Interpretation of Plasma Renin Concentration in Patients Receiving Aliskiren Therapy

Duncan J. Campbell

Aliskiren (Tekturna or Rasilez) is an orally active renin inhibitor approved for the treatment of hypertension. As with inhibition of the renin-angiotensin system by angiotensin-converting enzyme inhibitors or type 1 angiotensin II (Ang II) receptor blockers, aliskiren therapy is accompanied by a reactive rise in plasma renin concentration, although in contrast to angiotensin-converting enzyme inhibitor and angiotensin receptor blocker therapy, aliskiren reduces enzymatic plasma renin activity (PRA). A direct comparison of aliskiren and angiotensin receptor blocker therapies in hypertensive patients showed differences in the magnitude of the reactive renin response. In 1 study, 300 mg/d of aliskiren and 320 mg/d of valsartan were similarly hypotensive, whereas plasma renin concentration was 2-fold higher during aliskiren therapy. In a second study, 150 mg/d of aliskiren and 150 mg/d of irbesartan were similarly hypotensive, whereas plasma renin concentration was 1.4-fold higher during aliskiren therapy. Moreover, the renin response to 300 mg of aliskiren was 1.6-fold higher than the response to 320 mg of valsartan in sodium-replete normotensive volunteers.

Sealey and Laragh proposed that the exaggerated renin response to aliskiren therapy may paradoxically increase blood pressure in patients with a highly reactive renin concentration (renovascular, advanced, and malignant hypertension). Another concern expressed by several authors was the possible action of the increased renin concentrations on the putative renin receptor.

How should the exaggerated renin response to aliskiren therapy be interpreted? Renin secretion is subject to tonic negative feedback inhibition by Ang II, and the reactive increase in renin concentration provides a valuable, although indirect, measure of the reduction of Ang II levels by aliskiren. The renin response may also be due to unloading of the renal and extrarenal baroreceptors, although several studies show that aliskiren is not more hypotensive than angiotensin receptor blocker therapy. The kidney is an important site of the uptake of renin inhibitors, and autoradiographic studies show localization of aliskiren in the renal glomeruli, renal arteries, and capillaries but not in the renal tubules. It is, therefore, possible that aliskiren may act directly on the renin-secreting juxtaglomerular cell to influence prorenin processing and renin release by a mechanism independent of Ang II levels, although there is as yet no evidence for this. The reactive renin response may also reflect an effect of aliskiren on renin clearance, but no information is available that addresses this possibility. Finally, aliskiren may interfere with the renin assay by binding to prorenin and causing an overestimation of the renin concentration.

In this brief review, I summarize some of the properties of aliskiren and the mechanisms that may account for the exaggerated renin response to aliskiren therapy. I review evidence that an important contributor to the exaggerated renin response is the interference by the renin inhibitor in the renin assay causing overestimation of the renin concentration. Contrary to the suggestion of Sealey and Laragh, the reactive renin response may limit the effect of aliskiren therapy on Ang II levels, the exaggerated renin response may not represent an increase in enzymatically active renin molecules in plasma. An important consequence of the overestimation of renin concentration is that the impact of the renin inhibitor on angiotensin peptide formation in vivo may be less than that indicated by the renin response.

Aliskiren

Aliskiren has the chemical structure 2(S),4(S),5(S),7(S)-N-(2-carbamoyl-2-methylpropyl)-5-amino-4-hydroxy-2,7-diisopropyl-8-(4-methoxy-3-[3-methoxypropoxy]-phenyl)-octanamide and a molecular mass of 551.8 g/mol. It is a competitive renin inhibitor with a high species specificity and is reported to have an IC50 of 0.6 nmol/L for both purified human renin and human plasma renin. Aliskiren is a rather hydrophilic molecule (log octagonal/water partition coefficient = 2.45 at pH 7.4) with high aqueous solubility (>350 mg/mL at pH 7.4). The distribution volume of intravenously administered aliskiren is reported to be 135 L in normal volunteers, indicating extensive tissue uptake of the drug. The absorbed fraction of orally administered aliskiren is estimated to be ≈5%, with 90% of the drug excreted...
unchanged by the fecal route. In contrast to other renin inhibitors with similar affinity for renin, aliskiren has a long plasma half-life, reported to be 24 to 70 hours. Steady-state plasma aliskiren levels are achieved after 5 to 8 days of daily dosing. The long plasma half-life and very low urinary excretion (<1%) suggest that binding of the drug to plasma proteins may be higher than the 49.5% reported for human plasma by Azizi et al.

