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Research Article

In Vitro and *in Vivo* Investigations of Dihydropyridine-Based Chemical Delivery Systems for Anticonvulsants

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A dihydropyridine-based chemical delivery system (CDS), intended to improve drug delivery to the brain, was investigated with a series of analogues of the anticonvulsant stiripentol. *In vitro* experiments demonstrated that the rates of hydrolysis of the corresponding pyridinium conjugates were influenced markedly by small changes in the structure of the drug moiety to be released. Thus, allylic esters were hydrolyzed rapidly to drug in all aqueous media, while the analogous saturated esters and an allylic amide derivative were almost totally stable. The mechanism of hydrolysis, which is particular to this series of CDS conjugates, appeared to occur via ionization to a resonance-stabilized carbocation intermediate. The same CDS compounds were investigated *in vivo* and compared to the corresponding drugs after intravenous administration. Only those CDS compounds that were found to hydrolyze *in vitro* released appreciable amounts of drug *in vivo*. Prolonged release of the drug from the CDS in the brain could be demonstrated for these compounds, but the gain in the ratio of brain-to-plasma AUC when the CDS was administered depended on the innate distribution characteristics of the drug. Thus, the drug D3, which had a high brain-to-plasma AUC ratio, did not show an improvement in this ratio when administered as CDS3. In contrast, stiripentol with a poor brain-to-plasma AUC ratio showed a two- to threefold increase in this ratio when administered as a CDS. These investigations highlight the need for a thorough understanding of the mechanism of drug release and the importance of the pharmacokinetic properties of the drug in designing a carrier system for delivery of drugs to the brain.

KEY WORDS: stiripentol; anticonvulsants; pharmacokinetics; drug targeting; *in vitro/in vivo* correlation; chemical delivery system.

INTRODUCTION

Many drugs whose desired site of action is in the central nervous system have dose-limiting systemic side effects (1). Some of these drugs do not penetrate the blood–brain barrier easily and require high plasma concentrations in order to generate effective concentrations in the brain (2). Others have specific, undesirable pharmacological effects at relatively low concentrations in the systemic circulation (3).

One approach which has been advocated to overcome this problem of selective delivery of drugs to the brain is the chemical delivery system (CDS) developed by Bodor (4). According to the theory of this approach (5), the drug is conjugated, through an ester or amide linkage, to a dihydropyridine ring system. The heterocyclic moiety is oxidized readily to the corresponding pyridinium species in a process which may occur throughout the body and is possibly enzyme mediated. The pyridinium conjugate is eliminated rapidly from the systemic circulation due to its positive charge. In the brain, however, the charged pyridinium conjugate is "locked in" and so can be hydrolyzed slowly to release drug selectively into the brain at a sustained concentration. The CDS approach has now been investigated for a number of different drugs, with varying degrees of success (6–10).

Previously, in studying the usefulness of this CDS approach, a single drug has been selected and different dihydropyridine carriers have been attached in an effort to optimize delivery to the brain. This is a useful approach for studying the delivery of an existing drug to the brain, but it provides a little information as to the characteristics which make one drug a better candidate than another for CDS-mediated delivery to the brain. The influence of the drug moiety on the potential success of the CDS approach has not been systematically investigated in these studies.

In the present study, a single dihydropyridine carrier was used to form a CDS with each of a series of structurally related anticonvulsants based on stiripentol (STP), a drug currently undergoing clinical trials in Europe and the United States (11). The oxidation and hydrolysis reactions of these

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CDS compounds were investigated in different biological media *in vitro*. The results of these studies were compared to the abilities of the CDS compounds to deliver the drug selectively to the brain *in vivo*. The unconjugated drugs themselves served as references against which the distribution and bioavailability of the carrier-delivered drug could be measured.

The objectives of the study were to investigate the nature of the relationship between rates of oxidation and hydrolysis *in vitro* and the performance of this series of CDS conjugates *in vivo*. In particular, we wished to study the role of the drug moiety itself in determining the chemical delivery of native drug.

MATERIALS AND METHODS

The five CDS compounds (CDS0, CDS1, CDS2, CDS3, and CDS4), their corresponding quaternary pyridinium species (Q0, Q1, Q2, Q3, and Q4), and the drugs to be released from the CDS (STP, D1, D2, D3 and D4) (Fig. 1) were kindly supplied by Biocodex Laboratories, Compiègne, France. Of the CDS compounds, CDS0 and CDS2 were stable on storage at −20°C. CDS compounds 1, 3, and 4 were received as a mixture of regioisomeric dihydropyridines and other unidentified compounds, as determined by HPLC with diode array detection and by LC-MS. In each case, purification of the desired 1,4-regioisomer was achieved by chromatography on a neutral alumina column (containing less than 1% water) using dry ether as eluent. The major (and least polar) 1,4-isomer was eluted first and was collected in a light-protected flask cooled in liquid nitrogen. The solvent was removed under reduced pressure and the resulting pale yellow oil was maintained under high vacuum until yellow rods formed. The purity of this material (typically >94%) was assessed by HPLC and the product stored at −70°C until used. Upon exposure of the crystalline material to air, a yellow oil re-formed and darkened rapidly. The identity of the 1,4-regioisomer was confirmed by UV spectroscopy (λ_{max} = 356 nm, with no band at 260 nm) and by NMR spectroscopy. All other chemicals were of reagent or HPLC grade as appropriate. The rats used were adult, male Sprague–Dawley, weighing 220 to 250 g.

