Monocarboxylate Transporters in Drug Disposition: Role in the Toxicokinetics and Toxicodynamics of the Drug of Abuse GHB

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Monocarboxylate Transporters (MCT)

- Two families of transport proteins with substrates consisting of important metabolic products such as lactate, pyruvate and ketone bodies, as well as monocarboxylate drugs.

  SLC16 - proton-coupled

  SLC5 – sodium-dependent
Relatedness of human MCT isoforms

- The natural substrates for MCT5, MCT6, MCT7, MCT9 and MCT11-MCT14 are unknown but probably do not include lactate and pyruvate.

- MCT8 is a thyroid hormone transporter and MCT10 (TAT1) is a neutral amino acid transporter.

- Known to transport lactate, pyruvate and ketone bodies.

Andrew Halestrap, Department of Biochemistry, University of Bristol
Proton-linked transport of monocarboxylates such as lactate, pyruvate and ketone bodies across membranes plays a critical role in the metabolism and pH regulation of most cells.

SLC16A1- MCT1  SLC16A3-MCT4
SLC16A7-MCT2  SLC16A8-MCT3

MCT1-4  H⁺-coupled
Topology of MCT1

Confirmed by selective proteolysis and labelling of red blood cells

- 12 transmembrane domains (highly conserved)
- Intracellular N- and C- termini
- A large intracellular loop between domains 6 and 7

http://www.bristol.ac.uk/biochemistry/halestrap/mct.html
The lactate carrier is closely associated with another membrane protein of the immunoglobulin superfamily.
MCTs 1, 2 & 4 function in conjunction with an ancillary protein

- **Basigin (CD147)**, a glycoprotein that belongs to a small subset of the immunoglobulin superfamily, is necessary for the function of MCT1 and MCT4; it is the target for organomercurial inhibition of these MCTs.

- The ancillary protein for MCT2 is **Embigin (gp70)**.

- Interaction is necessary for membrane trafficking, and the direct protein-protein interaction in the membrane is necessary for activity.
Substrates of MCT1-4

Natural Substrates
- A variety of physiologically relevant substrates (e.g. lactate, pyruvate, butyrate)

Clinically relevant drugs
- NSAIDs (salicylic acid, ibuprofen)
- Beta-lactam antibiotics (phenethicillin, propicillin, carindacillin)
- HMG-CoA reductase inhibitors (simvastatin, atorvastatin)
- Valproic acid
- XP13512 (gabapentin prodrug)
- 6-mercaptopurine
- Quercetin, caffeic acid

Carindacillin
Kinetics of L-lactate transport into Xenopus oocytes transfected with MCT1, MCT2 and MCT4

Stefan Bröer, Angelika Bröer, Hans-Peter Schneider, Roger Dallwig and Joachim W. Deitmer, Tübingen

Uptake measured with [14C]-lactate
Tissue Distribution of MCTs

- **MCT1**: expressed ubiquitously in almost every tissue and serves as the carrier for lactate flux across the plasma membrane of most cells.

**MCT2-4: more tissue-specific role**

- **MCT2**: major MCT in rodent neurons, but in human brain its expression is minimal; liver expression.

- **MCT3**: expressed in the retinal pigment epithelium; located at the basolateral membrane, where it functions in tandem with MCT1 on the apical side.

- **MCT4**: expression is limited to highly glycolytic tissues (skeletal muscle, heart and liver); works synergistically with MCT1.
Relative gene expression in HeLa, HEK293, HL-60, K562, Saos-2, Caco-2 and HepG2 cells

Plus excellent correlation of mRNA and protein expression

Ahlin, Artursson  Drug Metab Disp, 2009
MCT Transporters in the Human Intestine

⇒ MCT-1: Highly expressed in all segments of the small intestine and colon

Cundy KC, Xenoprot, March 8, 2005
XP13512 undergoes enzymatic hydrolysis \textit{in vivo} to liberate gabapentin, isobutyrate, acetaldehyde, and CO\textsubscript{2}
Mean concentrations of gabapentin in blood after oral dosing of approximately equimolar doses of (A) XP13512 capsules (n = 8 per dose level) or (B) oral gabapentin (n = 10 per dose level).

