Elimination of a Quaternary Pyridinium Salt Delivered as Its Dihydropyridine Derivative from Brain of Mice

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Abstract □ 1-Methylpyridine-2-carbaldehyde oxime, a quaternary pyridinium salt, can be delivered efficiently through the blood–brain barrier in its dihydropyridine prodrug form. This redox system was used to study the elimination rate from the brain of a small quaternary salt. It was found that the oxime is eliminated relatively fast from the brain, which supports a hypothesis for the existence of an active transport mechanism for eliminating organic ions from the brain. The possibilities of using the pyridinium salt ⇌ dihydropyridine redox system for specific delivery of drugs to the brain are discussed.

Keyphrases □ Pyridinium salt, substituted quaternary—and prodrug, elimination rate from brain, mice □ Prodrugs—dihydropyridine derivative of quaternary pyridinium salt, elimination rate from brain, mice □ Elimination rate—substituted quaternary pyridinium salt and prodrug from brain, mice

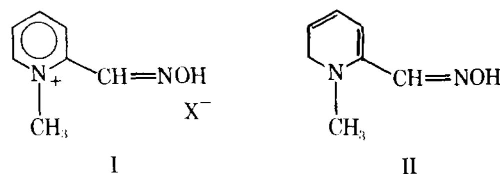
Many valuable chemotherapeutic agents are of limited use when the disease site lies within the central nervous system (CNS). The rates at which drugs or metabolic substrates penetrate the blood–brain barrier depend on their plasma concentration, cerebral blood flow, and barrier permeability. Although the blood–brain barrier is not a simple, anatomically well-defined unitary physical entity (1), it is generally accepted that lipid solubility, degree of ionic dissociation or protonation, and carrier-mediated or active transport govern the movement of molecules in and out of the brain, *i.e., the permeability of the blood–brain* barrier.

Changes in permeability can be caused by several pathological and toxicological processes (2). A general increase in the barrier permeability, such as a nonspecific breakdown of the barrier has, however, severe consequences including cerebral edema. The passage of molecules into the brain also can be enhanced (3) by intrathecal injections and drug latentiation.

BACKGROUND

Since highly selective permeability changes are desired, drug latentiation, or the use of prodrugs, presents the most hopeful approach for delivery of molecules otherwise unable to penetrate the blood–brain barrier into the brain.

A dihydropyridine–pyridinium salt-type redox system recently was developed (4–6) to alter the delivery and distribution characteristics of quaternary pyridinium ring-containing drugs. The approach was used successfully to improve delivery of 1-methylpyridine-2-carbaldehyde oxime chloride (I) as its dihydropyridine derivative, 1-methyl-1,6-dihydropyridine-2-carbaldehyde oxime (II). Because of its lipophilic character, II distributes quickly into tissues and organs unaccessible for I and is then rapidly oxidized ($t_{1/2}$ = 1 min) to the active I form at the site(s) of action (7).

