Mechanisms for Transport of Methotrexate across Apical and Basolateral Membranes in Human Intestinal Epithelial Caco-2 Cells

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Introduction

Various experiments have been conducted to investigate the transport mechanisms of methotrexate across the intestinal epithelial cell monolayer in Caco-2 cells. These experiments have included the analysis of transport across the apical membrane, the basolateral membrane, and both the apical and basolateral membranes. The results of these experiments have provided valuable insights into the mechanisms of transport and have contributed to our understanding of the physiological processes involved.

Materials and Methods

1. Materials

Key words

Materials for Transport of Methotrexate across Apical and Basolateral Membranes in Human Intestinal Epithelial Caco-2 Cells
2. Cell culture

5. Transcellular transport of folic acid and methotrexate

3. Real-time PCR assay of the mRNA of various transporters

4. Cellular uptake of folic acid and methotrexate

Cell culture

10. N. H 4 0ɾ )0
10. N. TPEJVN QZSVWBUF
6. Pharmacokinetic analysis

\[ \frac{dX_A}{dt} = - \frac{C_{\text{L}A|C}}{V_A} X_A + \frac{C_{\text{L}A|B}}{V_C} X_C - \frac{C_{\text{L}A|B}}{V_A} X_A + \frac{C_{\text{L}A|C}}{V_A} X_A \]

\[ \frac{dX_B}{dt} = - \frac{C_{\text{L}B|C}}{V_A} X_B + \frac{C_{\text{L}B|B}}{V_C} X_C + \frac{C_{\text{L}B|B}}{V_A} X_A - \frac{C_{\text{L}B|C}}{V_A} X_A \]

\[ \frac{dX_C}{dt} = \frac{C_{\text{L}C|C}}{V_A} X_A + \frac{C_{\text{L}C|B}}{V_C} X_B - \frac{(C_{\text{L}C|C} + C_{\text{L}C|D})}{V_C} X_C \]

7. Statistical analysis

1. Expression of mRNA of transporters in Caco-2 and LS 180 cells

Results

2. Apical uptake of folic acid and methotrexate in Caco-2 cells

\[ dX_A = - \frac{C_{\text{L}A|C}}{V_A} X_A + \frac{C_{\text{L}A|B}}{V_C} X_C - \frac{C_{\text{L}A|B}}{V_A} X_A + \frac{C_{\text{L}A|C}}{V_A} X_A \]

\[ dX_B = - \frac{C_{\text{L}B|C}}{V_A} X_B + \frac{C_{\text{L}B|B}}{V_C} X_C + \frac{C_{\text{L}B|B}}{V_A} X_A - \frac{C_{\text{L}B|C}}{V_A} X_A \]

\[ dX_C = \frac{C_{\text{L}C|C}}{V_A} X_A + \frac{C_{\text{L}C|B}}{V_C} X_B - \frac{(C_{\text{L}C|C} + C_{\text{L}C|D})}{V_C} X_C \]
Figure 1.

Figure 2.

Figure 3.

Figure 4.
3. Transcellular transport and membrane transport clearance of folic acid in Caco-2 cell monolayers

![Graphs A, B, C](image)

Table 1

<table>
<thead>
<tr>
<th>Condition</th>
<th>Uptake of Methotrexate (%) of Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100</td>
</tr>
<tr>
<td>+1 μM Folic acid</td>
<td>95</td>
</tr>
<tr>
<td>+1 μM Leucovorin</td>
<td>90</td>
</tr>
<tr>
<td>+1 μM Pemetrexed</td>
<td>85</td>
</tr>
</tbody>
</table>

* p < 0.05; ** p < 0.01; *** p < 0.001
4. Transcellular transport and membrane transport clearance of methotrexate in Caco-2 cell monolayers

Table 1. Comparison of the membrane transport clearance in Caco-2 cell monolayers

<table>
<thead>
<tr>
<th></th>
<th>pH 4.5</th>
<th>pH 5.5</th>
<th>pH 6.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL&lt;sub&gt;A→C&lt;/sub&gt; (μl/min/cm²)</td>
<td>2.225 ± 0.111</td>
<td>1.567 ± 0.039</td>
<td>1.088 ± 0.021*</td>
</tr>
<tr>
<td>CL&lt;sub&gt;C→A&lt;/sub&gt; (μl/min/cm²)</td>
<td>0.241 ± 0.017</td>
<td>0.140 ± 0.006*</td>
<td>0.135 ± 0.005*</td>
</tr>
<tr>
<td>CL&lt;sub&gt;A→B&lt;/sub&gt; (μl/min/cm²)</td>
<td>0.070 ± 0.002</td>
<td>0.056 ± 0.001</td>
<td>0.103 ± 0.006</td>
</tr>
<tr>
<td>CL&lt;sub&gt;C→B&lt;/sub&gt; (μl/min/cm²)</td>
<td>0.026 ± 0.002</td>
<td>0.018 ± 0.002*</td>
<td>0.016 ± 0.001*</td>
</tr>
</tbody>
</table>

* p < 0.05; significant differences compared to other pH values

Discussion

The data in Table 1 indicate that the transcellular transport clearance of methotrexate is pH-dependent. At pH 4.5, the Clearance (CL) values are significantly higher compared to pH 5.5 and 6.5. This suggests that the pH conditions affect the transport efficiency across the Caco-2 cell monolayers. The membrane transport clearance also shows a decrease with increasing pH, which could be due to changes in the membrane permeability or drug dissociation.
Table 2

<table>
<thead>
<tr>
<th>Methotrexate concentration</th>
<th>$C_{A\rightarrow C}$</th>
<th>$C_{C\rightarrow A}$</th>
<th>$C_{A\rightarrow B}$</th>
<th>$C_{C\rightarrow B}$</th>
<th>$C_{B\rightarrow C}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 μM</td>
<td>1.745 ± 0.119</td>
<td>0.153 ± 0.013</td>
<td>0.063 ± 0.004</td>
<td>0.028 ± 0.002</td>
<td>0.159 ± 0.014</td>
</tr>
<tr>
<td>3 μM</td>
<td>0.629 ± 0.019*</td>
<td>0.096 ± 0.005</td>
<td>0.074 ± 0.002*</td>
<td>0.024 ± 0.001</td>
<td>0.076 ± 0.009*</td>
</tr>
<tr>
<td>10 μM</td>
<td>0.262 ± 0.005*</td>
<td>0.079 ± 0.002*</td>
<td>0.066 ± 0.002</td>
<td>0.018 ± 0.001*</td>
<td>0.062 ± 0.004*</td>
</tr>
</tbody>
</table>

* Denotes statistical significance where $p < 0.05$; $\approx$ indicates non-significant difference.
Acknowledgements

References

[References text]