FACILITATED TRANSPORT OF BENZYLPCNICILLIN THROUGH THE BLOOD-BRAIN BARRIER IN RATS

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The unidirectional influx of benzylpenicillin through the blood-brain barrier was examined with an in situ rat brain perfusion technique using purified [3H]benzylpenicillin. The major transport system of benzylpenicillin through the blood-brain barrier was via a saturable process with a one-half saturation concentration of approximately 8-30 μM. This transport system was significantly inhibited by probenecid (100 μM) and ceftriaxone (2 mM), indicating that the transport system may be shared by some organic anions including third generation cephalosporin antibiotics. These findings suggest that concomitant administration of β-lactam antibiotics could produce a drug interaction to alter the drug penetration into the central nervous system.

KEYWORDS—β-lactam antibiotics; benzylpenicillin; blood-brain barrier transport; bacterial meningitis

INTRODUCTION

Although β-lactam antibiotics are widely used in the treatment of bacterial meningitis, their pharmacokinetic behavior in the central nervous system (CNS) is not fully clarified. Accumulating evidence indicates that the active transport system in the choroid plexus plays an important role in regulating the concentration of β-lactam antibiotics in the cerebrospinal fluid (T. Nohjoh, H. Suzuki, Y. Sawada, Y. Sugiyama, T. Iga and M. Hanano, manuscript submitted to J. Pharmacol. Exp. Ther.; H. Suzuki, Y. Sawada, Y. Sugiyama, T. Iga, M. Hanano and R. Spector, manuscript submitted to J. Pharmacol. Exp. Ther.).1-4) However, little information has been available concerning the entry of these drugs into the CNS.

The recent introduction of an in situ brain perfusion technique by Takasato et al.5) made it possible to precisely determine the permeability-surface area (PS) product of β-lactam antibiotics through the blood-brain barrier (BBB). Using this experimental technique, Spector6) reported that the transport of ceftriaxone from blood to brain is facilitated, in large part, by a probenecid-sensitive mechanism, while we found that the mechanism is not shared by imipenem, a novel carbapenem.

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The purpose of the present study is to characterize further the transport system by which β-lactam antibiotics are transferred from blood to brain, using the in situ brain perfusion technique. Benzylpenicillin, a prototypic β-lactam antibiotic, was used as a model compound.

MATERIALS AND METHODS

Materials—-[Carboxyl-14C]Inulin (3.32 μCi/mg) and [phenyl-4(n)-3H]benzylpenicillin (18.9 Ci/mmol) were purchased from ICN Radiochemicals (Irvine, CA) and Amersham International Ltd. (Buckinghamshire, England), respectively. Unlabeled benzylpenicillin sodium salt and probenecid were purchased from Sigma Chemical Co. (St. Louis, MO). Ceftriaxone was purchased from Nippon Roche (Tokyo, Japan).

Purity of the Isotopes—Purity of [14C]Inulin (> 99%) was established prior to use by gel chromatography with a Sephadex G-25 fine column as described by Cohen. [3H]Benzylpenicillin was purified with the following two kinds of chromatography systems (Systems (A) and (B)) immediately before use.

System (A): [3H]Benzylpenicillin was purified by high-performance liquid chromatography (HPLC). A variable wavelength UV detector (model 638-41, Hitachi Ltd., Tokyo, Japan) was set at 230 nm. The solvent delivery system used was a Liquid Chromatograph (model 655A-11, Hitachi Ltd.). Injections were made with a Rheodyne model 7125 valve and a 200 μl injection loop. Chromatography was performed on a reversed-phase, Shim-pack CLC-ODS, 5 μm C18, 250 mm X 4.6 mm I.D., analytical column (Shimadzu Corp., Kyoto, Japan). The mobile phase consisted of phosphate buffer solution (0.03 M Na2HPO4-0.02 M NaH2PO4, pH 7.8):acetonitrile = 7:3. The flow rate was 1 ml/min. Each 100 μl fraction was collected and analyzed for radioactivity. The purified [3H]benzylpenicillin was found to be more than 99% pure by silica gel thin-layer chromatography in acetone:toluene:glacial acetic acid = 66:33:0.1; methanol:isopropanol = 7:3; acetone:glacial acetic acid =19:1, by paper chromatography in n-butanol:ethanol:water = 40:10:50,2-4 and by the HPLC described above. However, we could detect a break-down product of [3H]benzylpenicillin (<1%) by silica gel thin-layer chromatography in acetone:toluene:glacial acetic acid = 66:33:0.1.

System (B): To eliminate this break-down product of [3H]benzylpenicillin, we also purified [3H]benzylpenicillin by silica gel thin-layer chromatography in acetone:toluene:glacial acetic acid = 66:33:0.1, prior to the purification with the HPLC. We observed no break-down product of [3H]benzylpenicillin after the final purification by all the chromatography systems described above.

