Ex Vivo Equilibrium Analysis of the Renin-Angiotensin-System: Novel Perspectives for Clinical Diagnostics

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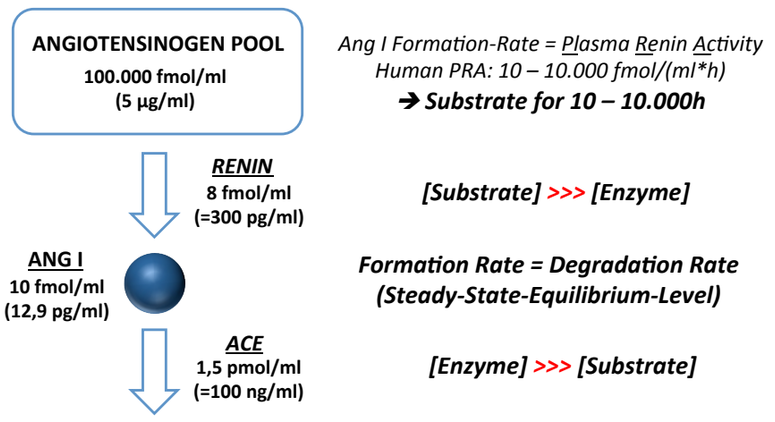
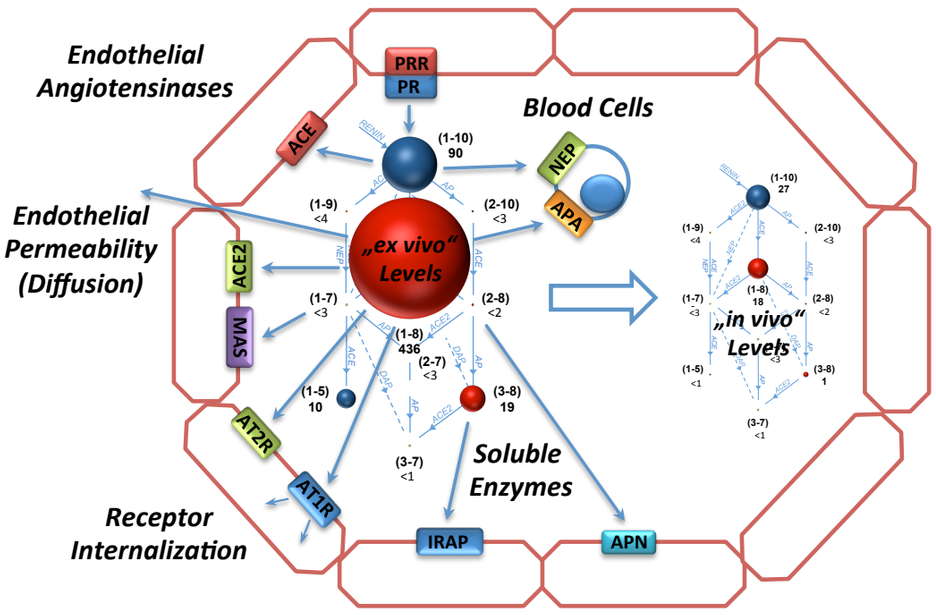
Background

Renin-mediated formation of angiotensin I from angiotensinogen is a well-characterized enzymatic reaction. In human plasma, this rate-limiting step of the RAS occurs is affected by the concentration of angiotensinogen and renin and maintains a constant flow of peptides into and through the RAS. The concentrations of different angiotensins are established by an equilibrium between their formation and their degradation/absorption rates. The resulting steady-state angiotensin levels are affected by local molecular factors including angiotensin receptors and angiotensinases, which might either be present in plasma or attached to blood cells and endothelial surfaces. The very high plasma concentration of angiotensinogen in combination with the reported values for PRA suggest long-lasting and stable Ang I formation by renin without significantly reducing angiotensinogen levels. This phenomenon can be utilized for the generation of an ex vivo situation that is characterized by significantly higher but stable angiotensin peptide levels. Ex vivo equilibrium angiotensin levels provide an integrated picture about plasma angiotensinase activities and therefore represent a powerful diagnostic tool for analyzing the systemic RAS in clinical samples.



In vivo vs. Ex vivo Angiotensin Levels: Proof-of-Concept in Clinical Samples

In Vivo Angiotensin Levels (Angiotensinase Inhibitor Stabilized Plasma)

