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Affinity of antineoplastic amino acid drugs for the large neutral amino acid transporter of the blood-brain barrier

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Summary. The relative affinity of six anticancer amino acid drugs for the neutral amino acid carrier of the blood-brain barrier was examined in rats using an in situ brain perfusion technique. Affinity was evaluated from the concentration-dependent inhibition of L-[^{14}C]-leucine uptake into rat brain during perfusion at tracer leucine concentrations and in the absence of competing amino acids. Of the six drugs tested, five, including melphalan, azaserine, acivicin, 6-diazo-5-oxo-L-norleucine, and buthionine sulfoximine, exhibited only low affinity for the carrier, displaying transport inhibition constants (K_i, concentrations producing 50% inhibition) ranging from 0.09 to 4.7 mM. However, one agent – D,L-2-amino-7-bis[(2-chloroethyl)amino]-1,2,3,4-tetrahydro-2-naphthoic acid (D,L-NAM) – demonstrated remarkably high affinity for the carrier, showing a K_i value of ~0.2 μM. The relative affinity ($1/K_i$) of D,L-NAM was >100-fold that of the other drugs and >10-fold that of any compound previously tested. As the blood-brain barrier penetrability of most endogenous neutral amino acids is related to their carrier affinity, the results suggest that D,L-NAM may be a promising agent which may show enhanced uptake and distribution to brain tumors.

Introduction

Chemotherapy has been proven to be minimally effective in the treatment of primary or metastatic brain tumors [15, 19]. Brain metastases often fail to respond to treatment even when there is marked regression of peripheral loci of the disease [30]. One reason often cited for the failure of brain tumor chemotherapy is inadequate drug delivery to critical tumor sites [9]. Many chemotherapeutic drugs are hydrophilic and do not readily cross the blood-brain barrier (BBB), which is located at the cerebral capillaries and is formed by a continuous layer of endothelial cells joined by tight junctions (zonulae occludens) [3]. Although the integrity of the barrier may be compromised in some brain tumors, studies suggest that tumor blood vessels provide a significant diffusion restriction for drug uptake in many instances [10, 13]. Furthermore, drug accumulation can be limited in the critical proliferating edge of a tumor by surrounding brain tissue, which often displays an intact BBB and can serve as a diffusional sink [20, 34].

Improvement of drug delivery to brain tumors and surrounding tissue is being approached in several ways. Lipid-soluble drugs exhibiting greater BBB passive permeability are being prepared and tested for activity [9]. In addition, attempts are being made to circumvent the barrier either by direct application of drug to the tumor [17, 32, 42], by drug injection into the CSF [33], or by transient "opening" of the BBB via brief intracarotid hypertonic infusion [23]. An alternative approach is to design drugs that are shuttled into the brain by the natural cerebrovascular transport carriers for essential nutrients, such as amino acids, glucose, and choline [38]. As brain tumors often show enhanced nutrient uptake and accumulation [26, 35], the latter approach may enable some selectivity of drug delivery to tumor as compared with surrounding brain tissue.

In a previous study, we demonstrated that melphalan, a nitrogen mustard derivative of L-phenylalanine (Fig. 1), is transported into the brain by the large neutral amino acid carrier of the BBB [11]. The large neutral amino acid carrier (L system) is located at the brain capillaries and accepts a wide variety of ligands displaying alpha-amino acid functional group [25, 38, 39]. Melphalan has previously been proven to be effective in the treatment of multiple myeloma [8] and of carcinoma of the breast and ovary [7, 29]. Although melphalan is transported into the brain, its affinity for the BBB neutral amino acid carrier is quite low and it thus attains only limited concentrations within the central nervous system following systemic administration [11].

