PHARMACOKINETICS OF THE BICYCLIC PEPTIDE TACHYKININ NK₂ RECEPTOR ANTAGONIST MEN 11420 (NEPADUTANT) IN RATS

ANNALISA LIPPI, MARCO CRISCUOLI, MARCO GUELFI, PAOLO SANTICOLI, AND CARLO ALBERTO MAGGI

Department of Pharmacology, Menarini Ricerche

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ABSTRACT:

The pharmacokinetics of MEN 11420 [nepadutant, c\([[\beta-D-Glc-
N\alpha)Asn-Asp-Trp-Phe-Dpr-Leu\]c\([2\beta-5\beta])\]], a potent glycosyl-
lated analogue of the selective, bicyclic peptide, tachykinin NK₂
receptor antagonist MEN 10627 \[c\([[\text{Met-Asp-Trp-Phe-Dpr-
Leu}\]c\([2\beta-5\beta])\]]\], were studied in rats after different routes of
administration. The plasma concentration profile for MEN 11420
after iv administration (1 mg/kg) was compared with that for the
parent compound MEN 10627. The mean plasma half-life (44
min) and AUC value (285 \mu g min/ml) for MEN 11420 were almost
3-fold greater than those for MEN 10627, and the systemic
clearance was reduced to one third. The absolute bioavailability
of MEN 11420 after intranasal (1 mg/kg) or ip (1 mg/kg) admin-
istration was virtually complete. However, bioavailability was
only approximately 5% after intrarectal treatment (5 mg/kg) and
was too low to be quantified (<3%) after sublingual (1 mg/kg) or
oral (10 mg/kg) doses. The urinary excretion of unchanged com-
 pound, after an iv dose of 1 mg/kg, was approximately 34% of
the dose for MEN 11420 but was <2% for MEN 10627. This is in
agreement with in vitro data showing that MEN 11420 is more
resistant to hydrolytic and oxidative metabolism than is MEN
10627. It is concluded that the hydrophilic modification of MEN
10627 to produce MEN 11420 resulted in marked improvement in
the pharmacokinetic and metabolic characteristics of the pep-
tide.

Neurokinin A, a member of the tachykinin peptide family, is widely
distributed in the mammalian nervous system and exerts different
biological effects, mainly by activating the tachykinin NK₂ receptor
(Buck et al., 1984; Masu et al., 1987; Regoli et al., 1987; Lee et al.,
1986). Therefore, tachykinin NK₂ receptor antagonists are candidates
for the treatment of many pathological conditions putatively mediated
by endogenous neurokinin A, e.g. bronchial hyperreactivity, irritable
bowel syndrome, and cystitis (Maggi et al., 1993; Quartara et al.,
1995).

Recently, the bicyclic hexapeptide MEN 10627 \[c\([[\text{Met-Asp-Trp-
Phe-Dpr-Leu}\]c\([2\beta-5\beta])\]]\) was reported to be a potent and selective
tachykinin NK₂ receptor antagonist (Maggi et al., 1994; Pavone et al.,
1995), but limited water solubility and marked lipophilicity hindered
its preclinical development. MEN 11420 [nepadutant, c\([[\beta-D-Glc-
N\alpha)Asn-Asp-Trp-Phe-Dpr-Leu\]c\([2\beta-5\beta])\]] (fig. 1) is derived from
MEN 10627 by substitution of a N-glycosylated asparagine for a
methionine residue. This modification increased water solubility 80-
fold, compared with the parent compound, while maintaining potency
and selectivity as an antagonist at the tachykinin NK₂ receptor
(Catalito et al., 1998).

In the present study, we compared the pharmacokinetics of MEN
11420 administered iv with those of MEN 10627, to evaluate how
their chemical differences affect the in vivo disposition of the com-
pounds. Moreover, the bioavailability of MEN 11420 administered by
different routes was examined, to evaluate the feasibility of noninjec-
tion administration of this peptide.

Materials and Methods

Drugs. MEN 11420 and MEN 10627 were synthesized within the Chem-
istry Department of Menarini Ricerche (Florence, Italy), by solid-phase meth-
ods (Pavone et al., 1995).