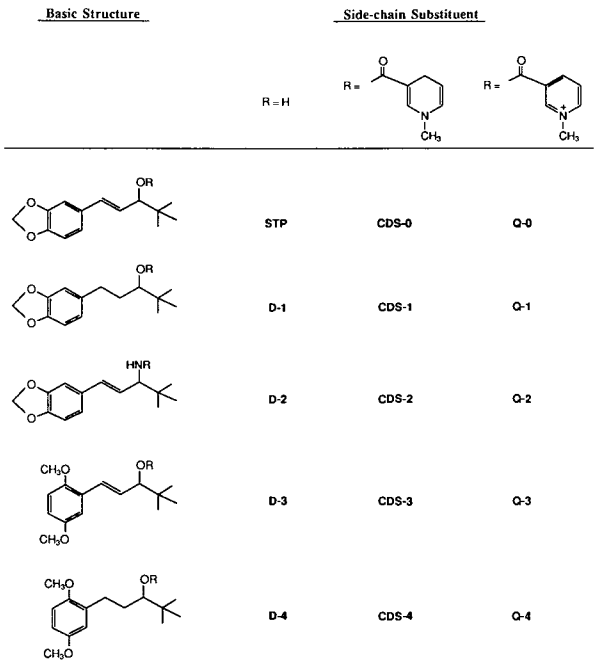

Fig. 1. Structures and nomenclature of CDS compounds and their corresponding quaternary and drug analogues.

Assays

All assays of CDS, quaternary pyridinium salts, and drug analogues were performed by reverse-phase HPLC using a Waters or Beckman C18 column and precolumn. The mobile phase was a mixture of acetonitrile and water, the proportion of the organic component varying from 60 to 70% and the flow rate from 1 to 1.5 ml/min as appropriate. For assay of the quaternary compounds and for CDS2 and D2, the aqueous component consisted of a 20 m*M* phosphate buffer, pH 7. Tetrabutylammonium dihydrogen phosphate (3 m*M*) and sodium 1-octanesulfonic acid (10 m*M*) were included in the mobile phase for assay of the quaternary compounds. The wavelengths for detection were 264, 288, 268, 254, and 290 nm in order of the CDS number.

In Vitro Studies

Each of the CDS compounds was incubated for 4 hr at 37°C in buffer (pH 7.4), freshly prepared rat brain and liver homogenates (4% in buffer), and whole rat blood (EDTA used as anticoagulant). Samples of 100 µl were withdrawn from the incubation medium at various time intervals and added to 400 µl of acetonitrile. The resulting precipitate was removed by centrifugation (Model 235B microcentrifuge, Fisher Scientific, USA) and the supernatant was injected directly into the HPLC system and assayed for the CDS and for the corresponding quaternary and drug. Experiments were performed in duplicate and average values used in calculating rates of disappearance. Similar incubations were performed with the quaternary compounds, when the medium was assayed for the remaining quaternary and for the corresponding native drug.

In addition, the mechanism of the hydrolysis reaction was investigated by incubating Q0 and Q3 in unbuffered $H_2{}^{18}O$ (Aldrich Co., 97 atom% excess ^{18}O). The pH of the solution was 7.0. The mixture was allowed to react for 10 min at 37°C and extracted into ethyl acetate. The organic extracts were separated and evaporated to dryness under nitrogen. The drug in each case was derivatized with 50 µl *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA, Pierce Chemical Co., Rockford, IL) and analyzed by gas chromatography–mass spectrometry (12).

In Vivo Studies

The day prior to the experiment, rats were anesthetized with an intraperitoneal dose of ketamine and cannulated in the jugular vein. The cannula was tested for patency immediately before and after dosing.

Dosing solutions of the CDS compounds and of the corresponding drug were prepared in PEG 400 at a concentration to allow a dosing volume of 2 ml/kg. All dosing solutions were assayed for stability and content before and after use.

CDS2 proved to be unstable in PEG 400 and therefore CDS2 and D2 were administered in DMSO at a dosing volume of 0.5 ml/kg. The CDS compounds were administered at doses of 20 to 60 mg/kg (107 to 162 μmol/kg) to each of five or six animals. The corresponding drugs were administered at doses of 6 mg/kg (23.6 to 25.8 μmol/kg) to another five or six animals. Doses were chosen to provide similar plasma concentrations of the drug following administration of the CDS or of the drug itself, based on preliminary experiments. In addition, CDS0 was administered at a dose of 100 mg/kg (272 μmol/kg) and STP at 15 mg/kg (64 μmol/kg). The doses were administered over approximately 1 min and the cannulae were flushed with saline immediately thereafter. At 5- to 20-min intervals after dosing, blood samples were withdrawn from the jugular cannulae. The initial portion which was contaminated with saline was discarded. The anticoagulant used was EDTA. Blood samples were centrifuged for 10 min and the resulting specimens of plasma were frozen at −20°C for subsequent analysis.

At various times after dosing, the animals were decapitated. The brain was removed, rinsed clean of blood, and sliced sagitally. One-half of the brain was weighed and homogenized with 4 vol of methanol. The homogenate was spun in a microcentrifuge for 2 min and 100 μl of the supernatant injected directly onto the HPLC. The time from decapitation to assay typically was less than 10 min.

Plasma was assayed by adding 100 μl of each sample to 200 μl of acetonitrile and 10 μl of the internal standard solution (*N*-propyl-*m*-bromicinnanamide, 10 μg/ml). The mixture was vortexed, then spun in a microcentrifuge, and 100 μl of the supernatant was injected into the HPLC. Brain and plasma were assayed for the CDS and for the corresponding quaternary species and drug. Standards were prepared in blank brain homogenate, prepared as for the samples, or in blank rat plasma. The assays had coefficients of variation of 5 to 10%, with lower limits of detection of approximately 1 μ*M* for the CDS and Q compounds and for D1 and 0.1 μ*M* for the other drugs.

Data Analysis

First-order rate constants were calculated for the disappearance of the substrates (CDS or quaternary species) *in vitro* and for the appearance of the corresponding products (quaternary species or native drug). These rate constants were used, where possible, to calculate half-lives for the processes of oxidation and hydrolysis.

