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In-Silico Analysis of Hepcidin-Ferroportin Axis as Potential Target in Anemia Due to Chronic Disease

KEYWORDS: Anemia, Hepcidin, Ferroportin, Amygdalin, Wheatgrass

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ABSTRACT *Anemia due to inflammation is prevalent in patients with chronic disease including cancer. It is associated with high morbidity and lower quality of life. Intracellular retention of iron within the cells of reticulo-endothelial system is the characteristic feature. This sequestration is primarily due to Hepcidin induced internalization followed by degradation of iron exporting protein Ferroportin resulting in blockade of iron efflux from these cells. Current modality of ACD (Anemia of chronic Disease) management including intravenous infusion of iron and/or erythropoietin are either ineffective or leads to manifestation of adverse reactions. Therefore, search for newer alternative therapies and novel strategies to target hepcidin-ferroportin axis is the need of the day. Uses of wheatgrass juice for rectification of anemia and cancer control have been claimed earlier. Wheatgrass is a potent source of many vitamins, minerals and glycoside e.g. amygdaline. In the present study we proposed an in-silico model for targeting ferroportin binding domain of hepcidin with amygdalin using bioinformatics tools. The result from Hepcidin-Ferroportin interaction study revealed the major interacting residues on hepcidin which interact more efficiently with amygdalin. This suggests a competitive binding of amygdalin with hepcidin which may prevent ferroportin degradation leading to rectification of anemia by allowing Fe efflux from RES available for EPO independent hemopoiesis.*

INTRODUCTION

Anemia of chronic inflammation (ACI) is the most frequent anemia in hospitalized patients with chronic diseases including cancer and is associated with significant morbidity. In several cancer patients the causative mechanism of anemia is incompletely defined; therefore the term "anemia of chronic disease" is used. Several cytokines, including tumor necrosis factor (TNF), Interferon Gamma (IF γ) and Interleukin-1 (IL-1), released into the blood of cancer patients can suppress erythropoiesis by affecting red cell production and impaired iron utilization[1]. Inflammatory cytokines promote the production of WBC's. Bone marrow produces both RBC's and WBC's from the same precursor stem cells. Therefore, the up regulation of WBC's causes fewer stem cells to differentiate into red blood cells. This may be an important cause for the effective inhibition of erythropoiesis, even when erythropoietin levels are normal[2]. Management of ACD using intravenous iron and erythropoiesis stimulating agents are ineffective for some patients and are not without adverse effects, driving the need for new alternative therapies.[3] Uses of wheatgrass juice for rectification of anemia and cancer control have been claimed earlier. Supplementation of fresh wheatgrass juice leads to changes in various interleukins 3, 6, GMCSF and TNF-a without significant alterations in EPO [4]. These cytokines are made by T lymphocytes, monocytes, resident macrophages, other tissues e.g. liver cells, and bone marrow stromal cells. Defective iron utilization, the hallmark of anemia of chronic illness, is due to the retention of iron within cells of the reticuloendothelial system (RES), thus making the metal unavailable for efficient erythropoiesis. [5] This reticuloendothelial iron sequestration is primarily mediated by excess levels of the inflammation induced iron regulatory protein hepcidin which blocks the release of iron from macrophages and interrupts intestinal iron absorption.[6] It internalizes and degrades the Ferroportin, only known iron exporting protein, resulting in blockade of iron efflux from these cells.

Besides this our previous study on in-vitro hematopoietic stem cell model showed formation of higher CFU-e in wheatgrass juice supplemented sera as compared to unsupplemented sera.[7] This lead to develop a hypothesis that besides the interleukins and growth factor there is some chemical entity which may be playing a role in inducing erythropoiesis by an EPO independent mechanism. Therefore the current study was undertaken to address this hypothesis using bioinformatics tools.

Materials and Methods

For this study the following offline and online bioinformatics tools were used

Offline tool:

Free trial version MOLEGRO virtual docker (4.1.0) for docking and calculating binding free energy

Online tools:

I-Tasser (http://zhanglab.ccmb.med.umich.edu/I-TASSER/) for Ab-initio structure prediction

HEX (http://hexserver.loria.fr/) for protein-protein interaction

Protein Data Bank (http://www.rcsb.org/pdb/home/home.do)

Target protein: Hepcidin (PDB ID: 3H0T)

PUBCHEM (https://pubchem.ncbi.nlm.nih.gov/)

Target ligand: Amygdalin (CID 34751)

Methodology

Picture 1: Screenshot of I-Tasser homepage

Amino acid sequence retrieved from NCBI for ferroportin is as follows:

>FDCITTGYAYTQGLSGSILS

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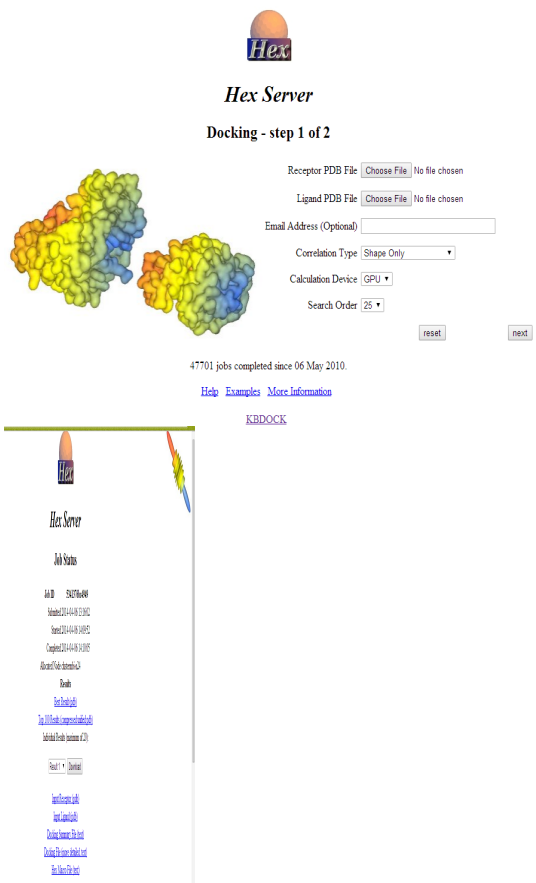
The sequence in Fasta format was pasted in the defined box and uploaded. The predicted structure was released with best five models with C-score, estimated accuracy and RMSD.

MD simulation

The predicted structure was optimized by running molecular dynamics simulation at 37°C, pH 7.4 and normal saline environment for 20ps using YASARA. The trajectory was build for RMSD of C-a and backbone. The average structure was generated and compared with the energy minimized initial structure to assess the overall deviation in the conformation. RMSF was calculated to check the deviation in specific amino acid residue. The average structure was considered for further protein-protein interaction study using Hex online server.

Picture 2: Screenshot of HEX online server



In the HEX server receptor PDB file (Hepcidin-3H0T-c) and ligand PBD file (predicted Ferroportin) were uploaded with default setting. The results of receptor-ligand complex were retrieved as best result (.pdb file). Post processing of hepcidin-ferroportin complex downloaded from HEX server for energy calculation and interaction study to identify interacting residues.

Docking using MOLEGRO Virtual Docker

The following work flow was adopted to carry out docking studies with hepcidin Structure retrieved from Protein Data Bank (PDB ID: 3H0T-c chain)

Structure refinement was done using MOLEGRO (3H0T-c chain)

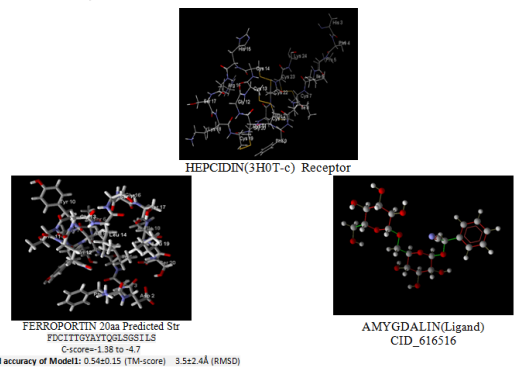
Structure of Amygdalin (CID 34751) was retrieved from PUBCHEM

Docking of Target with ligand was carried out using docking wizard against the whole protein as it was small in size and ferroportin interaction showed involvement of majority of amino acid residues

Post processing of docking results e.g. binding free energy; H-bond, selection of best pose was done

Result

Picture 3: Screenshot showing the structural details of receptor and ligands:



MD simulation

The predicted structure of ferroportin when simulated for 20ns under biological condition using YASARA we found significant deviation in C-α and backbone ranging from 1.1 to 4.7Å till 10ns which later on stabilized and remained below 3. The RMSD plot for individual amino acid revealed highest deviation in terminal two amino acids (Phe1, and Ser20) on either end. Besides that THR 6 showed high RMSD as compared to its neighboring amino acids.

Chart 1: Trajectory file for ferroportin plotting RMSD(C-a) vs time in picoseconds

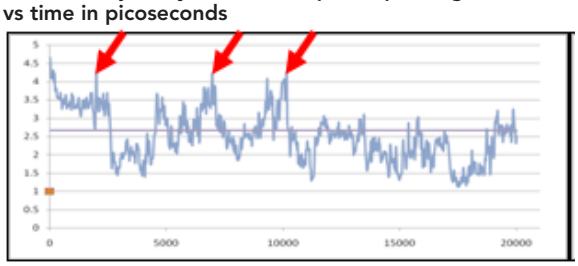
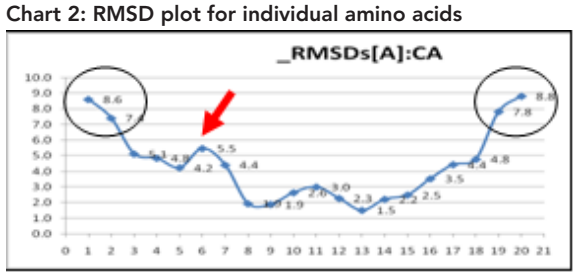
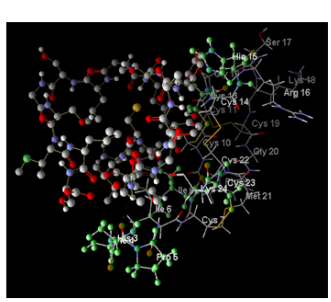
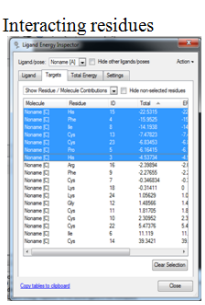