Animals and Treatments. Male Sprague-Dawley rats (Charles River,
Calco, Italy) weighing 300–400 g were used. They were fasted overnight
before oral treatment. The day before pharmacokinetic studies, the rats were
anesthetized with a combination of Hypnorm (1 ml/kg, ip; 24 \mu g/ml fentanyl
base and 1.2 mg/ml fluanisone) and pentobarbital sodium (1 ml/kg, iv; 30
mg/ml), and a Silastic catheter was implanted in a jugular vein. The cannula,
filled with heparinized saline solution and closed with a pin, was exteriorized
in the back of the neck and used for both iv administration and sample
collection.

MEN 11420 was dissolved in physiological saline solution and administered
to the animals according to the schemes presented in table 1. The solutions
were administered orally by using a gastric tube, intranasally by instilling one
half of the dose in each nostril, sublingually by sowing droplets under the
tongue, and intrarectally by infusing the dose, approximately 6 cm from the
anus, through a round-bottomed gastric probe. Anesthesia (with the pentobar-
bital concentration reduced to 20 mg/ml) was used for the intranasal, sublin-
gual, and intrarectal administrations, to avoid immediate rejection of the
administered dose by the animals. Rats recovered from anesthesia after ap-
proximately 60 min. MEN 10627 was dissolved in dimethylsulfoxide and administered iv at a dose of 1 mg/kg, in a volume of 0.1 ml/kg.

Blood samples (500 \mu l) were collected from the jugular vein catheter into
plastic tubes containing 10 \mu l of 0.5% (w/v) sodium heparin in saline solution,
at the time points shown in the figures. Plasma obtained after centrifugation
was stored at −20°C before analysis. To limit fluid depletion, 0.5 ml of saline
solution was infused through the catheter 1, 2, 3, and 4 hr after treatment.

To evaluate urinary excretion after iv administration, two additional groups
The overall sensitivity of the method was 25 ng/ml for both MEN 11420 and 5000 or 25, 50, 100, 200, and 500 ng/ml levels of the peptide, respectively.

Procedures were prepared by adding to drug-free plasma 200, 500, 1000, 2500, and 5000 units of pepsin from porcine stomach mucosa were dissolved in 100 ml of 7 mM HCl containing 0.2% (w/v) NaCl. MEN 11420 and MEN 10627 were added to the different media at a final concentration of 10 μg/ml. Mixtures were incubated at 37°C and, at different times, 50-μl samples were deproteinized with 250 μl of acetonitrile, vortex-mixed, and centrifuged at 2000 g for approximately 5 min. Two hundred microliters of supernatant were evaporated to dryness under an air stream, the residue was dissolved in 100 μl of distilled water, and 20 μl was injected for HPLC analysis of unchanged peptide.

HPLC Analysis. The HPLC system consisted of a model 465 autosampler (Kontron Instruments SpA, Milan, Italy) and a model LC-9A pump (Shimadzu Co., Kyoto, Japan) connected to a Nucleosil 100 C18 reverse-phase column (150 × 4.6 mm; particle size, 5 μm; Knauer, Berlin, Germany) protected by a precolumn. The detection system consisted of a fluorescence spectrophotometer (650–105; Perkin Elmer-Hitachi, Tokyo, Japan) set at an excitation wavelength of 280 nm and an emission wavelength of 350 nm. Signals from the detector were analyzed by class LC-10 software (Shimadzu Co.) on a model ProLinea 3/25s Compaq personal computer. The mobile phase was composed of water, acetonitrile, and methanol, each containing 0.1% trifluoroacetic acid, in the ratio of 60:30:10 (v/v) for MEN 11420 analysis and in the ratio of 50:40:10 (v/v) for MEN 10627 analysis. The flow rate was set at 1 ml/min. MEN 11420 and MEN 10627 showed retention times of approximately 8 and 9.5 min, respectively.

