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Transport of Methylmercury through the Epithelial Type Amino Acid Transporter System B^0

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Abstract

Background: System B^0 is a sodium dependent transporter that transports wide variety of neutral amino acids in the intestinal and renal proximal tubular epithelial cells. Methylmercury (MeHg) readily and non-enzymatically reacts with cysteine to form conjugate structurally similar to the amino acid methionine. **Objective:** In this study, we investigated the molecular mechanism of absorptive transport of MeHg in intestine using Xenopus oocytes expressing hB^0AT1 and the uptake of metylmercry-Cys (MeHg-Cys) by heterodimeric amino acids transporter. **Methodology:** We confirmed the uptake of [^{14}C] L-Leucine a potent substrate for the hB^0AT1 amino acids transporter. The uptake of [^{14}C] L-leucine by hB^0AT1 was inhibited by MeHg-Cys conjugate, leucine, cysteine, methinine and phenylalanine in concentration–dependent manner. The IC_{50} of MeHg-Cys conjugate was significantly lower than that of leucine, cysteine, methinine and phenylalanine, indicating that hB^0AT1 is a high affinity MeHg transporter. To assess MeHg-Cys conjugate transport, we measured [^{14}C] MeHg uptake in Xenopus oocytes expressing hB^0AT1 in presence or absence of sodium. The [^{14}C] MeHg was transport only in the presence of cysteine and the transport was significantly sodium dependent and inhibited by a system B^0 inhibitor 2-aminobicyclo-[2,21]- haptane-2-carboxylic acid (BCH). **Result:** The current findings indicate that hB^0AT1 and heterodimeric amino acids absorb MeHg in the form of cysteine conjugate from the intestinal lumen across the brush-border membrane in to the cells and is supposed to be plays a critical role in the pathogenesis of Minamata disease and present results descried a major molecular mechanism by which MeHg is transported across cell membranes and indicate that metal complexes may form a novel class of substrates for amino acid carriers. **Conclusion:** In this experiment the results also suggest that uptake of Methionine and MeHg-Cys by heterodimeric amino acid transporter is significantly correlated where the uptake of Methionine and MeHg-Cys between heterodimeric amino acid transporter and hB^0AT1 is not correlated. *[Journal of National Institute of Neurosciences Bangladesh, 2019;5(2): 127-136]*

Keywords: Amino acids transport; Methionine; Methyl-mercury-Cysteine conjugate

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Introduction

Methylmercury (MeHg) is a powerful neurotoxicant in all animals, including humans[1-4]. The developing brain is particularly sensitive to the effects of MeHg that can exist in the environment in several forms: elemental, inorganic (Hg_2^+), and/or organic [mainly as methylmercury (CH_3Hg^+)]. CH_3Hg^+ this is the principal form of mercury to which humans are most often exposed, primarily through the consumption of contaminated water and/or fish. Following ingestion of CH_3Hg^+, approximately 90.0% is absorbed by the gastrointestinal tract[5]. In systemic circulation, each methylmercuric ion forms a strong bond with the reduced sulfur atom of several thiol-containing molecules, such as albumin, Cys, glutathione (GSH), homocysteine (Hcy), and N-acetylcysteine (NAC)[6]. Some of these mercuric conjugates serve as transportable substrates of certain carrier proteins in target organs such as the kidneys, liver, and brain[7]. Because the chemical structures of CH_3Hg-S-Cys and the Hcy S-conjugate of CH_3Hg^+ (CH_3Hg-S-Hcy) are similar to that of the amino acid methionine, it has been postulated that these two conjugates may act as mimics of methionine to gain access to certain compartments of the body[8-9]. Indeed, several studies have provided indirect evidence for the involvement of molecular mimicry in the transport of CH_3Hg-S-Cys[10-13]. More direct evidence for this theory comes from a recent study in Xenopus laevis oocytes in which CH_3Hg-S-Cys was transported by the Na^+-independent amino acid transporter, system L[14] and Na^+-dependent system $B^{0,+}$[15].

Epithelial resorption of amino acids across the apical membrane in the kidney and intestine is thought to be carried out by four different transporters[16]. Anionic amino acids are taken up by a Na^+-dependent aspartate/glutamate transporter[17], which has been designated system X-AG. Cationic amino acids are taken by system $b^{0,+}$; the molecular correlate of this transporter being the heteromeric amino acid transporter r ABT/ $b^{0,+}AT$[18]. Most of neutral amino acids are thought to be transported by system B^0[19-20]. System B^0 has been characterized in jejunal brush border vesicle bovine epithelial cells[21-24] and Caco-2 cells[25]. These studies suggest that systemB^0 is a Na^+- dependent, chloride-independent transporter[26]. Hartnup disorder, a autosomal recessive defect result from impaired transport of neutral amino acid across epithelial cells of renal proximal tubules and intestinal mucosa[27-28]. Currently the sodium-dependent amino acid transporter B^0AT1 was cloned, called SLC6A19 and robust expression was observed at renal proximal tubules and intestinal mucosa. There finding indicate that SLC6A19 was the primary candidate for the gene causing Hartnup disorder[29].

