DuP 532, an angiotensin II receptor antagonist: First administration and comparison with losartan

We investigated the tolerability and angiotensin II antagonist activity of oral DuP 532 in healthy male subjects. DuP 532 (1 to 200 mg) was well tolerated, with no effect on blood pressure or heart rate. Compared with losartan (100 mg), DuP 532 (200 mg) was a weak antagonist of pressor responses to intravenous angiotensin II. Maximum inhibition of diastolic pressor response was 86% (95% confidence interval [CI], 84%, 88%) approximately 4.6 hours after losartan and 48% (95% CI, 38%, 56%) 8.7 hours after DuP 532. Twenty-four hours after dosing, inhibition by losartan and DuP 532 was similar (40% to 45%). DUP 532 is extensively bound in human plasma, with an in vitro free fraction of 0.06. Although DuP 532 and EXP3174 (losartan's active metabolite) have similar AT_1-receptor potency, and plasma concentrations of DuP 532 were much greater than losartan/EXP3174, the level of antagonism was much less for DuP 532. These results indicate that multiple factors determine the in vivo potency of angiotensin II antagonists, including affinity for and distribution to the receptor as modulated by plasma binding. (Clin Pharmacol Ther 1997;61:59-69.)

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Losartan was the first orally active, nonpeptide AT_1-selective angiotensin II antagonist to be evaluated in humans.[1-5] This article describes the initial clinical experience with DuP 532,[6,7] a structurally related angiotensin II antagonist. Unlike losartan,[2,5] DuP 532 is a carboxylic acid that does not have an active metabolite (Fig. 1).[8] In preclinical studies, compared with losartan, DuP 532 showed similar potency and duration of angiotensin II antagonist activity.[6] Like EXP3174, the active metabolite of losartan, DuP 532 is an insurmountable antagonist.[6,7,9] Losartan, EXP3174, and DuP 532 were all shown to be extensively protein bound (≥99%),[10] with the greatest binding evident for DuP 532. In vitro, such binding manifests pharmacologically as a reduction in the apparent potency at the AT_1-receptor when inhibition is determined in the presence of human serum albumin. Thus, k_i values increased from 1.2 to 5300 nmol/L for DuP 532, from 24 to 400 nmol/L for losartan, and from 1 to 200 nmol/L for EXP3174.[7] Further, DuP 532 was not metabolized in human and rat microsomal preparations but had a low apparent oral bioavailability in animals.[8] Because of these pharmacokinetic differences, it was of interest to compare this compound to losartan as an angiotensin II antagonist in the same clinical paradigm used to establish the activity of losartan. This article summarizes the tolerability results from a rising-dose phase I study, a crossover study of the antagonism by DUP 532 and losartan of pressor responses of angiotensin II, and results of in vitro protein binding studies in plasma from several species, including humans.

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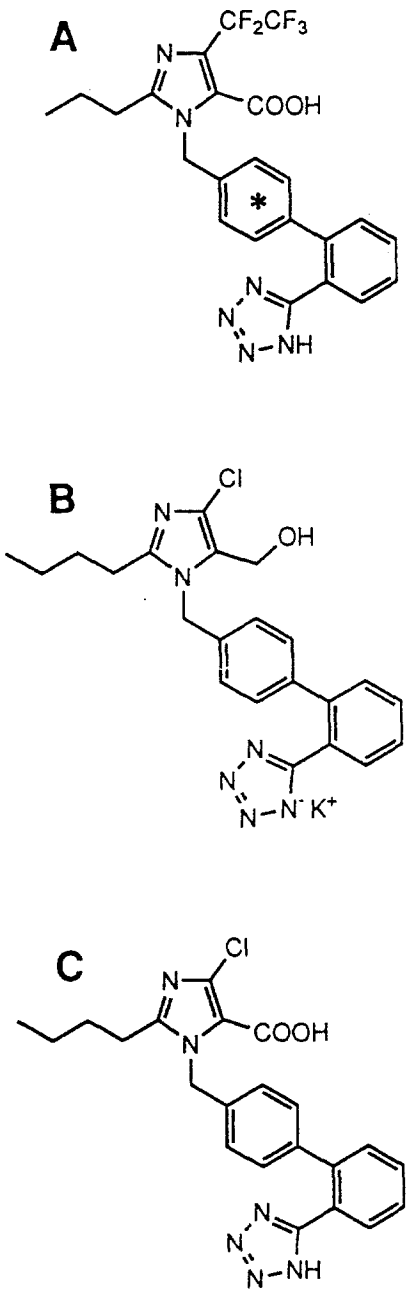

