at a dose of 2.5 mg/kg. The increase of this index under the influence of 5 mg/kg flavonoid was observed in the 4th and 5th half-hour samples by 28.1% ($P < 0.05$) and 30.4% ($P < 0.05$), respectively. At a dose of 10 mg/kg corvitin significantly increased secretion of total free BAs in the half-hour samples of bile from the 1st to 5th by 60.1% ($P < 0.001$), 54.9% ($P < 0.01$), 46.4% ($P < 0.01$), 51.9% ($P < 0.001$) and 60.4% ($P < 0.001$), respectively (Fig. 2).

Under physiological conditions, free BAs are not commonly found in human bile whereas in rats with no gallbladder they can be secreted in a small amount (near 10%) into the bile and transported to the intestine [23]. BAs biosynthesis occurs in hepatocytes and results in the formation of the so-called "primary BAs" such as CA (3α, 7α, 12α-trihydroxycholanic acid) and CDCA (3α, 7α-dihydroxycholanic acid) [6]. No changes in the level of biliary CA efflux were observed in rats treated with corvitin at a dose of 2.5 mg/kg. The total content of CDCA+DCA in this group of rats was increased only in the 3rd half-hour sample by 18.8% ($P < 0.05$). The increase of CA production was observed after corvitin injection at a dose of 5 mg/kg – by 22.7% ($P < 0.05$ and, 30% ($P < 0.05$) in the 4th and 5th half-hour samples, respectively. The total content of CDCA+DCA was increased in the 3rd, 4th and 5th half-hour samples by 20.5% ($P < 0.01$), 20.5% ($P < 0.05$) and 25% ($P < 0.01$), respectively (Table 1). The secretory response of the liver to corvitin treatment at a dose of 10 mg/kg was also significantly increased. Total secretion of CA was increased compared with the control by 23.9% ($P < 0.05$) in the 1st, by 20.4% ($P < 0.05$) in the 2nd, by 15.3% ($P < 0.05$) in the 3rd, by 20.7% ($P < 0.05$) in the 4th, by 36% ($P < 0.001$) in the 5th half-hour samples. After corvitin (10 mg/kg) treatment the secretion of CDCA+DCA into bile was enhanced in half-hour samples from 1st to 5th by

