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The Role of the Equilibrative Nucleoside Transporter 1 on Tissue and Fetal Distribution of Ribavirin in the Mouse

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Abstract

Ribavirin is used for the treatment of hepatitis C virus (HCV) infection. The equilibrative nucleoside transporter 1 (ENT1) expressed in hepatocytes transports ribavirin into the liver, the site of efficacy of the drug. However, it is still unclear whether ENT1 plays a dominant role in the hepatic distribution of the drug *in vivo*. In addition, due to fetal toxicity, administration of ribavirin to pregnant women with HCV infection is contraindicated. ENT1 might play a role in the fetal distribution and therefore the fetal toxicity of ribavirin. The aim of the present study was to investigate the *in vivo* contribution of ENT1 to the tissue distribution of ribavirin. When compared with that in Ent1(+/+) mice, ribavirin tissue to plasma concentration ratio (including phosphorylated metabolites) in Ent1(–/–) mice at 15 min and 6 hr after intravenous [³H]-ribavirin (3 mg/kg) administration was consistently and significantly decreased in the liver and the pancreas. Likewise, when compared with the Ent1(+/+) mice, the fetal distribution of ribavirin at 15 min after administration was significantly reduced in Ent1(–/–) fetuses and placenta. In contrast, there was no significant difference between Ent1(+/+), Ent1(+/-), and Ent1(–/–) mice in the fetal or placental to maternal plasma ribavirin concentration ratio at 2 hr after ribavirin administration. The findings in the present study suggest that ENT1 plays a pivotal role in the distribution of ribavirin into tissues including the liver and pancreas, but affects only the rate but not the extent of ribavirin distribution into the fetus.

Keywords

ribavirin; equilibrative nucleoside transporter; hepatitis C; tissue distribution; fetal distribution

Introduction

Ribavirin is a nucleoside analog drug used in the treatment of chronic hepatitis C virus (HCV) infection, usually in combination with other drugs such as sofosbuvir [1]. Ribavirin is a pro-drug and is phosphorylated intracellularly to its active and polar phosphorylated metabolites, ribavirin 5'-monophosphate (RMP) and ribavirin 5'-triphosphate (RTP) which are either retained in the cells or dephosphorylated back to ribavirin [2–4]. Ribavirin's entry

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Conflict of Interest

The authors have declared that there is no conflict of interest.

into cells is mediated primarily by the nucleoside transporters, equilibrative nucleoside transporter (ENT) 1, ENT2, concentrative nucleoside transporter (CNT) 2, and CNT3 [5–7]. ENT1 and ENT2 are equilibrative nucleoside transporters, while CNT2 and CNT3 are concentrative, Na⁺-dependent nucleoside transporters [8–10]. We have previously shown that ENT1 is the primary nucleoside transporter in the *in vitro* uptake of ribavirin into human hepatocytes [11], the efficacy site of the drug, and into erythrocytes [12], a major toxicity site of the drug. However, to our knowledge, the contribution of ENT1 on the tissue distribution of ribavirin *in vivo* has not been evaluated. Therefore, in the present study, we investigated the role of Ent1 in the tissue distribution of ribavirin using Ent1(+/+) and Ent1(–/–) mice.

Ribavirin causes significant fetal toxicity when administered to pregnant mice resulting in impaired craniofacial and limb bone development, as well as increased fetal resorption [13, 14]. For this reason, administration of ribavirin to pregnant women with hepatitis C is contraindicated [15]. ENT1/Ent1 is expressed in the human and rat placenta [7, 11, 16]. Hence, ENT1 could contribute to the trans-placental distribution of ribavirin, and therefore eventual toxicity of the drug. However, it is still unclear that whether ENT1 is the major player in the fetal distribution and toxicity of ribavirin. In the present study, we also measured fetal and placental concentration of ribavirin in Ent1(+/+), Ent1(+/-), and Ent1(-/-) mice to evaluate the role of Ent1 in fetal distribution of the drug.

Materials and Methods

Materials

Ribavirin was purchased from Sigma-Aldrich (St. Louis, MO). [³H]-Ribavirin (3.6 Ci/mmol), RMP, RTP, and [¹⁴C]-mannitol were purchased from Moravak Biochemicals (Brea, CA). All other chemicals were of reagent or analytical grade and purchased through other commercial suppliers.

