Characterization of the Hepatic Canalicular Membrane Transport of a Model Oligopeptide: Ditekiren

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Accepted for publication December 9, 1996

ABSTRACT

Many small oligopeptides are rapidly excreted unchanged into bile, which requires vectorial transport across the hepatocyte. To characterize the involved carrier system(s) at the canalicular membrane, studies were undertaken with vesicle preparations from the rat and the model pseudohexapeptide ditekiren. The initial uptake rate into inside-out-oriented vesicles was found to be ATP- and temperature-dependent and saturable. Kinetic analysis indicated the involvement of three processes: (1) an ATP-dependent carrier-mediated process (Km = 19.1 ± 4.26 μM; mean ± S.E.M.), Vmax = 140 ± 29.4 pmol/mg of protein/15 sec), (2) an ATP-independent carrier-mediated transporter (Km = 17.2 ± 9.58 μM, Vmax = 62.9 ± 24.5 pmol/mg of protein/15 sec) and (3) a nonsaturable component. ATP-dependent uptake was inhibited by several other oligopeptides, which in the case of EMD 51921 was competitive. Cis-inhibition studies with known substrates for the canalicular bile salt (taurocholate), multispecific organic anion (glutathione disulfide) and P-glycoprotein (daunomycin, nicardipine, cyclosporin A) transporters indicated a major role for the latter carrier system. Inhibition of the initial uptake rate of ditekiren by daunomycin was found to be competitive in nature (Ki = 16 μM). These findings indicate that the biliary excretion of ditekiren and possibly other hydrophobic oligopeptides is mediated, in part, by P-glycoprotein and suggest a possible physiological role for this hepatic transporter.

The potential of small peptides as therapeutic agents is limited by a number of factors. For example, even when the problem of proteolytic degradation by peptidases is overcome, difficulties remain in obtaining and maintaining an appropriate target organ level within the body, especially after oral administration. One reason for this is the rapid hepatic clearance of oligopeptides from the splanchic circulation, which, in many instances, can be attributed to biliary excretion of the intact drug (Ruwart, 1995). Such elimination indicates vectorial translocation of the oligopeptide across the sinusoidal and canalicular membrane surfaces of the hepatocyte. Previous rat liver perfusion studies using ditekiren [U-71038; Boc-Pro-Phe-N-MeHis-Leu\(\end{equation}\)CHOHCH2]Val-Ile-(aminomethylpyridine)] as a model compound indicated a potential role for carrier-mediated transport at both of these membranes (Adeoyin et al., 1993). In particular, extensive biliary excretion occurred against a concentration gradient and was markedly dose-dependent.

A number of export transporters located at the canalicular membrane have been shown to be involved in the biliary secretion of both endogenous and exogenous compounds and have been primarily classified according to their biochemical characteristics, such as substrate specificity and the involved driving force(s) (Meier, 1995). In many cases, the involved proteins appear to be members of the ABC superfamily of transporters because several of these processes exhibit ATP dependency (Greenberger and Ishikawa, 1994). For example, bile acids such as taurocholate are translocated across the canalicular membrane by a specific cBST, which is a saturable, vanadate-sensitive and unidirectional process (Arias et al., 1993; Gatmaitan and Arias, 1995; Vore, 1993) that is distinct from a similar but electrogenically driven system (Kast et al., 1994). An ATP-dependent nonbile acid organic transporter, or cMOAT, is also present at the canalculus and appears to be responsible for the transport of a large number of compounds, including cysteinyl leukotrienes (leukotriene C4), glutathione disulfide and glutathione S-conjugates and glucuronides of xenobiotics (Arias et al., 1993; Gatmaitan and Arias, 1995; Vore, 1993). A saturable but electropotential-dependent process has also been demonstrated for the transport of such compounds (Adachi et al., 1991; Fernández-Checa et al., 1992). Finally, organic cations are considered to be secreted into bile via an ATP-dependent P-glycoprotein-type transporter present at the canalicular membrane (Arias et al., 1993; Gatmaitan and Arias, 1995; Vore, 1993).

