# Correction of Lipid Metabolism with Chenodeoxycholic Acid and Galenic Preparations in Experiment

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In a rabbit model of cholestasis (40% ligation of the common bile duct), the size and number of cholesterol calculi in the gallbladder drop markedly after therapy with chenodeoxycholic acid (1.5 mg/kg once daily for 2-3 months) or with the new galenic preparation ekvalen (daily administration in a dose of 1.5 ml/kg in a 16-18% aqueous ethanol solution for 7 days at 3-4-day intervals during a 1-3-month period). Normalization of lipid metabolism in the enterohepatic system is documented.

**Key Words:** *cholestasis; lipid metabolism; chenodeoxycholic acid; galenic preparations*

Much more young people have been suffering from cholelithiasis during the last three years [4,5]. Surgery, including endoscopic cholecystotomy and lithotripsy, is performed on prescription and does not solve the social problem of cholelithiasis. Pain recurrence after surgery and the development of postcholecystomic syndrome prompt an extensive investigation of this disease.

Lipid metabolism disorders are thought to be the major cause of biliary cholesterol calculi [6,7]. Anticholelithiasis therapy is aimed primarily at normalizing lipid metabolism (cholesterol in particular) and restoring the bile acid balance with conventional drugs (chenofalk). Much less is known about the effects of galenic preparations.

In the present study we compared the lipid metabolism in rabbits with modeled cholestasis after monotherapy with chenodeoxycholic acid (CDA) and new experimental galenic preparations.

## MATERIALS AND METHODS

Experiments were performed on female rabbits weighing 2.0-2.5 kg. The animals were divided into 5 groups: group 1 rabbits served as the control, group 2 consisted of rabbits with calculous cholecystitis (CC), rabbits with CC treated with CDA comprised group 3, group 4 CC rabbits were treated with extract from the following medicinal plants: *Chelidonium majus, Taraxacum officinale, Polygonum aviculare, Stigmatum maydis, Hypericum perforatum, Viola tricolor,* and *Anisum vulgare* prepared on a 70% aqueous ethanol solution according to recipe 142 [8], and group 5 included rabbits with CC treated with extract from *Arctium lappa, Equisetum arvense, Achillea millefolium, Leonurus quinquelobatus* prepared according to recipe [3].

Calculous cholecystitis was induced by 40% ligation of the common bile duct in the duodenal region under sodium thiopental anesthesia (0.3-0.5 ml of 2% solution) and premedication with 1% Promedol. In some animals, the presence of cholesterol bile calculi in the gallbladder on days 15-30 after surgery was confirmed by ultrasound investigation using a USI Toshiba Sonolayer Sal 38 apparatus, after which monotherapy was prescribed.

Chenodeoxycholic acid was obtained as described elsewhere [2]. It was dissolved in sunflower oil and administered intragastrally via a gastral tube in a daily dose of 1.5 mg/kg for 2 or 3 months. The galenic preparations were administered intragastrally as 16-18% aqueous ethanol solutions in a daily dose of 1.5 ml/kg for 7 days at 3-4-day intervals during a 1-3-month period. The effectiveness of therapy was evaluated from ultrasound investigation, morpholog-

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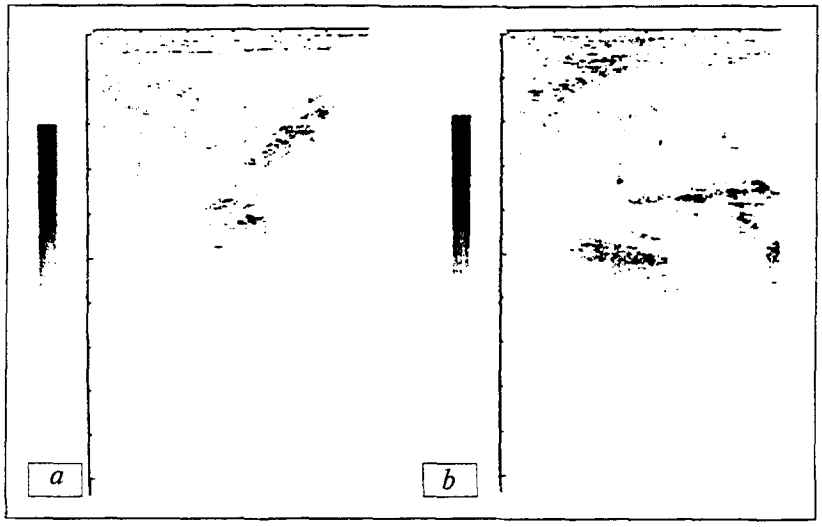


