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Organic anion transporting polypeptides expressed in liver and brain mediate uptake of microcystin

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Abstract

Microcystins are toxins produced by freshwater cyanobacteria. They are cyclic heptapeptides that exhibit hepato- and neurotoxicity. However, the transport systems that mediate uptake of microcystins into hepatocytes and across the blood–brain barrier have not yet been identified. Using the *Xenopus laevis* oocyte expression system we tested whether members of the organic anion transporting polypeptide superfamily (rodent: Oatps; human: OATPs) are involved in transport of the most common microcystin variant microcystin-LR by measuring uptake of a radiolabeled derivative dihydromicrocystin-LR. Among the tested Oatps/OATPs, rat Oatp1b2, human OATP1B1, human OATP1B3, and human OATP1A2 transported microcystin-LR 2- to 5-fold above water-injected control oocytes. This microcystin-LR transport was inhibited by co-incubation with the known Oatp/OATP substrates taurocholate (TC) and bromosulfophthalein (BSP). Microcystin-LR transport mediated by the human OATPs was further characterized and showed saturability with increasing microcystin-LR concentrations. The apparent K_m values amounted to 7 ± 3 μM for OATP1B1, 9 ± 3 μM for OATP1B3, and 20 ± 8 μM for OATP1A2. No microcystin-LR transport was observed in oocytes expressing Oatp1a1, Oatp1a4, and OATP2B1. These results may explain some of the observed organ-specific toxicity of microcystin-LR. Oatp1b2, OATP1B1, and OATP1B3 are responsible for microcystin transport into hepatocytes, whereas OATP1A2 mediates microcystin-LR transport across the blood–brain barrier.

Keywords: Environmental toxins; Natural toxins; Microcystin; Cyanobacteria; Blood–brain barrier; Hepatocyte; Organic anion transport; Oatp; OATP

Introduction

Microcystins are naturally occurring toxins produced by freshwater cyanobacteria and can be found in lakes, ponds, and rivers that are often used for recreational activities (Chorus et al., 2000; Watanabe et al., 1996). Thus, besides menacing terrestrial and aquatic wildlife and livestock (Carmichael, 1994; Fischer and Dietrich, 2000; Schwimmer and Schwimmer, 1968), cyanobacteria also represent a threat to human health (Jochimsen et al., 1998; Teixeira et al., 1993). In 1996, a hemodialysis unit in Caruaru, Brazil, used water for dialysis from a reservoir, which was contaminated with blue-green algae and contained microcystins. Sixty of the 126 intoxicated patients died of severe microcystin-induced neurotoxicity or toxic liver failure (Jochimsen et al., 1998; Pouria et al., 1998). Microcystins act by inhibiting Ser/Thr protein phosphatase-1 and -2A (Mackintosh et al., 1990), which leads to disruption of the cytoskeleton and subsequent cell death (Eriksson et al., 1989).

Target organs of microcystin are mainly the liver and the brain. This requires uptake of microcystin across the sinusoidal (basolateral) plasma membrane of hepatocytes and its transport across the blood–brain barrier. In rat hepatocytes, basolateral uptake of microcystin was inhibited by cholate, taurocholate (TC), and bromosulfophthalein (BSP) (Eriksson et al., 1990; Runnegar et al., 1995). All

Abbreviations: OATP, human organic anion transporting polypeptide; Oatp, rodent organic anion transporting polypeptide; BSP, bromosulfophthalein; TC, taurocholate.

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latter compounds are substrates of the organic anion transporting polypeptide (rodent: Oatps; human: OATPs) superfamily of membrane transporters (Hagenbuch and Meier, 2003, 2004) that are classified within the *SLCO* family of solute carriers (Human Gene Nomenclature Committee Database) (Hagenbuch and Meier, 2004). Oatps/OATPs mediate Na^+-independent uptake of a wide variety of amphipathic organic compounds including bile salts, organic anionic dyes, steroids and steroid conjugates, drugs, various peptides, and toxins (Hagenbuch and Meier, 2003). Oatps/OATPs that are expressed in the brain (blood–brain barrier, choroid plexus) as well as in the liver include rat Oatp1a1 (Jacquemin et al., 1994), rat Oatp1a4 (Abe et al., 1998; Noé et al., 1997), human OATP1A2 (Kullak-Ublick et al., 1995), and human OATP2B1 (Kullak-Ublick et al., 2001). Rat Oatp1b2 (Cattori et al., 2000), human OATP1B1 (Abe et al., 1999; Hsiang et al., 1999; König et al., 2000b), and human OATP1B3 (König et al., 2000a) are primarily expressed in the liver. In the present study, we used the radiolabeled dihydromicrocystin-LR epimer [L-MeAla^7]microcystin-LR (Meriluoto et al., 1990), which was previously shown to have similar characteristics as native microcystins (Meriluoto et al., 1990). The study served to investigate whether the hepato- and neurotoxicity observed after microcystin intoxication (Jochimsen et al., 1998; Pouria et al., 1998) could be explained by microcystin transport mediated by members of the Oatp/OATP superfamily that are expressed in hepatocytes and in the blood–brain barrier.

