EXPERIMENTAL STUDIES

THE INDUCING EFFECT OF S-NITROSOGLUTATHIONE ON THE EXPRESSION AND ACTIVITY OF ORGANIC ANION TRANSPORTING POLYPEPTIDE 1B1 (OATP1B1) IN HepG2 CELLS

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The effect of the nitric oxide donor S-nitrosoglutathione on the level and activity of organic anion transporting polypeptide 1B1 (OATP1B1), as well as the expression of the *SLCO1B1* gene encoding the transporter protein, was investigated in HepG2 cells. The study has shown that treatment of cells with S-nitrosoglutathione for 3 h did not influence the content and activity of OATP1B1. Incubation with S-nitrosoglutathione (10–500 μM) for 24 h increased *SLCO1B1* expression, the content of OATP1B1, and activity of the transporter protein. Induction of the OATP1B1 protein by the NO donor was suppressed by the soluble guanylate cyclase (sGC) inhibitor, 10 μM ODQ (1H-[1,2,4]oxadiazolo-[4,3-a]quinoxaline-1-OH). Thus, the study has shown that S-nitrosoglutathione, acting through the NO-sGC-cGMP signaling pathway, increased *SLCO1B1* gene expression, accompanied by the increase in the transporter protein content and its activity in cells.

Keywords: OATP1B1; *SLCO1B1* gene; S-nitrosoglutathione; NO-sGC-cGMP signaling pathway

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INTRODUCTION

Transporter proteins involved in translocation of endo- and exobiotics play one of the key roles in pharmacokinetic processes (absorption, distribution, and excretion); they also participate in the transfer of small electroneutral molecules of through biological membranes [1, 2].

All transporter proteins are divided into two large groups: ABC transporters and SLC transporters. ABC transporters (ATP-binding cassette transporters) use the energy of ATP to transfer substrates through the bilipid membrane from the cytoplasm to the extracellular space and biological body fluids (the so-called efflux transporters). SLC proteins (solute carriers) use the difference in electrochemical potentials or ion gradient to transfer their substrates. That is why they are classified as secondary active transporters. SLC transporters mainly mediate the entry of substances into cells, i.e. they are influx carriers, although some can function as efflux transporters or mediate bidirectional transport of substances. To date, more than 550 SLC transporters have been identified in humans; they are divided into families. The family of organic anion transporting polypeptides (OATP1) is one of the most studied and clinically significant; it includes 3 subfamilies: OATP1A, OATP1B, and OATP1C [3].

The human OATP1B1 protein (OATP2, OATP-C, LST-1, SLC21A6) encoded by the *SLCO1B1* gene is a 691-amino acid glycoprotein with an apparent molecular mass of 84 kDa, which decreases to about 54 kDa after deglycosylation [4, 5].

OATP1B1 is localized in hepatocytes, primarily on the basolateral membrane, the basement membrane of Sertoli cells, and diffusely in Leydig cells [4]. *SLCO1B1* mRNA has also been detected in enterocytes, breast cancer cells [6], ovarian carcinoma cells [1], hepatocellular carcinoma, and other tumor cells [7]. In all these cells, OATP1B1 performs a single function: it mediates influx of substrate substances from the blood and intercellular space into the cells [8].

Expression of *SLCO1B1*/OATP1B1 is reduced in hepatocellular carcinoma cells [9, 10]. This may be one of the mechanisms underlying their resistance to chemotherapy: a reduced content of the influx transporter reduces the penetration of cytostatics (OATP1B1 substrates) into tumor cells [11]. Therefore, in order to increase the effectiveness of chemotherapy, it is promising to search for substances that increase the content and activity of OATP1B1 in hepatocellular carcinoma cells.

We have previously shown that the NO donor, S-nitrosoglutathione, can increase the activity and amount of the ABC transporter protein — P-glycoprotein [12]. Therefore, we suggested that S-nitrosoglutathione could modulate other transporter proteins, particularly, OATP1B1.

The aim of this study was to evaluate the effect of S-nitrosoglutathione on the expression and activity of OATP1B1 in the HepG2 cells and to study its possible mechanisms.

MATERIALS AND METHODS

Cell Cultivation

The study was performed using a HepG2 cell culture (Institute of Cytology, Russian Academy of Sciences, St. Petersburg). The cells were grown at 37°C and 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM) containing glucose (4.5 g/l), 4 mM L-glutamine, 10% fetal bovine serum, penicillin (100 U/ml), and streptomycin (100 μg/ml) (all components from Sigma-Aldrich, Germany). The cells were cultured in 96-well and 6-well plates (Corning, USA).

S-nitrosoglutathione (Sigma-Aldrich, Germany) was added to the culture medium at concentrations of 1 μM, 10 μM, 50 μM, 100 μM, and 500 μM, and the cells were incubated for 3 h and 24 h. Water for injection (S-nitrosoglutathione solvent) was added to the control cells in an equivalent volume. To assess the role of the NO-sGC-cGMP signaling pathway in the mechanism of S-nitrosoglutathione action on OATP1B1, a sGC inhibitor, 1H-[1,2,4]oxadiazolo-[4,3-a]quinoxaline-1-OH (ODQ, Sigma-Aldrich, USA), was added to the culture medium at a concentration of 10 μM 30 min before adding the NO donor [13].

mRNA Quantification by Real-time PCR

The *SLCO1B1* gene expression in HepG2 cells was evaluated using real-time PCR. For this purpose, cells were cultured in 6-well plates (Corning). After the monolayer formation, they were scrapped from the wells. Total RNA was isolated using the RNeasy Mini Kit (QIAGEN, Germany) according to the manufacturer's instructions. The RNA concentration was determined spectrophotometrically using the NanoPhotometr NP80-Touch (Implen, Germany). The RT-PCR reaction was carried out by the one-step method using the BioMaster RT-PCR SYBR Blue kit (Biolabmix, Russia), according to the manufacturer's instructions. Reverse transcription conditions included: temperature 45°C, incubation time 10 min, number of cycles 1.

