INTERACTION OF L-CARNITINE AND ^{3}H-7-METHOXYTACRINE FROM THE ASPECT OF THE BIODISTRIBUTION AND EFFECT ON THE BRAIN ACETYLCHOLINESTERASE ACTIVITY

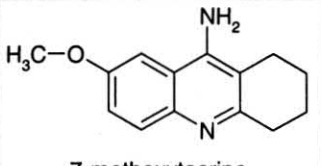
[1]Zbyněk SVOBODA, [2]Josef HERINK, [2]Josef BAJGAR, [1]Jaroslav KVĚTINA,
[3]Bohuslav ČERNÝ
[1]Institute of Experimental Biopharmaceutics, Joint Laboratory of the Czech Academy of Sciences and PRO.MED.CS Praha a. s.
[2]Department of Toxicology of Purkyně Military Medical Academy, Hradec Králové
[3]1[st] Faculty of Medicine, Charles University, Praha, Czech Republic

Summary

7-methoxytacrine (MEOTA) is a reversible inhibitor of acetylcholinesterase and it belongs to a group of drugs, which are known as activators of cognitive functions and could be useful in the therapy of some neurodegenerative disorders such as Alzheimer's disease. The lower toxicity is its advantage in comparison with tacrine. L-carnitine (CRT) was used to increase penetration of MEOTA through the blood brain barrier in rat. ^{3}H-MEOTA was administered intravenously (5 mg/kg), orally (5 and 0.5 mg/kg) or intramuscularly (100 mg/kg) to rats in order to evaluate its pharmacokinetic properties. The intramuscular administration was chosen for the biodistribution study, where MEOTA was administered in two groups of rats: one group was administered only with MEOTA and the second group was administered CRT (300 mg/kg) orally for 3 consecutive days before MEOTA. Levels of MEOTA were assessed in blood and in the brain tissue samples. In the next experimental step it was evaluated inhibition of acetylcholinesterase (AChE) activity in separate brain areas following administration of MEOTA in interaction with CRT. The results showed that MEOTA crossed blood brain barrier and its levels in the brain were weakly increased after CRT repeated administration. On the other hand the repeated administration of CRT enhanced significantly the inhibiting effect of 7-MEOTA on the brain AChE activity. A comparison of the brain tissue levels of MEOTA under the influence of CRT with the inhibiting effect on the AChE activity in the brain showed that the higher inhibiting effect of MEOTA on AChE activity with CRT is not caused by a global increasing of its concentration in the brain tissue, but it is probably due to an interaction of MEOTA and CRT on active centre of the corresponding enzyme.

Introduction

7-methoxytacrine (MEOTA), a tacrine derivative (synthesised in Purkyně Military Medical Academy, Hradec Králové, Czech Republic), is a substance with demonstrated inhibitory activity on acetylcholinesterase (1). An advantage in comparison with tacrine, which was already approved for treatment of Alzheimer's disease (2, 3), is its lower hepatotoxicity.



7-methoxytacrine

L-carnitine (CRT) is an endogenous compound that facilitates the transport of long-chain fatty acids into the mitochondrial matrix for β-oxidation (4).

An attempt has been made at targeted biodistribution of MEOTA into the cerebral tissue by means of a potentially targeting effect of CRT aiming to increase the therapeutic index of MEOTA (primarily to limit peripheral undesirable effects) and also to closely analyze its central effect by means of examining the changes in the activities of enzymes of cholinergic transfer in interaction with CRT.

Methods

Chemicals:

7-methoxytacrine (Purkyně Military Medical Academy, Hradec Králové, Czech Republic) [^{3}H]-7--methoxytacrine (specific activity 128,5 GBq/mmol, radiochemical purity > 98%, tritiated in the 1[st] Faculty of Medicine, Charles University, Praha, Czech Republic)
L-carnitine hydrochloride (Sigma)

Animals:

Male Wistar Han II rats from the conventional breed Konárovice n. L. (average weight 275 ± 46 g).

Animals were fasted with water *ad libitum* for 12 hours prior to administration.

Working procedure:

A. Pharmacokinetics of MEOTA

Rats were administered [^{3}H]-MEOTA (5 MBq/kg) intravenously (5 mg/kg, n = 7), orally (0.5 mg/kg, n = 6 a 5 mg/kg, n = 6), and intramuscularly (100 mg/kg, n = 6). Blood samples were withdrawn from the cannulated jugular vein at the following time intervals: 10, 30 min., 1, 2, 4, 6, 8, 10, 24, 48, and 72 hours after administration. Cannulation was performed in ether anaesthesia; withdrawals were then carried out in fully conscious animals not requiring fixation.