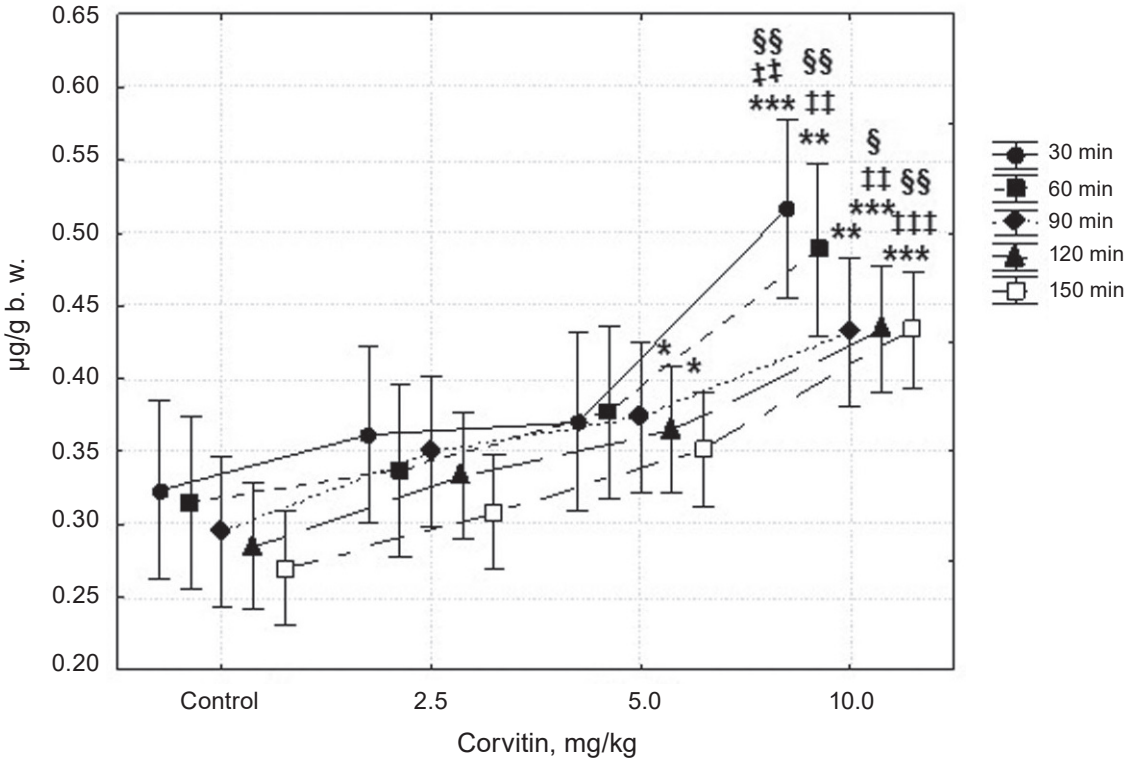

*Fig. 2. The content of total free BAs in bile under the effect of corvitin (2.5, 5 and 10 mg/kg). Mean ± SEM; n = 6; *P < 0.05, **P < 0.01, ***P < 0.001 versus control group; ‡‡P < 0.01, ‡‡‡P < 0.001 corvitin 2.5 mg/kg versus corvitin 10 mg/kg; §P < 0.05, §§P < 0.01 corvitin 5 mg/kg versus corvitin 10 mg/kg; observed power (at alpha 0.06) = 0.99; partial eta = 0.52*

Table 1. The content of free BAs (μg/g b.w.) in half-hour samples of bile collected in rats treated by corvitin (2.5, 5 and 10 mg/kg)

Animal group	Sample	Cholic acid, μg/g b.w.	Chenodeoxycholic acid + deoxycholic acid, μg/g b.w.
Control	1	0.24 ± 0.01	0.090 ± 0.003
Corvitin 2.5 mg/kg		0.26 ± 0.02	0.100 ± 0.007
Corvitin 5 mg/kg		0.24 ± 0.02	0.100 ± 0.004
Corvitin 10 mg/kg		0.30 ± 0.03*	0.180 ± 0.015***
Control	2	0.23 ± 0.01	0.090 ± 0.005
Corvitin 2.5 mg/kg		0.24 ± 0.02	0.090 ± 0.003
Corvitin 5 mg/kg		0.24 ± 0.01	0.100 ± 0.004
Corvitin 10 mg/kg		0.28 ± 0.04*	0.16 ± 0.15***
Control	3	0.22 ± 0.01	0.083 ± 0.005
Corvitin 2.5 mg/kg		0.25 ± 0.02	0.100 ± 0.004
Corvitin 5 mg/kg		0.25 ± 0.02	0.100 ± 0.006**
Corvitin 10 mg/kg		0.25 ± 0.03*	0.15 ± 0.01***
Control	4	0.22 ± 0.01	0.083 ± 0.005
Corvitin 2.5 mg/kg		0.25 ± 0.02	0.090 ± 0.003
Corvitin 5 mg/kg		0.27 ± 0.01*	0.100 ± 0.002*
Corvitin 10 mg/kg		0.26 ± 0.03*	0.14 ± 0.01***
Control	5	0.20 ± 0.02	0.080 ± 0.005
Corvitin 2.5 mg/kg		0.25 ± 0.01	0.090 ± 0.005
Corvitin 5 mg/kg		0.26 ± 0.01*	0.100 ± 0.003**
Corvitin 10 mg/kg		0.27 ± 0.02**	0.140 ± 0.008***

Statistically signifcant difference *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

94.4% ($P < 0.001$), 77.8% ($P < 0.001$), 80.7% ($P < 0.001$), 68.7% ($P < 0.001$) and 75% ($P < 0.001$), respectively (Table 1).