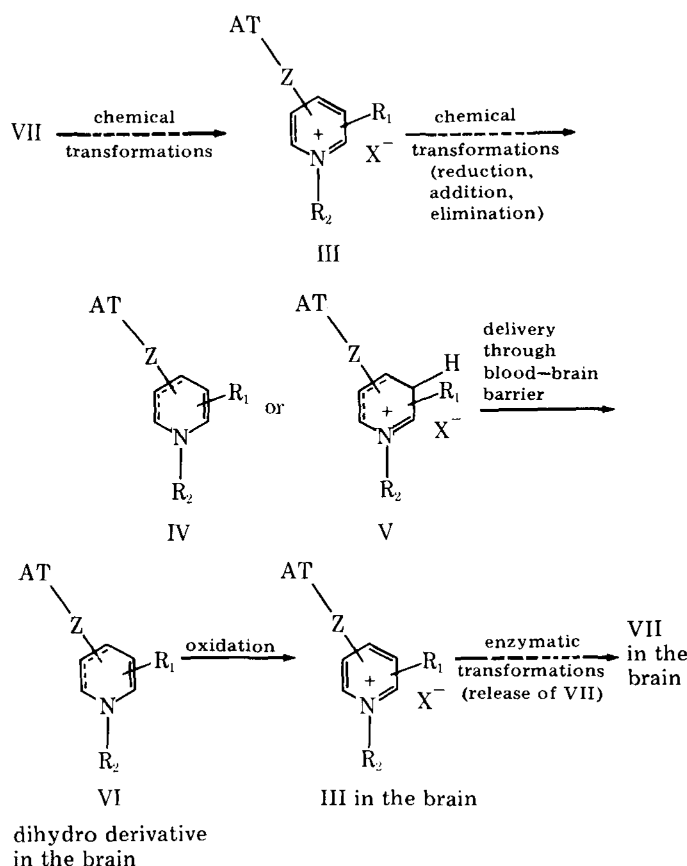


A highly successful delivery of a quaternary pyridinium salt, I, into the brain was achieved using the prodrug II (8). A dramatic increase in drug delivery to the brain and subsequent reactivation of blocked brain cholinesterase was realized. The importance of the reactivation of brain cholinesterase after exposure to organophosphates is well documented (9), from the point of view of both survival and psychiatric problems.

The treatment of neoplasms or infections in the CNS is hindered by the relative impermeability of the blood–brain barrier to most chemotherapeutic agents. To overcome this problem, the redox system could be used for specific delivery of a drug into the brain. Conceivably, a generalized version of the dihydropyridine ⇌ pyridinium salt approach can be extended to delivery into the brain of many drugs containing quaternary heteroaromatic structure or with the dihydropyridine–pyridinium part as a carrier system.

The hypothesis (4) is illustrated in Scheme I, where VII is an antitumor agent (AT), Z is the link between VII and the carrier pyridinium part, and R_1 is a substituent to control the release rate of VII from the quaternary derivative (III) delivered in this way to the brain. Examples for the link Z are: CH_2O, COO, COHNCRHO, and CH=NO.

It is important to note the possibility of a specific delivery to the brain. If the blood–brain barrier would hinder the efflux of the quaternary derivative formed in the brain after delivery, a slow infusion procedure would result in a specific higher concentration of the drug in the brain against the concentration gradient, since the quaternary salt formed in blood would be rapidly excreted, thus avoiding systemic toxicity.



Scheme I

Table I—Distribution of I at Various Time Intervals after Intravenous Delivery as I and as II to White Mice [a]

Minutes	Concentration of I, % Dose/g of Tissue ± SE [b]					
	Blood [c]	Lungs	Kidney	Diaphragm	Heart	Brain
			I			
10	1.22 ± 0.22	1.88 ± 0.31	3.52 ± 0.47	0.79 ± 0.08	2.11 ± 0.13	0.35 ± 0.04
30	0.62 ± 0.16	1.49 ± 0.29	2.2 ± 0.13	0.67 ± 0.23	1.02 ± 0.15	0.38 ± 0.02
75	0.41 ± 0.06	0.97 ± 0.12	1.39 ± 0.16	0.17 ± 0.07	0.49 ± 0.08	0.35 ± 0.03
			II			
10	0.85 ± 0.1	3.55 ± 0.32	2.56 ± 0.28	3.77 ± 0.25	7.06 ± 1.01	2.65 ± 0.19
20	0.61 ± 0.08	1.89 ± 0.14	6.23 ± 0.68	2.70 ± 0.24	4.83 ± 0.64	1.47 ± 0.22
30	0.74 ± 0.05	1.85 ± 0.15	4.03 ± 1.07	2.85 ± 0.16	4.39 ± 0.23	1.40 ± 0.14
75	0.70 ± 0.07	0.50 ± 0.06	1.12 ± 0.08	1.08 ± 0.20	0.66 ± 0.08	0.54 ± 0.08
150	0.56 ± 0.10	0.40 ± 0.03	0.67 ± 0.03	0.61 ± 0.09	0.47 ± 0.05	0.24 ± 0.01
225	0.26 ± 0.03	0.24 ± 0.01	0.65 ± 0.09	0.28 ± 0.02	0.35 ± 0.06	0.21 ± 0.04

[a] Dose: 5 mg of ^{14}C-I or ^{14}C-II/kg. Specific activity: 1 mCi/mmole. [b] Each point is the average of six animals. [c] Expressed as percent of dose per milliliter.

