Clinical Trial

Multiple Ascending Dose Study With the New Renin Inhibitor VTP-27999

Nephrocentric Consequences of Too Much Renin Inhibition


Abstract—This study compared the pharmacodynamic/pharmacokinetic profile of the new renin inhibitor VTP-27999 in salt-depleted healthy volunteers, administered once daily (75, 150, 300, and 600 mg) for 10 days, versus placebo and 300 mg aliskiren. VTP-27999 was well tolerated with no significant safety issues. It was rapidly absorbed, attaining maximum plasma concentrations at 1 to 4 hours after dosing, with a terminal half-life of 24 to 30 hours. Plasma renin activity remained suppressed during the 24-hour dosing interval at all doses. VTP-27999 administration resulted in a dose-dependent induction of renin, increasing the concentration of plasma renin maximally 350-fold. This induction was greater than with aliskiren, indicating greater intrarenal renin inhibition. VTP-27999 decreased plasma angiotensin II and aldosterone. At 24 hours and later time points after dosing on day 10 in the 600-mg group, angiotensin II and aldosterone levels were increased, and plasma renin activity was also increased at 48 and 72 hours, compared with baseline. VTP-27999 decreased urinary aldosterone excretion versus placebo on day 1. On day 10, urinary aldosterone excretion was higher in the 300- and 600-mg VTP-27999 dose groups compared with baseline. VTP-27999 decreased blood pressure to the same degree as aliskiren. In conclusion, excessive intrarenal renin inhibition, obtained at VTP-27999 doses of 300 mg and higher, is accompanied by plasma renin rises, that after stopping drug intake, exceed the capacity of extrarenal VTP-27999 to block fully the enzymatic reaction. This results in significant rises of angiotensin II and aldosterone. Therefore, renin inhibition has an upper limit. (Hypertension. 2014;63:942-950.)

Key Words: aldosterone ■ angiotensins ■ blood pressure ■ humans ■ pharmacokinetics ■ renin

The renin–angiotensin–aldosterone system (RAAS) is a hormone system that regulates blood pressure, plasma sodium and potassium levels, and extracellular fluid volume in the body. The RAAS sequentially processes angiotensinogen to angiotensin II (Ang II), a peptide hormone that is a potent vasoconstrictor. Renin catalyzes the first and rate-limiting step of the RAAS cascade and is a specific protease for angiotensinogen. Direct renin inhibition leads to a decrease in plasma renin activity (PRA) and is expected to decrease Ang II and aldosterone levels. Plasma renin concentration (PRC) and prorenin levels are expected to increase through a compensatory feedback induction mechanism. The only direct renin inhibitor currently available for clinical use is aliskiren, marketed at 150 and 300 mg/d. Its use at doses >300 mg/d is hampered by the occurrence of gastrointestinal side effects including cramping and diarrhea.

VTP-27999 is a highly potent direct renin inhibitor being developed for the treatment of chronic renal disease and end-organ protection.1,4 Its oral bioavailability is ≈10-fold higher than that of aliskiren. This study examines the safety and tolerability, pharmacokinetics, and pharmacodynamics of VTP-27999 compared with a placebo and to the full marketed dose of aliskiren, 300 mg/d, after 10 days of administration to salt-depleted healthy volunteers.

Materials and Methods

The Methods section is available in the online-only Data Supplement.

Results

Subject Demography and Disposition

A total of 37 subjects participated in the study (35 men, 2 women: 3 Asian, 8 black, and 26 white; age, 18–45 years;
mean age, 27 years; weight, 60–111 kg; and mean weight, 80 kg). Two subjects were discontinued from the study: 1 subject because of an adverse event (AE) unrelated to study medication and 1 subject who was discontinued by the investigator for having a prestudy measurement that did not meet entry criteria.

**Pharmacokinetics**

After oral administration in the fasted state, VTP-27999 was rapidly absorbed, attaining C<sub>max</sub> at 1 to 4 hours (Figure 1; Table S1 in the online-only Data Supplement), after which plasma concentrations decreased slowly in a multi-exponential manner. Administration of VTP-27999 exhibited dose-proportional pharmacokinetics, based on area under the curve (AUC<sub>0–24</sub>) and C<sub>max</sub> (Table S1). The t<sub>1/2</sub> for VTP-27999 ranged from 24 to 30 hours across all doses and steady-state levels were reached by day 7 (data not shown). Plasma VTP-27999 exposures (AUC) on day 10 were ≈2-fold higher than on day 1, also consistent with a t<sub>1/2</sub> of 24 to 30 hours. Alikiren pharmacokinetic parameters were in agreement with those reported previously, its t<sub>1/2</sub> being ≈30 to 40 hours.5 Urine was collected on days 1 and 10, and at steady state on day 10, between 5% and 10% of the daily dose was excreted unchanged into the urine (Table S2).

