INFLUENCE OF LONG-TERM PER ORAL TAURINE TREATMENT ON ACTIVITY OF LIVER ENZYMES IN MATURE RATS

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Taurine is sulfur-containing derivative of methionin and cysteine that plays a key role in glucose and lipid metabolism. Taurine regulates antioxidant defense system, membrane potential of mitochondria, and glycolysis. Thus, it might have a serious impact on cell metabolism in mammals. The aim of this work was to study the influence of long-term per oral taurine treatment (5, 10 and 20 mg/kg) on the antioxidant defense system, cholesterol content, activity of transaminases and lactate dehydrogenase in rat liver. To achieve that goal, mature Wistar rats (weight 140–160 g, age – 4 months) were divided in four groups – control, rats which once a day during 28 days were injected drinking water in esophagus, and three experimental groups, that were injected taurine solution in concentrations: I – 5 mg/kg, II – 10 mg/kg and III – 20 mg/kg of body mass. After 28 days, rats were decapitated under light chloroform narcosis and liver mass was determined. In liver, content of cholesterol, TBA-active products and activity of antioxidant defense enzymes, transaminases, and lactate dehydrogenase were measured.

Our results show that after a long-term per oral taurine treatment liver mass decreased by 27.5 % comparing to control group. Lactate dehydrogenase activity increased two times, while the activity of alanine aminotransferase and aspartate aminotransferase in mitochondrial fraction increased by 40–90 % in liver of animals of all experimental groups. Total activity of these enzymes in rat liver increased by 20–37 % in all experimental groups. Cholesterol content increased by 32.4 % in rats of experimental group II. This may indicate intensification of liver metabolism. The content of TBA-active products in submitochondrial and mitochondrial fractions increased more than two times in rats of experimental groups II and III. The activity of superoxide dismutase in the submitochondrial fraction increased by 49.8 and 36.5 % in liver of that rats. The activation of superoxide dismutase and a rise in content of TBA-active products may suggest a rise of free oxygen radicals production and inability of antioxidant defense system. These results may indicate a negative influence of taurine in doses higher than 5 mg/kg, since the balance between antioxidant protection and lipid peroxidation processes was violated.

**Keywords:** rats, liver, antioxidant defense, taurine, lactate dehydrogenase, transaminases
INTRODUCTION

Liver is a key organ in mammals, that can influence an intensity of energy metabolism of the whole organism [19]. Hepatocytes are main cell type in liver and it is a central link in biochemical transformations in the energetic metabolism [27]. Taurine that is a derivative of sulfur-containing aminoacids methionine and cysteine participates in regulation of liver metabolic activity [26].

Taurine is a component of bile acids that facilitates cholestasis, maintains activity of antioxidant defense system and electron transport chain [12]. Depletion of taurine results in a decrease of mitochondria quantity in hepatocytes, increase of peroxidative stress, and cell membrane damage, hepatocyte apoptosis, and liver fibrosis [4]. Surplus of taurine has a variable effect on liver metabolism. Per oral treatment of rats diabetic with taurine in doses 50–100 mg/kg results in activation of antioxidant defense system and a decrease in TBA-active products [12]. Treatment of rats with 1 % taurine solution for 15 days increased the content of TBA-active products and catalase activity in liver [16]. Per oral treatment for 28 days with taurine in doses 40 and 100 mg/kg increased lactate dehydrogenase activity and intensified mitochondrial respiration of hepatocytes [22, 23]. However, lipid peroxidation processes were also increased that resulted in the increase of TBA content [24]. Increase of AST activity in blood plasm points on hepatocyte membrane damage that may be caused by uncontrolled elevation of peroxidation processes [21].

Thus, it is necessary to indentify optimal doses of taurine that influence liver metabolism without negative consequences. This will enable development new pharmacological preparations and feed additives containing taurine. The aim of our work was to study influence of long-term per oral taurine treatment on the activity of liver enzymes of in mature rats.

