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Enhanced brain delivery of 2′-fluoro-5-methylarabinosyluracil [1]

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Summary

The dihydropyridine ⇌ pyridinium salt redox carrier based chemical delivery system (CDS) approach was applied to the antiviral agent 2′-fluoro-5-methylarabinosyluracil (FMAU) (**1**). Two CDSs were examined, having the redox carrier attached to the 3′-OH functionality and the 5′-OH group esterified with hexanoic (**2a**) and octanoic acid (**2b**), respectively. In vitro stability and in vivo distribution studies were performed. When **2a** was systemically administered to rats, sustained levels of the oxidized, trigonelline ester (**3a**) were found in brain. The parent drug was identified beginning at 4 h post-dosing. In the case of 2b, both the oxidized form (**3b**) and the native **1** were found in brain at relatively high concentrations. No drug (**1**) was detected in brain after administration of an equivalent amount (50 μmol/kg) of FMAU.

Introduction

Herpes encephalitis is a primary or reactivated infection of the central nervous system (CNS) caused by herpes simplex viruses (HSV-1 and HSV-2). The high rate of mortality (over 70% for untreated disease (Griffin, 1991)) and the severe sequels produced in survivors (Gutman et al., 1986) require prompt and prolonged therapy of the infection with antiviral drugs. Fortunately, HSV encephalitis is one of the few examples of viral diseases in which chemotherapy has been successfully applied to man. Several compounds, including vidarabine and acyclovir, have been shown to be effective in this respect. (Whitley et al., 1980, 1986). However, in their clear benefits, these drugs have serious limitations; the overall mortality rate is still 30–50% in the case of treatment with vidarabine and 13–28% in the case of acyclovir and a larger percentage of the survivors have varying degrees of neurologic damages. Finally, drug resistance has developed in many instances (Field, 1988).

The use of antiviral drugs, including the established agents and a number of novel drugs with

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remarkable in vitro activity is limited mainly because of their poor entry into the CNS, their inability to deliver sufficient concentrations for therapeutic efficacy and their toxic side-effects. The entry of the therapeutic agents and other xenobiotics from blood to CNS is restricted by the protective, lipoidal blood-brain barrier (BBB) (Pardridge et al., 1975; Rapoport, 1976). The penetration of antiviral agents across the BBB is dependent upon their lipid solubility at physiological pH, their protein bindings, the pH gradient over the barrier, the size and steric complexity of the molecule, changes in the integrity of the barrier which occur during the course of infection, etc. Generally, large and hydrophilic molecules cannot cross the BBB (Pardridge, 1981). However, active and passive transport mechanisms (Benet and Sheiner, 1985) and other factors (like the permeability of the BBB during infection) may influence drug levels in CNS. For instance, the brain penetration by some antiviral agents such as polar, hydrophilic nucleosides can be aided by active transport systems (Kalaria and Harik, 1986).

It is obvious that new, more efficient drugs are required for the treatment of encephalitis. Poorly explored, however, is the improvement of the biological membrane penetration properties of existing drugs. One of the best approaches in this direction is the application of the brain-specific chemical drug delivery system (CDS) approach (Bodor, 1981, 1985, 1987) to the antiviral agents. This system is based on a dihydropyridine ↔ pyridinium salt type molecular targetor, similar biochemically to the endogenous NADH ↔ NAD coenzyme system. The desired centrally mediated effects of the agents can be achieved without the exposure of the body to high drug levels which may be responsible for the toxic side effects. The CDS approach has been reviewed elsewhere and applied to a number of antiviral agents (Rand et al., 1986; El-Koussi and Bodor, 1987; Little et al., 1990; Bhagrath et al., 1991; Deyrup et al., 1991). The application of the CDS approach to 2′-fluoro-5-methyl-arabinosyluracil (FMAU), a representative of a family of fluorinated nucleoside analogs (Watanabe et al., 1979; Lopez et al., 1988), is described below.