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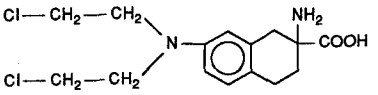
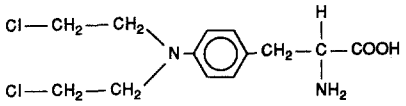
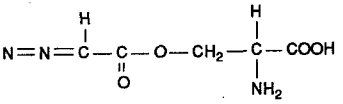
i. D,L-NAM



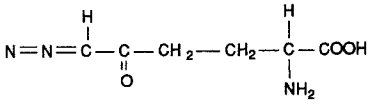
ii. Melphalan



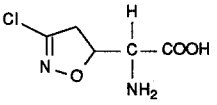
iii. Azaserine



iv. L-DON



v. Acivicin



vi. Buthionine Sulfoximine (BSO)

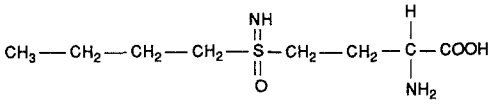


Fig. 1. Structures of chemotherapeutic amino acid drugs

In the present study, the relative affinity of six chemotherapeutic amino acid drugs for the neutral amino acid transporter of the BBB was examined in rats in an attempt to identify agents exhibiting greater affinity for the neutral amino acid transport protein. Affinity was evaluated according to the ability of drugs to inhibit brain uptake of L-[^{14}C]-leucine as measured using the in situ brain perfusion technique [38, 39]. Three agents that were tested – azaserine, 6-diazo-5-oxo-L-norleucine (L-DON), and acivicin (Fig. 1) – are glutamine antagonists- antimetabolites that have demonstrated significant peripheral chemotherapeutic activity [2, 24]. The fourth – buthionine sulfoximime (BSO) – is a glutathione synthesis inhibitor that has been proposed for use to limit the development of resistance to alkylating drugs [12]. The fifth – D,L-2-amino-7-bis[(2-chloroethyl)amino]-1,2,3,4-tetrahydro-2-naphthoic acid (NAM) – is an analog of melphalan that has been shown by Vistica and colleagues [14, 44] to possess enhanced affinity for the sodium-independent, large neutral amino acid transporter of murine L1210 leukemia cells. The last agent – melphalan – was studied to compare the affinity determined by transport inhibition to that obtained directly from [^{14}C]-melphalan uptake into the brain [11].

Materials and methods

Chemicals. Melphalan [4-bis(2-chloroethyl)amino-L-phenylalanine], azaserine (*O*-diazoacetyl-L-serine), DON (both D and L isomers), acivicin (alpha-amino-3-chloro-4,5-dihydro-5-isoxazoleacetic acid) and L-BSO were obtained from Sigma Chemical Co. (St. Louis, Mo.). D,L-NAM was synthesized and purified as described by Haines et al. [14] (estimated purity, >95%). L-[^{14}C(U)]-Leucine (sp. act., 322 μCi/μmol), [^{3}H]-diazepam, and [^{3}H]-methoxy-inulin were purchased from DuPont-New England Nuclear (Boston, Mass.). Radiochemical purity (>99%) was confirmed for all tracers by thin-layer or gel-permeation chromatography [37]. To minimize decomposition, drug solutions were prepared immediately prior to their use and were protected from light.

Brain perfusion. Drug inhibition of L-[^{14}C]-leucine transport into the brain was measured using the in situ brain perfusion technique of Takasato et al. [41]. This method involves complete, unilateral perfusion of rat brain with physiologic saline or blood and enables the determination of influx in the virtual absence of endogenous competitors at constant drug concentrations [37].

Male Sprague-Dawley rats weighing between 260 and 340 g were anesthetized with sodium pentobarbital (40 mg/kg i.p.). The right external carotid artery was cannulated (PE-50), and the right pterygopalatine, occipital, and superior thyroid arteries were cauterized and cut [41]. Perfusion was begun by first ligating the right common carotid artery just proximal to the bifurcation of the external carotid artery and then infusing HCO_3-buffered physiologic saline (pH ~7.4) into the external carotid artery at 5–15 ml/min using a pump (Harvard Apparatus, South Natick, Mass.). In most experiments, the heart was stopped just prior to perfusion (2–3 s) by severing the left ventricle so as to eliminate potential flow contributions from the systemic circulation. With the ventricle severed, a higher infusion rate (~10 ml/min) was generally required to obtain brain flow rates (~10×10^{-2} ml/s/g) comparable to those reported by Takasato et al. [41].