MATERIALS AND METHODS

Experiments were carried out using male Wistar rats. The rats were 4 months old with body mass 140–160 g at the beginning of the experiment. All manipulations with animals were carried out according to the European Convention “For the protection of vertebrate animals used for experimental and other scientific purposes” and Ukrainian Law “On protecting animals of ill-treatment”. Approval for study was obtained from the Ethics Committee of the Biological faculty of Ivan Franko National University of Lviv (Ethical Committee Approval, protocol No 8-03 by March 03, 2018). Animals were randomly divided into four groups: one control group (n = 4) and the experimental groups. During 28 day period, animals were daily injected in esophagus: control group – drinking water, group I, II and III-solution of taurine (5, 10 and 20 mg/kg of body weight, respectively) (n = 4). On 29th day, the animals were decapitated under light chloroform anesthesia. Liver was extirpated, weighted and homogenized in solution of such composition: sucrose – 250 mM, EGTA – 1 mM, HEPES – 10 mM; pH 7.2 [9]. The homogenate was centrifuged for 15 min at 1,000 g. After that, supernatant was discarded and centrifuged for 20 min at 9,000 g. After second centrifugation supernatant was discarded and precipitate was resuspended in homogenating solution. In supernatant and resuspended precipitate, the activities of transaminases (aspartate (AST) and alanine (ALT) transaminase $\lambda_{abs} = 412$ nm) were determined according to Reitman and Frankel [6].
Activity of such antioxidant defense enzymes in mitochondrial and subcellular fraction were measured: superoxide dismutase with nitroblue tetrazolium (SOD; $\lambda_{abs} = 540$ nm; UI/mg of protein [1]), glutathione peroxidase – with Elman reagent (GPO; $\lambda_{abs} = 412$ nm; $\mu$mol GSH/min×mg of protein [17]), catalase – with ammonium molybdate and hydrogen peroxide (CAT; $\lambda_{abs} = 410$ nm; $\mu$mol H$_2$O$_2$/min×mg of protein [11]). The content of TBA-active products was determined using phosphorwolframic and thiobarbituric acids ($\mu$mol/mg of protein [10]). Protein content was measured using Lowry method [14].

In supernatant, the activity of lactate dehydrogenase was defined by kinetic method (LDH; UI/mg of protein; $\lambda_{abs} = 340$ nm [5]). In the subcellular fraction, cholesterol content was measured by the enzymatic method ($\lambda_{abs} = 500$ nm; $\mu$mol/mg of protein [6]).

The inter relations between superoxide dismutase and catalase (SOD/CAT), glutathione peroxidase and catalase activity (GPO/CAT) and superoxide dismutase and glutathione peroxidase activities (SOD/GPO) were calculated. Ratio between the activity of antioxidant defense enzymes and content of TBA-active products was calculated (index of antioxidant status):

$$\text{AOD/TBA} = \frac{\text{SOD} \times \text{CAT} \times \text{GPO}}{\text{TBA}}$$

where SOD, CAT and GPO are activities of corresponding enzymes of antioxidant defense, TBA – content of TBA-active products.

The results were analyzed by variation statistics using Microsoft Excel 2010. Results were presented in the form of arithmetic mean and arithmetic mean error (М±m), significance of the data difference was calculated using the paired samples Student t-test for independent samples [15].

RESULTS AND DISCUSSION

The liver mass in animals of the control group was 8.04±0.48 g. In rats of I and III experimental groups, the liver mass did not differ significantly from the control values (7.71±0.46 and 8.37±0.61 g, n = 4). The mass of liver in animals of II experimental group was 6.30±0.34 g that lower by 27.5% (P <0.05, n = 4) than in control.

Literature data showed that a prolonged oral administration of taurine in doses of 40 and 100 mg/kg leads to an increase in the LDH activity in rat liver [23]. It was found that at lower doses of taurine (5–20 mg/kg), the activity of this enzyme increased by 1.65–2 times compared to control (Table 1).

Cholesterol content in liver of I and III experimental groups did not differ from the control indicators, and in the II experimental group it was 32.4% higher than in the control group. It was found that taurine increased bile acid content in liver [13]. It is likely that an increase in cholesterol content is due to the intensification of bile acid synthesis. In addition, an increase in the LDH activity in rat liver leads to an increase in concentration of pyruvate, that can be used for synthesis of cholesterol [18].

Total activity of AST in animals liver in the I and II experimental groups was higher by 21.4 and 19.7% than in the control group. The activity of the mitochondrial form of AST (mAST) was higher by 93.3 and 80.0% compared to control. Despite the fact that in the liver of rats of the III experimental group the activity of AST remains on the control level, the activity of its mitochondrial isozyme was higher by 83.3%, and the cytoplas-
mic one was lower by 20.6% than in the liver of animals of the control group. Thus, the total activity of AST in the liver of rats of all experimental groups was increased by one to the activity of mAST (Fig. 1).