Studies on the structure-activity relationships of pyrimidine nucleoside analogs indicated that the anti-HSV effect was greatly increased by 2′-fluoroarabinosyl group substitution and 5-iodo or 5-methyl group substitution (Watanabe et al., 1984; Fox et al., 1985; Perlman et al., 1985). FMAU proved to be the most potent anti-HSV (both 1 and 2) drug in a mouse model (Schinazi et al., 1986). However, FMAU caused neurotoxicity in humans (Fanucchi et al., 1985); encephalopathy occurred at doses as low as 0.8 mg/kg per day (five intravenous doses). In preliminary studies in patients with advanced cancer, FMAU produced irreversible neurologic damage at doses greater than 32 mg/m^2 per day. Other side effects at these doses included diarrhea, nausea and blood count depression (Fanucchi et al., 1985; Schinazi et al., 1986). Unfortunately, repeated and relatively high doses of this agent are needed in order to attain an effective CNS concentration in spite of a low ED_{50}.

The CDS approach has been expected to result in sustained active brain levels of drug without the necessity of administration of high and repeated doses. The peripheral toxicity which can be a result of incorporation of FMAU into mammalian cell DNA besides the viral DNA (Grant et al., 1982; Chou et al., 1987) could also be reduced. Furthermore, since the majority of a drug delivered using the CDS method is masked as an inactive conjugate, central toxicities may also well be mitigated. In this context, the active parent drug is released slowly and in a sustained manner.

Materials and Methods

All materials were reagent grade. 2′-Fluoro-5-methylarabinosyluracil (FMAU) (**1**) was provided by Bristol-Myers or synthesized according to the literature (Watanabe et al., 1979). The two examined CDSs, 3′-(1,4-dihydro-1-methylnicotinoyl)-5′-hexanoyl-FMAU (**2a**) and 3′-(1,4-dihydro-1-methylnicotinoyl)-5′-octanoyl-FMAU (**2b**), were synthesized in our laboratories by using conventional methods (Bodor et al., unpublished data). Briefly, the 5′-OH functionality of **1** was pro-

tected as a silyl ether using *tert*-butyldimethylsilyl chloride, the 3′-OH group was acylated with nicotinic anhydride, and the silyl group was then deprotected using tetrabutylammonium fluoride in acetic acid-tetrahydrofuran and esterified with hexanoic or octanoic anhydrides. The pyridine nitrogens of the resulting 3′,5′-diesters were alkylated with methyl iodide and the formed quaternary salts reduced regioselectively with sodium dithionite produced the 1,4-dihydropyridine derivatives 2**a** and 2**b**.

Analytical methodology

High-performance liquid chromatography (HPLC) was used for quantitative analyses of the dihydropyridines (**2a**, **2b**), pyridinium salts (**3a**, **3b**) and FMAU (**1**). Two HPLC systems were used. The HPLC system used for quantitation of FMAU (**I**) consisted of a SpectraPhysics SP 8810 pump, an SP8880 autosampler, an SP-Spectra 100 UV detector (265 nm) and an SP-Chromoset integrator. A Spherisorb ODS-2 5 μm reverse-phase column fitted with a guard column (pellicular ODS) was used. The mobile phase consisted of 0.05 M KH_2PO_4 buffer/ methanol/ water (50:15:35), the flow rate was 0.8 ml/min, and the analyses were conducted at ambient temperatures. The retention time of **1** was 11.9–12.5 min. For the analysis of the CDSs and the corresponding oxidized salts, the HPLC system (II) consisted of a SpectraPhysics SP8810 pump, an SP8780 autosampler and an SP4290 integrator and two LDC/Milton Roy detectors (250 and 362 nm). A Spherisorb C8 column, jacketed and maintained at 17.5°C was used. The mobile phase consisted of 0.05 M ammonium acetate/ acetonitrile (25:75) containing 5 mM tetraethylammonium perchlorate (TEAP) for **2a** and **3a** (retention times 8.3–8.7 and 5.0–5.5 min for **2a** and **3a**, respectively; flow rate 1 ml/min), and of 0.05 M ammonium acetate/ acetonitrile (25:75) containing 10 mM TEAP for **2b** and **3b** (retention times 8.4–8.9 and 6.0–6.5 min, respectively; flow rate 1 ml/min).