Primer sequences were selected using the DNASTAR Lasregene, PrimerSelect software. The following *SLCO1B1* primers were used: 5′-GGTGAATGCCCAAGAGATGAG-3′ (forward) and 5′-TGGAAACCCAGTGCAAGTGATT-3′ (reverse) (Eurogen, Russia). The mRNA results were normalized to expression of the reference *GAPDH* gene, encoding glyceraldehyde-3-phosphate dehydrogenase. The following *GAPDH* primers were used: 5′-GTCCCTCTGACTTCAACAGCG-3′ (forward) and 5′-ACCACCCTGTTGCTGTAGCCAA-3′ (reverse) (Eurogen). The analysis was carried out under the following temperature cycling conditions: denaturation — heating the reaction mixture to 95°C, cooling — 53°C, elongation — 72°C. For the work, 0.2 ml test tubes in strips of 8 pieces were used. The analysis was performed using an Applied Biosystems Quant Studio 5 nucleic acid amplifier with hybridization-fluorescence detection of PCR products in real time (Life Technologies Holdings Pte. Ltd., Singapore) with QuantStudio Design and Analysis software. The expression level of *SLCO1B1* relative to the reference gene (*GAPDH*) was calculated using LinRegPCR v.11.0 software (Heart Failure Research Center, the Netherlands).

Determination of the Relative Amount of the OATP1B1 Protein by Western Blot

To assess the relative amount of OATP1B1, the cells were cultured in 6-well plates. After exposure, the cells were removed from the wells with a trypsin-EDTA solution (0.25% trypsin and 0.2% EDTA, Sigma-Aldrich, Germany), washed three times with a phosphate buffer solution (Bio-Rad, USA). The cells were then lysed with NP40 Cell Lysis Buffer Thermo (Thermo Fisher Scientific, USA) containing a mixture of proteinase inhibitors (Sigma-Aldrich, Germany) (10^7 cells per 100 μl buffer) for 30 min at 4°C and constant stirring. The resulting lysate was centrifuged at 5000 *g* (CM-50, Eppendorf, Germany). The supernatant was used for analysis.

Next, 30 μg of cell lysate supernatant proteins were subjected to electrophoresis using 7.5% TGX Stain-Free FastCast Acrylamide Kit (Bio-Rad) in the Laemmli buffer system (Bio-Rad).

Samples were mixed with the Laemmli buffer (Bio-Rad) containing 50 mM β-mercaptoethanol (Bio-Rad) at a ratio of 1:3 and incubated for 10 min at 70°C. Electrophoresis was performed at 100 V for 90 min.

Proteins were transferred to a nitrocellulose membrane (Trans-Blot Turbo Mini-Size nitrocellulose, Bio-Rad) using a Mini Trans-Blot (Bio-Rad) for 7 min at 25 V and 1.3 A.

Proteins on the membrane were blocked with 1% Casein Blocker (Bio-Rad) containing 0.1% Tween for 1 h at room temperature.

Detection of OATP1B1 was performed using primary mouse monoclonal antibodies (OATP2 Polyclonal Antibody, Invitrogen, USA) at a dilution of 1:200 in a blocking solution for 2 h at 37°C. Visualization of primary antibodies was performed using secondary rabbit antibodies (Rabbit-anti-Mouse IgG (H+L) Secondary Antibody, HRP, Invitrogen) at a dilution of 1:4000 and incubation for 1 h at room temperature.

Chemiluminescence was detected using ChemiDocXRS+ (Bio-Rad). The intensity of the resulting bands was analyzed densitometrically using ImageLab software (Bio-Rad).

The molecular mass of OATP1B1 was confirmed by comparison with molecular mass markers (Precision plus protein standards Dual Color, Bio-Rad).

The OATP1B1 content was estimated relative to the GAPDH protein by using primary antibodies GAPDH Loading Control Monoclonal Antibody (GA1R), DyLight 68 (Invitrogen; dilution 1:1000), and secondary rabbit antibodies — Rabbit-anti-Mouse IgG (H+L) Secondary Antibody, HRP (Invitrogen; dilution 1:4000).

Determination of OATP1B1 Activity by HPLC MS/MS

OATP1B1 activity was analyzed by the penetration of the transporter substrate into HepG2 cells. To estimate the effect of S-nitrosoglutathione on OATP1B1 activity, the cells were cultured in 24-well plates. After reaching a monolayer, S-nitrosoglutathione was added to the cells at concentrations of 1 μM, 10 μM, 50 μM, 100 μM, and 500 μM and incubated at 37°C for 24 h. After incubation, transport experiments were performed. For this, the cells were washed once with a transport medium heated to 37°C. The medium contained Hanks' solution (Sigma-Aldrich, Germany) buffered with 25 mM Hepes, pH 7.4 (Sigma-Aldrich, Germany), and 1% dimethyl sulfoxide (PanEco, Russia). Then, the OATP1B1 substrate, atorvastatin (1 μM, Sigma-Aldrich, USA), was added to the cells, which were incubated for 5 min. The reaction was stopped by removing the transport medium followed by cell washing with 500 μl of the ice-cold transport medium. The cells were then washed three times with 500 μl of ice-cold phosphate buffer (PanEco). Cells were lysed by freeze-thaw three times (cells were frozen at -80°C and then thawed at room temperature). The concentration of atorvastatin in the resulting cell lysates was determined by HPLC-MS/MS [14].