Cundy et al., J Clin Pharmacol 2008;48:1378-1388
Sodium-coupled monocarboxylate transporter (SMCT)

SMCT1 (SLC5A8, high affinity)
SMCT2 (SLC5A12, low affinity)

SMCT1:

- **Expression:**
  - kidney (apical, tubular epithelial cells), GI (apical, colonic and intestinal epithelial cells), thyroid gland, brain, salivary gland, retina

- **Substrates:**
  - short chain fatty acids (butyrate, lactate, acetate, propionate, pyruvate), medium-chain fatty acids (hexanoate, pentanoate, heptanoate, octanoate), ketone bodies (β-hydroxybutyrate and acetoacetate), nicotinate, salicylate, and 5-aminosalicylate
γ-Hydroxybutyric Acid:
MCT and SMCT substrate
Gamma-hydroxybutyric acid (GHB)

- Naturally occurring short-chain fatty acid formed from GABA
  - MW 104
  - pKa 4.7
  - Highly hydrophilic
  - Negligible protein binding

- Clinical uses:
  - Narcolepsy (USA)
  - Alcohol withdrawal (Europe)

- Marketed as Xyrem®
GHB Toxicity and Abuse Potential

• Wide spread abuse of GHB (Liquid Ecstasy) and its precursors (1,4-butanediol and gamma-butyrolactone):
  – Body building (growth hormone releasing effects)
  – Recreational use for euphoric effects (club drug)
  – Drug-facilitated sexual assault

• Typical recreational dose: 1 – 3 grams typically in liquid form

• GHB overdoses have resulted in 1084 ER visits in 2006 (DAWN, NIDA)
  – Likely an underestimate due to detection difficulties and lack of reporting
  – Second most common drug detected in urine of young people presenting with drug-induced coma, just behind cocaine

• Adverse effects characterized by CNS depression and cardiovascular effects:
  – Respiratory depression, seizures, nausea, vomiting, unconsciousness, coma and death

• Current treatment strategies involve supportive care
Pharmacokinetics of GHB

- GHB is eliminated by metabolism to CO₂
- Nonlinear PK of GHB has been demonstrated in rats (Lettieri and Fung, 1978) and humans (Palatini, et al. 1993)
- Urine concentrations are low; only 3-6% of GHB total dose
- Urine concentrations of GHB are very high in GHB overdosed patients [Sporer et al. 2003]
The goal of this research is to identify specific therapeutic interventions for the treatment of GHB overdoses.
Toxicokinetics of GHB

Urine concentrations are low (3-6% of dose) after low doses but much higher after overdoses.

Hypothesis:
GHB is actively reabsorbed by the kidney by MCTs resulting in nonlinear renal clearance
Non-linear Renal Clearance

\[ \text{CL}_t = \frac{K_{0,\text{GHB}}}{C_{ss,\text{GHB}}} \]

\[ \text{CL}_R = \frac{A_{e,\text{urine}}/t}{C_{ss,\text{GHB}}} \]

\[ \text{CL}_m = \text{CL}_t - \text{CL}_R \]

** P< 0.01, *** P<0.001

Morris et al, J Pharmacol Exp Ther, 2005
# GHB Renal Clearance

<table>
<thead>
<tr>
<th>Dose (mg/kg/h)</th>
<th>Cp (mg/ml)</th>
<th>CLr (ml/kg/h)</th>
<th>CLt (ml/kg/h)</th>
<th>fe(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>108.3 (n=3)</td>
<td>0.23±0.03</td>
<td>14.9±5.1</td>
<td>523±96</td>
<td>2.9±1.0</td>
</tr>
<tr>
<td>127.0 (n=4)</td>
<td>0.43±0.05*</td>
<td>51.8±13.0</td>
<td>302±40</td>
<td>17.1±3.6*</td>
</tr>
<tr>
<td>208.3 (n=4)</td>
<td>0.67±0.12***</td>
<td>97.1±43.1</td>
<td>312±50</td>
<td>32.1±16.2***</td>
</tr>
</tbody>
</table>

Compared to low dose

* p< 0.05; ** p<0.01; *** p<0.001

One-way ANOVA followed by Dunnett’s test
Dose-dependent increases in $\text{Cl}_R$ suggest active renal reabsorption of GHB.
Expression of mRNA of MCTs in HK-2 cells and human kidney cortex

Wang, Q et al., Mol Pharmaceutics, 2006
MCT1 in human kidney HK-2 cells

MCT1 (FITC-conjugated antibody) is distributed predominantly on the basal membrane of HK-2 cells.

