Antihypertensive Effects of Fasidotril, a Dual Inhibitor of Neprilysin and Angiotensin-Converting Enzyme, in Rats and Humans

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Abstract—The aim of this study was to assess the antihypertensive activity of fasidotril, a dual inhibitor of neprilysin (NEP) and angiotensin I–converting enzyme (ACE), in various models of hypertension in rats (spontaneously hypertensive rats [SHR]; renovascular Goldblatt 2-kidney, 1-clip rats; and deoxycorticosterone acetate [DOCA]-salt hypertensive rats) and in patients with mild-to-moderate essential hypertension. Fasidotril treatment (100 mg/kg PO twice daily for 3 weeks) resulted in a progressive and sustained decrease in systolic blood pressure (220 to 230 mm Hg) in SHR and Goldblatt rats compared with vehicle-treated rats and prevented the progressive rise in blood pressure in DOCA-salt hypertensive rats. After a 4-week placebo run-in period, 57 patients with essential hypertension were included in a randomized double-blind, placebo-controlled, parallel-group study and received orally either fasidotril (100 mg twice daily) or placebo for 6 weeks. Blood pressure was measured during the 6 hours after the first intake and then at trough (12 hours after the last intake) on days 7, 28, and 42. The first dose of fasidotril had no significant effect on blood pressure. After 42 days, compared with placebo, fasidotril lowered supine systolic and diastolic blood pressures by 7.4/5.4 mm Hg and standing blood pressure by 7.6/6.8 mm Hg. Fasidotril, a dual NEP/ACE inhibitor, was an effective oral antihypertensive agent during chronic treatment in high-renin renovascular rats, normal-renin SHR, and low-renin DOCA-salt hypertensive rats and in patients with essential hypertension. (Hypertension. 2000;35:1148-1153.)

Key Words: hypertension, essential enzyme angiotensin-converting enzyme neprilysin angiotensin-converting enzyme inhibitors

The renin-angiotensin system and natriuretic peptides are both involved in the regulation of blood pressure (BP) and electrolyte balance, and their pharmacological manipulation may be useful in the treatment of hypertension. Inhibition of angiotensin II formation by inhibition of angiotensin I–converting enzyme (ACE, EC 3.4.15.11) is well known to be an effective therapeutic means in hypertensive states. Inhibition of neprilysin (NEP, EC 3.4.24.11, also known as neutral endopeptidase, atriopeptidase, or enkephalinase), an enzyme implicated in the degradation of natriuretic peptides, represents a more recent approach to elicit BP reduction in hypertensive states by potentiating the diuretic, natriuretic, and vasorelaxant effects of natriuretic peptides.1,2

Animal studies have reported differences in the antihypertensive profile of ACE and NEP inhibitors according to the experimental model. Indeed, ACE inhibitors are effective in renin-dependent models, such as the 2-kidney, 1-clip Goldblatt model, and in spontaneously hypertensive rats (SHR), but they are ineffective in deoxycorticosterone acetate (DOCA)-salt hypertensive rats, a volume-dependent model.3 In contrast, NEP inhibitors are effective in DOCA-salt rats but ineffective in Goldblatt rats and SHR.4,5

In humans, the antihypertensive activity of pure NEP inhibitors remains uncertain. Their acute administration does not lower BP.6,7 Some studies have reported a significant decrease in systolic BP (SBP) and diastolic BP (DBP) after chronic administration,8–10 whereas others11,12 have observed only marginal or negligible effects. Therefore, combination of NEP and ACE inhibition might be useful in the treatment of hypertension, independently of the activity of the renin-angiotensin system or the degree of salt retention. The synergism of such a combination has been shown in animal models4 and in hypertensive patients,12 in whom coadministration of a NEP inhibitor and an ACE inhibitor produced depressor effects greater than those elicited by either selective inhibitor alone.