The biochemical analysis of the bile demonstrated that under the effect of flavonoid the main fraction in the increased content of total free cholates was CDCA+DCA.

At a dose of 10 mg/kg, flavonoid significantly increased the level of free BAs in bile, especially CDCA+DCA (about 65%), reducing the concentration of a conjugated BAs and conjugation ratio [16]. This result indicates a significant activation by corvitin (10 mg/kg) of alternative (acidic) way of primary BAs synthesis from cholesterol, which in humans provides a synthesis of 18% of BAs, whereas in rodents – up to 50% of those. Depending on the dose, the tested drug may differently affect the efficiency of polyenzyme systems which provide biosynthesis and conjugation of BAs in rats.

Effect of corvitin on total conjugated BAs content. The application of corvitin (at all tested doses) also increased the biliary secretion of total taurine- and glycine-conjugated BAs in comparison with control data. In the rats treated with 2.5 mg/kg flavonoid, we observed an augmentation of conjugated BAs production in the half-hour samples of bile from 3rd to 5th by 34% ($P < 0.001$), 33.6% ($P < 0.01$), 37% ($P < 0.01$), respectively. At a dose of 5 mg/kg the tested drug increased the bile secretion of the total conjugated cholates by 28.4% ($P < 0.05$), 60.9% ($P < 0.001$), 58.6% ($P < 0.001$), 64.9% ($P < 0.001$) in the 1st, 3rd, 4th and 5th samples, respectively. In response to 10 mg/kg corvitin, the secretion of total conjugated BAs exceeded the control data in the 2nd half-hour sample by 55.9% ($P < 0.05$), in the 3rd half-hour one by 31.2% ($P < 0.001$), in the 4th half-hour by 41.5% ($P < 0.001$), in the 5th half-hour by 47.3% ($P < 0.001$) compared with control group

(Fig. 3). Among all groups, the secretory effect of corvitin was maximal at a dose of 5 mg/kg (Fig. 3). The main components in the increased content of total conjugated cholates in bile were taurine-conjugated BAs. It should be noted that the prevalence of taurine-conjugated BAs over glycine-conjugated BAs is thought to be a rats' distinctive feature, which was confirmed in our study. The production of TCA under the influence of 2.5 mg/kg corvitin was signifcantly increased versus control data in half-hour samples of bile from the 2nd to 5th by 23% ($P < 0.01$), 34.8% ($P < 0.001$), 35.5% ($P < 0.001$) and 41.4% ($P < 0.001$), respectively. Corvitin (5 mg/kg) enhanced secretion of TCA in bile compared with the control level by 30% ($P < 0.05$), 45% ($P < 0.001$), 56% ($P < 0.001$), 54.9% ($P < 0.001$), 65% ($P < 0.001$) in the 1st, 2nd, 3rd, 4th, 5th half-hour samples, respectively. At a dose of 10 mg/kg the drug significantly increased the TCA content versus control only in the 3rd, 4th, and 5th half-hour samples of bile by 23.4% ($P < 0.05$), 25.1% ($P < 0.01$) and 34% ($P < 0.01$), respectively (Table 2). Corvitin-treated rats exhibited an increase in biliary TCDCA+TDCA efflux during the experiment. Increased level of TCDCA+TDCA in response to 2.5 mg/kg corvitin was observed in the 2nd, 3rd, 4th, 5th half-hour samples by 21.9% ($P < 0.05$), 71.4% ($P < 0.001$), 55.8 % ($P < 0.05$), 49% ($P < 0.05$), respectively as compared with the results in the control group (Table 1). At a dose of 5 mg/kg corvitin, this index exceeded that in the control group by 51.5% ($P < 0.01$), 82% ($P < 0.001$), 120.6% ($P < 0.001$), 112.5% ($P < 0.001$), 119.2% ($P < 0.001$) in the samples from 1st to 5th, respectively. Corvitin administration at a dose of 10 mg/kg also resulted in the increase in TCDCA+TDCA content in the bile by 20.1% ($P < 0.05$), 30.9% ($P < 0.05$), 31.4% ($P < 0.05$) and 41.3% ($P < 0.05$) in half-hour samples from 2nd to 5th, respectively. The quantity of GCA in rats bile was not changed under the influence of 2.5 and 5.0 mg/kg corvitin. Treatment with flavonoid at a dose of 10 mg/kg caused a slight decrease in GCA production in the half-hours samples from 2nd to 5th by 22.2% ($P < 0.05$), by 20% ($P < 0.05$), by 21.2% ($P < 0.05$) and by 26.4% ($P < 0.01$), respectively. The