The effects of aliskiren therapy on plasma angiotensin levels are less than that predicted by the plasma levels of aliskiren and its IC50 for renin inhibition. After administration of 40 mg/d of aliskiren for 8 days, peak plasma aliskiren levels of 9 nmol/L (15-fold higher than the IC50) produced only a transient 50% reduction in angiotensin I (Ang I) and Ang II levels, although plasma renin levels increased 2- to 3-fold. By 3 to 6 hours after the 40-mg dose, plasma Ang II and Ang I levels had returned to control, although renin levels remained 3-fold elevated, and the plasma aliskiren concentration (4 to 6 nmol/L) was still 7- to 10-fold higher than the IC50. This discrepancy between plasma aliskiren concentrations and plasma angiotensin levels may be attributed to lower aliskiren levels in tissues, which are the main sites of angiotensin peptide formation. However, a more likely explanation for the incomplete reduction of plasma angiotensin levels by aliskiren concentrations in considerable excess of its reported IC50 is its extensive binding to plasma proteins, such that the concentration of free drug was much less than the measured plasma concentration. In support of this proposal, Nussberger measured IC50 values of 10 to 14 nmol/L of aliskiren for inhibition of renin in human plasma (Nussberger, personal communication, 2007) using the antibody-capture method of PRA assay (described below). This 20-fold difference between the IC50 values of 0.6 nmol/L reported by Wood et al. and 10 to 14 nmol/L measured by Nussberger is consistent with ~95% binding of aliskiren to plasma proteins and explains the apparent discrepancy between plasma concentrations of aliskiren and its effects on angiotensin levels.

The Relationship Between Renin and Prorenin
Renin is synthesized as the inactive zymogen prorenin, which contains a prosegment that masks the active site (Figure 1). Plasma concentrations of prorenin are ~10-fold higher than renin concentrations. Plasma prorenin may be converted to renin by a 2-step process, whereby the prosegment of prorenin is unfolded and then cleaved. Both cooling and low pH promote unfolding of the prosegment, whereas refolding of the prosegment is promoted by 37°C. Cryoactivation of plasma prorenin occurs when plasma is cooled to between 4°C and −5°C and is attributed to unfolding of the prosegment of prorenin, with subsequent cleavage by plasma proteases. To minimize cryoactivation of prorenin it is important to avoid the cooling of blood and plasma after collection, to snap-freeze plasma for storage, and to rapidly thaw the plasma for renin assay. Spontaneous activation of prorenin over time has been detected at room temperature but is virtually absent at 37°C.

Measurement of Renin
The PRA assay measures the enzymatic activity of uninhibited renin, whereas the immunoradiometric assay (IRMA) measures the concentration of renin molecules, both active and inhibited. Given the extensive binding of renin inhibitors to plasma proteins, care must be taken to avoid displacement of the protein-bound inhibitor during the PRA assay, because displacement of the renin inhibitor causes overestimation of the degree of renin inhibition. Displacement of the renin inhibitor can be reduced by an antibody-capture method of PRA assay, whereby excess Ang I antibody is added to the assay to protect Ang I as it is generated, thereby avoiding the use of peptidase inhibitors and a change in pH that displaces the protein-bound renin inhibitor. However, although the antibody-capture method of PRA assay provides valuable information, it can overestimate the extent of renin inhibition in vivo, as indicated by plasma Ang I and Ang II levels, and cannot replace the measurement of angiotensin levels for the reliable evaluation of the efficacy of renin inhibition in vivo.

The renin IRMA uses 2 monoclonal antibodies that bind to 2 different parts of the renin molecule in a sandwich assay (Figure 1). A capture antibody binds to a site that is equally exposed on renin and prorenin molecules, whereas a detection antibody binds to a part of the renin molecule that is normally masked by the prosegment of prorenin and does not bind to renin if the prosegment is properly folded (Figure 1). The renin IRMA may inadvertently measure prorenin if the prosegment becomes unfolded, as may occur when prorenin is subjected to cooling or low pH. Importantly for the measurement of renin during renin inhibitor therapy, renin inhibitor molecules may bind to the active site of prorenin molecules with an unfolded prosegment, thereby preventing refolding of the prosegment and making the prorenin recognizable by the renin IRMA (Figure 1). This property of renin

![Diagramatic representation of the different conformations of prorenin and renin, their recognition by the capture and detection antibodies of the renin immunoradiometric assay, and their binding of aliskiren. All of the renin forms are recognized by the capture antibody. A, Prorenin with prosegment folded across its active site is not recognized by the detection antibody for renin. B, Prorenin with an unfolded prosegment is recognized by the detection antibody. In addition, aliskiren can bind to prorenin with an unfolded prosegment and prevent the prosegment from refolding properly. C, Cleavage of the prosegment converts prorenin to renin, which is recognized by the detection antibody, and also binds to aliskiren. This is a simplified version of diagrams published by Derkx et al. and Danser and Deinum.]