To extract atorvastatin from the samples, 300 μl of acetonitrile containing an internal standard (10 ng/ml valsartan, Sigma, USA) was added to 300 μl of cell lysate and mixed using a Vortex shaker (Heidolph, Germany). The samples were then centrifuged at 21,000 *g* for 10 min at 4°C using an Avanti JXN-3 high-speed centrifuge (Beckman Coulter, USA). The supernatant was transferred to 1 ml vials (Thermo Fisher Scientific) and placed in an autosampler. The analysis was performed on an Ultimate 3000 high-performance liquid chromatograph (Thermo Fisher Scientific) with a TSQ Fortis tandem mass-selective detector (Thermo Fisher Scientific), a gradient pump, a degasser, and an autosampler.

The chromatographic analysis conditions were as follows: Selectra C18 Guard Cartridges SLC-18GDC46-5UM precolumn, UCT Selectra C18 4.6 mm × 100 mm, 5 μm, 100 Å chromatographic column, and column thermostatting at 35°C.

A gradient elution mode was used. The molecules were ionized using electrospray positive ionization mode at atmospheric pressure. The spray voltage was 3500 V. The substance was detected in the following mode: sheath gas — 50 l/min, auxiliary gas —- 10 l/min, sweep gas — 1 l/min, the temperature of the ion transfer tube was 300°C, the evaporator temperature was 350°C. The substance was detected according to the following conditions: atorvastatin — positive ionization mode, 559.30 *m/z* → 466.20 *m/z*, collision energy — 17 V, source fragmentation — 0, CID gas (collision-induced dissociation gas) — 2 mTorr; 559.30 *m/z* → 440.20 *m/z*, collision energy — 32 V, source fragmentation — 0, CID gas — 2 mTorr; valsartan 436.2 m/z → 206.3* m/z (used for quantitative analysis), collision energy — 27 V, source fragmentation — 0, CID gas — 2 mTorr; valsartan 436.2 *m/z* → 234.9 *m/z*, collision energy — 18 V, lens voltage — 104 V, source fragmentation — 0, CID gas — 2 mTorr; flow rate 0.3 ml/min; samples were injected into the chromatograph in a volume of 2 μl. The analysis was carried out for 10 min. Under these conditions, the retention time for atorvastatin and valsartan was 4.53 min and 4.45 min, respectively. The results were recalculated per protein content and expressed in pmol/mg/min.

The obtained results were analyzed using StatSoft Statistica 13.0, Microsoft Excel, GraphPad Prism8. The statistical significance of differences was assessed by ANOVA, the exact value of the Fisher criterion (F) was indicated in the text, the critical level of significance was taken at $p<0.05$.

Paired comparisons with the control were performed using the Dunnett test. The results in the table and figures are presented as the arithmetic mean and standard deviation (M±SD). Differences were considered statistically significant at $p<0.05$.

RESULTS

The Effect of S-Nitrosoglutathione on the SLCO1B1 Gene Expression

At the first stage of the study, we have investigated *SLCO1B1* expression in HepG2 cells. Cell incubation with all tested concentrations of S-nitrosoglutathione (1–500 μM) for 3 h insignificantly influenced the studied parameter ($F = 2.85$, $p = 0.08$) (Fig. 1a). Incubation for 24 h significantly increased *SLCO1B1* expression ($F = 225$, $p<0.0001$); in the presence of 10 μM S-nitrosoglutathione *SLCO1B1* expression demonstrated a 40-fold increase ($p<0.0001$), while in the presence of 100 μM and 500 μM S-nitrosoglutathione a ten-fold increase ($p = 0.0003$) in *SLCO1B1* expression was observed (Fig. 1b).