The mechanism or mechanisms by which oligopeptides are

ABBREVIATIONS: HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; cBST, canalicular bile salt transporter; cMOAT, organic anion multispecific organic anion transporter; Ntcp, Na+-taurocholate cotransporting polypeptide; Oatp1, organic anion transporting polypeptide.

Received for publication August 6, 1996

1 This work was supported in part by United States Public Health Service Grant GM31304.
translocated across the canalicular membrane and secreted into bile against a concentration gradient are not known. It could involve one or more of the established transporters, or a distinctly different system or systems may be responsible. Regardless, further understanding of this potentially rate-limiting step in the rapid removal process would be useful in the design of therapeutic peptides. Accordingly, we undertook characterization of the transport of a model pseudohexapeptide, ditekiren, by rat hepatic canalicular membrane vesicles.

**Methods**

**Chemicals.** Unlabeled ditekiren, U-71013 [Boc-Pro-Phe-N-MeHis-Leu][CHOH-NH]Ile-(aminomethylpyridine), U-77456 [Tham-Pro-Phe-N-MeHis-Leu][CHOHCH]Val-Ile-(aminomethylpyridine-N-oxide)] and radiolabeled (\[^{3}H\]prolyl) ditekiren were obtained from The Upjohn Co. (Kalamazoo, MI). Radiopurity of the labeled peptide was >98% by high performance liquid chromatography (specific activity, 32.7 Ci/mol). Angiopeptin (Nal-Cys-Tyr-Trp-Lys-Val-Cys-Thr-NH\(_2\)) and cyclosporin A were kindly provided by the Henri Beaufour Institute USA (Washington, DC) and Sandoz Pharmaceutical Corp. (East Hanover, NJ), respectively. EMD-55068 [6-aminoxanoyl-Phe-Gly-(4-amino-5-cyclohexyl-3-hydroxypentanoyl)-Ile-(N-2-amino-5,6-dimethyl-3-pyrazinylmethylamidyl)] and EMD-51921 [Boc-Phe-Gly-(aminomethylpyridine-3-hydroxyoctanoyl)-Ile-(N-4-amino-2-methyl-5-pyrimidinylmethylamidyl)] were gifts from E. Merck Co. (Darmstadt, Germany). ATP (ATP-disodium salt from equine muscle), 5′-adenylylimidodiphosphate, creatine phosphate, daunomycin hydrochloride, glutathione disulfide, nicardipine hydrochloride, serpentine, rhodamine-123, sodium taurocholate, verapamil hydrochloride and vinblastine sulfate were purchased from Sigma Chemical Co. (St. Louis, MO). Creatine phosphokinase was obtained from Boehringer-Mannheim Biochemicals (Indianapolis, IN), and sodium orthovanadate was from Aldrich Chemical Co. (Milwaukee, WI). \[^{3}H\]Taurocholic acid (2.0 Ci/mmol, radiopurity >98%) was purchased from DuPont-New England Nuclear (Boston, MA). All other chemicals were of reagent grade and were from Fisher Scientific Co. (Fair Lawn, NJ) or Sigma.

**Preparation and characterization of canalicular membrane vesicles.** Livers were obtained from male Wistar rats (200–250 g; Harlan Sprague-Dawley, Indianapolis, IN), and canalicular plasma membrane vesicles were prepared according to a two-step method described by Kobayashi et al. (1990). This consisted of an initial Percoll gradient procedure to obtain a mixed hepatic plasma membrane fraction from which a canalicular-enriched preparation was obtained using sucrose-density gradient centrifugation. The vesicles were suspended in buffer A (0.25 M sucrose, 10 mM HEPES/Tris and 0.2 mM CaCl\(_2\), pH 7.4) at a protein concentration of 2 to 3 mg/ml and stored at −70°C for ≥14 days before use in transport studies.

