Aliskiren (Tektura 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 proposed the renin response to aliskiren therapy may paradoxically increase blood pressure by counteracting the effect of renin inhibition on Ang II levels and may thereby account for the failure of 600 mg of aliskiren to reduce blood pressure at >300 mg of aliskiren. They also proposed the renin response to aliskiren therapy may paradoxically increase the impact 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
hydrophilic molecule (log octagonal/water partition coeffi-
cient [logPoct/water]=2.45 at pH 7.4) with high aqueous solu-
bility (>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 concentrations 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 the ~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. Prosegment of prorenin may be converted to renin by a 2-step process, whereby the prosegment of prorenin is unfolded and then cleaved. Cooling and low pH promote unfolding of the prosegment, and 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 block Ang I as it is generated, thereby avoiding the use of pepstatin A 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 inhibitor therapy.

The renin IRMA uses 2 monoclonal antibodies that bind to 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

![Diagram](https://via.placeholder.com/150)
Overestimation of Renin Concentration

There are 2 commercial renin IRMAs, 1 produced by Cisbio International and 1 produced by Nichols Institute, now sold by Diagnostics Systems Laboratories. The Cisbio IRMA involves a 3-hour incubation at room temperature, whereas the Nichols IRMA involves a 24-hour incubation at room temperature. Ménard et al compared the 2 renin IRMAs and found that, although the Cisbio and Nichols assays gave similar results for subjects before and after valsartan administration, the Nichols assay gave \( \approx 2 \) -fold higher renin concentrations than the Cisbio assay after aliskiren administration. They showed the renin concentrations measured when the Cisbio assay was incubated for 24 hours were 2-fold higher than when the assay was incubated for 3 hours, thereby indicating that the conditions of incubation can artificially elevate the measured renin concentration. Ménard et al also measured the renin concentration of plasmas to which aliskiren was added before the assay (Figure 2). For the Cisbio assay incubated for 3 hours, no increase in renin concentration was seen until aliskiren concentrations were \( > 1000 \) nmol/L, whereas the Nichols assay showed artifactual elevation of the renin concentration at 10 to 100 nmol/L of aliskiren (Figure 2). The Nichols IRMA has been shown to overestimate plasma renin concentration in the absence of renin inhibitor therapy. Deinum et al showed the Nichols assay, when performed at 22°C for 24 hours, measures \( \approx 5\% \) of plasma prorenin as renin because of unfolding of the prosegment. However, these authors did not detect any evidence for unfolding of the prosegment when the assay was performed at 37°C for 6 hours. This overestimation of renin concentration may also be less for the automated Nichols Advantage chemiluminescent immunoassay performed at 37°C, although no data have been reported about the effect of renin inhibitors on this assay.

The studies by Ménard et al clearly demonstrate that the 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, 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, but an exaggerated renin response was also observed in studies that used the Cisbio IRMA. 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 that the prosegment does not unfold during 6 hours at 37°C. Moreover, Ménard et al 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. Given that peak plasma aliskiren concentrations are much less than 1000 nmol/L, 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. 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. 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 therapy, it is likely that the reactive renin response was overestimated and the effect of aliskiren on angiotensin levels in vivo was less than that indicated by the renin response.

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