Investigating Polyunsaturated Fatty Acids as Potential Inhibitors of SARS-CoV-2 Cell Entry

Lilian Toma* and Praveen Nekkar Rao

School of Pharmacy, University of Waterloo, Ontario, Canada, N2L 3G1

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ABSTRACT

Purpose: Coronavirus disease 2019 (COVID-19) made a devastating worldwide impact, and new variants of severe acute respiratory syndrome virus 2 (SARS-CoV-2) continue to surface. SARS-CoV-2 initiates cell entry through binding of its Spike protein to human angiotensin converting enzyme 2 (ACE2).[1] The aim of this study was to investigate the ability of polyunsaturated fatty acids to inhibit SARS-CoV-2 Spike receptor binding domain (RBD) and ACE2 binding.

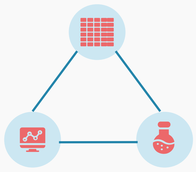
Methods: Molecular docking studies (BIOVIA Discovery Studio) were conducted to investigate the interactions of the fatty acids at the binding interface (PDB ID: 6LZG). Enzyme-linked immunosorbent assays (ELISA) (Cayman Chemical) were used to investigate ACE2/Spike-S1-RBD binding inhibition by the fatty acids at 10, 25, and 50 μM. Human ACE inhibition activity by the fatty acids (1, 5, 10 μM) was measured using an ACE inhibitor assay kit (BPS Bioscience). To investigate toxicity of the fatty acids, Calu-3 (human lung adenocarcinoma) cells were treated with the fatty acids (10 & 25 μM) for 24 hours, and quantified with cell-counting kit 8 (Dojindo).

IN VITRO ASSAYS

1. ACE2:SARS-CoV-2 Spike RBD Inhibitor Screening Assays.
2. ACE Inhibitor Assays.
3. Cell Viability Assays.

COMPUTATIONAL MODELING

Molecular docking studies investigated the interactions of PUFAs & derivatives at the binding interface.



CHEMICAL SYNTHESIS

Synthesized PUFA derivatives using organic chemistry techniques.

Results: The molecular docking studies showed a trend of hydrogen bonding between the fatty acids with Lys353 (ACE2), a main virus-binding hotspot.[2] In the ELISA assay, α-linolenic acid significantly reduced ACE2/Spike-S1-RBD interactions (20% inhibition at 50 μM, $p < 0.05$), whereas oleic acid and linoleic acid exhibited weaker inhibition (< 10% at 50 μM). In the ACE inhibition assay, no fatty acids significantly inhibited ACE at 1, 5, and 10 μM (inhibition range: 0-2%). The fatty acids did not show significant toxicity to Calu-3 cells at 10 and 25 μM (cell viability range: 93-125%).

Conclusion: The results of these studies suggest that the fatty acids tested have potential in preventing SARS-CoV-2 Spike and ACE2 interactions. Additional studies are underway to obtain structure-activity data which will be useful for designing novel viral entry inhibitors.

HYPOTHESIS & OBJECTIVES

We hypothesize PUFAs and their derivatives can inhibit SARS-CoV-2 Spike protein & ACE2 binding. Our objectives were to:

I. Synthesize a library of PUFA derivatives.
II. Evaluate their ability to prevent SARS-CoV-2 Spike protein & ACE2 interaction using in vitro studies.
III. Investigate the interactions of PUFAs/PUFA derivatives at the SARS-CoV-2 Spike & ACE2 binding interface with molecular docking studies.

RESULTS

ACE Inhibitor Assay

Compound	Concentration (μM)	Inhibition (%)	Compound	Concentration (μM)	Inhibition (%)
LNA	1	NA	ARA	1	NA
	5	NA		5	NA
	10	NA		10	NA
ALA	1	NA	DHA	1	NA
	5	NA		5	NA
	10	2		10	NA
OA	1	NA	EPA	1	NA
	5	NA		5	NA
	10	NA		10	NA
Positive Control	N/A	100			

Table 1: Results of the ACE Inhibitor Assay, a fluorometric assay from BPS Bioscience (n=3). "NA" signifies "Not Active." LNA = linoleic acid, ALA = α-linolenic acid, OA = oleic acid, ARA = arachidonic acid, DHA = docosahexaenoic acid, EPA = eicosapentaenoic acid.

