Applications Brief

Ramipril:
Autoradiographic studies on transfer and binding sites of (3H) – Ramiprilat in the rat brain
INSTRUCTION

RAMIPRIL (HOE 498) is a new angiotensin converting enzyme (ACE) inhibitor. The compound is an esterified prodrug which, upon absorption is hydrolysed to the pharmacological active metabolite, the dicarboxylic acid RAMIPRLAT (Fig. 1).

Inhibition of the converting enzyme (CE) has proved to be an effective therapeutic principle in the treatment of hypertension. While the role of the peripheral renin angiotensin system (RAS) is well documented, questions remain still open concerning the brain RAS. Inhibition of CE in the brain has been implicated in the antihypertensive action of some CE-inhibitors (1),(6). Since the central nervous system is separated from the peripheral one by the blood brain barrier, the ability of the CE-inhibitor to cross this natural barrier to reach the brain is an essential prerequisite for efficacy.

It was the aim of the study to investigate if there is a transfer from blood to brain after intravenous administration of RAMIPRLAT, which was labeled with tritium for this purpose. Autoradiography served as detection method. The results were compared with those obtained after in vitro incubation of brain sections with 3H-RAMIPRLAT.

Fig. 1: Deesterification of RAMIPRIL to the active moiety RAMIPRLAT

MATERIAL AND METHODS

Radioactive labeled compound
HOE498 - diacid- (azabicyclooctyl-7.8 - 3H -) [RAMIPRLAT]
• denotes the position of the tritium label

IN VIVO - STUDIES

Dose
10 ng RAMIPRLAT- 3H/kg b.w. (approx. 50 MBq/kg);
three times intravenously via a lateral tail vein; dose interval 2 hours. RAMIPRLAT-3H was dissolved in a small volume of methanol (200 ul) and was administered in a PBS-buffer solution (0.17 molar, pH 7.4).

Animals: female Wistar rats (WISKf SPF 71, Hoechst AG); 195 g

Time points of investigation: 5 min, 30 min, 24 h, 48 h after the last dose

IN VITRO - STUDIES

Sample preparation
Freshly prepared (-15°C) brain sections at different transverse and sagittal brain levels were incubated at 4°C for 5 min in 170 nM PBS-buffer solution (pH 7.4). For the following different parts of the study the incubation period was 2 hours at room temperature (approx. 22°C).

Total binding: Incubation in PBS-buffer (50 ml) containing 1 nM RAMIPRLAT - 3H

Nonspecific binding: Incubation in PBS-buffer (50 ml) containing 1 nM RAMIPRLAT - 3H and 100 nM non labelled ligand

Washings: three times for 15 sec. in ice cold PBS-buffer and once for 10 sec. in distilled water (4°C) after finishing incubation

ANALYTICAL METHOD

Quantitative studies of the radioactivity distribution in the brain by means of videodensitometric analysis of the autoradiograms (ASBA Picture Analysis System, Leica AG, CH) prepared from 12 um freeze sections (Frigocut 2800 E, Leica Instruments GmbH, FRG) at different brain levels using Ultrofilm 3H, (Leica Instruments GmbH, FRG) and the vacuum contact method (2).
RESULTS

Radioactivity appeared shortly after the last intravenous dose of 3H-Ramiprilat in the brain. It was detected in different defined brain regions listed together with the quantitative data measured by videodensitometry in Tab. 1. The corresponding autoradiograms are shown in Fig. 2. The choroid plexus showed the highest radioactivity concentrations. They were distinctly higher - about 64 times on the average - than in the other brain parts. In most of the regions investigated, the radioactivity still increased for a short time after application and then remained relatively constant over the whole study period of 48 hours (Fig. 3). In the CSF of the ventricles the radioactivity was low at all time points and similar to that of the surrounding brain tissue. Notable concentrations were observed in the ependymal lining of the ventricles (Fig. 4).