Lipophilicity

The relative lipophilicity of the CDSs as compared to the parent drug was determined by using a chromatographic method. By using HPLC, the change in the retention time for **1**, **2a** and **2b** with changes in the acetonitrile/water composition of the mobile phase was recorded. The range of mobile phase concentrations was 70:30 acetonitrile/water to 40:60 acetonitrile/water. For each compound, at each mobile phase composition, the capacity factor (log k') was determined by using the following equation:

$$\log k' = \log[(t_R - t_0)/t_0]$$

where t_R denotes the retention time of the compound and t_0 is that of an unretained peak (formaldehyde). The log k' values were then plotted against the percentage of water in the mobile phase and a straight line was obtained. Extrapolation to a completely aqueous mobile phase gave the extrapolated log k', used as a lipophilicity index. The correlations obtained for these lines were 0.991 for **1**, 0.998 for **2a** and 0.997 for **2b**.

Chemical oxidation

*Oxidation of **2a** and **2b** by hydrogen peroxide:* 10 ml of 30% H_2O_2 solution were added to 50 ml of a 1% solution of **2a** and **2b** in methanol. The mixture was stirred and the decrease in the absorbance at 360 nm monitored.

*Oxidation of **2a** or **2b** by silver nitrate:* 1 ml of a 5% solution of **2a** or **2b** in methanol was added to the methanolic solution of $AgNO_3$. A silver mirror formed, and the 360 nm absorbance disappeared.