**Changes in Plasma RAAS Components**

As expected with the low-salt diet, baseline PRA, PRC, plasma Ang II, and aldosterone levels were increased when compared with reference values from healthy volunteers on a normal sodium diet.6 PRA declined rapidly and dose dependently after dosing on day 1, reaching a blockade of >90% within 15 minutes at all doses of VTP-27999 (Figures 2 and 3). Although PRA started to rise again after 4 to 6 hours, suppression was maintained for 24 hours post dose on day 1 at all doses of VTP-27999 and with alikiren, with little difference in the PRA values between the various VTP-27999 doses and alikiren during the 24 hours. There was little change in PRA during the 24 hours with placebo. On day 10, predose PRA was below baseline (and similar to the 24-hour value on day 1) in all active dose groups, and there was an acute drop in PRA after dosing. The PRA returned to day 10 predose levels by 12 hours and remained stable during the subsequent 60 hours (through 72 hours post dose) except for the 600-mg group, in which the PRA continued to rise through 48 hours post dose, ending at 1.5 to 2× the day 10 predose level. There was a minor decrease in day 10, predose PRA in the placebo group (perhaps reflecting temporal changes during clinic confinement) with no intraday reduction.

**Figure 1.** Drug levels (pharmacokinetics [PK]) on treatment days 1 (top) and 10 (bottom), and after stopping treatment. Data are mean±SEM of n=4 to 6.

**Figure 2.** Plasma renin activity (PRA) and the plasma concentration of renin (PRC) and prorenin, angiotensin II, and aldosterone, respectively, on treatment days 1 (left) and 10 (right), and after stopping treatment. Data are mean±SEM of n=4 to 6. Note that the PRC scale is 10-fold greater for day 10 compared with day 1.
Plasma Ang II levels largely followed PRA (Figures 2 and 3; Figure S1) on day 1 except that recovery to baseline levels occurred by 24 hours. On day 10, the predose Ang II levels were similar to baseline in all dose groups. After dosing on day 10, Ang II again decreased after VTP-27999 or aliskiren administration and returned to day 10, predose levels by 6 hours. At 72 hours in the 600-mg group, and similar to the PRA values, the mean Ang II value was higher than baseline by 2- to 3-fold.

For plasma aldosterone, the degree of suppression on day 1 was less than that for PRA or Ang II, and the levels generally recovered by 24 hours (Figures 2 and 3). The day-1 plasma
aldosterone levels were higher in the placebo group than the drug-treated groups at all time points, including the baseline time point, for unknown reasons, but the values were stable during the 24 hours. On day 10, aldosterone levels were lower in the placebo group than on day 1, and similar to the day-1 baseline values for the other groups. In the VTP-27999 75- and 150-mg groups and the aliskiren group, baseline aldosterone levels on day 10 were similar to those on day 1. In the 300- and 600-mg dose groups, levels tended to be somewhat higher at predose on day 10. The aldosterone levels for all groups returned to day 10, predose levels by 6 hours and remained stable through 72 hours post dose except for the 600-mg group. In that group, and similar to the PRA and Ang II values, the aldosterone levels continued to rise through the 72-hour final measurement.

VTP-27999 dose dependently increased PRC and prorenin on day 1, with up to a 35-fold increase over baseline for the $C_{\text{max}}$ of PRC in the 600-mg VTP-27999 group (Figures 2 and 4). Peak levels were reached after 6 and >12 hours, respectively, for PRC and prorenin, with persistent elevations through hour 24. Substantial further rises in PRC (≤350-fold for the $C_{\text{max}}$ of PRC at 600 mg VTP-27999) occurred during treatment on day 10, when the placebo group showed a minor reduction in PRC and no change in prorenin levels. Note that in Figure 2, the PRC scale for day 10 is 10-fold greater than the day-1 scale. After stopping treatment, PRC decreased roughly in parallel with plasma VTP-27999 concentrations, albeit somewhat more slowly (see terminal slopes in Figure 4). However, at 72 hours after the last dose of drug, the PRC levels were still ≥10-fold higher for aliskiren and the lower VTP-27999 dose groups, and to ≤50-fold higher for the 600-mg dose group compared with the baseline levels on day 1. Prorenin levels increased in a dose-dependent manner, and this was particularly apparent after 10 days of dosing (Figures 2 and 4). The prorenin levels remained high after stopping treatment and started to decrease only after 48 hours consistent with the known longer half-life of prorenin.

Drug levels correlated positively with PRC (Figure 5). PRC variation versus drug levels diminished when comparing $AUC_{0-24}$ for both drug and PRC (Figure 5). To measure the relative level of inhibition of renin in the circulation, the PRA values were divided by the PRC values, and the PRA/PRC ratio was then normalized such that 100% was set to the predose ratio in the placebo group on day 1 (Figure 6). Renin was inhibited between 90% and 99.8% on day 1 in a dose- and exposure-dependent fashion and between 95% and 99.9% on day 10. Even at 72 hours after the last dose, renin was inhibited by 90% to 98%, with the greatest inhibition being in the 600-mg group.

Changes in Urinary RAAS Components

The urinary renin excretion rate increased dose dependently during VTP-27999 treatment on day 1, and the increases were even more prominent on day 10 (Figure 7). Note the different scales used on the graphs for the 2 different days. These changes generally reflected the changes seen in PRC and were greatest in the VTP-27999 600-mg dose group. Changes in urinary renin resulting from aliskiren administration were comparable with those observed with 75 and 150 mg VTP-27999.