The question then arises: Is the blood–brain barrier a one-way street?

It was suggested that restriction of intravascular materials from entry into the brain is not the only major role of the blood–brain barrier. More important, perhaps, is its capability of excreting detoxification and waste products into the blood (10). Although drugs pass from blood into cerebrospinal fluid at rates that apparently parallel their lipid solubility, they pass in the reverse direction at rates only partly dependent on this property. Although the rapid elimination of lipid-insoluble substances, such as mannitol, sucrose, and inulin, can occur through the arachnoid villi, an active transport system is also conceivable (11). Based on *in vitro* studies (3), it was concluded that an active transport system apparently pumps organic cations, such as hexamethonium, decamethonium, and N'-methylnicotinamide, from the cerebrospinal fluid to the blood.

Exactly the opposite conclusion was reached by Ross and Froden (12). They studied the elimination of a piperidinium quaternary salt, apparently formed *in situ* after delivery of a haloalkylamine, which undergoes cyclization to the quaternary salt, in the brain, as well. A significantly slower elimination from the brain of the formed piperidinium salt was observed. It was concluded that the outflow rate of the quaternary salt corresponded to the inflow rate. Similar results, however, were obtained for erythrocytes; the efflux of the quaternary salt was very slow (13).

Based on the success in direct delivery of I into the brain of mice, this model was chosen to verify the influx–efflux properties of I.

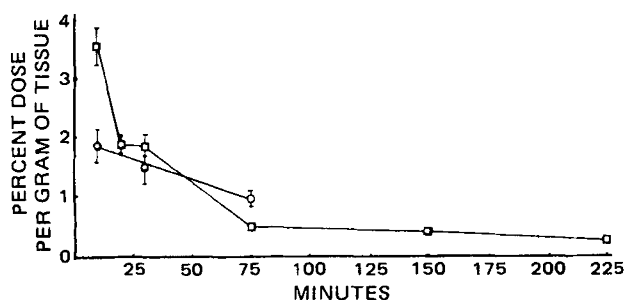


Figure 1—*Concentration of I in the lung tissues of mice as a function of time after administration of 5.0 mg/kg iv of I (O) and II (□).*

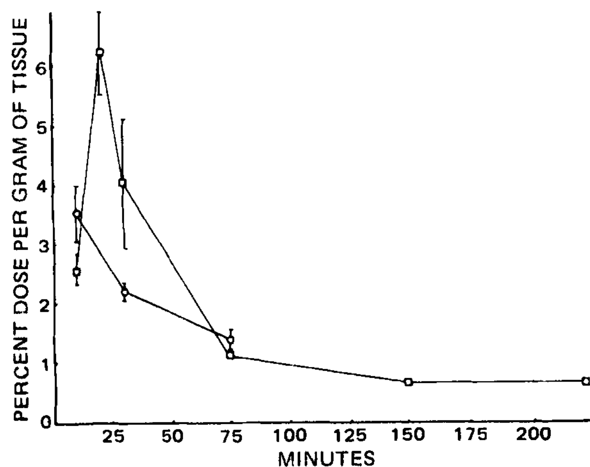


Figure 2—*Concentration of I in the kidney tissues of mice as a function of time after administration of 5.0 mg/kg iv of I (O) and II (□).*

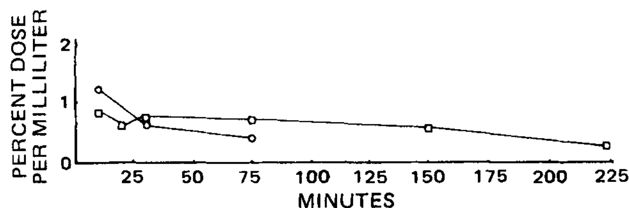


Figure 3—*Concentration of I in the blood of mice as a function of time after adminstration of 5.0 mg/kg iv of I (O) and II (□).*