**Table 1. Biochemical parameters in rat liver during long-term taurine treatment**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Animal group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>LDH, UI/mg of protein</td>
<td>2.73±0.33</td>
</tr>
<tr>
<td>Cholesterol content, µmol/mg of protein</td>
<td>69.4±2.7</td>
</tr>
<tr>
<td>AST, µcatal/mg of protein</td>
<td>0.217±0.005</td>
</tr>
<tr>
<td>cAST, µcatal/mg of protein</td>
<td>0.187±0.007</td>
</tr>
<tr>
<td>mAST, µcatal/mg of protein</td>
<td>0.030±0.001</td>
</tr>
<tr>
<td>ALT, µcatal/mg of protein</td>
<td>0.229±0.008</td>
</tr>
<tr>
<td>cALT, µcatal/mg of protein</td>
<td>0.184±0.007</td>
</tr>
<tr>
<td>mALT, µcatal/mg of protein</td>
<td>0.045±0.003</td>
</tr>
</tbody>
</table>

**Comments:** here and below * – statistically significant difference compared to values of the control group with P<0.05; ** – with P<0.01; *** – with P<0.001

**Fig. 1.** The effect of long-term oral administration of taurine on percentage of mitochondrial (mAST) and cytoplasmic (cAST) isozymes activity of aspartate aminotransferase on the background of changes in the total activity of AST (tAST)

**Рис. 1.** Вплив тривалого перорального введення таурину на відсоткове співвідношення активностей ізозимів мітохондріальної (мАСТ) та цитоплазматичної (цАСТ) аспартатамінотрансфераз на тлі зміни загальної активності АСТ (зАСТ)
Elevated mAST activity indicates an increase in the aspartate and α-ketoglutarate synthesis. In its turn, α-ketoglutarate can be included in the Krebs cycle or used as a co-substrate in the cytoplasmic oxygenase reactions [25].

The total ALT activity was increased by 37.1 and 28.0 % in rat liver of I and II experimental groups, respectively. The activity of cytoplasmic isozyme was increased (cALT, by 27.2 and 24.5 %), and of the mitochondrial isozyme (mALT; by 77.8 and 42.2 %, respectively, Table 1). Although the activity of ALT was not increased in liver of animals of III experimental group, the activity of its mitochondrial isozyme was increased by 35.6 %. An increase of the total activity of ALT in the I and III study group was due to growth of mALT (Fig. 2). In II experimental group, the ratio between isozyme activity remained unchanged.

Fig. 2. Change in ratio between isozyme activity of mitochondrial (mALT) and cytoplasmic (cALT) alanine aminotransferase on the background of a change in total activity ALT (tALT) after a long-term per oral injection of taurine

An increase in mALT activity in animals of all experimental groups indicates an intensification of the production of pyruvate and glutamate [2]. Glutamate is used in mitochondria as a substrate for transamination and formation of aspartate [7]. Pyruvate is oxidized by the pyruvate dehydrogenase and is included into Krebs cycle, or into synthesis of cholesterol, or glucose in the hepatocyte cytoplasm [8]. The latter is less likely, since in animals of all experimental groups the concentration of glucose in blood was lower than in control animals [20]. Increased concentration of pyruvate can intensify the rate of Krebs cycle reactions and increase the transport of electrons in the respiratory chain. This caused intensification of peroxide lipid oxidation and increased activity of antioxidant enzymes [18].

It was shown that oral treatment with taurine for 28 days in doses of 40 and 100 mg/kg increased the activity of SOD in rats liver [24]. An injection of taurine in esophagus in
10 and 20 mg/kg doses during the same period increased the activity of SOD in the submithochondrial fraction of rat liver by 49.8 and 36.5 % (Table 2). The activity of GPO and KAT in the submithochondrial fraction of liver remained at the control level in animals of all experimental groups. Nevertheless, the GPO/ CAT ratio decreased equally – by 10.6 % – in rats in the I and II experimental groups, that may indicate an increase in production of the hydrogen peroxide. The growth of SOD activity in the submithochondrial liver fraction of II and III experimental groups led to an increase by 40 and 46.7 % of SOD / KAT ratio and by 55.9 and 48.4 % of the SOD / GPO ratio. That might indicate an increase in the production of superoxide anion radical. Despite an increase in SOD activity, the content of TBA-active products increased 2.8 and 3.0 times in rats of the II and III experimental groups. A 2–3 times decrease of AOD/TBA coefficient in rats of these groups indicates an inability to utilize formed free oxygen radicals. In the submithochondrial fraction of rat liver of I experimental group, the content of TBA-active products was 21.1 % lower than in the control. This may suggest that in low doses taurine inhibits lipid peroxidation in rat liver, and in high doses – it intensifies it. The latter might by a result of intensification of oxidative metabolism that leads to the activation of superoxide dismutase [18].

