STUDY PROTOCOL

Effect of ultraviolet radiation on coronaviruses

UV has long been known to inactivate viruses while preserving their ability to be used as antigens in the preparation of vaccines. Viral genomes are susceptible to UV-induced damage, which can cause lethal breaks and mutations within viral genomes. Thus, viruses can be potentially killed by damage to their nucleic acids while, at the same time, leaving antigenic surface components (proteins, glycoproteins, and/or fatty acids) intact. Killed viruses can be recognized by the immune system generating humoral and cellular immunity against the virus, which can protect the host against future infections.

Severe acute respiratory syndrome (SARS) is a respiratory illness that has recently been reported in Asia, North America, and Europe. It has been assossiated with a new coronavirus, which resembles some sequence homology to bovine, mouse and avian coronaviruses. The new coronavirus (SARS-CoV) is a member of the Coronaviridae family of enveloped, plus-stranded RNA viruses, which have a broad host range. The current hypothesis is that SARS-CoV originated from certain animal species such as masked palm civets, raccoon dogs and ferret badgers. After transmission to humans, it seems that SARC-CoV accumulated certain mutations and deletions that allowed it to efficiently spread in humans and cause severe atypical pneumonia.

Certain coronavirus infections in humans and birds cause respiratory disease, while other coronavirus infections in rodents, cats, pigs, and cattle lead to enteric disease. The 27 kb - 32 kb genomes of coronaviruses, the largest known RNA viruses, encode multiple putative proteins, including 4 major structural proteins; nucleocapsid (N), spike (S), membrane (M), and small envelope (E).The spike protein, a glycoprotein projection on the viral surface, is critical for viral attachment and entry into the host cell. In addition, variations of S protein among strains of coronavirus are responsible for host range and tissue tropism.

Among all known coronaviruses, only respiratory bovine coronaviruses isolated by investigators at Louisiana State University (Drs. H. Storz and K. G. Kousoulas) are known to be associated with severe upper respiratory disease and pneumonia in cattle. LSU investigators have described two different epizootics of acute upper respiratory disease and pneumonia affecting more than 100 animals each in 1997 and 1998, respectively. They found that bovine coronaviruses were by far the most prevalent virus present in tracheal washes as well as in lung tissues of animals that died from pneumonia. More than 200 different strains were isolated from different animals as well as from animals at different points during disease progression. Thus, bovine coronaviruses that cause pneumonia in cattle may serve as a suitable virus model system for SARS-CoV.

Purpose

This study was conducted to determine the extent of inactivation by ultraviolet radiation of bovine corona virus lung isolate (BCV-LUN), which is known to be associated with lethal pneumonia in cattle, as a model for severe acute respiratory syndrome (SARS).

Design

The device utilized in this study is known as the Energex Hemo-Modulator, (Energex Systems, Inc. , Emerson, NJ.) a blood irradiator that produces UV energy ranging from 200nm – 400nm. Different amounts of viral loads in buffered growth medium were treated with ultraviolet radiation and the infectious titers were determined before and after irradiation to estimate the extent of inactivation.

Study protocol

1. BCoV-LUN at titers of 7 and 5 logs was spiked into 50 ml samples of buffered growth medium (DMEM+10 fetal calf serum) at a hundred-fold dilution to obtain a final titer of 5 and 3 logs.
2. The spiked samples were treated under standard UV radiation protocol and they were collected in 3 equal fractions after treatment.
3. BCoV titers were determined before treatment and after UV iradiation in each of the 3 fractions collected. As a control, BCoV titers were determined in sham-irradiated samples.
4. BCoV-LUN Infectious titers were assessed by plaque formation on human rectal tumor cell line 18G (HRT-18G) using 10-fold serial dilutions.
5. BCoV-LUN infectious titers were also assessed by indirect immunofluorescence assays using monoclonal antibodies against the spike glycoprotein.

Results

A

Control (5-logs) No treatment	Control (5-logs) Through machine, no UV	Control (5-logs) N1	Control (5-logs) N2	Control (5-logs) N3
311 PFU	296 PFU	27 PFU	17 PFU	14 PFU

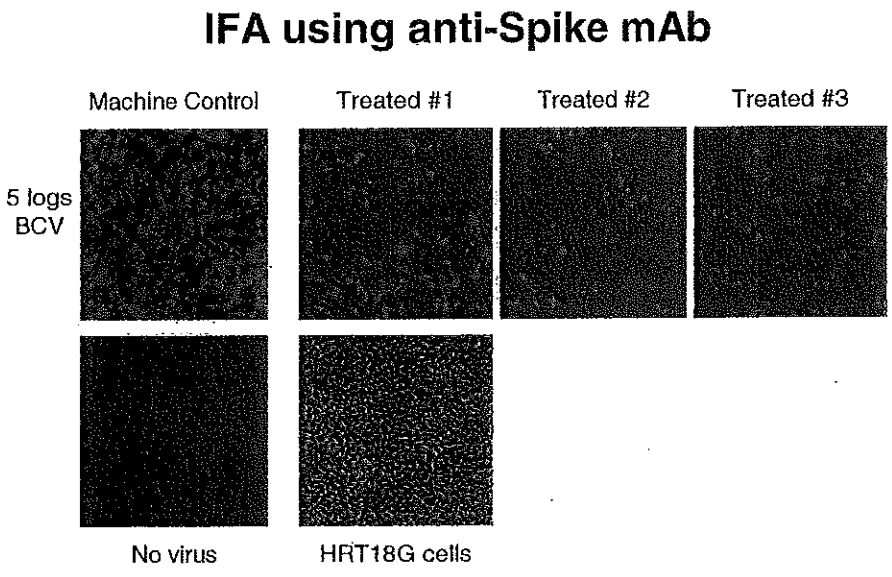
B

Control (3-logs) No treatment	Control (3-logs) Through machine, no UV	Control (3-logs) N1	Control (3-logs) N2	Control (3-logs) N3
14 PFU	9 PFU	0	0	1

Significant reduction of infectious BCV-LUN was observed after single pass UV irradiation in the hemo-modulator. PFU shown in table A were used to derive the average PFU reduction after UV irradiation. The average of N1, N2 and N3 values is 19 PFU (at the 10^{-2} dilution), which is approximately 15-times less than the average of 311 and 296 PFU obtained in the absence of UV irradiation. Titers at 3-logs were considered too low for determination of infectious virus reduction.

Secondary confirmation of the observed PFU reduction was provided by indirect immunofluorescence assays (IFA) using monoclonal antibody against the spike glycoprotein of BCoV-LUN. IFA results were in agreement with the 15-fold PFU reduction obtained by determining actual viral plaques in HRT-18G cells.

Figure 1: Reduction of BCV-LUN Spike Glycoprotein Expression after Single UV Irradiation.



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