Most of the MeHg in tissue is normally complexed with water soluble sulfhydryl-containing molecule, primarily with L-cysteine. Mercuric sulfur bonds form spontaneously under physiological condition and they have high thermodynamic stability although these bonds are kinetically labile[30-31]. The widespread tissue distribution of the MeHg leads to the hypothesis that MeHg may be a substrate for the transporter. In support of this hypothesis, hB^0AT1, a neutral amino acid transporter is a good candidate as of its high expression in the luminal membrane of the intestine and renal proximal tubules. In this study, we examined the transport of MeHg in hB^0AT1 using Xenopus oocytes expression system.

This study focused exclusively on the Na^+- dependent component of the MeHg transport and showed that hB^0AT1 is a high affinity transporter for MeHg and the uptake was inhibited by system B^0 inhibitor, BCH. Moreover, [^{14}C] L- leucine uptake was inhibited by MeHg –cysteine in a concentration -dependent manner, suggesting that this complex is transported by a neutral amino acid carrier. We also examined the transport rate of MeHg in system L, LAT1 and LAT2, system y^+L, y^+LAT1, hAsc-1, system b^{0+}, rBAT/BAT1.The present results showed that hB^0AT1 transporters can transport metal complex with high catalytic efficiency by hB^0AT1, indicating that these proteins may provide the route of MeHg entry into intestine and enter the circulation and may account for the rapid and widespread tissue distribution of MeHg. These observations offer important insight into the molecular mechanisms of MeHg disposition, toxicity, and potential therapies, as well as, provide new understanding in the mechanism of uptake of MeHg.

Experimental Methods and Materials

Materials and animals: Methylmercury (π) chloride [methyl^{14}C] purchased from American Radiolabeled Chemical Inc. St Louis, Mo. [^{14}C]L-Leucine , [^{14}C]L-Methionine were purchased from Perkin Elmer Life Science Inc. (Boston, MA), Methylmercury-Cystiene conjugate purchased from U.S.A. BCH, L-Cysteine , L-Methionine, L-Leucine, L-Phenylalanine were purchased from Sigma (St Louis, Mo). Non radio labeled Metylmercury (π) Chloride was purchased from Wako, pure Chemical industries, Ltd. T7 m MASSGE m MACHINE kit (Ambion.) poly adenylation of c RNA using the poly (A) tailing kit (Ambion). Mature Xenopus laevis were purchased from Saitama

Experimental animal, Saitama, Japan. Animal were maintained under a cons.

Synthesis of cRNA: cRNAs for hB^0AT1 were obtained by in vitro transcription using the T7 m MASSGE m MACHINE kit (Ambion) and poly adenylation of c RNA using the poly(A) tailing kit (Ambion). The cRNA for hLAT1[32], hLAT2[33], y^+LAT1[34], hAsc-1[35] was obtained as described elsewhere.

Xenopus laevis oocyte expression: *Xenopus laevis* oocyte expression studies were performed as described elsewhere with minor modification (36-37). Briefly, oocyte were treated with collagenase A (2mg/ml)(Roche Molecular Biochemical's, Mannheim Germany) for 30 to 50 min at room temperature in ca^+-free medium (82.5 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, and 5mM HEEPS, pH 7.5) to remove follicular layer and then maintained in modified Barths solution (88 mM NaCl,1 mM KCl, 0.33 mM $Ca(NO_3)_2$, 0.41 mM $CaCl_2$,0.82 mM $MgSO_4$, 2.4 mM $NaHCO_3$, and 10 mM HEEPS, pH 7.5). Oocytes were injected with 50ng of c RNA encoding hB^0AT1. For coexpression of LAT1 and 4F2hc, LAT2 and 4F2hc,y+ LAT1 and 4F2hc, hAsc-1 and 4F2hc in X. *laevis oocytes*, defolliculated oocytes were injected with LAT1 cRNA (15ng) and 4F2hc(10ng) to give a molar ratio of 1:132. After injection of cRNAs, the oocytes were incubated in modified Barth's solution art 18^0c with a daily change of culture medium for 3 days. Healthy oocytes with a clean brown animal half and distinct equator line were selected for experiments.

Uptake Measurement: Uptake measurements were performed 3 days after injection of cRNAs of hB^0AT1 and 2 days for other transporters (hLAT1, hLAT2, y+LAT1, hAsc-1). Groups of six to eight oocytes were washed in the uptake solution and then incubated in 500µl of uptake solution (containing 100 mM $NaCl_2$,2 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEEPS, and 5 mM Tris, pH 7.4) or Groups of six to eight oocytes were washed in the uptake solution and then incubated at room temperature in 500µl of uptake solution (100 mM chlorine, 2 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEEPS, and 5 mM Tris, pH 7.4) containing either ^{14}C MeHgCL or ^{14}CMeHg-Cys complex- for 30 mins[36]. The oocytes were then washed five times with ice-cold uptake solution. Single oocyte were placed into scintillation vials and lysed by adding of 250 µl of 10% SDS. After lysis, 3 ml of scintillation fluid was added, and the radioactivity was determined by liquid scintillation spectrometry, and the values are expressed in picomoles per oocyte per minute[37].

