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EFFECT OF 7-DAY INTRODUCTION OF GLUTATHIONE ON ACTIVITIES OF H_2S-PRODUCING ENZYMES IN THE LIVER OF RATS UNDER EXPERIMENTAL NEPHROPATHY CONDITIONS

Introduction. *Kidney diseases are a worldwide health problem. The renal dysfunctions cause the production of reactive oxygen species and can co-exist with different liver disease, or stimulate their development, so scientists are becoming more interested in the study of the influence of antioxidants such as glutathione on metabolic pathways of the body under conditions of different disease. Hydrogen sulfide has anti-inflammatory, anti-oxidant and anti-apoptotic effects that are mediated by its ability to downregulate the synthesis of lipid peroxides and reactive oxygen species-producing enzymes and may play an important role in the mechanism of development of nephropathy.*

The aim of the study *– to learn the effect of glutathione introduction within 7 days on the system of H_2S production in the liver of rats under conditions of experimental nephropathy.*

Research Methods. *The experiment was conducted on albino mature male rats. The animals in experimental group were administered a single intraperitoneal dose of folic acid (250 mg/kg). Glutathione was introduced intragastral (100 mg/kg) within 7 days after intoxication. The activity of H_2S-producing enzymes, the concentration and production of H_2S were measured in the liver.*

Results and Discussion. *Under conditions of experimental nephropathy there was a decrease in the concentration and production of hydrogen sulfide compared with the control group. The introduction of glutathione increased the content of hydrogen sulfide and promoted the growth of the activities of H_2S-producing enzymes in the liver of rats.*

Conclusions. *It was found that the content and production of hydrogen sulfide in the group of animals with nephropathy were diminished by a decrease in the activities of hepatic H_2S-producing enzymes. The introduction of glutathione increased the content of hydrogen sulfide by stimulation the activities of cystathionine-β-synthase and cysteinaminotransferase in the liver of rats. As reasons for this effect, antioxidant properties of glutathione and the possibility of including tripeptide as a source of cysteine in the synthesis of hydrogen sulfide are considered.*

KEY WORDS: **nephropathy; hydrogen sulfide; glutathione.**

INTRODUCTION. Renal disease is a common worldwide health problem. Kidney disease can lead to the oxidative stress related to an imbalance between free radical production and decreased antioxidant capacity. The renal dysfunctions can co-exist with a different liver disease, or stimulate their development.

H_2S has been established [1, 2] to possess potent antioxidant and anti-inflammatory properties suggesting that it could protect or retard progression of some disease. Hepatic H_2S metabolism affects glucose metabolism, insulin sensitivity, lipoprotein synthesis, mitochondrial biogenetics and biogenesis [3, 4]. Malfunction of H_2S metabolism may be involved in many diseases. It is known that plasma H_2S level markedly reduced in patients with nephropathy, so the use of antioxidants is important for the protection of the body by renal disease.

Therefore, investigating the association between kidney disease and metabolism of H_2S in the liver and influence of glutathione (GSH) as one of the antioxidants [5–7] that may take part in the metabolism of H_2S has important practical value because the understanding of metabolic pathways by pathophysiological conditions will help to improve therapeutic strategies for patients with kidney and liver diseases.

The aim of the study – to determine the effect of glutathione introduction within 7 days on the system of H_2S-producing enzymes, concentration and production H_2S in the liver of rats under conditions of experimental nephropathy.

RESEARCH METHODS. The experiment was conducted on 83 male albino rats with the body weight of 0.16–0.18 kg. Experimental nephropathy was modeled by injection of a single intraperitoneal dose of folic acid (250 mg/kg) [8]. In order to confirm the pathology the kidneys were examined by morphometric analysis. Glutathione was introduced daily (100 mg/kg) by intragastric way for 7 days following after the injection of folic acid. Animals were devided into 3 groups: I – control group (n=36), II – nephropathy (7^{th} day (n=24)), III – nephropathy + 7 days of glutathione introduction (n=23). Rats were kept under the standard vivarium conditions at constant temperature and basic allowance. Animals were narcotized with chloroform and sacrificed on the next day after the last glutathione introduction. All manipulations with animals were carried out according to European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes and law of Ukraine "On protection of animals from cruelty".