Urinary angiotensinogen and prorenin excretion rates were unaffected by drug treatment (data not shown).

The urinary aldosterone excretion rate was decreased during all treatments on day 1 versus placebo, particularly in the 0- to 6-hour interval (Figure 7). On day 10, urinary aldosterone excretion rates for the 2 lower doses of VTP-27999 and aliskiren were again below the placebo group for 0 to 6 hours and were near placebo for the remainder of the day. Conversely, aldosterone excretion rates on day 10 were above placebo for the 300- and 600-mg doses of VTP-27999. The aldosterone urinary excretion rates reflected the elevated plasma aldosterone levels for these 2 groups.

On day 1, 24-hour urinary sodium excretion rates (normalized for creatinine excretion) were, as expected, higher at all doses of VTP-27999 and aliskiren than with placebo (Table S3). On day 10, these rates had decreased and were similar to that observed in the placebo group.

Safety Profile and Hemodynamic Effects

Two of the 37 subjects who received study medication were discontinued from the study. One subject was withdrawn for an AE of sinus tachycardia that was also present pre treatment; the investigator considered this AE to be because of anxiety and unrelated to study medication (VTP-27999 75 mg). One subject was discontinued by the investigator after 1 dose of placebo when it was noticed that the subject’s predose ECG did not meet entry criteria. There were no serious AEs. Eleven of the 25 subjects who received VTP-27999 reported ≥1 AE (Table S4). AEs were mild to moderate in intensity and all were reported as resolved by the follow-up visit on day 17. The greatest number of AEs was reported at the 600-mg dose of VTP-27999; the majority of which were nausea and vomiting. There were no important findings on physical examinations, ECGs, or laboratory tests; serum potassium levels were not elevated (Table S5).

In these normotensive but sodium-depleted subjects, VTP-27999 and aliskiren decreased blood pressure on days 1 and 10 compared with placebo (Figure 8). However, because of the small sample size and the inherent variability in blood pressure within and between days, the relative effects on blood pressure between the different doses of VTP-27999 and aliskiren could not be differentiated. Heart rates tended to be somewhat elevated in all dose groups on day 10 versus placebo, with a more substantial increase in the heart rate in the 600-mg dose group.

Discussion

This is the first description of the pharmacokinetic and pharmacodynamic characteristics of a new renin inhibitor, VTP-27999, in human subjects. VTP-27999 is rapidly absorbed, reaching $C_{\text{max}}$ between 1 and 4 hours after oral dosing, and it had a multi-exponential decay curve from plasma. There was a rapid distribution phase followed by a terminal removal phase with a $t_{1/2}$ of 24 to 30 hours. The pharmacokinetics were dose-proportional, and the day-10 pharmacokinetic parameters were as predicted from the day 1 kinetics; day 10 $C_{\text{max}}$ values were ≥50% greater than the day 1 values, and the AUC was approximately doubled. Relatively little of the drug was excreted in the urine (<10%), and thus the liver
and metabolism are apparently the major routes of clearance. With the long terminal $t_{1/2}$ and dose proportionate exposure, the pharmacokinetics supports once a day dosing for VTP-27999.

VTP-27999 was generally safe and well tolerated in these salt-restricted, healthy, normal volunteers. Nausea and some vomiting were observed in the 600-mg dose group, but this was not observed at the lower doses of VTP-27999, and no subject discontinued drug because of tolerability issues. There were no clinically significant changes in laboratory values or ECGs, and more specifically, no significant changes in serum

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**Figure 4.** Plasma prorenin, renin, and drug concentrations on treatment days 1 and 10, and after stopping treatment. Each panel represents a different dose of drug. Data are mean±SEM of n=4 to 6. PRC indicates plasma renin concentration.
Changes in blood pressure are not easily seen in normotensive volunteers, even when following a low-salt diet, and differences in blood pressure in drug-treated versus placebo only could be observed. There were no drug-related discontinuations or significant AEs.

The pharmacodynamics of VTP-27999 demonstrated a rapid onset and almost complete inhibition of PRA; >90% at 15 minutes after dosing on day 1 at all dose levels, and significant inhibition of PRA was maintained for 24 hours on day 1. There was also substantial inhibition of PRA at all dose levels on day 10, supporting once a day dosing, consistent with the pharmacokinetics. There was a clear dose–response for PRC and a significant induction between days 1 and 10, with a 2- to 10-fold higher \( C_{\text{max}} \) on day 10 compared with day 1 for the different doses of drug. With the PRC providing the clearest dose–response, one can compare the relative pharmacodynamic potency of orally administered VTP-27999 with aliskiren. With the day-1 PRC measurements, on an oral dose basis, VTP-27999 was ≈2- to 3-fold more potent than aliskiren, and on day 10, it was ≈2-fold more potent. Given the comparable IC\(_{50}\) of both inhibitors,\(^3,4\) it can be concluded that the greater oral potency of VTP-27999 is related to its greater bioavailability.