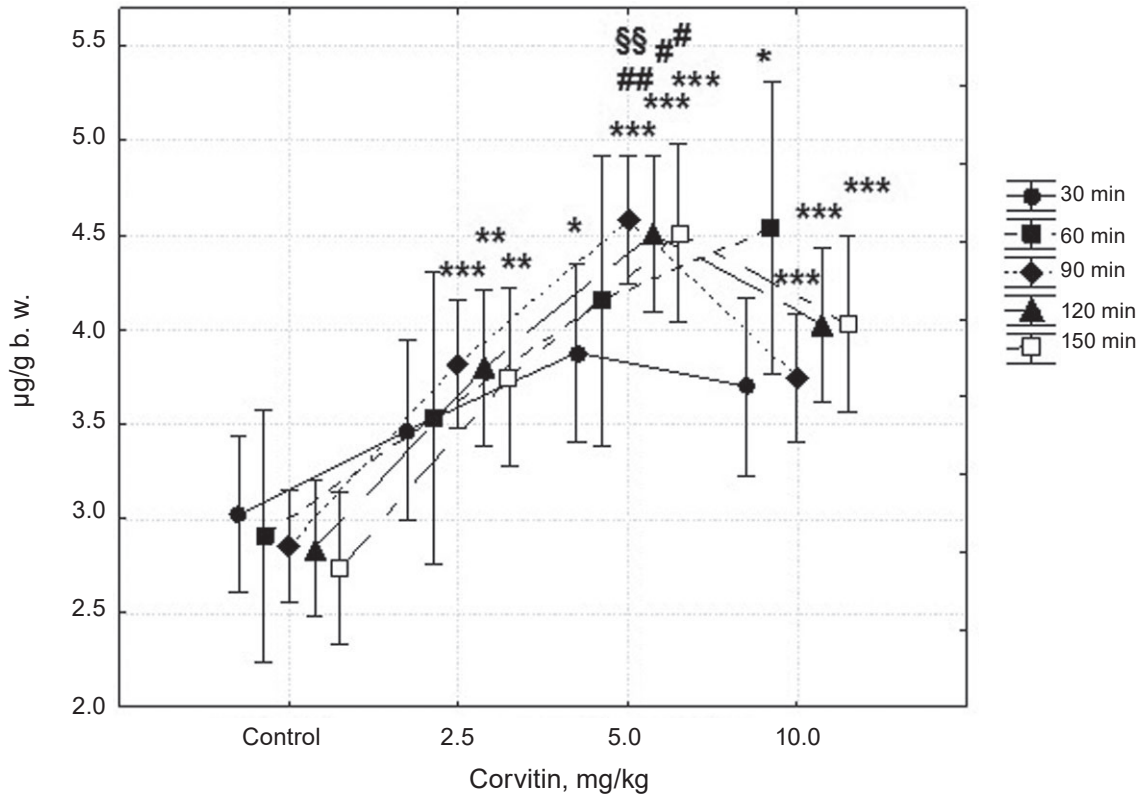

*Fig. 3. Effect of corvitin (2.5, 5 and 10 mg/kg) on total conjugated BAs production. Secreted bile was collected during 2.5 h. Mean ± SEM; n = 6; **P < 0.01, ***P < 0.001 versus control group; #P < 0.05, ##P < 0.01 corvitin 2.5 mg/kg versus corvitin 5 mg/kg; §§P < 0.01 corvitin 5 mg/kg versus corvitin 10 mg/kg; observed power (at alpha 0.05) = 0.997; partial eta = 0.52*