In the mitochondrial fraction of all experimental group rats, the activity of SOD and CAT remained at the control level. In animals of I experimental group, GPO activity was 20.4 % higher than in control. At the same time, the content of TBA-active products increases by 2–2.5 times and AOP/TBC ratio reduced by 75.4 and 37.6 % in liver of the mitochondrial fraction of animals of the II and III experimental groups. This indicates an increase in the processes of lipid peroxide oxidation, releasing oxygen free radicals, and, possibly, the intensification of transport of electrons through the respiratory chain. In liver mitochondrial fraction of III experimental group rats, the HPA / CAT ratio reduced by 25.0 % that may indicate an increased production of \( \text{H}_2\text{O}_2 \).

Consequently, a long-term oral treatment of taurine at 5–20 mg/kg doses led to increased the activity of LDH in liver. This may indicate an increase in the production of lactate by other tissues, since liver is a central organ where lactate is transformed into pyruvate [13]. In turn, pyruvate which is produced by LDH in liver, can be used for synthesis of cholesterol or be included in Krebs cycle, thus, increasing concentration of \( \text{NADH}^+ \) and \( \text{FADH}_2 \) [18] (Fig. 3).
increase in the content of TBA-active products in the submithondrial fraction, as superoxide anion is released during the oxidation of the substrates. In addition, the elevation of α-ketoglutarate synthesis may result in the increase of mALT activity [2].

In conclusion, long-term per oral taurine treatment in dose 5 mg/kg for 28 days resulted in transaminase (AST and ALT) and LDH activity rise. That might led to inten-

Table 2. Influence of long-term taurine treatment on antioxidant defense in rat liver

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Animal group</th>
<th>Control</th>
<th>I (5 mg/kg)</th>
<th>II (10 mg/kg)</th>
<th>III (20 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<tr>
<td>Submitochondrial fraction</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOD, UI/mg of protein</td>
<td></td>
<td>2.19±0.26</td>
<td>2.36±0.17</td>
<td>3.28±0.10**</td>
<td>2.99±0.10*</td>
</tr>
<tr>
<td>GPO, μmol GSH/min×mg of protein</td>
<td></td>
<td>0.230±0.014</td>
<td>0.214±0.015</td>
<td>0.218±0.006</td>
<td>0.214±0.015</td>
</tr>
<tr>
<td>CAT, μmol H₂O₂/min×mg of protein</td>
<td></td>
<td>14.94±0.76</td>
<td>15.75±0.52</td>
<td>16.02±0.11</td>
<td>13.94±0.70</td>
</tr>
<tr>
<td>TBA-active products, mmol/mg of protein</td>
<td></td>
<td>0.147±0.007</td>
<td>0.116±0.007*</td>
<td>0.593±0.032*</td>
<td>0.568±0.040*</td>
</tr>
<tr>
<td>SOD/CAT, UI</td>
<td></td>
<td>0.15±0.02</td>
<td>0.15±0.01</td>
<td>0.21±0.01*</td>
<td>0.22±0.01*</td>
</tr>
<tr>
<td>SOD/GPO, UI</td>
<td></td>
<td>9.52±1.11</td>
<td>10.85±0.03</td>
<td>14.84±0.63**</td>
<td>14.13±1.26*</td>
</tr>
<tr>
<td>GPO/CAT, UI</td>
<td></td>
<td>15.5±0.2</td>
<td>13.8±0.2*</td>
<td>13.8±0.2*</td>
<td>15.65±1.0</td>
</tr>
<tr>
<td>AOD/TBA, UI</td>
<td></td>
<td>53.33±10.13</td>
<td>69.81±3.60</td>
<td>19.77±1.24*</td>
<td>15.81±2.43*</td>
</tr>
<tr>
<td>Mitochondrial fraction</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOD, UI/mg of protein</td>
<td></td>
<td>0.39±0.03</td>
<td>0.45±0.03</td>
<td>0.45±0.03</td>
<td>0.39±0.03</td>
</tr>
<tr>
<td>GPO, μmol GSH/min×mg of protein</td>
<td></td>
<td>0.054±0.002</td>
<td>0.065±0.004*</td>
<td>0.056±0.003</td>
<td>0.060±0.001</td>
</tr>
<tr>
<td>CAT, μmol H₂O₂/min×mg of protein</td>
<td></td>
<td>2.21±0.08</td>
<td>2.67±0.21</td>
<td>2.48±0.11</td>
<td>2.29±0.06</td>
</tr>
<tr>
<td>TBA-active products, mmol/mg of protein</td>
<td></td>
<td>0.047±0.001</td>
<td>0.045±0.004</td>
<td>0.112±0.008**</td>
<td>0.093±0.005**</td>
</tr>
<tr>
<td>SOD/CAT, UI</td>
<td></td>
<td>0.18±0.01</td>
<td>0.17±0.01</td>
<td>0.18±0.01</td>
<td>0.13±0.01</td>
</tr>
<tr>
<td>SOD/GPO, UI</td>
<td></td>
<td>7.11±0.35</td>
<td>6.80±0.24</td>
<td>8.06±0.24</td>
<td>6.58±0.61</td>
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<tr>
<td>GPO/CAT, UI</td>
<td></td>
<td>0.025±0.001</td>
<td>0.025±0.001</td>
<td>0.023±0.001</td>
<td>0.020±0.001**</td>
</tr>
<tr>
<td>AOD/TBA, UI</td>
<td></td>
<td>1.00±0.12</td>
<td>1.76±0.23*</td>
<td>0.57±0.05*</td>
<td>0.727±0.03*</td>
</tr>
</tbody>
</table>
sification of liver metabolism. After treatment with taurine solution in doses 10 and 20 mg/kg, the activity of transaminases and LDH were higher than in control, and the content of TBA-products was increased.

