ABSTRACT:

Corticotropin-releasing factor (CRF) is known to play an important role in the body response to stress. Butyl-[2,5-dimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl]-ethylamine (CP-154,526) is a CRF, antagonist showing anxiolytic activities in rats in behavioral models, suggesting that CP-154,526 crosses the blood-brain barrier. However, there is no direct evidence for this. This study determined the pharmacokinetic profile of CP-154,526 in rats after i.v. and p.o. application. After i.v. bolus, the concentration declined in a biphasic manner, the first half-life being 0.9 h and the terminal one being 51 h. Systemic clearance was 36 ml/min/kg, and declined in a biphasic manner, the first half-life being 0.9 h and the terminal one being 51 h. Systemic clearance was 36 ml/min/kg, and the volume of distribution was 106 l/kg. Oral bioavailability reached 27%. To study brain pharmacokinetics, rats were given a single dose of CP-154,526 p.o. or i.v. and sacrificed after different post-treatment times. Plasma, cortex, striatum, hypothalamus, hippocampus, and cerebellum concentrations were measured. After i.v. bolus, maximal brain concentration was reached after 20 min. The hypothalamus displayed significantly lower concentrations compared with the other brain tissues. In the p.o. study, the maximal plasma concentration was reached after 30 min, whereas the maximal brain concentration was observed after 1 h and remained stable until 2 h post-treatment, without significant differences between the brain tissues. The unidirectional brain extraction ratio was 27 and 9% at vascular concentrations of 0.08 and 16 nmol/ml, respectively. These results demonstrate a large brain penetration of CP-154,526 after i.v. and p.o. applications and a comparable distribution among the brain regions, except for the hypothalamus, which displayed lower concentrations after i.v. bolus.

BRAIN PHARMACOKINETICS OF A NONPEPTIDIC CORTICOTROPIN-RELEASING FACTOR RECEPTOR ANTAGONIST

CAROLINE KELLER, ARMIN BRUELISAUER, MICHEL LEMAIRE, AND ALBERT ENZ

Novartis Pharma AG, Basel, Switzerland

(Received June 11, 2001; accepted November 7, 2001)

This paper is available online at http://dmd.aspetjournals.org

Materials and Methods

Test Compound. The first part of this study was performed with 3H]CP-154,526 and the second part with nonlabeled substance. The labeling was carried out by the Isotope Laboratories of Novartis Pharma (Basel, Switzerland).
was calculated as \(100 \cdot \left(\frac{\text{H}^{3}\text{C}}{\text{H}^{14}\text{C}} \text{ dpm}_{\text{oral}} - \frac{\text{H}^{3}\text{C}}{\text{H}^{14}\text{C}} \text{ dpm}_{\text{injection}}\right)\). The brain extraction ratio \(E\) was calculated from \(E = \text{BUI} \cdot 0.73\), where 0.73 represents the brain extraction ratio of \(1^{14}\text{C}\)-butanol (Pardridge et al., 1985).

**Sample Analysis.** The concentration of \(1^{14}\text{C}\)-CP-154,526 in blood was determined by LC-reversed isotope dilution. The procedure involved the addition of 13.7 nmol of nonradioabeled CP-154,526 to each blood sample as an internal standard. After adding 1 ml of water, 100 \(\mu\)l of buffer, pH 9 (concentrated 5 times; Merck) and 4 ml of diethyl ether (Merck), the samples were shaken for 30 min and centrifuged (6000g, 5 min, 20°C). The supernatant was evaporated in a vacuum centrifuge (Univapo 150H; Zivy, Oberwil, Switzerland). The residue was reconstituted in 250 \(\mu\)l of mobile phase/water and centrifuged (3000g; 60 s). The supernatant (200 \(\mu\)l) was analyzed by HPLC (MCT2; Kontron Instruments, Zürich, Switzerland) on a Waters Symmetry Shield RP-8 column (5 \(\mu\m), 30 \times 3.9 \text{ mm}, 60°C; Milford MA) with 0.1% tetramethylammonium hydrogen sulfate/acetonitrile (60:40, v/v; Merck) as mobile phase. The flow rate was 1.2 ml/min; the effluent was monitored at 295 nm. The peak corresponding to the unchanged \(1^{14}\text{C}\)-CP-154,526 was collected in a polyethylene vial by a fraction collector (SuperFrac; Amersham Biosciences AB, Uppsala, Sweden) and subjected to radioactivity determination.

