METHADONE INHIBITS RHODAMINE123 TRANSPORT IN CACO-2 CELLS

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ABSTRACT:

This study investigated the effects of racemic methadone (MET) on P-glycoprotein (P-gp) activity in cell culture. MET showed no differential rates of passage between the basolateral to apical (B to A) and apical to basolateral (A to B) direction across Caco-2 cell monolayers in a transwell system. MET transport in either direction was not importantly influenced by the P-gp inhibitor verapamil. However, MET was a potent inhibitor (IC_{50} = 7.5 μM) of rhodamine123 B to A transport across Caco-2 cell monolayers, causing a reduction to 25% of control at 100 μM MET. In this model of Caco-2 monolayers, rates of MET passage between B to A and A to B directions could not be distinguished. However, MET can inhibit P-gp activity at intraluminal concentrations that might be achieved clinically. This may lead to increased bioavailability of coadministered compounds.

Methadone (MET\textsuperscript{1}) is a synthetic opioid used for maintenance therapy of opiate dependence. MET is metabolized by hepatic and intestinal CYP3A4 (Moody et al., 1997; Foster et al., 1999) and has been shown to inhibit hepatic CYP2D6 and CYP3A4 (Wu et al., 1993; Iriarte et al., 1997). In addition, interactions of MET with P-glycoprotein (P-gp), the product of the human MDRI gene (Fardel et al., 1996), have been suggested (Callaghan and Riordan, 1993; Bouer et al., 1999; Thompson et al., 2000). P-gp is an ATP-dependent drug efflux pump constitutively expressed in several human tissues, including the epithelia of the small intestine and blood brain barrier, liver, and kidney (Sugawara et al., 1988; Cordon-Cardo et al., 1989), as well as in cancer cells (Roninson, 1992). The human colon adenocarcinoma cell line Caco-2 resembles small intestinal epithelial cells (Pinto et al., 1983; Quaroni and Hochman, 1996) and is frequently used for studies modeling intestinal drug transport. This study was designed to investigate the effects of MET on P-gp activity in Caco-2 cells in order to anticipate P-gp related drug-drug interactions.

Materials and Methods

Chemicals. Drugs and chemicals were purchased from commercial sources or were kindly provided by their pharmaceutical manufacturers.

Cell Lines. The human colon adenocarcinoma cell line Caco-2 was kindly provided by Douglas Jefferson, Ph.D. (Tufts University School of Medicine and the New England Medical Center, Boston, MA) and used at passages 30 to 40. Cells were grown in Dulbecco’s modified Eagle’s medium (Invitrogen, Rockville, MD) supplemented with 10% fetal bovine serum, 0.1 mM nonessential amino acids (Invitrogen), 100 units/ml penicillin, and 0.1 mg/ml streptomycin.

Transport Experiments. Caco-2 cells were seeded at 2 × 10^6/cm² in polycarbonate membrane transwell plates (3-μm pore size) (Corning Costar Corp., Cambridge, MA) and grown in a humidified chamber (37°C, 5% CO₂) with media changes every 3 to 5 days. Experiments were conducted on day 16 to 20 after seeding. Drug solutions were prepared in Hanks’ balanced salt solution (Invitrogen) containing 10 mM Hepes, 4.2 mM NaHCO₃, and 0.5% dimethyl sulfoxide.

P-gp inhibition. Hanks’ balanced salt solution containing Rh123 (5 μM) was added to the apical (A) or basolateral (B) chamber, with the inhibitor (100 μM verapamil or 1–100 μM MET) present in both chambers. Cells were incubated for 90 min, and Rh123 concentrations in the chamber initially not containing Rh123 were determined by fluorescence spectrophotometry at 500/550 nm (excitation/emission). Rh123 B to A transport across Caco-2 monolayers was linear between 0.3 and 100 μM Rh123, indicating that the 5 μM concentration used in the transport study was within the linear range. The duration of incubation (90 min) also was within the linear range.