For each administration of drug or CDS, data from all rats were pooled to provide brain concentrations from single animals and average plasma concentrations (n = 2 to 6) for the different chemical species at various times after administration. A bi- or monoexponential model, as appropriate, was fitted to the pooled brain and plasma data. The terminal rate constant was used to calculate a half-life for the different species in brain and plasma. The area under the concentration–time curve (AUC) for CDS and drug in brain and plasma was calculated using the trapezoidal rule, extrapolating to infinity. The fraction of the total AUC beyond the last sample time was less than 30% in all cases. The ratio of AUC in brain to that in plasma was calculated for each drug when administered as the CDS or as the native drug itself. Also, the AUC for the drug in plasma was used to calculate the fraction of the dose of each CDS which was converted to the drug in the body. This fraction is a measure of the bioavailability of the drug in the systemic circulation when administered as the CDS.

RESULTS

The results for each CDS, quaternary species, and corresponding native drug are described separately in this section. Comparisons among the CDS compounds and among the different native drugs are made in the Discussion.

CDS0, Q0, STP

In Vitro. CDS0 decomposed relatively slowly in buffer (Table I), and only 13% could be accounted for as STP after 4 hr at 37°C. Q0 could not be detected as an intermediate because it was very unstable in buffer (half-life = 0.3 min) and underwent quantitative hydrolysis to STP. CDS0 disappeared more rapidly in liver and brain homogenates and afforded a higher yield of STP than was the case in buffer, indicating that the oxidation reaction may have an enzymatic component in these tissues.

Mass spectral analysis of the product of hydrolysis of Q0 in $H_2{}^{18}O$ revealed that the STP had incorporated one atom of ^{18}O, which was present at the same level of enrichment as the medium. Thus, the hydrolysis of Q0 to STP and trigenolline appears to proceed via a resonance-stabilized carbocation intermediate (Fig. 2), rather than by nucleophilic attack at the ester carbonyl group. A similar phenomenon has been observed with the hydrolysis of other allylic esters (13).

Table I. *In Vitro* Disappearance Half-Lives and Percentage Yield of Quaternary or Drug for Different CDS Compounds in Various Media[a]

	CDS0			CDS1			CDS2			CDS3			CDS4		
		Yield			Yield			Yield			Yield			Yield	
Medium	$t_{1/2}$ (min)	Q0	STP	$t_{1/2}$ (min)	Q1	D1	$t_{1/2}$ (min)	Q2	D2	$t_{1/2}$ (min)	Q3	D3	$t_{1/2}$ (min)	Q4	D4
Buffer	234	0	12.7	300	20.2	0	7.8	34.2	0	192	0	25.8	210	46.8	0
Blood	624	0	13.9	474	32.7	0	18.4	50.4	0	318	0	13.2	786	33.9	0
Liver	48	0	29.2	72	89.7	0	7.0	75.2	0	84	0	42.7	48	57.4	0
Brain	120	0	23.0	120	62.9	0	7.8	70.7	0	96	0	29.5	108	72.5	0

[a] Yield, after 4 hr of incubation, expressed as a percentage of the quantity of CDS originally present. Buffer pH 7.4, fresh rat blood, and liver and brain homogenates (4% in buffer), all at 37°C.

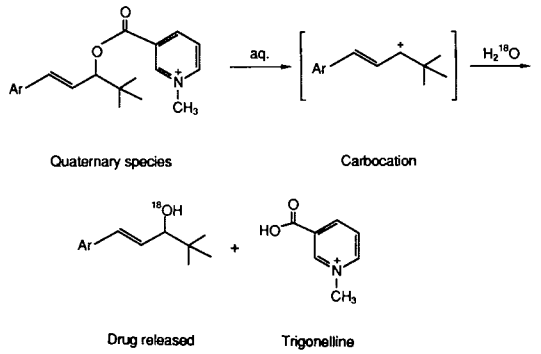

Fig. 2. Mechanism of hydrolysis of allylic ester quaternary compounds via carbocation to release drug and trigenolline.

In Vivo. CDS0 penetrated the brain readily and yielded a brain-to-plasma AUC ratio of 1.04 at 40 mg/kg and 2.35 at 100 mg/kg. Its half-life in plasma also varied with dose (Table II). The corresponding quaternary Q0 could not be detected in either brain or plasma following administration of CDS0, since it appears that this intermediate hydrolyzes almost instantaneously *in vivo* to release STP. Figures 3 and 4 demonstrate that CDS0 provided a sustained concentration of STP in both brain and plasma. The half-life of STP in brain and plasma was longer when CDS0 was administered than when STP itself was administered (Table III). Also, the ratio of brain AUC to plasma AUC for STP was greater following administration of CDS0. The percentage of the dose of CDS0 which was available in the systemic circulation as STP was 13% (100 mg/kg or 272 μmol/kg) or 20% (40 mg/kg or 109 μmol/kg) (Table III).

The doses of STP were chosen to approximately match STP plasma concentrations following administration of CDS0. The dose-normalized AUC values for STP in both plasma and brain were equal (data not shown) at the two doses of STP and the clearance and half-life values in Table III show no signs of nonlinearity in the kinetics or distribution of this drug over the concentration range studied.

CDS1, Q1, D1

In Vitro. CDS1 disappeared slowly in buffer and blood,

Table II. Pharmacokinetic and Brain Distribution Parameters for CDS Compounds and Their Quaternary Metabolites (Q)

		CDS			Q,
CDS	Dose (μmol/kg)	$t_{1/2}$ (plasma) (min)	$t_{1/2}$ (brain) (min)	Brain/plasma AUC ratio	$t_{1/2}$ (plasma) (min)[a]
CDS0	109	30.3	36.1	1.04	—
CDS0	272	69.4	29.4	2.35	—
CDS1	112	66.0	46.5	1.36	58.7
CDS2	113	79.7	11.8	0.96	69.5
CDS3	162	38.1	24.4	1.94	—
CDS4	107	31.6	6.24	1.07	52.4

[a] Q0 and Q3 could not be quantified in plasma or brain due to their rapid hydrolysis to release STP and D3, respectively. Although Q1, Q2, and Q4 could be measured in brain, the concentrations did not decline during the sampling period and a half-life could not be determined. Therefore only plasma half-life values are shown.

