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

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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 adminstration 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 possibilities for specific delivery to the brain, skin, eye or to other organs and sites[1-3]. 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 bidirectional movement of the drug in and out of the brain with a dihydropyridine $\rightleftharpoons$ 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 lipoidal compound, dihydropyridine. In addition, this system has been well characterized because of its biological importance in the $NAD^+ \rightleftharpoons$ NADH coenzyme pair. It has been applied to brain specific sustained release of 1-methylpyridinium-2-carbaldoxime[4-6], testosterone[7], dopamine[8,9], phenylethylamine[10] and others.

The present work was designed to study the rates of elimination of two drug delivery carriers of the dihydropyridine derivatives, 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-(ethoxycarbonyl)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 C_{18} 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 sulphonic acid 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 - 5×10^{-4} M ammonium phosphate dibasic in the mixture acetonitrile/methanol/water (20 : 10 : 70).

4 - 0.2 % w/v solution of dioctyl 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 C_{18} 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 C_{18} 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 Adminstration of Dihydropyridine Carrier - Ethanol Couples:

A group of 44 male Sprague Dawley rats of average weight 200 ± 20 g was anethetized 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 Adminstration 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 filteration 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 Adminstration 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 $\{D\text{-}QC\}^+$ 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 adminstration of this {D-DHC}, it is quickly distributed (K_0) throughout the body, including the brain (K_1^1) and in the blood (K_1^2) where it is oxidized to the original $\{D\text{-}QC\}^+$ quaternary salt. $\{D\text{-}QC\}^+$, due to its ionic hydrophilic nature, should be eliminated fast from the blood, while BBB should prevent its elimination from the brain ($K_2 >> K_3$, $K_2 << K_7$). Enzymatic cleavage of $\{D\text{-}QC\}^+$, that is "locked in" the brain will result in a sustained delivery of the drug {D} followed by its normal elimination (K_5^1) and metabolism. The oxidized cleaved carrier $\{QC\}^+$ and its metabolites must be eliminated rapidly from the brain ($K_6^1 >> K_3$) 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. adminstration 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. adminstration.

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

From Fig. 1, it seems that after i.v. adminstration 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^{o}C).

The distributions of V and VI in the brain and blood at various time intervals after i.v. adminstration of IV to rate 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

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.

Time,	Concentration of II[@]		Concentration of III[@]	
hr	in brain[@@]	in blood[@@]	in brain[@@]	in blood[@@]
0.5	46.10 ± 0.80	4.49 ± 0.97	20.40 ± 1.79	9.37 ± 1.08
1.0	35.20 ± 1.11	3.78 ± 0.02	11.14 ± 0.97	8.63 ± 1.09
2.0	27.60 ± 0.97	2.60 ± 0.47	6.80 ± 0.51	6.21 ± 0.42
4.0	7.73 ± 1.23	0.00	6.35 ± 0.80	3.37 ± 0.54
8.0	0.00	0.00	3.20 ± 0.26	0.88 ± 0.16
12.0	0.00	0.00	2.54 ± 0.15	0.62 ± 0.06
24.0	0.00	0.00	0.46 ± 0.08	0.00
48.0	0.00	0.00	0.00	0.00
60.0	0.00	0.00	0.00	0.00

@ 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.

Organ	Compound	K, hours^{-1}	r	a	$t_{1/2}$,hr
brain	II	0.50 ± 0.059	0.9867	62.41	1.38
	III	0.13 ± 0.009	0.9915	10.51	5.34
blood	II	0.37 ± 0.007	0.9998	5 .41	1.90
	III	0.25 ± 0.025	0.9817	10.02	2.72

