Enhanced Delivery of 5-Iodo-2′-Deoxyuridine to the Brain Parenchyma

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5′-Ester derivatives of 5-iodo-2′-deoxyuridine (IDU) with varying degrees of lipophilicity were examined to evaluate the effectiveness of lipophilic ester prodrugs for enhanced and sustained delivery of IDU to the brain parenchyma. Approximately 1.0% (1.0 ± 0.19; n = 4) of the total radioactivity was found in the brain at 30 min following intravenous administration of the lipophilic benzoyl-5′-ester of ^{125}I-labeled IDU, whereas IDU per se yielded only 0.01% (0.01 ± 0.06; n = 4). Since the IDU 5′-esters generated significantly higher levels of IDU in the brain, an HPLC analysis of IDU in the presence of 5′-esters and the metabolite 5-iodouracil was developed to characterize IDU uptake in the brain. The drug was detected at levels of 6.6 and 9.5 μg/g of brain tissue at 3 hr following intravenous administration of valeryl and benzoyl IDU, respectively, at a dose level of 40 mg/kg IDU equivalent each. IDU, on the other hand, when injected at a similar dose level, produced concentration levels below 0.01 μg/g of brain tissue, which was too low to be detected accurately by the HPLC assay. These results suggest that the 5′-ester derivatives cross the blood–brain barrier effectively and generate significantly higher brain levels of the parent drug in the brain parenchyma. The regenerated hydrophilic drug because of its polarity is "locked in" the brain and is subsequently metabolized by pyrimidine phosphorylase to 5-iodouracil. A higher concentration of IDU was generated following administration of the benzoyl ester probably because the ester itself is slowly hydrolyzed by the brain cholinesterases, thereby competitively inhibiting the metabolism of IDU to 5-iodouracil by brain pyrimidine phosphorylase. 5′-Benzoyl IDU appears to be a promising bioreversible analogue which can provide enhanced and sustained delivery of IDU to the brain parenchyma.

KEY WORDS: 5′-ester prodrugs; 5-iodo-2′-deoxyuridine; distribution; brain uptake; metabolism; competitive inhibition; sustained brain levels.

INTRODUCTION

5-Iodo-2′-deoxyuridine (Idoxuridine; IDU), a thymidine analogue, is readily incorporated into viral DNA following its conversion to triphosphate by virus-induced thymidine kinase (1,2). This potent antiviral agent has found widespread application in the chemotherapy of ocular herpes simplex infections (3). Despite its proven efficacy, the drug has been of questionable effectiveness in the treatment of herpes encephalitis (4–6). Such ineffectiveness may be attributed mainly to its hydrophilic and hydrogen bonding properties, which restrict trancellular transport into the brain parenchyma because of the highly lipoidal nature of the blood–brain barrier (BBB) and the presence of tight junctions in the BBB (7,8). In addition to its restrictive BBB permeability, IDU concentration in the brain parenchyma is also reduced due to its rapid metabolism to 5-iodouracil (IU) by pyrimidine nucleoside phosphorylase (9). Several reports have shown that the chemical conversion of a water-soluble compound to a lipid-soluble molecule can result in a log-order increase in blood–brain barrier permeability (10,11). In an attempt to improve IDU delivery across lipoidal brain capillary endothelium, a series of 5′-ester prodrugs of IDU was synthesized. In an earlier report, we have discussed the synthesis, physicochemical properties, and protein binding of these ester prodrugs (12,13).

The present report is directed toward the delivery of these ester prodrugs to the brain parenchyma. The 5′-IDU ester, following intravenous administration, will be distributed rapidly to various tissues and, due to its increased lipophilicity, will also cross the blood–brain barrier readily and enter into the brain parenchyma. The esters will then be hydrolyzed by the cholinesterases in systemic circulation to the corresponding parent compound, which because of its hydrophilic character may undergo rapid clearance from the systemic circulation. On the other hand, the polarity of IDU should prevent its rapid efflux from the brain parenchyma following its regeneration from 5′ esters. The concentration of IDU in the brain will increase with time until it reaches a maximum value, which depends primarily on the relative rates of entry of the ester to the brain, the rate of IDU regeneration inside the brain parenchyma, and the rate of its breakdown by pyrimidine nucleoside phosphorylase.

Two ester derivatives (one each from the aliphatic group, i.e., IDU-5′-valeryl ester, and the aromatic group, i.e., IDU-5′-benzoyl ester) were chosen for the *in vivo* study. A solution of the 5′ derivative in 5% dimethyl sulfoxide and 95% phosphate-buffered saline (PBS) was administered through the jugular vein at 40 mg/kg IDU equivalent dose. At different time points the animals were sacrificed and the blood and brain samples were analyzed for unhydrolyzed IDU-5′ ester, regenerated IDU, and IU metabolite by the HPLC method described in a previous report (12).