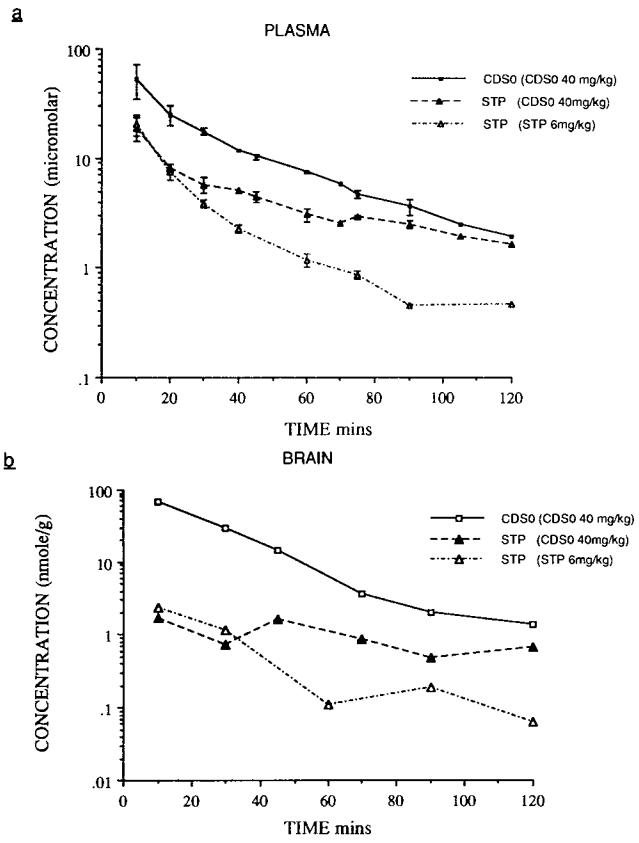

Fig. 3. Concentration–time profiles in plasma (a) and brain (b) of CDS0 and STP after administration either of 40 mg/kg (109 μmol/kg) of CDS0 or of 6 mg/kg (25.6 μmol/kg) of STP. Plasma data are means, with error bars for standard deviations, for plasma samples at that sampling time.

but more rapidly and to give a higher yield of Q1 in brain and liver homogenates (Table I). Q1 was stable in all media examined and no D1 was detected in incubations with either CDS1 or Q1.

In Vivo. CDS1 penetrated the brain readily and generated a high concentration of the quaternary Q1 (>20 nmol/g) which remained relatively constant during the period of sampling (Fig. 5b). In plasma, the concentration of Q1 declined in parallel to that of CDS1. No D1 could be detected in the brain and only a trace quantity of D1 could be detected in early plasma samples (Fig. 5a). The percentage of the dose of CDS1 converted to D1 in the systemic circulation was estimated to be 3.3% (Table III).

When administered as such, D1 was detectable in only the earliest brain sample, although detectable levels were present in plasma throughout the sampling period (data not shown; pharmacokinetic parameters in Table III).

CDS2, Q2, D2

In Vitro. CDS2 underwent rapid oxidation in all media studied ($t_{1/2}$ < 20 min), with a similar disappearance half-life in buffer and tissue homogenates. The yield of Q2 in the biological media was higher than that in buffer (Table I). Q2 was stable in all media and no hydrolysis to D2 could be detected.

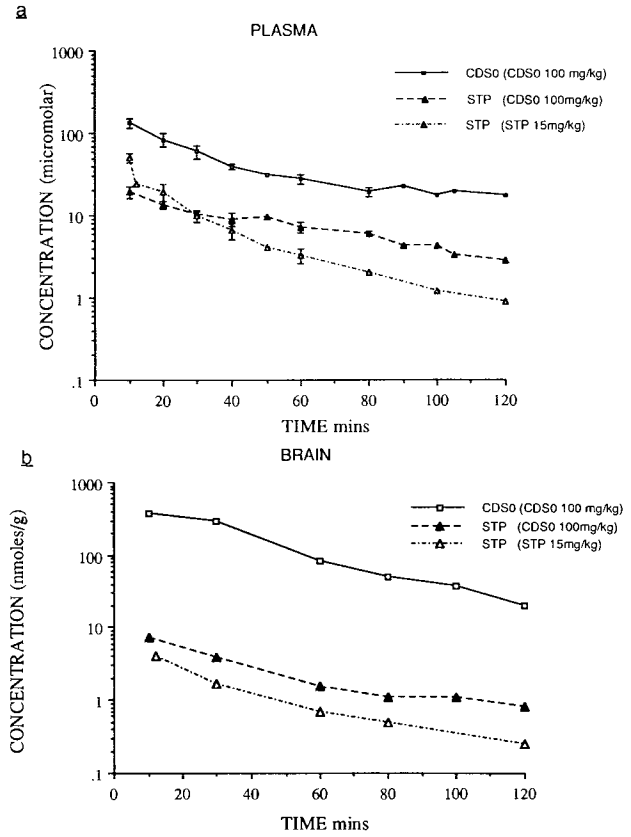

Fig. 4. Concentration–time profiles in plasma (a) and brain (b) of CDS0 and STP after administration either of 100 mg/kg (272 μmol/kg) of CDS0 or of 15 mg/kg (64 μmol/kg) of STP. Plasma data are means, with error bars for standard deviations, for plasma samples at that sampling time.