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

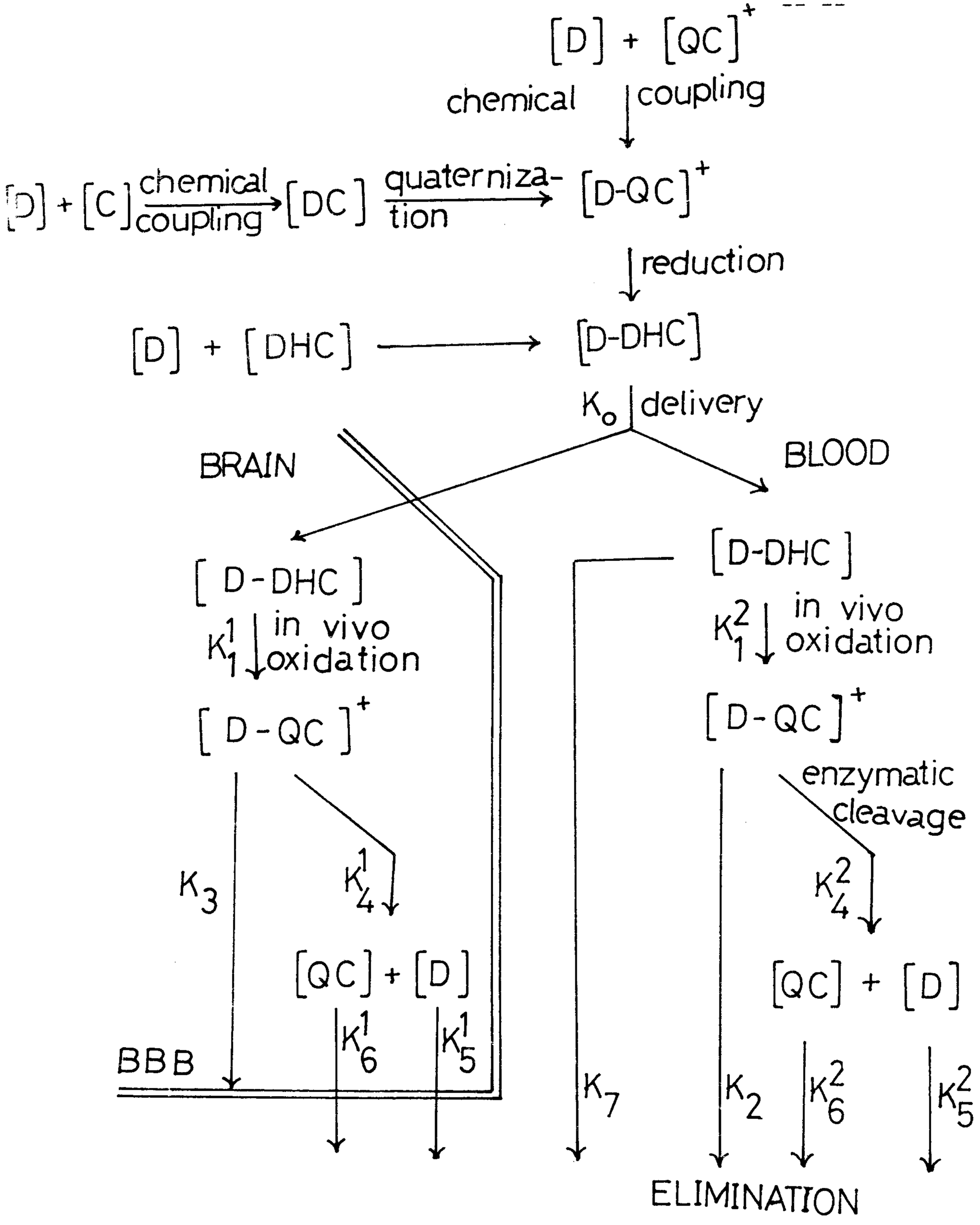
Time, hr	Concentration of V[@]		Concentration of VI[@]	
	in brain[@@]	in blood[@@]	in brain[@@]	in blood[@@]
0.25	0.00	0.00	9.41 ± 0.56	11.10 ± 1.20
0.50	0.00	0.00	7.01 ± 2.86	7.67 ± 1.32
1.0	0.00	0.00	4.63 ± 1.81	4.64 ± 1.95
2.0	0.00	0.00	3.75 ± 1.87	3.99 ± 1.48
4.0	0.00	0.00	1.53 ± 1.02	0.45 ± 0.61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

@ Concentrations in μg/ml blood or in μg/g brain tissue.