MATERIALS AND METHODS

Adult male Sprague–Dawley rats weighing about 300–350 g were obtained from Harlan Sprague Dawley, Inc., Indianapolis, Indiana. Ester prodrugs of IDU were synthesized as described earlier (13) by esterification of the 5′-hydroxyl group of IDU using a 1.1 molar excess of appropriate acid chloride in a pyridine–dimethyl foramide solution (1:1).

Materials

[5-^{125}I]Iododeoxyuridine (0.5 mCi/ml) was obtained from New England Nuclear Research (Du Pont).

Methods

Preparation of Radiolabeled Compounds

^{125}I-Labeled ester prodrugs of IDU were synthesized in a similar manner as described in our earlier communication

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(13) except that IDU was mixed with 0.2 ml of [^{125}I]IDU (0.5 mCi/ml) before the reaction was initiated by the addition of acid chloride. Following the addition of a 10% molar excess of appropriate acid chloride, the reaction was continued and the final product was purified as described earlier (13). The specific activity of the ester prodrugs was found to be 0.16 μCi/mg for 5′-valeryl and 0.1 μCi/mg for 5′-benzoyl IDU. The purity of both esters was ascertained by TLC and HPLC assays.

Distribution of IDU and Its Ester Prodrugs

Distribution studies of IDU and its ester prodrugs were studied with radiolabeled compounds at two time points. One hundred microliters of a 10% (w/v) solution of radiolabeled compounds in 5% dimethyl sulfoxide/95% PBS was injected (bolus) through the jugular vein at a dose of 40 mg/kg to a group of rats weighing 300–350 g. At predetermined time points (15 and 30 min postadministration) the animals were sacrificed by decapitation. An aliquot (0.2 ml) of blood was taken and the whole organs such as spleen, kidneys, lungs, liver, and brain were removed as quickly as possible. Portions of these organs were counted for radioactivity following a thorough wash in cold 0.9% NaCl solution. Total radioactivity in the organs were determined by multiplying the total counts per unit weight by the total weight of the tissues. The total radioactivity in the blood was calculated assuming that the blood volume in an average rat of 300 g is 16.25 ml.

Brain Uptake Studies of IDU/IDU 5′ Ester in Rat

The rats were anesthetized with an intraperitoneal injection of a 100 mg/ml mixture of ketamine–xylazine (10:1) at a dose of 1 μl/g of body weight. A 10% (w/v) solution in 5% dimethyl sulfoxide/95% PBS of IDU and its ester derivatives (5′-valeryl and 5′-benzoyl esters) was injected (bolus) through the jugular vein at a dose of 40 mg/kg IDU equivalent to a group of rats. At different time points, 0.5 ml of blood was withdrawn and centrifuged at 6000 rpm for 20 min. An aliquot of the plasma (0.2 ml) was added immediately to 3 vol of methanol. The animals were sacrificed by decapitation and the brains were collected. The brains were washed thoroughly with buffer, blotted with filter paper, and weighed. The tissue samples were then homogenized with 0.5 ml of buffer. Three milliliters of methanol was added and the mixture was rehomogenized thoroughly. The supernatant obtained by centrifugation was subsequently filtered and the filtrate was analyzed by HPLC. Quantitation was done by using a recovery standard curve from experiments where known amounts of the substrates were added to either brain homogenate or blood and then treated in the same manner.

RESULTS AND DISCUSSION

For *in vivo* studies, 5′-benzoyl and 5′-valeryl derivatives were selected mainly because of their increased lipophilicity and high degree of specificity for brain cholinesterases compared to other ester derivatives (12). The results from the distribution studies with radiolabeled compounds are presented in Table I. The data clearly demonstrate that the modification of IDU with a lipophilic group enhances IDU transport through the blood–brain barrier. Both 5′-valeryl and 5′-benzoyl derivatives exhibit an enhanced brain uptake following intravenous administration (Table I). Almost 3.5- to 5-fold higher radioactivity is observed with the ester derivatives compared to IDU within the first 15 min, and the ratio increased further, to 8- to 10-fold, respectively, during the next 15 min. With IDU, a very small amount (0.13%) of total radioactivity is found in the first 15 min, which decreases further, to 0.098%, over the next 15 min. Such a decrease in radioactivity, although insignificant, could be due to its rapid metabolism by brain pyrimidine nucleoside phosphorylase to 5-iodouracil followed by its elimination (8). The higher percentage of total radioactivity in both blood and liver (30 min) might be due to the presence of 5-iodouracil or free iodine. In comparison, maximum brain uptake is observed with benzoyl ester, despite the fact both benzoyl and valeryl esters have similar octanol/water partition coefficients. This could be due to the differences in plasma concentration resulting from their different elimination rates from systemic circulation and also their different susceptibilities toward esterases (12). During *in vivo* stability studies of the ester derivatives in plasma, the benzoyl ester was found to be about 30 times more stable than the valeryl ester (13). However, unlike IDU, the radioactivity in the brain increases (1.5 times) with time (at 30 min), which suggests that the unhydrolyzed esters are still available in systemic circulation for brain uptake. Chemical modification not only enhances IDU penetration through the blood–brain barrier, but also might decrease the overall drug clearance from the systemic circulation. The total radioactivity found in the brain may not necessarily be