<table>
<thead>
<tr>
<th>REGION OF INTEREST (ROI)</th>
<th>IN VIVO</th>
<th>IN VITRO</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 min</td>
<td>30 min</td>
</tr>
<tr>
<td>Nuc. caudatus / Putamen</td>
<td>4.9</td>
<td>5.3</td>
</tr>
<tr>
<td>Thalamus</td>
<td>4.3</td>
<td>4.6</td>
</tr>
<tr>
<td>Massa intermedia</td>
<td>9.7</td>
<td>4.5</td>
</tr>
<tr>
<td>Globus pallidus</td>
<td>9.7</td>
<td>4.5</td>
</tr>
<tr>
<td>Nuc. entopoduncularis</td>
<td>4.3</td>
<td>3.3</td>
</tr>
<tr>
<td>Cortex cerebri</td>
<td>4.3</td>
<td>3.3</td>
</tr>
<tr>
<td>Plexus choroiodeus of:</td>
<td>242</td>
<td>283</td>
</tr>
<tr>
<td>Ventricle lateralis</td>
<td>295</td>
<td>291</td>
</tr>
<tr>
<td>Ventricle tertius</td>
<td>124</td>
<td>308</td>
</tr>
<tr>
<td>Ventricle quartus</td>
<td>124</td>
<td>308</td>
</tr>
<tr>
<td>Substantia nigra</td>
<td>1.2</td>
<td>1.8</td>
</tr>
<tr>
<td>Blood vessels</td>
<td>21</td>
<td>24</td>
</tr>
</tbody>
</table>

ng RAMIPRILAT - $^3$H/g wet tissue (mean values)

** no optical densities more than the background
n.e. not estimated; TB = total binding; NSB = non specific binding

Tab.1: Concentrations in different brain regions after multiple intravenous administration of approx. 10 µg/kg to female rats or in-vitro incubation of brain sections in radioactively labelled compound containing buffer solutions.

Fig. 3: Time course of radioactivity in different parts of the rat brain after multiple intravenous administration of approx. 10 µg/kg [3H-RAMIPRILAT]

Abbreviations used in text and figures:

- Nc/P — Nucleus caudatus / Putamen
- Ne — Nucleus entopoduncularis
- Cc — Cortex cerebri
- Bv — Blood vessel
- Sn — Substantia nigra
- Vl — Ventricle lateralis
- Pp — Plexus choroiodeus
- Th — Nucleus thalami
- Vqu — Ventricle quartus
- EL — Ependymal lining
- CSF — Cerebrospinal fluid

Fig. 4: Autoradiogram (left hand side) of the corresponding HE stained brain section (right hand side) demonstrating the distribution of 3H-RAMIPRILAT in the choroid plexus and the ependymal lining of the lateral ventricle, 48 h after administration. The autoradiogram is representative for the whole study. Enlargement: 60 x
DISCUSSION

Most of the brain areas in which the in vivo autoradiographic studies revealed radioactivity are known from in vitro receptor binding studies for their high ACE content (Fig. 2); (3), (4), (5). The different intensities of the grey levels in the autoradiograms depend upon the dose which was very much higher in the in vitro study. Other regions as e.g. the thalamus showed only radioactivity in the in vivo study. It is supposed that the radioactivity was washed out by the in vitro working up procedure. The long lasting concentrations in the described brain regions under in vivo conditions indicate a tight binding of the labelled ligand to receptor sites. The non-specific binding was very low (Fig. 2).

RAMIPRILAT is very rapidly formed from RAMIPRIL by hydrolysis in rat serum, and is the main metabolite in blood of rats. Metabolites derived from RAMIPRILAT were detected only sporadically and in very low amounts and not until increasing time. The radioactivity which was found immediately after administration of 3H-RAMIPRILAT is therefore assumed to correspond to RAMIPRILAT. From the low concentrations in the CSF it can be concluded that 3H-RAMIPRILAT will penetrate the blood brain barrier rather than the blood CSF barrier.