Chart 2: RMSD plot for individual amino acids



Picture 4: Screenshot showing HEX DOCKING





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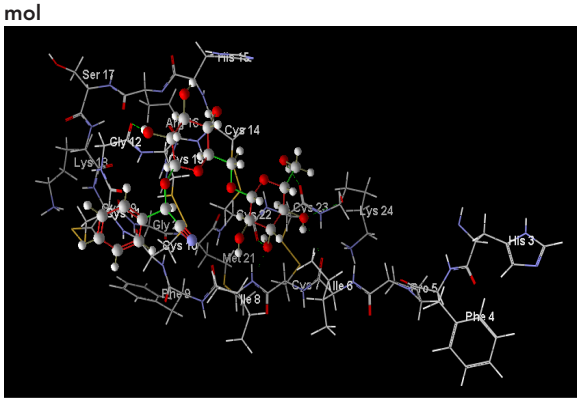
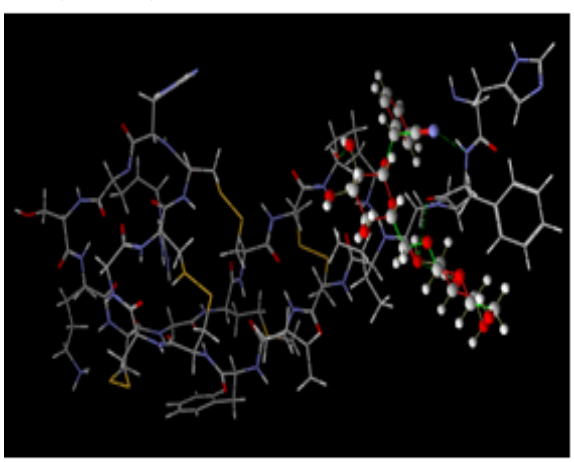
HEX docking reveals interaction of majority of amino acid residues of hepcidin with ferroportin with major interactions involving 3, 4,5,8,13,15 and 23 as shown in shaded zone.

Docking of Hepcidin & Amygdalin revealed strong interaction. The post processing of docking results gave two major poses as follows:

Picture 5: Screenshot of pose1showing interacting residues (6-8; 10-15; 22-24) with moledock score-74.3 Kcal/mol



Picture 6: Screenshot of pose2 showing interacting residues (3-6 & 24) with moledock score -71.1 Kcal/mol



These in-silico results are suggestive of a strong molecular interaction of Hepcidin with amygdalin stabilized by H-bonding which is more stronger than ferroportin. This prompted us to predict a competitive binding of amygdalin with hepcidin than with ferroportin.

DISCUSSION:

A characteristic feature of ACD is functional deficiency of iron, which is associated with increased serum hepcidin levels. These functional iron deficiency in ACD, are rectified by iron supplementation either alone or in combination with erythropoiesis stimulating agents (ESA). Reports generated from past studies have shown that treatment of ACD using ESAs and iron therapy have limited effectiveness and may have potentially serious adverse events [3]. Inspite of this they do not address the root cause of the Pathophysiology of ACD, i.e. excess hepcidin and reduced ferroportin activity. Research in the last decade suggest that the functional iron deficiency in ACD can be attributed to over expression of Hepcidin, and suggest that targeting hepcidin may be a useful therapeutic approach to treat ACD. The design of hepcidin inhibitors has been directed elucidating the molecular mechanisms of hepcidin regulation and action. In the current study we judiciously targeted hepcidin - ferroportin interaction site as the potential binding site to increase the effectiveness of inhibitor binding. The blind docking of ligand with the whole hepcidin molecule revealed interaction of the same residues which interacted with the ferroportin but with more efficiency and strongly with H-bonding.

CONCLUSION:

The current study suggestive of amygdalin, a unique constituent of wheatgrass juice, as a potential ligand (inhibitor) which can competitively bind to ferroportin binding site on hepcidin and thereby may prevent the ferroportin inhibition by hepcidin in inflammatory condition. At the same time wheatgrass juice which is loaded with many phyto-nutrients, vitamins and minerals may pose to be a healthy supplement for rectifying other nutritional deficiencies due to chronic disease e.g. cancer.

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