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Free Fatty Acids from Fish Oil Inactivate SARS-CoV-2

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Abstract

Aims: A natural component of fish oils, free fatty acids (FFA) inactivate enveloped viruses, some bacteria and fungi. We test the hypothesis that free fatty acids made by hydrolysis from fish oil inactivate SARS-CoV-2 in vitro. We also study an existing food additive containing 2% FFA in cod liver oil.

Methods: Virucidal effect of 0.1%, 1% and 2% free fatty acids from fish oil on SARS-CoV-2 was measured *in vitro*. A survey was conducted of 42 users of a marketed food additive containing 2% free fatty acids in cod liver oil.

Results: 1% and 2% free fatty acids reduce SARS-CoV-2 viral concentration more than 99.9% compared to vehicle control ($p<0.0001$). The food additive with 2% free fatty acids in cod liver oil was well tolerated by users with 10/42 reporting mild irritation or dryness in throat.

Conclusion: Cod liver oil and other fish oils naturally contain free fatty acids and have been used as food additives for centuries. 1-2% FFA inactivate SARS-CoV-2 in vitro and are well tolerated in cod liver oil as mouth wash. Widely available in industrial quantities, free fatty acids in fish oil may have potential as mouth/throat wash to reduce concentration of SARS-CoV-2 viruses in saliva and upper respiratory mucosa and reduce transmission. Clinical trials are in preparation.

Keywords

SARS-CoV-2 , Covid-19, Free fatty acids, fish oil , Coronavirus, enveloped viruses, mouth wash, virucidal

Introduction

Cod liver oil and other fishoils have traditionally been used as food additive, including as remedy to prevent respiratory infections and colds in northern European countries. Fish oils contain free fatty acids as a natural degradation product. Several researchers [1-7] have demonstrated that free fatty acids inactivate enveloped viruses as well as some bacteria and fungi. Coronaviruses are have a lipid envelope and this opens the potential for using widely available and natural free fatty acids and fish oil to inactivate SARS-CoV-2.

A food additive with 2% free fatty acids in cod liver oil is marketed in Iceland and has been used as mouth- and throat-wash during the pandemic, including by many frontline health workers. We report results on virucidal effect of free fatty acids on SARS-CoV-2 in vitro and a survey of individuals who used the marketed mouth wash containing free fatty acids in cod liver oil.

Methods

We examined the virucidal effect of 0.1%, 1% and 2% free fatty acids from fish oil on SARS-CoV-2 in vitro and compared with the vehicle control. Free fatty acids are made by hydrolysis from cod liver oil in a GMP approved facility at Lysi hf, Reykjavík, Iceland.

Virus, media and cells

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2, USA-WA1/2020 strain) was obtained from the World Reference Center for Emerging Viruses and Arboviruses (WRCEVA) at the University of Texas Medical Branch (UTMB, Galveston, TX). A working stock was prepared prior to testing by passaging in Vero 76 cells.

Culture media for prepared stock (test media) was MEM with 2% fetal bovine serum and 50 µg/mL gentamicin.

Virucidal assay

A sample of free fatty acids was pre-warmed in a 37°C incubator, then dissolved 1 part in 1 part 100% ethanol. Test media was also pre-warmed in a 37°C incubator to prepare compound dilutions of 4%, 2%, and 0.2%. Compound dilutions were added in six replicates to equal volume of SARS-CoV-2 virus solution, to get final test concentrations of 2%, 1%, and 0.1%. Vehicle not containing compound was prepared as described above, subsituting test media for the free fatty acids, and tested in parallel. Test media only was also tested for each prepared concentration to serve as toxicity controls. Solution and virus were incubated at room temperature 10 minutes. The solution was then neutralized by 1/10 dilution in test media containing 10% FBS. The entire assay was repeated as described above as an independent assay replicate on a separate day .

Virus quantification

Neutralized samples were pooled for virus quantification so that triplicate samples were combined for each test concentration. Since there were 6 replicates from each experiment for each free fatty acid concentration, 3 each were combined into two pools for quantification, giving two replicates of data from each concentration. Surviving virus was quantified by standard end-point dilution assay. Pooled samples were serially diluted 1/10 in test medium, then 100 µL of each dilution were plated into quadruplicate wells of 96-well plates containing 80-90% confluent Vero 76 cells. Plates were incubated at 37 ± 2°C with 5% CO2 for 5 days. Each well was then scored for presence or absence of virus. The end-point titers (CCID50) values were calculated using the Reed-Muench (Reed et al 1938) equation.