The full pharmacodynamic data set demonstrates an interesting and surprising relationship between the dose of VTP-27999 administered, the intrarenal inhibition of renin, and the blockade of the systemic RAAS. Because the majority of renin is synthesized and secreted from juxtaglomerular cells in the renal glomeruli, and because renin secretion by the kidney is downregulated by Ang II, plasma levels of renin are inversely related to, and are a reflection of, inhibition of renin in the kidney.\(^5\)

Biochemical data obtained from day 10 and out to 72 hours after the day 10 dose are consistent with the 600-mg dose of VTP-27999 being less effective than 300 mg on decreasing PRA, Ang II production, and aldosterone production at 48 and 72 hours after dosing. After the 600-mg dose, the rise in the PRC \( C_{\text{max}} \) on day 10 was ≤350-fold compared with the day 1 baseline values. PRA remained below baseline (ie, pre-dose level) during the full 24 hours after the last dose, and thereafter started to rise. Ang II and aldosterone also started to rise 24 hours after the last dose in the 600-mg cohort, even reaching plasma concentrations that were several fold above baseline. These rises were not seen for the other doses of VTP-27999 or for aliskiren, except for a 50% rise in plasma aldosterone in the VTP-27999 300 mg cohort at 72 hours after the day 10 dose.

Thus, a dissociation occurs in the 600-mg dose group at the later time points (>24 hours after dosing) between the intrarenal blockade of renin, which increases the synthesis and secretion of renin, and the systemic blockade of renin. This dissociation occurs at times when there are declining plasma levels of the renin inhibitor. After the last dose of drug on day 10, the decrease in PRC generally paralleled the decrease in renin inhibitor concentration (Figure 3). However, in the case of 600-mg VTP-27999 cohort, PRC decreased significantly...
more slowly than VTP-27999 (≈1/3 as fast) after stopping treatment, and this is the likely cause of the PRA and plasma Ang II and aldosterone overshoot. Renin is still inhibited 98% to 99% at these times (Figure 6), but because the renin levels are so elevated, the absolute PRA values are increased 1.5- to 2-fold.

When high doses of VTP-27999, aliskiren, and other renin inhibitors, such as ciprokiren and remikiren, are administered, all of which are known to accumulate in the kidney,9,10 the degree of renal/juxtaglomerular RAAS blockade may be greater than that outside the kidney. Under such conditions, there is a sustained stimulation of renal renin release secondary to the intrarenal inhibition of renin and subsequent decrease of Ang II at the level of the juxtaglomerular cells, even when extrarenal renin inhibitor levels are no longer sufficient to fully block renin systemically. However, once released into the circulation, these VTP-27999–bound renin molecules face declining VTP-27999 concentrations in plasma, and thus dissociation of the renin–VTP-27999 complex is likely to occur.

The capacity of the human body to counteract RAAS blockade through the synthesis and secretion of renin did not reach its limit in the present study, and interestingly, the renin levels observed at 600 mg VTP-27999 were comparable with those in patients with renin-producing tumors.11 If a stimulation for renin secretion persists during a long period of time, the increased secretory capacity involves increased synthesis of prorenin through hypertrophy of juxtaglomerular cells and metaplastic transformation of preglomerular vascular smooth muscle cells into renin-producing cells.12,13 On day 10, the PRC AUC values were 2 to 10× higher than the AUC values on day 1. Thus, there is a large increase in renin secretion with drug treatment and also with drug treatment between days 1 and 10. This is almost certainly because of both strong inhibition of intrarenal renin and an increase in the overall secretory capacity, particularly at the higher doses of VTP-27999. This hypertrophy and metaplastic transformation may also contribute to the overshoot or escape of the systemic RAAS. For the same level of decrease in intrarenal Ang II, there will be a greater capacity for renin production and release by the increased mass of the juxtaglomerular cells in the glomeruli.

When the degree of renal RAAS blockade exceeds that outside the kidney, plasma renin will increase as will the PRA, and this rise in PRA will lead to a counter-regulatory increase in the concentrations of Ang II and aldosterone (escape). It is likely that this would occur with aliskiren too, had it been able to be tested at higher doses. Our findings are reminiscent of the nephrocentric view of angiotensin-converting enzyme inhibition noted 25 years ago in patients with congestive heart failure.14 It was asked why the kidneys continue to release renin in such patients; the answer being that they do everything possible to preserve renal function and glomerular filtration, apparently at the expense of the hemodynamic burden on the heart. This situation mimics our findings, although obtained in healthy volunteers, at the highest dose of VTP-27999 where the kidneys respond to renal RAAS suppression by releasing large quantities of renin, resulting in elevations of PRA, Ang II, and aldosterone. Such elevated Ang II levels might also, either directly or indirectly (via the facilitation of norepinephrine release), be responsible for the rise in heart rate that was observed at the highest VTP-27999 dose.
The PRC correlated with the plasma levels VTP-27999, and if assessments are limited to the PRC-VTP-27999 concentration relationship on day 1 (i.e., before the induction of renin synthesis) or day 10 (i.e., after the induction of renin synthesis), the variation in PRC levels for a given drug concentration varies between 30- and 100-fold. There are several potential reasons for this variation. First, there are biological interindividual variations in renin levels. Second, peak levels for renin occurred 2 to 4 hours after peak levels of VTP-27999. When the time/drug level/PRC relationship was plotted, there was clear hysteresis for the relationship between drug levels and the PRCs (plots not shown). To compensate for this hysteresis, we compared the AUC0–24 for VTP-27999 versus the AUC 0–24 for PRC, and did this for both on days 1 and 10. By doing this, the variation in the PRC AUC for a given VTP-27999 AUC was reduced to <10.

