IN VIVO ELIMINATION OF DIHYDROPYRIDINE DRUG DELIVERY CARRIERS FROM BRAIN AND BLOOD OF RATS

N. Bodor, M.E. El-Kommos and Ch. Nath
Department of Medicinal Chemistry, College of Pharmacy
University of Florida, Gainesville, U.S.A. and Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Assiut University, Assiut, Egypt.

ABSTRACT

The dihydropyridine pyridinium salt redox delivery system, used for specific delivery and sustained release of drugs in the brain was studied. The rates of elimination of the ester and acid metabolites of 1-methyl-3-ethoxycarbonyl-1,4-dihydropyridine and of 1-(2-ethoxycarbonyl)ethyl-1,4-dihydronicotinamide from brain and blood of rats after systemic administration of dihydro carrier-ethanol couples were investigated, taking ethanol as a model drug. It was found that the quaternary species are eliminated relatively fast, which supports the hypothesis for existence of an active transport mechanism for eliminating organic ions from the brain. The results can serve as a primary evidence for the suggestion that the carrier and its metabolites will not impair central nervous system functions.

INTRODUCTION

The delivery of drugs specifically to the brain or any other organ or site is often seriously limited by the lipoidal biological barriers e.g. the functional barrier of the tightly joined endothelial capillary wall, referred to as the blood brain barrier (BBB). Among the various possible ways to achieve site-
specific or organ-specific delivery, the so-called "Chemical 
Delivery System" (CDS) is the most flexible and offers possibili-
ties for specific delivery to the brain, skin, eye or to other 
organs and sites. Properly designed, the drug delivery system 
should concentrate the desired active agent at its site of action 
and reduce its concentration in other locations. The net result 
is an improvement in the efficacy of the drug and decrease in 
its toxicity.

This concept has led to the successful site-specific and 
controlled delivery of various drugs by affecting the bidirec-
tional movement of the drug in and out of the brain with a dihy-
dropyridine = pyridinium redox carrier system. This carrier 
can function as a site-specific and sustained release CDS. The 
success of this system is related to its ability to transiently 
convert a highly polar compound, pyridinium salt, into a limi-
ted compound, dihydropyridine. In addition, this system has been 
well characterized because of its biological importance in the 
NAD+ = NADH coenzyme pair. It has been applied to brain spe-
cific sustained release of 1-methylpyridinium-2-carboxaldehyde4-6, 
testosterone7, dopamine8,9, phenylethylamine10 and others.

The present work was designed to study the rates of elimina-
tion of two drug delivery carriers of the dihydropyridine deriva-
tives, taking ethanol as a model drug. The carriers chosen were 
1-methyl-3-carboxy-1,4-dihydropyridine and 1-(2-carboxy) ethyl-
1,4-dihydronicotinamide.

EXPERIMENTAL

Materials:

1-methyl-3-\{ethoxycarbonyl\}1,4-dihydropyridine (I), 1-methyl-3-\{ethoxycarbo-
nyl\}pyridinium iodide (II), 1-methyl-3-carboxy pyridinium iodide (III), 
1-(2-ethoxycarbonyl)ethyl-1,4-dihydronicotinamide (IV), 1-(2-ethoxycarbonyl)ethyl
nicotinamide (V) and 1-(2-carboxy)ethyl nicotinamide (VI) were prepared by Marcus Brewster and Jirina Vlasak (Pharmatec Inc., Gainesville, Florida, U.S.A.) and checked for purity by UV, TLC and NMR.

Procedures:

High pressure liquid chromatographic methods were developed for analysis of the quaternary esters II; V and acids III; VI. The equipment used consisted of a Beckman Model 112 solvent delivery system, Model 210 sample injection valve and analytical UV detector Model 153, operated at 254 nm. Two columns were used; 25 cm x 4.1 mm (internal diameter) Versapack C18 column (Alltech/Applied Science Labs) and 25 cm x 5.0 mm (internal diameter) Partisil-10 SCX column (Phenomenex). Both columns were operated at ambient temperature. Four mobile phases were used in this study:

1 - 0.001 M pentane sulphonate sodium salt in acetonitrile/0.01 M ammonium phosphate dibasic (1:1) with added 5% tetrahydrofuran.
2 - 0.003 M phosphoric acid in the mixture acetonitrile/methanol/water (5:10:85).
3 - 5 x 10^{-4} M ammonium phosphate dibasic in the mixture acetonitrile/methanol/water (20:10:70).
4 - 0.2% w/v solution of diocetyl sulfosuccinate sodium in the mixture methanol/acetonitrile/formic acid/water (30:15:1:54).