Mouse Husbandry

All animal procedures were reviewed and approved by the University of Washington Institutional Animal Care and Use Committee (IACUC). Ent1(+/+), Ent1(+/-) and Ent1(-/-) colonies were maintained as previously described [12].

Tissue Distribution Study

The Ent1(+/+) and Ent1(–/–) mice in the present study were in a 50% C57BL/6 and 50% SV140 background strain. The groups in the tissue distribution study were not balanced by sex. This was a result of the random selection of animals used in this study. There are numerous previous reports of no sex differences in both the pharmacokinetics and tissue distribution of ribavirin in C57BL/6 mice [17, 18]. Therefore, we do not anticipate that the sex imbalances in the present study contributes significantly to the difference in ribavirin distribution between the Ent1(+/+) and Ent1(–/–) animals. [³H]-Ribavirin in vehicle (0.9% saline) was administered intravenously (in the retro-orbital sinus; 3 mg/kg, 1.1 mCi/kg) under ketamine/xylazine (130 and 8.8 mg/kg, respectively) anesthesia. The animals were sacrificed by exsanguination after pentobarbital overdose at either 15 min or 6 hr after

ribavirin injection. These time points were chosen to capture the initial distribution (15 min) and elimination (6 hr) of ribavirin respectively. Approximately 2 min prior to the sampling point, [^{14}C]-mannitol (a marker of tissue vascular volume) was administered intravenously (retro-orbital sinus; tracer dose, 0.05 $\mu\text{Ci/g}$) [19]. Blood was sampled from each animal in heparinized microhematocrit tubes immediately prior to euthanasia. The total blood volume sampled from each animal was less than 1% of the total body weight. The blood samples were immediately centrifuged for 1 min at $5000 \times g$. The hematocrit was measured, and the hematocrit tube scored at the buffy-coat/plasma interface. The plasma or packed erythrocytes (5–10 μL , leaving behind the interface) were transferred to pre-weighed microhematocrit tubes containing 100 μL deionized water (dH_2O), and immediately froze in liquid nitrogen. Whole tissues (pancreas, spleen, kidney, muscle [thigh], liver, heart, lung, brain, colon and small intestine) were collected at necropsy, blotted dry, and immediately frozen in liquid nitrogen. In separate experiments, the animals were immediately placed in metabolic cages to collect urine and feces. The contents of the bladder were collected using a syringe with a 26 G needle and pooled with the urine collected from the metabolic cages. The cages were washed down with dH_2O , and the cage wash stored and analyzed separately from the urine. All samples were stored at -80°C until further analysis.

Fetal Distribution Study

The fetal distribution of ribavirin was investigated using methodology similar to that previously described [20]. This methodology enabled us to control for any variability in the maternal plasma concentration of ribavirin, because a single dam carrying pups of multiple genotypes would expose each pup to the same maternal plasma concentration of ribavirin. [^3H]-Ribavirin (3 mg/kg, 0.4 mCi/kg) was administered intravenously (as above) to pregnant (gestational day 17) Ent1(+/-) mice mated with Ent1(+/-) males. Gestational date was determined by visual confirmation of a vaginal plug on day 0. The animals were euthanized at 15 min or 2 hr after drug administration by exsanguination by cardiac puncture under pentobarbital anesthesia. The 2 hr (rather than the 6 hr time point used in non-pregnant animals) was chosen to ensure that the radioactivity in the placenta and the fetus was measurable and yet capture the elimination of ribavirin. Plasma and erythrocyte ribavirin concentrations and hematocrit were determined as described in above. The fetuses and placenta (Ent1(+/+), Ent1(+/-) and Ent1(-/-)) were collected at necropsy and genotyped as previously described [12].

Direct Counting Analysis of Total [^3H] and [^{14}C] Radioactivity

Total radioactivity in plasma, erythrocytes, or urine was measured as described previously [21]. For analysis of tissue radioactivity content, each tissue was thawed at room temperature, weighed, and transferred to a 15 mL homogenization tube. One to three mL PBS was added, and the tissue was homogenized with a hand held tissue homogenizer (Omni International, Kennesaw, GA) using disposable tips to prevent sample to sample contamination. One-hundred μL of the homogenate was transferred to tared 20 mL scintillation vials using a positive displacement pipette and the mass determined. One mL of BioSol tissue solubilizer (National Diagnostics, Atlanta, GA) was added and the samples incubated and shaken for 3 hr at 55°C and 200 rpm. After cooling, the samples were decolorized by the addition of 300 μL 30% H_2O_2 . Ten mL of scintillation fluid was added