PRA also correlated strongly with Ang II and less with aldosterone (Figure S1).

Prorenin, like renin, increased dose dependently, the only difference being that this rise required >12 hours on day 1, because of the fact that it depends on the actual induction of prorenin synthesis, because prorenin is not stored in the kidney. On day 10, prorenin was elevated compared with day 1 predose values, and there was little change in prorenin for 24 hours, reflecting the longer half-life of prorenin in the circulation. This was followed by a slow decline in concentration during the subsequent 2 days. Changes in urinary renin and aldosterone levels generally reflected the alterations in their levels in plasma at the various doses and various times of collection, suggesting that urinary renin originates largely, if not completely, from filtration from the systemic circulation into the urine and not from direct secretion from the kidney into the urine.

**Perspectives**

The data obtained in the present study support that VTP-27999 is a potent and generally well-tolerated, renin inhibitor, capable of effectively suppressing the RAAS with once a day dosing, and on an oral dose-basis, VTP-27999 is approximately twice as potent as aliskiren. VTP-27999, which is known to be sequestered in the kidney, has a temporal asynergism, dependent on the tissue and plasma levels of the inhibitor, between intrarenal renin inhibition, with its consequent stimulation of renin synthesis and release, and extrarenal inhibition with its consequent decrease in PRA, Ang II, and aldosterone. With VTP-2799 at doses >300 mg/d, the high level of intrarenal renin inhibition, with subsequent dramatic increases in renin release, results in systemic renin levels that are greater than the systemic levels of VTP-27999 can fully inhibit at 24 to 72 hours after the last dose. This raises the interesting possibility of VTP-27999 at the highest dose having superior renal renin inhibition and protection while having increased systemic

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**Figure 8.** Change from baseline in systolic blood pressure (SBP; A), diastolic blood pressure (DBP; B), and heart rate (HR; C) on days 1 and 10, and after stopping treatment. Data are mean±SEM of n=4 to 6.
PRA, Ang II, and aldosterone levels. This study also demonstrates once more the variability of local renin–angiotensin systems when stressed by a renin inhibitor, and the need for more studies investigating, with an appropriate and complex technology, the pharmacodynamics of the RAAS, within and outside the kidneys. The dose of VTP-27999 to optimally balance the antiproteinuric, nsprefrptive, antihypertensive, and cardioprotective effects remains to be determined.

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**References**

**Novelty and Significance**

**What is New?**
- VTP-27999 is a safe and generally well-tolerated renin inhibitor, capable of effectively suppressing angiotensin and aldosterone production in humans at doses of ≤300 mg, and it is approximately twice as potent as aliskiren on a dose basis.
- VTP-27999 dose dependently increases renin production, and when given at doses >300 mg, these increases are so great that 24 to 72 hours after a day 10 dose, they overwhelm the ability of VTP-27999 to inhibit systemic renin.

**What is Relevant?**
- High renin inhibitor doses, accumulating in the kidney, are likely to suppress the renal renin–angiotensin–aldosterone system (RAAS) more effectively than they suppress extrarenal RAAS. Although this may amplify the beneficial renal effects, it simultaneously results in extrarenal RAAS stimulation. Thus, high levels of renin inhibition have counteracting effects, and more is not necessarily better. To what degree this kidney-specific RAAS suppression also applies to other RAAS blockers, particular when given in combination, needs to be explored.

**Summary**
VTP-27999, a new renin inhibitor, is very efficacious, safe, and generally well tolerated after 10 days of dosing, and it is 2-fold more potent, on a dose basis, than aliskiren. When testing VTP-27999 in salt-depleted healthy volunteers, it became clear that at the highest dose of VTP-27999 tested, 600 mg, it blocks the renal renin–angiotensin system more effectively than the circulating RAAS. As a consequence, after 10 days of dosing and at 24 to 72 hours after drug intake, the accompanying plasma renin rise exceeds the capacity of extrarenal VTP-27999 to block fully the enzymatic reaction. Although intrarenal renin–angiotensin system is inhibited at these times, extrarenal renin–angiotensin system activation occurs, increasing circulating concentrations of angiotensin II and aldosterone. Renin inhibition has an upper limit and confronting the large variability in the individual responses of renin release; these drugs should be dosed optimally and not necessarily maximally.
Multiple Ascending Dose Study With the New Renin Inhibitor VTP-27999: Nephrocentric Consequences of Too Much Renin Inhibition


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DOSE-FINDING STUDY WITH THE NEW RENIN INHIBITOR VTP-27999:
NEPHROCENTRIC CONSEQUENCES OF TOO MUCH RENIN INHIBITION

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SUPPLEMENTAL INFORMATION

Material and Methods

Study Protocol

This was a randomized, double-blind, placebo- and active-controlled study in which once-daily doses of 75, 150, 300, or 600 mg VTP-27999 were administered orally as 75 mg tablets for 10 days to healthy subjects (males and females not of childbearing potential, aged between 18-45 years, and with a BMI ranging from 18-32 kg/m²) in 4 sequential ascending dose cohorts. The study was approved by the PRACS Institute, Ltd. Institutional Review Board. All subjects provided written, informed consent and the study was conducted in accordance with accepted standards of Good Clinical Practice (GCP), as defined in the International Conference on Harmonization (ICH) E6 Guideline for Good Clinical Practice, in agreement with the latest revision of the Declaration of Helsinki.

