ANGIOTENSIN CONVERTING ENZYME HUB CAPACITY - A SIMPLE MODEL OF RELATING PEPTIDE FLUX AND RENIN INFLUENCE

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ABSTRACT: As the Vitamin C, Thiamine, and Steroids in Sepsis (VICTAS) Trial was not designed to measure Renin-Angiotensin-Aldosterone System components, this hub model was developed to limit eight components to a system with one entrance and two exits to facilitate the calculation of angiotensinogen levels. Bootstrapping the bioactive peptide egress rate constant using control subjects and incorporating the previously established renin relationship, the model was used to develop a contingency test to index and classify component relationships.

INTRODUCTION

Under classical physiologic conditions, angiotensin converting enzyme (ACE, EC 3.4.15.1) is a critical hub node for the Renin-Angiotensin-Aldosterone System (RAAS) and the conservation of the precursor angiotensinogen (Ao) consumption for a direct chain of conversions to angiotensin-(1-5) [Ang-(1-5)]. Angiotensin I [Ang-(1-10) or Ang I] is generated by renin [EC 3.4.23.15] from Ao, then ACE yields the first N-terminally intact bioactive peptide (with direct receptor action, B) in this cascade, angiotensin II [Ang-(1-8) or Ang II], which is converted to the next B, angiotensin-(1-7) [Ang-(1-7)], then ACE generates Ang-(1-5) (Chappell, 2016) (Figure 1).

We recently completed a post-hoc analysis of the RAAS in a subset of patients from the VICTAS septic shock trial that revealed a strong association of renin to mortality (Busse et al., 2023). However, plasma samples were not collected with a renin inhibitor, so Ang I or intact Ao levels were not assessed in the study. To determine these components, we developed a conservation model that utilized the RAAS data from the VICTAS trial, which may be applied to other situations to calculate an unknown member of the RAAS (Westwood et al., 2008).

METHODS

If we consider ACE and its substrates and products from Figure 1 as a hub control volume extended (ACE hub, gray arrows) from Ang-(1-7), we can write the conservation equation 1 [this model assumes essentially all Ang I generated by renin is converted to Ang II by ACE, then directly converted to Ang-(1-7) by ACE2 [EC 3.4.17.23] and is acted upon by ACE to generate Ang-(1-5)]. There is an egress construct in the ACE hub linear model for bioactive peptides with rate B* acting on Ang II (encompassing direct receptor binding or conversion to Ang-(1-7) with receptor binding and/or any other non-ACE hub flux), which we modeled as equivalent to that of ACE [Q0, Q1, Q2 and Q3]. Under the pseudo-steady state hypothesis (PSSH), we can set the accumulation term to zero, and solve for Ao (equation 2).

Given the ACE hub (Figure 1), the conservation of Ang-(1-7) can be described:

$$\frac{dAng-(1-7)}{dt} = Renin[Ao] - ACE[Ang-(1-7)] - B*[Ang\ II] = 0 \qquad (1)$$

Enzyme concentrations were used as proxy for rate constants. The PSSH yields the key to estimating intact Ao level:

$$[Ao]_{calc} = \frac{ACE[Ang-(1-7)]+B*[AngII]}{Renin} \qquad (2)$$

Calculated Ao values were evaluated to find 'far out' extreme outliers (outer fence) (Tukey, 1977). These 'wild shots' were Winsorized (W) by setting them to the outer fence value (Tukey, 1962).

Area under the curve (AUC) for receiver operating characteristic (ROC) curves (Fawcett, 2006) were calculated by renin concentration percentile divisible by 5 (%ile/5) vs. whether Ao_{calc}, AngII and Ang-(1-7) concentrations were in normal range or not (Chappell, 2016) (Figure 2).

All calculations were performed in Excel (Microsoft Corp., WA), Prism (GraphPad Software, MA) was used for plotting data in figures and statistical analyses.

RESULTS

Using ACE quartiles as a surrogate for B* yielded output values with a correlation matrix for the four sets of calculations with all coefficients being greater than 0.995. The field solution was repeated for 20 control subjects, with $Q1_{ACE}$ yielding an interquartile range of Ao_{ctrl} calculated values matching the range of normal range values for Ao (Chappell, 2016). For all subsequent plots B* = $Q1_{ACE}$. Ao_{calc}, Ang II and

Ang-(1-7) [r^2= (0.64, 0.04 and 0.24) log-log line (with 95% CI bands), respectively; n=84; Ao_{ctrl}, r^2=0.59, n=20; Figure 3] concentrations were plotted against renin. W signal clipping only effected the uppermost [Ao] values ($r^2_{noW\text{-}Ao}$ = 0.67).

ROC curves were plotted relative to renin %ile/5 for Ao_{calc}, Ang II and Ang-(1-7) in Figure 4. W had no effect on ROC analysis.

The three AUC values were 0.65±0.032 ($\bar{x} \pm$ SD). To achieve an FP rate = 0 and TP rate = 1 would result in an AUC = 1 yielding a perfect positive performance (Fawcett, 2006).