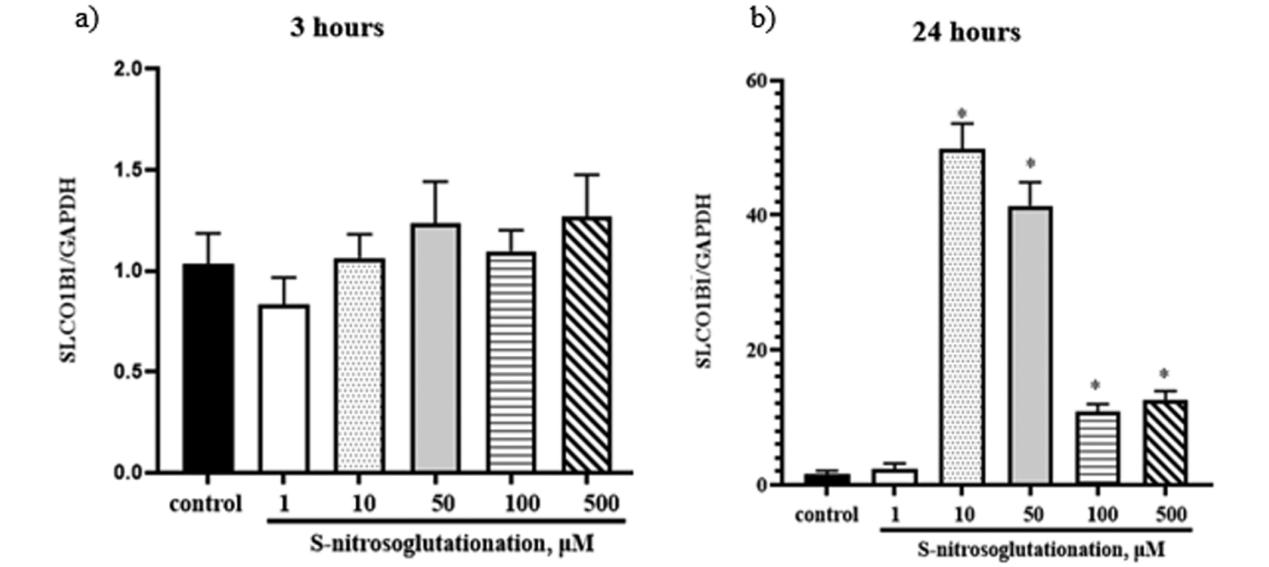

Figure 1. Relative abundance of amplicons of the *SLCO1B1* gene in HepG2 cells exposed to S-nitrosoglutathione for 3 h (**a**) and 24 h (**b**) and evaluated by real-time quantitative PCR analysis. * – $p<0.001$ relative to control.

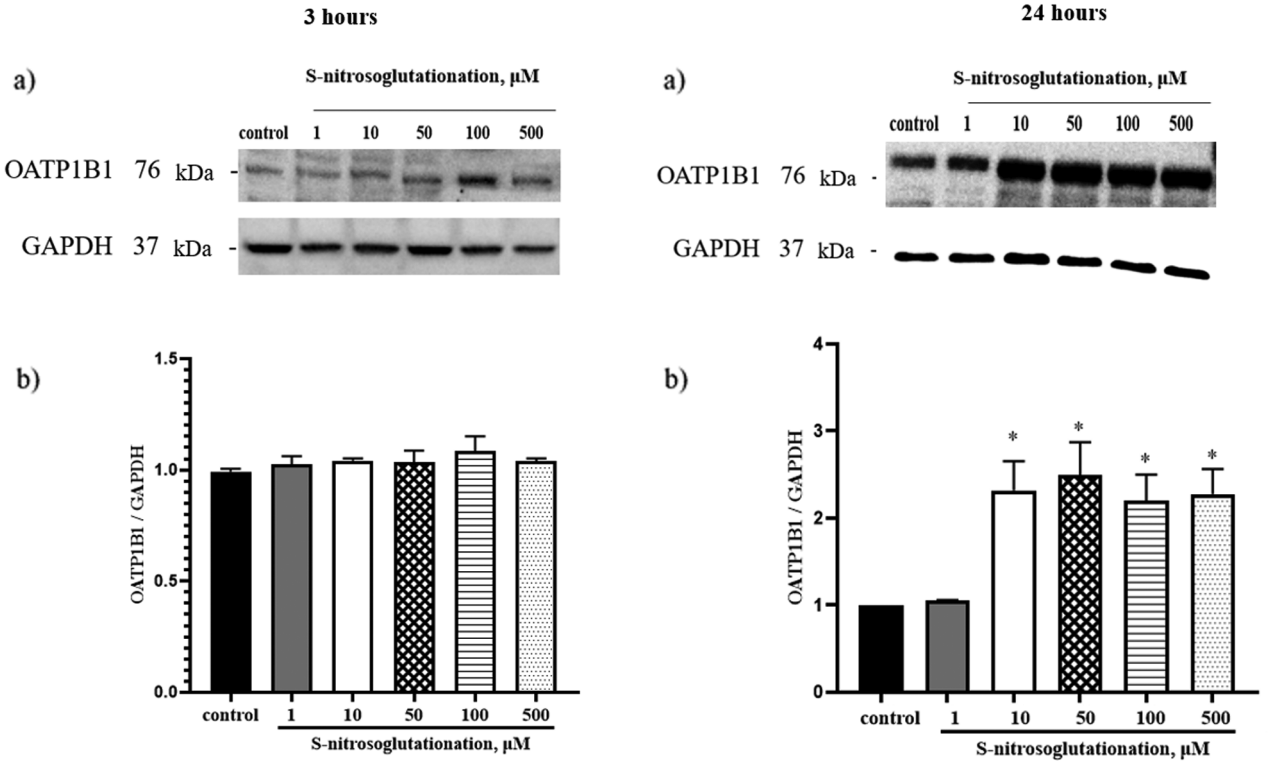

Figure 2. The relative content of organic anion transporting polypeptide 1B1 (OATP1B1) in HepG2 cells treated with 1–500 μM S-nitrosoglutathione for 3 h or 24 h. * – $p<0.001$ relative to control. Photographs of the bands were obtained using ChemiDocXRSt (**a**). Densitometric analysis was performed using ImageLab software (**b**).

