Investigating the Angiotensin Converting Enzyme (ACE) Inhibiting Properties of Naturally Occuring Terpenes Using *in silico* Models

Mifsud, S.[a], Shoemake, C.[a], Attard, E.[b], Sant Fournier, M.A.[a], Azzopardi, L.M.[a]

[a]Department of Pharmacy, [b]Institute of Agriculture, University of Malta

Introduction

Angiotensin Converting Enzyme (ACE) inhibitors are used primarily in treatment of hypertension and congestive heart failure, in some cases as the drugs of first choice.

Physiologically, the Renin-Angiotensin-Aldosterone System is activated in hypotensive situations resulting in decreased sodium concentration in the renal distal tubule, decreased blood volume and renal sympathetic nerve stimulation. In response, the kidneys release renin which cleaves the liver-derived angiotensinogen into angiotensin I. Angiotensin I is then converted to angiotensin 11 via the ACE in the pulmonary circulation as well as in the endothelium of blood vessels in many parts of the body. This results in an increase blood pressure.
ACE inhibition prevents the conversion of angiotensin 1 into angiotensin 11[1].

Physiological Effects Following Endogenous Angiotensin 11 Release
Vasoconstriction = Increased Blood Pressure
Constriction of Efferent Arterioles of Kidney = Increased Glomerular Perfusion
Cardiac Ventricular Remodeling = Ventricular Hypertrophy & Congestive Heart Failure
Stimulation of Adrenal Cortex Aldosterone Secretion = Renal Retention of Na^+ and Cl^-
Posterior Pituitary Stimulation with Vasopressin Release = Water Retention
Decrease in Renal Protein Kinase C Release

Clinical Effects Following ACE Administration
Lowering of Arteriole Resistance
Increased Venous Capacity
Increased Cardiac Output, Cardiac Index, Stroke Work & Volume
Lowered Renovascular Resistance = Reduction in Progress of Diabetic Nephropathy
Increased Natriuresis

Adverse Effects Associated With ACE Administration
Hypotension
Cough (due to increased Bradykinin levels)
Hyperkalaemia (Andiotensin 11 suppression decreases aldosterone (responsible for potassium secretion) levels.
Headache
Dizziness
Fatigue
Nausea
Renal Impairment
Rashes & Taste Disturbances (more prevalent with Captopril & attrubuted to sufhydryl moiety)
Renal Impairment (Reduced [Angiotensin 11] reduces Glomerular Filtration Rate)
Angioedema (Due to increased bradykinin levels)

The importance of this class of drugs in contemporary cardioprotective antihypertensive aramamentaria makes the ACE a target for novel drug design studies which seek to identify molecules capable of superior inhibition for as minimal a side effect profile as possible.
In vitro and clinical studies are indicative of the fact that the hydroethanolic extract of *Crataegus monogyna*, the Common Hawthorne, which is composed of ursolic, moronic, oleanolic and betulinic acids as well as of α- and β- amyrin, have ACE inhibitory ability. Obtained IC50 values were 335 µg/ml and 3.61 µM for the hydroethanolic extract and oleanolic acid respectively in comparison with 46.9 nM for captopril[2].
This represents the *raison d'etre* for this *in silico* study which sought to understand the binding modality of these naturally occurring terpenes within the ACE ligand binding pocket (ACE_LBP), to establish their binding affinity for the ACE, and to compare these to those of established ACE inhibitors captopril, enalapril and lisinpril. This study sought furthermore to identify critical and destabilising contacts forged by the established ACE inhibitor moieties and their cognate amino acid side chains on the ACE-LBP. This in order to serve as a basis for a drug design exercise that could lead to the identification of a novel analog series of semi-synthetic molecules with quantified ACE inhibiting properties and acceptable ADME/tox properties which would consequently have the desired drug-like qualities to make them eligible for synthesis and further evaluation.

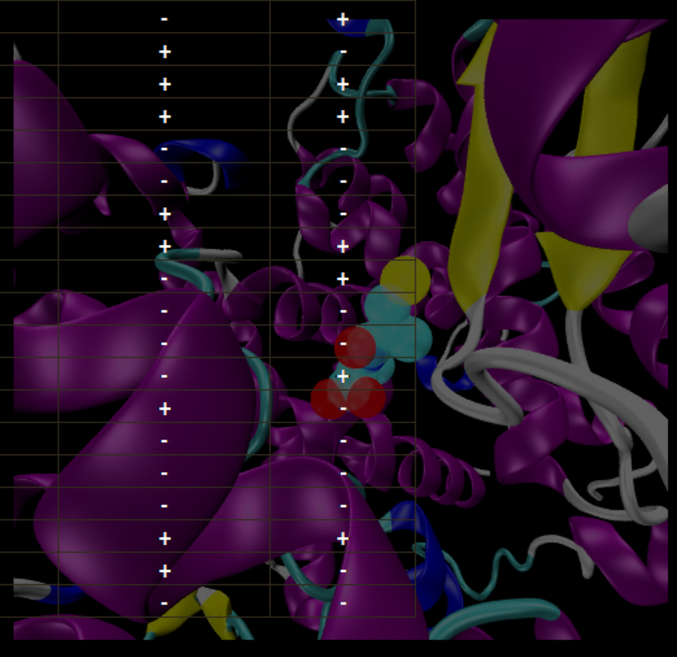
Methods

- The Protein Data Bank (pdb) crystallographic deposition by Natesh et al. (1UZF) describing the binding modality of captopril with the ACE and resolved to 2Å was used as a template[3].
- The amino acid perimeter forming the ligand binding pocket of the ACE was identified
- The bioactive conformation of captopril was extracted
- The critical stabilising interactions between the ACE and captopril were individualised
- The structures of ursolic, moronic, oleanolic and betulinic acids and of α- and β- amyrin were drawn and structurally optimised in Sybyl®[4].
- They were overlaid onto the bioactive conformation of captopril so as to ensure that the essential binding moieties were proximal to the ligand binding pocket amino acid side chains identified as critical for ligand binding and stabilisation within the binding pocket
- In silico LBA of captopril and ursolic, moronic, oleanolic and betulinic acids and of α- and β- amyrin were estimated and compared