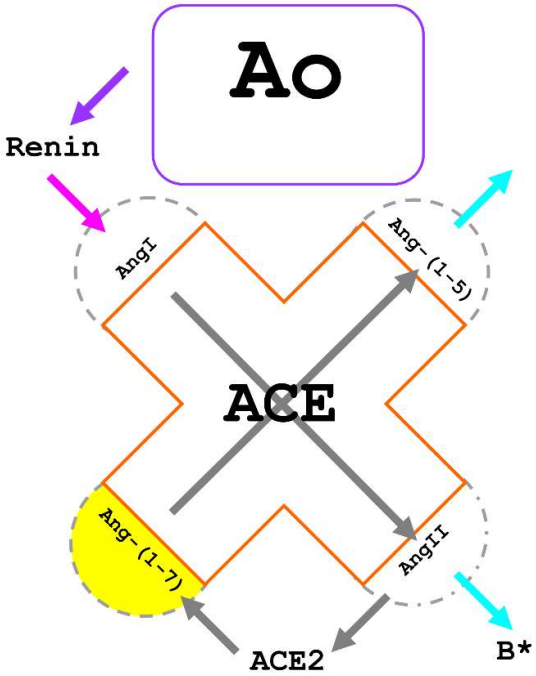

Figure 1. Simple model for ACE hub with the control volume extended to substrates and products, for development of conservation equation mathematical model for RAAS components. Graphic shows the mostly linear path of major angiotensin peptides under classical physiologic conditions.

For each **[renin]** %ile/5	Are **[Ao]**, **[AngII]** and **[Ang-(1-7)]** in normal range?		
		Yes	No
[renin] %ile	below	**TP**	**FP**
	above	**FN**	**TN**

Figure 2. Receiver operating characteristics for renin, relative to the two major bioactive RAS peptides and the system precursor protein. Contingency used to analyze data shown in Figure 4.

DISCUSSION

While the correlations between renin concentrations vs. Ao_{calc}, Ang-(1-7) and Ang II concentrations denote rank logarithmic decay {[Ao_{calc}, Ang-(1-7), Ang II], [0.64, 0.24, 0.04]; r^2 = 1}, the receiver operating characteristic AUC values have a small rank slope {[Ang-(1-7), Ao_{calc}, Ang II], [0.69,0.64,0.63]; equation 3, r^2 = 0.87}.

$$AUC = -0.03(rank) + 0.71 \qquad (3)$$

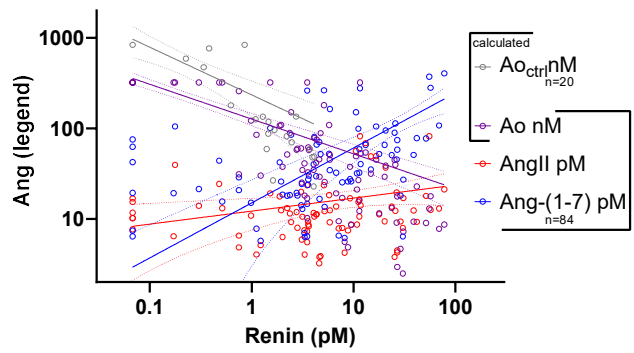

Figure 3. Log-log plot of Ao_{ctrl}, Ao_{calc}, Ang II and Ang-(1-7) concentrations vs. renin concentration.

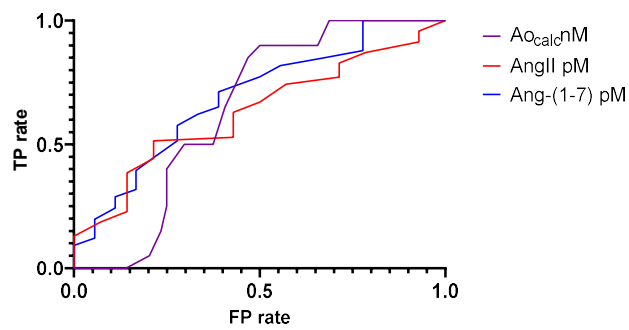

Figure 4. ROC curves for Ao_{calc}, Ang II and Ang-(1-7) concentration contingency with renin concentration as described in Figure 2.

This model may represent a seed method for the description of the complex interplay between not only this select catalog of RAAS components, but also may be generalized as a paradigm to explore greater utility in more complex systems.

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ABBREVIATIONS

VICTAS, Vitamin C, Thiamine, and Steroids in Sepsis; ACE, angiotensin converting enzyme; RAAS, Renin-Angiotensin-Aldosterone System; Ao, angiotensinogen; Ang-(1-5), angiotensin-(1-5); Ang I or Ang-(1-10), Angiotensin I; Ang II or Ang-(1-8), angiotensin II; B, N-terminally intact bioactive peptide (with direct receptor action); Ang-(1-7), angiotensin-(1-7); ACE hub, ACE and its substrates and products as a hub control volume extended; B*, egress rate construct in the ACE hub linear model for bioactive peptides, PSSH, pseudo-steady state hypothesis; W, Winsorized; AUC, area under the curve, ROC, receiver operating characteristic; Ao_{calc}, calculated Ao values; Ao_{ctrl}, calculated Ao values for control subjects.

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