The Effect of S-Nitrosoglutathione on the Relative Content of the OATP1B1 Transporter Protein

Similar results have been obtained during evaluation of the S-nitrosoglutathione effect on the relative content of the OATP1B1 protein. The 3-h incubation of cells with all tested concentrations of S-nitrosoglutathione (1–500 μM) insignificantly influenced the relative content of OATP1B1 ($F = 3.18$, $p = 0.09$) (Fig. 2a). Incubation for 24 h with 1 μM S-nitrosoglutathione had no effect on the OATP1B1 content, while higher concentrations of S-nitrosoglutathione increased the studied parameter: at 10 μM, 50 μM, 100 μM, and 500 μM S-nitrosoglutathione the OATP1B1 content increased ($F = 19.0$, $p < 0.0001$) by 136.7% ($p = 0.0003$), 154.1% ($p < 0.0001$), 124.5% ($p = 0.0006$), and 131.6% ($p = 0.0004$), respectively (Fig. 2b).

The Effect of S-Nitrosoglutathione on the OATP1B1 Activity

The 24-h incubation with 10 μM, 50 μM, and 100 μM S-nitrosoglutathione increased atorvastatin penetration into HepG2 cells ($F = 10.4$, $p < 0.0005$) by 27.8% ($p=0.02$), 41.8% ($p=0.001$), 21.8% ($p=0.06$), respectively; however, the effect was not observer after 24-incubation with 500 μM S-nitrosoglutathione ($p>0.5$) (Table 1).

The results obtained indicate an increase in OATP1B1 activity induced by S-nitrosoglutathione.

The increase in the relative amount of OATP1B1 was accompanied by an increase in the activity of the transporter protein.

The Mechanism of OATP1B1 Regulation by S-Nitrosoglutathione

Incubation of HepG2 cells with the sGC inhibitor, ODQ, alone did not influence the relative content of the OATP1B1 protein (Fig. 3a). At the same time, when the sGC inhibitor ODQ was added to S-nitrosoglutathione, it prevented an increase in the OATP1B1 content so that the transporter level did not differ from the control values (Fig. 3b). The obtained results indicate the involvement of the NO-sGC-cGMP biochemical cascade in the regulation of the relative content of OATP1B1 induced by S-nitrosoglutathione.

DISCUSSION

HepG2 is a human hepatocellular carcinoma cell line with epithelial phenotype; it was derived from the liver tissue of a 15-year-old white male [15]. The HepG2 cell line is one of the most widely used cancer cell lines in many biomedical studies due to its versatility. As a human endodermal cell line, HepG2 is used as a model for toxicological studies, including toxicogenomic screens using CRISPR-Cas9, oncology, liver diseases, gene regulation mechanisms, biomarker discovery, and drug metabolism, including those involving the OATP1B1 transporter

Table 1. The effect of S-nitrosoglutathione on atorvastatin accumulation inside HepG2 cells (pmol/mg/min, M±SD, n=3)

	Control	**S-nitrosoglutathione, μM**				
		1	**10**	**50**	**100**	**500**
Atorvastatin accumulation, pmol/mg/min	4.78±0.49	4.62±0.36	6.11±0.13* p=0.02	6.76±0.77* p=0.001	5.82±0.41# p=0.07	4.79±0.42

* - $p<0.05$ versus control, # - $p=0.06$ versus control.