The overlay of MCT1 (red, stained with rhodamine-conjugated antibody) and Na+/K+-ATPase (green). The two proteins are distributed on the same side of the cell membrane, and they are partially co-localized.

Rat kidney expression of MCT1 and MCT2

Western blot analysis

Wang et al., JPET 318:751-761, 2006
Uptake of L-lactate decreased as pH increased

Uptake of L-lactate was not affected by the presence or absence of sodium ion

Uptake of L-lactate was inhibited by CHC (α-cyano-hydroxycinnamate 2mM), a MCT inhibitor
GHB transport in HK-2 cells

Parameters | Mean ± SD
--- | ---
$V_{\text{max}}$ (pmol/mg/min) | 27.6 ± 9.3
$K_m$ (mM) | 2.07 ± 0.79
P (µL/mg/min) | 0.54 ± 0.15

Graphs and images:
- Uptake (pmol/mg/min) at pH 6.0 and pH 7.5 for control, CHC, w/o Na, and W/o Na CHC.
- Bar graph showing MCT1 and Actin expression levels.
- Graph showing % of Control for GHB and Lactate with significance markers.

Legend:
- Black: control
- Red: CHC
- Green: w/o Na
- Yellow: W/o Na CHC
MCTs Involved in GHB Uptake

- RNA interference studies using siRNA
- Significant decreases in MCT1 and MCT2 mediated GHB uptake in HK-2 cells
- MCT1 represents an important transporter for GHB uptake

Wang Q et al. (2007) Pharm Res
Effects of Inhibitors on Uptake of GHB by HK-2 Cells

*** p< 0.001

TEA: Triethylamine (2mM);
CHC: Alpha-cyano-4-hydroxycinnamate (2mM); pCMB= p=chloro-mercuribenzoic acid
Probenecid (0.1 mM), Salicylate (2mM), GHB (5mM), D, L-lactate (5mM)
MDA-MB231 cells: human breast-cancer cells lines; lack of MCT1 expression
---Garcia CK, et al, cells. 1994
Rat MCT1 expression
(Prof Tamai and Tsuji, Kanazawa Univ, Japan)

### Parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>MCT1 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_m$ (mM)</td>
<td>4.6 ± 1.5</td>
</tr>
<tr>
<td>$V_{max}$ (pmol/mg/s)</td>
<td>1350 ± 613</td>
</tr>
</tbody>
</table>
Effect of inhibitors on MCT1-mediated GHB uptake

1 min uptake by rat MCT1 gene-transfected MDA-MB231 cells at RT pH 6.0, GHB 5 µM, luteolin 0.05 mM, all other inhibitors 1 mM,

* p < 0.05, ** p < 0.01

Cui and Morris, DMD 2009
Hypothesis

Aim: Determine effects of MCT inhibitors on the TK and TD of GHB

Hypothesis: Inhibiting MCTs represents a potential strategy of treating overdoses of GHB
L-Lactate as a MCT Inhibitor
Effects of L-lactate on GHB PK