The activity of H_2S-producing enzymes (cystathionine-γ-lyase (CSE), cystathionine-β-synthase (CBS) and cysteinaminotransferase (CAT)), the concentration and production of H_2S [9, 10] were measured in the postmitochondrial fraction of the liver. The protein level was determined by Lowry method [11]. The type of distribution was estimated using Shapiro-Wilk test. Significant differences between group were evaluated by using Wilcoxon test with $p<0.05$ considered. All results are represented as median minimum-maximum values (Me[min-max]).

RESULTS AND DISCUSSION. Under the conditions of experimental nephropathy the H_2S concentration decreased by 45.3 % on 7 experimental days and production of hydrogen sulfide was decreased by 27.8 % compared to the control group (Table). H_2S-producing activities of CSE, CBS, and CAT in the liver of rat with nephropathy were reduced by 31 %, 32.12 % and 32.7 %, respectively, relative to control animals. Such changes can be caused by a violation of the regulation of intracellular metabolism under condition of developing a disease.

The introduction of glutathione led to increased production of H_2S by 36 % and concentration by 43 % compared to animals with nephropathy. Glutathione increased the content of hydrogen sulfide by increasing the activity of CBS by 27 % compared to the group of animals with nephropathy. And the activity of CAT was increased by 49.7 % after glutathione introduction within 7 days and in this case exceeded the activity of the control group of animals. At the same time, the activity CSE after the introduction of glutathione remained significantly lower compared to the control group.

Glutathione is involved in maintaining oxidative-reducing potential in the processes of detoxification of endogenous and exogenous xenobiotics directly and as a substrate for biotransformation enzymes, and its use is effective in conditions of nephropathy caused by high doses of folic acid. Antioxidant by binding of free radicals, inactivation of products of peroxide oxidation of lipids, protects cell membranes from the generation of free radicals under conditions of nephropathy.

It can be assumed that the cysteine present in glutathione is used in the synthesis reactions of hydrogen sulfide, and the glutathione as a strong antioxidant [4] contribute to the improvement of the investigated parameters in the liver of animals with experimental nephropathy. On the other hand, it was previously demonstrated that H_2S protects cells against oxidative stress via increasing GSH production [2].

It is, therefore, logical to expect (Fig.) that GSH also contributes to the increase of H_2S, and our experiment confirms this hypothesis.

Collectively, these results suggest that glutathione by increasing activities of H_2S-producing

Table – **Indicators of hydrogen sulfide production in the liver of rats under conditions of experimental nephropathy and introduction of glutathione (M±m)**

Investigated indicators	Control (n=36)	Nephropathy, 7day (n=24)	Nephropathy+glutathione, 7day (n=23)
H_2S-concentration, nmol/l	21.33±0.24	1.68±0.66**	20.49±0.61#
H_2S-production, nmol/min/mg of protein	38.20±0.27	27.56±0.91**	37.64±0.79#
CSE, nmol H_2S /min/mg of protein	3.31±0.06	2.26±0.11**	2.46±0.13**
CBS, nmol H_2S /min/mg of protein	2.49±0.06	1.69±0.15**	2.16±0.15##
CAT, nmol H_2S /min/mg of protein	2.69±0.05	1.81±0.10**	2.71±0.13#

Notes:
1. ** – $p<0.01$, relative to control.
2. ## – $p<0.05$, relative to animals with nephropathy.
3. # – $p<0.01$, relative to animals with nephropathy.
4. M±m; the data are presented as mean±SEM.