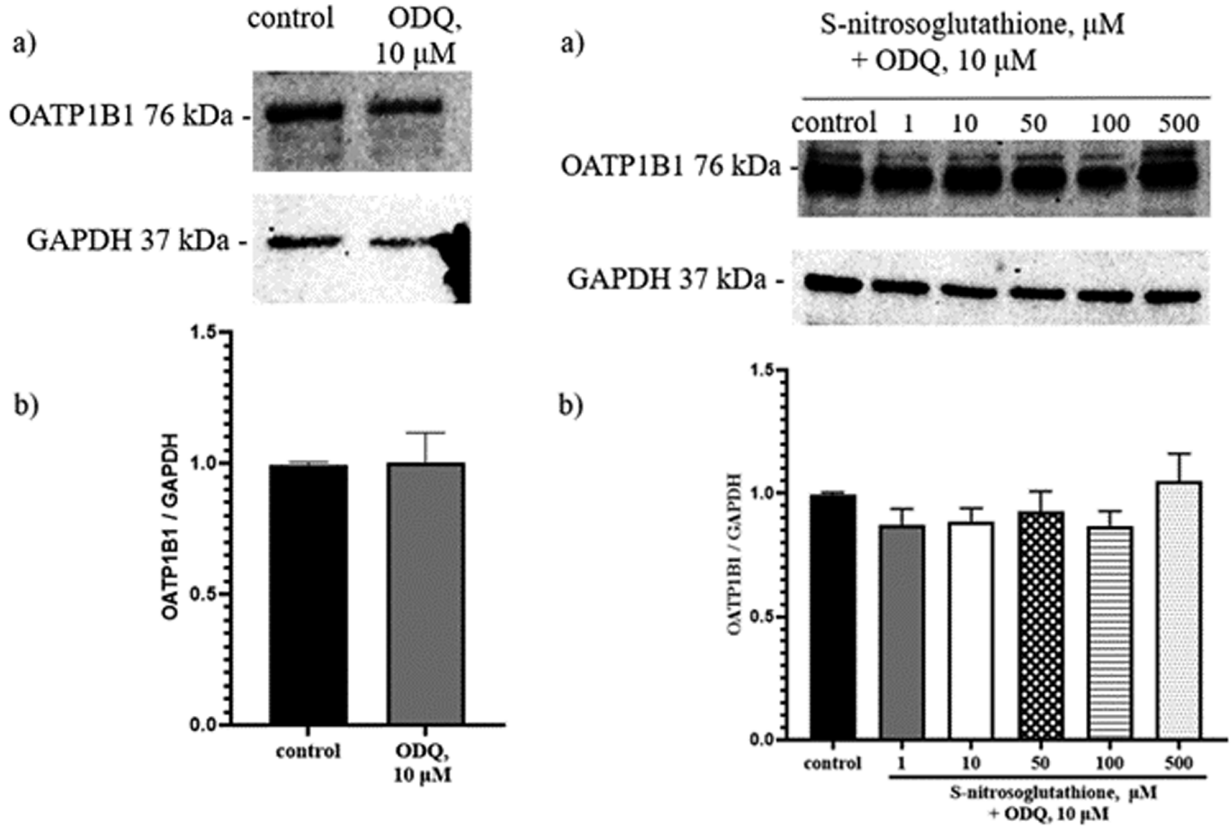

Figure 3. The relative amount of organic anion transporting polypeptide 1B1 (OATP1B1) in HepG2 cells treated with 10 μM ODQ alone and in combination with 1–500 μM S-nitrosoglutathione for 24 h. Photographs of the bands were obtained using ChemiDocXRSt (**a**). Densitometric analysis was performed using ImageLab software (**b**).

protein [16, 17]. That is why the HepG2 cell line was chosen to study the molecular mechanisms of OATP1B1 regulation upon exposure to the nitric oxide donor S-nitrosoglutathione.

OATP1B1 is an influx transporter protein that plays an important role in the penetration of its substrates into hepatocytes and a number of other cells. Substrates of this transporter include such drugs as statins, angiotensin-converting enzyme inhibitors, sartans, cytostatics and a number of other substances [18]. OATP1B1 is involved in the transport of bilirubin and a decrease in its activity can lead to the development of jaundice [19]. OATP1B1 expression in tumor cells promotes the penetration of cytostatic substrates into cells; it is reduced in hepatocellular carcinoma cells [11]. Therefore, the use of OATP1B1 inducers could theoretically increase the effectiveness of antitumor therapy [20, 21].

The present study has shown that the NO donor, S-nitrosoglutathione, increased OATP1B1 expression and activity in HepG2 cells treated with it for 24 h. S-nitrosoglutathione increased the *SLCO1B1* mRNA and the relative content of its protein product, OATP1B1, as well as the penetration of the OATP1B1 substrate (atorvastatin) into HepG2 cells thus indicating an increase in the transporter activity.

At present, several mechanisms of regulation have been described for OATP1B1 [1]; these include:

1) direct inhibition of OATP1B1;

2) transcriptional regulation;

3) post-transcriptional regulation;

4) post-translational regulation (glycosylation, ubiquitination, regulation by protein kinases);

5) polymorphism of the *SLCO1B1* gene.

In the range of concentrations 10–500 μM, S-nitrosoglutathione increased the *SLCO1B1* mRNA level and the amount of OATP1B1 protein. This indicates that in HepG2 cells the increase in the OATP1B1 protein content is regulated by the expression of the SLCO1B1 gene.

At the same time, treatment with 500 μM S-nitrosoglutathione increased *SLCO1B1* expression and the OATP1B1 content but did not change the transporter activity. This latter may be probably associated with a structural modification of the transporter protein (nitrosylation of its amino acid residues) under conditions of NO hyperproduction [12].

S-nitrosoglutathione is an endogenous S-nitrosylated derivative of glutathione [22], which is involved in various biochemical processes that control homeostasis. S-nitrosoglutathione is believed to be a NO donor; it can activate the classical sGC-cGMP-dependent signaling pathway or cause nitrosylation of certain proteins [23].

Taking these circumstances into account, in the final part of the study, we have evaluated the role of the NO-sGC-cGMP signaling pathway in the S-nitrosoglutathione induction of OATP1B1. For this purpose we used a specific sGC blocker, ODQ, as the inhibitor of the NO-cGMP signaling pathway. ODQ is known to oxidize the heme moiety in sGC without affecting the catalytic domain, thereby reducing sGC activity [24]. In our experiments ODQ alone did not influence the OATP1B1 content, but in combination with S-nitrosoglutathione treatment (1–500 μM) for 24 h, it prevented an increase in the relative content of OATP1B1. The obtained results indicate involvement of the NO-sGC-cGMP biochemical cascade in the regulation of the relative content of OATP1B1 during cell treatment with S-nitrosoglutathione.

A limitation of this study is that the study was performed on one HepG2 cell line and the OATP1B1 activity has been assessed by the penetration of atorvastatin into the cells, which is also a substrate for another transporter, OATP1B3 [25]. However, at the moment there are no selective substrates of OATP1B1, and to study the effect on OATP1B1, it is necessary to use recombinant cell lines selectively overexpressing the studied transporter. The disadvantage of this approach is the lack of regulatory pathways in these cell lines, which are present in hepatocytes. Alternatively or simultaneously it is possible to use an OATP1B3 inhibitor in such experiments, but there are also not so many of these substances and their selectivity is questionable.