The composition of the saline perfusate was 128 mM NaCl, 24 mM $NaHCO_3$, 4.2 mM KCl, 1.5 mM $CaCl_2$, 0.9 mM $MgCl_2$, 2.4 mM NaH_2PO_4, and 9 mM D-glucose [22]. To this were added ~0.3 μCi L-[^{14}C(U)]-leucine/ml, ~0.3 μCi [^{3}H]-diazepam/ml (to measure cerebral perfusion fluid flow), and 0–30 mM unlabeled anticancer drug (for competition). In some experiments, [^{3}H]-inulin was added in place of [^{3}H]-diazepam for quantification of cerebral vascular volume [41]. All solutions were filtered, warmed to 37° C, and oxygenated with 95% air 5% CO_2 prior to their use. The body temperature of the rats was maintained at 36–37° C throughout all experiments using a heat lamp.

Perfusions were stopped after 30 s. The brain was removed from the skull and dissected on ice [37]. Tissue samples were collected from the right cerebral hemisphere after removal of the meninges and surface blood vessels. Two 20- to 30-μl aliquots of perfusion fluid were also collected for determination of perfusate tracer concentrations. Samples were weighed, digested overnight in 1 M piperidine (~1 ml/sample), and dissolved in scintillation cocktail (~10 ml/sample; Ready Solv-MP, Beckman, Fullerton, Calif.). ^{3}H and ^{14}C dpm values were determined after correction for background, quench, and efficiency using a standard dual-label liquid scintillation counting technique.

Calculations. Sample dpm values were first expressed per gram of tissue wet weight or per milliliter of perfusion fluid. Cerebrovascular permeability-surface area products (PA) were then calculated for L-[^{14}C]-leucine uptake into the brain as [38].

$$PA = -F \ln [1-(Q^*_{br}/c^*_{pf}T F)], \quad (1)$$

where Q^*_{br} represents the quantity of ^{14}C tracer per gram of brain tissue (corrected for residual intravascular activity), c^*_{pf} stands for the perfusate [^{14}C]-leucine concentration (dpm/ml), F indicates the cerebral perfusion fluid flow (ml s^{-1} g^{-1}), and T represents the net time of perfusion (corrected for the small delay required for perfusion fluid to reach the brain after the pump has been started; $T = T_{total}$- 3 s). Equation 1 assumes unidirectional uptake kinetics and corrects for flow limitations in brain transfer [22, 37, 41]. The ^{14}C content of the brain (dpm/g) was corrected for intravascular tracer by subtracting the product of the brain vascular volume (V_v) and the perfusate L-[^{14}C]-leucine concentration ($Q^*_{br} = Q^*_{tot} - V_v c^*_{pf}$) [41]. Cerebral vascular volume was determined from the brain-to-perfusate concentration ratio for [^{3}H]-inulin and equaled on average ~8 μl/g in the cerebral cortex. Cerebral perfusion fluid flow was quantitated using [^{3}H]-diazepam [41]. Influx (J_{in}) was calculated as $J_{in} = PA \times c_{pf}$, where c_{pf} represents the perfusate concen-

Table 1. Control values for cerebrovascular PA for L-[^{14}C]-leucine, cerebral perfusion fluid flow, and leucine extraction during perfusion of rat brain at differing rates with physiologic saline fluid that did not contain competing amino acids or drugs

Infusion rate (ml/min)	PA for L-[^{14}C]-leucine (ml s^{-1} $g^{-1} \times 10^2$)	Cerebral perfusion fluid flow (ml s^{-1} $g^{-1} \times 10^2$)	Brain extraction (%)
5	3.37±0.25*	5.40±0.49*	49±3*
10	3.90±0.12	12.14±0.53*	31±6*
15	3.92±0.21	16.21±1.28	21±0.9

Data represent mean values ± SE for 4–9 animals. All data were obtained for frontal cerebral cortex

* Differs significantly from the value at the next higher flow rate (P <0.05)

tration of L-leucine [37]. The perfusate leucine concentration was kept constant throughout the experiments.