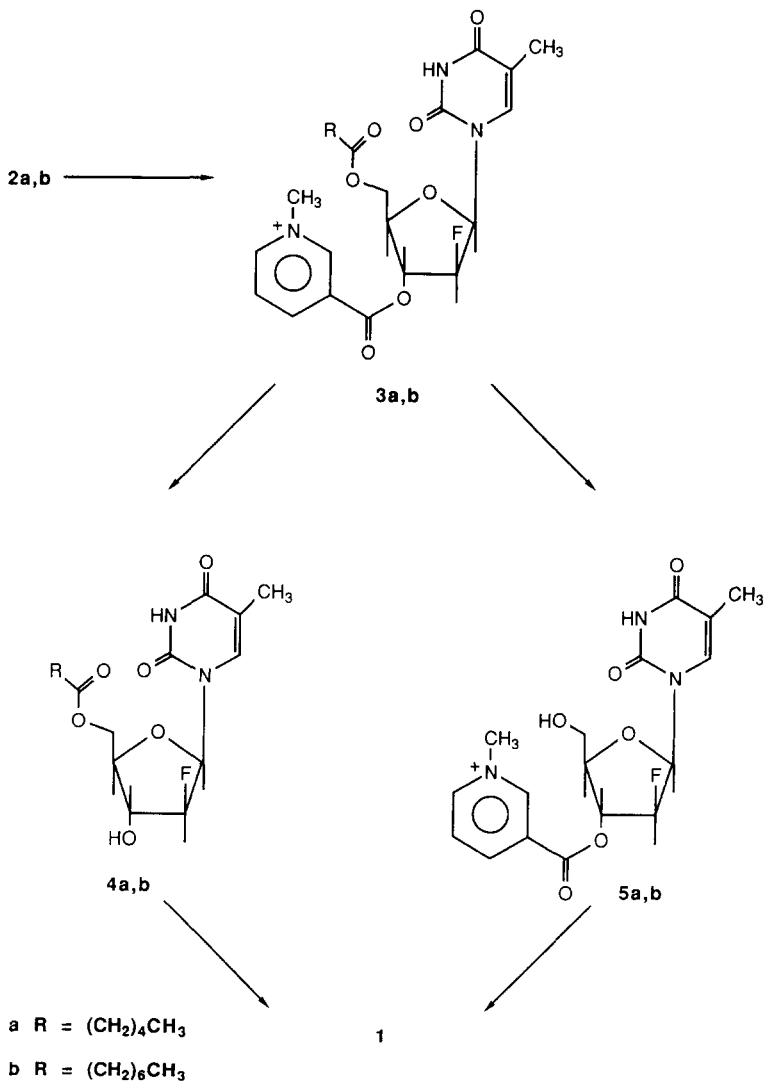
In vitro stability

The stability of **2a** and **2b** was determined in isotonic phosphate buffer (pH 7.4) in rat whole blood, 20% rat brain homogenate and in human whole blood. Freshly collected rat blood and brain tissue were used for determination. The brain was homogenized in ice-cold pH 7.4 phosphate buffer. To 5 ml aliquots of homogenates or buffer solution, prewarmed to 37°C, 200 μl of **2a** or **2b** stock solution (16.2 and 16.3 mg/ml **2a** or **2b** in acetonitrile) were added (zero time) and samples of 200 μl were then taken at various time intervals (from 30 s to 300 min) and added to 800 μl

of cold acetonitrile, containing 5% dimethyl sulfoxide. The mixtures were centrifuged, supernatants were removed and analyzed by HPLC. Each sampling was performed in duplicate. Standards of **2a** and **2b** were prepared from the stock solution and used to calculate the recovery from the matrices. It was found that 88% of CDS was recovered from supernatants. Pseudo-first-order rate constants for the disappearance of compounds were determined by linear regression analysis from plots of log peak area vs time. Reactions in biological media were followed for three half-lives and no nonlinearity was observed in the course of reaction.

In vivo distribution studies

Male Sprague-Dawley rats weighing 200 ± 20 g were used for distribution studies. CDSs **2a**, **2b** and parent drug **1**, were administered in the tail vein of conscious, restrained animals in a single dose of 50 μmol/kg dissolved in dimethyl sulfoxide which served as the vehicle (0.5 ml/kg). Ani-



Scheme 1.

mals were killed at 15 min, 1 h, 2 h, 4 h and 6 h following drug administration. Groups of five animals were used for each time point. Animals were decapitated and trunk blood was collected in heparinated tubes. Brain, liver, kidney, lung, testes, heart and fat were removed, weighed and frozen on dry ice. In preparing samples for analyses, each tissue (1 ml or 1 g) was homogenized in 1 ml water. To the homogenates, 4 ml of cold acetonitrile and 1 ml of saturated sodium chloride solution were added and the mixtures were vortexed and cooled to −15°C. The organic layer was separated, filtered, placed in autosampler vials and stored at −70°C until analyzed. The CDSs, parent drug and quaternary salt-type metabolites were determined by HPLC.

Results and Discussion

In vitro studies

Based on preliminary experiments (Bodor et al., unpublished data), two CDSs for FMAU (**2a**, **2b**, Scheme 1) have been examined for their brain-targeting potential. In both CDSs, the dihydrotrigonelline-type carrier was attached to the secondary 3′-OH functionality. The primary 5′-OH functionalities were acylated with long chain fatty acids (hexanoic, octanoic) in order to augment (with different magnitudes) the lipophilic character of the CDSs.

The previously examined 5′-pivalate CDS (Bodor et al., unpublished data) was not a viable candidate for further investigation since the enzymatic hydrolysis of the 5′-ester was inappropriately slow. Recent studies (Kawaguchi et al., 1988) on enzymatic hydrolysis of various 3′,5′-diester type prodrugs of 5-fluoro-2′-dioxyuridine (FUdR) suggested that the enzymatic activity depends on the nature of the acyl groups. It appears that longer chain esters, such as pentanoates and octanoates are more easily hydrolyzed than acetates, for example, but an interaction between the 3′- and 5′-ester group may affect their affinity to esterases. Also, different reactivity of the 3′- and 5′-esters has been achieved with animal and human plasma. By using the 5′-hexanoate and 5′-octanoate esters **2a** and **2b** it was hoped to develop a CDS which more rapidly hydrolyzed at both 3′ and 5′ positions resulting in the release of **1**.