CONCLUSIONS

Thus, the study has shown that S-nitrosoglutathione, acting through the NO-sGC-cGMP signaling pathway, increases the SLCO1B1 gene expression, causing an increase in the content of its protein product and as a consequence, its transporter activity. The data obtained on the inducing effect of S-nitrosoglutathione on OATP1B1 can be useful for increasing the effectiveness of hepatocellular carcinoma therapy by increasing the penetration of cytostatic OATP1B1 substrates into cancer cells.

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COMPLIANCE WITH ETHICAL STANDARDS

The study protocol was approved by the Bioethics Commission of Ryazan State Medical University (Protocol No. 87 dated 07.11.2023).

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

1. *Svoboda M., Riha J., Wlcek K., Jaeger W., Thalhammer T.* (2011) Organic anion transporting polypeptides (OATPs): regulation of expression and function. Curr. Drug Metab., **12**(2), 139–153. DOI: 10.2174/138920011795016863
2. *Schnegelberger R.D., Steiert B., Sandoval P.J., Hagenbuch B.* (2022) Using a competitive counterflow assay to identify novel cationic substrates of OATP1B1 and OATP1B3. Front. Physiol., **8**(13), 969363. DOI: 10.3389/fphys.2022.969363
3. *Liu X.* (2019) SLC family transporters. Adv. Exp. Med. Biol., **1141**, 101–202. DOI: 10.1007/978-981-13-7647-4_3
4. *Konig J., Cui Y., Nies A.T., Keppler D.* (2000) Localization and genomic organization of a new hepatocellular organic anion transporting polypeptide. J. Biol. Chem., **275**(30), 23161–23168. DOI: 10.1074/jbc.M001448200
5. *Hagenbuch B., Gui C.* (2008) Xenobiotic transporters of the human organic anion transporting polypeptides (OATP) family. Xenobiotica, **38**(7–8), 778–801. DOI: 10.1080/00498250801986951
6. *Banerjee N., Allen C., Bendayan R.* (2012) Differential role of organic anion-transporting polypeptides in estrone-3-sulphate uptake by breast epithelial cells and breast cancer cells. J. Pharmacol. Exp. Ther., **342**(2), 510–519. DOI: 10.1124/jpet.112.192344
7. *Wen J., Zhao M.* (2021) OATP1B1 Plays an important role in the transport and treatment efficacy of sorafenib in hepatocellular carcinoma. Dis. Markers, **26**, 9711179. DOI: 10.1155/2021/9711179
8. *Obaidat A., Roth M., Hagenbuch B.* (2012) The expression and function of organic anion transporting polypeptides in normal tissues and in cancer. Annu. Rev. Pharmacol. Toxicol., **52**, 135–151. DOI: 10.1146/annurev-pharmtox-010510-100556
9. *Libra A., Fernetti C., Lorusso V., Visigalli M., Anelli P.L., Staud F., Tiribelli C., Pascolo L.* (2006) Molecular determinants in the transport of a bile acid-derived diagnostic agent in tumoral and nontumoral cell lines of human liver. J. Pharmacol. Exp. Ther., **319**(2), 809–817. DOI: 10.1124/jpet.106.106591.
10. *Cui Y., König J., Nies A.T., Pfannschmidt M., Hergt M., Franke W.W., Alt W., Moll R., Keppler D.* (2003) Detection of the human organic anion transporters SLC21A6 (OATP2) and SLC21A8 (OATP8) in liver and hepatocellular carcinoma. Lab. Invest., **83**(4), 527–538. DOI: 10.1097/01.lab.0000065015.02412.48.
11. *Briz O., Serrano M.A., Rebollo N., Hagenbuch B., Meier P.J., Koepsell H., Marin J.J.* (2002) Carriers involved in targeting the cytostatic bile acid-cisplatin derivatives cis-diammine-chloro-cholylglycinate-platinum(II) and cis-diammine-bisursodeoxycholate-platinum(II) toward liver cells. Mol. Pharmacol., **61**(4), 853–860. DOI: 10.1124/mol.61.4.853.
12. *Shchulkin A.V., Abalenikhina Y.V., Sudakova E.A., Mylnikov P.Y., Yakusheva E.N.* (2022) Mechanisms of regulation of the P-glycoprotein transporter protein functioning under the action of nitric oxide. Biochemistry (Moscow), **87**(4), 366–379. DOI: 10.1134/S000629792204006X
13. *Hwang, T.L., Wu C.C., Teng C.M.* (1998) Comparison of two soluble guanylyl cyclase inhibitors, methylene blue and ODQ, on sodium nitroprusside-induced relaxation in guinea-pig trachea. Br. J. Pharmacol., **125**, 1158–1163. DOI: 10.1038/sj.bjp.0702181
14. *Erokhina P.D., Myl'nikov P.Yu., Ganina S.O., Konyakhin E.A., Shchul'kin A.V., Slepnev A.A., Yakusheva E.N.* (2022) Development and validation of the quantitative determination of atorvastatin in HepG2 cell line using high-performance liquid chromatography with mass-spectrometric detection. I.P. Pavlov Russian Medical Biological Herald, **30**(2), 149–158] DOI: 10.17816/PAVLOVJ100986
15. *López-Terrada D., Cheung S.W., Finegold M.J., Knowles B.B.* (2009) Hep G2 is a hepatoblastoma-derived cell line. Hum. Pathol., **40**(10), 1512–1515. DOI: 10.1016/j.humpath.2009.07.003
16. *Chatuphonprasert W., Tukum-Mee W., Wattanathorn J., Jarukamjorn K.* (2022) Impact of pineapple juice on expression of CYP3A4, NAT2, SULT1A1 and OATP1B1 mRNA in HepG2 cells. Pak. J. Biol. Sci., **25**(1), 15–22. DOI: 10.3923/pjbs.2022.15.22
17. *Choi J.M., Oh S.J., Lee S.Y., Im J.H., Oh J.M., Ryu C.S., Kwak H.C., Lee J.Y., Kang K.W., Kim S.K.* (2015) HepG2 cells as an *in vitro* model for evaluation of cytochrome P450 induction by xenobiotics. Arch. Pharm. Res., **38**(5), 691–704. DOI: 10.1007/s12272-014-0502-6
18. *Alam K., Crowe A., Wang X., Zhang P., Ding K., Li L., Yue W.* (2018) Regulation of organic anion transporting polypeptides (OATP) 1B1- and OATP1B3-mediated transport: an updated review in the context of OATP-mediated drug-drug interactions. Int. J. Mol. Sci., **19**(3), 855. DOI: 10.3390/ijms19030855
19. *Zhang W., He Y.J., Gan Z., Fan L., Li Q., Wang A., Liu Z.Q., Deng S., Huang Y.F., Xu L.Y., Zhou H.H.* (2007) OATP1B1 polymorphism is a major determinant of serum bilirubin level but not associated with rifampicin-mediated bilirubin elevation. Clin. Exp. Pharmacol. Physiol., **34**(12), 1240–1244. DOI: 10.1111/j.1440-1681.2007.04798.x
20. *Whisenant T.C., Nigam S.K.* (2022) Organic anion transporters (OAT) and other SLC22 transporters in progression of renal cell carcinoma. Cancers (Basel), **14**(19), 4772. DOI: 10.3390/cancers14194772
21. *Shi C., Wu J.B., Chu G.C., Li Q., Wang R., Zhang C., Zhang Y., Kim H.L., Wang J., Zhau H.E., Pan D., Chung L.W.* (2014) Hep-tamethine carbocyanine dye-mediated near-infrared imaging of canine and human cancers through the HIF-1α/OATPs signaling axis. Oncotarget., **5**(20), 10114–10126. DOI: 10.18632/oncotarget.2464
22. *Broniowska A.K., Diers A.R., Hogg N.* (2013) S-nitrosoglutathione. Biochim. Biophys. Acta, **1830**(5), 3173–3181. DOI: 10.1016/j.bbagen.2013.02.004
23. *Zhang Y., Sun C., Xiao G., Shan H., Tang L., Yi Y., Yu W., Gu Y.* (2019) S-nitrosylation of the peroxiredoxin-2 promotes S-nitrosoglutathione-mediated lung cancer cells apoptosis via AMPK-SIRT1 pathway. Cell Death Dis., **10**(5), 329. DOI: 10.1038/s41419-019-1561-x
24. *Zhao Y., Brandish P.E., di Valentin M., Schelvis J.P., Babcock G.T., Marletta M.A.* (2000) Inhibition of soluble guanylate cyclase by ODQ. Biochemistry, **39**(35), 10848–10854. DOI: 10.1021/bi9929296
25. *Garrison D.A., Talebi Z., Eisenmann E.D., Sparreboom A., Baker S.D.* (2020) Role of OATP1B1 and OATP1B3 in drug-drug interactions mediated by tyrosine kinase inhibitors. Pharmaceutics, **12**(9), 856. DOI: 10.3390/pharmaceutics12090856

