The Animal Biology, 2014, vol. 16, no. 2
UDC 636.22/28:619:539.1

BILE ACIDS IN ORGANISMS OF HEALTHY AND SICK NEWBORN CALVES AND AFTER APPLYING ENTEROSORBENTS

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Taking into consideration the crucial role of bile acids in the processes of digestion and assimilation of feed components of lipid nature, intermediate metabolism of bile acids in the body of newborn calves suffering from dyspepsia was investigated.

Analysis of the data shows that in calf's bile appear differences of the ratio of individual bile acids and a significant decrease in their total content. Seven fractions of conjugated and free bile acids, including the dominated taurocholic, taurohenodesoksicholic, taurodezoksicholic, glikocholic acid are identified. It was found that on the background of reduction of bile acids conjugated with taurine and glycine in gallbladder bile and liver of newborn calves suffering from acute indigestion, the level of free bile acids increases significantly. This indicates a decrease in biosynthetic and conjugating liver function of patients with dyspepsia in calves. In addition, the increasing toxic lithocholic acid. Analysis of extracts from the colon diseased calves showed that the total content of bile acids significantly increased. This indicates that the body of sick animals lose large amounts of the compounds and the significant variations in enterohepatycal cycle of the gastrointestinal tract.

In the bile and the contents of the colon in patients calves serum total bilirubin significantly increased. Thus, its content in bile of patients calves 1.2 times greater than in the control, and the contents of the colon is almost twice the control value. These data indicate that the body of sick calves under the influence of certain factors, including elevated levels of LHA, intensified hemoglobin break down simultaneously with the reduction of the term of erythrocytes half-life.

Apply for therapeutic purposes enterosorbents partially or fully stabilize certain level of bile-acid metabolism in the body of experimental animals and most of their recovery.

Keywords: BILE ACIDS, BILE, NEWBORN CALVES, ENTEROSORBENTS

Bile is a product of the exocrine activity of the liver. It contains a wide variety of organic and inorganic compounds. Bile acid represents 60% of organic compounds in bile. Its role is fat digestion, absorption of fat-soluble vitamins, bacteriostatic effect on the intestinal microflora, stimulation of intestinal motor function, exocrine pancreatic function, as well as maintenance of the colloidal state of the bile with cholesterol solubility in it. Bile acids synthesis occurs in liver cells and essentially depends on the cholesterol level [1-4].

Irrespective of the factors, the majority of lesions of the hepatobiliary system lead to significant changes in bile secretion which is complicated by the damage of small bile ducts. Biliary disorders of the liver are accompanied by destructive changes of hepatocyte cell membranes [5, 6].

Interest in the study of the bile acid content of biological substances is attributed to its important biological role, as well as the frequent development of complications occurring when its concentration is changed.

It is known that liver and intestine are affected in the development of toxic hepatitis, which causes disorders of biosynthesis and hepatic circulation of bile acids. Toxic hepatitis is accompanied by a breach of synthetic, conjugating and eliminative functions of liver (slightly apparent) and the development of intrahepatic cholestasis [6].

Therefore, the purpose of the study was to investigate bile acids in organisms of healthy and sick newborn calves and after applying enterosorbents.

Materials and Methods

Four groups of calves were formed for the study, 5 animals in each group at the age of 2-3 days, weighing 30-35 kg, clinically healthy and with dyspepsia, treated by enterosorbents "Enterosgel" in a dose of 15 g per day before colostrum ingestion during three days, or by "Polisorb" in a dose of 2 g twice a day. Preparations were mixed with 200 ml of isotonic NaCl, heated to a temperature of 37°C. Animals in the control group received the same volume of isotonic NaCl.

Bile taken directly from calves' gallbladders was used for the study. Preparation of liver tissue and colon contents samples for biochemical analysis was performed according to the recommendations described in the well-known textbooks [7-10].

Bile acids from the bile, liver, and calves' colon contents were determined by the published method [11, 12]. Extraction of bile acids from the abovementioned biomaterials was performed by the cooled mixture of ethylene-acetone (1: 3). The

extraction of bile acids was followed by their concentration in conical tubes and dry residue was diluted in microvolumes (10-50 ml) of ethanol-water mixture (6: 4) before the application.

The chromatographic analysis of bile extracts, liver, and calves' colon contents by individual bile acids was performed by the use of a solvent system with the following components: amyl ether acetic acid, butanol, toluene, acetic acid and distilled water in the ratio - 3: 1: 1: 3: 1. Identification of individual fractions of bile acids was conducted with the help of witnesses from various manufacturers, such as Sigma and Reanal (Hungary).