T a b l e 2. Total content of conjugated BAs (μg/g b.w.) in rats bile, collected in all 2.5 hours of the experiment after treatment by corvitin (2.5, 5 and 10 mg/kg)

Animal group	Half-hour samples	TCA, μg/g b.w.	TCDCA+TDCA, μg/g b.w.	GCA, μg/g b.w.	GCDCA+GDCA, μg/g b.w.
Control	1	1.70 ± 0.05	1.10 ± 0.06	1.70 ± 0.13	0.30 ± 0.02
Corvitin 2.5 mg/kg		2.10 ± 0.08**	1.30 ± 0.09*	1.8 ± 0.1	0.30 ± 0.02
Corvitin 5 mg/kg		2.30 ± 0.19*	1.60 ± 0.24*	1.7 ± 0.1	0.30 ± 0.02
Corvitin 10 mg/kg		1.80 ± 0.14	1.20 ± 0.05	1.40 ± 0.13	0.25 ± 0.02
Control	2	1.70 ± 0.06	1.10 ± 0.08	1.80 ± 0.11	0.25 ± 0.02
Corvitin 2.5 mg/kg		2.10 ± 0.07***	1.30 ± 0.06**	1.70 ± 0.07	0.30 ± 0.01*
Corvitin 5 mg/kg		2.50 ± 0.14***	1.90 ± 0.18***	1.90 ± 0.08	0.40 ± 0.02**
Corvitin 10 mg/kg		1.90 ± 0.13	1.30 ± 0.07*	1.4 ± 0.1*	0.30 ± 0.01
Control	3	1.70 ± 0.05	1.00 ± 0.06	2.10 ± 0.11	0.20 ± 0.01
Corvitin 2.5 mg/kg		2.30 ± 0.06***	1.75 ± 0.18**	1.90 ± 0.07	0.30 ± 0.02**
Corvitin 5 mg/kg		2.60 ± 0.12***	2.25 ± 0.17***	2.1 ± 0.1	0.40 ± 0.02***
Corvitin 10 mg/kg		2.10 ± 0.11**	1.30 ± 0.05**	1.60 ± 0.13*	0.30 ± 0.02**
Control	4	1.70 ± 0.06	1.00 ± 0.08	2.0 ± 0.1	0.20 ± 0.02
Corvitin 2.5 mg/kg		2.3 ± 0.1***	1.60 ± 0.13**	1.90 ± 0.07	0.30 ± 0.02**
5 mg/kg		2.60 ± 0.08***	2.20 ± 0.06***	2.00 ± 0.06	0.40 ± 0.03***
10 mg/kg		2.10 ± 0.12**	1.40 ± 0.09*	1.50 ± 0.11*	0.30 ± 0.01*
Control	5	1.60 ± 0.08	1.00 ± 0.09	2.10 ± 0.11	0.20 ± 0.02
Corvitin 2.5 mg/kg		2.20 ± 0.12***	1.55 ± 0.15**	1.9 ± 0.1	0.30 ± 0.02**
Corvitin 5 mg/kg		2.6 ± 0.1***	2.3 ± 0.1***	2.00 ± 0.06	0.40 ± 0.02***
Corvitin 10 mg/kg		2.10 ± 0.14**	1.50 ± 0.13*	1.4 ± 0.1**	0.30 ± 0.02*