B. Examination of changes in MEOTA biodistribution in interaction with CRT.

a) One group of rats (n = 6) was intramuscularly administered [^{3}H]-MEOTA in a dose of 100 mg/kg (5 MBq/kg).

b) The second group of rats (n = 6) received CRT in a dose of 300 mg/kg via a gastric tube for three days. On the third day, 30 min. after CTR administration, the rats were administered [3H]--MEOTA in a dose identical with that of the group described in a).

One hour after [^{3}H]-MEOTA administration, the rats of both groups were killed by exsanguination and blood and organ withdrawals (the liver, kidney, heart, hemisphere, cerebellum, medulla oblongata, and hypophysis) were carried out.

Radioactivity of plasma and tissue samples was measured with the use of liquid scintillation spectrometry on the tritium channel for a period of 10 min. using an apparatus BECKMAN LS 5000TD. Sigmafluor (Sigma) served as the scintillation solution. Prior to the measurements, tissue samples were dissolved in a tissue solvent NCS (Amersham).

C. Examination of the changes in the activity of the enzymes of cholinergic transfer in the CNS.

The procedures of administration of drugs and the MEOTA dose were identical with those shown in B. CRT was administered in doses of 100, 250, 300, and 400 mg/kg. Brain tissue withdrawals were performed after killing the animal by exsanguination. Changes in AChE activity were examined in the homogenates (1:10) with phosphate buffer (pH = 7.4) of the following parts of the rat brain: frontal cortex (FC), hippocampus (H), septum (S), and basal ganglia (BG). Activity was measured following the method of Ellman (5) and expressed in nmol/min/100 mg of the wet brain tissue.

Statistics:

Results were expressed as the mean ± standard deviation. F-test and Student t-test were employed for statistical calculations.

Results and Discussion

A long half-time of the pharmacokinetic elimination phase of MEOTA follows from the confrontation of the pharmacokinetic profiles (Figs. 1–4) obtained after individual routes of administration (i.v., p.o., i.m.). It varies from 38 to 40 hours. For further experiments (B., C.), the intramuscular method of administration was selected, where the smallest interindividual variability was found and MEOTA achieves the maximum plasma concentrations regularly one hour after administration (Fig. 4). Due to the fact that it was the sum of radioactivity in plasma samples that was measured, it is not clear whether it is the elimination of MEOTA alone, or also that of its metabolite.

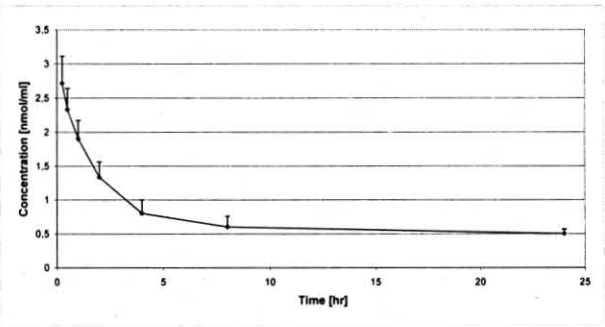


Fig. 1: Pharmacokinetics of 3H-7-methoxytacrine in rat (5 mg/kg, i.v.)

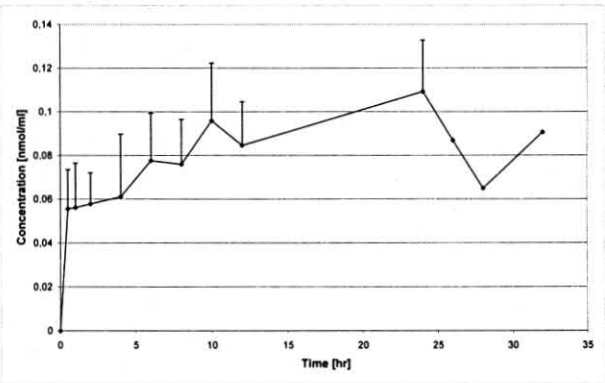


Fig. 2: Pharmacokinetics of 3H-7-methoxytacrine in rat (0.5 mg/kg, p.o.)

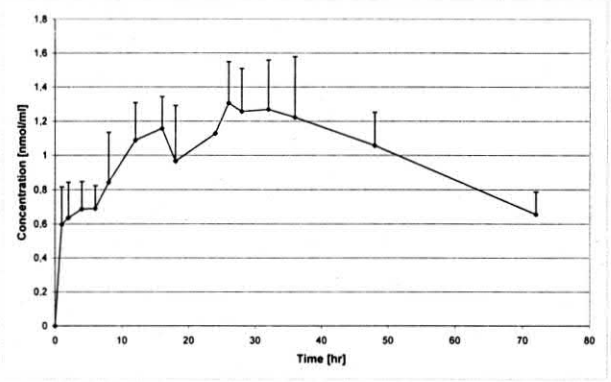


Fig. 3: Pharmacokinetics of 3H-7-methoxytacrine in rat (5 mg/kg, p.o.)

