COVID Information Commons (CIC) Research Lightning Talk

Transcript of a Presentation by Harvey Pollard (Uniformed Services University School of Medicine), December 9, 2024

Title: COVID-19 airway inflammation is due to Spike inhibition of CFTR signaling

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YouTube Recording with Slides

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Transcript

Slide 1

Thank you - good morning everybody, or good afternoon. The title of this talk is: “Inflammation in the COVID-19 Airway is due to CFTR Signaling by the SARS-CoV-2 Spike Protein.” Since we’ve all been living through the COVID pandemic, a lot of this will not be new to you. But CFTR may be new. It stands for - it’s the name of a protein mutation which causes a disease called Cystic Fibrosis. This is something we work on.

It’s hard to remember, actually, the name of this protein, because the people who were studying it in the beginning, at the hospital for sick kids in Toronto, had no idea what it did. They were listening to a rock station, CFTR, Canadian Frequency Toronto, 680 on the AM dial, and they decided to call it CFTR. That’s the way I remember the name - it’s a way of giving information about what people were doing in the old days when they didn’t know exactly what they were doing. In any event, the protein itself is really critical, as many of the proteins or genes that are found in rare diseases - they are rare because it’s difficult to live without them. We are joined by colleagues at USAMRI, the United State Army Medical Research Institute of Infectious Diseases, where the very worst viruses are studied. We were able to do this - this is a collaboration - and the paper that much of the data I’m going to talk about was recently published in scientific reports. Hung Caohuy et al is shown as a citation down at the bottom of this slide.

Slide 2

The goals of this project were to understand, first of all, the mechanism by which pro-inflammatory cytokine storm in the COVID-19 airway occurred. It's essentially lethal. Second, to find a therapeutic strategy to suppress the storm. By the way, if you see the spike trimer moving, it's supposed to be moving, it's not your eyes.

Slide 3

Firstly, we developed an in vitro assay for spike protein binding to ACE2. Then, we looked for candidate drugs that could block the interaction. Finally, we found the cardiac glycosides such as digitoxin, digoxin, or ouabain were potent inhibitors of Spike-ACE2 binding. After going through a lot of studies that were looking at the binding, we asked the question whether any of those drugs which were approved around the world - and licensed digoxin is licensed here in the United States - actually interfered with infection

Slide 4

This slide shows that the infectivity of data virus is inhibited by digitoxin and other cardiac glycosides in human lung cells - the A549 cell, very typical. Across the top is a series of dose response curves. The x-axis is the concentration of the drug and the y-axis in green is the inhibitory values, or K12 values, essentially. EC50 values for all three of the cardiac glycoside drugs. They're in concentration ranges used to treat heart failure, interestingly. The red color is cytotoxicity. It's about tenfold greater than the respective inhibitory EC50 values, which means that you can use these drugs without getting into inhibiting the cells. The two boxes below are the steroid nucleuses that go with these drugs minus the sugars. They're not very effective. That was published in scientific reports in 2021, so we're very happy to see that we could actually transmit some in vitro experiments into something that was approximating in vivo.

Slide 5

There's additional preliminary information about CFTR which I want to emphasize. It's a gene responsible for the control of inflammation in the rare genetic disease Cystic Fibrosis. And as we will see in the next few slides, it's essential for control of inflammation in the COVID-19 airway.

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I'm just going to read the first part because it says what I want to say and we'll go on. Early in the COVID-19 pandemic, it was found that the lethal cytokine storm was due to activation of the TNF alpha and NF Kappa B pathway, but how that happened no one knew.

In the middle of this slide, you can see a signaling pathway which starts with the TNF alpha, which then binds to a TNF receptor in the membrane of the cell. There, it actually connects up to the first intracellular adapter, a molecule called TRADD. The DD stands for Death Domain, you know, very unnerving. That molecule is a critical director to take all those signals from TNF

alpha to the IKK'some that then binds to, or phosphorelates to I kappa B alpha, releases enough Kappa B, which moves into the nucleus and generates all of these cytokines - IL-6, IL-8, TNF alpha, and a bunch of mRNAs, which are then synthesized and secreted into the airway and into the rest of the body. Actually, in the context of a cytokine storm, we, in studying this situation with Cystic Fibrosis, we found that there were some looking at countervailing candidate drugs like digitoxin - we've already shown you that they interfere with the infection. We found that digitoxin, digoxin, and ouabain basically block that process. You can see in red letters and little block, the T-Junction, that when you make a binary complex between TNF alpha and TNFR1 one, it wants to bind to TRADD but it can't. So that is- the first experiments were just done in plain old HeLa cells, and that was actually demonstrated where the problem lay. One function of CFTR is to block that molecule TRADD and thereby inhibit the cytokine storm in the CF airway, but in fact, since the digitoxin and the CFTR are all working in the same place, essentially, the drugs are phenocopying or mimicking the CFTR. We hypothesized that the COVID-19 cytokine storm was due to the loss of CFTR and that cardiac glycosides, which would basically mimic CFTR, might be therapeutic.