The concentration of \(1^{14}\text{C}\)-CP-154,526 in blood and brain compartments was also determined by LC/MS. Aliquots of plasma (100 \(\mu\)l) were extracted (3 \times 15 min on an HS 250 basic IKA shaker) with 500 \(\mu\)l of ethyl acetate (Merck). The combined extracts were then evaporated under a nitrogen stream and dissolved in acetonitrile (Merck) for analysis. The samples were homogenized in water (20% brain tissue) using an IKA Ultra-Turrax T8 homogenizer (30 s at position 5; Janke & Kuntel, Staufen, Germany), and aliquots were treated following the same procedure as the plasma samples. For the calibration curves, control biological matrices (plasma or brain homogenates) were spiked with CP-154,526 (six concentrations) and processed the same way as the samples. Two independent extractions were made for each sample. Quantification of the samples was made by electrospray ionization-LC/MS [HPLC pump, Flux instrument Rheos 4000 equipped with an ERC-3215ra degassed and controlled by the Janoe 1.8e software (Flux Instruments, Basel, Switzerland)]; MS apparatus, Finnigan Navigator with Masslab 2.0 for Windows NT program (Spectronex, Basel, Switzerland)]. The chromatographic separation was performed on a Macherey-Nagel Nucleos 100-5 C18 (125 \(\times\) 2-mm i.d.; Oesingen, Switzerland) analytical column, using acetonitrile/water (+ 0.5% formic acid) (7:3) as eluent at a flow rate of 500 \(\mu\)l/min. The MS conditions were as follow: positive ion mode; gas, nitrogen; capillary, 3.5 kV; cone, 0.39 V; source heater, 150°C; low mass resolution, 12.5; high mass resolution, 12.5; ion energy, 0.0 V; ion energy ramp, 0 V; multiplier, 650 V. Detection was made by selected ion recording at \(m/z\) 365.5, and quantification of the samples was performed using the Masslab software facilities. The detection limit was 20 fmol/injection.

**Data Analysis.** The peeling method was applied to describe the data by a biphasic experimental model with \(C = C_{1} \cdot e^{-\lambda_{1} t} + C_{2} \cdot e^{-\lambda_{2} t}\). The initial estimates of \(C_{1}, \lambda_{1}, C_{2}, \lambda_{2}\) were taken to generate the best fit using the computer software ELSFIT (Sheiner, 1981). The half-lives were calculated as \(t_{1/2}=\ln 2/\lambda\). Areas under the curve (AUC) and areas under the first-moment curve were calculated by the trapezoidal rule and extrapolated to infinite time. Total clearance (CL) was calculated as dose/AUC. The volume of distribution at steady state was calculated as \(V_{SS} = MRT \cdot CL\), where \(MRT\) is the mean residence time, calculated as areas under the first-moment curve/AUC.

**Results.** Based on the dose-normalized AUC ratios, an average bioavailability of 27 ± 6% was estimated. CP-154,526 displayed a large volume of distribution (\(V_{SS} = 105 \text{ l/kg}\)), and a systemic CL of 36 ml/min/kg. After i.v. bolus, the concentration of CP-154,526 in blood declined

