Mechanisms of 5-Fluorouracil (5-FU) Transport in Isolated Rabbit Choroid Plexus Tissue Slices

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INTRODUCTION

5-Fluorouracil (5-FU), a synthetic nucleobase analog, is one of the most widely used antineoplastic agents for the treatment of solid tumors including colorectal, breast and gynecological carcinomas. Similar to many antineoplastic agents, 5-FU produces a number of adverse effects. These include bone marrow suppression, gastrointestinal disturbances and neurotoxicities. The neurotoxicities associated with 5-FU administration include dizziness, speech disturbance, Parkinsonism, somnolence, confusion and vertigo (1,2). The most common neurologic effect is cerebellar ataxia. These adverse effects have a rapid onset and usually subside upon discontinuation of 5-FU.

Since 5-FU is hydrophilic (log $P_c = -0.93$) (3), specific transport systems appear to be responsible in part for its transport across biological membranes. In human erythrocytes, 5-FU is transported by nonfacilitated diffusion as well as a concentrative process (4). Several Na^+-dependent nucleobase transport systems are involved in the transport of 5-FU across various epithelia including rat jejunal tissue (5,6), LLC-PK1 cells (7), and guinea pig renal brush border membrane vesicles (8).

The choroid plexus functions to maintain the microenvironment of the central nervous system. Isolated rabbit choroid plexus slices have been extensively used to study the mechanisms of nucleoside (9–11) and nucleobase transport. A knowledge of the mechanisms of transport of 5-FU across the barriers of the central nervous system (CNS) is critical in understanding its neurotoxicities. Recently we characterized a broadly selective Na^+-nucleobase transporter in rabbit choroid plexus epithelium (blood-cerebrospinal fluid barrier) (12). Our preliminary data suggested that 5-FU interacts with this system. The objective of this study was to determine the mechanisms of 5-FU transport in the choroid plexus. Our data suggest that 5-FU is transported in rabbit choroid plexus by a saturable, Na^+-dependent transporter. This transporter may play a role in mediating the toxicities of 5-FU in the CNS.

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MATERIALS AND METHODS

Materials

[^{3}H]5-FU (15 Ci/mmol, sp. activity), [^{3}H]hypoxanthine (11.6 Ci/mmol, sp. activity), and [^{14}C]mannitol (56 mCi/mmol, sp. activity) were purchased from either Sigma Chemical Company (St. Louis, MO), NEN (Wilmington, DE) or Amersham Life Science (Arlington Heights, IL). 5-FU, hypoxanthine, adenine, xanthine, and uracil were purchased from either Sigma or Aldrich. All other chemicals were purchased from either Sigma, Fisher Scientific, or Aldrich. New Zealand White rabbits were purchased from Nitabell Rabbitry, Hayward, CA. Cytoscint ES scintillation fluid was purchased from ICN Biomedical Inc.

Preparation of ATP-depleted Choroid Plexus Tissue Slices

Choroid plexuses were excised from the lateral ventricles of New Zealand white rabbits and tissue slices were prepared as described previously (11,12). The tissue slices (2-3 mm) were then placed in buffer containing 2,4 dinitrophenol (DNP, 250 μM) for 20 minutes at 37°C for ATP depletion and stored on ice until uptake studies were performed.

Uptake of [^{3}H] 5-FU

Choroid plexus tissue slices were incubated at 37°C with 140 μl of reaction mixture containing [^{3}H]5-FU (0.2 μM), [^{14}C]mannitol (25.4 μM), DNP (250 μM), in either KCl or NaCl-containing buffer. The buffers were of the following compositions: KCl (150 mM) or NaCl (150 mM), mannitol (40 mM) and 4-2(2-hydroxyethyl)-1-piperazineethanesulfonic acid, (HEPES), (25 mM), pH 7.4 with 1 M Tris. The uptake was stopped by blotting on laboratory tissue (13). The choroid plexus tissue slices were then dried, weighed and dissolved. [^{3}H] and [^{14}C] were determined in the dissolved tissue samples and the corresponding reaction mix by dual isotope liquid scintillation counting.

Data Analysis

The radioactive content of each choroid plexus tissue slice was expressed as a volume of distribution (V_d) as described previously (11,14). Briefly, the following equation is used:

$$V_d = \frac{\text{dpm[}^3\text{H]-5-FU/g choroid plexus}}{\text{dpm[}^3\text{H]-5-FU/ml media}} - \frac{\text{dpm[}^{14}\text{C]-mannitol/g choroid plexus}}{\text{dpm[}^{14}\text{C]-mannitol/ml media}}$$

Statistical analysis was carried out by a Student's unpaired *t*-test. A probability, P, of less than 0.05 was considered significant. Data points were determined in triplicate for each experiment. Data, unless mentioned otherwise, were obtained from 3 experiments in choroid plexus tissue from separate animals. Michaelis-Menten parameters and IC_{50} values were obtained by fitting data to the appropriate equation. Fits were carried out using a nonlinear fitting routine of Kaleidagraph® (version 2.0, Synergy Software, Reading, PA) software.