Materials and methods

Animals. Female *Xenopus laevis* were purchased from the African Xenopus facility c.c, Noordhoek, Rep. South Africa.

Chemicals. [^{3}H]-dihydromicrocystin-LR (0.5 mCi/mmol) was prepared as described (Meriluoto et al., 1990). [^{3}H]Estrone-3-sulfate (53 Ci/mmol) was obtained from NEN Life Science Products (Boston, MA), [γ-^{32}P]ATP (3000 Ci/mmol) was purchased from Amersham (Buckinghamshire, UK).

Uptake studies in X. laevis oocytes. Complementary RNA was transcribed in vitro from linearized cDNA using the mMESSAGE mMACHINE T7 kit (Ambion, Austin, TX). *X. laevis* oocytes were prepared as described (Hagenbuch et al., 1996) and injected either with 50 nl of water or with 5 ng of cRNA in 50 nl of water. After 3 days in culture at 18 °C with a daily change of Barth's solution, uptake of radiolabeled substrate was measured at 25 °C in 100 μl of uptake solution containing 100 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, and 10 mM HEPES adjusted to pH 7.5 with Tris as described (Reichel et al., 1999).

Protein phosphatase-1 and -2A inhibition assay. Preparation of [γ-^{32}P]phosphorylase-a and the protein phosphatase-1 and -2A inhibition assay was carried out as described (Cohen et al., 1988; MacKintosh, 1993). Assays were performed in 60-μl reactions consisting of 20 μl *X. laevis* oocyte-extract, 20 μl assay buffer, and 20 μl [γ-^{32}P]phos-32P]phosphorylase-a substrate for 10 min at 30 °C. The reaction was stopped by addition of 180 μl of 20% (w/v) trichloro-acetic acid, kept on ice for 10 min, and centrifuged at 17500 × *g* for 5 min. For acid molybdate extraction of inorganic [^{32}P]phosphate, 200 μl of the supernatant was mixed with 200 μl 1.25 mM KH_2PO_4 in 0.5 M H_2SO_4, 500 μl isobutanol/heptane (1:2), and 100 μl ammonium molybdate (5% w/v). After separation of the organic and aqueous phase, 300 μl of the organic phase was added to 3 ml of scintillation fluid (Ready Safe, Beckman, USA) and subjected to β-scintillation spectrometry.

Statistical analysis. Data are expressed as mean ± SE. To compare uptake into water injected with cRNA-injected oocytes, the unpaired Student's *t* test was used. For comparison of protein phosphatase-1 and -2A inhibition, data (mean ± SD) were subjected to ANOVA followed by a Tukey's test, significance levels were determined at $P < 0.05$.

Results

Transport of microcystin by liver Oatps/OATPs

Several Oatps/OATPs are expressed in hepatocytes where they mediate uptake of numerous organic compounds from blood plasma. To determine whether one of these transporters could mediate uptake of microcystin-LR into hepatocytes, we expressed these transporters in the *X. laevis* oocyte expression system and measured uptake of radiolabeled microcystin-LR. As demonstrated in Fig. 1, rat Oatp1b2-expressing oocytes accumulated microcystin-LR about 2-fold. Furthermore, human OATP1B1 and OATP1B3 showed an approximately 3- to 4-fold higher uptake of microcystin-LR as compared to water-injected control oocytes. No significant microcystin-LR uptake was observed for rat Oatp1a1-, rat Oatp1a4-, and human OATP2B1-expressing oocytes although they were able to mediate uptake of the positive control estrone-3-sulfate (data not shown). To further characterize microcystin-LR transport mediated by the human OATPs, we performed inhibition experiments and saturation kinetics. Co-incubation of OATP1B1- and OATP1B3-expressing oocytes with microcystin-LR and two known OATP substrates, either 100 μM TC (Fig. 2, hatched bars) or 50 μM BSP (Fig. 2, open bars), reduced microcystin-LR uptake into the oocytes almost completely. Initial experiments demonstrated that microcystin-