Steady-state Plasma Concentrations

Lactate 1: 60.5 mg/hr/kg
Lactate 2: 121 mg/hr/kg
Lactate 3: 302.5 mg/hr/kg

* p< 0.05; ** p<0.01; *** p<0.001

Wang et al, Drug Metab Disp 36:2244. 2008
# Effects of L-lactate on GHB TK

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>L-lactate-1</th>
<th>L-lactate-2</th>
<th>L-lactate-3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plasma [GHB] (mg/ml)</strong></td>
<td>0.62 ± 0.11</td>
<td>0.48 ± 0.04**</td>
<td>0.46 ± 0.01**</td>
<td>0.38 ± 0.02**</td>
</tr>
<tr>
<td><strong>Total clearance (ml/hr/kg)</strong></td>
<td>340 ± 39</td>
<td>441 ± 44***</td>
<td>440 ± 24**</td>
<td>512 ± 52***</td>
</tr>
<tr>
<td><strong>Renal clearance (ml/hr/kg)</strong></td>
<td>96 ± 26</td>
<td>163 ± 48**</td>
<td>214 ± 21***</td>
<td>213 ± 27***</td>
</tr>
<tr>
<td><strong>Metabolic clearance (ml/hr/kg)</strong></td>
<td>238 ± 36</td>
<td>278 ± 42</td>
<td>226 ± 12</td>
<td>298 ± 42</td>
</tr>
<tr>
<td><strong>Plasma [Inulin] (mg/ml)</strong></td>
<td>0.19 ± 0.02</td>
<td>0.23 ± 0.04</td>
<td>0.19 ± 0.01</td>
<td>0.19 ± 0.01</td>
</tr>
<tr>
<td><strong>GFR (ml/hr/kg)</strong></td>
<td>584 ± 111</td>
<td>482 ± 54</td>
<td>603 ± 30</td>
<td>549 ± 16</td>
</tr>
<tr>
<td><strong>% of GHB reabsorbed</strong></td>
<td>80.3 ± 2.6</td>
<td>66.3 ± 10.2**</td>
<td>63.59 ± 5.1**</td>
<td>61.1 ± 4.8**</td>
</tr>
</tbody>
</table>

Wang et al, Drug Metab Disp 36:2244. 2008
Luteolin as a MCT inhibitor
Flavonoids as MCT1 Inhibitors

- Flavonoids phloretin and quercetin are inhibitors of L-lactate transport by MCT1.

- Flavonoids are inhibitors of various transporters: P-glycoprotein, MRP1, BCRP, OATP

Chemical structure of luteolin
Flavonoid Inhibition of GHB uptake by MCT1

Effects of flavonoids on the uptake of GHB by rMCT1 gene transfected cells and control cells. * p<0.05, **p<0.01.

Luteolin is a competitive inhibitor of GHB uptake

<table>
<thead>
<tr>
<th></th>
<th>Luteolin</th>
<th>Morin</th>
<th>Phloretin</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC₅₀(µM)</td>
<td>0.41±0.14</td>
<td>6.21±2.01</td>
<td>2.57±0.48</td>
</tr>
</tbody>
</table>

Line-weaver Burk plot. Open circles represent the uptake in the presence of Luteolin (50 µM); closed circles represent the uptake in the absence of Luteolin (50 µM).
Luteolin on GHB TK/TD

GHB iv bolus 1 g/kg
Luteolin iv bolus 10 mg/kg

N = 3 for control and luteolin treatment group

Proposed PK Model for Luteolin

Luteolin PK model:

\[ K_{10} K_{21} K_{12} \]

Plasma

\[ C_{Lut}, V_{Lut} \]

Tissue Compartment

IV Bolus

Proposed PK Model for Luteolin and GHB

Luteolin PK model:

GHB PK:
- 1 compartment kinetics
- Nonlinear PK
  1) metabolism
  2) renal reabsorption.

GHB PK model:
PK Model Fitting of Luteolin~GHB Interaction

Uncompetitive inhibition provides the best model fitting. True \( K_i = \frac{K_i'}{V_{c_{GHB}}} = 1.1 \) μM

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Fitted Value</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>( K_m ) (mg/ml)</td>
<td>0.063</td>
<td>fixed</td>
</tr>
<tr>
<td>( V_{max} ) (mg/min/kg)</td>
<td>2.27</td>
<td>fixed</td>
</tr>
<tr>
<td>( V_{c_{GHB}} ) (ml/kg)</td>
<td>578</td>
<td>3.63</td>
</tr>
<tr>
<td>GFR (ml/min/kg)</td>
<td>10</td>
<td>fixed</td>
</tr>
<tr>
<td>( V_{max_{ren}} ) (mg/min/kg)</td>
<td>49.2</td>
<td>38.5</td>
</tr>
<tr>
<td>( K_{ren} ) (mg/ml)</td>
<td>5.54</td>
<td>43.5</td>
</tr>
<tr>
<td>( K_i' ) (ug/kg)</td>
<td>63.3</td>
<td>35.2</td>
</tr>
</tbody>
</table>

Luteolin: 0 mg/kg iv
2 mg/kg iv
4 mg/kg iv
10 mg/kg iv
20 mg/kg iv
Simulation of Luteolin-GHB Interaction

➢ To determine the effect of varying doses of luteolin on GHB detoxification at different dose levels of GHB.