The degree of enrichment of the vesicle preparation was determined using various marker enzyme activities relative to their levels in the homogenate before purification: leucine aminopeptidase (Goldbarg and Rutenberg, 1958), alkaline phosphatase (Yachi et al., 1988), Mg\(^{++}\)-ATPase (Scharschmidt et al., 1979) and \(\gamma\)-glutamyltransferase (Orlowski and Meister, 1963) for canalicular plasma membranes; Na⁺,K⁺-ATPase (Scharschmidt et al., 1979) for basolateral plasma membranes; glucose-6-phosphatase (Baginski et al., 1974) for the endoplasmic reticulum; acid phosphatase (Walter and Schutt, 1974) for lysosomes; and succinate dehydrogenase (Gutman et al., 1971) for mitochondria. Protein concentrations were determined using a Coomassie Protein Assay Reagent (Pierce Chemical Co., Rockford, IL) and bovine serum albumin as the standard.

The proportion of inside-out vesicles in the vesicle preparation was estimated by the addition of neuraminidase to a suspension in the presence and absence of Triton-X (Steck and Kant, 1974). The sialic acid liberated from the intracellular membrane surface was determined using a thiobarbituric acid assay (Warren, 1959).

**Transport studies.** Membrane vesicle transport of \[^{3}H\]ditekiren was determined by a rapid filtration technique. Frozen vesicles were quick-thawed by immersion in a 37°C water bath, resuspended by being passed through a 25-gauge hypodermic needle and then kept on ice until use. A 10-μl aliquot of the suspended vesicles (20–30 μg of protein) that had been preincubated for 5 min at 37°C was added to 50 μl of standard medium containing \[^{3}H\]ditekiren (0.22 μCi; 0.011 μM), 1 to 25 μM unlabeled ditekiren (depending on the particular study), 5 mM ATP and an ATP-regenerating system (3 mM creatine phosphate and 100 μg/ml creatine phosphokinase) in buffer B (0.25 M sucrose, 10 mM HEPES/Tris, 10 mM MgCl\(_2\) and 0.2 mM CaCl\(_2\), pH 7.4) that had been preincubated for 10 min at 37°C. Radiolabeled ditekiren uptake was terminated after the desired time period by the addition of 1 ml ice-cold buffer C (0.25 M sucrose, 10 mM HEPES/Tris, 10 mM MgCl\(_2\), 0.2 mM CaCl\(_2\), 100 mM NaCl, and 50 μM ditekiren, pH 7.4). The suspension was immediately filtered through a 1.2-μm GF/C glass fiber filter (Whatman International Ltd., Maidstone, UK) that had been previously presoaked with buffer C. The filter was then washed three times with 9 ml of ice-cold buffer C and subsequently dissolved in 5 ml of BCS scintillation fluid (Amersham Corp., Arlington Heights, IL). The vesicle-associated radioactivity was determined in a 1219-Rackbeta liquid scintillation counter (Pharmacia-LKB Nuclear, Gaithersburg, MD) using an automatic quench correction procedure. All incubations were performed in triplicate, and the reported results (mean ± S.E.M.) represent the mean observations from at least three different vesicle preparations.

Non-specific binding of \[^{3}H\]ditekiren to the filter was corrected for by the addition of buffer A rather than the vesicle suspension and subtraction of the resulting value from the measured uptake. Total transport was determined by incubating \[^{3}H\]ditekiren in the standard ATP-regenerating medium at 37°C. Transport studies were also performed at 4°C to determine transport by a non-carrier-mediated process. Subtraction of this linear component, reflecting passive diffusion and non-specific membrane binding, from the total transport values provided a measurement of carrier-mediated transport. Similar studies performed in the absence of ATP and the ATP-regenerating system in buffer B allowed separation of the overall active uptake process into ATP-dependent and ATP-independent components. The concentrations of ATP, ADP and ATP metabolite(s) in the incubation medium were determined according to high performance liquid chromatography as previously described (Hill et al., 1987).

To determine whether the ATP-dependent uptake of \[^{3}H\]ditekiren represented transmembrane movement rather than binding to the membrane surface, uptake studies were performed after preincubation of the vesicles at 25°C for 30 min in the presence of different concentrations (0–0.33 M) of raffinose (Nishida et al., 1992). Inhibition studies were performed by coinoculation of the putative inhibitor with 1 μM unlabeled ditekiren and the radiolabeled oligopeptide.