The concentration dependence of L-leucine influx into the brain at physiologic L-leucine concentrations (<1 mM) and in the absence of competitors has been shown to follow Michaelis-Menten kinetics:

$$J_{in} = V_{max}c_{cap}/(K_m + c_{pf}) \quad (2)$$

where V_{max} is the maximal transport rate (μmol s^{-1} g^{-1}) of the saturable carrier and K_m is the half-saturation constant [37]. Since $PA = J_{in}/c_{cap}$, Eq. 2 can be simplified to:

$$PA_o = V_{max}/(K_m + c_{pf}), \quad (3)$$

where PA_o signifies uptake in the absence of competitors and c_{pf} represents the perfusate total (labeled plus unlabeled) L-leucine concentration. In the presence of competitors, PA is given as [28, 38]:

$$PA_i = V_{max}/[K_m(1 + \Sigma(c_i/K_i)) + c_{pf}], \quad (4)$$

where PA_i signifies uptake in the presence of competitors, c_i represents the perfusate concentration of each competitor, and K_i indicates the corresponding inhibition constant. K_i is defined as the competitor concentration that produces a 50% reduction in saturable influx when uptake is measured at tracer concentrations (L-[^{14}C]-leucine) and in the absence of other competitors [36]. K_i has been observed to approximate closely the K_m for BBB transport of several physiologic neutral amino acids [28, 38, 39].

Because no unlabeled L-leucine was added to the perfusate and because the specific activity of the tracer leucine was high, $c_{pf} << K_m$, and Eqs. 3 and 4 could be simplified and expressed as:

$$\%\ Inhibition = 100\,(PA_o\text{-}PA_i)/PA_o \approx 100c_i/(c_i + 2NK_i). \quad (5)$$

K_i values were obtained by fitting Eq. 5 to the data using weighted nonlinear least-squares regression. Inhibitor concentrations (c_i) in all analyses were limited such that the percentage of inhibition was ≤90% and, thus, contributions from the nonsaturable component of uptake could be ignored [37].

Statistical analysis. All data represent mean values (±SE) unless otherwise indicated. Statistical significance was determined using one-way analysis of variance and the Bonferroni multiple-comparison test [21]. Weighted regressions were performed on a DEC-10 computer using the MLAB program [37].

Results

Control values obtained for the BBB transport of L-[^{14}C]-leucine into rat cerebral cortex from physiologic saline are summarized in Table 1. In the absence of competing amino acids or drugs, the PA for L-[^{14}C]-leucine equalled 3.3–4.0 × 10^{-2} ml s^{-1} g^{-1} and varied minimally (<20%) with flow over the measured infusion range (5–15 ml/min). As PAs at the highest infusion rates were considered to be the most accurate (i.e., having lowest extractions and exhibiting the least potential for error due to unlabeled amino acid efflux from the brain [37, 38]), the 10- and 15-ml/min values were pooled and used as PA_o in all subsequent calculations (PA_o = 3.91 ± 0.11 × 10^{-2} ml s^{-1} g^{-1}, n = 11). Preliminary experiments verified that the brain intravascular volume and the cerebrovascular permeability to small nonelectrolytes (thiourea and sucrose) were normal in perfused animals (data not shown), suggesting that the BBB was intact and that no damage had occurred during perfusion. In most experiments, the heart was stopped just prior to perfusion so as to eliminate potential mixing and flow contributions from the systemic circulation [37, 41].