In Vivo. CDS2 could be measured in plasma throughout the sampling period but was not detectable in brain after 1 hr (Fig. 6). The decline of Q2 concentrations in plasma was in parallel to the decline of CDS2. In the brain, Q2 rapidly attained high levels (>100 nmol/g) which did not fall during the period of observation. D2 could not be detected in the brain after administration of CDS2 and was detectable in only a few plasma samples. Nearly 4% of the dose of CDS2 was converted to D2 in the systemic circulation (Table III), as estimated from these sparse data.

When D2 itself was administered, the brain-to-plasma AUC ratio was nearly 3 (data not shown; pharmacokinetic parameters in Table III).

CDS3, Q3, D3

In Vitro. CDS3 behaved in a similar fashion to CDS0, disappearing more rapidly and with a higher yield of D3 in liver and brain homogenates than in buffer or blood (Table I). Q3 disappeared rapidly from buffer (half-life = 1.2 min) and was converted quantitatively to D3. The mechanism of the hydrolysis reaction was the same as that for Q0 (Fig. 2), in that the D3 released from CDS3 in a medium enriched with $H_2{}^{18}O$ was labeled with one atom of ^{18}O.

In Vivo. CDS3 penetrated the brain readily, with a brain-to-plasma AUC ratio of nearly 2 (Table II). As for Q0, the quaternary Q3 was not detectable due to its instability. When CDS3 was administered, sustained levels of D3 were obtained in both brain and plasma (Fig. 7). The brain-to-plasma AUC ratio was greater than unity but only slightly greater than the corresponding ratio when the drug itself was administered (Table III). Approximately 10% of the dose of CDS3 was available in the body as D3, based on plasma data.

When administered as such D3 was present in the brain at similar concentrations to those in plasma, but levels of D3 declined more rapidly in the former tissue (Fig. 7).

CDS4, Q4, D4

In Vitro. CDS4 behaved in a similar fashion to CDS1, disappearing more rapidly and with a higher yield of Q4 in liver and brain homogenates than in buffer or blood (Table I). Q4 was stable in all media studied and no D4 was detectable.

In Vivo. CDS4 was eliminated very rapidly from the body and was not detectable in either brain or plasma 1 hr after administration. The quaternary Q4 declined in parallel with CDS4 in plasma, but persistently high levels were observed in the brain (Fig. 8). When CDS4 was administered, D4 could be detected at very low concentrations in the plasma and not at all in the brain. It was not possible to determine a half-life for the D4 in plasma that was released from CDS4 due to the variability in the concentration values observed. The fraction of the dose of CDS4 which was available in the plasma as D4 was estimated to be 2.3%.

When D4 was administered, it was also eliminated rapidly from brain and plasma, concentrations falling below detection limits after 90 min (data not shown; pharmacokinetic parameters in Table III). The brain-to-plasma AUC ratio was approximately 0.8.

DISCUSSION

A series of five CDS compounds has been synthesized and evaluated as delivery systems for their respective native drugs. It appears that each different drug moiety endows the CDS with unique properties, and it is instructive to make some comparisons among the series with regard to their *in vitro* and *in vivo* behavior. Such information is important in optimizing the selection of drugs for use with a CDS carrier.

A comparison of CDS0 with CDS1 shows that a minor modification to the drug structure (saturation of the side-chain C=C double bond) can have a dramatic effect on the rate of hydrolysis of the quaternary compound *in vitro* and on the ability of the CDS to release the native drug *in vivo*. Thus, 10 to 30% of CDS0 was converted to STP *in vitro*, presumably *via* the readily hydrolyzed Q0. In contrast, CDS1, the saturated analogue of CDS0, yielded none of the corresponding drug, D1, *in vitro* and very little *in vivo*. Likewise, comparison of CDS3 and CDS4, a pair analogous to CDS0 and CDS1, shows that only the unsaturated compound released the native drug, presumably *via* the short-lived Q3.

Comparison of CDS0 and CDS2 showed that replacement of the ester moiety in the former by an amide linkage had a major effect both *in vitro* and *in vivo*. Thus, CDS2

Table III. Pharmacokinetic and Brain Distribution Parameters for Drugs After Administration of Native Drug (D) or of CDS Compound

Dose (μmol/kg)	$t_{1/2}$ (plasma) (min)	$t_{1/2}$ (brain) (min)	Brain/plasma AUC ratio	Clearance (ml/min/kg)	Percentage of CDS as D
STP (25.6)	26.8	25.6	0.111	36.9	
STP (64.0)	24.5	27.9	0.109	38.6	
CDS0 (109)	51.7	73.7	0.278	—	20.4
CDS0 (272)	47.5	42.8	0.339	—	12.9
D1 (25.4)	39.6	—	0.115[a]	28.6	
CDS1[b] (112)	—	—	0	—	3.3
D2 (25.8)	51.0	28.8	2.98	42.3	
CDS2[c] (113)	174	—	0	—	3.7
D3 (24.0)	49.1	22.1	0.988	67.0	
CDS3 (162)	54.1	87.2	1.03	—	10.0
D4 (23.6)	46.5	4.47	0.83	102	
CDS4[d] (53.5)	—	—	0	—	2.3

[a] D1 could be detected in only the 10-min brain sample. The brain-to-plasma concentration ratio is given instead of the brain/plasma AUC ratio.

[b] D1 could be detected in only a few plasma sample and not at all in the brain after administration of CDS1. The plasma half-life could not be determined and the percentage of CDS1 converted to D1 is calculated from an estimated AUC, using the measured concentrations and the elimination rate constant from administration of D1.

[c] No D2 could be detected in the brain after administration of CDS2. The plasma half-life and percentage of dose available as D2 are estimated from only three plasma samples in which the drug could be detected.