TABLE 1

Lipophilicity indices (extrapolated capacity factors, log k′) of FMAU and CDSs

Compound	Log k'	r [a]
FMAU (**1**)	0.042	0.992
CDS **2a**	2.380	0.998
CDS **2b**	2.920	0.997

[a] Correlation coefficient.

Lipophilicity is an essential factor which controls the interaction of drugs with biological systems. Lipophilic character is essential for CDS penetration of the blood-brain barrier. Therefore, the relative lipophilicity of the CDSs was examined and compared with FMAU using their retention characteristics on a reversed-phase HPLC column. The capacity factor (log k') relative to an unretained marker (formaldehyde) was calculated for various mobile phase combinations of acetonitrile : water. The log k' values, extrapolated to a mobile phase of 100% water, are analogous to the R_m values determined by thin-layer chromatography (TLC) (Biagi et al., 1965) and can be used as lipophilicity indices (Yamana et al., 1977). As expected, the dihydropyridine derivatives were much more lipophilic than the FMAU (Table 1). The most lipoidal compound, **2b**, the octanoyl ester was 695 times more lipophilic than FMAU, while **2a** was a 567 times more lipophilic compound (**2b** was 1.2 times more lipophilic than **2a**, due to the longer alkyl chain of the 5′-ester group). These results indicate that both CDSs should be able to penetrate the BBB.

The chemical oxidation studies indicated that both CDSs were easily oxidized to the corresponding quaternary pyridinium salts (**3a** and **3b**, Scheme 1) in the presence of hydrogen peroxide or silver nitrate. The oxidation of the dihydropyridines is important, being one of the main operation associated with CDS functioning. Systemic (intravenous) administration of the lipophilic CDS results in CNS distribution, as well as entry into other peripheral compartments. The CDS is ex-

pected then to be oxidized to the polar quaternary salt form which is 'locked in' the CNS behind the BBB while it is lost from periphery. By enzymatic hydrolysis the quaternary salt slowly releases the drug at the needed site, while the carrier, being a small molecule, can be easily eliminated from brain.

The in vitro stabilities of both the CDSs (**2a** and **2b**) and their oxidized forms (**3a** and **3b**) were studied. In Scheme 1, the main expected degradation pathways of the CDSs **2a**, **2b** are indicated: oxidation to the quaternary salts **3a**, **3b** then their hydrolysis to **1**. Intermediates **4a**, **4b** and **5a**, **5b** can also result during this process. Other possible transformations include the hydrolysis of the 3'- and 5'-esters before oxidation and water addition to the dihydropyridine nucleus, resulting in 6-hydroxy-1,4,5,6-tetrahydropyridine derivatives. However, this latter process, which occurs mainly under acid-catalyzed conditions, is less probable at physiological pH of 7.4. The in vitro studies were performed in isotonic pH 7.4 buffer, rat whole blood and 20% brain homogenate and human blood (for **2a** and **2b** only).

