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Effect of tamoxifen, raloxifen and tibolon on bile components in ovariectomized rats

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Abstract

The aim of the study was to investigate the effect of ovariectomy on the bile composition in order to estimate the ability of selective estrogen receptor modulators (SERMS) (tibolon, tamoxifen, raloxifen) to modify the ovariectomy-induced disorders. The study was carried out on the ovariectomized female Wistar rats. Tibolon (1 mg kg^{-1} 24 h^{-1}), tamoxifen (5 mg kg^{-1} 24 h^{-1}) and raloxifen (10 mg kg^{-1} 24 h^{-1}) were administered for 42 days. Under anesthesia bile was collected during 6 h period. The ovariectomy increased significantly the excretion of biliary acids and calcium in bile and decreased the excretion of cholesterol and chloride. In rats treated with tamoxifen and raloxifen the excretion and concentration of cholesterol in bile were significantly reduced in comparison with ovariectomized rats. In rats treated with tibolon these values were increased. Moreover in rats treated with tamoxifen and raloxifen the concentrations of calcium in bile were significantly reduced. Tibolon had no significant effect on bile calcium concentrations. The therapy with tamoxifen, raloxifen and tibolon decreased the serum cholesterol concentrations, whereas the bile acid concentrations were increased in comparison with ovariectomized control. The drugs studied had no significant effect on calcium and chloride serum concentrations. Our results suggest that the therapy with tamoxifen and raloxifen may have the positive effect on bile composition in ovariectomized rats and probably may prevent the gallstone formation.

Keywords: SERMS; Bile; Gallstones

1. Introduction

The selective estrogen receptor modulators (SERMS) represent a major therapeutic advance for clinical practice. Unlike estrogens, which are uniformly agonists, and antiestrogens, which are uniformly antagonists, the SERMS exert selective agonist or antagonist effects on various estrogen target tissues. The SERMS are chemically diverse compounds that lack the steroid structure of estrogens but possess a tertiary structure that allows them to bind to the estrogen receptor [1,2].

In the classic model of estrogen action, the nuclear estrogen receptor resides in the nuclei of target cells in an inactive form. Binding to an agonist, such as estradiol, alters the physiochemical properties of the estrogen receptor, allowing the receptor dimmer to interact with specific DNA sequences (estrogen response elements) within the promoters of responsive genes. The DNA-bound estrogen receptor then regulates target gene transcription, either positively or negatively [3,4]. However, the recognition that tamoxifen and other SERMS have tissue-specific agonist-antagonist activity led to the realization that the classic model was

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incomplete and that estrogen action was more complex than had been thought. The mechanisms of the tissue selective, mixed agonist-antagonist action of SERMS, although still only partly understood, are gradually becoming clearer. SERMS are widely used in breast cancer therapy and in the treatment of osteoporosis in postmenopausal women. Decreases in estrogens induced by ovariectomy or aging lead to loss of skeletal mass, osteoporotic fractures as well as to disturbances in lipid metabolism. In previous study we have shown that tamoxifen prevented the bone mass reduction induced by ovariectomy and exerted the positive effect lipid metabolism [5]. Therefore this therapy may be the alternative form of treatment in postmenopausal women with the contradictions to hormonal replacement.

Although different estrogen regimens or dosages would not necessarily have provided similar results, the trial results bring into sharper focus the risks of hormone-replacement therapy in its association with an increase in stroke, pulmonary embolism, and breast cancer risk [6,7]. The estrogen replacement may be also associated with enhanced risk of gallstones [8,9]. It seems also that therapy with SERMS may have beneficial effect of estrogens without some adverse events.

The aim of the study was to evaluate the effect of tibolon, tamoxifen and raloxifen on bile composition in ovariectomized rats as the model of estrogen deficiency.

2. Materials and methods

Female Wistar rats were used in this experiment. They were allowed to acclimatize for a minimum of 10 days prior to the study. The rats were housed in the room maintained at 21 ± 1 °C with 12 h light-dark cycle with the light cycle beginning at 6:00 a.m. At 3 months of age, 40 females (body weight 238.3 ± 10.4 g) were anesthetized with a combination of ketamine (Ketolar) and xylazin (Rompun) and were ovariectomized (40 individuals) or sham-operated (10 individuals). After surgery the rats were allowed a recovery period till the beginning of the experiment. The rats were divided into five groups (n = 10) as follows:

(1) ovariectomized control,
(2) sham-operated controls,
(3) tibolon 1 mg kg^{-1} 24 h^{-1} orally,
(4) tamoxifen 5 mg kg^{-1} 24 h^{-1} orally,
(5) raloxifen 10 mg kg^{-1} 24 h^{-1} orally.