Subjects visited the clinic on Day -5 to receive dietary instructions and low salt meals (10 mEq/day) for outpatient consumption. Subjects were admitted to the clinic on Day -1 to undergo baseline assessments and were verified to have a spot urinary Na⁺/creatinine ratio of <30 mEq/g creatinine before they could continue in the study. Subjects were randomized within each dose cohort to receive VTP-27999, placebo or aliskiren 300 mg, all administered orally once daily in the fasted state at approximately 08:00 hours. VTP-27999 75 mg tablets and a matching placebo were supplied to the clinical site by Vitae Pharmaceuticals, Inc. Aliskiren 300 mg tablets were purchased by the site pharmacy from a commercial source. The 75 mg and 150 mg cohorts each included 8 subjects (6 on VTP-27999, 1 on aliskiren and 1 on placebo matching VTP-27999). The 300 mg and 600 mg cohorts each included 10 subjects (6 on VTP-27999, 2 on aliskiren and 2 on placebo). Dosing began on the morning of Day 1 and continued through Day 10. Subjects were released from the clinic on Day 13 and returned for a safety visit on Day 17±2 days, after which they were discharged from the study.

Tolerability and Safety Assessments

Routine safety assessments included vital signs, physical examination, standard laboratory tests, and 12-lead ECGs. Adverse events (AE) were monitored throughout the study.

Pharmacokinetic and Pharmacodynamic Assessments

Blood samples for the determination of plasma VTP-27999 and aliskiren concentrations were collected at 0 hour (pre-dose), at 0.25, 0.5, 1, 2, 4, 6, 8, 12, and 24 hours after dosing on Days 1 and 10, predose on Days 5 and 7, and at 48 and 72 hours after dosing on Day 10. Samples were processed on ice in the presence of a serine protease inhibitor and stored at -70°C until shipment to the bioanalytical laboratory.

Blood samples for determination of plasma renin activity (PRA), plasma renin concentration (PRC), plasma prorenin concentration, Ang II, and aldosterone were obtained as described previously¹ at -30 and -15 minutes (pre-dose) and at 0.5, 2, 4, 6, 12, and 24 hours post-dose on Day 1 and Day 10, pre-dose on Day 5 and Day 7, and at 48 and 72 h after the last dose on Day 10. PRA, PRC, prorenin, and aldosterone samples were processed at room temperature and stored at -70°C until analysis. Ang II samples were placed on ice, processed at 4°C and stored at -70°C until analysis.
Urine was collected for the measurement of VTP-27999, Na⁺, creatinine, renin, angiotensinogen, aldosterone, Ang II, and prorenin over the intervals of 0-6, 6-12, and 12-24 hours post-dose on Days 1 and 10. Urine samples were stored at -20°C until analysis.

Blood pressure and heart rate were measured in triplicate, after quiet semi-supine rest before each blood sample collection, using an automated device.

Bioanalytical Methods

Plasma VTP-27999 concentrations were measured using a solid phase extraction method and LC-MS/MS analysis developed and validated by Cetero Research, Houston, TX, USA. The standard curve ranged from 1-200 ng/mL. The analytes and internal standard peak areas were exported to Watson Laboratory Information Management System (LIMS) to create calibration curves using weighted (1/x²) least squares regression fit to a linear model. The concentrations of the standards, QCs, and samples were calculated using Watson LIMS. Plasma aliskiren concentrations were determined using a of solid phase extraction method and LC-MS/MS analysis developed at Vitae Pharmaceuticals. The standard curve ranged from 1-500 ng/mL. The analyte and internal standard peak areas were calculated by Analyst 1.4.2® Software to create a calibration curve using weighted (1/x²) least squares regression fit to a linear model. The concentrations of the standards, QCs, and samples were calculated using Analyst 1.4.2® Software.

PRA was determined by measuring Ang I generation during incubation of plasma at 37°C and pH 7.4. PRC was measured with an immunoradiometric kit (Renin III, Cisbio, Gif-sur-Yvette, France).² Urinary renin was measured with the same renin kit after concentrating the samples as described before.² Plasma Ang II was measured by radioimmunoassay after SepPak extraction as described before. Plasma aldosterone and free urine aldosterone were measured by solid phase radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA, USA).₄ Plasma prorenin was measured with a direct prorenin enzyme-linked immunosorbent assay (Molecular Innovations, Novi, MI, USA).⁵ Urinary angiotensinogen was measured as the maximum quantity of Ang I that was generated during incubation with excess recombinant renin.