Fig. 1. Chemical structures of DUP 532 (**A**), losartan (**B**), and EXP3174 (**C**). *Asterisk* (*) indicates ring location of ^{14}C for protein binding studies.

METHODS

Study designs. Two studies were conducted to investigate the tolerability and activity of oral DuP 532 in healthy male subjects. The first study was a single-rising-dose, placebo-controlled, double-blind five-period study in two alternating panels of 10 subjects. Each panel was studied on alternating days, with dose advancement between panels based on clinical tolerability. The dose was advanced until a maximum dose of 200 mg had been received or until symptoms, blood pressure effects, or abnormal laboratory tests precluded a further increase in dose. The first panel received doses of 0.1, 5, 25, and 100 mg; the second panel received doses of 1, 10, 50, and 200 mg. Within each panel, the allocation schedule was designed so that all subjects received all doses of DuP 532 for that panel, as well as placebo. One week separated each treatment period for each subject.

The second study was a two-part study (A and B) designed to investigate the ability of maximally tolerated doses of DuP 532 from the first study to antagonize pressor responses to exogenous angiotensin II. The methods used were similar to those previously used to study losartan.[1,2] Part A of the study was an exploratory, single-blind, nonrandomized rising-dose study of four healthy volunteers. Oral doses of DuP 532 (10, 50, 100 and 200 mg) were advanced at weekly intervals to confirm safety results of the rising-dose tolerability study and to select doses for the second part of the study.

Part B of this study was to be a double-blind, placebo-controlled, four-period crossover study of eight additional healthy volunteers receiving oral doses of placebo, 100 mg losartan, and two doses of DuP 532 to compare the duration and extent of angiotensin antagonism by the two angiotensin II antagonists. However, preliminary data from the tolerability study and antagonism results of part A (not shown) indicated that DuP 532 might be less active than anticipated and had a very long half-life, so that a 1-week interval after a single dose might not be sufficient to ensure washout of drug effect. Therefore part B was modified to a three-period study in six subjects with losartan and placebo in random sequence in the first two periods, preceding the 200 mg dose of DuP 532.

The study protocols were reviewed and approved by the ethical review committee of the Centre Hospitalier Universitaire Vaudois (Lausanne, Switzerland). All subjects provided written informed consent for their participation in the study.

Subjects and procedures. Healthy male subjects between the ages of 18 and 40 years were enrolled in these studies. For the first (i.e., tolerability) study, subjects were provided a diet that contained 130 to 150 mmol sodium and 80 to 100 mmol potassium every 24 hours for 4 days before each dosing day and

on the dosing days. For the second (i.e., antagonism) study, subjects were instructed to follow a diet that contained approximately 180 mmol sodium every 24 hours. Before the start of the second study, an intravenous bolus dose of angiotensin II that increased reclining systolic blood pressure approximately 25 mmHg, was identified for each subject by monitoring the blood pressure response to angiotensin II at the finger with a Finapres recorder.[10]

The subjects fasted from 8 pm the night before until 4 (tolerability study) or 6 (antagonism study) hours after dosing on each study day. For the antagonism study, individual responses to the selected angiotensin II dose were confirmed before each dose. Study drug was then given with 200 ml water. In the tolerability study, blood pressure and heart rate were measured with an automatic sphygmomanometer (Dinamap) and recorded frequently from predose until 12 hours after dosing. In the second study, resting blood pressure and the onset and duration of angiotensin II blockade were assessed by continuous monitoring of supine blood pressure and heart rate and responses to the selected angiotensin II dose with the Finapres machine.[1] Eight bolus doses of angiotensin II were given at 0.5- to 2-hour intervals during the first 12 hours after administration of the study drug and then again 24 hours after study drug. Two angiotensin II responses were averaged 24 hours after dosing.