**Fig. 1.** Ultrasound investigation of the gallbladder of a rabbit with calculous cholecystitis before (*a*) and after (*b*) treatment with ekvalen. *a*) the gallbladder is pear-shaped, its size is increased 3-fold, the walls are rough, 3.5-mm thick, numerous calculi are present in the lumen, the largest calculus has a diameter of 12 mm, bile echogenicity is increased; *b*) the gallbladder is boomerang-shaped (as in intact rabbits), its size is increased 1.4-fold, the walls are smooth, not thickened (less than 2 mm); bile echogenicity is normal, occasional 1-3 mm calculi are present.

ical studies of bile and gallbladder, and biochemical parameters of lipid metabolism.

The bile acid spectrum, lipid fractions, and fatty acids were analyzed by thin layer and gas chromatography [1]. The results were statistically processed using Student's *t* test.

## RESULTS

The animals developed chronic cholecystitis within 14-30 days after surgery. Calculous cholecystitis (the presence of 2-11-mm cholesterol calculi in the gallbladder bile, Fig. 1, *a*) was diagnosed in 63% of

**TABLE 1.** Effect of Monotherapy with Chenodeoxycholic Acid (CDA) and Galenic Preparations on Lipid Metabolism of Rabbits with Calculous Cholecystitis (CC) (*M*±*m*)

| Parameter | Group 1 (control, *n*=9) | Group 2 (CC, *n*=10) | Group 3 (CDA, *n*=10) | Group 4 (medicinal plants, recipe 142, *n*=9) | Group 5 (ekvalen, *n*=12) |
|---|---|---|---|---|---|
| **Gallbladder bile, mmol/liter** | | | | | |
| Bile acids | 103.5±6.6 | 38.1±5.1* | 140.5±12.6** | 60.6±9.5+ | 87.4±9.5** |
| Cholesterol | 5.9±0.6 | 19.3±1.5* | 10.4±2.6+ | 12.8±1.4+ | 6.7±0.5** |
| Phospholipids | 14.3±1.5 | 6.9±1.0* | 16.5±3.3** | 9.6±1.7+ | 10.6±1.1+ |
| Lithogenic index | 0.75±0.06 | 4.14±0.38* | 0.96±0.11** | 2.86±0.79+ | 0.86±0.09** |
| **Enterocytes, mg/g** | | | | | |
| Free fatty acids | 0.70±0.22 | 1.66±0.32* | 0.92±0.20** | 1.22±0.33 | 0.92±0.16** |
| Esterified cholesterol | 0.20±0.04 | 1.95±0.40* | 0.28±0.09** | 0.69±0.19** | 0.48±0.09+ |
| Triacylglycerides | 9.41±1.23 | 7.53±1.24 | 14.3±2.54** | 10.61±2.13 | 8.83±1.17 |
| Phospholipids | 11.73±0.72 | 7.25±0.58* | 17.51±2.45+ | 10.57±2.05 | 13.51±1.73** |
| **Hepatocytes, mg/g** | | | | | |
| Free fatty acids | 1.00±0.25 | 2.65±0.34** | 1.32±0.33** | 1.45±0.24** | 1.22±0.19** |
| Esterified cholesterol | 2.33±0.24 | 4.95±1.11* | 3.10±0.38** | 3.61±0.64* | 2.05±0.36** |
| Triacylglycerides | 15.47±1.66 | 19.93±3.87 | 14.62±2.16 | 17.33±1.84 | 13.65±1.26 |
| Phospholipids | 17.92±0.82 | 7.24±0.75* | 13.82±3.29+ | 11.28±1.26* | 14.84±1.37** |
| **Arachidonic acid in esterified cholesterol, %** | | | | | |
| Serum | 4.5±0.9 | 14.3±3.5* | 9.6±2.2* | 9.5±1.6* | 6.3±1.1** |
| Enterocytes | 5.6±1.3 | 19.5±5.4* | 11.6±3.2+ | 15.0±2.2* | 12.4±2.6* |
| Hepatocytes | 3.2±0.7 | 8.8±1.1* | 5.0±1.2** | 6.6±0.9* | 4.7±0.8** |