For the analysis of II in blood and brain samples, system (1) was used with Versapack C18 column. At a flow rate of 2.0 ml/min, II had a retention time of 9.2 min. For analysis of III in brain samples, system (2) was used with partisil-10 SCX column. At a flow rate of 2.0 ml/min, III had a retention time of 14.8 min. For analysis of III in blood samples, system (3) was used with partisil-10 SCX column. At a flow rate of 1.0 ml/min, III had a retention time of 5.6 min.

For analysis of V and VI in blood and brain samples, system (4) was used with Versapack C18 column. At a flow rate of 3.0 ml/min, VI had a retention time of 5.0 min and V had a retention time of 10.3 min.
In Vivo Administration of Dihydropyridine Carrier - Ethanol Couples:

A group of 44 male Sprague Dawley rats of average weight 200 + 20 g was anesthetized with Inovar-Vet (50 µl/rat). Four of them were injected with dimethylsulfoxide (0.5 ml/Kg body weight) via the jugular vein by infusion at a rate of 0.1 ml/min. The other rats were injected by the same method with the dihydro carrier - ethanol couple (50 mg/Kg body weight) dissolved in dimethylsulfoxide (0.5 ml/Kg body weight). At 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, 12.0, 24.0, 48.0 and 60.0 hour intervals after treatment four rats at a time were subjected to the following procedure: 3 ml of blood were collected into heparinized syringes via cardiac puncture and after decapitation, the brains were dissected from the cranium. Whole blood and brain samples were immediately frozen until processed.

Study of the Rate of Elimination of II and III from Brain and Blood after Administration of I:

The brains were weighed, homogenized with 2.0 ml of distilled water and the homogenate mixed with 8.0 ml of 25% acetonitrile in methanol. The mixture was centrifuged for 20 min at a speed of 90,000 cps in Clay Adams Centrifuge. The supernatent solution was filtered through Millipore filter type SJHV, pore size 0.45 µm. For analysis of II, 100 µl were injected into HPLC and analysed using system (1). For analysis of III, 50 µl were injected into HPLC using system (2).

Blood samples were analysed for II by mixing 1.0 ml of the sample with 4.0 ml of acetonitrile, centrifugation and filtration of the mixture as above. Of the filtrate, 100 µl were injected into HPLC and analysed using system (1). For determination of III in blood, 1.0 ml sample was mixed with 4.0 ml of methanol, the mixture centrifuged and filtered as above, then 20 µl were injected into HPLC using system (3).
Study of the Rate of Elimination of V and VI from Brain and Blood after Administration of VI:

1.0 ml of blood sample was shaken with 4.0 ml of 0.5 % w/v solution of ascorbic acid in methanol, centrifuged, filtered as usual, and 20 μl of the filtered supernatent solution were injected into HPLC and analysed using system (4).

The brains were weighed, homogenized with 1.0 ml distilled water, mixed with 4.0 ml of 0.5 % w/v solution of ascorbic acid in methanol. The mixture was centrifuged and filtered as usual, 20 μl of the filtered supernatent solution were injected into HPLC and analysed using system (4).

RESULTS AND DISCUSSION

The concept of CDS is based on the sequences, illustrated in Scheme 1. The drug \{D\} is either coupled to a tertiary carrier \{QC\}+ directly, and the obtained \{D-QC\}+ is reduced chemically to the lipoidal dihydro form \{D-DHC\}. Alternatively, the drug \{D\} can be directly coupled with the dihydro carrier \{DHC\}. After in vivo administration of this \{D-DHC\}, it is quickly distributed (K0) throughout the body, including the brain (K1) and in the blood (K2) where it is oxidized to the original \{D-QC\}+ quaternary salt. \{D-QC\}+, due to its ionic hydrophilic nature, should be eliminated fast from the blood, while BBB should prevent its elimination from the brain (K2 >> K3, K2 << K7). Enzymatic cleavage of \{D-QC\}+, that is "locked in" the brain will result in a sustained delivery of the drug \{D\} followed by its normal elimination (K5) and metabolism. The oxidized cleaved carrier \{QC\}+ and its metabolites must be eliminated rapidly from the brain (K5 >> K3) for proper and specific delivery. Metabolism of the two investigated carrier - drug systems is illustrated in Scheme 2.
The distributions of II and III at various time intervals in the brain and blood after i.v. administration of I to rats are summarized in Tables I and II and Fig. 1 and 2. It is evident from data of Tables I and II that:

a - Comparatively higher concentrations of II and III were found in the brains of rats in comparison with blood. This means that significant amount of I reaches the brain shortly after i.v. administration.

b - The carrier is eliminated fairly rapidly from the brain and from the blood after its liberation from the quaternary derivative \[ \text{(D-QC)}^+ \], formed as a result of the oxidative transformation of the dihydropyridine part (see Scheme 1).