The drugs were administered orally in balls once a day. The first and second groups received the vehicle. The treatment was continued for 42 days. Twenty-four hours before experiment the animals were starved with free access to water. After 42 days of drugs administration under anesthesia with ethyl urethane (1.7 g/kg i.p.) the right external jugular vein and common bile duct were cannulated and polyethylene catheter was introduced into the bile duct. Bile was collected during 6 h period. Bile was analyzed for total cholesterol, total pool of bile acids, calcium and chloride. The above components were also analyzed in serum.

2.1. Biochemical analysis

Blood was obtained by cardiac puncture, allowed to clot at 4 °C and then centrifuged at 3000 × g for 10 min. The bile acid concentration in bile and serum was determined using enzymatic assay kit Mercotest (Merk, Germany). The measurement was performed using UV–vis Marcel Pro Spectrophotometer (Marcell Serwis, Warszawa, Poland) at a wavelength of 500 nm.

The concentration of cholesterol in bile and serum was determined using enzymatic cholesterol assay Biochemtest (POCH, Gliwice, Poland). The measurement was performed using UV–vis Marcel Pro Spectrophotometer (Marcell Serwis, Warszawa, Poland) at a wavelength of 500 nm.

The concentration of calcium in bile and serum was determined using Wapń-Biochemtest kit (POCH, Gliwice, Poland). The measurement was performed using UV–vis Marcel Pro Spectrophotometer (Marcell Serwis, Warszawa, Poland) at a wavelength of 575 nm.

The concentration of chloride in bile and serum was determined using Chlorki MPK-Biochemtest kit (POCH, Gliwice, Poland). The measurement was performed using UV–vis Marcel Pro Spectrophotometer (Marcell Serwis, Warszawa, Poland) at a wavelength of 457 nm.

The differences between groups were statistically evaluated using Cochran–Cox test.

3. Results

As shown in Table 1 there were no differences in bile excretion between sham-operated and ovariectomized rats. In rats treated with tamoxifen, raloxifen and tibolon we observed the significant reduction of excreted bile. Moreover in rats treated with tamoxifen and raloxifen the excretion of cholesterol was significantly reduced in comparison with ovariectomized rats (Table 1).

In ovarictomized rats the increased excretion of calcium in bile was observed. The administration of drugs studied significantly reduced the excretion of calcium (Table 1).

As shown in Table 2 the administration of tamoxifen and raloxifen significantly reduced the concentration of cholesterol in bile. In rats treated with tibolon these values were increased. Moreover the administration of tamoxifen and tibolon significantly increased the concentration of bile acids in bile as compared with ovariectomized control.

In ovariectomized rats the increased concentration of calcium in bile was observed as compared with sham-operated control. In animals treated with tamoxifen, raloxifen and tibolon these values were reduced (Table 2).

In ovariectomized rats the increased concentration of serum cholesterol was observed. As shown in Table 3, the

Table 1
The excretion of bile, cholesterol, bile acids, calcium and chloride during 6 h of study

Animals	Bile (g/100 g b.w.)	Cholesterol (μmol/100 g b.w.)	Bile acids (μmol/100 g b.w.)	Calcium (μmol/100 g b.w.)	Chloride (μmol/100 g b.w.)
Sham-operated control	1.321 ± 0.154	0.568 ± 0.077	10.890 ± 1.227	1.992 ± 0.348	123.524 ± 12.042
Ovariectomized control	1.299 ± 0.149	0.443 ± 0.058**	14.428 ± 1.566**	2.648 ± 0.415**	105.762 ± 11.158**
Rats treated with tamoxifen	0.826 ± 0.096*	0.131 ± 0.034*	11.783 ± 1.324*	1.227 ± 0.205*	70.260 ± 9.041*
Rats treated with raloxifen	0.922 ± 0.114*	0.215 ± 0.028*	10.460 ± 1.119*	1.437 ± 0.219*	77.781 ± 8.187*
Rats treated with tibolon	0.911 ± 0.102*	0.458 ± 0.072	15.838 ± 1.713	1.878 ± 0.377*	83.596 ± 9.429*

* $P < 0.05$ vs. ovariectomized control.
** $P < 0.05$ vs. sham-operated control.