Material. DuP 532 (4-pentafluoroethyl-2-propyl-1-[(2′-(1H-tetrazol-5-yl)biphenyl-4-yl)methyl] imidazole-5-carboxylic acid; Fig. 1), losartan, and EXP3174 were synthesized by the DuPont Merck Pharmaceutical Co. (Wilmington, Del.), ^{14}C-DuP 532 (specific activity 38.9 mCi/mmole; radiochemical purity >99% [stated]) was synthesized by New England Nuclear Co. (Boston, Mass.). Human serum albumin and α_1-acid glycoprotein were bought from Calbiochem Corp. (San Diego, Calif.). Frozen human plasma from male, nonsmoking white donors was bought from Biological Specialty Corp. (Lansdale, Pa.). All buffer salts and solvents were obtained from commercial sources and were the highest grade available. The micropartition devices were bought from Amicon (Centrifree, Danvers, Conn.) and were used after pretreatment according to the manufacturer's recommendation.

Plasma protein binding determinations. Plasma protein binding and binding to purified albumin or α_1-acid glycoprotein was determined as described[11] with use of ultrafiltration. All determinations were done in triplicate. Plasma binding was also determined with rats, rhesus monkeys, and beagle dogs.

Blood/plasma distribution ratio determinations. The in vitro blood/plasma ratio for ^{14}C-DuP 532 was determined as described[12] with use of fresh beagle dog and rat blood.

Plasma drug levels: Plasma renin activity (PRA) and angiotensin II concentration. In both studies, blood was drawn at specified times for measurement of plasma drug levels, PRA, and plasma angiotensin II concentration up to 24 hours after dosing. Total concentrations of DuP 532, losartan, and the active metabolite of losartan (EXP3174) were measured by HPLC with ultraviolet detection).[13,14] PRA and angiotensin II were measured according to established methods.[15,16]

Data analysis. Responses to angiotensin II were determined from the Finapres tracing by subtracting the systolic or diastolic blood pressure value immediately preceding the injection of angiotensin II from the respective maximum systolic or diastolic blood pressure value after the agonist injection (noted within 1 to 3 minutes). Baseline for assessment of treatment effects was defined on each study day as the angiotensin II response before administration of study drug. Heart rate responses were expressed as the difference between heart rate before angiotensin II and the minimum heart rate after agonist injection. Antagonism of angiotensin II responses was assessed from the ratios of the posttreatment angiotensin II responses to the baseline response, with the smallest ratio indicating the maximum treatment effect (i.e., greatest inhibition). To assess angiotensin II antagonism, four parameters were derived from these measurements: maximum inhibition, inhibition at 24 hours after dosing, area under the inhibition versus time curve (AUC), and time to maximum inhibition.

To account for potential measurement-to-measurement fluctuations in the observed level of inhibition of responses to angiotensin II, maximum inhibition was defined based on a moving average through the 12-hour postdose measurements. For heart rate, the maximum drug effect was defined as the greatest decrease after the angiotensin II infusion after ingestion of study medication, also based on moving averages. AUC from 0 to 24 hours was derived for both blood pressure and heart rate responses to angiotensin II. AUC was calculated with use of the trapezoidal rule and was based on the proportion of response relative to baseline (defined as the ratio of posttreatment angiotensin II response

to baseline angiotensin II response). This method results in a greater numerical area when there is no inhibition (i.e., ratio approaches 1) compared to inhibition (ratio less than 1). Time to maximum inhibition was also determined, defined as the average of the two time points used to calculate the moving average defining the maximum effect. Geometric means were calculated for ratios of maximum and 24-hour inhibition.

Treatment effects were tested with an ANOVA model[17]; data from all three periods were included. Ninety-five percent confidence intervals were calculated for the mean of each treatment group with the mean square error from the ANOVA model on the basis of the logs of ratios (when calculated), then exponentiated. Pairwise differences between treatments were assessed with the 95% confidence intervals on the difference in treatment mean values. These intervals also used the mean square error from the ANOVA model.