Figure 2 illustrates the relative ability of various antitumor amino acid drugs to inhibit L-[^{14}C]-leucine uptake across the BBB. The percentage of inhibition was expressed as $100 \times [(PA_o\text{–}PA_i)/PA_o]$, where PA_i is the BBB PA for L-[^{14}C]-leucine in the presence of drug competitor. Drugs differed widely in their ability to inhibit BBB L-[^{14}C]-leucine transport. The most potent, D,L-NAM, re-

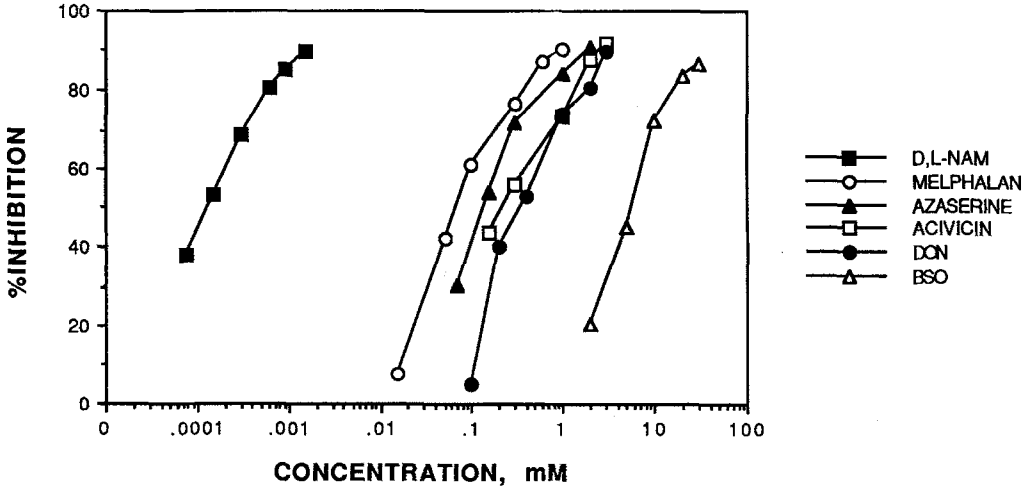


Fig. 2. Inhibition of brain L-[^{14}C]-leucine uptake by chemotherapeutic amino acid drugs. Points represent mean values for 3–6 animals. Inhibition is expressed as the percentage of reduction in the PA for L-[^{14}C]-leucine. The saline perfusate contained only tracer levels of L-leucine and no competing amino acids aside from the chemotherapeutic amino acid drug. SEs (not shown) were ≤15% of the respective means

Table 2. K_i values for drug inhibition of L-[^{14}]-leucine transport into rat brain

Amino acid drug	K_i (mM)[a]	Apparent affinity (mM^{-1})[b]
D,L-NAM	0.0002±0.00001	5000
Melphalan	0.09 ±0.01	11
Azaserine	0.16 ±0.01	6.3
Acivicin	0.29 ±0.02	3.4
L-DON	0.37 ±0.02	2.7
BSO	4.7 ±0.27	0.21

Data represent mean values ± SE for 17–18 animals

[a] Calculated by least-squares regression from the concentration dependence of inhibition as described in Materials and methods

[b] Calculated as the inverse of K_i. For comparison, the K_m and apparent affinity for L-phenylalanine, the natural amino acid showing the highest affinity for the carrier, are 0.01 mM and 100 mM^{-1}, respectively [22]

quired a concentration of <0.5 μM to produce ~50% inhibition, whereas the least potent, BSO, required a concentration of >4 mM to obtain the same effect.

K_i values were calculated by fitting Eq. 5 to the data and are listed along with the estimated affinities (affinity ≈ $1/K_i$ or K_m) in Table 2. The apparent affinity for D,L-NAM was >100-fold that of the other amino acid drugs and >10-fold that of any compound previously tested. In comparison, L-phenylalanine – the endogenous amino acid exhibiting the highest affinity for the carrier – displays a K_m value of ~10 μM [22], whereas L-leucine shows a value of ~25 μM [37, 39]. Most endogenous low-affinity amino acids exhibit K_i or K_m values of 0.1–10 mM (L-valine, L-threonine, L-cysteine, L-glutamine, L-asparagine, L-alanine, and L-serine) [38]. L-Naphthylalanine displays an estimated K_i value of ~3 μM [39].