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So the question was, does spike protein exposure reduce CFTR? The way we actually did this experiment was use a human lung organoid prepared at the air-water interface. You put some basal cells which we got from a colleague into this air-water interface and over the course of 28 days of farming, you grow a beautiful epithelium. You can see goblet cells, ancillary cells, and other sorts of cells verified by immunofluorescence below. On the right hand side we used Western blot analyses of spike protein effects on levels of CFTR. Along the horizontal axis we increased the concentration in the first upper column, A, the ancestral or original spike protein from Wuhan. It causes a dose dependent, significant dose dependent, loss of CFTR. We then looked at a much more biologically potent strain. This is the South African Beta strain, much more potent, as it turned out, as a COVID virus. Much more potent at reducing CFTR. You see the difference in the bar graphs and in the Western plots. So we concluded spike protein exposure does reduce CFTR levels.

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The next question was if spike proteins activate pro-inflammatory NF kappa B signaling. Well, in the graph I showed of the signaling pathways is a series of proteins. Starting from the top, TNF Alpha, TRADD, and so forth. These get activated if CFTR is lost. As you increase the concentration of the spike protein, again, we're using the Wuhan strain here, all of those red arrows on the left actually show places where an increase is happening. If you look over, you'll see that TRADD is actually increasing. In the end, IL-8, which is one of the cytokines we measured, is also increasing. So yes, the spike protein activates pro-inflammatory NF kappa B signaling.

Slide 9

This is where our USAMRI colleagues came in handy. Do the cardiac glycosides protect epithelial cells from CFTR lost by native virus? On the left hand side, you'll see a Western plot.

Various things are added right in the middle. Where it says 'virus only', you'll see that the level of intensity of that CFTR band, in this case is the dimer, is reduced substantially compared with the medium control and that digitoxin and ouabain raise the levels. The bar graph basically shows very similar things. These are all based on four independent experiments. The real situation here is that it's all very well for us to actually do experiments with spike proteins, but it's very important to verify that this is happening in real viruses and real diseases. This was something that we were interested to show and to validate.

Slide 10

The next question is "How does spike only bind to ACE2, yet also affect CFTR levels?" How could that be? One possibility was that maybe the CFTR that existed was bound to ACE2 in the cell membrane. It turns out, yes, it's bound to ACE2. The left hand side of this figure shows that, in fact, CFTR and ACE2 are actually found both in the basal cells that make up the differentiated epithelium and in the undifferentiated epithelium. CFTR is there, ACE2 and actually everything else you need to get a nice infection going (so long as you can call an infection nice). On the right hand side, it shows that if you take an antibody against ACE2 and immuno-precipitate it, then test that immuno-precipitate for bound CFTR, sure enough, you can find plenty of CFTR. Those are two duplicate experiments, the NRS is simply normal rat serum. You can also find immunoprecipitated CFTR in just the basal cells, so it's basically accessible in all the different parts of the epithelium.

Slide 11

I want to pause here to remind or to emphasize to everyone that CFTR is a cyclic AMP activated chloride channel. That channel is critical for keeping the airway hydrated. When the channel isn't there, the airway dries out and inflammation occurs. In this particular case, we have the CFTR protein in the cell surface. Up is the airway, down is the interior of the cell. Norepinephrine or epinephrine can bind to a protein - another protein that binds to CFTR is called the beta 2 adrenergic receptor. This generates cyclic AMP, Protein Kinase A is activated. It then labels the phosphorelates with CFTR. The channel activity is activated. You can see a chloride molecule moving out.

Why do I show this? Well, if the protein is being lost, you expect that the channel activity would also be lost. It's a sort of test. Is it really true that the spike protein reduces and removes everything associated with the CFTR?

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On the right hand side is a classical chamber experiment essentially. But we could actually look over to the horizontal axis on the left hand side - it is a time course and the vertical axis is the the activity of transport of chloride from one side of the liquid interface to the air side. If you add some IBMX or something to stabilize the [inaudible], the green - the channel opens up. You can see a very nice signal. If you turn and add an inhibitor of CFTR, it will go down, but if you add a spike protein, you lose about 50% of the signal. In this experiment, the spike is Wuhan, so it's the least potent of these, but if you add ouabin or digitoxin or digoxin to this cell culture that's

culturing over the course of 24 hours, the spike effect is lost. On the right hand side, you can see that story with the bar graphs, but just below where it says "CFTR", you can see that when you've added the spike, you get even less CFTR. So the spike protein causes loss of the CFTR protein in the same system and the CFTR protein loss is rescued by treatment of the epithelia with all three cardiac glycosides. The loss of channel activity, loss of protein, all occur at the same time. That means we feel we've robustly addressed the concept that the spike protein, and the virus for that matter, has reduced CFTR levels.

Slide 13

Our conclusions are that the data support the hypothesis that the COVID-19 cytokine storm is due to loss of CFTR. The second conclusion is that the loss of CFTR activates TRADD to drive TNFa/NF kappa B signaling. The digitoxin, digoxin, and ouabain were maybe therapeutic, first by blocking infection, by inhibiting the virus from binding to ACE2, and second by inhibiting inflammation, by inhibiting TRADD, and thus substituting for the lost CFTR. We suggest that the cardiac glycosite advantage is that it is one family of drugs with two simultaneous candidate therapeutic activities for COVID-19.

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We have to have these acknowledgements. Partly, our lab at the Uniform Services and also the Herbert and Florez labs at USAMRIID. We also have to have a Department of Defense disclaimer which talks about the fact that you we're not trying to sell anybody anything. We thank you for that.

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Thank you - I look forward to any questions that might be there later in the presentation.