Pharmacokinetic Analysis. Individual plasma concentration-time curves were analyzed with the fitting software EasyFit for Macintosh (Istituto M. Negri, Milan, Italy), to derive kinetic parameters. The models applied were as follows: biexponential decay for iv administration, monoeXponential increase followed by monoeXponential decay for intranasal and ip administration, and monoeXponential increase followed by biexponential decay for intrarectal administration. The weighing functions 1/y(estimated y) or 1/y² (estimated y) were used, depending on the degree of minimization of the set of SE values for the estimated parameters that they allowed. Absolute bioavailability was calculated as the ratio between the mean AUC value for any other route and the estimated parameters that they allowed. Absolute bioavailability was calculated as the ratio between the mean AUC value for any other route and the

Statistical Analysis. Means were compared by one-way analysis of variance, followed by the Tukey test (Instat for Macintosh; GraphPad Software, San Diego CA).

Results

Plasma Levels. Intravenous Administration. As shown in fig. 2, the plasma levels of MEN 11420 were measurable for at least 360 min after treatment, whereas those of MEN 10627 were measurable only up to 60 min, with the concentration at 90 min being below the sensitivity limit (25 ng/ml). Individual fitting of the concentration-time data according to a bicompartamental model showed that the apparent distribution and elimination phases for MEN 11420 were considerably longer than those measured for MEN 10627 (in partic-
ular, the mean terminal half-life was 44 min, compared with 16 min). The AUC value was also doubled and the systemic clearance value was reduced to one third. Only the value for the apparent volume of distribution remained unchanged (table 2).

Intranasal Administration. The plasma levels of MEN 11420 after intranasal administration were measurable up to 480 min after the 1 mg/kg intranasal dose. The compound entered the systemic circulation rather rapidly, peaking at approximately 15–30 min (fig. 3). Absorption through the nasal mucosa continued for 60 min, as indicated by the lasting plateau of the maximal concentrations and by the value of the apparent elimination half-life, which was significantly longer than after iv administration (table 3). Intranasal bioavailability was virtually complete. This allowed the calculation of the apparent volume of distribution and the total body clearance, which were the same as those measured after iv administration.

Intraperitoneal Administration. As shown in fig. 3, the plasma levels of MEN 11420 were measurable up to 240 min after administration of a 1 mg/kg dose. The maximum concentration was reached at 15 min, and the apparent elimination phase was comparable to that measured after iv administration (table 3). The bioavailability approached 100%. As a consequence, as after intranasal treatment, the apparent volume of distribution and the total body clearance could be calculated without correction factors, and they were not statistically different from those calculated after iv administration.

Intrarectal Administration. Fig. 3 presents the plasma concentration-time curve for MEN 11420 after intrarectal administration of a dose of 5 mg/kg. The plasma levels were measurable up to 240 min after treatment, and the peak concentration was reached at 15 min. Decay of plasma levels was biphasic, and half-life values were not significantly different from those calculated after iv treatment (table 3). A bioavailability of approximately 5% was calculated for this route of administration.

Oral Administration. After oral treatment with a 10 mg/kg dose, the plasma levels of MEN 11420 were equal to or below the limit of detection; therefore, it was not possible to obtain an estimate of oral bioavailability. Considering that the administered dose was twice the intrarectal dose, it can be inferred that the oral bioavailability was <3%.

Sublingual Administration. Administration of MEN 11420 (1 mg/kg) by sowing the solution as droplets under the tongue, to increase the absorption area, did not produce measurable plasma levels at any time between 0 and 6 hr after treatment.

Urinary Recovery. The urinary excretion of unchanged MEN 11420 and MEN 10627 was measurable during the first 6 hr after iv treatment (1 mg/kg) and amounted to 34 ± 9 and 1.2 ± 0.4% of the administered dose, respectively (mean ± SE, N = 4). Neither peptide was detectable in the 6–24-hr urine samples.

Biliary Excretion. Table 4 reports the amounts of MEN 11420 found in bile after intraduodenal (10 mg/kg) and ip (1 mg/kg) treatment. In the first case, the compound (which was not detectable in plasma, similar to oral administration) was detected in bile at only approximately 2% of the administered dose. After ip administration, when bioavailability was virtually complete, biliary excretion amounted to approximately 60%.

Bile samples obtained after intraduodenal administration were also assayed after incubation with β-glucuronidase (type XA, from Escherichia coli) at 37°C for 18 hr. No increase in the amount of measurable MEN 11420 was observed (data not shown).