**Note.** $p<0.05$: *compared with group 1, **group 2, and +groups 1 and 2.

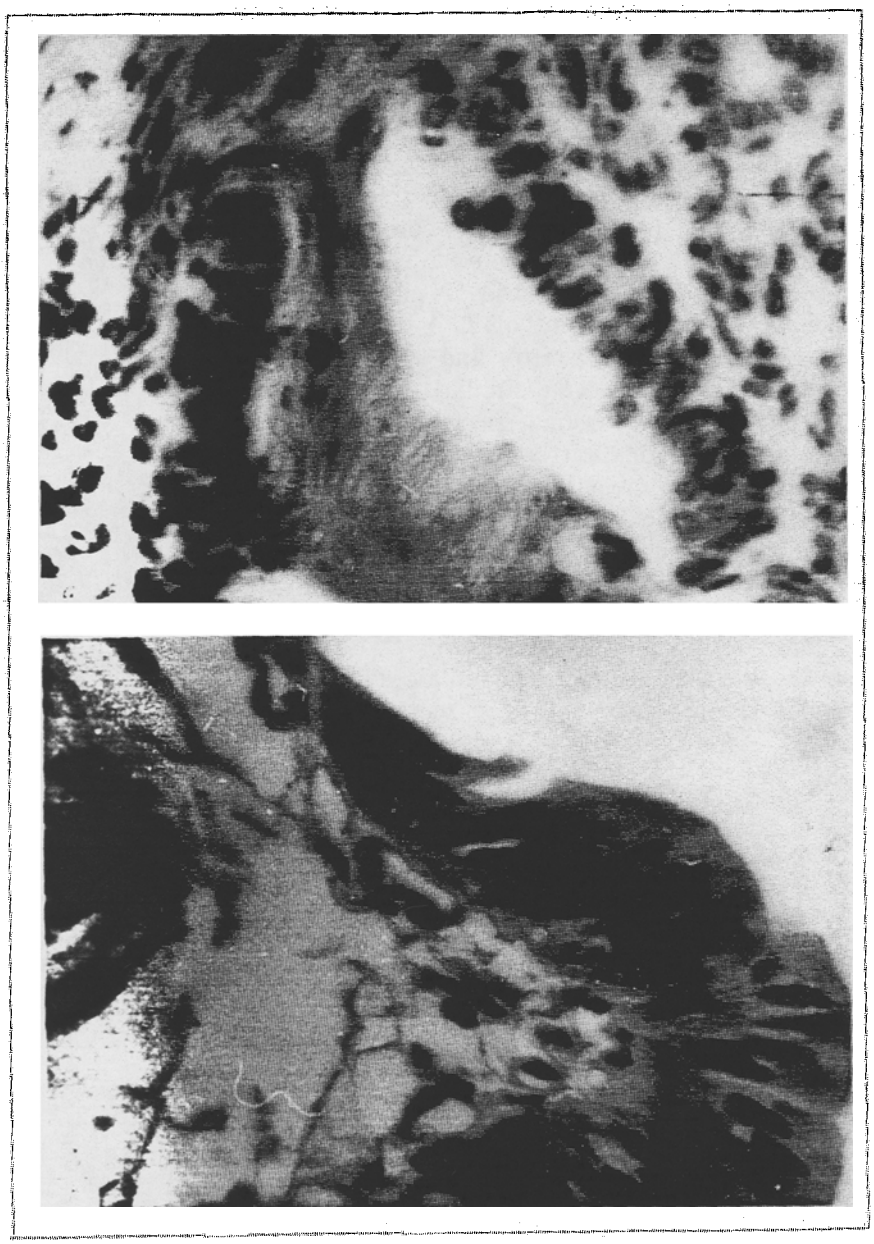

Fig. 2. Morphological study of the stroma of the gallbladder mucosa in a rabbit with calculous cholecystitis before (*a*) and after (*b*) ekvalen therapy. Hematoxylin and eosin staining. *a*) decreased number of mucosal rugae, desquamation and micropolypoidal outgrowth of the epithelium, fibrous areas and thickened vascular walls × 140; *b*) occasional cells infiltrating the mucosa slight swelling of the endothelium, occasional cholestatic granules, and the absence of pronounced structural changes. × 100.