Table 2 lists the calculated pseudo-first order rate constants and half-lives. The data indicate a generally faster degradation of the dihydropyridine derivatives **2a** and **2b** in biological materials than in phosphate buffer pH 7.4. The stability for both compounds was much lower in whole rat blood compared to human blood (~ 14 times less stable for **2a** and 19 times less stable for **2b**). This observation is consistent with previously noted species specificity; namely, that esterases from rodent sources such as rat and mouse showed a higher degree of hydrolytic activity compared to esterase from dog and human sources (Von Daehne et al., 1970). The main degradation product, both in buffer and in blood, was the parent FMAU. Some oxidation products were detected in human blood, where the hydrolysis was slower. Interestingly, while in whole rat blood and, especially in human blood **2b** was more stable than **2a**, in aqueous buffer the order of the stability was reversed. In rat brain homogenate, both **2a** and **2b** were more stable than in rat blood. The main transformation observed in the brain was the oxidation of the dihydropyridine derivatives to the respective quaternary salts **3a** and **3b**, with other hydrolysis products also observed. Since **2b** was oxidized faster (~ 7.7 times) to the locked in species, the pyridinium salt **3b**, than **2a** was converted to **3a**, it appears to be a better candidate for delivering FMAU to the brain. (When the oxidation is completed, the efflux from the CNS to the blood stream is slowed dramatically.) However, the oxidation rate of **2a** does not exclude it from further consideration.

TABLE 2

Pseudo-first-order rate constants (k) and half-lives ($t_{1/2}$) for the disappearance of FMAU CDSs from various media

Media	Compound	k ($\times 10^3$) (min^{-1})	$t_{1/2}$ (min)	r [a]
Isotonic pH 7.4 phosphate buffer	**2a**	11.08	62.55	0.969
	2b	22.30	31.10	0.983
Whole rat blood	**2a**	306.07	2.23	0.981
	2b	270.36	2.56	0.986
Whole human blood	**2a**	22.63	30.60	0.989
	2b	14.07	49.25	0.986
20% Rat brain homogenate	**2a**	15.90	43.60	0.999
	2b	42.72	16.23	0.997

[a] Correlation coefficient.

TABLE 3

Pseudo-first order rate constants (k) and half lives ($t_{1/2}$) for the disappearance of FMAU-pyridinium salt-type derivatives from various media

Media	Compound	k ($\times 10^3$) (min^{-1})	$t_{1/2}$ (min)
Phosphate buffer, pH 7.4	**3a**	44.66	15.52
	3b	46.80	14.81
Whole rat blood	**3a**	364.80	1.90
	3b	558.90	1.24
20% rat brain homogenate	**3a**	19.10	36.29
	3b	38.81	17.86

The stability of the quaternary salts in biological matrices is also of interest. The results of these determinations (Bodor et al., unpublished data) are presented in Table 3. As in the case of the dihydropyridine derivatives the esterase present in rat blood was a more efficient catalyst for

TABLE 4

In vivo distribution of ***3a*** *following intravenous administration of 50 μmol / kg (24 mg / kg) CDS* ***2a***

Time (min)	Wet tissue concentration (μg/g)							
	Blood	Brain	Liver	Kidney	Lung	Heart	Fat	Testes
15	0.53	3.87	3.83	5.11	6.18	6.25	33.8	1.1
60	[a]	2.95	2.35	0.51	3.52	3.63	29.1	1.96
120	0.48	2.69	3.04	[a]	3.07	2.21	26.8	0.95
240	[a]	0.97	1.67	0.51	1.77	[a]	25.6	2.23
360	[a]	0.48	1.76	[a]	1.55	[a]	13.7	0.47

[a] Below detection limit.

TABLE 5

In vivo distribution of FMAU (1) following intravenous administration of 50 μmol / kg (24 mg / kg) ***2a***

Time (min)	Wet tissue concentration (μg/g)							
	Blood	Brain	Liver	Kidney	Lung	Heart	Fat	Testes
15	2.79	[a]	3.38	1.80	8.48	[a]	0.93	0.7
60	[a]	[a]	3.20	2.13	6.18	1.48	0.54	1.9
120	[a]	[a]	[a]	1.18	6.32	0.88	[a]	1.9
240	[a]	1.85	[a]	0.67	4.25	1.09	[a]	0.7
360	[a]	3.47	[a]	[a]	8.03	1.98	[a]	[a]

[a] Below detection limit.