<table>
<thead>
<tr>
<th>Application</th>
<th>Parameter</th>
<th>Unit</th>
<th>Mean ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral</td>
<td>(C_{\text{max}})</td>
<td>nmol/ml</td>
<td>0.101 ± 0.035</td>
</tr>
<tr>
<td></td>
<td>(T_{\text{max}})</td>
<td>h</td>
<td>3.3 ± 1.2</td>
</tr>
<tr>
<td>Intravenous</td>
<td>AUC</td>
<td>nmol/ml · h</td>
<td>1.70 ± 0.19</td>
</tr>
<tr>
<td></td>
<td>(t_{1/2})</td>
<td>% of dose</td>
<td>27 ± 6</td>
</tr>
<tr>
<td></td>
<td>(t_{1/2;1})</td>
<td>h</td>
<td>0.87 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>(t_{1/2;2})</td>
<td>h</td>
<td>51 ± 15</td>
</tr>
<tr>
<td></td>
<td>CL</td>
<td>ml/min/kg</td>
<td>35.9 ± 3.6</td>
</tr>
<tr>
<td></td>
<td>(V_{e})</td>
<td>l/kg</td>
<td>2.55 ± 0.39</td>
</tr>
<tr>
<td></td>
<td>(V_{ss})</td>
<td>l/kg</td>
<td>105 ± 30</td>
</tr>
</tbody>
</table>

\(t_{1/2;1}\) first half-life; \(t_{1/2;2}\) terminal half-life; \(V_{e}\) volume of initial dilution compartment.

was 10 standard and a radiochemical purity of

was 10 standard and a radiochemical purity of

TABLE 1

Pharmacokinetic parameters of CP-154,526 after p.o. (13.7 \(\mu\m/kg\)) and i.v. application (2.7 \(\mu\m/kg\)).
biphasically with a first half-life of 0.9 h and a terminal half-life of 51 h (Table 1; Fig. 2).

The unidirectional brain extraction of \([3^H]\)CP-154,526 was 27 and 9 \(\pm\) 2% after the intracarotid injection of Ringer’s buffer containing 0.08 or 16.4 nmol/ml of the compound, respectively. At both concentrations, the brain extraction was strongly decreased when the compound was dissolved in rat plasma to the low, but significant, values of 3 to 4%.

Brain pharmacokinetic was studied by comparing plasma levels of CP-154,526 with the concentration in five brain tissues, namely the cortex, hypothalamus, hippocampus, striatum, and cerebellum. After i.v. application, brain maximal concentrations were reached after ca. 20 min, and no significant differences were observed between the five studied brain tissues, except for the hypothalamus, which exhibited slightly lower concentrations, and for the cerebellum, which showed higher concentrations until 20 min post-treatment (Table 2; Fig. 3A). After i.v. application, the concentration ratio of brain part/plasma reached the maximum of about 2.5 after 1 to 2 h and then slowly decreased in all brain tissues, except again for hypothalamus, where the ratio never exceeded 1 (Fig. 4).

After p.o. application, maximal plasma concentration was reached after ca. 30 min. Brain concentrations increased rapidly to reach maximal concentration after about 1 h and remained stable until 2 h post-treatment (Fig. 3B). No significant differences could be observed between the brain regions (Table 3). No differences in the ratios of brain tissues/plasma were noted between the brain tissues after p.o. application.

**Discussion**

CP-154,526 displayed a high tissue distribution, revealed by the large volume of distribution (105 l/kg). In accordance with this, an extensive brain distribution with high brain/blood concentrations ratios in both i.v. and p.o. applications could be demonstrated. The brain extraction ratios (9 to 27%) indicate a large and saturable brain penetration of CP-154,526, and the decrease of these ratios to 3 to 4% when using rat plasma as vehicle indicates high plasma protein binding. Since a fairly high systemic clearance (CL = 36 ml/min/kg) was observed, the slow elimination (\(t_{1/2} = 51\) h) may be attributed to the large volume of distribution.