A result was determined to be "statistically significant" when the statistical test yielded a two-tailed probability of 0.05 or less. Statistical analyses were restricted to the PRA data from the first study and angiotensin II antagonism data from part B of the second study.

RESULTS

Subject characteristics and clinical results

A total of 35 subjects (age range, 22 to 45 years) were evaluated in both studies. The study treatments were generally well tolerated. Adverse events were mild and self-limited, although one subject was discontinued from the first study for a migraine headache during placebo, another was discontinued for orthostatic hypotension considered to be unrelated to DuP 532 (0.1 mg), and a third was discontinued for faintness after the 100 mg dose of DuP 532. No subjects in the second study discontinued dosing because of adverse events.

Fig. 2 summarizes supine blood pressure and heart rate changes after the 100 and 200 mg doses of DuP 532 in the tolerability study. Clinically apparent mean changes in supine and standing blood pressure and heart rate were not identified in the healthy subjects in this study.

Plasma drug concentrations

Study 1. In the first study, total plasma concentrations of DuP 532 were measured at defined intervals after dosing on most study days. Fig. 3 shows the concentrations of DuP 532 for two periods in two representative subjects who had received 50 mg DuP 532 1 week before these measurements (i.e., period 3): 200 mg at 0 hours and placebo 1 week later. Measurable levels of DuP 532 were present before dosing on both sampling days. The drug appeared to be absorbed slowly because maximum measured concentrations were achieved at 6 and 30 hours after the 200 mg dose in the two subjects. Plasma levels of DuP 532 could be detected throughout the sampling period 1 week after administration of the 200 mg dose, after administration of placebo on that day. These data show an extremely long half-life for DuP 532 in humans after a single dose.

Study 2. In the second study, respective plasma concentrations of losartan, its active metabolite (EXP3174), and DuP 532 were measured before and 2, 4, 8, and 24 hours after the 100 mg dose of losartan and the 200 mg dose of DuP 532. Fig. 4 shows the mean plasma concentrations achieved in this study. Plasma concentration profiles of DuP 532 were clearly different from losartan, with much higher drug concentrations and a very long half-life (half-life of losartan averages 1 to 2 hours; half-life of EXP3174 averages 6 to 9 hours).[18]

In vitro protein binding and blood/plasma ratio studies

The binding of DuP 532 in vitro to plasma, purified albumin, and α_1-acid glycoprotein (orosomucoid) was measured by ultrafiltration with ^{14}C-DuP 532. DuP 532 was extensively bound in the plasma of all species examined, with 0.06% ± 0.02%, 0.21% ± 0.01%, 0.56% ± 0.05%, and 0.17% ± 0.09% free (unbound, mean ± SD, n = 4 samples analyzed in triplicate) at 1.0 μg/ml in human, rhesus monkey, beagle dog, and Sprague Dawley rat plasma, respectively. Binding was significantly greater in human plasma than in plasma from dogs and rhesus monkeys ($p < 0.001$) and nearly significantly greater in human compared with rat plasma ($p = 0.066$). This extreme degree of plasma binding (i.e., >99.4%) results primarily from binding to albumin because physiologic concentrations (4.5 gm/dl) of rat and human albumin bind 99.82% (0.18% ± 0.01% free) and 99.62% (0.38% ± 0.08% free) of added DuP 532, respectively. Binding to orosomucoid was insignificant, with 73.18% ± 1.51% and 80.99% ± 1.36% free at physiologic concentrations (1 mg/ml). The blood/plasma distribution ratio, assessed with fresh dog