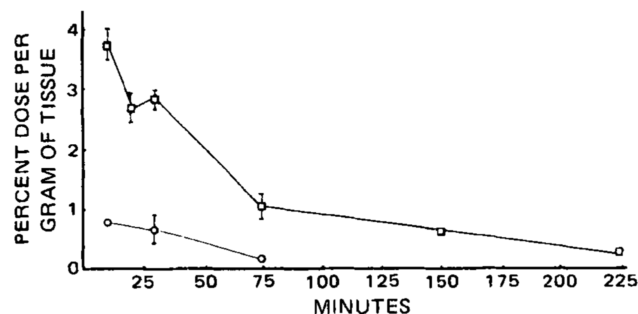


Figure 4—*Concentration of I in the diaphragm tissues of mice as a function of time after administration of 5.0 mg/kg iv of I (O) and II (□).*

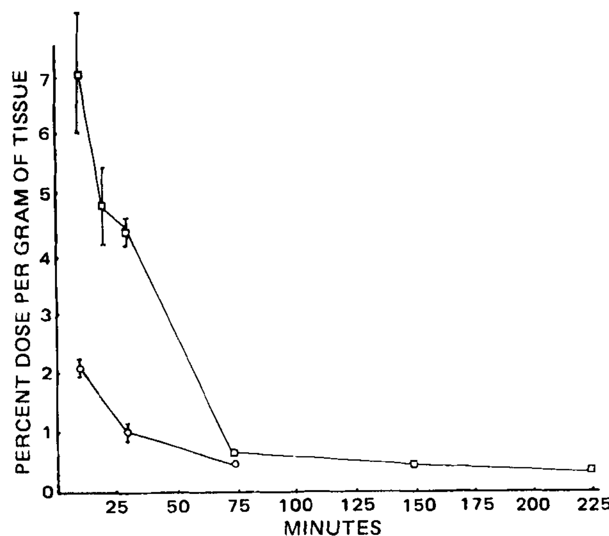


Figure 5—*Concentration of I in the heart tissues of mice as a function of time after administration of 5.0 mg/kg iv of I (O) and II (□).*

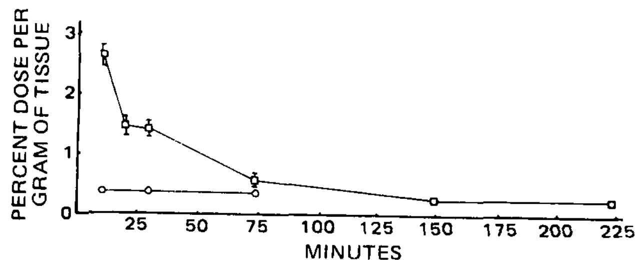


Figure 6—*Concentration of I in the brain tissues of mice as a function of time after administration of 5.0 mg/kg iv of I (○) and II (□).*

EXPERIMENTAL

Materials—^{14}C-Methyl iodide was used as received[1]. ^{14}C-Methyl-labeled I iodide and ^{14}C-II chloride were synthesized as previously described (6). Homogenization was carried out with a motor-driven glass homogenizer. Radioactive counting was performed in a liquid scintillation system[2].

Procedure—Radioactive I and II were dissolved separately in the appropriate amount of 0.9% sterile saline, and 0.2 ml was injected into the tail vein of two groups of white mice (ICR) weighing 28 ± 5 g. A group of 18 mice was given ^{14}C-I, and another group of 36 was given ^{14}C-II. Six animals from the ^{14}C-I group were decapitated after 10, 20, and 75 min; six from the ^{14}C-II group were decapitated at 10, 20, 30, 75, 150, and 225 min following administration.