Because ACE and NEP are both metallopeptidases sharing multiple structural analogies, catalytic mechanisms, and mul-
tiple substrates, it has been possible to design dual NEP/ACE inhibitors that bear similar inhibitory activities against both enzymes within a single molecule with \( K_i \) in the nanomolar range.\(^{13,14}\) Fasidotril (BP 1.137, previously named alatriopril or aladotril) was the first of such compounds to be disclosed.\(^4\) It is a prodrug that is converted in the body into fasidotrilat, the active form. Fasidotrilat has a \( K_i \) of 5.1 nmol/L against human NEP (recombinant human enzyme) and of 9.8 nmol/L against human ACE (human kidney membrane).\(^4\) Similar inhibitory potencies were found for both enzymes in the mouse, but in the rat, fasidotrilat displayed a weaker potency against ACE (\( K_i \ 30 \)nmol/L). Fasidotril exerted typical actions of ACE inhibitors and NEP inhibitors, such as dose-dependent inhibition of angiotensin I–induced hypertension, protection of atrial natriuretic factor, and enhancement of diuresis, natriuresis, and cGMP urinary excretion in rats submitted to volume expansion.\(^4\) Previous studies in healthy volunteers have shown that fasidotril was well tolerated in single and multiple oral doses up to 1200 mg daily.

The objective of the present study was to assess the antihypertensive activity of fasidotril during chronic treatment in various experimental models of rat hypertension and in patients with essential hypertension.

Methods

Fasidotril (\( \text{N}(\text{S})-\alpha-(\text{mercaptomethyl})-3,4-(\text{methyleneoxy})-\text{hydroxycinnamoyl})-\text{l}-\text{alanine}, \text{benzyl ester}, \text{acetyl ester}) \) was supplied by Laboratoire Bioprojet, Paris, France.

Measurement of ACE and NEP Activities

Measurements of ACE and NEP activities were performed in mice and humans. In male mice receiving a single oral dose of fasidotril (either 1 mg/kg or 10 mg/kg), ACE activity was evaluated in the plasma and lungs up to 5 hours after treatment. Six healthy male volunteers, aged 18 to 40 years, received fasidotril at a dose of 100 mg twice daily for 8 days. Plasma ACE and NEP activities were evaluated at various times after each ingestion, during 12 hours at days 1 and 8. Each subject gave his written informed consent to participate in the study, which was approved by the Ethical Committee of Haute Normandie (France).

NEP activity was evaluated with 25 \( \mu \text{mol/L} \) succinyl-Ala-Ala-Phe-amidomethylcoumarin as the substrate,\(^{19}\) and ACE activity was evaluated with 0.2 \( \mu \text{mol/L} \) \( \alpha \)-amino-benzoyl-glycyl-\( p \)-nitrophenylalanine as the substrate.\(^{18}\)

Hypertensive Rats

Experimental procedures followed in the present study conformed with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication No. 85-23, revised 1985). Three hypertensive rat models were used. Male SHR (13 weeks old) were purchased from Ifca-Credo, l’Arbresle, France. Renovascular hypertension was induced in male Wistar rats by the 2-kidney, 1-clip method: a silver clip (0.20-mm inside diameter) was placed on the left renal artery with the animals under ether anesthesia, and the animals were studied 4 to 5 weeks after clipping. The selective ACE inhibitor captopril (10 mg/kg PO twice daily) was given to 10 Goldblatt rats who had received vehicle treatment, and SBP was measured 2 hours after the third captopril administration.

Hypertensive Patients

Patients, aged 18 to 70 years, with mild-to-moderate essential hypertension were included in a multicenter, randomized, double-blind, placebo-controlled, parallel-group study. A 4-week single-blind placebo run-in period was followed by a 6-week double-blind treatment period comparing fasidotril (100 mg PO twice daily) with placebo. The study protocol had been approved by the Ethical Committee of Hôpital Broussais (Paris, France), and all patients gave their written informed consent.

Patients were excluded if they had significant concurrent medical conditions or laboratory abnormalities or if they were taking treatment with nonsteroidal anti-inflammatory drugs other than low doses of aspirin. Any previous antihypertensive therapy was stopped before entry into the 4-week single-blind placebo run-in period. Patients with supine DBP between 95 and 114 mm Hg and supine SBP <220 mm Hg at the end of the run-in period were randomized to double-blind treatment. A total of 57 patients were randomized, of whom 50 completed the study (27 in the fasidotril group and 23 in the placebo group). The patients were studied on a sodium-free diet.