Coloring of bile acids on thin-layer chromatograms was performed by complex coloring agent, which included the following components: 1 gram of phosphomolybdic acid diluted in a mixture of 15 ml glacial acetic acid + 5 ml of 50% trichloroacetic acid and 1 ml of concentrated sulfuric acid.

Quantitative assessment of the content of certain bile acids was carried out by using densitometer K-1 m in the reflected rays (L-620 nm) and the corresponding calibration curves for individual witnesses.

Statistical analysis of the results of experiments was undertaken on the basis of Student's t-test [13].

Results and discussion

The chromatographic analysis of extracts of bile, liver, and colon contents in calves identified seven factions of free and conjugated bile acids (Table. 1-3). The highest content of bile acids was found in the bile of animals of the control group.

Table 1

The content of bile acids in the gallbladder bile of healthy and sick newborn calves and after application of Enterosorbents mg% (M ± m, n = 5)

Bile acids	Calves			
	Healthy (control)	Sick	Enterosgel	Polisorb
TCA	318.8±18.1	196.4±16.1*	252.7±21.1**	271.3±19.2
TCDCA + TDCA	294.5±12.3	186.5±22.4*	180.4±11.3**	278.5±14.4**
GCA	959.4±51.3	357.5±17.6*	804.4±7.1**	476.5±19.4**
GCDCA + GDCA	893.5±31.2	289.4±9.9*	695.2±20.3**	392.5±16.5**
CA	103.2±12.4	175.5±11.8*	130.7±14.5**	73.4±6.3**
CDCA + DCA	90.4±5.9	153.0±9.3*	89.7±5.6**	52.6±5.5**
LCA	0.89±0.07	10.1±0.9*	2.95±0.43**	3.45±0.33**

Note: In Tables 1-3 BA - bile acid TCA - taurocholic acid; TCDCA + TDCA - taurochenodeoxycholic acid + taurodeoxycholic acid; GCA - glycocholic acid; GCDCA + GDCA - glycochenodeoxycholic acid + glycodeoxycholic acid; CA – cholic acid; CDCA + DCA - chenodeoxycholic acid + deoxycholic acid; LCA - lithocholic acid. This and the following tables show the probability * - $p < 0.05$ in control group, ** - $p < 0.01$ in sick calves, n = 5

Table 2

The content of bile acids in the liver of healthy and sick newborn calves and after application of Enterosorbents, 100 g wet tissue (M ± m, n = 5)

Bile acids	Calves			
	Healthy (control)	Sick	Enterosgel	Polisorb
TCA	3.45±0.31	1.56±0.12*	2.81±0.22**	3.28±0.27**
TCDCA + TDCA	2.98±0.18	1.70±0.07*	2.33±0.17**	2.85±0.13**
GCA	5.8±0.30	2.44±0.22*	4.60±0.31**	3.47±0.29**
GCDCA + GDCA	5.50±0.33	2.10±0.15*	4.50±0.35**	3.30±0.27**
CA	2.75±0.23	1.53±0.25*	1.95±0.17**	2.10±0.22**
CDCA + DCA	1.94±0.21	1.50±0.13*	1.55±0.16**	0.93±0.23**
LCA	0.55±0.05	1.96±0.16*	1.07±0.29**	0.90±0.14**

Table 3

The content of bile acids in the colon of healthy and sick newborn calves and after application of Enterosorbents mg% (M ± m, n = 5)

Bile acids	Calves			
	Healthy (control)	Sick	Enterosgel	Polisorb
TCA	4.70±0.28	27.60±2.30*	11.63±2.20**	9.45±0.81**
TCDCA + TDCA	6.15±1.20	30.30±1.60*	15.3±2.60**	10.00±0.51**
GCA	6.40±0.93	43.70±3.90*	10.80±1.10**	15.60±2.45**
GCDCA + GDCA	7.50±0.90	48.60±4.10*	12.40±0.84**	17.80±1.90**
CA	3.30±0.32	16.60±2.70*	5.10±0.12**	3.55±0.37**
CDCA + DCA	4.20±0.26	17.70±2.40*	6.60±0.79**	4.45±0.41**
LCA	1.07±0.13	13.80±1.30*	3.93±0.42**	2.52±0.22**

Compared to healthy animals, significant differences of bile-acid spectrum were found in the bile of sick animals, which was characterized by a marked

decline in the content of conjugated bile acids. Thus, in the control group the TCA was 62.6% TCDCA + TDCA were 63.3; GCA was 37.2; GCDCA + GDCA were 32.3%. At the same time compared to the control group the level of free bile acids increased significantly in the gallbladder bile: CA by 70.0%; CDCA + DCA by 69.2% and lithocholic acid by 11.3 times.