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AKTIVNISTь ЕНЗИМІВ ПЕЧІНКИ СТАТЕВОЗРІЛИХ ЩУРІВ ЗА ТРИВАЛОГО ПЕРОРАЛЬНОГО ВВЕДЕННЯ ТАУРИНУ

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Таурин – сірковмісна похідна метіоніну і цистеїну, що відіграє ключову роль у метаболізмі глюкози та ліпідів. Відомо, що таурин регулює активність антиоксидантної системи, мембранний потенціал мітохондрій і гліколіз. Таким чином, таурин відіграє ключову роль у метаболізмі клітин організму ссавців. Метою роботи було дослідити вплив тривалого перорального введення таурину дозами 5, 10 та 20 мг/кг на стан антиоксидантного захисту, вміст холестерину, активність ензимів трансамінування та лактатдегідрогенази у печінці щурів. Для досягнення мети статевозрілих самців щурів лінії Wistar (масою 140–160 г та віком 4 місяці) розділяли на чотири групи – контрольну, яким протягом 28 діб щоденно вводили у стравохід питну воду (контроль), та три дослідні, яким вводили таурин у дозах: І дослідна – 5 мг/кг, ІІ дослідна – 10 мг/кг і ІІІ дослідна групи – 20 мг/кг маси тіла. У печінці щурів визначали: масу, активність ензимів антиоксидантного захисту, трансамінування, лактатдегідрогенази, вміст холестерину і ТБК-активних продуктів.

У результаті досліджень виявлено, що тривале пероральне введення таурину приводить до зниження маси печінки на 27,5 % у щурів ІІ дослідної групи. Активність лактатдегідрогенази зростає у два рази та мітохондріальних ензимів трансамінування – на 40–90 %, у тварин усіх дослідних груп. Загальна активність аспартат-і аланін амінотрансфераз зростає на 20–37 % у всіх дослідних групах. У тварин І ІІ дослідної групи на 32,4 % зростає вміст холестерину. Це може вказувати на інтенсифікацію метаболізму печінки. Водночас у ІІ та ІІІ дослідних групах зростає більш як удвічі вміст ТБК-активних продуктів у субмітохондріальній і мітохондріальній фракції. Активність супероксиддисмутази у субмітохондріальній фракції –
зростає на 49,8 і 36,5 %. Активація супероксиддисмутази та зростання вмісту ТБК-активних продуктів може вказувати на зростання продукції вільних радикалів окис-гену і нездатність антиоксидантної системи захисту утилізувати їх. Наші результа-ти можуть свідчити про негативний вплив доз таурину, вищих, ніж 5 мг/кг, оскільки порушується баланс між антиоксидантним захистом і процесами пероксидного окиснення ліпідів.

Ключові слова: щури, печінка, антиоксидантний захист, таурин, лактатдегідро-геназа, ензими трансамінування