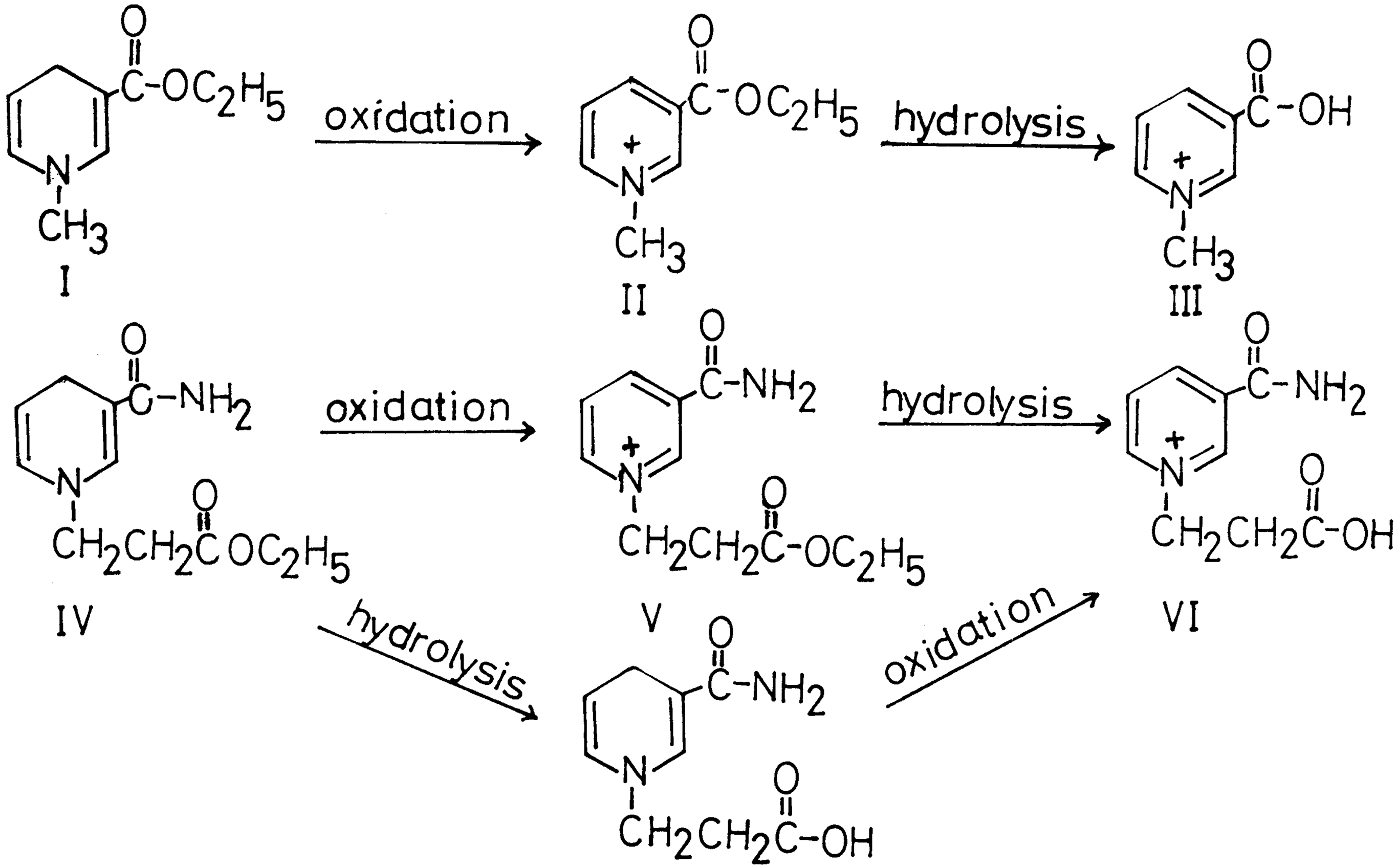
@@ Each point is the mean of four animals ± SD.

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

Parameter	Brain data	Blood data
K, $hours^{-1}$	0.25 ± 0.056	0.80 ± 0.0999
r	0.9114	0.9779
a	6.92	12.86
$t_{1/2}$, hr	2.80	0.86



Scheme 1. Chemical delivery of drugs to the brain.



Scheme 2. Metabolic pathways of investigated drug - carrier couples.