The vesicular transport of \[^{3}H\]taurocholate (10 μM) was determined in a similar fashion to that of ditekiren but with 1 mM taurocholate used instead of 50 μM ditekiren in the buffer C stop solution and with a 0.45-μm-pore cellulose nitrate membrane filter (Millipore, Bedford, MA).

The concentration dependency of the initial uptake rate of \[^{3}H\]ditekiren by canalicular membrane-enriched vesicles was analyzed through the simultaneous fitting of three equations to the data.
Uptake for this bile acid (data not shown).

An "overshoot" phenomenon was observed in the time course of ATP-dependent transport of taurocholate, when a classic functional potential was confirmed by measuring the location of substrate from the incubation medium into the vesicle. Porters that normally function in an export fashion to translocate substance from the incubation medium into the vesicle. The vesicle preparation was markedly enriched (45–163-fold) relative to the four marker enzymes associated with the hepatic canalicular membrane. In contrast, Na+,K+-ATPase, which is exclusively localized in the basolateral membrane, was absent, and there was minimal contamination by enzymes indicative of various intracellular organelles. Approximately one-third (32.3 ± 3.8%, n = 3) of the canalicular vesicles had an inside-out orientation, thus allowing transporters that normally function in an export fashion to translocate substrate from the incubation medium into the vesicle. Such functional potential was confirmed by measuring the ATP-dependent transport of taurocholate, when a classic "overshoot" phenomenon was observed in the time course of uptake for this bile acid (data not shown).

Initial studies showed that the time profile of [3H]ditekiren uptake also indicated an ATP-dependent component that was initially rapid, reached a maximum at ~1 to 2 min, and then declined (fig. 1). This profile was similar to the loss of ATP in the incubation medium (data not shown). Furthermore, the uptake of the oligopeptide was found to be dependent on the ATP concentration in the incubation medium, and this could be described by a Michaelis-Menten relationship with a $K_m$ value of 129 μM and with maximal stimulation being attained at an ATP concentration of ~1 mM. Additional confirmation for the critical role of ATP was also obtained by the addition (5 mM) of ATP, ADP, AMP, GTP and a nonhydrolyzable ATP analog, 5'-adenylylimido-diphosphate, to the incubation medium in the absence of an ATP-regenerating system. Only ATP significantly enhanced, by ~2-fold, the initial uptake rate of [3H]ditekiren. In addition, vanadate (100 μM), which inhibits P-type ATPase activity, reduced the ATP-dependent uptake of [3H]ditekiren by more than one third (P < .05). Accordingly, all subsequent studies were undertaken at an initial medium ATP concentration of 5 mM along with an ATP-regenerating system. Under these conditions, [3H]ditekiren uptake was linear over ≥20 sec, and therefore subsequent initial rate of uptake studies routinely used a 15-sec incubation period.

![Fig. 1. Time course of the initial [3H]ditekiren (9 μM) uptake rate by canalicular membrane vesicles at 37°C. ATP-dependent uptake (▲) represents the different between transport in the presence (●) and absence (○) of ATP. Data represents the mean ± S.E.M. values for three different vesicle preparations.](image1)

![Fig. 2. Effect of intravesicular osmolarity on the initial [3H]ditekiren (7.5 μM) uptake rate by canalicular membrane vesicles at 37°C in the presence (●) and absence (○) of ATP. Data represent the mean ± S.E.M. values for three different vesicle preparations.](image2)
ATP and an ATP-regenerating system was nonlinear with respect to the ditekiren concentration in the incubation medium (fig. 3A). This was in contrast to the linear relationship observed when the incubation medium was maintained at 4°C. The latter uptake was considered to reflect passive diffusion of ditekiren into the vesicle and nonspecific binding. Accordingly, the difference between the initial uptake rates at the two temperatures was considered to indicate carrier-mediated uptake. The ATP-dependent component of such transport was estimated by also measuring uptake in the absence of ATP and its regenerating system and subtracting this from the carrier-mediated process (fig. 3B). Both the ATP-dependent ($K_m = 19.1 \pm 4.26 \mu M$; mean $\pm$ S.E.M., $V_{max} = 140 \pm 29.4$ pmol/mg of protein/15 sec) and ATP-independent ($K_m = 17.2 \pm 9.55 \mu M$, $V_{max} = 62.9 \pm 24.5$ pmol/mg of protein/15 sec) processes were concentration-dependent and exhibited saturable-type kinetics. Based on the $V_{max}/K_m$ ratios at low oligopeptide concentration, the ATP-dependent process ($7.33 \pm 1.45 \mu l/mg$ of protein/15 sec) was about twice as effective at transporting ditekiren as that of the ATP-independent transporter ($3.66 \pm 5.06 \mu l/mg$ of protein/15 sec) and comparable in magnitude to the nonsaturable component ($7.51 \pm 2.05 \mu l/mg$ of protein/15 sec).