and the total radioactivity determined by dual-channel liquid scintillation counting. The total radioactivity (expressed as $\mu\text{Ci/g}$ tissue) was corrected for the contribution of $[^3\text{H}]$ -radioactivity in the vascular space. That is, we determined the tissue vascular volume using $[^{14}\text{C}]$ -mannitol. We then subtracted the radioactivity derived from the blood, which was calculated from the blood radioactivity concentration (calculated from the plasma and erythrocyte total radioactivity concentrations, and the hematocrit) and the tissue blood volume from the tissue total radioactivity. This is a more accurate measurement of the radioactivity for tissue that have a high blood volume (i.e. liver and kidney), as it corrects for the contribution of the radioactivity in the blood.

HPLC Analysis to Determine Ribavirin Composition

Plasma and tissue samples were analyzed by HPLC as previously described [12, 21]. We have previously confirmed that RMP and RTP were not detected in the mouse plasma at either 15 min or ~3 hr after intravenous dosing [21], and this was consistent with observations in humans [22]. The phosphorylated nucleotides $[^3\text{H}]$ -RMP and $[^3\text{H}]$ -RTP (Moravsek, Brea, CA) exhibited spontaneous degradation to ribavirin in the presence of tissue homogenate. Therefore, the tissue samples were incubated for 30 min at 37 °C with 10 U alkaline phosphatase to dephosphorylate the ribavirin nucleotides (RMP, ribavirin 5'-diphosphate (RDP) and RTP) to ribavirin. In preliminary experiments, RTP and RMP spiked liver homogenate were completely converted to ribavirin after 30-min treatment with alkaline phosphatase (data not shown). In all samples, the protein in each sample was precipitated with 6% perchloric acid, neutralized with 2 M K_2HPO_4 , and then centrifuged at $20,000 \times g$ for 10 min at 4 °C. The supernatant was analyzed by HPLC and fraction collecting as previously described [21]. Fractions were analyzed by scintillation counting as described above.

Data Analysis

In calculating the mass balance of total radioactivity in each tissue, we multiplied the tissue radioactivity concentration by the total tissue mass. For the tissue in which we only sampled the tissue as opposed to harvesting the entire organ (i.e. blood and skeletal muscle), we used literature values for organ percent body mass of 7% (blood) and 38.7% (skeletal muscle) [23].

Significant differences between $\text{Ent1}(+/+)$ and $\text{Ent1}(-/-)$ tissue (ribavirin plus phosphorylated metabolites) to plasma concentration ratios were tested using the two-sided Student's t-test. Since the intracellular phosphorylated metabolites are formed only after ribavirin enters the cells, the summation of ribavirin plus its phosphorylated metabolites reflect the total uptake of ribavirin into the cells. Differences between $\text{Ent1}(+/+)$, $\text{Ent1}(+/-)$ and $\text{Ent1}(-/-)$ animals was determined using the Kruskal-Wallis-based multiple comparison test.

Results

Ribavirin Total Radioactivity in Tissues

Table 1 shows the total radioactivity in tissues of Ent1(+/+) and Ent1(-/-) mice. The total radioactivity in various tissues was corrected for the total radioactivity present in the vascular volume of each tissue using [¹⁴C]-mannitol (see Methods). In the Ent1(+/+) mice, the liver and the skeletal muscles were the major sites of total ribavirin radioactivity (ribavirin plus metabolites) distribution irrespective of the time of sampling (Table 1). The remaining tissues received a minority of the drug. Interestingly, the pancreas and the small intestine were the next highest site of distribution of total ribavirin radioactivity. On the other hand, total radioactivity in the liver in Ent1(-/-) mice was less than that in Ent1(+/+) mice, whereas total radioactivity in the skeletal muscle was similar to that in Ent1(+/+) animals at 15 min after administration (Table 1). The percent of the total radioactivity in the liver decreased at 6 hr after administration, but did not decrease in the skeletal muscle. Total radioactivity in the remaining tissues (expressed as a percentage of the dose) had substantially lower amounts of total radioactivity (Table 1).