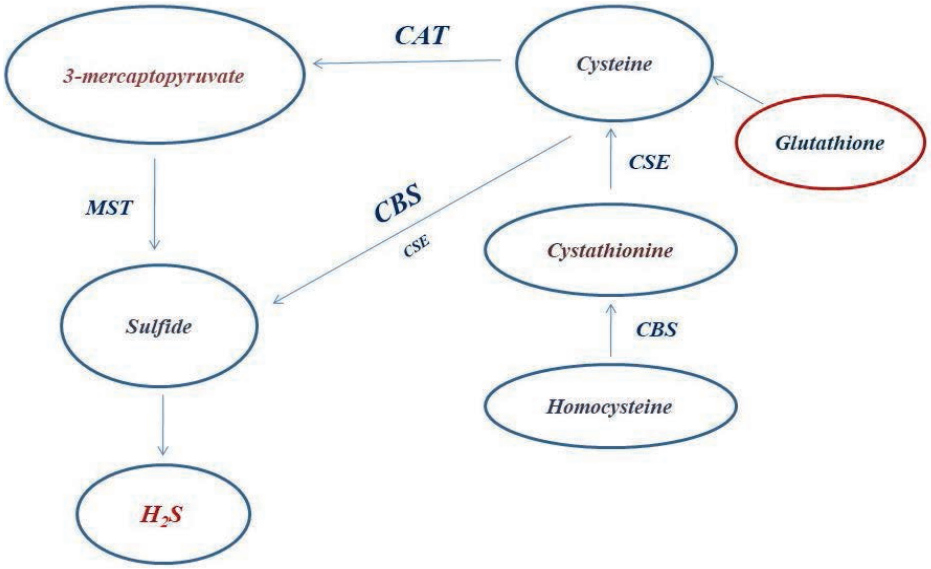

Fig. Scheme of cysteine involvement in hydrogen sulfide synthesis.

Note. CSE – cystathionine-γ-lyase, CBS – cystathionine-β-synthase, CAT – cysteinaminotransferase, MST – 3-mercaptopyruvate sulfurtransferase.

enzymes has an influence on the production of H_2S. The regulation of H_2S metabolism by GSH-dependent pathways will greatly improve our understanding of general metabolism changes in the body and also provide new insights into the control of the state of the liver diseases by nephropathy.

CONCLUSIONS. The production and concentration of H_2S by experimental nephropathy in the liver of rats are diminished due to decreased activities of H_2S-producing enzymes. The introduction of glutathione increased the content of hydrogen sulfide by stimulation the activities of cystathionine-β-synthase and cysteinaminotransferase in the liver of rats. As reasons for this effect, antioxidant properties of glutathione and the possibility of including tripeptide as a source of cysteine in the synthesis of hydrogen sulfide are considered.

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БУКОВИНСЬКИЙ ДЕРЖАВНИЙ МЕДИЧНИЙ УНІВЕРСИТЕТ, ЧЕРНІВЦІ

ВПЛИВ СЕМИДЕННОГО ВВЕДЕННЯ ГЛУТАТІОНУ НА АКТИВНІСТЬ ЕНЗИМІВ, ЩО ПРОДУКУЮТЬ H_2S, У ПЕЧІНЦІ ЩУРІВ ЗА УМОВ ЕКСПЕРИМЕНТАЛЬНОЇ НЕФРОПАТІЇ

Резюме

Вступ. *Захворювання нирок – одна з найпоширеніших та найактуальніших проблем сучасної медицини. Порушення функцій нирок викликає продукування активних форм кисню, може поєднуватися з різними захворюваннями печінки або стимулювати їх розвиток, тому науковці дедалі більше цікавляться вивченням впливу антиоксидантів, таких, як глутатіон, на метаболічні шляхи організму за умов різних захворювань. Сірководень (гідроген сульфід, H_2S) має протизапальні, антиоксидантні й антиапоптозні властивості, які забезпечуються його здатністю знижувати синтез пероксидів ліпідів і регулювати активність ензимів, що продукують активні форми кисню, а також відіграє важливу роль у механізмі розвитку нефропатії.*

Мета дослідження *– вивчити вплив семиденного введення глутатіону на систему продукування H_2S у печінці щурів за умов експериментальної нефропатії.*