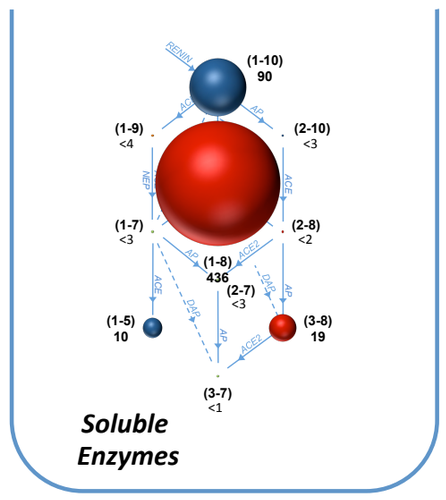


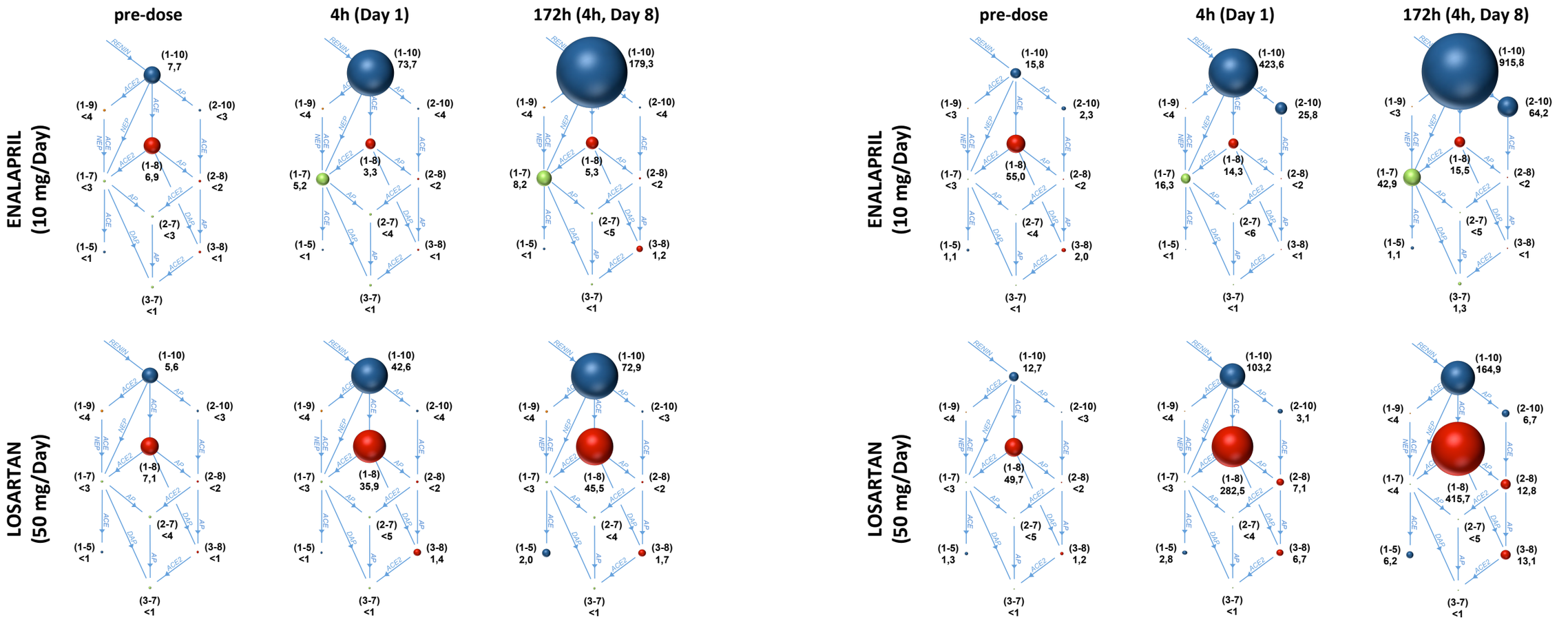
Blood Collection
Plasma Isolation

Removal of Tissue and Blood Components
➔ New Microenvironment
➔ New Equilibrium Levels

Formation Rate = Degradation Rate

Ex Vivo Angiotensin Levels (Heparin Plasma or Serum)





12 healthy volunteers were consecutively treated with indicated RAS-Blockers (Enalapril, Losartan) for a period of 8 days followed by a drug washout phase of 13 days. Daily doses were either 10 mg Enalapril (ACEI) or 50 mg Losartan (ARB). Heparin plasma samples (Ex vivo Equilibrium Angiotensin Levels, right panel) and angiotensinase inhibitor stabilized plasma samples (In vivo Circulating Angiotensin Levels, left panel) were collected on day 1 and day 8 of treatment periods at 4h post drug administration. Samples were analyzed by LC-MS/MS analysis and MEDIAN values (n=12) of angiotensin levels are displayed in RAS-Fingerprint graphs.

Conclusions

- Ex Vivo Levels and In Vivo Levels: Formation Rate = Degradation/Absorption Rate
- Ex Vivo Levels >>> In Vivo Levels
- In Vivo Angiotensin Levels = Remnants of Ex Vivo Levels?
- Ex Vivo Levels reflect In Vivo Levels!

Ex vivo RAS-Fingerprint: Advantages

➔ Standard Sampling (Li-Heparin Plasma, Na-Heparin Plasma, Serum)

➔ Biobank Compatibility (Frozen and long-term stored samples)

➔ High Information Content (10 Angiotensin Peptides, Enzyme-Activity Screen)

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