The mass balances of total radioactivity at 15 min and 6 hr after administration in the Ent1(+/+) animals were different from those in the Ent1(-/-) animals (Table 1). There appeared to be an increase in the amount of total radioactivity eliminated in the urine in Ent1(-/-) mice (Table 1). We were unable to formally test these differences, because the 3 animals in each genotype were housed in a common metabolic cage to collect pooled urine for 6 hr. On the other hand, we previously investigated the urinary excretion of ribavirin in Ent1(+/+) and Ent1(-/-) in detail, and reported that there is no significant differences in 0–48 hr urinary excretion of ribavirin total radioactivity [21].

Ribavirin Tissue Distribution

Figure 1A and 1B show the tissue:plasma concentration ratio of ribavirin in Ent1(+/+) and Ent1(-/-) mice at 15 min and 6 hr after intravenous administration of the drug. The concentration of ribavirin in various tissues was determined after correcting for the total radioactivity present in the vascular volume of each tissue using [¹⁴C]-mannitol (see Methods). The ribavirin tissue to plasma concentration ratio in Ent1(-/-) mice at 15 min after administration was significantly decreased in the liver, lung and pancreas as compared to tissue from the Ent1(+/+) animals (Figure 1A). Additionally, the kidney to plasma concentration ratio in the Ent1(-/-) mice was significantly increased as compared to that in Ent1(+/+) animals (Figure 1A). The distribution of ribavirin at 6 hr after administration was significantly decreased in the Ent1(-/-) liver, pancreas, heart, proximal small intestine segment, skeletal muscle, and brain as compared to the Ent1(+/+) animals (Figure 1B). In contrast to the 15 min data, the kidney to plasma concentration ratio in Ent1(+/+) mice at 6 hr after administration was similar to that in Ent1(-/-) mice (Figure 1A and 1B).

In Ent1(+/+) mice, the tissue distribution of ribavirin in the liver and distal small intestine at 6 hr after administration was significantly lower than that at 15 min after administration. In contrast, the tissue distribution of ribavirin in the pancreas and brain at 6 hr after administration was significantly higher than that at 15 min after administration (Figure 1). In

Ent1(−/−) mice, except for the kidney, there was no time-dependent change in the tissue distribution of ribavirin (Figure 1).

Ribavirin Fetal Distribution

Figure 2A and 2B show the blood contamination-corrected tissue (placenta and fetus):maternal plasma concentration ratio of ribavirin in Ent1(+/+), Ent1(+/-), and Ent1(−/−) mice at 15 min and 2 hr after intravenous administration of the drug. The 2 hr (rather than the 6 hr time point used in non-pregnant animals) was chosen to ensure that the radioactivity in the placenta and the fetus was measurable and yet capture the elimination of ribavirin. The tissue (either fetus or placenta) to maternal plasma concentration ratio at 15 min after intravenous administration was lower than unity for each genotype. The distribution of ribavirin in Ent1(+/-) and Ent1(−/−) fetuses and placenta at 15 min after administration was significantly reduced when compared with that in Ent1(+/-) fetuses (Figure 2A).

The tissue to maternal plasma concentration ratio at 2 hr after intravenous administration was approximately unity for each tissue and genotype. There were no significant differences in the tissue (either fetus or placenta) to maternal plasma concentration ratios between the Ent1(+/-) or the Ent1(−/−) animals and the Ent1(+/-) animals (Figure 2B).

The tissue:maternal plasma concentration ratio of ribavirin in both placenta and fetus at 2 hr after administration was significantly higher than that at 15 min after administration in Ent1(+/+), Ent1(+/-), and Ent1(−/−) mice (Figure 2).

Discussion

In the present study, there was incomplete mass balance of the dose in the Ent1(−/−) animals (Table I). This findings suggest that a significant proportion of the dose (~35–40%) has distributed into organs or tissues that were not collected. We did not collect the skin or tissue from the bone, lymph nodes, fat, or urogenital system. The dose has possibly distributed into these tissues, especially when considering that organs such as skin account for about 17% of the mouse total body mass. In addition, there may be heterogeneous distribution of ribavirin into the skeletal muscles in the Ent1(−/−) mice, as there have been reported widely variable regional values for skeletal blood flow depending on muscle location, activity and animal anesthetic state [23]. This could impact our estimation of the total mass of drug that distributed into all skeletal muscle, as we only sampled a portion (the muscles of the upper thigh) of skeletal muscles of each animal. However, this incomplete mass balance does not detract from the conclusions made below regarding tissue distribution of ribavirin (see below), because the latter is based on the individual tissue:plasma concentration ratio and is independent of the mass balance of the drug.