Functional Expression in HEK 293 cells: Because of the difficulty in evaluating amino acid transport mediated by hBAT1 in *Xenopus* oocytes, where endogenous transporters activated by rBAT are expressed abundantly[38-39], HEK293 cells were used for functional expression of rBAT/hBAT1. HEK 293 cells were obtained from the American Type Culture Collection. The cells were grown in DMEM (life technologies) supplemented with sodium bicarbonate 1.5g/litre, non-essential amino acid 0.1mM, sodium pyruvate 1mM, and 10.0% (vol/vol) FBS in a humidified atmosphere of 5% CO_2 at 37º C. The cells were grown for 3 days to confluence, trypsinized and centrifuge at 200×g rpm for 5 minutes, and resuspended in culture medium. Cells were maintained in the growth medium (DMEM medium supplemented with 10% fetal bovine serum) at 37º C in 5% CO_2. The cells were collected and seeded on 24-well plates (1×105 cells/well) in the fresh growth medium. For transfection, 20µg of plasmids were diluted into 1ml DMEM without serum and mixed with serum followed by incubating 30 minutes at room temperature. The mixture was applied to HEK293 cells maintained in a tissue culture dish (90 mm diameter) with 70 to 90% confluences. At 24 hours after transfection, the transfected cells were collected and seeded on a 24-well plate (2x 105/ well) in fresh culture media. The uptake measurements were performed at 48 hours after seeding. After removal of growth medium, the cells were washed twice with regular uptake solution [Dulbecco's modified phosphate buffered saline (PBS) supplemented with 5 mmol/L glucose] and preincubated for 10 minutes at 37º C[40]. Then the medium was replaced by regular uptake solution containing ^{14}C L-Cystiene. Amino acid uptake was terminated by removing uptake medium followed by washing three times with ice-cold regular uptake solution. Then cells were solubilized with 0.1N NaOH, and radioactivity was counted. For the uptake measurements, the regular uptake solution was used unless other-wise indicated.

Inhibition Study: For the inhibition experiments, oocytes expressing hB^0AT1 were incubated for 1hr in ND96 solution containing 10µM [^{14}C]L-Leucine in presence or absence of amino acids , amino acids analogue BCH and MeHg-Cys conjugate at different concentration except for BCH (1mM).The inhibitor were directly dissolved in ND96 solution from the stock solutions.

Statistical Analysis: Value are shown as mean ± S.E.M. (n=6-8). Statistical differences were analyzed by Student's unpaired t test. Correlation coefficient was measured by Spearman's test.

Results

hB0AT1 is a Na^+- dependent transporter that transports almost all of the neutral amino acids. Among them Leucine is the best substrate. Expression of hB^0AT1 in *Xenopus* oocytes resulted in a significant increase of leucine uptake activity compared with control water injected oocytes. After injection the oocytes were incubated for three days and [^{14}C] L- leucine uptake (10µM) was measured in the standard uptake solution. As shown in Fig 1. *Xenopus* oocytes that express hB^0AT1 exhibited a high level of L-[^{14}C] leucine uptake compared with water injected control oocytes.

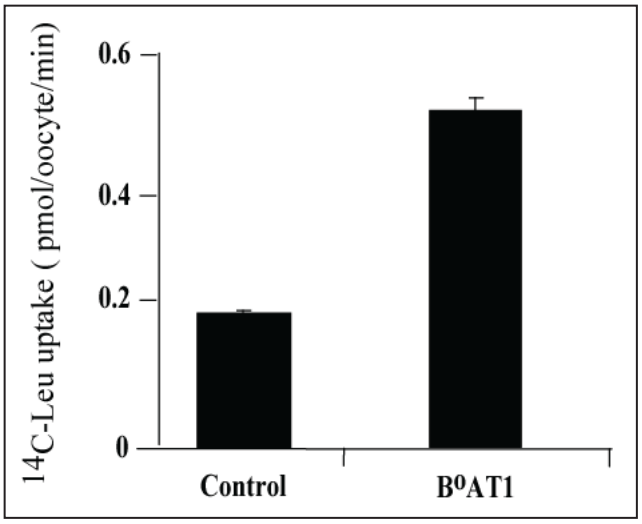


Figure 1: Functional expression of hB^0AT1. Oocyte were injected with hB^0AT1 cRNA or water injected in the controls. After three days incubation, uptake was performed in standard uptake solution. The uptake rates of [^{14}C]L-Leucine by control or hB^0AT1-expressed oocytes were measured for 30 min. Values are mean ± S.E.M of three independent experiments. The significance difference between control (water injected) and hB^0AT1cRNA-injected oocytes was determined by the unpair t- test ($p<.001$)

Xenopus oocytes expressing the hB0AT1 was examined for the uptake of [^{14}C] MeHg and compared with water injected control oocytes. There is no significant difference in the transport of MeHg between water and hB^0AT1 injected oocytes (Figure 2A). When the extracellular Na^+ was replaced with Cl- at equimolar concentration, the uptake of MeHg was not changed compare to control oocytes (Figure 2B), confirming that only the [^{14}C] MeHg cannot uptake by h B^0AT1 expressing oocytes.