Statistically signifcant difference *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. TCA – taurocholic acid; TCDCA+TDCA – taurochenodeoxycholic acid+taurodeoxycholic acid; GCA – glycocholic acid; GCDCA+GDCA – glycochenodeoxycholic acid+glycodeoxycholic acid

content of GCDCA+GDCA in bile, in response to corvitin treatment (2.5 mg/kg), was higher than in the control rats in the 2nd, 3rd, 4th and 5th half-hour samples by 24.8% ($P < 0.05$), 34.3% ($P < 0.01$), 34.9% ($P < 0.01$), 33% ($P < 0.05$), respectively. The value of stimulatory effect of 5 mg/kg corvitin on bile GCDCA+GDCA secretion was 42.8% ($P < 0.001$) in the 2nd half-hour, 68.6% ($P < 0.01$) in the 3rd half-hour, 78.9% ($P < 0.001$) in the 4th half-hour and 75.5% ($P < 0.001$) in the 5th half-hour samples. At a dose of 10 mg/kg flavonoid caused increase in GCDCA+GDCA content by 30.5 ($P < 0.05$) in the 3rd and by 19.6% ($P < 0.05$) in the 5th samples of bile compared with the control animals (Table 2).

The liver has a high capacity for conjugation and as a result newly synthesized BAs conjugate with glycine or taurine, that is the final step in primary BAs synthesis. BAs amphiphilic nature determines their surface-active properties and participation in the formation of lipid micelles and in fats digestion. Conjugated BAs are stronger detergents than unconjugated ones since they have reduced dissociation constant value and their molecules are completely dissociated at pH 6.0 in the proximal intestine. Conjugation enhances a high intraluminal BAs concentration and therefore efficient solubilization of lipids with low aqueous solubility such as saturated fatty acids and fat-soluble vitamins [24]. The relatively high concentration of BAs in the small intestinal lumen during digestion is the result of several factors. First, conjugated BAs are strong acids that are fully ionized at intestinal pH and are, therefore, impermeable to cell membranes, and the BA molecule is too large to pass across the para-

cellular junctions. Second, efficient conservation of BAs by active (carrier mediated) absorption from the small intestine results in a pool of BAs that cycles several times with each meal. Alternatively, unconjugated BAs are not well transported by canalicular transporters and, in some cases, may accumulate in hepatocytes causing direct injury and/or recruitment of inflammatory factors. Interestingly, that conjugation involves both a newly synthesized and deconjugated in the intestine BAs, that have returned to the liver via the enterohepatic circulation. At last, hepatic conjugation of BAs with taurine or glycine not only enhances resistance to precipitation by Ca^{2+} ions, but makes them less toxic and more soluble for improved secretion into bile [25]. In the present study we have shown that BAs conjugates with glycine and especially with taurine in the bile of rats of all tested groups after injection of corvitin, although the ratio was different depending on its dose.

The results described above show that corvitin (5 mg/kg) has the most effective influence on BAs conjugation with taurine, especially TCDCA+TDCA. Ikeda et al. showed that the intensity of BAs conjugation with taurine is determined by the activity of its transporter that delivers this amino acid from blood to the hepatocyte across the sinusoidal membrane [26]. Based on our results we suggest that corvitin activates taurine transport system, increasing the content of this amino acid in hepatocytes.

It is important to note that there is a tight interrelation between the level of BAs and secretion of bile lipids [27]. An increase in bile BAs secretion in all tested groups of animals is undoubtedly a positive result of our study because it indicates on possible elevation of bile emulsifying efficiency by corvitin.

Previously we showed that corvitin significantly increased blood flow in rat liver [13], that improves hepatic tissue respiration and provides a better energy supply of synthetic and metabolic processes. It is suggested that QUE is accumulated in the mitochondria increasing mitochondrial biogenesis [28-30] and improving mitochondrial bioenergetics [31]. This may contribute to the activation of synthetic processes in hepatocytes and to acceleration of bile excretion from the liver.

In conclusion, this study provided the first evidence showing that corvitin as the blocker of leukotriene synthesis modulates excretory function of the rat liver by increasing BAs content and enhancing BAs biotransformation by conjugation. Corvitin effects on the BAs pool depend on the doses used. We conclude that at doses of 2.5 and 5 mg/kg flavonoid affects more effectively BAs conjugation with glycine and especially with taurine than the conversion of cholesterol into free BAs. At a dose of 10 mg/kg corvitin mainly activates the transformation of cholesterol into free BAs but not BAs conjugation. The increased content of conjugated BAs after treatment with 2.5 and 5 mg /kg corvitin improves detergent properties of bile and the ability to emulsify dietary fats. The enhanced biliary secretion of free BAs under the influence of 10 mg/kg corvitin may indicate decreased BAs conjugation with taurine and glycine and impairment of fat digestion and absorption in rats intestine.