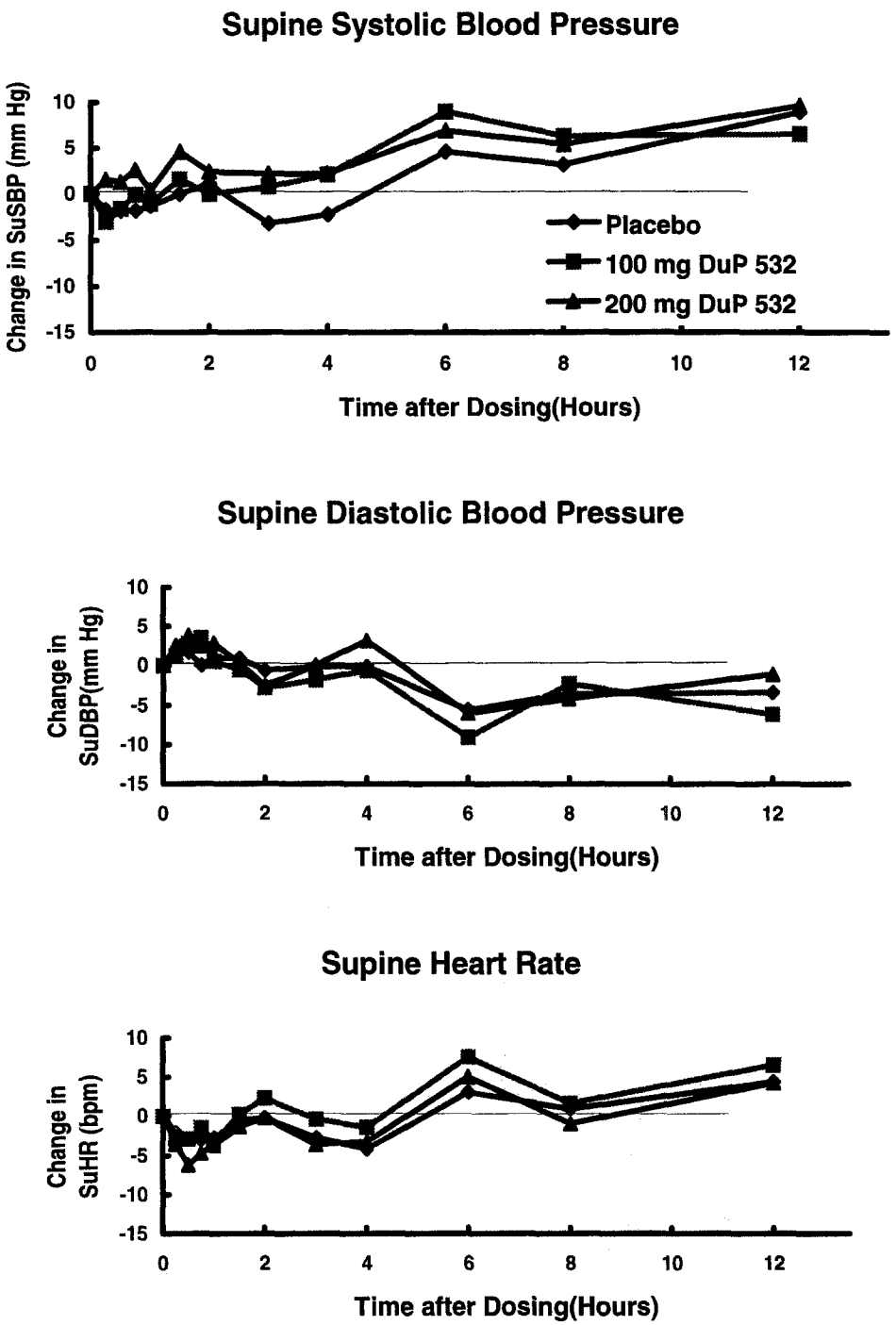

Fig. 2. Mean changes from predose values in supine systolic blood pressure **(top panel)**, diastolic blood pressure **(middle panel)**, and heart rate **(bottom panel)** in healthy male subjects given 100 mg DuP 532 (n = 9), 200 mg DuP 532 (n = 10), and placebo (n = 19).

and rat blood, was low, ranging from 0.44 ± 0.01 to 0.49 ± 0.03 for the rat and 0.56 ± 0.02 to 0.61 ± 0.03 for the dog at DuP 532 concentrations of 0.050 to 2.500 μg/ml.

Responses to angiotensin II infusions

Responses to bolus infusions of angiotensin II in the crossover phase (part B) of study 2 are summarized in Fig. 5, which shows the mean sys-

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