The blood was collected after decapitation. Then the kidneys, brain, heart, lungs, and diaphragm were excised, washed with normal saline, and kept at −18° until prepared for counting. Samples of 0.1–0.4 ml of whole blood taken from the decapitated upper thorax were discolored with hydrogen peroxide and homogenized with 10 ml of distilled water. A 1-ml aliquot was added to 10 ml of the scintillation cocktail and counted for 10 min. Tissue samples were thawed, dried, and weighed; then they were discolored with 10 ml of 30% H_2O_2 and homogenized with 10 ml of distilled water. A 3-ml aliquot was added to 10 ml of the scintillation cocktail and counted for 10 min.

RESULTS AND DISCUSSION

As expected (8), radiochromatographic analyses showed that the radioactivity in the various organs was due to I, even after administration of II, since the *in vivo* II → I transformation is a fast process, $t_{1/2}$ = 1.04 min (7). Further metabolism of the I formed was a much slower process. The results are summarized in Table I.

The elimination of I from various organs, when administered as I and as II, is compared in Figs. 1–6. Except in the blood, I resulting from delivery by II achieved a significantly higher concentration than when administered as itself. This result could be expected based on the lipophilic character of II since its pKa = 6.32 (6); thus, it is essentially in the free base form in the plasma. The results confirmed again that a significant amount of I reaches the brain shortly after II administration while I is unable to penetrate the blood–brain barrier.

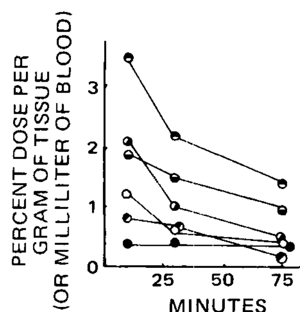


Figure 7—*Time dependence of relative distribution of I in various organs of mice after administration of 5 mg/kg iv of I, in blood (○), brain (●), heart (◐), diaphragm (◐), lungs (◒), and kidneys (◓).*

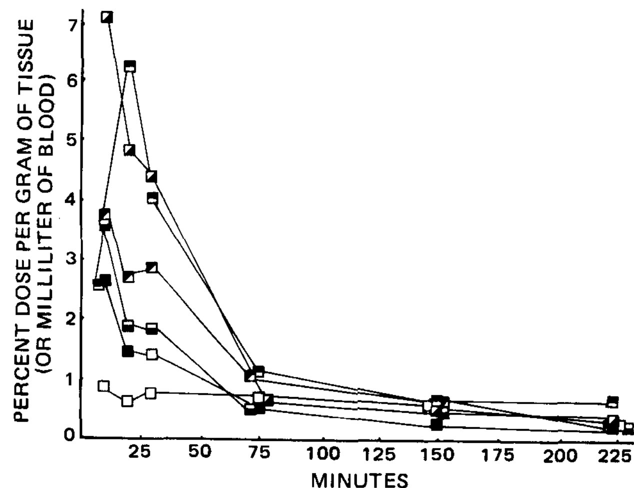


Figure 8—*Time dependence of relative distribution of I in various organs of mice, after administration of 5 mg/kg iv of II, in blood (□), brain (■), heart (◪), diaphragm (◩), lungs (⬓), and kidneys (⬒).*

The efflux of I from the various organs is compared in Figs. 7 and 8. The apparent elimination rate from the brain almost parallels the elimination rate from the other organs, except the blood.

In other words, the blood–brain barrier does not present any barrier properties for the efflux of I from the brain. The mechanism by which it is eliminated is unknown. It could be actively transported or eliminated through the arachnoid villi.

If it is accepted that larger, aliphatic quaternary salts are eliminated slowly (12) while, based on the present study, small pyridinium quaternary salts are excreted quickly from the brain, it seems possible that drugs could be delivered and concentrated specifically in the brain by using a carrier part. The carrier part would be eliminated quickly after its liberation from the quaternary derivative (III) formed as a result of the oxidative transformation of the dihydropyridine part.

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This paper is the fifth in the series: Improved Delivery through Biological Membranes. Previous paper: N. Bodor, K. B. Sloan, T. Higuchi, and K. Sasahara, *J. Med. Chem.*, **20,** 1435 (1977).

[1] New England Nuclear.

[2] An LS-150 Beckman liquid scintillation counter was used. Aquasol (New England Nuclear) was used as the scintillation cocktail.