Methionine is one of high affinity substrate for the system B^0AT1 and a molecular homologue of CH_3Hg-S-Cys which is a complex of MeHg and Cysteine Because of the similar chemical formulae for the MeHg-Cys and methionine (Figure 3A), we examined the methionine transport as confirmation of the functional insertion of h B^0AT1 in the plasma membrane of the oocytes. The uptake of methionine was significantly greater in the oocytes injected with cRNA encoding hB^0AT1 than in the water injected controls (Figure 3B). In blood cystiene is one of the major nonprotein thiols and MeHg associated with sulfhydryl group of the cys to make a complex of MeHg-Cys. The uptake of MeHg-Cys was examined in hB0AT1 expressing oocytes in presence or absence of Na^+ in the uptake solution. The transport of MeHg-Cys was significantly higher in hB^0AT1 cRNA injected oocytes than in the controls only in Na^+ dependent condition (Figure 3C). To facilitate the analysis of the data, the amount of transport activity in water injected control oocytes was subtracted from that in hB^0AT1 cRNA injected oocytes (Figure 3D).

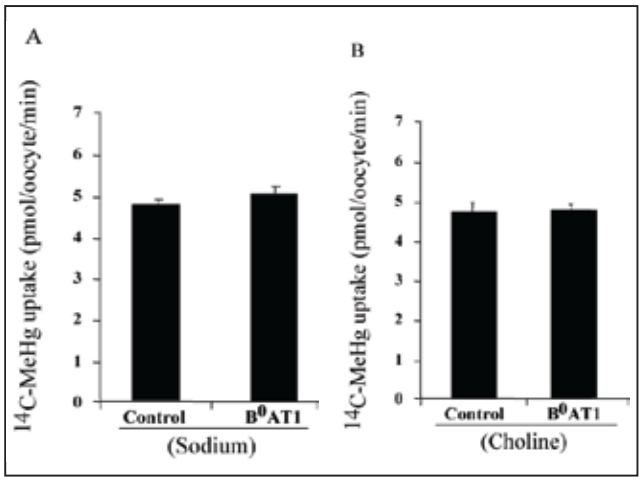
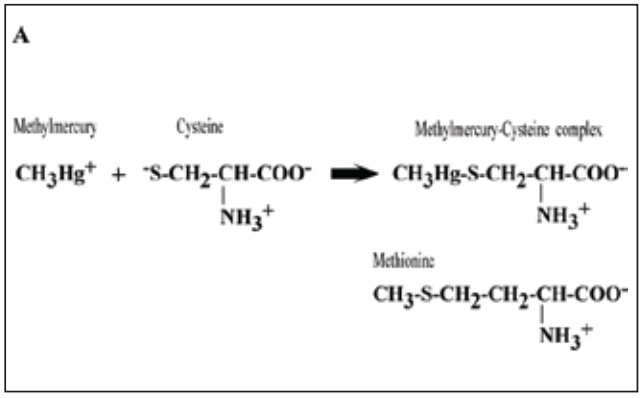


Figure 2: The uptake of [^{14}C] MeHg by B^0AT1 expressing *Xenopus* oocytes. The uptake of [^{14}C] MeHg was measured in water injected (control) or hB^0AT1 cRNA injected oocytes. They were incubated with 10µM [^{14}C] MeHg in the presence (A) or absence(B) of extracellular Na^+. Extracellular Na^+ was replaced with equimolar concentration of choline. The uptake was measured for 30mins at room temperature. Values are mean ± S.E.M of three independent experiments.