In Vitro Stability. MEN 11420 was stable for up to 6 hr (2 hr for simulated gastric fluid) at 37°C in the presence of rat plasma or crude homogenates obtained from rat liver, small intestine, or kidney. In comparison, MEN 10627 was degraded by >60% in the presence of liver or small intestine homogenates. When incubated for 1 hr with rat liver microsomes in the presence of NADPH, MEN 11420 remained virtually unchanged, whereas the recovery of MEN 10627 was <40% (table 5).

**Discussion**

The glycosylated peptide MEN 11420 showed marked improvement over MEN 10627 in terms of aqueous solubility, with only a minor loss in *in vitro* potency and no change in selectivity at tachykinin NK2 receptors. Furthermore, a potency increase of up to 10-fold, compared with the parent compound, was obtained in *in vivo* rat models (Catalioto et al., 1998). An obvious explanation for these findings is that the increase in hydrophilicity achieved with MEN
were able to degrade the peptide, whereas under the same conditions the catabolism of MEN 10627 was relatively extensive. This hypothesis is in keeping with the results of the present study; in particular, MEN 11420 exhibits a marked increase in half-life and a decrease in systemic clearance, resulting from the higher AUC value, in comparison with MEN 10627. The decrease in clearance with MEN 11420 may be related to the reduced efficacy of hepatic extraction, compared with MEN 10627; 34% of the dose of the more hydrophilic compound was recovered in the urine after iv administration, and approximately 60% was found in the bile after ip treatment. The respective values for MEN 10627 administered iv were approximately 1 and >90%. The latter value was obtained in previous experiments with 14C-labeled peptide and may be ascribed to MEN 10627 and its metabolites (Crea AEG, personal communication). The resistance to both hydrolytic and oxidative metabolic activities might contribute to the lower hepatic extractability of MEN 11420; neither liver homogenate nor liver microsomes in the presence of cofactors were able to degrade the peptide, whereas under the same in vitro conditions the catabolism of MEN 10627 was relatively extensive.

Both compounds undergo rapid distribution into the extravascular space, as shown by the early fast-decay phase. This was, however, limited, as indicated by the apparent volume of distribution values, which were similar for the two compounds and intermediate between plasma and body volumes. The absence of macroscopic differences in distribution, together with the substantial equipotency at tachykinin NK2 receptors, further indicates that the improved profile of in vivo activity of MEN 11420 is ascribable to the attainment of higher and more sustained plasma levels, compared with those observed after an equal dose of MEN 10627.

The bioavailability of MEN 11420 after intranasal administration approached 100%, agreeing with the results of in vivo pharmacological testing (Catalioto et al., 1998). This suggests that this route might be exploited as a convenient alternative to the injected administration of this peptide, which showed very low bioavailability through the oral and sublingual routes. The striking difference between the bioavailability values obtained after intranasal and sublingual administration was not unexpected, because there are several published reports on the differences in the absorption characteristics of these two mucosal surfaces, both in humans and in animal models. Compounds that are structurally very different from one another, such as sodium cromoglycate, nafarelin, and insulin, have been reported to be much better absorbed after intranasal administration than after sublingual administration (Fisher et al., 1985; Aungst et al., 1988; Chan et al., 1988).

The biliary excretion of only 2% of the intraduodenal dose, compared with 60% of the ip dose, confirms the very low bioavailability of MEN 11420 after enteral administration. This agrees with previous pharmacodynamic studies showing that approximately 100-fold higher doses are required for the intraduodenal route, compared with the iv route, to produce similar effects in anesthetized rats (Catalioto et al., 1998). For MEN 11420, the 100% bioavailability after ip administration indicates that low bioavailability after oral or intraduodenal administration is not the result of an hepatic first-pass effect, because drugs are conveyed to the liver after ip administration just as after oral treatment. On the other hand, the resistance of MEN 11420 to in vitro hydrolytic attack by simulated gastric fluid and small intestine homogenates suggests that presystemic metabolism does not play a significant role and that limited permeability through intestinal

