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Influence of luminal monosaccharides on secretion of glutathione conjugates from rat small intestine in vitro

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ABSTRACT

Intestinal efflux transporters can significantly reduce the absorption of the drug after peroral application. In this work we studied secretion of glutathione conjugates triggered by glucose at the luminal side of the intestine. Glucose stimulated secretion of DNPSG, NEMSG and CDNB. We used some different monosaccharides and determined that glucose, galactose and α-methylglucopyranoside trigger the secretion, while mannitol and fructose do not. We concluded that interaction with SGLT transporter is the key process necessary for this triggering. To determine which of possible glutathione conjugate transporters (MRP2, MRP4, BCRP or RLIP76) is responsible for the secretion of glutathione conjugates, we used benzbromarone, a MRP inhibitor, and sulfanitran and furosemide, two allosteric MRP2 activators. Benzbromarone inhibited glucose stimulated DNPSG secretion, while allosteric activators additionally increased the secretion. We concluded that MRP2 transporter is related to glucose stimulated DNPSG secretion. Regarding the work of Kubitz et al. we tested the effect of changed medium osmolarity on DNPSG transport and determined that hypoosmolar conditions trigger secretion of DNPSG. These findings suggest that intestinal MRP2 activity has no basal level, but can be stimulated by hypoosmolarity and SGLT transport.

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1. Introduction

MRP transporters are well known subfamily of efflux ABC transporters. The activity of these transporters can significantly influence pharmacokinetic parameters of substances, which are their substrates. MRP transporters are widely expressed in various tissues, but the extent of expression and cellular orientation are transporter specific. They are present in eliminatory organs such as the liver, the kidney and the intestine. Apical MRP transporters facilitate drug secretion into the bile, the renal tubules and into the intestinal lumen, while basolateral MRP transporters facilitate drug secretion into the blood stream. Substrates of MRP transporters include xenobiotics, i.e. anticancer and anti-HIV drugs, and endogenous substances, i.e. biliary acids and glutathione (GSH) conjugates. The latter are frequently employed as markers of MRP activity. Most commonly used are *S*-(2,4-dinitrophenyl)glutathione (DNPSG), *S*-(*N*-ethylsuccinimi-2-yl)glutathione (NEMSG) and leucotriene LTC_4. They are produced by conjugation of GSH with 1-chloro-2,4-dinitrobenzene (CDNB), *N*-ethylmaleimide (NEM) and endogenous arachidonic acid, respectively (Chan et al., 2004). GSH conjugates are also substrates for another efflux ABC transporter, BCRP, and a non-ABC transporter, RLIP76. Both transporters share tissue distribution and substrate specificity of MRP transporter family (Awasthi et al., 2002; Chan et al., 2004).

Several researchers have studied regulatory strategies of MRP transporters. Their activity is under constant translational, transcriptional and posttranscriptional control. Posttranscriptional level includes allosteric regulation and vesicular trafficking (Gerk and Vore, 2002). Zelcer and coworkers studied the mechanism of allosteric regulation of MRP2 transporter and suggested that the substrate and the allosteric binding site are very similar. Therefore, a drug can bind to one or to both sites, complex drug–drug interactions emerge. This kind of regulation is also pertaining to other MRP transporters, but affinities for substrates and allosteric activators are different (Zelcer et al., 2003). Cellular trafficking of MRP transporters containing vesicles is well documented in the liver and in the kidneys. It affects the amount of transporters in the cellular membrane by regulating rates of exocytotic incorporation of vesicles containing transporters into the apical or basolateral membrane and of their endocytotic retrieval into the intracellular pool (Gerk and Vore, 2002). The described regulatory strategies differ in stimuli and in response time (from days at transcriptional regulation to minutes and seconds in vesicular and allosteric regulation, respectively). Some stimuli can regulate MRP activity on multiple levels. For example expression of MRP2 transporters in hepatic cell culture can be increased by dexamethasone and by

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hypoosmolar medium on transcriptional level (Kubitz et al., 1999), but only the hypoosmolar medium triggers also the response on the vesicular level (Dombrowski et al., 2000). Different transporters and organs can also respond differently to the same stimuli. For example, cholestasis decreases hepatic MRP2 activity, while it increases the hepatic MRP3 and renal MRP2 activity (Tanaka et al., 2002).

Although MRP transporters are also expressed in the small intestine, no research has been done on cellular trafficking of MRP transporters in the intestinal mucosa. The aim of the present study was thus to investigate the regulation of MRP activity in the rat small intestine *in vitro* using DNPSG, NEMSG and CDNB as marker substrates.

2. Materials and methods

2.1. Materials

DNPSG was synthesized according to Hinchman (Hinchman et al., 1991). Product was dried for 24 h and its identification was performed on a Varian LC/MS-MS 1200L system. All other chemicals were purchased from Sigma and were of highest purity grade available.

2.2. Experimental methods

Rat intestine from male Wistar rats (250–320 g) was obtained, prepared and mounted in easy mount side-by-side diffusion chambers (Physiologic Instruments, San Diego, CA, USA) as described previously (Žakelj et al., 2004). The experiments conformed to the law for the protection of animals (Republic of Slovenia, EU) and are registered at the Veterinary Administration of the Republic of Slovenia.