Методи дослідження. *Експериментальну нефропатію моделювали шляхом внутрішньочеревного введення білим статевозрілим щурам-самцям фолієвої кислоти в дозі 250 мг/кг маси тіла. Глутатіон вводили інтрагастрально в дозі 100 мг/кг маси тіла впродовж 7-ми днів. У печінці визначали активність ензимів, що генерують H_2S, а також його концентрацію і продукцію.*

Результати й обговорення. *За умов експериментальної нефропатії спостерігали зниження концентрації та продукції гідроген сульфіду порівняно з контрольною групою тварин. Введення глутатіону сприяло зростанню активності ензимів, що генерують H_2S, та підвищенню його вмісту в печінці щурів із нефропатією.*

Висновки. *Встановлено, що вміст і продукція сірководню в групі тварин із нефропатією знижуються за рахунок пригнічення активності ензимів, що продукують H_2S, у печінці. Введення глутатіону сприяє нормалізації вмісту сірководню в печінці щурів шляхом підвищення активності цистатіонін-β-синтази і цистеїнамінотрансферази, що можна пояснити антиоксидантними властивостями глутатіону та ймовірним включенням трипептиду як джерела цистеїну в синтез газотрансмітера.*

КЛЮЧОВІ СЛОВА: **нефропатія; гідроген сульфід; глутатіон; ензими.**

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БУКОВИНСКИЙ ГОСУДАРСТВЕННЫЙ МЕДИЦИНСКИЙ УНИВЕРСИТЕТ, ЧЕРНОВЦЫ

ВЛИЯНИЕ СЕМИДНЕВНОГО ВВЕДЕНИЯ ГЛУТАТИОНА НА АКТИВНОСТЬ ЭНЗИМОВ, КОТОРЫЕ ПРОДУЦИРУЮТ H_2S, В ПЕЧЕНИ КРЫС В УСЛОВИЯХ ЭКСПЕРИМЕНТАЛЬНОЙ НЕФРОПАТИИ

Резюме

Вступление. *Заболевания почек – одна из самых распространенных и актуальных проблем современной медицины. Нарушение функции почек вызывает продуцирование активных форм кислорода, может совмещаться с различными заболеваниями печени или стимулировать их развитие, поэтому ученые все больше интересуются изучением влияния антиоксидантов, таких, как глутатион, на метаболические пути организма в условиях различных заболеваний. Сероводород (водород сульфид, H_2S) обладает противовоспалительными, антиоксидантными и антиапоптозными свойствами, которые обеспечиваются его способностью снижать синтез пероксидов липидов и регулировать активность энзимов, продуцирующих активные формы кислорода, а также играет важную роль в механизме развития нефропатии.*

Цель исследования *– изучить влияние семидневного введения глутатиона на систему продуцирования H_2S в печени крыс в условиях экспериментальной нефропатии.*

Методы исследования. *Экспериментальную нефропатию моделировали путем внутрибрюшного введения белым половозрелым крысам-самцам фолиевой кислоты в дозе 250 мг/кг массы тела. Глутатион вводили интрагастрально в дозе 100 мг/кг массы тела в течение 7-ми дней. В печени определяли активность энзимов, генерирующих H_2S, а также его концентрацию и продукцию.*

Результаты и обсуждение. *В условиях экспериментальной нефропатии наблюдали снижение концентрации и продукции водород сульфида по сравнению с контрольной группой животных. Введение глутатиона способствовало росту активности энзимов, генерирующих H_2S, и повышению его содержания в печени крыс с нефропатией.*

Выводы. *Установлено, что содержание и продукция сероводорода в группе животных с нефропатией снижаются за счет угнетения активности энзимов, продуцирующих H_2S, в печени. Введение глутатиона способствует нормализации содержания сероводорода в печени крыс путем повышения активности цистатионин-β-синтазы и цистеинаминотрансферазы, что можно объяснить антиоксидантными свойствами глутатиона и вероятным включением трипептида в качестве источника цистеина в синтез газотрансмитера.*

КЛЮЧЕВЫЕ СЛОВА: **нефропатия; водород сульфид; глутатион; энзимы.**

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