[d] No D4 could be detected in the brain after administration of CDS4. D4 could be detected in plasma samples, but at a low and variable concentration. Thus, a half-life could not be determined and the percentage of the dose available as D4 is calculated from an estimated AUC using the elimination rate constant from administration of D4.

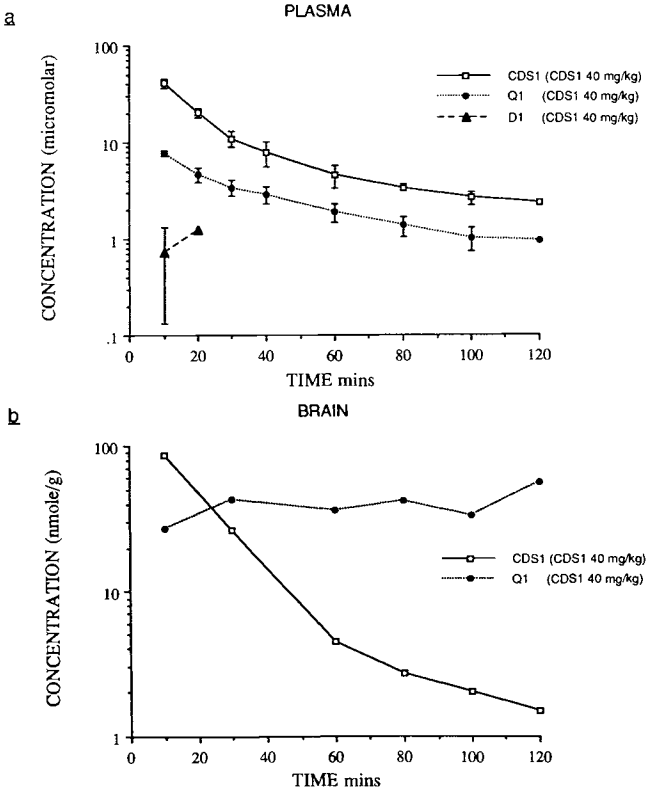

Fig. 5. Concentration–time profiles in plasma (a) and brain (b) of CDS1, Q1, and D1 after administration of 40 mg/kg (112 μmol/kg) of CDS1. Plasma data are means, with error bars for standard deviations, for plasma samples at that sampling time.

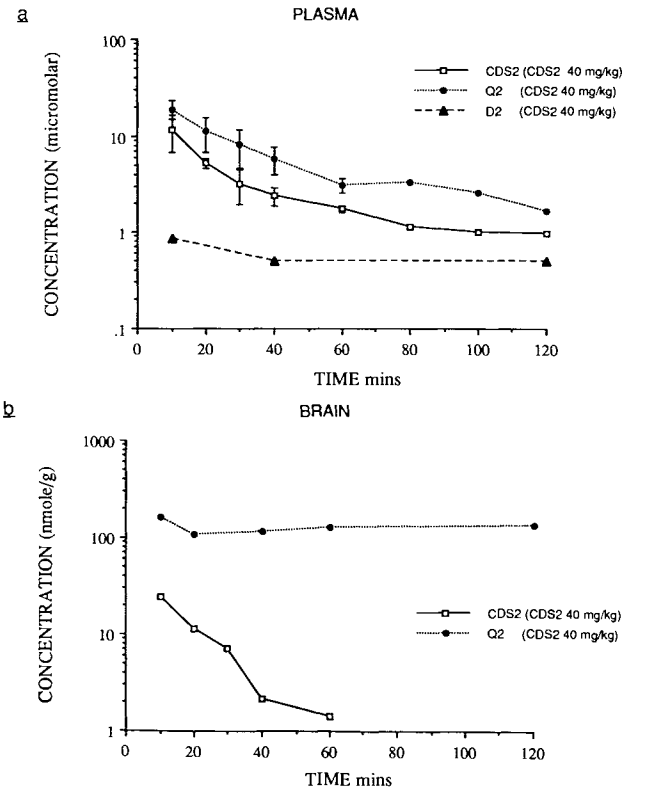

Fig. 6. Concentration–time profiles in plasma (a) and brain (b) of CDS2, Q2, and D2 after administration of 40 mg/kg (113 μmol/kg) of CDS2. Plasma data are means, with error bars for standard deviations, for plasma samples at that sampling time.

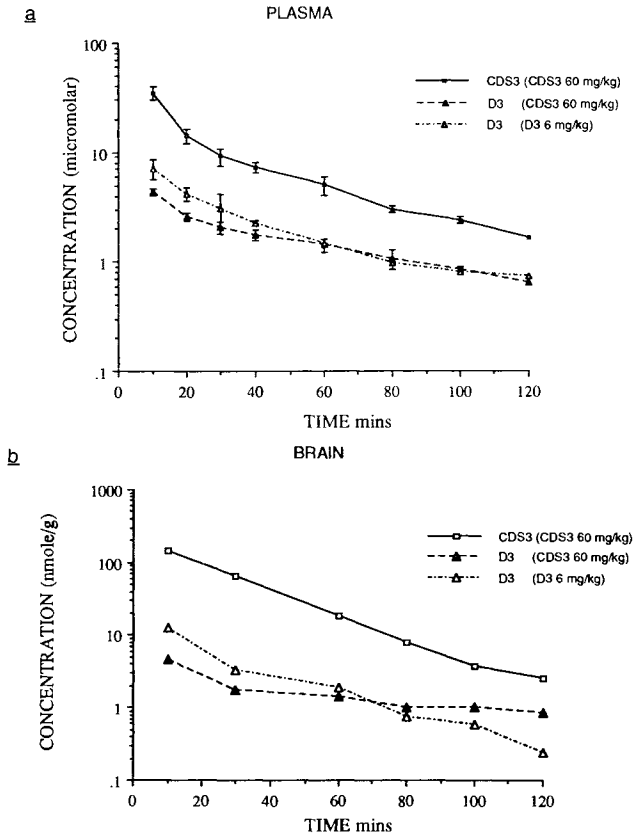

Fig. 7. Concentration–time profiles in plasma (a) and brain (b) of CDS3 and D3 after administration either of 60 mg/kg (162 μmol/kg) of CDS3 or of 6 mg/kg (24.0 μmol/kg) of D3. Plasma data are means, with error bars for standard deviations, for plasma samples at that sampling time.