Specificity of the ATP-dependent transport at a ditekiren concentration of 1 $\mu M$ was investigated by determining the ability of other oligopeptides to inhibit the process when present in 100-fold excess. Related compounds as well as other renin inhibitor peptides and angiopeptin reduced transport to varying extents (table 1). EMD-55068 was the most effective inhibitor, inhibiting $[^3H]$ditekiren transport by ~80%. A related compound, EMD-51921, and angiopeptin were also inhibitory but to a lesser extent. In contrast, other similar peptides that had been synthesized by The Upjohn Co., such as U-77436 and U-71013, did not statistically affect $[^3H]$ditekiren transport, although the data suggested a trend with regard to the latter compound. No inhibition was observed with the dipeptide Ala-Asp (data not shown). Additional studies with EMD-51921 indicated that the inhibition of $[^3H]$ditekiren was competitive with a $K_i$ value of 46 $\mu M$ (fig. 4A).

To determine whether ditekiren transport involved one of the known canalicular membrane transport systems, the effect of prototypic substrates (100 $\mu M$) of these processes on ATP-dependent $[^3H]$ditekiren (1 $\mu M$) transport was investigated. Glutathione disulfide, which is transported by cMOAT, had no effect on the initial uptake rate of ditekiren. Taurocholate, however, caused marked inhibition of transport ($6.33 \pm 0.98$ vs. $2.29 \pm 1.11$ pmol/mg of protein/15 sec, $P < .01$), but this was found to be noncompetitive in nature. Similar kinetics were also obtained when the ability of ditekiren to reduce taurocholate transport was studied (data not shown). In contrast, daunomycin reduced the initial transport rate of ditekiren by approximately two thirds ($6.96 \pm 1.02$ vs. $2.30 \pm 1.23$ pmol/mg of protein/15 sec); furthermore, such inhibition was competitive (fig. 4B), with a $K_i$ value of ~16 $\mu M$. Because these findings suggested the involvement of a P-glycoprotein-type transporter, the effects of typical substrates of this system on $[^3H]$ditekiren transport were subsequently studied. Both nicardipine (100 $\mu M$) and cyclosporin A (10 $\mu M$) markedly reduced the ATP-dependent transport of ditekiren (1 $\mu M$) (table 2). More modest (40–60%) inhibition was noted with vinblastine, verapamil, rhodamine-123 and reserpine (all at a concentration of 100 $\mu M$).

### TABLE 1

Effect of other oligopeptides on ATP-dependent, ditekiren transport by canalicular membrane vesicles

<table>
<thead>
<tr>
<th>Peptide</th>
<th>ATP-dependent initial uptake rate</th>
<th>Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (pmol/mg of protein/15 sec)</td>
<td>Oligopeptide (pmol/mg of protein/15 sec)</td>
</tr>
<tr>
<td>EMD-55068</td>
<td>$8.06 \pm 2.22$</td>
<td>$0.87 \pm 0.60$</td>
</tr>
<tr>
<td>EMD-51921</td>
<td>$6.96 \pm 0.85$</td>
<td>$2.59 \pm 0.84$</td>
</tr>
<tr>
<td>Angiopeptin</td>
<td>$9.09 \pm 1.67$</td>
<td>$3.77 \pm 1.42$</td>
</tr>
<tr>
<td>U-71013</td>
<td>$8.97 \pm 1.34$</td>
<td>$4.74 \pm 2.38$</td>
</tr>
<tr>
<td>U-77436</td>
<td>$8.24 \pm 1.68$</td>
<td>$7.56 \pm 1.43$</td>
</tr>
</tbody>
</table>

Values are mean $\pm$ S.E.M. for five separate membrane preparations.