them. Morphological studies of the gallbladder of CC rabbits revealed a decrease in the number of mucosal rugae, desquamation of the epithelium and its micropolypoidal outgrowth, formation of fibrous areas, and thickening of the walls of blood vessels [1]. The imbalance of bile lipids (Table 1, group 2) manifested itself in a 1.8-fold decline in the content of phospholipids, primarily of phosphatidylcholine, a 3-fold decrease in the concentration of bile acids, and a 3.1-fold increase in the content of cholesterol (both free and esterified) with a resultant 3-fold increase in the lithogenic index [1]. In the liver and intestine cells, the content of essential arachidonic acid in esterified cholesterol increased 2.5- to 3.5-fold.

In CDA-treated animals, the gallbladder rugae were partially restored, the number of infiltrating cells dropped, the degree of endothelial swelling decreased, but numerous cholestatic granules were preserved. The number of 2-4-mm bile calculi decreased, while the levels of bile acids and phospholipids increased, the cholesterol content dropped, and the lithogenic index returned to the baseline value. The levels of free fatty acids and esterified cholesterol in hepatocytes and enterocytes declined, blood phospholipids and triacylglycerides rose, and the content of 20:4 acid in esterified cholesterol in hepatocytes and enterocytes decreased (group 3). At the same time, 7 out of 15 lipid metabolism indexes were significantly different from the control. Similar results (10 out of 15 indexes) were obtained in groups 1 and 4 animals (galenic preparation based on medicinal plants recommended for the treatment of cholelithiasis [8]).

In all animals treated with the galenic preparation ekvalen, occasional microcalculi composed of cholesterol monohydrate were found in the gallbladder (Fig. 1, *b*). The gallbladder mucosa was characterized by the presence of occasional infiltrating cells and cholestatic granules, slight endothelial swelling, and the absence of pronounced structural changes (Fig. 2, *b*). The levels of lipid components in the gallbladder bile were similar to those in the control (groups 5 and 1). The lipid levels in hepatocytes and enterocytes, the content of 20:4 acid in esterified blood and liver cholesterol, and 13 out of 15 lipid metabolism indexes were similar to the control.

Thus, the conventional species of medicinal plants recommended for the treatment of cholelithiasis, on which our experimental galenic preparation was based, proved to be ineffective in a rabbit model of cholelithiasis. Ekvalen, a new combination of medicinal plants, effectively normalized lipid metabolism in rabbits with CC. A comparison of lipid metabolism parameters in rabbits treated with CDA or ekvalen showed that these agents act unilaterally on the gallbladder morphology and lipid metabolism. The effect of ekvalen proved to be specific towards lipid metabolism, cholesterol in particular.

## REFERENCES

1. Yu. A. Bogdarin, *Peculiarities of Lipid Composition in the Enterohepatic System and Its Correction in Cholelithiasis, D. Sci. Dissertation* [in Russian], Gorky (1990).
2. Yu. A. Bogdarin, A. V. Galkin, I. B. Denisova, and A. S. Vizgalova, *Method of Preparation of Individual Bile Acids*, Russian Federation Patent No. 166568 November 22, 1991.
3. Yu. A. Bogdarin, *Drug for the Prevention and Treatment of Cholecystitis and Cholelithiasis*, Russian Federation Patent No. 5068531, October 17, 1994.
4. Yu. P. Ipatov, *Clinical Significance of X-Ray and Ultrasound Diagnostics of Gastroduodenal and Pancreatobiliar Disorders in Children, MD. Dissertation* [in Russian], Gorky (1989).
5. D. I. Krivitskii, N. N. Gvozdyak, H. G. Polyakov, and A. A. Nikitenko, *Vestn. Khir.*, **134**, No. 4, 46-49 (1985).
6. A. S. Loginov, S. M. Chebanov, Yu. Kh. Marakhovskii, and I. I. Goncharik, *Byull. Eksp. Biol. Med.*, **108**, No. 8, 251-254 (1989).
7. Kh. Kh. Mansurov, in: *Problems of Gastroenterology* [in Russian], V. Kh. Vasilenko (Ed.), Vol. 7, Dushanbe (1987), pp. 9-20.
8. S. Ya. Sokolov and I. P. Zamotaev, *A Reference Book of Medicinal Plants* [in Russian], Moscow (1989).