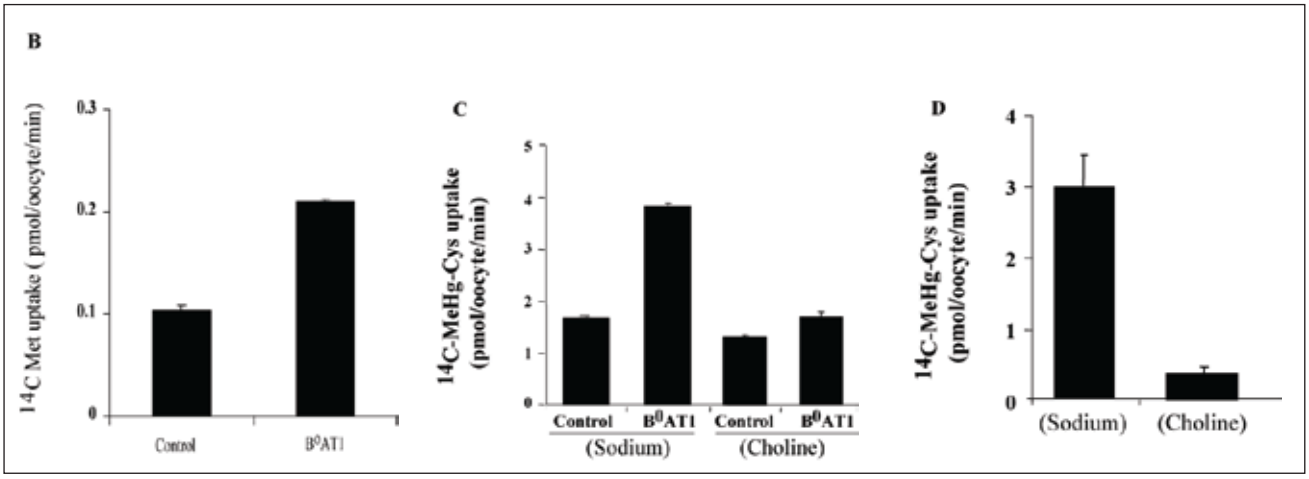


Figure 3: The uptake of [^{14}C] Met and [^{14}C] MeHg-Cys in h B^{0}AT1 expressing oocytes. Chemical formulae of methylmercury-cysteine complex and methionine (A). [^{14}C] Methionine 10µM uptake was determined after three days incubation in standard uptake solution (B).Ion dependence uptake of 10µM [^{14}C] MeHg-Cys via hB^{0}AT1 was determined in the presence or absence of extracellular Na^+. Extracellular Na^+ was replaced with equimolar concentration of choline. Each Bar represeants the mean ±S.E.M of three independent experiments(C).The transport activity of 10µM [^{14}C] MeHg-Cys in each solution were subtracted(D).The significance difference between control (water injected) and hB^{0}AT1cRNA-injected oocytes was determined by the un pair t- test ($p<0.001$).

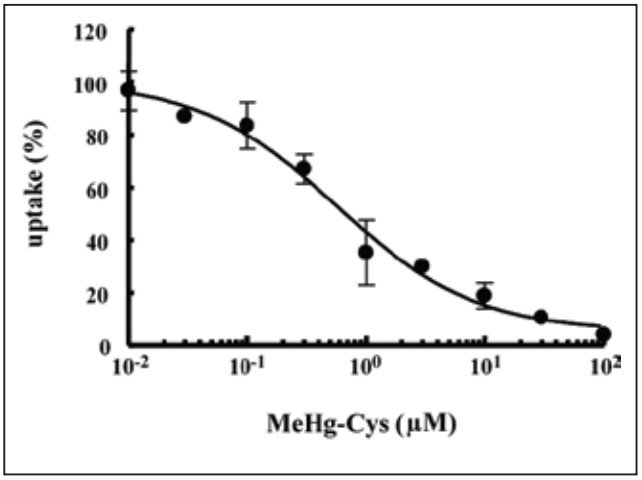


Figure 4. The concentration dependent inhibition of [^{14}C] L-Leucine uptake by MeHg-Cys. The 10µM [^{14}C] L-Leucine uptake was measured for 30 min with Na^+ containing uptake solution in the presence of varied concentrations of MeHg-Cys and expressed as a percentage of control L-Leucine uptake in the absence of MeHg-Cys. Each bar represents the mean ±S.E.M of three independent experiments

The effect of MeHg-Cys on the L-leucine transport was investigated by measuring the [^{14}C] L-Leucine uptake (10µM) in the presence of varied concentration of MeHg-Cys in hB^{0}AT1 expressing oocytes. As shown in the Figure 4 [^{14}C] L-leucine uptake was inhibited in a concentration-dependent manner with IC_{50} value of 0.8 ± 0.21 µM (mean ± S.E. of three separate experiments). Similarly [^{14}C] L-leucine uptake (10µM) was measured in the presence of non-radiolabeled compounds L-Leucine, L-Phenylalanine, L-Methionine, L-Cysteine at different concentration in the Na^+ containing uptake solution and IC_{50} value was calculated (Table 1). The IC_{50} of MeHg-Cys conjugate was significantly lower than that of Leucine, Cysteine, Methionine and Phenylalanine, indicating that hB^{0}AT1 is a high affinity MeHg transporter.

Table 1. IC_{50} value of amino acids and MeHg-Cys for [^{14}C] Leucine uptake mediated by hB^{0}AT1 expressing oocyte

Substrates	IC_{50} (µM)
Leucine	7.5 ± 0.43
Phenylalaline	3.12 ± 0.57
Cysteine	7.5 ± 0.70
Methionine	26.9 ± 0.84
MeHg-cys	0.8 ± 0.21

B^{0}AT1 expressing oocytes were incubated in a solution containnig 10(µM)[^{14}C] Leucine in presence of various concentration of amino acids and MeHg-cys . Each value represents the mean ± S.E.M of six to eight oocytes from 3 separated experiment.

The effect of amino acid analogue system B⁰ specific inhibitor BCH (2-aminobicyclo- (2,2,1)-heptane-2-carboxylic acid) was observed in [^{14}C]MeHg-Cys uptake expressing hB^{0}AT1 oocytes (Figure 5A). [^{14}C] MeHg-Cys uptake (10µM) was measured in presence or absence of 1mM of BCH in the Na^+ containing uptake solution.

The uptake of [^{14}C] MeHg-Cys in water injected control oocyte was subtracted from hB^{0}AT1 expressing oocytes and shown in Fig. 5B. The significant

inhibition of [^{14}C] MeHg-Cys uptake was observed in presence of BCH.

