SYN-010, a Proprietary Modified-Release Formulation of Lovastatin Lactone, may Improve Constipation by Inhibiting Enzymes in the Archaeal Methanogenesis Pathway: Results of Computational *M. smithii* Enzyme-Ligand Docking Experiments

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ABSTRACT

BACKGROUND: Evidence supports a causal role for methane, produced by *M. smithii* in the intestine, in constipation-related disorders. A recent phase 2 clinical trial (NCT02495623) with lovastatin in patients with constipation-predominant irritable bowel syndrome showed a reduction in clinical symptoms and breath methane levels compared to placebo. While the cholesterol-lowering effect of statins is mediated through the inhibition of HMG-CoA reductase, an alternative mechanism of action may be responsible for the anti-methanogenesis effect, specifically, direct inhibition of enzymes in the archaeal methanogenesis pathway. We consequently conducted protein-ligand docking experiments to evaluate this possibility. METHODS: *M. smithii* F420-dependent methylenetetrahydromethanopterin dehydrogenase (mtd), a key methanogenesis enzyme with a known nucleotide sequence but no tertiary protein structural information, was modeled using Protein Databank (PDB) templates employing the Eidogen Sertanty's STRUCTFAST™ algorithm. Once models were developed, ligand binding sites were identified by inference from the respective PDB templates used in modeling, and from the Eidogen SiteSeeker algorithm which looks for concave surface features sufficiently exposed to enable ligand binding, and also consideris evolutionary conservation of sequence. The ligands for docking were the F420-coenzyme (as natural ligand), lovastatin and simvastatin, both in their respective lactone and β-hydroxyacid forms. BIOVIA's (Accelrys') Pipeline Pilot technology was used for ligand preparation. Each representation was then docked into each site and scored using open-source AutoDock Vina. A total of 88 ligand variations were systematically docked across the extracted 12 sites for a total of 1,056 docking simulations. Because the docking process scores ligand conformations based on ligand conformation and ligand-to-receptor interactions within a grid box, after the 1,056 docking simulations were complete, we rescored all docked ligand variations against their respective full model structures. RESULTS: 1) Generally, for each sequence/site the lactone form statins had more favorable site interactions (i.e., lower docking scores) compared to F420. 2) The statin lactone forms generally had more favorable docking scores, even relative to the native template PDB ligands. 3) The statin β-hydroxy acid forms had less favorable docking scores, and typically scored in the middle with some of the F420 tautomeric forms. CONCLUSION: The lactone forms of statins exhibit preferential binding over the native-F420 coenzyme ligand in silico and thus could inhibit the activity of the key *M. smithii* methanogenesis enzyme mtd in vivo. Statin lactones may thus exert a methane-reducing effect that is distinct from cholesterol lowering activity, which requires HMGR inhibition by statin β-hydroxyacid forms.

BACKGROUND

Irritable bowel syndrome (IBS) affects as many as 45 million people in the United States, and between 10 to 15% of the worldwide population. Approximately one third of patients with IBS have IBS with constipation (IBS-C).[1]

Observational studies have shown a strong causal association between methane production and delayed intestinal transit.[1] The only organisms known to produce methane are methanogenic archaea. *Methanobrevibacter smithii* is well adapted to the human intestine, where it is the dominant methanogen.

Here we report the results of protein-ligand docking experiments to explore whether lactone forms of statins directly inhibit enzymes in the archaeal methanogenesis pathway and have the potential to treat IBS-C.

METHODS

Protein-ligand docking experiments were conducted to investigate whether, in addition to their cholesterol-lowering effects, HMG-CoA reductase inhibitors (specifically lovastatin lactone) directly inhibit enzymes in the archaeal methanogenesis pathway. *Methanobrevibacter smithii* F420-dependent methylenetetrahydromethanopterin dehydrogenase (mtd), a key methanogenesis enzyme with a known sequence but no tertiary protein structural information, was modeled using Protein Databank (PDB) templates employing Eidogen STRUCTFAST[1,2] technology.

METHODS

Ligand binding sites were identified by inference from the respective PDB templates used in modeling and from the Eidogen SiteSeeker algorithm,[3] were used to develop models for A5UMI1 and Q02394.¶
Other sites were manually inferred within PyMOL v1.8 after aligning models and templates containing their respective co-complexed ligands. Residues on model structures with a 7Å cutoff of co-complexed ligands within the templates were exported and also processed as sites.
The ligands for docking were the F420-coenzyme (as natural ligand), lovastatin and simvastatin, both in their respective lactone and β-hydroxyacid forms.

Ligands were carefully prepared considering different protonation states, isomers, and tautomers. Charges were standardized, missing hydrogens added, ionization states enumerated, functional groups ionized, tautomers and isomers generated, and starting-point 3D coordinates for each ligand using BIOVIA's (Accelrys') Pipeline Pilot technology v8.5 generated.[4] Ligands were finally prepared into mol2 format[5] and then docked into each identified site and scored using AutoDock Vina v1.1.2.[6]

A total of 88 ligand variations were systematically docked across the extracted 12 sites for a total of 1,056 docking simulations. Because the docking process scores ligand conformations based on ligand conformation and ligand-to-receptor interactions within a grid box, after the 1,056 docking simulations were complete, all docked ligand variations against their respective full model structures were rescored.