Table I. Percentage of Radioactivity[a] in Different Organs at 15 and 30 min Following Intravenous Administration of IDU, Benzoyl-IDU, and Valeryl-IDU Through Rat Jugular Vein

	IDU		Benzoyl IDU		Valeryl IDU	
Organ	15 min	30 min	15 min	30 min	15 min	30 min
Brain	0.130 ± 0.08	0.098 ± 0.06	0.67 ± 0.12	0.99 ± 0.19	0.45 ± 0.12	0.72 ± 0.15
Blood	10.40 ± 2.39	12.90 ± 4.52	15.87 ± 1.89	19.65 ± 3.12	13.92 ± 2.14	10.58 ± 1.97
Liver	7.96 ± 1.87	7.40 ± 3.22	13.92 ± 4.19	9.84 ± 4.54	9.64 ± 2.26	6.00 ± 2.15
Spleen	0.21 ± 0.11	0.27 ± 0.17	0.42 ± 0.28	0.39 ± 0.21	0.31 ± 0.17	0.3 ± 0.11
Kidney	2.52 ± 1.18	2.00 ± 1.22	3.30 ± 2.85	4.60 ± 3.53	3.30 ± 2.89	1.62 ± 1.11
Lungs	0.96 ± 0.25	1.00 ± 0.55	2.90 ± 0.41	2.20 ± 0.41	2.70 ± 0.75	2.56 ± 0.89

[a] Mean ± SE; $n = 4$.

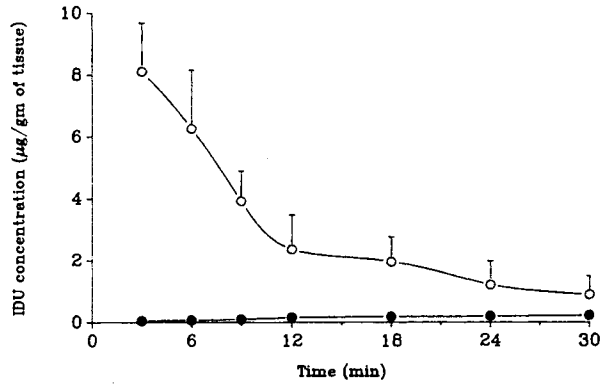

Fig. 1. The concentration of IDU in plasma (○——○) and in brain (●——●) following intrajugular administration of 40 mg/kg IDU (mean ± SE; n = 4).

due only to the intact ester derivatives; it could also be derived from regenerated parent drug and 5-iodouracil metabolite. In order to determine the parent IDU concentration in the brain parenchyma as a function of time, further studies were carried out using HPLC analysis.

Figures 1 and 2 illustrate the appearance of IDU in brain following intravenous administration through the jugular vein of both IDU and valeryl IDU. This study revealed that valeryl IDU rapidly and efficiently penetrated the BBB and was readily hydrolyzed to generate IDU in brain. IDU, on the other hand, was found to produce negligible brain concentrations (<0.01 μg/g of tissue), confirming the poor BBB permeation of the molecule. Spiking brain homogenate with IDU led to an increase in the height of observed peak, suggesting that the sample peak indeed corresponds to IDU. Further, time-dependent uptake studies were carried out with two 5′-ester derivatives (valeryl and benzoyl).

Plots of IDU concentrations remaining in plasma and brain following intravenous administration of IDU through jugular vein have been illustrated in Fig. 1. No IDU could be detected accurately in the brain within the sensitivity limits of the procedure (<0.01 μg/g of tissue). This study was carried out for 30 min because the IDU concentration in plasma was relatively high at early time points and then quickly diminished during that time period, with a terminal half-life of 6.35 min. On the contrary, the concentration of IDU increased steadily in the brain, reaching a maximum concentration at 30 min, following the administration of valeryl IDU (Fig. 2). The brain IDU concentration, then, exhibited an elimination phase, which might be due to IDU efflux or metabolism or both. The formation of 5-iodouracil and a steady increase in its concentration following valeryl IDU administration suggest that a decrease in IDU concentration was due at least in part to further metabolism of IDU to 5-iodouracil (Fig. 2). However, the apparent first-order rate constant of the declining IDU phase was found to be 1.56×10^{-2} min^{-1} (Fig. 2). The obtained IDU half-life of 44.5 min from the brain parenchyma indicates that IDU elimination from the brain is about seven times slower compared to the blood, probably because of its high polarity, diminishing efflux from the brain.