TABLE 6

In vivo distribution of ***2b*** *following intravenous administration of 50 μmol / kg (25.4 mg / kg) CDS* ***2b***

Time (min)	Wet tissue concentration (μg/g)							
	Blood	Brain	Liver	Kidney	Lung	Heart	Fat	Testes
15	[a]	5.84	[a]	3.37	296.00	9.43	17.54	7.60
60	[a]	[a]	[a]	2.17	93.90	14.47	8.61	2.20
120	[a]	[a]	[a]	0.47	72.30	11.75	16.49	[a]
240	[a]	[a]	[a]	[a]	23.70	11.83	10.90	[a]
360	[a]	[a]	[a]	[a]	15.50	8.63	11.70	[a]

[a] Below detection limit.

TABLE 7

In vivo distribution of ***3b*** *following intravenous administration of 50 μmol / kg (25.4 mg / kg) CDS* ***2b***

Time (min)	Wet tissue concentration (μg/g)							
	Blood	Brain	Liver	Kidney	Lung	Heart	Fat	Testes
15	[a]	7.33	10.7	4.18	28.12	8.97	19.33	7.9
60	[a]	3.83	7.26	3.98	19.19	8.59	14.83	7.4
120	[a]	1.22	3.81	[a]	17.64	5.65	18.74	1.0
240	[a]	2.41	[a]	7.18	12.68	11.41	15.45	1.7
360	[a]	[a]	[a]	12.41	11.10	[a]	8.77	[a]

[a] Below detection limit.

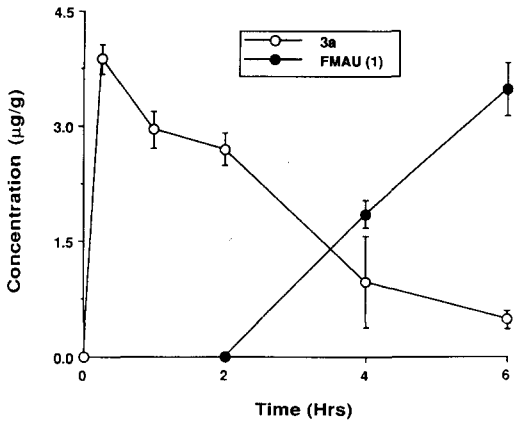


Fig. 1. Distribution of **1** and **3a** in the brain after i.v. administration of 24 mg/kg **2a**.

degradation as compared to the chemical hydrolyses which take place in isotonic buffer. In both cases, there was no noticeable difference between the stability of **3a** and **3b**. In 20% brain homogenate, **3a** was about twice as stable as **3b**. The products of degradation were mainly the parent drug **1**, which resulted from hydrolysis, but other quaternary salt-type derivatives, probably the products of the partial hydrolysis (**5**), were also identified. The release of the parent drug by enzymatic hydrolysis of the quaternary salts in the CNS is the ultimate step of the CDS approach. Based on these in vitro experiments, both examined CDSs appeared to possess the required properties for delivering FMAU to the brain.

In vivo distribution study

To validate the delivery of FMAU by using CDSs, a tissue distribution study was performed in rats. Doses of 50 μmol/kg of **2a** and **2b** were administered i.v. and at various times post-injection the animals were killed, blood and organs collected, the samples analyzed by HPLC for the dihydro derivatives (**2a**, **2b**), quaternary salt metabolites (**3a**, **3b**) and parent drug (**1**).