A mercury sphygmomanometer was used for measurements of supine BP in triplicate after patients were recumbent for 10 minutes and after patients had been standing for 2 minutes. Under each condition, the average of the 3 measurements was taken. BPs and HR were measured in the morning at 12 hours after the last capsule ingestion) at baseline and after 7, 28, and 42 days of treatment (D0, D7, D28, and D42, respectively). In addition, BPs and HR were measured repeatedly during the 6 hours after drug or placebo intake, at D0 and D42.

In addition, urinary cGMP was determined in a subgroup of 13 randomly selected patients who were investigated in 2 centers for clinical investigation. Nine patients received fasidotril, and 4 received placebo. Urinary cGMP was determined by radioimmunoassay (Biosys), as previously described.\(^{16}\) On D0 and D42, before oral dosing, patients voided their bladders to complete a 1-hour urine collection (U0, from 8 AM to 9 AM). Two further 3-hour urine collections were completed after drug intake (U1, from 9 AM to 12 AM; U2, from 12 AM to 3 PM). Urinary cGMP and creatinine were determined for each urine sample, and urinary cGMP was expressed as nmol/mg creatinine.

Statistical Analysis

In rats, data were analyzed by a 2-way ANOVA with repeated measures, followed by the Newman-Keuls test. In humans, data were analyzed by a 2-way ANOVA with repeated measures and adjustment on basal values, followed, in case of a significant group-time interaction, by the test of the group effect at a fixed level of the time factor. Values were expressed as mean±SEM. Differences were considered statistically significant at a value of \( P<0.05 \).

Results

Effect of Fasidotril Treatment on ACE and NEP Activities

In mice, fasidotril treatment (1 or 10 mg/kg) resulted in a dose-dependent inhibition of plasma ACE activity. Maximal inhibition was seen 0.5 hours after intake and reached 50%
with the low dosage and 82% with the high dosage. Three hours after intake, plasma ACE activity was still inhibited by 50% with the high dosage but had returned to basal value with the low dosage. Concomitant measurements of lung ACE activity showed a dissociation between the degrees of enzyme inhibition in plasma and tissue, with a progressive increase in lung ACE inhibition, which reached, 5 hours after intake, 43% with the low dosage and 62% with the high dosage.

In healthy men, the percentage of inhibition in plasma was between 75% and 90% for NEP and between 40% to 50% for ACE during the 4 hours after the first intake of 100 mg fasidotril. Afterward, the percentage of inhibition was attenuated but was still 34% for NEP after 12 hours. At day 8, the ACE or NEP inhibition profiles in plasma were similar to those at day 1.

Effects of Fasidotril on BP in Hypertensive Rats
In each model of hypertension, the values of SBP before therapy were not significantly different between vehicle-treated rats and fasidotril-treated rats. As shown in Figure 1, fasidotril treatment resulted in significant decreases in SBP in SHR and Goldblatt rats and prevented the increase in SBP in DOCA-salt rats compared with vehicle-treated rats.

In SHR and Goldblatt rats receiving the vehicle, SBP remained relatively constant during the treatment period. In SHR receiving fasidotril, SBP values were 205±4 mm Hg before therapy, 196±5 mm Hg at day 7, 185±4 mm Hg at day 14, and 163±5 mm Hg at day 21. At the end of the treatment period (day 21), the difference in SBP between fasidotril and vehicle treatment reached −21 mm Hg in SHR and −27 mm Hg in Goldblatt rats (Figure 1). Captopril (10 mg/kg PO twice daily), administered to Goldblatt rats at the end of the 3-week vehicle-treatment period, significantly lowered SBP, from 190±6 to 151±6 mm Hg the day after the initiation of therapy.