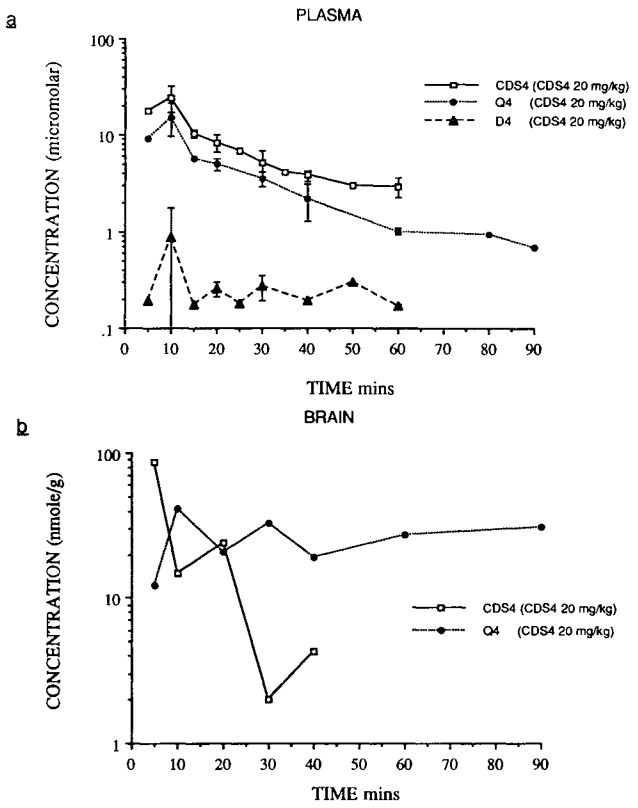

Fig. 8. Concentration–time profiles in plasma (a) and brain (b) of CDS4, Q4, and D4 after administration of 20 mg/kg (53.5 μmol/kg) of CDS4. Plasma data are means, with error bars for standard deviations, for plasma samples at that sampling time.

released no D2 *in vitro* and a negligible amount *in vivo*. The reason for this marked selectivity can be found in the mechanism of the hydrolysis reaction. Allylic esters, but not allylic amides, may ionize to generate resonance-stabilized carbocation intermediates (12) (Fig. 2). Therefore, Q1, Q2, and Q4 are refractory to hydrolytic cleavage by this mechanism. They also appear to be poor substrates for enzyme-mediated hydrolysis, possibly due to steric hindrance by the t-butyl group present in these analogues. The proposed mechanism by which the hydrolysis of the quaternary intermediates studied here occurs probably is not general for other CDS-type conjugates. Nevertheless, this study emphasizes the need to understand that mechanism in order to rationalize the observed rates of release of native drug from CDS-type carriers.

The *in vitro* results correlated well with the behavior of the CDS compounds *in vivo*. This illustrates the usefulness of conducting *in vitro* assessments of the oxidation of the CDS compounds and of the hydrolysis of the corresponding quaternary moieties. Thus, those compounds which formed a readily hyrodrolyzable quaternary *in vitro* (CDS0 and CDS3) released appreciable quantities of the drug (STP or D3) in the brain when administered intravenously. The other CS compounds (CDS1, CDS2, and CDS4) gave rise to quaternary compounds which were stable toward hydrolysis and released only trace amounts of drug (D1, D2, or D4) *in vivo*. None of the latter three CDS compounds produced a measurable concentration of native drug in the brain, despite the presence of high and sustained levels of the quaternary compounds in that tissue. The kinetics of the quaternary compounds in brain and plasma confirm that the dihydropyridine–pyridinium CDS can achieve selective delivery of a drug precursor to the brain. However, this is of little benefit if no drug is released in brain tissue (14). It is worth noting that, at best, only 20% of a dose of CDS could be accounted for as the corresponding drug in these studies. The remainder of the administered dose is eliminated either as the intact CDS or as unknown metabolites.

Comparison of CDS0 and CDS3 illustrates two further important considerations for the investigation of site-specific drug delivery systems. Both succeeded in producing a sustained concentration of the drug in the target tissue, as shown by a comparison of half-life values for drug when administered as such or as a CDS carrier (Table III). However, only CDS0 provided a significant advantage, over administration of the drug itself, in brain-to-plasma distribution. This advantage may be expressed as the quotient of the brain-to-plasma. AUC ratio for the drug when the CDS was administered, to the same ratio when the native drug was administered (15). For CDS0 this index varied from 2.6 to 3.3, depending on the dose. The index for CDS3 was approximately equal to one, the theoretical minimum. This was due to the uniform distribution of D3 between brain and plasma, unlike STP, which did not distribute well into the brain.

From this observation it may be argued that selective delivery to the brain provides the most potential benefit for a drug which would not distribute into the brain following conventional administration. The theoretical basis for this argument has been described previously (15,16). However, it should be borne in mind that the ultimate aim of selective drug delivery is to provide effective drug concentrations at the target site, not simply to provide a relative gain in brain to plasma distribution. It is also possible that the slight difference in structure between STP and D3 altered the predominant site of drug release from the CDS, increasing the proportion of D3 released outside the brain.