P-gp substrates. Racemic MET (10 μM) was added to the A or B chamber, in the presence and absence of verapamil (100 μM) in both chambers. Cells were incubated for 90 and 180 min to assure measurable concentrations in the receiving chamber. MET concentrations were determined by high-performance liquid chromatography using a 3.9 × 300-mm μBondapak (C₁₈) column (Waters Associates, Milford, MA) and a mobile phase consisting of 45% acetonitrile and 55% 0.05 M KH₂PO₄ (pH 5.5) delivered at 2.4 ml/min, with UV detection at 220 nm. Retention times were 6.9 min for verapamil, 8.3 min for nefazodone (internal standard), and 12.8 min for methadone. The average CV for triplicate samples was 5.9% at 1.5 μM MET. The detection limit was 5 ng of MET.

Data Analysis. Statistical Analysis was performed using analysis of variance followed by Bonferroni t tests against the control group with a significance level of p < 0.05.

Since the baseline diffusion of Rh123 from A to B is consistently low in our cell line (approximately 1–2% of the corresponding B to A transport), it has not been considered in calculating the percentage of inhibition of B to A Rh123 transport.

IC_{50} values for Rh123 transport inhibition were determined using nonlinear regression (SigmaPlot 4.0, SPSS Inc., Chicago, IL) based on eqs. 1 and 2.

\[
f = 1 - \frac{I_{\text{max}} \cdot F}{F + IC}
\]

\[
IC_{50} = \frac{IC}{(2I_{\text{max}} - 1)^{\gamma}}
\]
Transport of MET and Rh123 (positive control) across Caco-2 cell monolayers in a transwell system in presence and absence of verapamil (100 μM)

Values are nanomoles of drug transported per square centimeter of membrane area. Data represent means of triplicate samples, standard deviations in parentheses.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>90 min</th>
<th>180 min</th>
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<tbody>
<tr>
<td></td>
<td>B → A&lt;sup&gt;a&lt;/sup&gt;</td>
<td>A → B&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Methadone (10 μM)</td>
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<tr>
<td>− Verapamil</td>
<td>0.71 (0.14)</td>
<td>0.64 (0.02)</td>
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<tr>
<td>+ Verapamil</td>
<td>0.64 (0.1)</td>
<td>0.84 (0.03)*</td>
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<tr>
<td>Rhodamine123 (5 μM)</td>
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<tr>
<td>− Verapamil</td>
<td>0.06 (0.01)</td>
<td>0.001 (0.0001)</td>
</tr>
<tr>
<td>+ Verapamil</td>
<td>0.003 (0.0003)*</td>
<td>0.001 (0.0002)</td>
</tr>
</tbody>
</table>

* Values statistically significant from the corresponding values in absence of verapamil (p < 0.05).

<sup>a</sup> Basolateral to apical chamber, indicative of active transport.
<sup>b</sup> Apical to basolateral chamber, indicative of passive diffusion.

Results and Discussion

Substrate. In a transwell system, transport of MET across a Caco-2 cell monolayer was evaluated in both directions, B to A (indicative of active transport) and A to B (indicative of passive diffusion). MET showed no differential transport compared with a ≥20-fold difference observed for the positive control Rh123, a known P-gp substrate (Table 1). The P-gp inhibitor verapamil (100 μM) reduced Rh123 B to A transport to 5% of control. Verapamil had no effect on the B to A transport of MET at either time point. A to B passage of MET appeared to be affected by verapamil, but in opposite directions at the 90- and 180-min points. Therefore, the findings, although statistically significant, do not reflect a consistent biological effect.

Inhibition. MET (100 μM) reduced B to A Rh123 transport across Caco-2 monolayers to 25% of control, while a reduction to 5% was achieved by the P-gp inhibitor verapamil (100 μM) used as a positive control (Fig. 1a). The corresponding A to B Rh123 transport was <10% of the B to A transport and was not affected by MET or verapamil (Fig. 1a). The inhibition was concentration-dependent, with an IC<sub>50</sub> of 7.5 μM (Fig. 1b).