SUMMARY

3H-RAMIPRILAT is able to penetrate the blood brain barrier and was localised in brain areas known for their high ACE content. The long lasting concentrations in these areas indicate a tight binding of the labelled ligand to receptor sites. Despite the high radioactivity in the choroid plexus, radioactivity in the CSF remained low. Having found that

REFERENCES


(2) G. Kloss, H.-M. Kellner, C. Köther Vakuum- Kontakt-Methode bei der Makroautoradiographie N. Naturforschung, 28 c, 468 - 468 a (1973)

(3) H.-M. Kellner, W. Esinger In vitro autoradiographic studies on the affinity of RAMIPRILAT to the angiotensin converting enzyme in the rat brain ISSX 2nd European Meeting on Foreign Compound Metabolism, Frankfurt/Main, March 29. - April 3, 1987


(5) F.A.O. Mendelsohn Localization of angiotensin converting enzyme in rat forebrain and other tissues by in vitro autoradiography using 125I-labelled MK 351 A


Acknowledgement: The authors wish to acknowledge the excellent technical assistance of Mrs. Chr. Zimmer, Hoechst AG
IN VIVO STUDY

A

Autoradiogram

Sagittal section 24 h p. appl.

B

Pseudocolour Computer Image (without cerebellum)

IN VIVO STUDY

C

Autoradiogram

Transversal section 48 h p. appl.

D

Pseudocolour Computer Image

IN VITRO STUDY

E

Autoradiogram

Total binding (TB)

F

Transversal sections; 2 hours incubation

Autoradiogram

Non specific binding (NSB)

Fig. 2: Autoradiograms of the radioactivity distribution in the rat brain under in vivo and in vitro study conditions as explained in material and methods. The pictures B and D are the corresponding pseudocolour computer images of the autoradiograms A and C.
For further information please contact your local Leica sales company

Australia: Gladesville/NSW  Phone +61 2 9897 9700  Fax +61 2 9897 8358
Austria: Vienne  Phone +43 1 486 80 50  Fax +43 1 486 80 30
Canada: Richmond Hill/Ontario  Phone +1 905 762 2000  Fax +1 905 762 8937
Denmark: Herlev  Phone +45 4404 0101  Fax +45 4404 0111
France: Rue-Maison  Phone +33 1 4732 0565  Fax +33 1 4732 9596
Germany: Bensheim  Phone +49 6251 1360  Fax +49 6251 136155
Italy: Milan  Phone +39 02 57 496 1  Fax +39 02 57 40 3273
Japan: Tokyo  Phone +81 3 5435 0603  Fax +81 3 5435 3615
Korea: Seoul  Phone +82 2 514 6542  Fax +82 2 514 6548
Netherlands: Rijswijk  Phone +31 70 4132130  Fax +31 70 4132139
Portugal: Lisbon  Phone +351 1 388 9112  Fax +351 1 3564 668
Republic of China: Hong Kong  Phone +852 2364 6699  Fax +852 2364 4183
Singapore: Singapore  Phone +65 77 97 823  Fax +65 77 30 628
Spain: Barcelona  Phone +34 93 494 0500  Fax +34 93 494 9532
Sweden: Solentuna  Phone +46 8 625 4045  Fax +46 0 625 4101
Switzerland: Glattbrugg  Phone +41 1 809 9944  Fax +41 1 809 9922
United Kingdom: Milton Keynes  Phone +44 1 908 246 246  Fax +44 1 908 609 992
USA: Barrickburn/Ilinois  Phone +1 847 405 0123  Fax +1 847 405 0164

... and more than 100 national distributors.

Leica Microsystems Nussloch GmbH
Heidelberger Strasse 17-19
D-69226 Nussloch

Telephone: (06224) 143-0
Fax: (06224) 143-200
eMail: histo_info@leica-microsystems.com
www.leica-microsystems.com