There was some evidence for stereospecificity of inhibition, as L-DON produced a reduction in L-[^{14}C]-leucine uptake into the brain that was greater than that induced by D-DON at the same concentration. The PA for L-[^{14}C]-leucine in the presence of 1 mM L-DON was $1.03 \pm 0.07 \times 10^{-2}$ ml s^{-1} g^{-1} ($n = 4$), whereas that obtained in the presence of 1 mM D-DON was approximately 65% greater ($1.68 \pm 0.01 \times 10^{-2}$ ml s^{-1} g^{-1}, $n = 2$). Previous studies have shown that the large, neutral BBB amino acid transporter prefers L-amino acids but that this preference is not absolute, as many D-amino acids exhibit some level of measurable affinity [25, 37].

Discussion

Greig et al. [11] first demonstrated that the anticancer alkylating drug melphalan is transported into the brain by the large, neutral amino acid carrier of the BBB. The results of the present study confirm that finding and show that five additional anticancer amino acid drugs exhibit affinity for and may be transported into the brain by the same carrier as well. Most of the drugs that were tested displayed relatively low affinity [K_i = ~0.1–5 mM (100–5000 μM)] and would not be expected to gain ready access to the brain from the circulation via this mechanism. However, one agent, D,L-NAM, exhibited remarkably high affinity for the carrier (K_i = ~0.2 μM) that was >100-fold that of melphalan and >10-fold that of any compound previously tested. The results suggest that D,L-NAM may be a promising candidate for further evaluation of enhanced uptake by brain and activity against brain tumors.

The large, neutral amino acid carrier of the BBB, like other L system transporters [27, 31], is known to accept a wide variety of ligands exhibiting the alpha-amino acid functional group [38, 39]. Among amino acids, affinity tends to be highest for those displaying large, hydrophobic side chains, such as L-phenylalanine, L-tryptophan, and L-leucine (BBB K_m = 10–30 μM), and lowest for those showing small, hydrophilic or charged side groups, such as L-alanine, L-serine, L-glutamate, and L-lysine (BBB K_m >1 mM) [38, 39]. The marked affinity of D,L-NAM is likely due in part to the extreme hydrophobicity of the naphthalene side chain, whose estimated lipid solubility is ~10-fold that of the phenyl group of phenylalanine [18]. In fact, D,L-NAM was developed from a study of the transport properties of several markedly hydrophobic amino acids [43, 44]. However, factors other than hydrophobicity may also influence affinity. For example, addition of the nitrogen mustard group to L-phenylalanine to form melphalan decreases the transport affinity at the BBB by >10 times [11, 38], whereas addition of the same group to the 7 position of 2-amino-tetrahydro-2-naphthoic acid enhances the affinity by >10 times [14, 43]. Furthermore, movement of the nitrogen mustard from the 7 to the 5 position of 2-amino-tetrahydro-2-naphthoic acid reduces the affinity by >98% [14]. Thus, the presence and the precise placement of the nitrogen mustard group on the amino acid are also critical factors that merit further evaluation.

K_i values for the other amino acid drugs tested lay within the range of 0.09–4.7 mM, as expected from their lower estimated side-chain hydrophobicities [18]. The K_i value for melphalan (~0.09 mM) compares well with the K_m (0.15 mM) value reported by Greig et al. [11] for [^{14}C]-melphalan transport into the brain and approximates that reported by Begleiter and colleagues [1] (K_m = 0.08 mM) for system L transport of melphalan in L5178Y lymphoblasts. Such close agreement supports the use of K_i values to obtain initial estimates of transport affinity at the BBB.

Although the mechanisms of transport and the relative affinities of several antitumor amino acid drugs have previously been examined in cultured cell lines, this study is the first to examine the affinity of a number of such agents at the BBB in vivo. Chastain and Borchardt [4] evaluated the BBB transport of acivicin using a cultured brain-microvessel preparation and found that uptake was saturable, exhibiting a K_m value of 0.24 mM. However, in their in vitro preparation, acivicin transport was only slightly inhibited by L-leucine (<20% at 0.1–1 mM) and 2.75 mM acivicin did not reduce but instead enhanced L-[^{3}H]-leucine transfer. The latter paradoxical finding was attributed by the authors to the inhibition of endothelial cell protein synthesis by acivicin, as previous studies had shown that protein synthesis inhibitors increase leucine transport activity in cultured endothelial cells. Such an effect was unlikely in our in vivo experiments, as drug exposure and leucine transport were examined over only a very brief interval (~30 s). The transport kinetics of acivi-