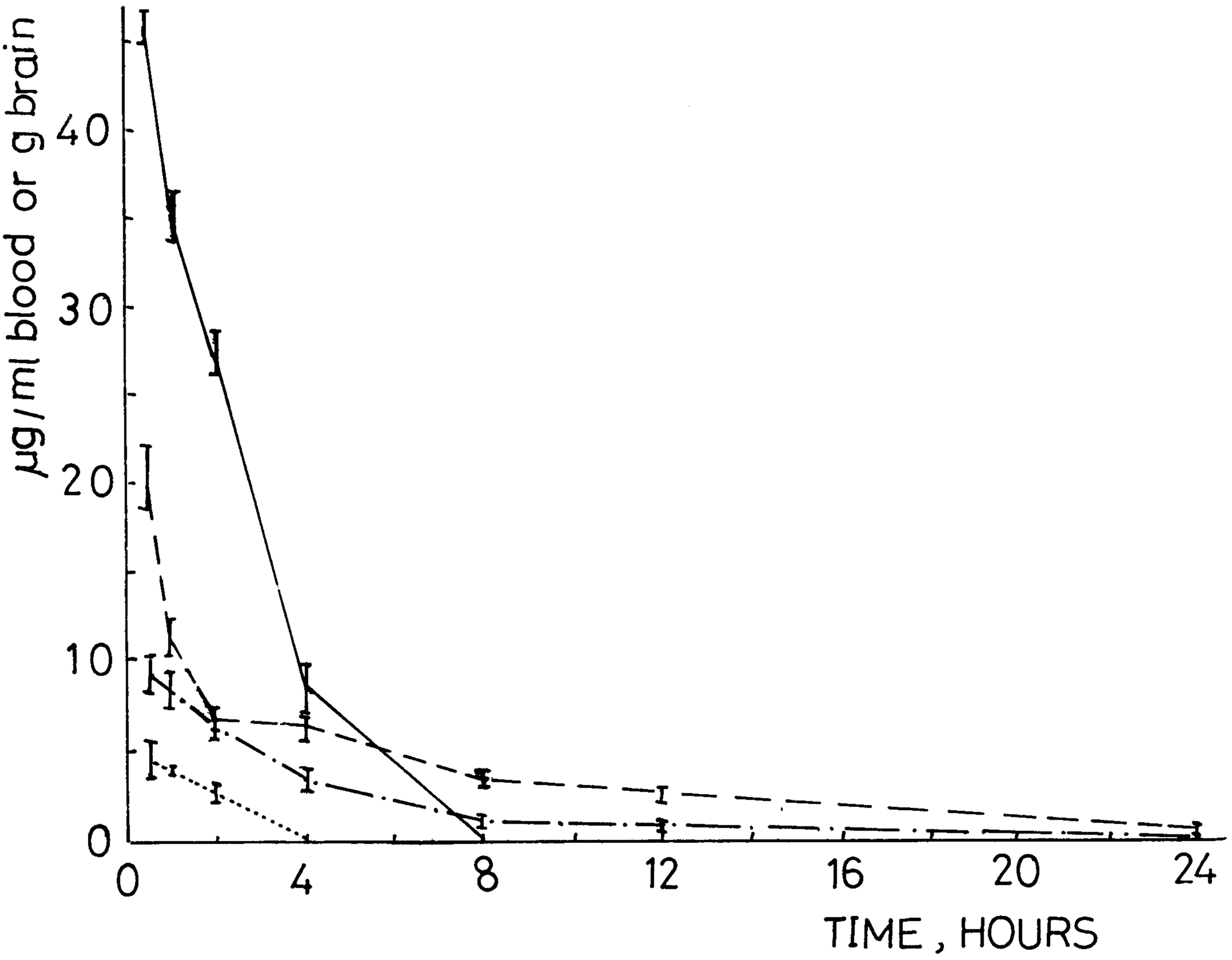


Fig. 1: Changes in the brain and blood levels of II and III after systemic administration of I.

———— elimination of II from brain.
— — — — elimination of III from brain.
..... elimination of II from blood.
—.—.— elimination of III from blood.