Since ribavirin phosphates spontaneously degraded to ribavirin, we measured the combined concentration of ribavirin plus its phosphorylated metabolites when computing the tissue:plasma concentration ratio of the drug. However, this does not in any way detract from the interpretation of the uptake of ribavirin since the phosphorylated metabolites are formed only after ribavirin has been taken up into the tissues and are trapped there due to their hydrophilicity. This assumption is supported by the fact that we did not observed the

presence of any phosphorylated metabolites in the plasma (see Methods) [21, 22]. Thus, this combined radioactivity reflects the total uptake (and therefore the transport) of ribavirin into the tissues. Alternatively stated, the amount of total ribavirin (unchanged plus phosphorylated) in the tissue can be used as a measure of transport (via nucleoside transporters) as well as passive diffusion of ribavirin at the tissue:plasma/blood interface.

We deliberately chose two time points, 15 min and 6 hr, to measure the tissue distribution of ribavirin to capture data during the distribution (15 min) and elimination (6 hr) phase of ribavirin [21]. The tissue:plasma concentration ratios of ribavirin (and its phosphorylated metabolites) in Ent1(+/+) mice were different from that in Ent1(-/-) mice (Figure 1A and 1B). It is unlikely that these differences are due to difference in the plasma concentration of ribavirin as we have previously shown that the plasma concentrations of ribavirin after intravenous administration to Ent1(+/+) mice are not significantly different from that in Ent1(-/-) mice [21]. Therefore, the observed differences in tissue:plasma concentration of ribavirin between Ent1(-/-) and Ent1(+/+) mice can be attributed to the role of Ent1 in the tissue distribution of ribavirin. For example, in the liver and pancreas, the tissue:plasma concentration ratio of ribavirin in Ent1(+/+) mice was higher than that in Ent1(-/-) mice (Figure 1A and 1B); therefore, Ent1 plays a pivotal role in the distribution of the drug in the liver and pancreas. This observation is consistent with data which suggest that Ent1 plays an important role in the efficacy and toxicity of nucleoside drugs that target these organs. For example, Nordh et al. summarized the potential predictive value of human ENT1 expression in pancreatic tumor cells in patients treated with gemcitabine [24]. Nine of 10 studies they evaluated showed a statistically significant higher overall survival in patients with high/positive human ENT1 expression as compared with those with low/negative expression. Additionally, 7 of 10 studies also reported disease-free survival (DFS) as an outcome, and 6 of 7 studies had statistically longer DFS in the high/positive ENT groups [24].

The kidney:plasma concentration ratio of ribavirin in Ent1(+/+) mice was lower than that in Ent1(-/-) mice at 15 min after administration but not at 6 hr (Figure 1A and 1B). This finding suggests that ribavirin accumulated in the kidney by a concentrative process when ENT1 is absent. In Ent1(+/+) mice, ribavirin might actively be reabsorbed from the lumen into the renal tubular epithelial cells by Cnt2 [25, 26], since Cnt1 does not transport ribavirin and Cnt3 mRNA is not detectable in the mouse kidney [27] (our unpublished data). Then, the drug could be transported across the basolateral membrane of these cells by Ent1. In our preliminary study, the mRNA level of Cnt2 in the Ent1(-/-) mouse kidney was not changed as compared with Ent1(+/+) mouse kidney (unpublished data). Therefore, in Ent1(-/-) mice, ribavirin may be sequestered to very high concentrations in the kidney epithelial cells, whereas the ribavirin may rapidly re-equilibrate out of the cell into the blood in Ent1(+/+) mice. This difference may be quite large at early time-points, when the urine concentration of ribavirin is greatest. Additionally, when the entire organ has reached distributional equilibrium with the plasma (at 6 hr after administration), we may be unable to detect the “localized” difference in ribavirin concentrations in the tubular epithelial cells between the Ent1(+/+) and Ent1(-/-) mice. One of the explanations could be that the kidney is a heterogeneous organ (we collected whole kidney, and homogenized it to measure the ribavirin accumulation in this tissue), with many cell types that do not express Ent1. Therefore, the diffusion of ribavirin into the vast majority of non-Ent expressing cells in the

kidney “overwhelm” the difference in ribavirin concentrations in the tubular epithelial cells. Further studies will be needed to clarify the mechanisms of the ribavirin distribution into the kidney.