Normoosmolar (300 mOsm) and hypoosmolar (200 mOsm) Ringer buffer differed in concentration of NaCl used. For hyperosmolar (400 mOsm) Ringer buffer, osmolarity was increased using mannitol or glucose at mucosal and mannitol at serosal side of the tissue. Mucosal acceptor solution additionally contained 10 mM of appropriate monosaccharides in Ringer buffer depending on experiment and 1 mM acivicin, irreversible gama-glutamyltransferase inhibitor, to prevent the metabolism of GSH conjugates (Hinchman et al., 1998). Serosal solutions contained 10 mM glucose in Ringer buffer. Unless otherwise stated, the concentrations of DNPSG and NEMSG in the donor solutions were 1 mM, while CDNB was used in 0.1 mM concentration because of its toxicity. The solutions of NEMSG were prepared by the in-situ reaction (Fig. 1) simply by dissolving appropriate amounts of GSH and NEM in the Ringer buffer. In experiments with allosteric MRP2 activators, sulfanitran (0.25 mM) and furosemide (0.5 mM) in Ringer buffer were present in mucosal solutions during the entire experiment.

Experiments were performed in serosal to mucosal (S–M) and in mucosal to serosal (M–S) direction. Tissue viability and integrity were controlled throughout the experiments by monitoring the trans-tissue potential difference, the short circuit current and the trans-tissue electrical resistance with a multichannel voltage–current clamp (model VCC MC8, Physiologic Instruments) as described previously (Žakelj et al., 2004). The trans-tissue potential difference is a highly reliable parameter for the determination of the tissue viability (Polentarutti et al., 1999). At the end of the experiments, the trans-tissue potential difference after the addition of glucose to the mucosal compartment (final concentration was 25 mM) was also measured. Segments with inappropriate viability were excluded from the statistical analysis.

2.3. Analytical procedures

All analytes (CDNB, DNPSG and NEMSG) from the transport experiments were analyzed by HPLC system (Series 1100, Hewlett Packard, Waldbron, Germany). For chromatographic separation of samples with CDNB and DNPSG Phenomenex Onyx Monolithic C18 (100 mm × 3 mm) column was used, while separation of samples with NEMSG was performed on Phenomenex Gemini C18 (50 mm × 4.6 mm) column. For determination of DNPSG and CDNB temperature of 35 °C and flow rate of 4 mL/min were applied. Gradient elution with acetonitrile and 10 mM phosphate buffer (pH 3.5) was used. The analysis started with 5% acetonitrile, which was raised linearly to 45% in 3 min. Wavelengths of 250 nm and 335 nm were used for the detection of CDNB (retention time 1.46 min) and DNPSG (retention time 2.63 min), respectively. NEMSG was analyzed at 40 °C, flow rate 3 mL/min. Elution was isocratic and mobile phase consisted of 5% acetonitrile and 95% 10 mM phosphate buffer (pH 2.0). Detection wavelength was 200 nm. The analyte produced two peaks at 0.83 and 0.95 min with a resolution of 1.8. The dual peak phenomenon was already described (Kuninori and Nishiyama, 1991) and is caused by the resolution of two NEMSG diastereomeres. Volume of injection was 100 μL for all analyses.

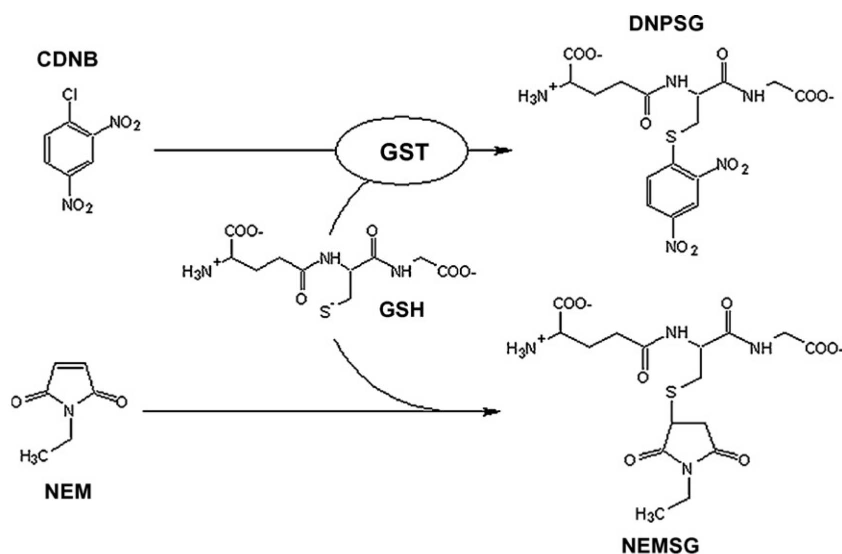

Fig. 1. GSH conjugates and their precursors. CDNB and NEM are precursors of GSH conjugates DNPSG and NEMSG, respectively. While NEM and GSH react quickly (Gregory, 1955), conjugation of CDNB with GSH must be catalyzed by glutathione-*S*-transferase (GST).

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