Heterodimeric amino acid transporters play pivotal role in the transepithelial transport of neutral and basic amino acids at renal proximal tubules and intestinal epithelia. Methionine is a common transportable substrate in these transporters. The uptake of methionine and MeHg-Cys complex, structurally similar compounds was examined in apical and basolateral membrane transporter. The uptake was markedly enhanced in oocytes injecting with cRNA for hB0AT1, LAT1/4F2hc, LAT2/4F2hc, and y+LAT1/4Fhc but not highly enhanced in oocytes injecting with cRNA for h Asc-1/4F2hc. However Methionine and MeHg-Cys complex uptake was markedly enhanced in BAT1/rBAT co-transfected in HEK293 cells. MeHg-Cys and methionine uptake is correlated among examined amino acids transporter. As shown in Figure 6A and B the uptake of MeHg-Cys and methionine is directly correlated in heterodimeric amino acids transpoter (LAT1/ 4F2hc, LAT2/ 4F2hc, y+ LAT1/ 4F2hc, hAsc-1/ 4F2hc and BAT1/r BAT but no positive correlation was observed between the heterodimeric amino acid transporters with hB0AT1 (Figure 6C)

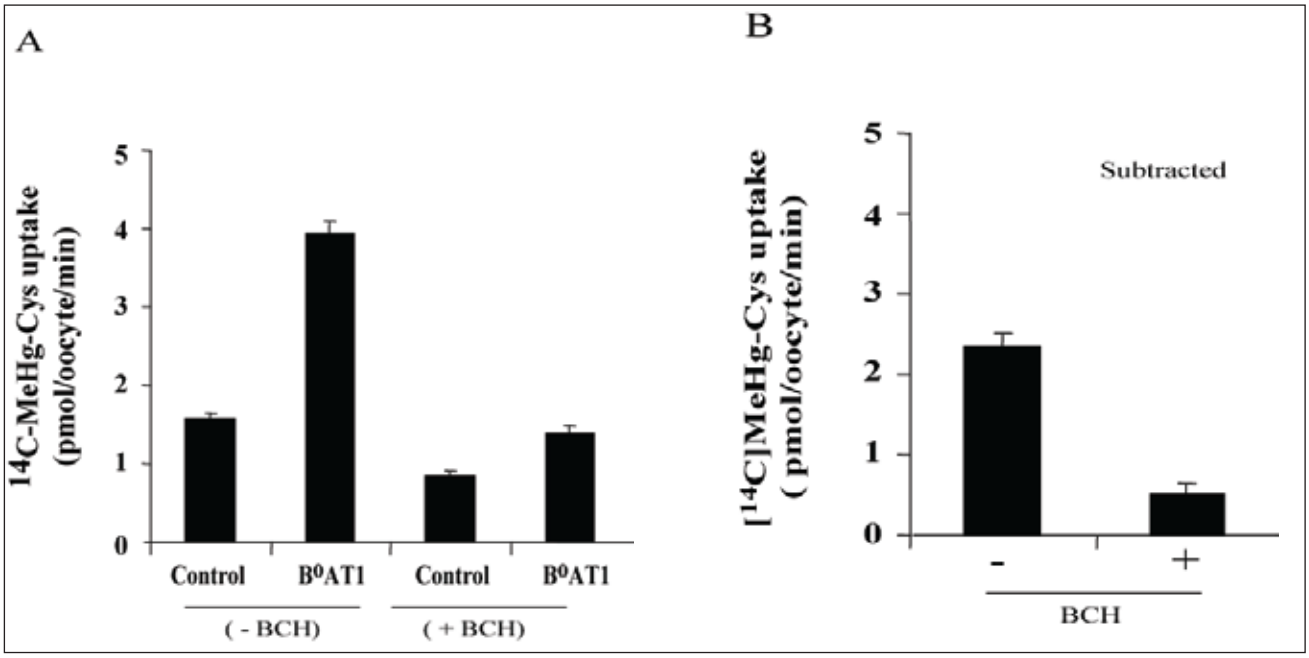
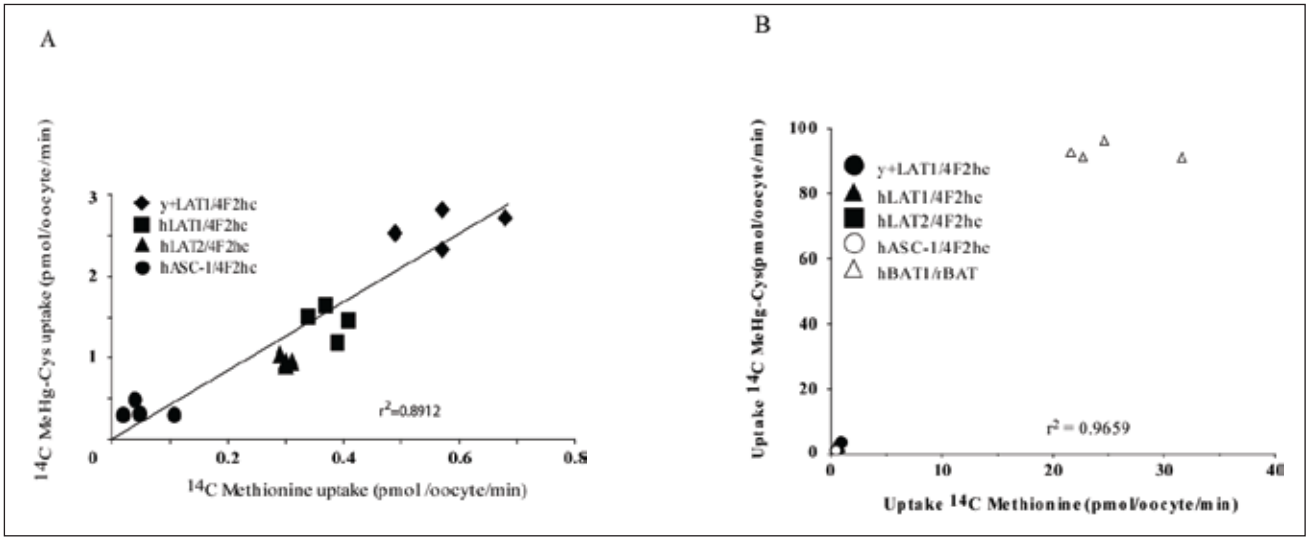