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ИНДУЦИРУЮЩЕЕ ВЛИЯНИЕ S-НИТРОЗОГЛУТАТИОНА НА ЭКСПРЕССИЮ И АКТИВНОСТЬ ПОЛИПЕПТИДА, ТРАНСПОРТИРУЮЩЕГО ОРГАНИЧЕСКИЕ АНИОНЫ 1B1 В КЛЕТКАХ ЛИНИИ HepG2

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В исследовании на клетках линии HepG2 изучено влияние донора оксида азота S-нитрозоглутатиона на количество и активность полипептида, транспортирующего органические анионы 1B1 (OATP1B1), а также экспрессию гена *SLCO1B1*, кодирующего белок-транспортер. В ходе работы было установлено, что при воздействии в течение 3 ч S-нитрозоглутатион не влиял на количество и активность OATP1B1. При инкубации в течение 24 ч S-нитрозоглутатион (10–500 мкМ) увеличивал экспрессию *SLCO1B1* и количество OATP1B1, что сопровождалось повышением активности белка-транспортера. Индукцию белка OATP1B1 под действием донора NO подавлял ингибитор растворимой гуанилатциклазы (рГЦ) — ODQ (1H-[1,2,4]оксадиазоло-[4,3-a]хиноксалин-1-OH) в концентрации 10 мкМ. Таким образом, в ходе выполненного исследования установлено, что S-нитрозоглутатион, действуя через сигнальный путь NO-рГЦ-cGMP, повышает экспрессию гена *SLCO1B1*, что вызывает увеличение количества белка-транспортера и, как следствие, его активности.

Полный текст статьи на русском языке доступен на сайте журнала (http://pbmc.ibmc.msk.ru).

Ключевые слова: OATP1B1; ген *SLCO1B1*; S-нитрозоглутатион; NO-рГЦ-цГМФ-сигнальный путь

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