* $P < .05$. 

![Fig. 3. Effect of ditekiren concentration on the initial uptake rate of $[^3H]$ditekiren by canalicular membrane vesicles. A, Total uptake of $[^3H]$ditekiren in the presence of ATP at 37°C (●) and 4°C (▲). B, Carrier-mediated transport (●) obtained from the difference in uptake at 4°C and 37°C; ATP-dependent transport (■) obtained from the difference in uptake at 37°C in the presence and absence of ATP; ATP-independent transport (▲) obtained from the difference between active and ATP-dependent transport at 37°C. Data represent mean ± S.E.M. values for five separate vesicle preparations.](image-url)
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initial uptake into inside-out vesicles prepared from rat he-
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being the rate-limiting step in the excretion process.
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concerning the translocation of oligopeptides across the can-
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process is involved, the nature of which appears to depend on
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(i.e., across the sinusoidal membrane) has recently been in-
of a number of oligopeptides from the blood into hepatocytes
in vivo
is rapidly excreted intact into the bile
TABLE 2
<table>
<thead>
<tr>
<th>Substrate</th>
<th>ATP-dependent initial uptake rate</th>
<th>Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>P-glycoprotein</td>
</tr>
<tr>
<td></td>
<td>pmol/mg of protein/15 sec</td>
<td>%</td>
</tr>
<tr>
<td>Cyclosporin A</td>
<td>8.06 ± 2.22</td>
<td>1.02 ± 1.93</td>
</tr>
<tr>
<td>Nicardipine</td>
<td>8.06 ± 2.22</td>
<td>2.28 ± 1.37</td>
</tr>
<tr>
<td>Daunomycin</td>
<td>6.96 ± 1.02</td>
<td>3.30 ± 1.23</td>
</tr>
<tr>
<td>Verapamil</td>
<td>6.83 ± 1.64</td>
<td>2.22 ± 0.43</td>
</tr>
<tr>
<td>Reserpine</td>
<td>8.19 ± 1.99</td>
<td>2.39 ± 0.98</td>
</tr>
<tr>
<td>Rhodamine</td>
<td>8.99 ± 1.78</td>
<td>4.67 ± 1.55</td>
</tr>
<tr>
<td>Vinblastine</td>
<td>6.30 ± 1.06</td>
<td>3.84 ± 0.99</td>
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</table>

Values are mean ± S.E.M. for five separate membrane preparations.
° p < .01.
* p < .05.

Discussion
Biliary excretion is a major pathway for the elimination of
many oligopeptides, including those with renin-inhibiting
activity such as ditekiren (Ruwart, 1995). In the case of this
pseudohexapeptide, >90% of an administered dose in the rat
is rapidly excreted intact into the bile in vivo (Greenfield
et al., 1989); similar results were obtained in an isolated per-
fused liver preparation (Adedoyin et al., 1993). The transport
of a number of oligopeptides from the blood into hepatocytes
(i.e., across the sinusoidal membrane) has recently been in-
vestigated. In many instances, a carrier-mediated uptake
process is involved, the nature of which appears to depend on
the structure and lipophilicity of the compound; however, the
roles of recently identified specific transporter proteins such
as Ntcp and Oatp1 (Meier, 1995) have yet to be defined
(Ziegler et al., 1996). Considerably less information exists
concerning the translocation of oligopeptides across the can-
alcular membrane into bile, despite the likelihood of this
being the rate-limiting step in the excretion process.
The experimental findings using ditekiren as a model oli-
gopeptide indicate that several processes contribute to its
initial uptake into inside-out vesicles prepared from rat he-
aptic canalicular membrane and, presumably, its biliary ex-
cretion. First, a concentration-independent process was
present that was identified through the study of uptake at
4°C and is consistent with passive diffusion into the canalic-
ules and/or nonspecific binding. This is not unexpected
given the high lipophilicity of ditekiren (e.g., the log-
arthim of its partition coefficient between octanol and pH 7.4
buffer is >4) (Burton et al., 1991). In addition, temperature-
and concentration-dependent transport into an osmotically
sensitive, intravesicular space occurred. This appeared to
involve two separate systems that could be differentiated
according to their ATP-dependency using several different
experimental approaches. On the basis of the kinetics of the
initial uptake process (i.e., Vmax/Km), the transport efficiency
of the ATP-independent carrier-mediated system was ap-
proximately half that of the ATP-dependent process. Studies
to further characterize the non-ATP-dependent transport
were not undertaken other than to note that it was not
influenced by the external buffer Na+ concentration or pH
over the range of 6.6 to 7.9. This probably precludes a poten-
tial-dependent mechanism for such transport because
ditekiren is a bivalent cation with pKs values of 3.95 and
6.3.2