Results

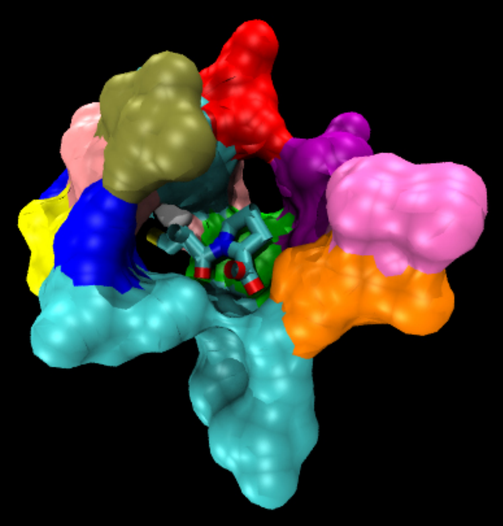
An analysis of the Ligand (Captopril)-Protein contacts as outlined in the pbd deposition 1UZF was crucial in establishing an optimum binding modality for the terpenes considered in this study. Attention was given to the preservation of the observed contacts, specifically the hydrogen bonding contacts, in establishing an optimum docking modality for these molecules.

Ligand:Protein Contacts (1UZF) Preserved in the Docking Exercise for the Molecules Considered in this Study					
Residue	Distance (Å)	Hydrogen Bonds	Aromatic Bonds	Hydrophobic Bonds	Direct Bonds
GLN 281	3.1	+	-	-	+
THR 282	6.5	-	-	+	-
HIS 353	2.5	+	-	+	+
ALA 354	3.7	-	-	+	+
SER 355	4.3	-	-	-	-
ALA 356	5	-	-	-	-
VAL 380	5	-	-	+	-
HIS 383	3.7	-	-	+	+
GLU 384	3.1	-	-	-	+
HIS 387	3.5	-	-	-	-
GLU 411	4.4	-	-	-	-
ASP 415	5.6	-	-	-	+
PHE 457	3.7	-	-	+	-
LYS 511	2.7	+	-	-	-
HIS 513	2.7	+	-	-	-
TYR 520	2.7	+	-	-	-
TYR 523	3.3	-	-	+	+
PHE 527	4.6	-	-	+	-
Zn 701	2.3	-	-	-	-



in silico estimations of ligand binding affinity between the ACE and captopril, and the terpenes considered in this study as determined through implementation of the SCORE algorithm[5] showed similar or superior binding affinity of the terpenes as compared to that of captopril.

Ligand	*in silico* pKd
Captopril	6.3
Ursolic Acid	6.2
Moronic Acid	6
Oleanolic Acid	7.4
Betulinic Acid	7
α-Amyrin	6.9
β-Amyrin	6.5



CONCLUSIONS

These preliminary results seem to corroborate the *in vitro* hypothesis that the hydroethanolic extracts of *Crataegus monogyna* bind with high affinity to the ACE and that these could be used as leads in the context of a semi-synthetic receptor based design study.

This hypothesis will be further tested in the second phase of this study in which, using Ligbuilder®[6], an analog series of molecules will be built, docked into the ACE_LBP, and evaluated for affinity and ADME/tox properties.

Comparative molecular dynamics simulation studies on *apo*- and *holo*- (bound to captopril and the designed molecules) forms of ACE will be carried out in the third and final phase of the study in order to evaluate, at an atomic level, the ACE domain fluctuations of importance, and to determine whether these latter are ligand dependent. Based on these results recommendations as to whether the designed ligands should be synthesised and further tested *in vitro* or not will be made.

Bibliography

1. Brunton LB, Lazo JS, Parker KL, editors. Goodman & Gilman's The Pharmacological Basis of Therapeutics. 11th ed. New York: McGraw-Hill; 2007.

2. Attard E, Attard H, The Potential Angiotensin-Converting Enzyme Inhibitory Activity of Oleanolic Acid in the Hydroethanolic Extract of Crataegus Monogyna Jacq. Natural Product Communications 2006 1(5): 381-385.

3. Bernstein FC, Koetzle TF, Williams GJ, Meyer EE.Jr., Brice JF, Rodgers JR, Kennard O, Shimanouchi T, Tasumi M, The Protein Data Bank: A Computer-based Archival File For Macromolecular Structures. J. Mol. Biol. 1977 112: 535.

4. SYBYL 7.3, Tripos International, 1699 South Hanley Rd., St. Louis, Missouri, 63144, USA

5. Wang R, Liu L, Lai L, Tang Y, SCORE: A New Empirical Method for Estimating the Binding Affinity of a Protein-Ligand Complex. J. Mol. Model. 1998; 4:379-394.

6. Wang R, Gao Y, Lai L, LigBuilder: A Multi-Purpose Program for Structure-Based Drug Design. J. Mol. Model. 2000; 6:498 – 516.