Benzoyl IDU, because of the presence of sterically hindered group, undergoes hydrolysis very slowly by cholinesterases present in the brain. Its *in vitro* half-life in plasma was found to be 30 times more than valeryl IDU (13). Despite its slow hydrolysis, it was rapidly eliminated from the systemic circulation. Within 30–40 min, most of the benzoyl IDU was eliminated from the circulation following its intravenous administration (Fig. 3). Only 30–35% of its initial concentration was found to be hydrolyzed to IDU by brain enzymes during a 3-hr period (Fig. 3). The concentration of IDU, however, remained fairly constant within the range of 7–10 μg/g of brain tissue (much higher than the 1.0 μg/g needed to cause 50% inhibition of HSV-1 replication) without any detectable 5-iodouracil (Fig. 3). This observation could be explained from our results obtained with *in vitro* studies (9). In those studies, we found that benzoyl ester was slowly metabolized and, in its presence, the pyrimidine phosphorylase-mediated metabolism of IDU is completely inhibited. Valeryl IDU was also capable of producing such inhibition but it failed to generate a steady level of IDU because it was very unstable due to rapid hydrolysis by the brain esterases. In systemic circulation, the ester can be eliminated by excretion in the urine unchanged or it can undergo metabolism, forming IDU. The generated IDU could be either excreted directly from the systemic circulation or further cleaved to 5-iodouracil, which would subsequently undergo renal excretion. In con-

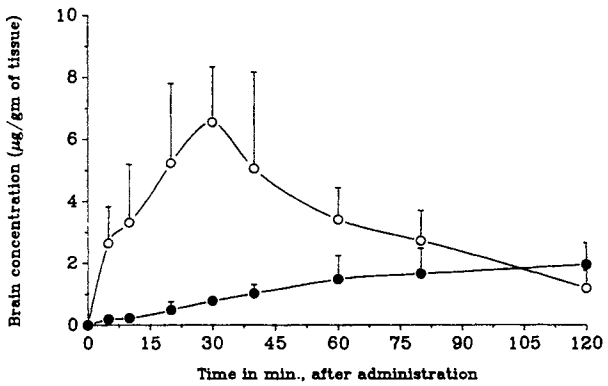

Fig. 2. Concentration of 5-iodouracil (●——●) and IDU (○——○) in brain following administration of valeryl-IDU through jugular vein at a dose of 40 mg/kg IDU equivalent (mean ± SE; n = 4).

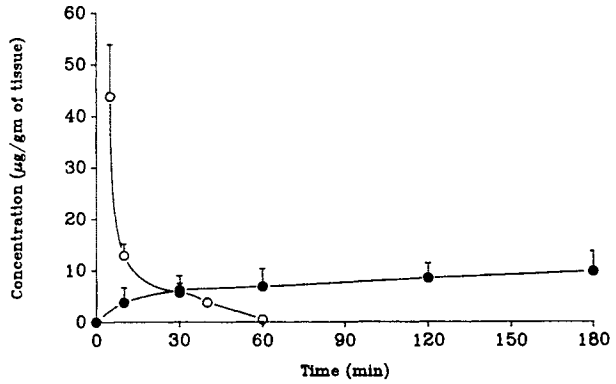

Fig. 3. Concentration of IDU in brain (●——●) and benzoyl IDU in plasma (○——○) following administration of benzoyl-IDU through the jugular vein at a dose of 40 mg/kg IDU equivalent (mean ± SE; n = 4).

clusion, the lipophilicity of the 5′-ester derivatives appears to enhance IDU concentration in the brain. Following hydrolysis by cholinesterases in the brain tissue, the corresponding hydrophilic parent compound IDU is formed. The parent antiviral agent is essentially "locked in" the brain and probably undergoes further metabolism to 5-iodouracil. However, as long as unhydrolyzed IDU ester remains present, it might prevent the hydrolysis of IDU to 5-iodouracil. Benzoyl-5′-IDU ester is capable of generating a steady 7–10 μg/g brain tissue concentration of IDU for at least 3 hr. Long-term studies will be conducted to evaluate fully the clinical potential of benzoyl-5′-IDU in HSV-1 encephalopathies.

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