Several ATP-dependent transporters are now known to be
present in the canalicular membrane (Arias et al., 1993;
Gatmanian and Arias, 1995; Vore, 1993). One of these, cBST,
is involved in the secretion of bile acids such as taurocholate
(Meier, 1995). Although a high taurocholate concentration
(100 μM) markedly inhibited ditekiren vesicular uptake and
the reverse phenomenon also occurred (i.e., the oligopeptide
reduced taurocholate transport), in neither case was the in-
hibition competitive in nature. Because the presence of bile
acids is known to alter the function of other ATP-dependent
transporters (e.g., P-glycoprotein) without themselves being
substrates (Mazzanti et al., 1994), it was considered unlikely
that cBST was involved in ATP-dependent transport of
ditekiren. A similar conclusion was made with respect to a
second major canalicular transporter that has relatively
broad specificity for organic anions other than bile acids (i.e.,
cMOAT) (Arias et al., 1993; Gatmanian and Arias, 1995; Vore,

2 Personal communication, Dr. M. Ruwart, Upjohn Co.
1993). This was based on the observation that glutathione disulfide, a prototypic substrate for this carrier protein, did not inhibit diitekiren transport, even at high concentrations. However, competitive inhibition was noted when the canalicular vesicles were incubated in the presence of daunomycin with a $K_a$ value (16 $\mu$M) similar to the $K_m$ value for ATP-dependent transport of this drug by canalicular membrane vesicles (Bohme et al., 1993). In addition, other established substrates/inhibitors of P-glycoprotein, such as cyclosporin A, nocardipine, reserpine, rhodamine-123 and verapamil, also reduced diitekiren uptake, suggesting that this transporter was importantly involved in the uptake of the oligopeptide.

P-glycoprotein localized in the liver is primarily the product of the $mdr1a$ gene and functions as an efflux pump at the canalicular membrane for a broad range of hydrophobic, cat-ionic substrates with molecular weights of 400 to 1200 and containing at least two planar rings (Arias et al., 1993; Gaitman and Arias, 1995; Vore, 1993); the physicochemical properties of diitekiren fulfill these criteria. The importance of P-glycoprotein-mediated transport was first recognized with regard to the phenomenon of multidrug resistance in tumor cells (Gottesman and Pastan, 1993); however, the presence of the transporter in various normal tissues (Gottesman and Pastan, 1993) has led to speculation concerning its possible physiological substrate(s). Several members of the ABC superfamily in both prokaryotic and eukaryotic cells appear to be importantly involved in the transport of oligopeptides (e.g., the gene products of the Opp operon that translocate oligopeptides in bacteria; that of STE6 in yeast, etc.), which are presently unknown but could involve lipophilicity, and angiopeptin. However, the determinants of this selectivity are other renin inhibitors (EMD-51921 and EMD-55068) and U-77436 are not as effective inhibitors of ditekiren transport and that closely related analogs such as U-71013 and U-71038, a renin inhibitor pseudoxephalopeptidase. Drug Metab. Dispos. 21: 184–188, 1993.


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