¶ A5UMI1 (275 amino acid residues) F420-dependent methylenetetrahydromethanopterin dehydrogenase of M. smithii and Q02394 (358 amino acid residues) F420-dependent methylenetetrahydromethanopterin (methylene-H(4)MPT) dehydrogenase (mtd) of Methanopyrus kandleri are evolutionary related enzymes that only leverage F420 as a coenzyme, which assisted our computational analyses by avoiding issues associated with an NADP induced fit.

RESULTS

Protein Modeling and Site Identification

Three different PDB templates that had sufficient sequence homology to model the **Q02394** sequence were identified. The top three PDBs showing significant sequence homology to **Q02394** included: 3F47 (57%), 3H65 (57%), and 4JJF (52%). Each template was used to model **Q02394.**

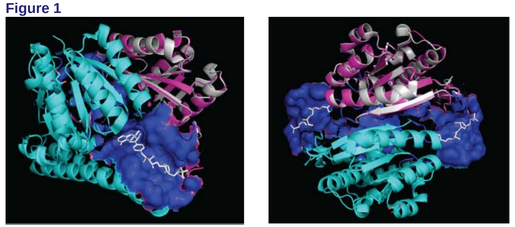
The modeling of sequence **A5UMI1** was straightforward given its high 52% sequence homology to 3IQZ. The Eidogen SiteSeeker algorithm identified only one site when template chains A, C, D were used, while two sites were identified in models leveraging template chains B, E, F.

The H4M site was modeled manually (Figure1).

Four ligand sites from the **A5UMI1** modeling and six sites from **Q02394** modeling were used in the docking simulations.

RESULTS

Figure 1

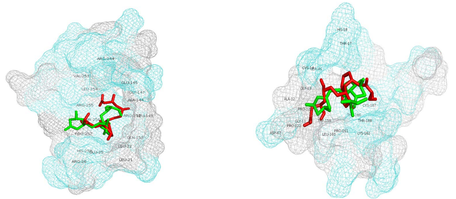


*Modeled quaternary structure of **A5UMI1**/3IQZB (cyan) and **A5UMI1**/3IQZF (pink) after respective alignments onto chain-B and chain-F of 3IQZ within PyMOL. 3IQZ's chain-F is highlighted in silver. Dual chain model site residues (blue surface) were inferred from residues in chain-B and chain-F models that are within 7 Å of the 3IQZ ligand (H4M - white). 3IQZ's chain-B and chain-F form a quaternary structure with two different H4M binding sites (right).*

Ligand Processing and Docking

Key ligands included lovastatin (lactone and hydroxyacid forms), F420, and simvastatin (lactone and hydroxyacid forms). Process ligands found in PB3 templates were used to model sequences and computationally processed prior to docking. A total of 88 ligand variations were docked into 10 identified binding sites across all models for a total of 880 docking simulations. The top two scoring sites were **A5UMI1_3IQZB** and **Q02394_4JJF**. These were used to rank order each ligand (Figure 2).

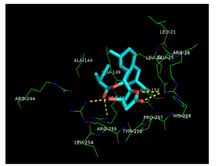
Figure 2 Lovastatin-Lactone and Acid Poses



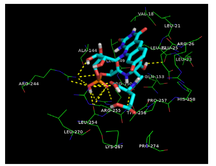
A5UMI1_3IQZB_SiteSeeker2 **Q02394**_4JJF_SiteSeeker

1. Consistent with Sharma *et al.*,[7] the lactone form statins docked into each site with favorable site interactions (i.e. lower docking scores) as compared to F420 for the same sequence/site grouping.
2. The statin lactone forms generally had more favorable docking scores, even relative to the native template PDB ligands.
3. The statin acid forms had less favorable docking scores and typically scored in the middle with some of the F420 forms.
4. The F420 scores were generally the lowest for each sequence/site models of **A5UM1 and Q02394**.

Figure 3 Lovastatin and F420 docked into A5UMI1_3IQZB_SiteSeeker2



Lovastatin-lactone
Calculated affinity: -7.2 (kcal/mol); AutoDock4.1 Score: 14.3



F420
Calculated affinity: -6.99 (kcal/mol); AutoDock4.1Score: 63.3

CONCLUSIONS

- Statin binding is likely for the two key targets: **A5UMI1** and **Q02394**
- Lactone forms of statins exhibit preferential binding over the native-F420 coenzyme ligand in silico, and thus could inhibit the activity of the key *M. smithii* methanogenesis enzyme mtd *in vivo*.
- Statin lactones may exert a methane-reducing effect which is distinct from their cholesterol lowering activity

SYN-010 is a proprietary, modified-release formulation of lovastatin lactone intended to reduce methane production in the gut while minimizing disruption to the microbiome with the potential to treat the underlying cause of irritable bowel syndrome with constipation (IBS-C). A phase 2 clinical trial has been completed and results will be presented at DDW 2016.

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Disclosures: K. G., J. K-K., and J. S. are employees of Synthetic Biologics, Inc. (SB). V.W. is a consultant to SB. M.P. is a member of the SB IBS Advisory Board. SB sponsored the modeling study performed by Eidogen-Sertanty, Inc.