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RESULTS

In the absence of a Na^+-gradient (150 mM KCl buffer), 5-FU accumulated in the choroid plexus tissue slices and reached an equilibrium (V_d = 2.09 ± 0.32) in approximately five minutes (Figure 1). In the presence of an initial inwardly-directed Na^+-gradient (150 mM), 5-FU accumulated temporarily in the tissue slices above the equilibrium value (V_d = 4.06 ± 0.68) at 1 minute exhibiting an apparent "overshoot" phenomenon.

Kinetic experiments were performed in which the rate of 5-FU uptake (at 45 s) as a function of concentration was determined in the presence of an initial inwardly-directed Na^+-gradient (150 mM). The data (not shown) are consistent with a single saturable process. The data from each of the three experiments were fit to a Michaelis-Menten equation. The K_m and V_{max} (mean ± S.E.) were 8.63 ± 2.6 μM and 0.42 ± 0.12 nmol/g/sec, respectively.

The effect of various purine and pyrimidine nucleobases on Na^+-dependent 5-FU uptake was examined (Figure 2). At concentrations of 100 μM, unlabeled 5-FU, hypoxanthine, adenine, uracil and xanthine significantly inhibited Na^+-dependent 5-FU uptake at 45 s ($p < 0.05$). The substrate selectivity of Na^+-dependent 5-FU transport appears to be similar to that of the recently described Na^+-dependent nucleobase transporter in the rabbit choroid plexus (12). Further studies indicated that 5-FU is a potent inhibitor of Na^+-dependent hypoxanthine transport (IC_{50} = 3.56 ± 0.45 μM) (Figure 3). Inhibition curves were monophasic which is consistent with a single Na^+-dependent transport mechanism.

DISCUSSION

Early studies demonstrated that high concentrations of 5-FU (5 mM) inhibited the active transport of [^{14}C] uracil in rat intestine (15). However, the active transport of uracil or other nucleobases was not fully characterized in terms of driving force. Since then, a number of studies have characterized nucleobase transport in a variety of cells including intestinal cells (5,6), LLC-PK_1 cells (7) and guinea pig brush border membrane vesicles (8).

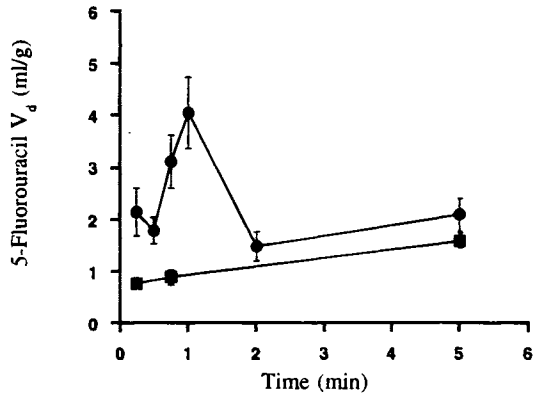

Fig. 1. 5-FU uptake (0.2 μM) in ATP-depleted rabbit choroid plexus tissue slices. The uptake of 5-FU was examined in the absence (squares) and presence (circles) of an initial inwardly-directed Na^+-gradient. Each data point represents the uptake of 5-FU (mean ± S.E.) from three experiments.

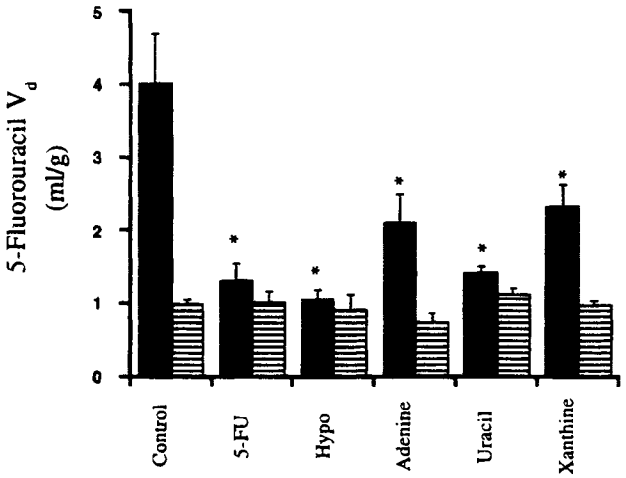

Fig. 2. The effect of purine and pyrimidine nucleobases on 5-FU (0.2 μM) uptake (at 45 s) in ATP-depleted rabbit choroid plexus tissue slices. At concentrations of 100 μM, 5-FU, hypoxanthine (hypo), adenine, uracil and xanthine significantly ($p < 0.05$) inhibited Na^+-dependent 5-FU uptake. Solid and hatched bars represent data obtained in rabbit choroid plexus slices in the presence and absence of an inwardly-directed Na^+-gradient, respectively. Bars represent the mean (±S.E.) of data obtained in three separate experiments.