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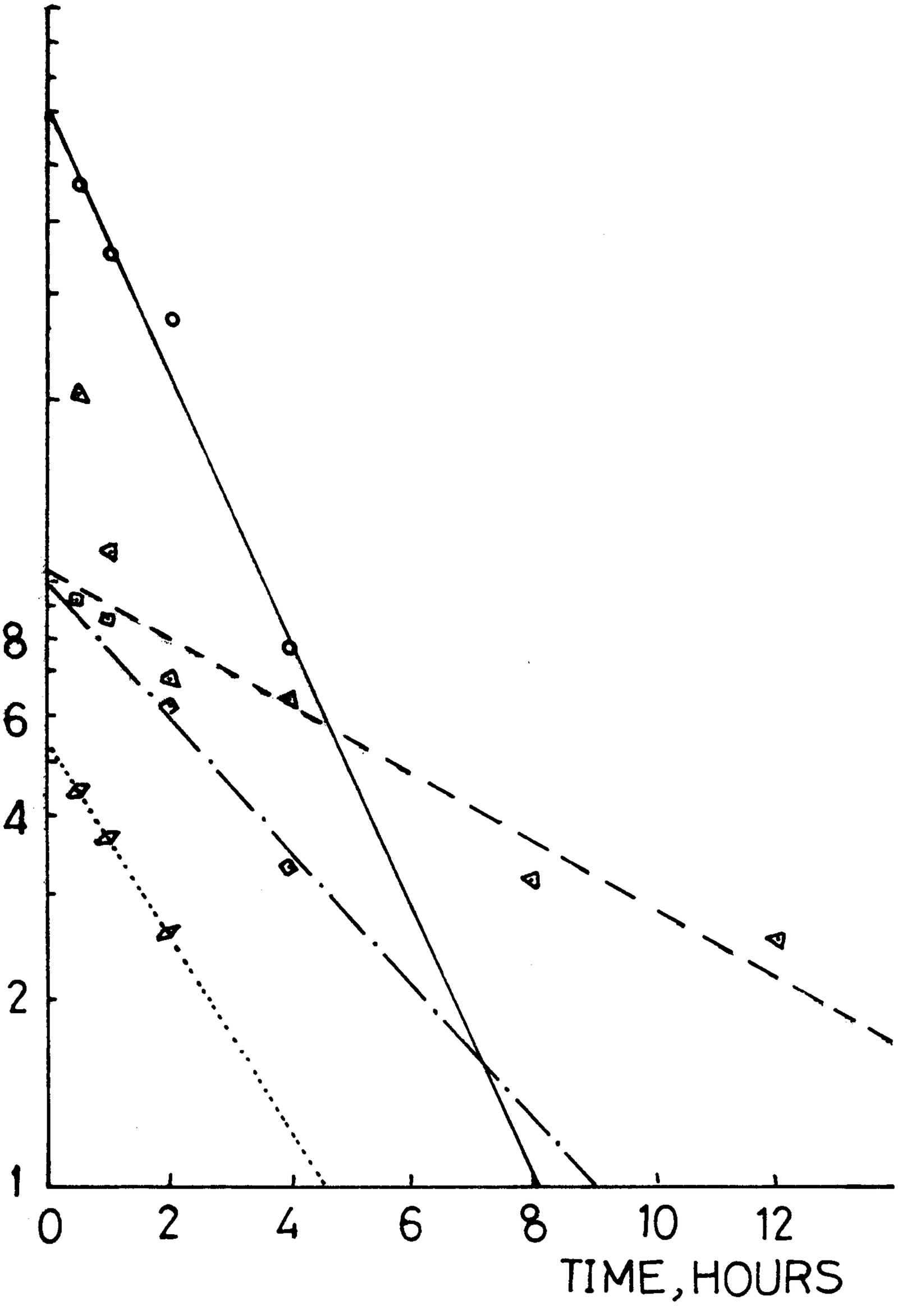

Fig. 2: Semilogarithmic plot of brain and blood levels of II and III as a function of time after systemic administration of I.

—⊙—⊙— elimination of II from brain.
—△- - -△- elimination of III from brain.
..... elimination of II from blood.
□·—□—·— elimination of III from blood.