From Fig. 1, it seems that after i.v. administration of I, it is concentrated in the brain, where it is oxidized rapidly to the ester II. Some of the resulting ester may eliminate as it is from the brain and some hydrolyses into the quaternary acid III, and this may explain the two-phase elimination of the acid from the brain. These results support the hypothesis for active transport mechanism for eliminating organic ions from the brain. In the blood, the two quaternary compounds II and III eliminate relatively fast \( t_{1/2} = 1.90 \) and \( 2.72 \) hours respectively. It was found also that the rate of in vivo elimination of the ester II from the blood is the same as that obtained for the hydrolysis of II in vitro (in blood at \( 37^\circ \)C).

The distributions of V and VI in the brain and blood at various time intervals after i.v. administration of IV to rats are summarized in Tables III and IV and Fig. 3. These results show that the ester V is not detected neither in the brain nor in the blood suggesting that in the nicotinamide series the dihydro carrier drug couple is hydrolyzed before being oxidized to the quaternary acid VI (see Scheme 2), probably because the long alkyl side
In Vivo Elimination of Dihydropyridine Drug Delivery Carriers from Brain and Blood of Rats.

chain on ring nitrogen renders the molecule more resistant to oxidation.

To sum up, all these findings can serve as a primary evidence for the suggestion that the carrier and its metabolites will be eliminated fairly rapidly from the brain and thus will not be expected to impair central nervous system functions.
Table I. Changes in the brain and blood levels of II and III with time after systemic administration of I.

<table>
<thead>
<tr>
<th>Time, hr</th>
<th>Concentration of II</th>
<th>Concentration of III</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>in brain</td>
<td>in blood</td>
</tr>
<tr>
<td>0.5</td>
<td>46.10 ± 0.80</td>
<td>4.49 ± 0.97</td>
</tr>
<tr>
<td>1.0</td>
<td>35.20 ± 1.11</td>
<td>3.78 ± 0.02</td>
</tr>
<tr>
<td>2.0</td>
<td>27.60 ± 0.97</td>
<td>2.60 ± 0.47</td>
</tr>
<tr>
<td>4.0</td>
<td>7.73 ± 1.23</td>
<td>0.00</td>
</tr>
<tr>
<td>8.0</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>12.0</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>24.0</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>48.0</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>60.0</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

@ Concentrations in µg/ml blood or in µg/g brain tissue.
@@ Each point is the mean of four animals ± SD.

Table II. Rates of in vivo elimination of II and III from brain and blood of rats after systemic administration of I.

<table>
<thead>
<tr>
<th>Organ</th>
<th>Compound</th>
<th>K, hours⁻¹</th>
<th>r</th>
<th>a</th>
<th>t₁/₂, hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>brain</td>
<td>II</td>
<td>0.50 ± 0.059</td>
<td>0.9867</td>
<td>62.41</td>
<td>1.38</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>0.13 ± 0.009</td>
<td>0.9915</td>
<td>10.51</td>
<td>5.34</td>
</tr>
<tr>
<td>blood</td>
<td>II</td>
<td>0.37 ± 0.007</td>
<td>0.9998</td>
<td>5.41</td>
<td>1.90</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>0.25 ± 0.025</td>
<td>0.9817</td>
<td>10.02</td>
<td>2.72</td>
</tr>
</tbody>
</table>
In Vivo Elimination of Dihydropyridine Drug Delivery Carriers from Brain and Blood of Rats.

Table III. Changes in the Brain and Blood Levels of V and VI with time after Systemic Administration of IV.

<table>
<thead>
<tr>
<th>Time, hr</th>
<th>Concentration of V in brain</th>
<th>Concentration of V in blood</th>
<th>Concentration of VI in brain</th>
<th>Concentration of VI in blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>0.00</td>
<td>0.00</td>
<td>9.41 ± 0.56</td>
<td>11.10 ± 1.20</td>
</tr>
<tr>
<td>0.50</td>
<td>0.00</td>
<td>0.00</td>
<td>7.01 ± 2.86</td>
<td>7.67 ± 1.32</td>
</tr>
<tr>
<td>1.0</td>
<td>0.00</td>
<td>0.00</td>
<td>4.63 ± 1.81</td>
<td>4.64 ± 1.95</td>
</tr>
<tr>
<td>2.0</td>
<td>0.00</td>
<td>0.00</td>
<td>3.75 ± 1.87</td>
<td>3.99 ± 1.48</td>
</tr>
<tr>
<td>4.0</td>
<td>0.00</td>
<td>0.00</td>
<td>1.53 ± 1.02</td>
<td>0.45 ± 0.61</td>
</tr>
<tr>
<td>8.0</td>
<td>0.00</td>
<td>0.00</td>
<td>1.28 ± 0.52</td>
<td>1.80 ± 2.55</td>
</tr>
<tr>
<td>12.0</td>
<td>0.00</td>
<td>0.00</td>
<td>2.47 ± 1.11</td>
<td>0.00</td>
</tr>
<tr>
<td>24.0</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>48.0</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>60.0</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

@ Concentrations in µg/ml blood or in µg/g brain tissue.
@@ Each point is the mean of four animals ± SD.