The results of these experiments are given in Tables 4–9 and Figs 1 and 2. (S.E. values were calculated but are not included in tables). In the case of CDS **2a**, the dihydropyridine was not identified in any tissue except for the lung where it was present in rather high concentration at the 15 min time point, and in trace amounts even after 4 h (data not shown). The fast oxidation of **2a** explains its rapid disappearance. On the other hand, the quaternary salt **3a** was found in variable concentration in all analyzed tissues. It is significant that sustained brain levels of **3a** were registered during the experimental time course (Fig 1, Table 4). The parent drug was detected in blood only at 15 min after administration (Table 5). Interestingly, in brain (Fig. 1) FMAU appeared only at the 4 h time point and its level increased at 6 h. This time course can be explained by the slow release of the parent drug from the quaternary salt **3a** and probably from the putative metabolite **5a**. When the CDS **2b** was administered to rats, the distribution looked rather different. The dihydro derivative was detected in brain (5.84 μg/g) only at the 15 min time interval. No CDS could be detected in blood and liver, but rather high concentrations were found in lung, heart and fat and lower amounts in kidney and testes (Table 6). The high lipophilicity of **2b** explains this extensive distribution. The quaternary salt was not identified in blood, but it was present in the brain for as long as 4 h (Fig. 2). In lung, fat, kidney and heart **2b** was detected in relatively high concentrations. It is important that the quaternary salt was lost from the liver after 2 h (Table 7). Fig. 2 indicates that high and sustained levels of FMAU were detected in brain even after the disappearance of the quaternary

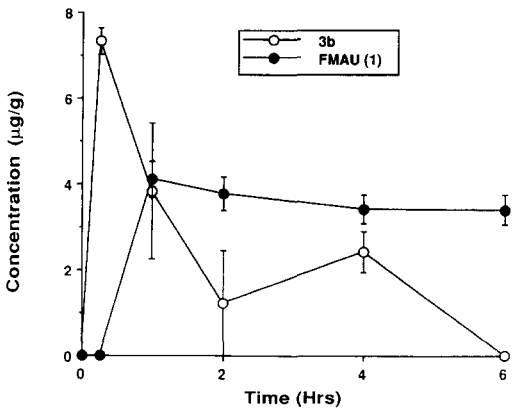


Fig. 2. Distribution of **1** and **3b** in the brain after i.v. administration of 25.4 mg/kg **2b**.

TABLE 8

In vivo distribution of FMAU (1) following intravenous administration of 50 μmol / kg (24 mg / kg) 2b

Time (min)	Wet tissue concentration (μg/g)							
	Blood	Brain	Liver	Kidney	Lung	Heart	Fat	Testes
15	[a]	[a]	3.11	3.37	8.48	[a]	[a]	0.8
60	[a]	4.11	4.45	2.12	6.18	[a]	[a]	2.0
120	[a]	3.78	5.20	0.47	6.32	[a]	[a]	0.8
240	[a]	3.42	1.23	[a]	4.25	[a]	[a]	[a]
360	[a]	3.40	5.44	[a]	8.03	[a]	[a]	[a]

[a] Below detection limit.

salt. This can be explained by the transformation of **3b** in another quaternary salt-type metabolite (**5b**) which continued to release **1**. The brain : blood ratio of **1** is high as a result of this manipulation. FMAU also was detected in other tissues as indicated in Table 8.

The distribution of FMAU when the drug itself was administered to rats in equimolar amounts with the CDSs **2a** and **2b** is indicated in Table 9. Although **1** was present in blood and most of the tissues, its brain concentrations were below the detection limit.

In conclusion, the two investigated CDSs of FMAU, and especially **2b**, markedly improved both the levels of the antiviral agent and the duration of its presence in the brain of rats. The high CNS levels obtained were not accompanied by any overt toxic side effects. From these data **2b** may be considered as a good candidate for further investigation.

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TABLE 9

Distribution for FMAU (1) following intravenous administration of 50 μmol / kg (13 mg / kg) of 1

Time (min)	Wet tissue concentration (μg/g)							
	Blood	Brain	Liver	Kidney	Lung	Heart	Fat	Testes
15	3.39	[a]	4.42	13.00	3.49	[a]	0.71	1.0
60	1.40	[a]	2.80	3.67	2.58	[a]	0.75	2.0
120	5.36	[a]	[a]	0.34	4.44	[a]	[a]	[a]
240	[a]	[a]	[a]	[a]	3.83	[a]	[a]	[a]
360	5.75	[a]	[a]	[a]	2.75	[a]	[a]	[a]

[a] Below detection limit.

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