In the current study, we experimentally imposed an initial inwardly-directed Na^+-gradient which resulted in an "overshoot phenomenon". This "overshoot phenomenon" provides direct evidence (Figure 1) that 5-FU transport is coupled to a Na^+-gradient. These data represent the first demonstration of Na^+-dependent 5-FU transport in the choroid plexus. The data are consistent with previous studies in LLC-PK_1 cells (7), guinea pig brush border membrane vesicles (8, 16) and choroid plexus tissue slices (12) demonstrating that 5-FU inhibits Na^+-hypoxanthine transport.

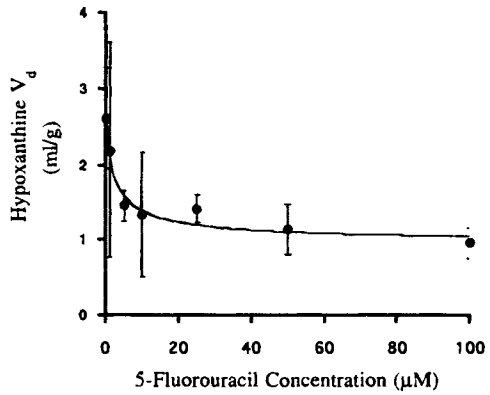

Fig. 3. [^{3}H]hypoxanthine (0.24 μM) uptake (at 30 s) in the presence of increasing concentrations of 5-FU in ATP-depleted rabbit choroid plexus tissue slices. The uptake of [^{3}H]hypoxanthine (0.24 μM) was determined in the presence of an initial inwardly-directed Na^+-gradient (150 mM) and in the presence of varying concentrations of 5-FU. The concentration of 5-FU producing 50% inhibition of Na^+-dependent hypoxanthine transport (IC_{50}) was derived from individual curves. The values represent IC_{50} of three separate experiments (mean ± S.E.).

The substrate selectivity of the Na^+-dependent 5-FU transporter in the choroid plexus is similar to that of the Na^+-dependent hypoxanthine transporter (12), but differs from transport systems characterized in LLC-PK_1 cells (7) and guinea pig brush border membranes (8). The purine nucleobases, adenine and xanthine did not inhibit hypoxanthine uptake in LLC-PK_1 cells nor did adenine inhibit Na^+-dependent hypoxanthine uptake in guinea pig brush border membrane vesicles. However, these nucleobases appear to interact with the Na^+-dependent 5-FU transport in rabbit choroid plexus.

Data obtained in this study indicate that 5-FU is a potent inhibitor of hypoxanthine uptake. The IC_{50} for the inhibition of Na^+-stimulated hypoxanthine uptake by 5-FU in choroid plexus (3.56 μM) agrees well with the K_m of 5-FU (8.63 μM). These data together with the similar substrate selectivity suggest that 5-FU is transported by the Na^+-dependent nucleobase transporter in choroid plexus (12). 5-FU appears to have a higher affinity (K_m = 8.63 μM) for the Na^+-dependent nucleobase transport system in choroid plexus than does hypoxanthine (K_m = 31 μM) (12). Since the Michaelis-Menten constant falls within the usual plasma concentration range of 5-FU (10 nM − 1 mM) after clinical doses (17), the data suggest that the Na^+-nucleobase transporter may play a role in the transport of 5-FU across the blood-cerebrospinal fluid barrier of the CNS following clinical doses.

In conclusion, this study demonstrates that 5-FU transport in choroid plexus is mediated by a Na^+-dependent, saturable transporter which is selective for both pyrimidine as well as purine nucleobases (adenine, 5-FU, hypoxanthine, uracil and xanthine). The substrate selectivity and the good agreement between the K_m of Na^+-dependent 5-FU transport (8.63 μM) and its K_i in inhibiting Na^+-dependent hypoxanthine transport (3.56 μM) suggest that 5-FU is transported by the recently characterized Na^+-dependent hypoxanthine transporter in choroid plexus. This transporter may play a role in the transport of 5-FU in the central nervous system thus mediating its neurotoxicity. However, additional studies are needed to determine the sidedness of the Na^+-nucleobase transporter (basolateral versus brush border membranes) and to elucidate the net direction of transepithelial flux of 5-FU.

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