A reduced level of total biliary acids was noted in the analysis of liver extracts of sick calves. The content of all fractions of conjugated bile acids in this tissue was significantly lower than the stated value. Only LCA level significantly increased 3.5-fold among free bile acids. The total content of chelates in the liver was reduced, which generally indicated a decline in protein synthesis and conjugation of liver function of newborn calves with acute digestive disorders.

Bile acid content significantly increased compared with stated values in the colon contents in diseased calves with both conjugated and free bile acids. Especially the TCA content was undergoing great changes – a 5.9-fold increase, GCA and GCDCA + GDCA contents were boosted almost 6.8-foldand lithocholic acid content - 13-fold compared with the control group. This significant difference in the level of cholic acid colon contents may indicate on its considerable loss in the body of sick calves, as well as irregularities in the enterohepatic circulation of these compounds.

Use of enterosorbents "Enterosgel" and "Polisorb" in complex treatment of acute digestive disorders of newborn calves improved bile producing and bile excreting liver functions, activated protein synthesis processes, normalized the digestion process and enhanced the clinical state of the body.

Total bilirubin level was significantly elevated in the bile and colon contents of calves (Table 4). Thus, its content in the bile of sick calves was 1.2-fold higher than in the control group and colon content was almost twice higher than the stated value. This data attests to the fact that the bodies of sick calves under the influence of certain factors, including increased levels of LCA, are subject to intensified process of hemoglobin splitting simultaneously with a reduction in lifespan of the red blood cells. Thus, changes in the proportion of free and conjugated bile acids in the bile, the liver tissue and colon contents of calves with acute indigestion showed significant deviation in the intermediate metabolism of studied compounds. Disorders in the intermediate metabolism of bile acids in the body of calves may influence functional disorders of the gastrointestinal tract.

Table 4

Number of total bilirubin in the bile and colon contents in healthy and sick newborn calves and after application of Enterosorbents mg% (M ± m, n = 5)

Indicators	Calves			
	Healthy (control)	Sick	Enterosgel	Polisorb
Bile	38.6±2.3	48.2±3.0*	39.3±2.5**	43.2±2.67**
The contents of the colon	4.45±0.28	8.26±0.74*	5.23±0.46**	5.45±0.36**

The use of Polisorb and Enterosgel preparations during the treatment of calves promotes their faster recovery and may be partly caused by the normalization of certain parts of the bile-acid metabolism in the body of laboratory animals. In particular, under the influence of Polisorb in the liver, and especially in bile of sick calves, the level of TCA increased by 41% and the amount of TCDCA + TDCA - by 40% compared to their content in the control group.

Considering the changes in the level of free bile acids in the liver, bile, and colon contents of calves under the influence of these drugs, it should be emphasized that their action differed only in intensity. So, the free LCD level in bile calves decreased by 2.4-fold while using Polisorb, whereas the use of Enterosgel led to the decline in its level only by 1.3-fold($r \leq 0,05$). In the same way, the mix level of free CDCA + DCA in bile of the calves changed during the test of these medications. Changes in the free bile acids exchange in calves also suggested improvement in the efficiency of multienzyme of liver cells that provide their conjugation with amino acids under the influence of applied Enterosorbents.

It is worth noting that preparations used for the treatment of calves could significantly affect the lithocholic acid level. Thus, with the help of the applied preparations the level of bile acids was reduced in the liver by almost half and in the bile - threefold. This may be the result of binding lithocholic acid with studied acid chelators that limited its reuptake in the intestines of diseased calves. This was partly confirmed by the fact that applications of the studied preparations reduce levels of the free lithocholic acid in colon contents [14].

Conclusions

Polisorb and Enterosgel preparations can actively influence the biosynthesis and conjugation of bile acids in the liver cells, change their bile level, which is probably largely caused by the binding of metabolites of the studied preparations in the intestine and inhibition of reuptake, most importantly to eliminate the toxic effects of lithocholic acid.

Prospects for further research.

The results obtained will be used for further examination of Enterosorbents influence on binding lithocholic toxic acids in the liver cells, change their levels in bile and gut, as well as other mechanisms of stabilizing action of Enterosorbents.

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