Figure 5: Inhibition of [^{14}C] MeHg-Cys uptake by amino acid analog BCH in oocytes. Control (water injected) and hB^{0}AT1 cRNA injected oocytes were incubated for three days and 10µM [^{14}C] MeHg-Cys uptake was measured in the absence and presence of 1mM system B^0 specific inhibitor BCH (A). The transport activity of 10µM [^{14}C] MeHg-Cys in control and hB^{0}AT1 were subtracted (B).



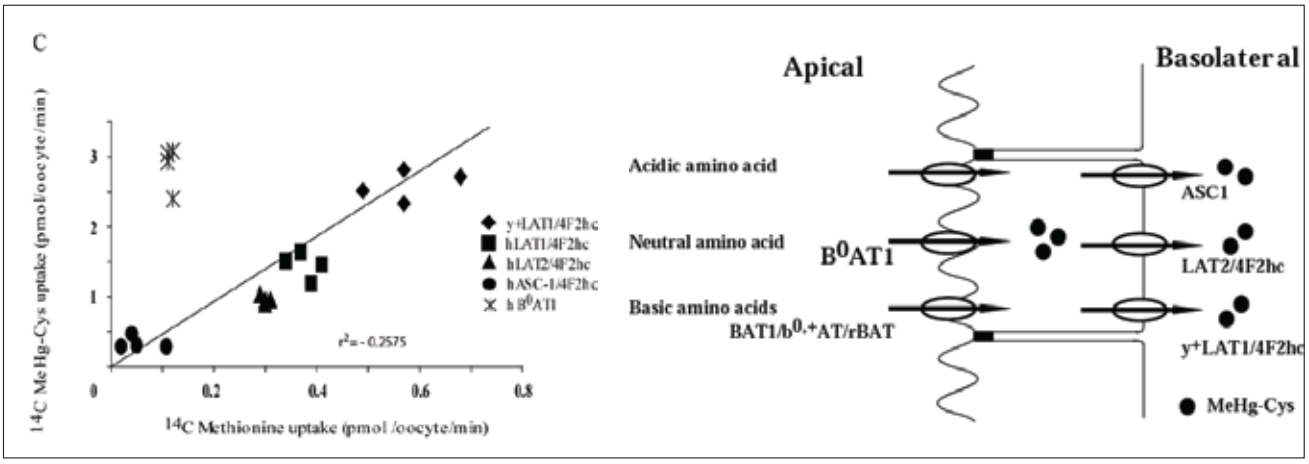


Figure 7: Transepithelial transport of MeHg-Cys in kidney and small intestine. Transporter in apical and basolateral membrane in kidney and small intestine plays a critical role in the absorption of amino acid. MeHg-Cys structurally similar to amino acid methionine are also transported by these transporters

Discussion

Methylmercury is the most frequently encountered organic mercuric compound in the environment that form mainly as a result of methylation of inorganic form of mercury by microorganisms in soil and water. The mercuric ions have a greater predilection to bond to reduce sulfur atoms, especially thiols containing molecules, such as glutathione, cysteine, homocysteine, N-acetylcysteine, metallothionein, and albumin. During the initial hours after the exposure of inorganic mercury, there is a rapid decrease in the plasma level with a rapid increase in kidney and liver. Mercuric S-conjugates of small endogenous thiols like glutathione and cysteine are primarily the transportable forms in the kidney and liver. The kidney are the primary target organ for inorganic mercury to taken up, accumulated and express toxicity[41]. The molecular mechanisms of methylmercury are not yet completely understood, but several studies showing the importance of amino acid transporter for its pharmacokinetic behaviors.

Cellular requirements of amino acid are accomplished by several families of amino acid transporter that expressed differentially and can accept a variety of structurally related amino acids or amino acid analogues as substrate. Amino acid transporter distributed in the apical and basolateral membrane of the renal proximal tubules and small intestine are in concert to play a critical role in the distribution of amino acids in a sodium-dependent and sodium-independent manner. System $b^{0,+}$, B^0, X-AG are located in apical membrane whereas system L and y^+L are heterodimeric amino acid transporters in the basolateral membrane.