Table 2
The mean concentration of cholesterol, bile acids, calcium and chloride in collected bile

Animals	Cholesterol (μmol/1 g)	Bile acids (μmol/1 g)	Calcium (μmol/1 g)	Chloride (μmol/1 g)
Sham-operated control	0.434 ± 0.055	8.247 ± 1.004	1.508 ± 0.321	93.517 ± 11.004
Ovariectomized control	0.337 ± 0.041**	11.105 ± 1.378**	2.038 ± 0.278**	81.425 ± 10.101**
Rats treated with tamoxifen	0.165 ± 0.024*	14.260 ± 1.807*	1.485 ± 0.280*	85.524 ± 9.969
Rats treated with raloxifen	0.227 ± 0.036*	11.350 ± 1.406	1.559 ± 0.301*	84.357 ± 9.224
Rats treated with tibolon	0.553 ± 0.059*	15.978 ± 1.826*	1.895 ± 0.338	84.321 ± 11.105

* $P < 0.05$ vs. ovariectomized control.
** $P < 0.05$ vs. sham-operated control.

administration of tamoxifen raloxifen and tibolon significantly reduced the serum concentration of cholesterol. Moreover the drugs studied significantly increased the serum concentration of bile acids, but had no effect on serum concentrations of calcium and chloride (Table 3).

4. Discussion

In our study, we have examined the influence of tibolon, tamoxifen and raloxifen on excretion of bile and some of its components in ovariectomized rats. Recent reports suggest that SERMS administration may be the alternative form of therapy in cases in which the hormonal replacement is contraindicated. The therapy with estrogen may be associated with increased risk of breast cancer, stroke, and pulmonary embolism as well as with gallstones [10–14]. The aim of this study was to evaluate the influence of SERMS on excretion and concentration of cholesterol, biliary acids and calcium in bile, factors which may be associated with the pathogenesis of gallstones. The therapy with tamoxifen and raloxifen significantly reduced the excretion and concentration of cholesterol and calcium in bile. These properties may suggest the beneficial effect of tamoxifen and raloxifen on gallstone generation. Risk factors associated with gallbladder disease include older age, female sex, white ethnicity/race, obesity, rapid weight loss, and among women greater parity, use of oral estrogen-containing contraceptives, and postmenopausal estrogen therapy [15,16]. Some follow studies suggest that estrogens increase the risk for gallbladder disease by as much as two- to four-fold [17,18].

In the Coronary Drug Project [19], which studied prevention of secondary coronary heart disease, high-dose estrogen therapy conferred a 65% increased risk for clinical gallbladder disease. In the study of Henriksson et al. [20], men with prostate cancer were randomly assigned to receive intramuscular and oral estrogens or to undergo orchiectomy. At 1-year follow-up, 5 of 28 men who had received estrogens had developed gallstones compared with none of the 26 men who had undergone orchiectomy. Eighty-four matched pairs of postmenopausal women in an earlier, 10-year study [21] were randomly assigned to receive high-dose estrogens; the women receiving postmenopausal hormone

Table 3
The concentration of cholesterol, bile acids, calcium and chloride in serum

Animals	Cholesterol (μmol/dm^3)	Bile acids (μmol/dm^3)	Calcium (μmol/dm^3)	Chloride (μmol/dm^3)
Sham-operated control	2.166 ± 0.149	105.105 ± 12.611	2.332 ± 0.138	92.749 ± 10.896
Ovariectomized control	2.555 ± 0.322**	85.915 ± 9.829**	2.021 ± 0.222	88.217 ± 10.294
Rats treated with tamoxifen	1.119 ± 0.113*	120.472 ± 13.344*	1.982 ± 0.098	86.441 ± 10.117
Rats treated with raloxifen	1.187 ± 0.131*	125.041 ± 14.267*	1.990 ± 0.230	89.041 ± 10.374
Rats treated with tibolon	0.848 ± 0.115*	131.365 ± 13.419*	2.066 ± 0.277	85.302 ± 9.947

* $P < 0.05$ vs. ovariectomized control.
** $P < 0.05$ vs. sham-operated control.

therapy had a no significantly higher incidence of gallstones than the placebo recipients. Most gallstones are composed partially or entirely of cholesterol, and three factors are required for the formation of cholesterol gallstones: supersaturation of bile with cholesterol, bile stasis, and destabilization of bile [22]. Cholesterol gallstones are formed when bile that is supersaturated with cholesterol becomes destabilized. By decreasing biliary acid synthesis and secretion, oral estrogen therapy alters the composition of bile, resulting in increased concentrations of biliary cholesterol. Whether progestins affect the risk for clinical gallbladder disease is less clear; progestins may inhibit gallbladder motility [23] and decrease cholesterol 7 α-hydroxylase activities, which, in turn, may affect the rate of cholesterol catabolism to bile acids [24]. The effect of orally and transdermally administered estrogens on the risk for gallbladder disease and biliary tract surgery may also be different. Compared with oral estrogens, transdermally administered estrogens, which avoid first-pass liver metabolism, have delayed or decreased effects on lipids [25] and may not induce lithogenic bile [26]. The results of present study suggest that tamoxifen and raloxifen may reduce the concentrations of cholesterol and calcium in bile. It seems also that the administration of these drugs may be the alternative form of therapy in postmenopausal women, which may also eliminate the disorders in bile composition predisposing to gallstones formation.

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