**Table 2**

<table>
<thead>
<tr>
<th>Time</th>
<th>Plasma</th>
<th>Cortex</th>
<th>Striatum</th>
<th>Cerebellum</th>
<th>Hippocampus</th>
<th>Hypothalamus</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.08</td>
<td>3.51 ± 0.38</td>
<td>1.60 ± 0.23</td>
<td>1.16 ± 0.26</td>
<td>2.46 ± 0.45&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.41 ± 0.42</td>
<td>0.86 ± 0.34</td>
</tr>
<tr>
<td>0.17</td>
<td>1.96 ± 0.30</td>
<td>1.29 ± 0.04</td>
<td>1.07 ± 0.12</td>
<td>1.82 ± 0.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.18 ± 0.08</td>
<td>0.95 ± 0.24</td>
</tr>
<tr>
<td>0.33</td>
<td>1.38 ± 0.28</td>
<td>1.68 ± 0.26</td>
<td>1.25 ± 0.21</td>
<td>2.29 ± 0.56&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.39 ± 0.36</td>
<td>1.09 ± 0.17&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.50</td>
<td>0.99 ± 0.28</td>
<td>1.37 ± 0.33</td>
<td>0.90 ± 0.21</td>
<td>1.53 ± 0.48</td>
<td>1.03 ± 0.30</td>
<td>0.75 ± 0.36&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>1</td>
<td>0.37 ± 0.03</td>
<td>0.91 ± 0.17</td>
<td>0.63 ± 0.19</td>
<td>0.89 ± 0.17</td>
<td>0.72 ± 0.09</td>
<td>0.29 ± 0.10&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>0.16 ± 0.04</td>
<td>0.35 ± 0.08</td>
<td>0.38 ± 0.08</td>
<td>0.37 ± 0.07</td>
<td>0.36 ± 0.09</td>
<td>0.16 ± 0.13&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>0.05 ± 0.03</td>
<td>0.09 ± 0.04</td>
<td>0.05 ± 0.04</td>
<td>0.10 ± 0.03</td>
<td>0.08 ± 0.04</td>
<td>0.04 ± 0.01</td>
</tr>
<tr>
<td>AUC</td>
<td>1812 ± 346</td>
<td>2393 ± 459</td>
<td>1877 ± 457</td>
<td>2742 ± 606</td>
<td>2060 ± 548</td>
<td>1154 ± 505</td>
</tr>
</tbody>
</table>

<sup>a</sup> <sub>p < 0.05 concentration significantly different from the other brain tissues.</sub>
receptors (Chalmers et al., 1995, 1996). It is not clear whether the
whereas the cerebellar cortex displays a higher density of CRF 1
receptors in comparison with other brain tissues, like the brain cortex,
tissues with high amounts of myelin.

The rat hypothalamus is known to have a lower density of CRF 1
receptors in comparison with other brain tissues, like the brain cortex,
tissues with high amounts of myelin. Myelin is also distributed differently
within the brain and, therefore, increases the lipophilia of the brain
regions where it is present in higher concentrations. This means that
a lipophilic substance will accumulate more and stay longer in the
regions with high amounts of myelin.

The rat hypothalamus is known to have a lower density of CRF 1
receptors in comparison with other brain tissues, like the brain cortex,
whereas the cerebellar cortex displays a higher density of CRF 1
receptors (Chalmers et al., 1995, 1996). It is not clear whether the
concentrations of CP-154,526 are a reflection of the receptor’s den-
sities, but since CP-154,526 is a highly specific CRF 1 receptor antagon-
ist (Schulz et al., 1996), the possible consequences of the lower
hypothalamus and higher cerebellum concentrations of CP-154,526
on its pharmacological activity have to be examined.

In the p.o. study, the concentration ratios still increased 8 h after
application (Fig. 4), but no significant differences within the brain
regions were observed. Differences between brain tissues seen only in
the i.v. trial, where the concentrations are about 10-fold higher than in
the p.o. experiment, suggest that a saturation occurs at high concen-
trations.

In summary, CP-154,526 has been proven to cross the blood-brain
barrier in a saturable way well. Correlative studies between behavioral
and brain concentrations should be undertaken to determine the min-
imal brain concentration required for activity, especially for p.o.
studies, where the oral bioavailability of 27% can lead to subthera-
peutic concentrations in the brain if not taken into account.

Acknowlegments. We thank Pierreette Guntz, Stefan Lehmann, and
Dieter Lötischer for technical assistance.

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Acknowledgments. We thank Pierrette Guntz, Stefan Lehmann, and
Dieter Lötischer for technical assistance.

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