Numerous sets of recent findings both in vivo and in vitro indicate that mercuric conjugates of cysteine are the primary species of inorganic mercury transported at the luminal membrane of proximal tubular cells. Methylmercury-cysteine conjugate have been implicated as potential mimics of methionine and methionine is a major substrate for the amino acid transporters. This study provides one of the direct evidence using the hB⁰AT1 that amino acid transporter are involve in metal transport. Moreover, our result provides additional evidence that hB⁰AT1 is the high affinity methylmercury-cysteine transporter among the transporters studied. In this study, we use the Xenopus oocyte system injected with cRNA of hB⁰AT1. To confirm the hB⁰AT1 expression in the current oocyte model, we measured the uptake of amino acid leucine in water and hB⁰AT1 injected oocytes. The transport of leucine was significantly greater in the hB⁰AT1 cRNA injected oocytes than in control, reflecting the presence of a functional hB⁰AT1. Methionine and methylmercury-cysteine are equally the good substrate for this transporter, indeed transport rate were higher for methylmercury-cysteine, whereas methylmercury itself was not a transportable substrate for hB⁰AT1. It is possible that some of the measured uptake mediated by the endogenous amino acid transporter other than hB⁰AT1. We subtracted the water injected uptake from the uptake by hB⁰AT1-injected oocytes; it became obvious that a large fraction of the uptake was mediated by the amino acid transporter hB⁰AT1.

Concentration dependent inhibition of methylmercury-cysteine, L-Leucine, L-Phenylalanine, L-Methionine, L-Cysteine on the uptake of [^{14}C] L-leucine provides additional support in the context of IC50 value where

Methylmercury-Cysteine showed the lowest even than the leucine which is a good substrate for the system B^0. Inhibition of Methylmercury-Cysteine uptake by the amino acid analogue BCH provides further evidence of Methylmercury-Cysteine transported by the amino acid carrier. These findings demonstrate that the uptake of Methylmercury-Cysteine complex is a saturable and inhibited by large neutral amino acid and amino acid analogue BCH. Interestingly, in water injected oocytes, the uptake of Methylmercury-Cysteine was inhibited by BCH. This reduction is most likely due to the inhibition of the endogenous amino acid transporters and the presence of these transporters does not alter the significance of the current findings.

Various amino acid transport systems contribute to the trans epithelial transport of amino acid at the renal proximal tubules and intestinal epithelial cells. Acidic amino acids are absorbed from the luminal fluid via the sodium dependent system X-AG glutamate transporter EAAC1, neutral amino acid via the system B^0, B^0AT1 and cystine and basic amino acid absorbed via the system $b^{0,+}$, rBAT/BAT1. All of these transporters are located at the apical membrane. The heterodimeric amino acid transporters system L, LAT1 and LAT2, system y^+L, y^+LAT1, system Asc, hAsc-1 at the basolateral plasma membrane plays a important role for the exit path[42]. In this study, we examined the methionine and methylmercury-cysteine uptake with all of these transporters. The basolateral heterodimeric amino acid transporter shows a positive correlation in the uptake of methionine and methylmercury-cysteine, indeed, negative correlation was observed when the uptake of hB^0AT1 added. The positive correlation among the heterodimeric amino acid transporter showed a similar affinity for the uptake of methionine and methylmercury-cysteine whereas B^0AT1 has the higher affinity for the methylmercury-cysteine than the methionine indicating a big difference in the pharmacokinetics of methylmercury. It is important to note that the implications for this finding may spread beyond the scope of system B^0.

Methylmercury secreted from liver cells into bile as the glutathione complex is converted to cysteine complex which is promptly reabsorbed in the small intestine. Intestinal absorption of methylmercury complexes was studied by means of direct injection of mercury compounds into ligated intestinal segments of rats, showing that Methylmercury-Cys play important roles in the intestinal reabsorption of methylmercury during its enterohepatic circulation[43]. Methylmercury uptake requiring metabolic energy and could be inhibited by probenecid and ouabain[44]. Studied in the everted sac of rat small intestine showed that with an increase in pH the transport of $HgCl_2$ through the tissue increased. Similar pH dependence was observed in the uptake of HgO[45]. All of these studies suggested a neutral amino acid carrier mechanism may exist for the absorption of the methylmercury in the intestine. In the intestine a gradient of the expression of the hB^0AT1 was observed toward the tip of the villi and the function of which showed strong pH dependency. The localization of this transporter with highest affinity for the methylmercury complex can explain the mechanism of absorption of the complex.

The concentration of the free cysteine in plasma is in the range of 5-20 μM. Plasma glutathione has a rapid turnover giving the cysteine available for the conjugation with methylmercury. Methionine is a transportable substrate for many amino acid carriers, like system L, $b^{0,+}$, y^+L and A. Multi organ distribution of these transporters serve a mechanism for the entry of methylmercury-cysteine complex and contribute to the detrimental effects observed following exposure of methylmercury. The highest affinity for the methylmercury-cysteine complex and localization of hB^0AT1 in the intestinal villi and renal proximal tubular cells added a mechanism in the transport of this toxic metal. Current therapy for MeHg poisoning relies on chelators to enhance the elimination into urine or faeces. Addition of the inhibitor for the neutral amino acid carrier hB^0AT1 may be more effective in limiting tissue uptake.

Conclusion

In this experiment the results also suggest that uptake of Methionine and MeHg-Cys by heterodimeric amino acid transporter is significantly correlated where the uptake of Methionine and MeHg-Cys between heterodimeric amino acid transporter and hB^0AT1 is not correlated. Reabsorption of amino acids in kidney and intestine is mediated by transporters, which refer groups of amino acids with similar physio-chemical properties.

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