Pharmacokinetic Parameters

Peak concentration (C_max), concentration at trough (C_min), time to C_max (t_max), terminal half-life (t_1/2), renal clearance (C_l), and area under the concentration-time curve up to 24 h post-dose (AUC_0-24) for each individual plasma concentration-time profile were determined by a non-compartmental method, using WinNonlin® (Pharsight® Corporation, Mountain View, CA, USA), Version 5.0.1. Values below the lower limit of quantitation (BLQ) were presented as zero in the plasma concentration tables and were treated as zero when calculating the descriptive summary of the concentrations by time point. When calculating PK parameters, BLQ values were treated as zero when occurring at the beginning of the profile and missing when occurring at the end of the profile or between two values that were above lower limit of quantitation (LLOQ).

Statistical Analysis

All subjects who received at least one dose of study drug (VTP-27999, aliskiren or placebo) are included in the safety analyses. Concentration data from all subjects who received VTP-27999 were used in the calculation of PK parameters and no subjects were excluded from the final VTP-27999 plasma and urine PK analysis. One subject was excluded from the aliskiren PK analysis dataset because this subject inadvertently received placebo on Day 1. Only
subjects with samples from Day 1 and Day 10 were included in the pharmacodynamic (PD) dataset. Two subjects (one each for the aliskiren and placebo cohorts) were excluded from the PD analysis because they inadvertently had their doses switched on Day 1.

Safety data from all subjects who received at least one dose of study medication were reported and summarized. Plasma VTP-27999 PK parameters included $C_{\text{max}}$, $C_{\text{min}}$, $t_{\text{max}}$, $\text{AUC}_{0-24}$, and $t_{1/2}$. All available plasma VTP-27999 concentration and PK results were summarized using appropriate descriptive statistics. Mean and individual concentration versus time curves were plotted for each analyte. Descriptive statistics, including changes from baseline were calculated for all plasma PD analytes and BP (mean of triplicate readings at each time point), by dose and time point. Percent change from baseline was also calculated for plasma PD analytes. Descriptive statistics for urine excretion amount and rates for aldosterone and renin were calculated by dose and collection interval.

References

Table S1. Plasma Pharmacokinetic parameters for VTP-27999 and Aliskiren. Data are mean±SD or median and range. VTP=VTP-27999, ALI=aliskiren.

<table>
<thead>
<tr>
<th>Drug</th>
<th>N</th>
<th>$\text{AUC}_{0-24}$ (ng·hr/mL)</th>
<th>$C_{\text{max}}$ (ng/mL)</th>
<th>$t_{\text{max}}$ (hr)</th>
<th>$C_{\text{min}}$ (ng/mL)</th>
<th>$t_{1/2}$ (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Day 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>75 mg VTP</td>
<td>5</td>
<td>$602 \pm 291$</td>
<td>$202 \pm 96$</td>
<td>0.8 (0.3 - 2.1)</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>150 mg VTP</td>
<td>6</td>
<td>$1754 \pm 937$</td>
<td>$441 \pm 267$</td>
<td>1.0 (0.6 - 4.0)</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>300 mg VTP</td>
<td>6</td>
<td>$3453 \pm 979$</td>
<td>$786 \pm 266$</td>
<td>1.5 (1.0 - 2.0)</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>600 mg VTP</td>
<td>6</td>
<td>$8466 \pm 2031$</td>
<td>$1207 \pm 264$</td>
<td>3.0 (1.0 - 4.0)</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>300 mg ALI</td>
<td>5</td>
<td>$1123 \pm 543$</td>
<td>$269 \pm 209$</td>
<td>2.5 (0.5 – 4.0)</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td><strong>Day 10</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>75 mg VTP</td>
<td>5</td>
<td>$1408 \pm 489$</td>
<td>$320 \pm 216$</td>
<td>2.0 (1.0 – 4.0)</td>
<td>21 ± 3.4</td>
<td>30.2 ± 4.02</td>
</tr>
<tr>
<td>150 mg VTP</td>
<td>6</td>
<td>$3035 \pm 1485$</td>
<td>$676 \pm 468$</td>
<td>2.0 (0.5 – 4.0)</td>
<td>40 ± 18</td>
<td>29.4 ± 2.28</td>
</tr>
<tr>
<td>300 mg VTP</td>
<td>6</td>
<td>$6210 \pm 1481$</td>
<td>$1126 \pm 283$</td>
<td>2.0 (0.5 – 2.0)</td>
<td>65 ± 11</td>
<td>29.1 ± 3.49</td>
</tr>
<tr>
<td>600 mg VTP</td>
<td>6</td>
<td>$13663 \pm 3111$</td>
<td>$1736 \pm 320$</td>
<td>4.0 (4.0 – 4.1)</td>
<td>105 ± 23</td>
<td>23.8 ± 2.24</td>
</tr>
<tr>
<td>300 mg ALI</td>
<td>5</td>
<td>$2348 \pm 1813$</td>
<td>$411 \pm 520$</td>
<td>2.5 (0.5 – 4.0)</td>
<td>40 ± 25</td>
<td>33.6 ± 1.00</td>
</tr>
</tbody>
</table>
Table S2. Summary of urinary excretion (Ae(0-24)) and renal clearance (Clr) for VTP-27999.