GHB: 1000 mg/kg iv

GHB: 400 mg/kg iv

GHB: 200 mg/kg iv

Luteolin: 0 mg/kg iv
2 mg/kg iv
4 mg/kg iv
10 mg/kg iv
20 mg/kg iv

Simulation by ADAPT II with 10 % Variance
Renal Clearance Model

Felmlee et al., AAPS J, accepted
Mechanistic Model Fitting

Solid line is population model fitting; dotted are 10\textsuperscript{th} percentile; dashed are 90\textsuperscript{th} percentile.
MCT1 is a target for immunosuppression

- Novel mechanism of action for immunosuppressant agents through inhibition of MCT1
- Suppression of T-lymphocyte proliferation by inhibiting the MCT-mediated efflux of lactate

![Graph showing inhibition of lactate uptake](image)

**AR-C155858**

Transport by SMCT1 (SLC5A8)
Effect of inhibitors on the uptake of D-lactate and butyrate in Rat Thyroid FRTL-5 Cells (Cui and Morris, DMD, 2009)

pH 7.4, 5 min uptake by FRTL-5 at RT, concentration of inhibitors: 1 mM
* p < 0.05, ** p < 0.01
Concentration dependence of GHB uptake in Rat Thyroid FRTL-5 cells expressing SMCT1

Net uptake (Na⁺-Na⁻)

\[ v = \frac{V_{\text{max}} \times C}{K_m + C} \]

\[ v = \frac{V_{\text{max}} \times C}{K_m + C} + P \times C \]  

\[ v = P \times C \]

\[ v = \frac{V_{\text{max}_1} \times C}{K_{m1} + C} + \frac{V_{\text{max}_2} \times C}{K_{m2} + C} \]

\[ pH 7.4, 5\text{min uptake at RT} \]

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>( K_m ) (mM)</td>
<td>0.74 ± 0.30</td>
</tr>
<tr>
<td>( V_{\text{max}} ) (nmol·mg⁻¹·min⁻¹)</td>
<td>3.47 ± 1.43</td>
</tr>
<tr>
<td>( P ) (µl·mg⁻¹·min⁻¹)</td>
<td>0.24 ± 0.11</td>
</tr>
</tbody>
</table>
Effect of potential inhibitors on the sodium-dependent uptake of GHB

pH 7.4, GHB 10µM, 5min uptake by FRTL-5 at RT
Concentration of inhibitors: phloretin 0.25mM, luteolin 0.05mM, all others 1mM
* p < 0.05, ** p < 0.01
IC\textsubscript{50} values for the inhibition of GHB

<table>
<thead>
<tr>
<th>Inhibitors</th>
<th>IC\textsubscript{50} (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ibuprofen</td>
<td>31.6 ± 19.7</td>
</tr>
<tr>
<td>Ketoprofen</td>
<td>64.4 ± 23.4</td>
</tr>
<tr>
<td>Probenecid</td>
<td>379.7 ± 43.4</td>
</tr>
<tr>
<td>L-lactate</td>
<td>100.5 ± 37.3</td>
</tr>
</tbody>
</table>

\[
% \text{ Inhibition} (F) = \left( \frac{v_{Na^+} - v_{inh}}{v_{Na^+} - v_{Na^-}} \right) \times 100
\]

\[
F = \frac{I_{max1} \times C^r}{IC_{50}^r + C^r} \times 100
\]

pH7.4, GHB 10 µM, 5min uptake by FRTL-5 at RT
Summary

• GHB is a substrate for MCT1, 2 and 4, and for SMCT1.

• Reabsorption of GHB in the renal proximal tubule can be inhibited by MCT inhibitors, resulting in increased renal and total clearances.

• Currently, we are assessing the importance of MCTs in tissue distribution, including distribution into the brain.

• Inhibition of MCT-mediated transport (renal/brain) may represent a potential strategy for treating overdoses of this drug of abuse.
Acknowledgements

Qi Wang
Ke Hu
Inger Darling
Kathyrn Lam
Xiaodong Wang
Dapeng Cui
Bridget Morse
Dr. Kathleen Boje
Dr. Ho-Leung Fung
Acknowledgements

Funding: NIH DA14988 and DA023223, Western NY Kidney Foundation/Upstate NY Transplantation Service.

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