The present study showed that MET can significantly modulate P-gp activity in Caco-2 cells. MET was found to be a potent inhibitor of Rh123 transport with an IC<sub>50</sub> of 7.5 μM. Plasma concentrations of methadone achieved in maintenance therapy are in the range of 1 to 3 μM (Altice et al., 1999). However, intraluminal concentrations can be expected to transiently exceed plasma concentrations after oral administration of usual therapeutic doses. This finding is consistent with a previous study showing increased vinblastine accumulation in drug-resistant cells in the presence of MET (Callaghan and Riordan, 1993). Although the K<sub>m</sub> of Rh123 transport in this system is not established, transport linearity was demonstrated up to at least 100 μM Rh123. This suggests that the Rh123 concentration used in the present study is well below the transport K<sub>m</sub> and that IC<sub>50</sub> values generated using Rh123 as the index substrate are likely to apply to other substrates for transport.

In Caco-2 cells, no differential transport of MET was observed, and transport from B to A was not affected by verapamil. In contrast, Bouer et al. (1999) reported a 60% increase in MET transport across everted rat intestine in the presence of verapamil, and a study by Thompson et al. (2000) demonstrated an increased analgesic effect of MET in mdr1 knockout mice. The discrepancy could be attributable to

\[ f \text{ represents the fraction of control B to A transport, } I \text{ represents inhibitor concentration, } I_{\text{max}} \text{ represents maximal inhibitory effect, IC represents inhibitor concentration causing half-maximal inhibitory effect, } b \text{ is an exponent, and IC}_{50} \text{ is inhibitor concentration causing 50% reduction of B to A transport compared to control. Note: IC} = IC_{50} \text{ for } I_{max} = 1. \text{ These functions are based on the Hill equation (Segel, 1975) and can be used for an empirical determination of IC}_{50} \text{ (Venkatakrishnan et al., 1998).} \]
interspecies differences in substrate specificity of P-gp similar to that previously shown for the related opioid morphine. LLC-PK1 cells transfected with the mouse mdr1a gene showed differential transport of morphine, while cells transfected with human MDR1 did not (Schnikel et al., 1995). In a clinical study, coadministration of the P-gp inhibitor PSC833 did not affect morphine pharmacokinetics or cause adverse events in healthy volunteers (Drew et al., 2000), while in mdr1 knockout mice an increased analgesic effect of morphine was reported (Thompson et al., 2000). Also, regional differences in P-gp expression depending on section of the small intestine used may confound studies using rat intestine (Makhey et al., 1998). Finally, note that the density of P-gp in nonselected Caco-2 cells is considerably lower than in other cell lines such as NIH3T3/MDR1 cells (Ibrahim et al., 2000). Thus P-gp-mediated transport of a lipophilic substance such as methadone, which may be quantitatively important in other models or in vivo, may not be evident in the present model due to the greater quantitative importance of passive diffusion.

Caco-2 cells are an accepted in vitro model for intestinal transport (Artursson and Karlsson, 1991; Artursson et al., 1996; Makhey et al., 1998). However, expression of transporters other than P-gp (multidrug resistance protein, lung resistance protein, and dipeptide transporters) in that cell line (Bailey et al., 1996; Makhey et al., 1998) require the use of index compounds that are relatively specific for the transporter studied. Rh123 is frequently used as a P-gp index substrate. A good correlation of Rh123 transport with expression of P-gp, but not multidrug resistance-associated protein, was found in a drug screen in 58 different cell lines (Lee et al., 1994). Verapamil has been shown to inhibit P-gp in MDR-1-transfected Madin-Darby canine kidney cells that constitutively express only low levels of transport proteins (Braun et al., 2000); however, verapamil may also inhibit transporters other than P-gp (Aszalos et al., 1999).

Our data suggest a potential for MET to cause drug interactions by inhibition of drug transport, which may increase bioavailability and central nervous system penetration or reduce renal or biliary clearance of coadministered drugs that are substrates for P-gp or other transporters.

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References