<table>
<thead>
<tr>
<th>VTP-27999</th>
<th>Ae(0-24) (µg)</th>
<th>Clr (L/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>75 mg</td>
<td>1504 ± 651.4 (43.3)</td>
<td>2.8 ± 0.90 (32.6)</td>
</tr>
<tr>
<td>150 mg</td>
<td>4503 ± 848.9 (18.9)</td>
<td>3.3 ± 1.91 (59.0)</td>
</tr>
<tr>
<td>300 mg</td>
<td>13938 ± 5073 (36.4)</td>
<td>3.9 ± 0.68 (17.3)</td>
</tr>
<tr>
<td>600 mg</td>
<td>34062 ± 14422 (42.3)</td>
<td>3.9 ± 1.58 (40.3)</td>
</tr>
<tr>
<td><strong>Day 10</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>75 mg</td>
<td>3169 ± 893.6 (27.0)</td>
<td>2.3 ± 0.46 (19.6)</td>
</tr>
<tr>
<td>150 mg</td>
<td>8498 ± 2543 (29.9)</td>
<td>3.3 ± 1.55 (46.6)</td>
</tr>
<tr>
<td>300 mg</td>
<td>22259 ± 5969 (22.2)</td>
<td>3.6 ± 0.74 (20.5)</td>
</tr>
<tr>
<td>600 mg</td>
<td>57794 ± 18932 (32.8)</td>
<td>4.1 ± 0.62 (14.8)</td>
</tr>
</tbody>
</table>

Table S3. Renal excretion of sodium and creatinine. Data are mean±SD.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Na⁺ (mmol/24 hours)</th>
<th>Creatinine (mmol/24 hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 10</td>
</tr>
<tr>
<td>75 mg VTP-27999</td>
<td>51±14</td>
<td>29±2</td>
</tr>
<tr>
<td>150 mg VTP-27999</td>
<td>54±3</td>
<td>36±2</td>
</tr>
<tr>
<td>300 mg VTP-27999</td>
<td>98±13</td>
<td>32±1</td>
</tr>
<tr>
<td>600 mg VTP-27999</td>
<td>46±9</td>
<td>26±2</td>
</tr>
<tr>
<td>300 mg Aliskiren</td>
<td>42±3</td>
<td>22±4</td>
</tr>
<tr>
<td>Placebo</td>
<td>29±2</td>
<td>23±3</td>
</tr>
</tbody>
</table>
Table S4. Summary of number of subjects with treatment-emergent adverse events (AE), presented in order of frequency.

<table>
<thead>
<tr>
<th>Preferred Term</th>
<th>VTP-27999 Doses</th>
<th>Aliskiren</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>75 mg N=6</td>
<td>150 mg N=6</td>
<td>300 mg N=7</td>
</tr>
<tr>
<td>AEs (N)</td>
<td>2 (33%)</td>
<td>5 (33%)</td>
<td>3 (42%)</td>
</tr>
<tr>
<td>Subjects with AEs (N)</td>
<td>2 (33%)</td>
<td>2 (33%)</td>
<td>3 (42%)</td>
</tr>
<tr>
<td>Nausea</td>
<td>4 (67%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vomiting</td>
<td>2 (33%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dizziness</td>
<td>2 (33%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cold sweat</td>
<td>2 (33%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abdominal pain upper</td>
<td>2 (33%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphadenopathy</td>
<td>1 (17%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Procedural dizziness</td>
<td>1 (17%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dyspnoea</td>
<td>1 (17%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pallor</td>
<td>1 (17%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Palpitations</td>
<td>1 (14%)</td>
<td>1 (17%)</td>
<td></td>
</tr>
<tr>
<td>Asthenia</td>
<td>1 (14%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Musculoskeletal chest pain</td>
<td>1 (14%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Headache</td>
<td>1 (17%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry eye</td>
<td>1 (17%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oral pain</td>
<td>1 (17%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sinus tachycardia</td>
<td>1 (17%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cough</td>
<td>1 (17%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

N = number of subjects dosed with each treatment. Blank cell = no reported AE. One subject, randomized to placebo, received one dose of aliskiren on Day 1. Another subject, randomized to aliskiren, received placebo on Day 1. Both subjects were counted in the aliskiren group for the safety analysis, but were excluded from the PK/PD analysis.
Table S5. Serum potassium levels. Data are mean±SD.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>K⁺ (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day -1</td>
</tr>
<tr>
<td>75 mg VTP-27999</td>
<td>4.7±0.5</td>
</tr>
<tr>
<td>150 mg VTP-27999</td>
<td>4.4±0.3</td>
</tr>
<tr>
<td>300 mg VTP-27999</td>
<td>4.4±0.3</td>
</tr>
<tr>
<td>600 mg VTP-27999</td>
<td>4.3±0.2</td>
</tr>
<tr>
<td>300 mg Aliskiren</td>
<td>4.6±0.3</td>
</tr>
<tr>
<td>Placebo</td>
<td>4.7±0.3</td>
</tr>
</tbody>
</table>

Figure S1. Plasma renin activity (PRA) versus plasma angiotensin (Ang) II and aldosterone in subjects treated with placebo, VTP-27999 (75, 150, 300 or 600 mg) or 300 mg aliskiren.