ACE2:SARS-CoV-2 Spike RBD Inhibitor Assay

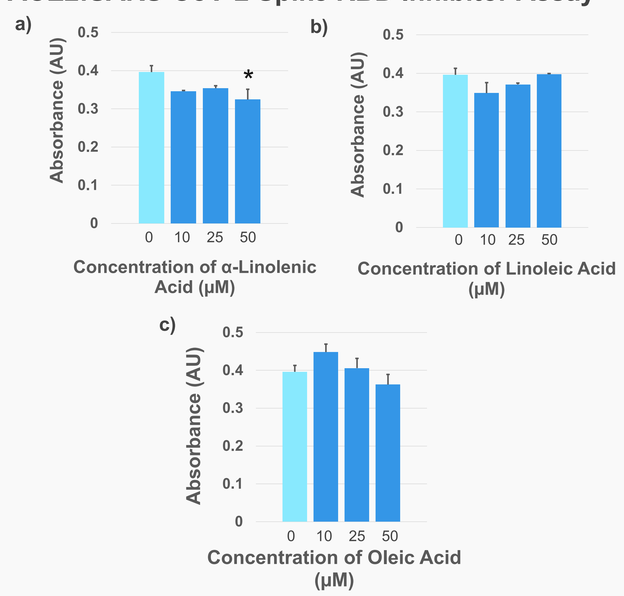


Figure 4a-c: Results of the ACE2:SARS-CoV-2 Spike RBD Assay, (n=2-3). Positive control showed 100% inhibition.

Molecular Docking Studies

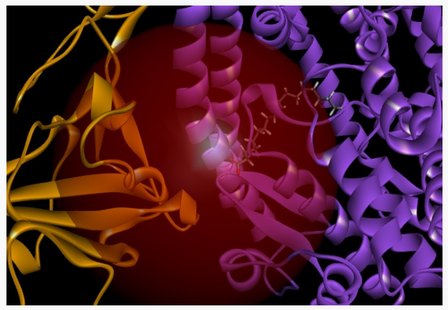


Figure 2: One of the predicted docking positions of oleic acid at the SARS-CoV-2 ACE2 interface (PDB id: 6LZG) using Discovery Studio. ACE2 is shown in purple, SARS-CoV-2 Spike RBD in yellow.

Calu-3 Cell Viability Assay

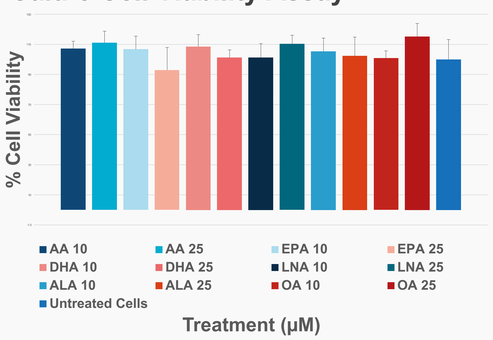


Figure 3: Calu-3 cell viability assay results.(n=6).

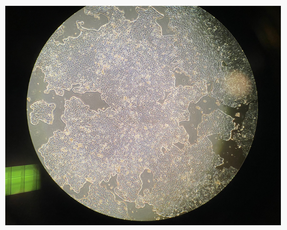


Figure 5: Light microscope image of Calu-3 (human lung adenocarcinoma) cells.

CONCLUSIONS

The results of these studies suggest that linolenic acid has potential in preventing SARS-CoV-2 Spike and ACE2 interactions, that the PUFAs do not inhibit ACE function, and that the PUFAs do not show toxicity to Calu-3 cells.

FUTURE DIRECTIONS

- Run these assays with PUFA derivatives and observe how the structural changes will impact biological activity.
- Conduct immunofluorescence assays to examine the impact of Spike protein on Calu-3 cell ACE2 expression, mitochondrial function, and cell permeability, and if the PUFAs/PUFA derivatives can mitigate these impacts.

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For further information, please contact: *ltoma@uwaterloo.ca*