cin and DON have been examined by Huber et al. [16] in several tumor cell lines and were found to be mediated by the L system carrier, displaying K_m values (0.15–0.9 mM) that were comparable with our K_i values of 0.29–0.37 mM. Interestingly, Huber et al. [16] found that the K_m value for L-DON was generally slightly greater than that for acivicin in most cell lines. We found the same trend at the BBB in vivo (Table 2). Haines et al. [14] previously reported a K_i value of ~0.2 μM for NAM in murine L1210 leukemic cells.

K_i values can be used to estimate the PA for BBB transfer in vivo using Eq. 3, provided that (1) V_{max} is known or can be approximated and (2) K_i is assumed to be equal to K_m. The latter assumption has been validated for several amino acids at the BBB [28, 38]. Assuming a V_{max} for NAM that is equal to that of melphalan (V_{max} = $\sim 0.9 \times 10^{-4}$ μmol s^{-1} g^{-1} [11]), we estimate that the BBB PA for NAM may be as high as $\sim 4 \times 10^{-1}$ ml s^{-1} g^{-1} for influx from saline in the absence of competitors. This value is >100-fold that measured by Greig et al. [11] for melphalan uptake under similar conditions. If the V_{max} for NAM is actually higher, lying more in the range of that for L-phenylalanine or L-leucine ($V_{max} = 7-10 \times 10^{-4}$ μmol s^{-1} g^{-1} [22, 37], the PA will be correspondingly greater as predicted by Eq. 3. Estimated permeabilities for the other five compounds are considerably lower, ranging between 0.2 and 60×10^{-4} ml s^{-1} g^{-1} depending on the assumed V_{max}. The calculated value for melphalan (PA = $\sim 10 \times 10^{-4}$ ml s^{-1} g^{-1}) agrees well with that measured by Greig et al. [11] using the in situ brain perfusion technique (PA = $9.6-10.8 \times 10^{-4}$ ml s^{-1} g^{-1}). Preliminary results obtained by Fekete et al. [6] suggest that brain BSO uptake from blood may be mediated by a saturable mechanism, as the PA for BSO decreased with increasing dose. Together, the results suggest that NAM may enter the brain much more readily than do the other amino acid drugs tested in the present study.

If brain uptake of antitumor amino acids is mediated by the cerebrovascular neutral amino acid transporter, then delivery to the brain would be predicted to be significantly influenced by competition from endogenous plasma amino acids [28, 38]. Competition is significant at the BBB because the carrier exhibits high affinity and is essentially saturated with plasma amino acids (as a group) at normal concentrations [38]. As a result, influx of a given amino acid depends both on the concentration of the amino acid and on the concentrations of all competitors. Competition may be beneficial and protect the brain from drugs such as acivicin, which exhibit dose-limiting CNS toxicity [24]. In fact, Williams et al. [45] recently used neutral amino acid infusions to limit brain acivicin uptake and to prevent acivicin toxicity in cats. However, competition may reduce the brain delivery of compounds that show little or no CNS toxicity, limiting their therapeutic effectiveness following systemic administration. This restrictive effect could be attenuated by lowering plasma amino acid concentrations either through the use of drugs (e.g., insulin) or through reduced dietary amino acid/protein intake.

Finally, the present results provide support for the idea that facilitated BBB transport may be a useful means of enhancing hydrophilic drug delivery to the brain [9]. An approach comparable with that described herein for the neutral amino acid carrier could be applied to other BBB carriers such as those for folates, monocarboxylic acids, and nucleic acid precursors [5, 40]. Similarly, the method need not be limited to anticancer agents but may also be useful for the enhancement of delivery to the brain of drugs for other disorders such as Alzheimer's disease and acquired immunodeficiency syndrome (AIDS).

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