In DOCA-salt rats, a progressive rise in SBP was observed in the control group over the study period (210±3 mm Hg at the onset of the experiment and 231±8 mm Hg at day 21). Fasidotril stabilized SBP throughout the whole treatment period (214±3 mm Hg before therapy and 207±7 mm Hg at day 21). At the end of the treatment period, the difference in SBP between fasidotril- and vehicle-treated rats reached −24 mm Hg (Figure 1). In the 3 experimental models, fasidotril treatment had no significant effect on HR.

Effects of Fasidotril on BP and HR in Hypertensive Patients
A total of 57 patients were randomized (30 in the fasidotril group and 27 in the placebo group), of whom 50 completed the study (27 in the fasidotril group and 23 in the placebo group). There were no significant differences between the fasidotril- and placebo-treated groups for age (49.4±1.9 and 51.5±2.2 years, respectively), gender (male/female ratio 16/14 and 18/9, respectively), weight (73.1±2.2 and 75.6±2.7 kg, respectively), race (percentage of white patients 87% and 96%, respectively) and for baseline supine SBP and DBP (156.6±2.3 mm Hg, respectively), and for baseline supine SBP and DBP (156.6±2.3 mm Hg, respectively [SBP]; 100.0±0.9 versus 103.1±1.2 mm Hg, respectively [DBP]).

BP measurements were made before drug intake at baseline (D0) and at trough (12 hours after the last capsule ingestion) at D7, D28, and D42. Fasidotril treatment resulted in a significant decrease in SBP and DBP in the supine and standing positions (Figure 2). The efficacy was maintained at the same level between D7 and D42. At D42, fasidotril significantly (P<0.05) lowered supine SBP/DBP by 17.6±2.7/9.8±1.4 mm Hg and standing SBP/DBP by 16.1±3.3/10.5±2.2 mm Hg, whereas placebo lowered supine SBP/DBP by 10.2±3.0/4.4±1.6 mm Hg and standing SBP/DBP by 8.4±3.5/3.7±2.6 mm Hg (Figure 2). Thus, compared with placebo, fasidotril lowered supine SBP/DBP by 7.4±5.4 mm Hg and standing SBP/DBP by 7.6±6.8 mm Hg.

Repeated measurements of BP during the 6 hours after drug intake at D0 and D42 are shown in Figure 3. At D0, there was a significant time effect for DBP (P<0.001) and SBP (P<0.05), consistent with the effect of rest on BP, but there was no significant treatment effect and no time-treatment interaction, indicating the lack of antihypertensive effect of the first dose of fasidotril. However, at D42, the curve analysis showed a significant time-treatment interaction (P=0.005 for DBP and P=0.03 for SBP). A significant time effect was observed in the placebo group (P<0.001 for DBP and P=0.004 for SBP) but not in the fasidotril group, showing the stability of the antihypertensive effect of fasidotril with no significant change in BP values between 0 and 6 hours.
Supine and standing HR values, measured at trough at D0, D7, D28, and D42, were not significantly different between the placebo and the fasidotril groups (not shown). Likewise, the repeated measurements of HR during the first 6 hours after drug intake, at D0 and D42, had a similar profile in the placebo and the fasidotril groups (not shown).

No significant difference between the 2 groups was observed for the occurrence of side effects. Orthostatic hypotension was observed in 1 patient in the fasidotril group and in 2 patients in the placebo group. Coughing was observed in 1 patient in the fasidotril group and 2 patients in the placebo group. No significant difference between the 2 groups was observed for biological parameters.

Effects of Fasidotril on Urinary cGMP in Hypertensive Patients

During the first and second collection periods (U1 and U2) after drug intake at D0, compared with placebo, fasidotril significantly increased urinary cGMP levels ($P<0.05$). After fasidotril intake at D0, urinary cGMP increased from $0.50 \pm 0.11$ nmol/mg creatinine (U0) to $0.82 \pm 0.17$ nmol/mg creatinine (U1) and then to $0.68 \pm 0.16$ nmol/mg creatinine (U2), whereas urinary cGMP did not change after placebo intake; values were $0.38 \pm 0.09$ nmol/mg creatinine at U0, $0.45 \pm 0.07$ nmol/mg creatinine at U1, and $0.32 \pm 0.04$ nmol/mg creatinine at U2. Similar results were observed at D42.