Ent1 also plays an acute role in the distribution of ribavirin into the fetus. This is highlighted by the “gene-dose” dependent decrease in the both the fetal and placental tissue to maternal plasma ribavirin concentration ratio at 15 min after intravenous administration (Figure 2A). Interestingly, this role of Ent1 was acute (see below for discussion), as we observed no significant differences in the tissue to maternal plasma ratio for both fetuses and the placenta at 2 hr after intravenous administration (Figure 2B). Therefore, it is unlikely that Ent1 plays a role in the fetal toxicity of ribavirin.

As described above, the impact of Ent1 on tissue distribution of ribavirin was time-dependent (Figure 1). In the Ent1(+/+) mice, the liver:plasma ribavirin concentration ratio decreases with time while for the brain and pancreas this ratio increases with time (Figure 1). At first sight this may seem surprising but this observation is probably explained by time-dependent distribution of ribavirin into various tissues. The liver is a highly perfused organ; therefore the impact of Ent1 will manifest early in time. In addition, as we have shown previously, Ent1 affects the rate of distribution of ribavirin into tissue but not always the extent of distribution [12]. Hence, with time, the difference in liver:plasma concentration ratio between Ent1(+/+) and Ent1(-/-) mice diminishes but does not completely dissipate. This is because ribavirin is extensively phosphorylated in the liver and these metabolites are likely trapped there. Therefore, the greater initial distribution of ribavirin into the Ent1(+/+) liver and the extensive phosphorylation in the liver results in a persistent difference in liver:plasma concentration ratio. In contrast, since the brain and pancreas have relatively lower perfusion [28], the impact of Ent1 in the tissue distribution of ribavirin manifests later in time. We predict that had we sampled for longer, this difference in brain and pancreas distribution of ribavirin between Ent1(+/+) and Ent1(-/-) mice would also diminish with time or be eliminated. We predict the latter for tissues where ribavirin is not extensively phosphorylated or the phosphorylated metabolites are rapidly and extensively dephosphorylated.

As in the case of the brain and pancreas, the placenta in mice is considered to be a relatively lower perfused organ [29]. The tissue (placenta and fetus):maternal plasma concentration of ribavirin (and its phosphorylated metabolites) at 2 hr after administration in Ent1(+/+), Ent1(+/-), and Ent1(-/-) mice was higher than that at 15 min (Figure 2); however, the tissue:maternal plasma concentration ratio of the drug at 2 hr after administration was about the unity. These results suggest that ribavirin is unlikely to be significantly phosphorylated in the placenta and fetus. Ent1 cannot transport phosphorylated metabolites; therefore, the tissue:plasma concentration of ribavirin (and its phosphorylated metabolites) should be higher than the unity if the ribavirin is phosphorylated in the tissue. That is, ribavirin's accumulation is less in the placenta and fetus in mice as compared with other tissues such as the liver and pancreas, and the accumulation of ribavirin in the fetus depends on the maternal plasma concentration of the drug.

In conclusion, the findings in the present study show that Ent1 plays an important role in the distribution of ribavirin into the tissues, especially liver and pancreas, which are the target of the nucleoside drugs *in vivo*. On the other hand, since Ent1 affects only the rate but not the extent of ribavirin distribution into the fetus, Ent1 is unlikely to be involved in the fetal toxicity of the drug. Further studies are needed to clarify the mechanism of fetal toxicity of ribavirin. Although our study was conducted after single dose administration of the drug, our conclusions should be valid for multiple dose administration provided the tissue distribution or intracellular phosphorylation/dephosphorylation ribavirin is not saturated. Studies in mice, after oral administration of ribavirin, support this assumption [21]. To our knowledge, this is the first report to show the role of ENT1 in the *in vivo* tissue and fetal distribution of ribavirin. Further studies may provide a better understanding of the contribution of other nucleoside transporters (i.e. CNT2) to the hepatic and pancreatic distribution of nucleoside drugs *in vivo*.