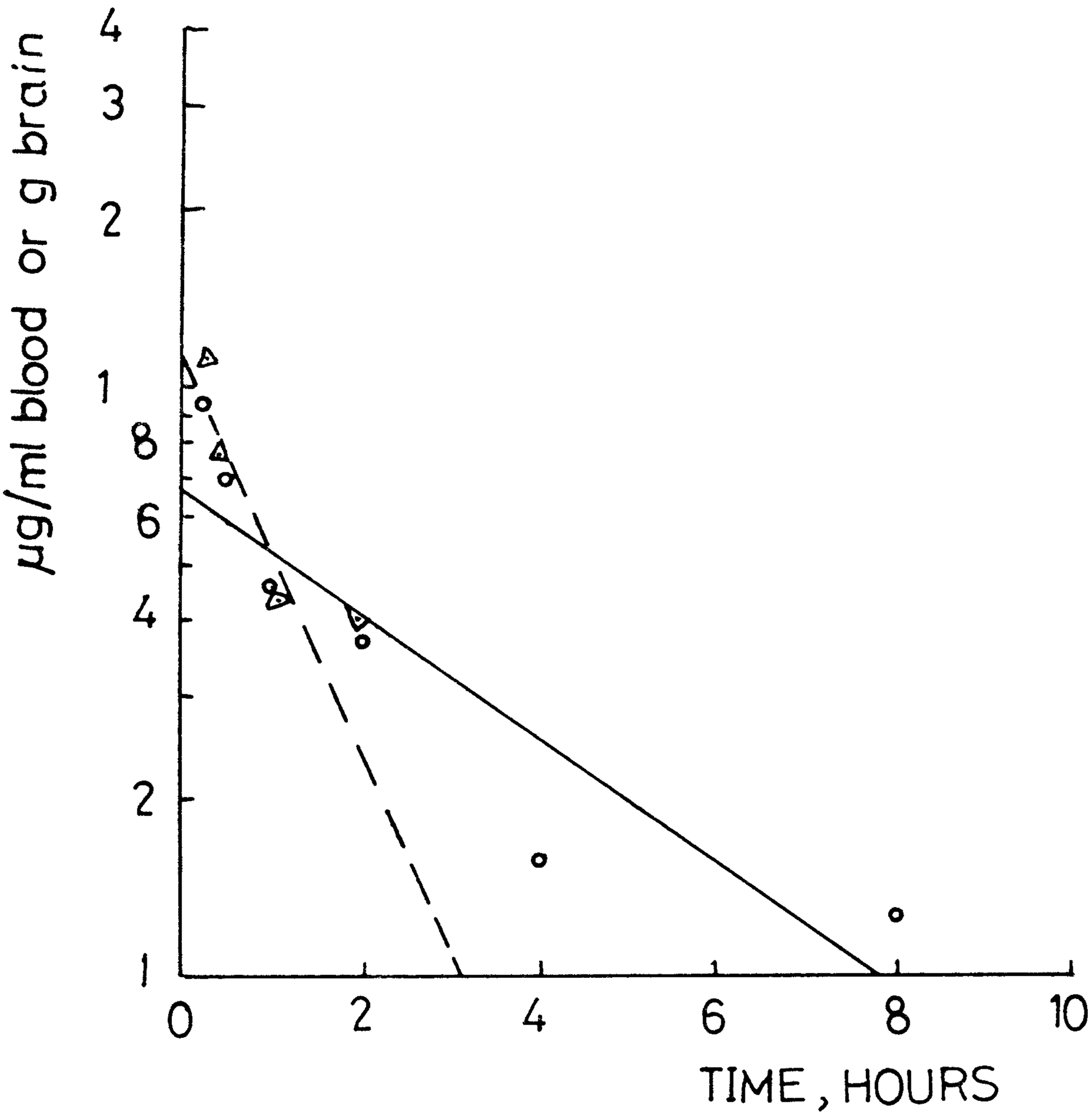

Fig. 3: Semilogarithmic plot of brain and blood levels of VI as a function of time after systemic administration of IV.

brain levels.

blood levels.

In Vivo Elimination of Dihydropyridine Drug Delivery Carriers from Brain and Blood of Rats.

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دراسة ازالة مشتقات الدايهيدروبيريدين حاملة الادوية للاعضاء من مخ ودم الفئران

نيكولاس بودر ، ميشيل ايليا القمص ، تشانديشوار ناث
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وقسم الكيمياء الصيدلية ـ كلية الصيدلة ـ جامعة اسيوط ـ مصر

فى هذا البحث تمت دراسة مشتقات الدايهيدروبيريدين التى تستخدم لحمل الادوية الى اعضاء معينة فى جسم الانسان (وبصفة خاصة الى المخ) حيث يتم توليد الدواء ببطء من المركب المزدوج والذى يتم حقنه فى الوريد . وقد تم حساب معدلات ازالة نواتج ايض المركبات المزدوجة والمحقونة (سواء كانت على هيئة استرات أو احماض) من مخ ودم الفئران بعد تتبع تركيزات نواتج الايض بعد الحقن بفترات زمنية مختلفة $\frac{1}{4}$ ، $\frac{1}{2}$ ، ١، ٢، ٤، ٨، ١٢، ٢٤، ٤٨، ٦٠ ساعة وقد تم تحليل كل هذه العينات بطريقة كروماتوجرافيا الضغط العالى السائلة مع مقارنتها بعينات اخذت من مخ ودم فئران لم تحقن بالمركبات المزدوجة .

وقد دلت نتائج البحث على أن نواتج ايض المركبات المزدوجة والتى تستخدم فيها مشتقات الدايهيدروبيريدين كمركبات حاملة للادوية الى الاعضاء تزول بسرعة معقولة من مخ ودم الفئران التى حقنت بالمركبات المزدوجة .

وهذه النتائج يمكن ان تكون برهانا على ان المركبات حاملة الادوية ونواتج ايضها لا تؤثر تأثيرا سيئا على المخ ولا على الجهاز العصبى المركزى مما يشجع على الاستمرار فى تشييد مركبات مزدوجة من الدايهيدروبيريدين مع الادوية المختلفة .

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