Discussion

The major finding of the present study is that a dual NEP/ACE inhibitor, fasidotril, was an effective antihypertensive agent during chronic treatment in patients with essential hypertension.

As a prerequisite to the clinical study, we evaluated the inhibitory potency of NEP and ACE after treatment with fasidotril and its chronic antihypertensive efficacy in different experimental rat models. In hypertensive rats, we selected a dose of 100 mg/kg fasidotril because it has been previously shown to inhibit the pressor response to angiotensin I, to potentiate the depressor effect of bradykinin, and to exert marked inhibition of plasma NEP and ACE activities. The present study showed that a 3-week treatment with fasidotril resulted in a progressive and sustained reduction of BP in SHR and 2-kidney, 2-clip Goldblatt rats and prevented the progression of hypertension in DOCA-salt rats. The antihypertensive action of other dual NEP/ACE inhibitors in SHR, Goldblatt, or DOCA-salt rats has mainly been demonstrated after acute administration. To the best of our knowledge, only SHR have previously been submitted to chronic treatment with NEP/ACE inhibitors.

Thus, the present study affords original data in favor of the efficacy of a chronic treatment with a dual NEP/ACE inhibitor in 2 other models: the DOCA-salt rat and the 2-kidney, 1-clip Goldblatt rat. The DOCA-salt rat is a well-established model of volume-dependent hypertension, characterized by low plasma renin activity and elevated atrial natriuretic peptide levels. That fasidotril treatment stabilized BP in DOCA-salt rats is consistent with the accelerated hypertension observed after administration of atrial natriuretic antibodies in this model. It should be noticed that
although fasidotril prevented the progression of hypertension in DOCA-salt rats under chronic intake, it did not lower BP, in contrast to its acute effects in this model (data not shown). The 2-kidney, 1-clip Goldblatt rat can be considered to be a renin-dependent model of hypertension 3 weeks after surgery, because captopril significantly lowered BP at that time. Thus, the antihypertensive efficacy of a chronic treatment with the NEP/ACE inhibitor fasidotril was demonstrated in high-renin renovascular Goldblatt rats, in normal-renin SHR, and in low-renin DOCA-salt hypertensive rats.

In patients with essential hypertension, fasidotril administered over a 6-week period significantly decreased DBP and SBP in the supine and standing positions. The antihypertensive efficacy was maintained at the same level between D7 and D42. The magnitude of the BP decrease was in the range of that observed with other classes of antihypertensive agents.\(^\text{14}\) NEP inhibition after fasidotril intake was confirmed by the increase in urinary cGMP (second messenger of natriuretic peptides), contrasting with the lack of change after placebo intake.\(^\text{13,25–27}\)

The contribution of NEP inhibition to the antihypertensive effect of fasidotril in patients is suggested by the lack of BP-lowering effect of fasidotril after the first intake, despite a significant long-term antihypertensive efficacy. The time-course antihypertensive effect of fasidotril is different from that of pure ACE inhibitors, which are able to lower BP after the first drug intake.\(^\text{5,10}\) Measurements of plasma ACE and NEP activities in healthy volunteers indicated that the fasidotril dosage used in hypertensive patients (100 mg twice daily) resulted in a strong inhibition of plasma NEP during the first hours after drug intake, whereas only a moderate inhibition of plasma ACE was observed during the same period. This is consistent with the in vitro data, which for fasidotril gave \(K_I\) values of 5.1 nmol/L against NEP and 9.8 nmol/L against ACE.\(^\text{14}\)

However, some limitations deserve to be discussed. We did not measure NEP and ACE inhibition in the hypertensive patients of the present study and did not compare fasidotril with a pure ACE inhibitor. The potency of plasma NEP or ACE inhibition strongly depends on the substrate used,\(^\text{28}\) influencing the ratio of NEP/ACE inhibition. Plasma enzyme inhibition does not necessarily reflect tissue inhibition, which may persist for longer periods,\(^\text{25}\) and our results obtained in mice clearly demonstrate the lack of correspondence between plasma and tissue ACE activities. In hypertensive patients, it has been demonstrated that after administration of captopril, BP remains low despite intermittent resumption of normal plasma ACE activity.\(^\text{29}\)