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Fig. 1. Ribavirin Distribution into Tissues after Correcting for Blood Contamination

(A) The tissue:plasma ribavirin concentration ratio at 15 min after intravenous injection of [3 H]-ribavirin in Ent1(+/+) and Ent1(-/-) mice. (B) The tissue:plasma ribavirin concentration ratio at 6 hr after intravenous injection of [3 H]-ribavirin in Ent1(+/+) and Ent1(-/-) mice. Values represent mean $\pm$ S.D. from 3 independent experiments. SI; small intestine. N.D.; not determined.

** $P < 0.01$, * $P < 0.05$; significantly different from Ent1(+/+) mice.

Fig. 2. Ribavirin Distribution into Placenta and Fetus after Correcting for Blood Contamination

(A) The fetal tissue:maternal plasma concentration ratio at 15 min after intravenous injection of [3 H]-ribavirin in Ent1(+/+), Ent1(+/-), and Ent1(-/-) mice. (B) The fetal tissue:maternal plasma concentration ratio at 2 hr after intravenous injection of [3 H]-ribavirin in Ent1(+/+), Ent1(+/-), and Ent1(-/-) mice. Filled, gray, and open columns indicate the tissue:plasma concentration ratios after dephosphorylation of the phosphorylated metabolites in Ent1(+/+), Ent1(+/-), and Ent1(-/-) mice, respectively. Values of tissue:maternal plasma concentration ratio at 15 min after administration (panel A) represent mean $\pm$ S.D. from 7 (Ent1(+/+)), 9 (Ent1(+/-)), and 7 (Ent1(-/-)) experiments. Values of tissue:maternal plasma concentration ratio at 2 hr after administration (panel B) represent mean $\pm$ S.D. from 4 (Ent1(+/+)), 8 (Ent1(+/-)), and 9 (Ent1(-/-)) experiments.

* $P < 0.05$; significantly different from Ent1(+/-) mice.

Table 1

Ribavirin Total Radioactivity in Tissues of Ent1(+/+) and Ent1(-/-) Mice After Correcting for Blood Contamination

Mass Balance Total Radioactivity Recovery (% of dose) [#]				
Ent1 genotype	15 min		6 hr	
	+/+	-/-	+/+	-/-
Blood	2.35 ± 0.39	2.20 ± 0.23	3.00 ± 0.48	1.72 ± 0.32 *
Pancreas	1.97 ± 0.06	3.16 ± 0.50 *	4.55 ± 1.06	1.05 ± 0.11 *
Spleen	1.36 ± 0.23	4.13 ± 0.52 *	0.81 ± 0.27	1.18 ± 0.66
Kidney	1.91 ± 0.39	4.01 ± 1.12 *	0.66 ± 0.03	0.25 ± 0.16 *
Liver	39.5 ± 5.1	10.6 ± 0.88 *	11.1 ± 3.7	3.11 ± 0.63 *
Heart	1.00 ± 0.17	1.80 ± 0.46 *	1.40 ± 0.12	0.70 ± 0.35 *
Lung	0.60 ± 0.19	0.82 ± 0.12	0.35 ± 0.04	0.18 ± 0.02 *
Brain	0.22 ± 0.04	0.21 ± 0.01	0.34 ± 0.05	0.28 ± 0.02
Skeletal Muscle	34.5 ± 13.8	22.3 ± 5.6	49.0 ± 6.8	17.7 ± 3.6 *
Colon	1.13 ± 0.11	1.42 ± 0.08 *	0.71 ± 0.14	0.24 ± 0.02 *
SI Proximal ^a	2.69 ± 0.74	5.23 ± 1.00 *	2.55 ± 0.16	1.08 ± 0.08 *
SI Medial ^a	1.92 ± 0.39	3.67 ± 0.52 *	0.91 ± 0.43	0.60 ± 0.18
SI Distal ^a	1.39 ± 0.22	2.28 ± 0.35 *	0.81 ± 0.27	0.48 ± 0.12
Sub-total Tissues	90.5 ± 18.3	61.8 ± 4.5	76.1 ± 12.1	28.6 ± 3.7
Urine ^b	N.D. ^c	N.D. ^c	11.7	27.9
Cage Wash ^b	N.D. ^c	N.D. ^c	5.29	6.27
Total	90.5	61.8	93.1	62.8

[#]Data shown are mean ± SD from 3 individual experiments.

^aSmall intestine, divided into three equal length segments

^bPooled from all animals by each genotype/group

^cN.D. Not determined

* $P < 0.05$, significantly different from Ent1(+/+) mice at same time-point.