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**Figure 1.** Diagrammatic representation of the different conformations of prorenin and renin, their recognition by the capture and detection antibodies of the renin immunoradiometric assay, and their binding of aliskiren. All of the renin forms are recognized by the capture antibody. A, Prorenin with prosegment folded across its active site is not recognized by the detection antibody for renin. B, Prorenin with an unfolded prosegment is recognized by the detection antibody. In addition, aliskiren can bind to prorenin with an unfolded prosegment and prevent the prosegment from refolding properly. C, Cleavage of the prosegment converts prorenin to renin, which is recognized by the detection antibody, and also binds to aliskiren. This is a simplified version of diagrams published by Derkx et al. and Danser and Deinum.
conditions of renin IRMA may lead to overestimation of plasma renin concentration when plasma contains a renin inhibitor. Although direct comparison of the 2 assays showed that the Nichols IRMA gave higher renin levels than the Cisbio IRMA for patients receiving aliskiren therapy,\textsuperscript{7} the exaggerated renin response to aliskiren therapy cannot be attributed solely to the use of the Nichols IRMA for renin measurement. The use of the Nichols IRMA may have contributed to the exaggerated renin response observed by Oparil et al,\textsuperscript{3} but an exaggerated renin response was also observed in studies that used the Cisbio IRMA.\textsuperscript{2,3} Other aspects of the renin IRMA methodology, other than the manufacturer of the assay, may contribute to overestimation of the renin concentration. For example, the methods of handling and centrifugation of blood and freezing and thawing of plasma may contribute to the overestimation of the renin concentration by promoting binding of a renin inhibitor to prorenin.

An important question is whether aliskiren binds to prorenin in vivo. Evidence against the binding of aliskiren to prorenin in vivo includes the observation by Deinum et al\textsuperscript{35} that the prosegment does not unfold during 6 hours at 37°C. Moreover, Ménard et al\textsuperscript{7} found that aliskiren concentrations of 1000 nmol/L did not influence the measured renin concentration during a 3-hour incubation at room temperature with the Cisbio assay. Peak plasma aliskiren levels after dosing for 8 days at 160 and 640 mg/d were \(\approx 25 \) (\(\approx 45\) nmol/L) and \(\approx 300\) ng/mL (\(\approx 554\) nmol/L), respectively, and had fallen by \(\approx 80\%\) at 24 hours after dosing.\textsuperscript{13} Given that peak plasma aliskiren concentrations are much less than 1000 nmol/L,\textsuperscript{13} it is unlikely that appreciable binding of aliskiren to prorenin occurs in vivo. Importantly, as mentioned earlier, the free aliskiren concentration is likely to be much less than the measured concentration because of the extensive binding of aliskiren to nonrenin proteins.

The question of whether aliskiren binds to prorenin in vivo is unlikely to be answered by the addition of aliskiren to plasma because of the difficulty in reproducing the kinetics of binding to renin, prorenin, and nonrenin proteins that occurs after oral administration. One possible approach to examining the contribution of its binding to prorenin in vitro and in vivo is to administer aliskiren to anephric subjects, who have nearly normal prorenin levels and markedly suppressed renin levels that should not respond to renin inhibition.\textsuperscript{26,37,38} Measurement of the time course of plasma renin concentration in plasma samples assayed immediately and plasma samples stored frozen before assay from anephric subjects administered aliskiren would help reveal whether aliskiren binds to prorenin in vivo and also during storage and freeze-thaw cycles of plasma.

In summary, the most reliable measure of renin inhibition in vivo is the change in plasma levels of Ang I and Ang II. Although the antibody-capture method of PRA assay provides a useful measure of renin inhibition, it may overestimate renin inhibition in vivo, as indicated by plasma angiotensin levels.\textsuperscript{3,11} The increase in plasma renin concentration is an indirect measure of renin inhibition in patients receiving aliskiren therapy, and it may be overestimated because of binding of aliskiren to prorenin. When interpreting plasma renin concentrations in patients receiving aliskiren therapy it is necessary to consider the type of assay, its manufacturer,
whether it is automated or manual, and the time and temperature of incubation. It is also necessary to consider whether appropriate precautions were taken during the collection and centrifugation of blood, during storage of plasma, and during any freeze-thaw cycles. Unfortunately, this information is usually not provided in published reports. Finally, it is necessary to consider the aliskiren dose and whether blood levels achieved are likely to bind to prorenin in vivo. In those studies reporting an exaggerated renin response to aliskiren levels achieved are likely to bind to prorenin in vivo was less than that indicated by the renin response.

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**References**

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