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УТВОРЕННЯ І СЕКРЕЦІЯ ЖОВЧІ В УМОВАХ БЛОКАДИ 5-ЛІПОКСИГЕНАЗНОГО ШЛЯХУ КОРВІТИНОМ У ЩУРІВ

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Корвітин – розчинна форма кверцетину, ефекти якого базуються на його здатності гальмувати активність 5-ліпоксигенази і таким чином пригнічувати утворення лейкотрієнів. Корвітин посилює кровотік у шлунку, підшлунковій залозі й печінці, однак його вплив на зовнішньосекреторну функцію печінки досі не вивчено. Ми дослідили вплив корвітину (2,5; 5 і 10 мг/кг, внутрішньопортально, болюсом) на формування жовчі, визначили біліарний вміст сумарних, вільних та кон'югованих жовчних кислот (ЖК). Вільні і кон'юговані ЖК було розділено методом тонкошарової хроматографії. Показано, що корвітин збільшував сумарний вміст ЖК у жовчі щурів усіх експериментальних

груп, про що свідчило збільшення їх секреції в жовч. У дозі 2,5 мг/кг флавоноїд не змінював вміст вільних ЖК, проте збільшував вміст кон'югованих холатів. Кількість як вільних, так і кон'югованих ЖК збільшувалася в щурів, які одержували 5 мг/кг корвітину. Збільшення дози флавоноїду до 10 мг/кг посилювало вихід у жовч вільних ЖК більшою мірою, ніж кон'югованих. Таким чином, пригнічення активності 5-ліпоксигенази корвітином впливає на утворення і секрецію в жовч сумарних, вільних і кон'югованих ЖК.

Ключові слова: корвітин, 5-ліпоксигеназа, лейкотрієни, секреція жовчі, вільні жирні кислоти, кон'юговані жирні кислоти.

ОБРАЗОВАНИЕ И СЕКРЕЦИЯ ЖЕЛЧИ В УСЛОВИЯХ БЛОКАДЫ 5-ЛИПОКСИГЕНАЗНОГО ПУТИ КОРВИТИНОМ У КРЫС

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Корвитин – растворимая форма кверцетина, эффекты которого базируются на его свойстве тормозить активность 5-липоксигеназы и таким образом угнетать образование лейкотриенов. Корвитин усиливает кровоток в желудке, поджелудочной железе и печени, однако его влияние на внешнесекреторную функцию печени до сих пор не изучено. Мы исследовали влияние корвитина (2.5; 5 и 10 мг/кг, внутрипортально, болюсом), на образование желчи и определили билиарное содержание суммарных, свободных и конъюгированных желчных кислот (ЖК). Свободные и конъюгированные ЖК разделяли методом тонкослойной хроматографии. Показано, что корвитин увеличивал суммарное содержание ЖК в желчи крыс всех экспериментальных групп, о чем свидетельствовало увеличение их секреции в желчь. В дозе 2,5 мг/кг флавоноид не изменял секрецию свободных ЖК, увеличивая в то же время содержание конъюгированных холатов. Содержание как свободных, так и конъюгированных ЖК увеличивалось у крыс, которые получали 5 мг/кг корвитина. Увеличение дозы флавоноида до 10 мг/кг усиливало выход в желчь свободных ЖК в большей степени, чем конъюгированных. Таким образом, ингибирование активности 5-липоксигеназы корвитином влияет на образование и секрецию в желчь суммарных, свободных и конъюгированных ЖК.

Ключевые слова: корвитин, 5-липоксигеназа, лейкотриены, секреция желчи, свободные ЖК, конъюгированные ЖК.

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