Controls

Vehicle controls were tested and the reduction of virus in test wells was compared to the vehicle controls to calculate as the log reduction value (LRV). Toxicity controls were tested with media not containing virus to observe if the samples were toxic to cells. Neutralization controls were tested to ensure that virus inactivation did not continue after the specified contact time, and that residual sample in the titer assay plates did not inhibit growth and detection of surviving virus. This was done by adding toxicity samples to titer test plates then spiking each well with a low amount of virus that would produce an observable amount of CPE during the incubation period.

Statistical analysis

Four total replicates of each concentration were tested and the mean and standard deviation were calculated. Test sample results were compared to vehicle controls by one-way ANOVA with Dunnett's multiple comparison tests using GraphPad Prism (version 8) software.

Table 1: Virucidal efficiency of free fatty acids against SARS-CoV-2 after a 10-minute incubation with virus at 22 ± 2°C

Compound	Concentration	Virus Titer[a]	LRV[b]
Free Fatty Acids	2%	1.2 ± 0.6****	3.0
Free Fatty Acids	1%	1.0 ± 0.5****	3.2
Free Fatty Acids	0.1%	4.1 ± 0.3	<1
Vehice Control	0%	4.2 ± 0.4	-

[a] Log_{10} $CCID_{50}$ of virus per 0.1mL

[b] LRV (log reduction value): reduction of virus compared to the vehicle control

****P < 0.0001 (n=4) by one-way ANOVA and Dunnett post-test compared with vehicle control.

For statistical analysis "<" signs were ignored.

Human study

49 volunteers who had used the marketed food additive containing 2% free fatty acids in cod liver oil (lemon extract added for taste; Lýsi hf. Reykjavík, Iceland) were asked to answer a survey. 42 answered the questionnaire, 29 men and 18 women, age 22-70 years. They had used the marketed food additive as mouth- and throat-wash, one teaspoon (5 ml) 2 or 4 times a day for 10 days.

Results

Free Fatty Acids is an effective virucidal after a 10-minute incubation with SARS-CoV-2 at room temperature, reducing virus from 4.2 log CCID50 per 0.1 mL in virus control samples to below the assay limit of detection (LRV>3.0, >99.9% of virus) when tested at 2% or 1% (P<0.0001, n=4). SARS-CoV-2 titer was not reduced when Free Fatty Acids was tested at 0.1%, (Table 1).

Free Fatty Acids showed some cytotoxicity in Vero 76 cells, affecting ability to detect virus CPE in cells and altering the limit of detection in one of the two assay replicates. Consequently, the limit of detection was either 0.7 or 1.7 log CCID50 per 0.1 mL in the 2% and 1% test samples. If virus was below the limit of detection, „<" signs were ignored for statistical analysis, and therefore LRV values are conservative.

Of the 42 participants who answered the questionnaire survey and had used the food additive, none reported serious adverse events. 10 individuals reported minor irritation or dryness in the throat and/or mild discomfort in the stomach after ingestion.

Discussion

Free fatty acids (FFA) are a potent virucidal agent against SARS-CoV-2. FFA are natural degradation prod-

uct and component of cod liver oil and other fish oils and as such safe, stable and available in industrial quantities [8]. Free fatty acids and fish oil have traditionally been used as a remedy against respiratory infections in nordic countries. The mechanism of free fatty acids inactivating the lipid envelope of enveloped viruses such as respiratory syncytial virus, herpes viruses and coronaviruses is well established [1].

We show for the first time that free fatty acids from fish oil are effective in inactivating SARS-CoV-2 upon contact. The effect is dose dependent, where 0.1% has no effect and 1% and 2% solutions are virucidal. 1% FFA in cod liver oil is listed in the European Pharmacopea and 2% FFA in cod liver oil is well tolerated as a marketed food additive in Iceland. This opens the way for development and testing of using free fatty acids to fight SARS-CoV-2 and other enveloped viruses and reduce their transmission. One possible approach is to wash the mouth and throat with fish oil containg free fatty acids in order to reduce viability of viruses in the saliva and on the mucosa. This might reduce the transmission from an infected person, and reduce the probability of an incoming virus surviving in a healthy persons. Clinical trials to test these hypotheses are in preparation.

Our group has tested preparations containing 30% free fatty acids in a skin ointment and suppositories. These have proven safe and non-irritating on skin and rectal mucosa in clinical trials [9-11].

In summary, we have demonstrated that free fatty acids from fish oil inactivate SARS-CoV-2 *in vitro*. They do so in concentration that is safe and within traditional usage. They offer a potential tool to fight the ongoing Covid-19 pandemic. Clinical trials are in preparation to test the efficacy of FFA in fish oil to reduce SARS-CoV-2 concentration in saliva and upper respiratory mucosa and reduce the risk of transmission.

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