This distinction between sustained and site-selective release of drug illustrates the need for a comparable dose of the parent drug for comparison with drug released from a potential carrier. The two CDS compounds derived from STP and D3 also show how the drug itself, and not just the putative chemical delivery system, can influence the potential advantage over conventional drug administration. The importance of drug selection for drug-targeting systems has been stressed in the theoretical literature on site-selective drug delivery (15,16). In these papers a high clearance is stressed as the most important characteristic of the candidate drug for the optimization of drug targeting. Although the compounds investigated in the present study have a range of clearance values (Table III), the influence of this parameter on the degree of selective delivery to the brain is confounded by other factors.

All of the above points show how the selection of the drug can have an important influence on the ability of a CDS to deliver the drug selectively to the brain. The dose-dependent behavior of CDS0 should also be noted as the optimum dose of a CDS may be difficult to estimate.

Finally, any approach to site-selective drug delivery requires that the delivery system itself is nontoxic. Although the present study did not rigorously evaluate the toxicity of the these compounds, signs of toxicity (ataxia, tremor, rigidity) were observed in several animals which received doses of the CDS conjugates. This toxicity could not be due to the native drug being released, as the concentrations of drug were equal to or below those achieved by administration of native drug itself. Routine toxicity testing in mice showed that CDS1, CDS3, and CDS4 were as much as five times more toxic than the corresponding native drugs (LD_{50} values; data not shown). Similar toxicity of some CDS compounds has been reported previously (17).

CONCLUSION

The present study is the first to investigate systematically the influence of drug structure on the utility of the dihydropyridine-based chemical delivery system proposed by Bodor (4). A comparison of different CDS compounds *in vitro* and *in vivo* demonstrates that minor modifications in drug structure can have a dramatic effect on the ability of a CDS to deliver drug selectively to the brain. The *in vitro* studies also showed how a detailed understanding of the mechanisms underlying the oxidation/hydrolysis reactions may allow for a more rational approach to the design of CDS/drug combinations. The *in vivo* studies agreed well with the results of the *in vitro* investigations and demonstrated that the relative improvement in delivery using a CDS, compared to conventional drug administration, may also depend on the selection of an appropriate drug candidate.

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REFERENCES

1. A. Goodman Gilman, L. S. Goodman, T. W. Rall, and F. Murad. *The Pharmacological Basis of Therapeutics,* 7th ed., Macmillan, New York, 1985.
2. W.-M. Wu, E. Pop, E. Shek, and N. Bodor. Brain specific delivery systems for beta-lactam antibiotics. *In vitro* and *in vivo* studies of some dihydropyridine and dihydroisoquinoline derivatives of benzylpenicillin in rats. *J. Med. Chem.* 32:1782–1788 (1989).
3. N. Bodor and M. Brewster. Problems of delivery of drugs to the brain. *Pharmacol. Ther.* 19:337–389 (1983).
4. N. Bodor. Targeting of drugs to the brain. *Methods Enzymol.* 112:381–396 (1985).
5. N. Bodor. Redox drug delivery systems for targeting drugs to the brain. *Ann. N.Y. Acad. Sci.* 507:289–306 (1987).
6. N. Bodor and H. H. Farag. Improved delivery through biological membranes. XIV. Brain specific, sustained delivery of testosterone using a redox delivery system. *J. Pharm. Sci.* 73:385–389 (1984).
7. W. R. Anderson, J. W. Simpkins, M. E. Brewster, and N. S. Bodor. Evidence for prolonged suppression of stress-induced release of adrenocorticotropic hormone and corticosterone with a brain enhanced dexamethasone-redox delivery system. *Neuroendocrinology* 50:9–16 (1989).
8. M. J. Phelan and N. Bodor. Improved delivery through biological membranes. XXXVII. Synthesis and stability of novel redox derivatives of naproxen and indomethacin. *Pharm. Res.* 6:667–676 (1989).
9. G. Mullersman, H. Derendorf, M. E. Brewster, K. S. Estes, and N. Bodor. High-performance liquid chromatographic assay of a central nervous system estradiol chemical delivery system and its application after intravenous administration. *Pharm. Res.* 5:172–177 (1988).
10. E. Palamino, D. Kessel, and J. P. Horwitz. A dihydropyridine carrier system for sustained delivery of 2′,3′-dideoxynucleosides to the brain. *J. Med. Chem.* 32:622–625 (1989).
11. M. Poisson, F. Huguet, A. Savattier, F. Bakri-Logeais, and G. Narcisse. A new type of anticonvulsant, Stiripentol. Pharmacological profile and neurochemical study. *Arzneim.-Forsch.* 34:199–204 (1984).
12. T. A. Moreland, J. Astoin, F. Lepage, F. Tombret, R. H. Levy, and T. A. Baillie. The metabolic fate of stiripentol in man. *Drug Metab. Disp.* 14:654–662 (1986).
13. J. Koskikallio. Alcoholysis, acidolysis and redistribution of esters. In S. Patai (ed.), *The Chemistry of Carboxylic Acids and Esters,* Interscience, New York, 1969, pp. 103–133.
14. A. Boddy and L. Aarons. Pharmacokinetic and pharmacodynamic aspects of site-specific drug delivery. *Adv. Drug Del. Rev.* 3:155–164 (1989).
15. C. A. Hunt, R. D. MacGregor, and R. A. Siegel. Engineering targeted *in vivo* drug delivery. I. The physiological and physicochemical principles governing opportunities and limitations. *Pharm. Res.* 3:333–344 (1986).
16. A. Boddy, L. Aarons, and K. Petrak. Efficiency of drug targeting: Steady-state considerations using a three-compartment model. *Pharm. Res.* 6:367–372 (1989).
17. E. Shek, J. Murakami, C. Nath, E. Pop, and N. S. Bodor. Improved anticonvulsant activity of phenytoin by a redox brain delivery system. III. Brain uptake and pharmacological effects. *J. Pharm. Sci.* 78:837–843 (1989).