Table IV. Rates of in vivo Elimination of VI from Brain and Blood of rats after Systemic Administration of IV.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Brain data</th>
<th>Blood data</th>
</tr>
</thead>
<tbody>
<tr>
<td>K, hours⁻¹</td>
<td>0.25 ± 0.056</td>
<td>0.80 ± 0.0999</td>
</tr>
<tr>
<td>r</td>
<td>0.9114</td>
<td>0.9779</td>
</tr>
<tr>
<td>a</td>
<td>6.92</td>
<td>12.86</td>
</tr>
<tr>
<td>t₁/₂,hr</td>
<td>2.80</td>
<td>0.86</td>
</tr>
</tbody>
</table>
Scheme 1. Chemical delivery of drugs to the brain.

In vivo Elimination of Phenytoinidine Drug Delivery Carriers

From Brain and Blood of rats.
Fig. 1: Changes in the brain and blood levels of II and III after systemic administration of I.

--- elimination of II from brain.
--- elimination of III from brain.
........... elimination of II from blood.
--- elimination of III from blood.
Fig. 2: Semilogarithmic plot of brain and blood levels of II and III as a function of time after systemic administration of I.

- - - - elimination of II from brain.
- - - - elimination of III from brain.
- - - - elimination of II from blood.
- - - - elimination of III from blood.
Fig. 3: Semilogarithmic plot of brain and blood levels of VI as a function of time after systemic administration of IV.

- ○ ○ ○ brain levels.
- △ △ △ blood levels.
REFERENCES


5) E. Shek, T. Higuchi and N. Bodor, ibid, 19, 108 (1976).

6) E. Shek, T. Higuchi and N. Bodor, ibid, 19, 113 (1976).


Acknowledgment

The authors wish to thank the assistance of Dr. Efraim Shek (Pharmatec Inc., Gainesville, Florida, U.S.A.).
دراسة ازالة مشتقات الدايبيدروبيريدين
حاملة الأدوية للإعفاء من مخ ودم الفشل

نيكولاس بودر ، ميشيل إيليا القمص ، تشانديوار نات
قسم الكيمياء الطبية - كلية الصيدلة - جامعة فلوريدا بالولايات المتحدة الأمريكية
وقسم الكيمياء الصيدلية - كلية الصيدلة - جامعة 서울 - مصر

في هذا البحث تم دراسة مشتقات الدايبيدروبيريدين التي تستخدم لحمل
الأدوية إلى أعضاء معينة في جسم الإنسان (وبيضة خاصة إلى المخ) حيث يتم
توليد الدواء ببضع من المركب المزدوج والذي يتم حقنه في الوريد. وقد تم
حساب معدلات ازالة نواتج أيبج المركبات المزدوجة والمحقنة (سواء كانت على
هيئة استرات أو احماض) من مخ ودم الفشل بعد تتبع تركيزات نواتج الأيبج
بعد الحقن بفترات زمنية مختلفة $\frac{1}{4}$، $\frac{3}{4}$، 1، 2، 4، 8، 12، 24، 48، 60 ساعة.
وقد تم تحليل كل هذه العينات بطريقة كروماتوغرافيا الغضب العالى السائلية
مع مقارنتها بعينات اخدة من مخ ودم فشل لم تحقن بالمركبات المزدوجة.

وقد دلت نتائج البحث أن نواتج أيبج المركبات المزدوجة والتي تم استخدام
فيها مشتقات الدايبيدروبيريدين كمركبات حاملة للأدوية إلى الأعضاء إلى
بسرعة معقولة من مخ ودم الفشل التي حقنت بالمركبات المزدوجة.

وهذه النتائج يمكن أن تكون برهاة على أن المركبات حاملة الأدوية
وتواجد أيبج لا تؤثر تأثيرا سلبيا على المخ ولا على الجهاز العصبي المركب-
رني مما يشجع علىستمر في تشييد مركبات مزدوجة من الدايبيدروبيريدي-
ن مع الأدوية المختلفة.

received in 28/4/1986 & accepted in 3/6/1986