The discrepancy between the acute and the long-term antihypertensive effects of fasidotril was also observed in the 2-kidney, 1-clip Goldblatt rat model. In this model, captopril (10 mg/kg PO twice daily) significantly lowered BP the day after starting treatment, whereas fasidotril (100 mg/kg PO twice daily) led to a progressive BP decrease, which was significant only at day 8 of treatment. A similar discrepancy in the time course of the antihypertensive effect between a dual NEP/ACE inhibitor and a pure ACE inhibitor has been reported by Wallis et al.\(^\text{25}\) These authors compared the effects of sampatrilat (dose range 50 to 200 mg once daily), which has an in vitro inhibitory profile similar to that of fasidotrilat, with the effects of the selective ACE inhibitor lisinopril (20 mg) over a 10-day treatment period. In contrast to lisinopril, sampatrilat did not lower BP at hour 6 after the first intake. A similar antihypertensive effect between the 2 drugs was observed only after 10 days. Taken together, these data suggest that ACE inhibition by fasidotril or sampatrilat\(^\text{25}\) is not strong enough to acutely lower BP and that slowly occurring mechanisms, in addition to those of pure ACE inhibition, are required to obtain a long-term antihypertensive effect with dual NEP/ACE inhibitors.

A likely mechanism is the inhibition, by natriuretic peptides, of the increase in renin secretion in response to ACE inhibitors,\(^\text{25,30}\) although this mechanism may be offset by the activation of the renin-angiotensin and sympathetic systems in response to the BP fall.\(^\text{10}\) The simultaneous inhibition of NEP and ACE pathways may potentiate the antihypertensive effect of fasidotril through other mechanisms. First, angiotensin II may antagonize the effects of natriuretic peptides through the downregulation of guanylate cyclase receptors and the upregulation of cGMP phosphodiesterase, both leading to a decrease in cGMP.\(^\text{31}\) Second, coinhibition of NEP and ACE may result in an increase in the effect of bradykinin that is greater than the effect elicited by either selective inhibitor alone.\(^\text{32,33}\)

The mechanism of the antihypertensive effect of dual NEP/ACE inhibitors is complex because of the lack of specificity of the 2 peptidases, resulting in alterations of various peptidergic systems. NEP, in addition to degrading natriuretic peptides, can also hydrolyze angiotensin,\(^\text{1–7}\) substance P, and endothelin 1, at least in vitro.\(^\text{34}\) Thus, dual NEP/ACE inhibition could lead to vasodilator and vasoconstrictor effects, which may depend on time and arterial territory, accounting for the progressive antihypertensive effect. Because angiotensin II is a substrate of NEP, a potentially detrimental effect of NEP inhibition is protection of the peptide, leading to an increase in angiotensin II plasma levels.\(^\text{10,26}\) However, treatment with a dual ACE/NEP inhibitor, through ACE inhibition, may attenuate this process by reducing the production of angiotensin II. Indeed, intravenous injection of candaxatrilat in healthy subjects caused an increase in BP, but the rise was prevented by enalapril pretreatment.\(^\text{37}\)

Dual NEP/ACE inhibitors can thus lower BP independently of the activity of the renin-angiotensin system or the degree of salt retention and may be more effective than the present monotherapy in treating a broader range of hypertensive patients. In this respect, it was recently demonstrated\(^\text{35}\) that an 8-week treatment with sampatrilat resulted in sustained antihypertensive actions in black hypertensive subjects shown to be insensitive to the long-lasting antihypertensive effects of ACE inhibitors.

In conclusion, the present study shows the efficacy of a chronic treatment with the dual NEP/ACE inhibitor fasidotrilat in hypertension. In rats, it exhibited a large spectrum of activity, combining the efficacy of ACE inhibitors and NEP inhibitors. In patients with essential hypertension, fasidotril produced a progressive and sustained decrease in SBP and DBP.


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References
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