### TABLE 3

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Intravenous, 1 mg/kg</th>
<th>Intranasal, 1 mg/kg</th>
<th>Intraperitoneal, 1 mg/kg</th>
<th>Intrarectal, 5 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximal plasma concentration (ng/ml)</td>
<td>2465 ± 260</td>
<td>4910 ± 342</td>
<td>1186 ± 131</td>
<td></td>
</tr>
<tr>
<td>Time of maximal concentration (min)*</td>
<td>23</td>
<td>15</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>( t_{1/2,x} ) (min)</td>
<td>46 ± 2.3</td>
<td>63 ± 5*</td>
<td>36 ± 3</td>
<td></td>
</tr>
<tr>
<td>( t_{1/2,M} ) (min)</td>
<td>44 ± 3</td>
<td>323 ± 51</td>
<td>339 ± 22</td>
<td></td>
</tr>
<tr>
<td>AUC (μg · min/ml)</td>
<td>285 ± 12</td>
<td>291 ± 27</td>
<td>152 ± 6</td>
<td></td>
</tr>
<tr>
<td>Volume of distribution (ml/kg)</td>
<td>225 ± 27</td>
<td>3.5 ± 0.2</td>
<td>3.0 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>Clearance (ml/min/kg)</td>
<td>0.2</td>
<td>0.5</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>Absolute bioavailability</td>
<td>100</td>
<td>113 ± 18</td>
<td>119 ± 8</td>
<td></td>
</tr>
</tbody>
</table>

Means ± SE are reported (\( N = 4 – 6 \)).

*Median.

\( p < 0.05 \) vs. iv administration.

### TABLE 4

**Biliary excretion of MEN 11420 after intraduodenal (10 mg/kg) and ip (1 mg/kg) administration**

<table>
<thead>
<tr>
<th>Sample Collection Interval</th>
<th>Recovered Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intraduodenal Route</td>
</tr>
<tr>
<td>hr</td>
<td>μg</td>
</tr>
<tr>
<td>0–2</td>
<td>57</td>
</tr>
<tr>
<td>2–4</td>
<td>17</td>
</tr>
<tr>
<td>4–8</td>
<td>8</td>
</tr>
<tr>
<td>0–8</td>
<td>82</td>
</tr>
</tbody>
</table>

Mean values from two rats are reported.

### TABLE 5

**In vitro metabolic stability of MEN 11420 and MEN 10627 in different media**

<table>
<thead>
<tr>
<th>Antagonist</th>
<th>Amount Remaining</th>
<th>Gastric Fluid</th>
<th>Rat Plasma</th>
<th>Rat Tissue Homogenates</th>
<th>Rat Liver Microsomes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>Liver</td>
<td>Kidneys</td>
<td>Small Intestine</td>
<td>+NADPH</td>
</tr>
<tr>
<td>MEN 11420</td>
<td>&gt;90</td>
<td>&gt;90</td>
<td>&gt;90</td>
<td>&gt;90</td>
<td>&gt;90</td>
</tr>
<tr>
<td>MEN 10627</td>
<td>ND*</td>
<td>38</td>
<td>&gt;90</td>
<td>31</td>
<td>35</td>
</tr>
</tbody>
</table>

Values are percentages of the original amounts remaining after 6-hr incubations with plasma and homogenates, after a 2-hr incubation with simulated gastric fluid, or after a 1-hr incubation with microsomal suspensions. Tests were performed in duplicate.

*ND, not done.
epithelia may be a main factor in determining enteral bioavailability. However, the possible role of anaerobic metabolism by enteral flora cannot be ruled out at present.

This study on the pharmacokinetics of MEN 11420 is, to our knowledge, the first report on the pharmacokinetics of a tachykinin NK₂ receptor antagonist. In addition to substantiating a rational explanation for the pharmacodynamic advantages of the glycosylated peptide MEN 11420, compared with MEN 10627, and producing useful indications regarding pharmaceutically exploitable routes for its administration, this study identifies crucial points that deserve further investigation. First, complete correlation between pharmacodynamic and pharmacokinetic data will require the establishment of 1) a different analytical method, allowing an approximately 100-fold increase in sensitivity, for adequate monitoring of low plasma levels and calculation of the true elimination half-life and 2) a study of the kinetics of distribution to the tissue districts supposedly involved in the pharmacological activity. Second, the fate of an oral dose at the preabsorption level should be investigated in greater detail, with particular attention to membrane permeability problems and bacterial metabolism in the intestinal lumen. Studies of these points are currently in progress in our laboratories.

References