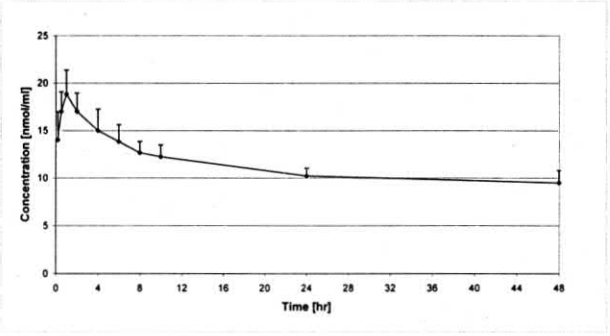


Fig. 4: Pharmacokinetics of 3H-7-methoxytacrine in rat (100 mg/kg, i.m.)

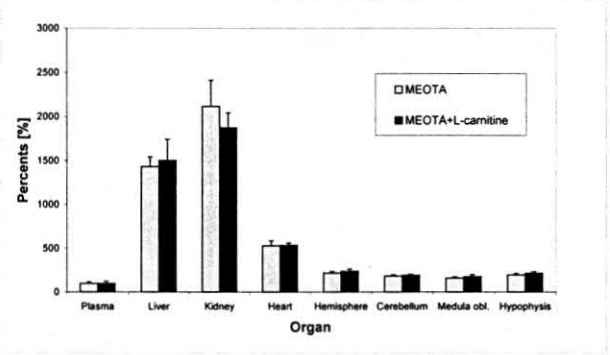


Fig. 5.: Influence of L-carnitine on biodistribution of 3H-7-methoxytacrine in percents (plasma = 100 %)

It has been found in the biodistributional part of the study that in the steady-state distributional pharmacokinetic stage MEOTA passes the haematoencephalic barrier and is relatively well distributed into the brain tissue (Fig. 5). Premedication with CRT for a period of three days then resulted in a weak increase in MEOTA concentration not only in the CNS, but also in the liver tissue, which, nevertheless, was not statistically significant.

Table 1

AChE activity influenced by MEOTA in interaction with CRT

Group	Frontal cortex	Hippocampus	Septum	Basal ganglia
MEOTA	270,2	168,5	586,6	1346,3
CRT100 + MEOTA	183,7*	190,0	426,5*	1057,5
CRT250 + MEOTA	167,5*	174,2	360,2*	642,3*
CRT300 + MEOTA	157,8*	161,3	320,2*	490,2*
CRT400 + MEOTA	172,2*	175,8	382,3*	525,5*

*$p < 0,05$

The interpretation of the interaction of MEOTA with CRT at the level of cerebral AChE (Tab. 1) is not simple; there appears a certain dose-dependence with the optimum at the selected "medium dose." As far as the differences in AChE activity in the individual cerebral zones are concerned, peroral premedication with CRT resulted in increased inhibitory potency of MEOTA: in the case of the smallest selected dose of CRT first in the frontal cortex and septum, in larger doses then in the frontal cortex, septum, and basal ganglia. The optimal dose range of CTR from the viewpoint of the inhibitory effect of MEOTA ranged between 250–300 mg/kg; on the other hand, the largest dose of 400 mg/kg showed already a smaller, though still significant effect.

Conclusions

1. Pharmacokinetic study showed relatively long elimination half-time of ^{3}H-MEOTA.
2. MEOTA alone penetrates into the CNS relatively well.
3. Premedication with CRT in rats results in a weak increase in MEOTA concentration in the cerebral tissue.
4. Premedication with CRT results in a significant strengthening of the inhibitory action of MEOTA on AChE in the CNS, particularly in doses of 250–300 mg/kg p.o.
5. A comparison of the cerebral levels of MEOTA under the influence of CRT with the inhibitory activity on cerebral AChE shows that a higher inhibitory efficacy of MEOTA on AChE in combination with CRT is not probably caused by a global increase in MEOTA concentration in the cerebral tissue, but rather a direct interaction of both drugs on the active centre of the appropriate enzyme.

Acknowledgement
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Correspondence: Zbyněk Svoboda
Institute of Experimental Biopharmaceutics
Joint Laboratory of the Czech Academy of Sciences and PRO.MED.CS Praha a. s.

Heyrovského 1207
500 02 Hradec Králové 2
Czech Republic
e-mail: svoboda@uebf.cas.cz

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