Transport of Benzylpenicillin through the BBB—The in situ brain perfusion technique5 was used except that male Wistar rats (240-260 g) under ethylcarbamate anesthesia (1.5 g/kg i.p.) were used. In brief, this technique involves cannulating the external carotid artery of the experimental animal and tying off all branches of the internal carotid artery. At time zero, the common carotid artery is ligated and the perfusion is continued with the bicarbonate-based balanced salt solution at 37°C for periods up to 60 sec. The perfusate was prepared containing (mM) NaCl, 142; NaHCO3, 28; KH2PO4, 4.2; CaSO4, 1.7; MgSO4, 1.0 and glucose 6.0. Six seconds are required for the perfusate to reach the brain, which were subtracted off the time of
the perfusion used in the calculations below. The perfusion rate was 70 μl/sec. At the end of the perfusion, the rat was decapitated and the cortex on the perfused side was removed, weighed, homogenized in the bicarbonate based balanced salt solution and then assayed for radioactivity. In all experiments in which [³H]benzylpenicillin (1.30 μCi/ml) was perfused, [¹⁴C]inulin (0.28 μCi/ml) was included in the perfusate as a reference for the intravascular space. The radioactivities of [³H] and [¹⁴C] in the samples were determined in a liquid scintillation spectrophotometer (model 3255, Packard Instruments Corp., Downers Grove, IL). The counting efficiency and crossover correction was determined by the external standard channels ratio technique. Cerebral perfusion flow, measured using diazepam, was 0.115 ± 0.008 ml/sec/g brain (mean ± S.E., n = 4) at a perfusate rate of 70 μl/sec (H. Suzuki, Y. Sawada, Y. Sugiyama, T. Iga, M. Hanano and R. Spector, manuscript submitted to J. Pharmacol. Exp. Ther.). The PS product for [³H]benzylpenicillin was calculated as described previously in detail.5,6

RESULTS AND DISCUSSION

The PS products for [³H]benzylpenicillin (8 μM), purified with systems (A) and (B), were 7.27 ± 0.50 x 10⁻⁵ sec⁻¹ (Table I) and 7.30 ± 0.45 x 10⁻⁵ sec⁻¹ (mean ± S.E., n = 4), respectively. Inasmuch as no significant difference was observed between these PS products (p > .05, by Student's t test), the break-down product of [³H]benzylpenicillin (<1%) does not interfere with the determination of the PS product for [³H]benzylpenicillin. Therefore, purified [³H]benzylpenicillin with system (A) was used in the following experiments.

The effect of inhibitors on the PS product for [³H]benzylpenicillin is shown in Table I. The PS product for [³H]benzylpenicillin was reduced by unlabeled benzylpenicillin with a one-half saturation concentration of approximately 8-30 μM. Furthermore, this transport system was significantly inhibited by probenecid (100 μM) and ceftriaxone (2 mM). These results are consistent with the previous observation by Fishman,8 who demonstrated the inhibitory effect of probenecid on the penetration of [¹⁴C]benzylpenicillin into the brain after systemic administration in rats.

Table I. PS Product for [³H]Benzylpenicillin under Various Conditions

<table>
<thead>
<tr>
<th>Conditions</th>
<th>PS product (% of control)</th>
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<tbody>
<tr>
<td></td>
<td>s⁻¹ x 10⁵</td>
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<tr>
<td>Control (0.07 μM)</td>
<td>9.14 ± 0.21 (100)</td>
</tr>
<tr>
<td>+ benzylpenicillin 8 μM</td>
<td>7.27 ± 0.50 (80)*</td>
</tr>
<tr>
<td>+ benzylpenicillin 30 μM</td>
<td>3.99 ± 0.39 (44)*</td>
</tr>
<tr>
<td>+ benzylpenicillin 2 mM</td>
<td>2.34 ± 0.25 (26)*</td>
</tr>
<tr>
<td>+ probenecid 100 μM</td>
<td>3.01 ± 0.22 (33)*</td>
</tr>
<tr>
<td>+ ceftriaxone 2 mM</td>
<td>4.30 ± 0.18 (47)*</td>
</tr>
</tbody>
</table>

The PS products for [³H]benzylpenicillin were determined with 0.07 μM [³H]benzylpenicillin. In some cases, inhibitors were added to the perfusate. Results are mean ± S.E. of four independent experiments. *Significantly lower than the control with p < .05 (Dunnett's test).
Transport of Penicillin through the BBB

From these observations, we concluded (1) that the major transport system of benzylpenicillin through the BBB is via a carrier-mediated system with a one-half saturation concentration much lower than the therapeutic plasma concentration and (2) that the transport system is shared by other third generation cephalosporin antibiotics such as ceftriaxone.6

These results may have some clinical implications for the penetration of β-lactam antibiotics into the CNS. In the treatment of bacterial meningitis, some β-lactam antibiotics can be concomitantly administered to cover the antibacterial spectrum.9) Previously, Okura et al.10) reported that latamoxef reduces the transfer of ampicillin into the cerebrospinal fluid in rabbits with staphylococcal meningitis. Latamoxef may competently inhibit the transport of ampicillin through the BBB. The results of the present study suggest that the concomitant administration of β-lactam antibiotics could produce a drug interaction to alter the drug penetration into the CNS.

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REFERENCES


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