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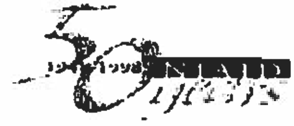

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☐ URGENT ☐ FOR REVIEW ☐ PLEASE COMMENT ☐ PLEASE REPLY ☐ PLEASE RECYCLE

NOTES/COMMENTS

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6534

G. Virucidal activity of ARB 03-~~5634~~

ASAP 10ppm 6534

Introduction

To determine if a colloidal silver product, ~~ARB 03-5634~~, could cause physical disruption of SARS coronavirus (SARSCoV), it was evaluated in a virucidal assay. The assay was done at room temperature to more accurately reflect the conditions under which the compound might be used (i.e., as a topical disinfectant or as a sanitizer/virucide).

Materials and Methods

Virus and Cells: SARSCoV was obtained from the Centers for Disease Control (CDC, Atlanta GA) and was grown in Vero 76 cells (American Type Culture Collection, Manassas, VA.). The cells were passaged in MEM containing 5% fetal bovine serum (Hyclone Laboratories, Logan, UT). When doing CPE inhibition assays, gentamicin was added to 50 μg/ml and serum was reduced to 2%.

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Compound: A colloidal silver product identified as AGX-32 (ARB no. 03-~~5634~~) was provided suspended in sterile water by Mr. Moeller of American Biotech Laboratories.

Virucidal Assay: To 90 μl of undiluted test mixture was added 10 μl of virus lysate that originally had a titer of $10^{6.5}$ $CCID_{50}$/0.1 ml. This mixture was incubated at room temperature (~25°C) for 1.0 h. Surviving virus was assayed in triplicate by cytopathic effect (CPE) assay in Vero 76 cells using a ten-fold dilution series. Virus was also incubated without test substance in water and in MEM supplemented with 2% FBS under the conditions described above and assayed in parallel by CPE assay. The latter treatments served as a virus controls. Water was used because the exact formulation of the test substance without the active ingredient could not be duplicated, but the solvent for the test substance was water.

Results and Discussion

Incubation in water resulted in less than a $\log_{10}$ drop in virus titer compared to virus in serum (Table 1). Treatment with compound resulted in a 1.17 $\log_{10}$ drop in titer. If this agent were to be considered for use as a disinfectant for eliminating fomites contaminated with SARSCoV, another study should be done to look at efficacy at time periods below 5 min. Good virucidal agents work within minutes. However, the data suggest that decreasing the time of exposure of SARSCoV to ARB 03-5634 might result no inactivation of virus at all.

Conclusions

The one-hour treatment with ARB 03-5634 resulted in marginal efficacy against the SARS virus when used in a virucidal assay at room temperature.

Table 1. Effects of ARB 03-5634 on reducing SARSCoV titers.

Treatment	Titer ($CCID_{50}$/0.1ml)	Log_{10} reduction
03-5634 6534	$10^{4.5}$	1.17
Virus in water	$10^{5.25}$	0.42
Virus in 2% FBS	$10^{5.67}$	0

Special Studies

B. Virucidal activity of ARB 03-6533

Introduction

To determine if a colloidal silver product, ARB 03-6533, could cause physical disruption of SARS coronavirus (SARSCoV), it was evaluated in a virucidal assay. This procedure was as recommended by Mr. William Moeller of American Biotech Laboratories (Provo, UT).

Materials and Methods

Virus and Cells: SARSCoV was obtained from the Centers for Disease Control (CDC, Atlanta GA) and was grown in Vero 76 cells (American Type Culture Collection, Manassas, VA.). The cells were passaged in MEM containing 5% fetal bovine serum (Hyclone Laboratories, Logan, UT). When doing CPE inhibition assays, gentamicin was added to 50 µg/ml and serum was reduced to 2%.

Compound: A colloidal silver product identified as AGX-32 (ARB no. 03-6533) was provided suspended in sterile water by Mr. Moeller of American Biotech Laboratories.

Virucidal Assay: Undiluted test mixture was added to an equal volume of virus lysate having a titer of $10^{5.5}$ $CCID_{50}$/ml and incubated at 37°C for 1.0 h. Surviving virus was assayed in triplicate by cytopathic effect (CPE) assay in Vero 76 cells using a ten-fold dilution series. Virus was also incubated without test substance in MEM supplemented with 2% FBS under the conditions described above and assayed in parallel by CPE assay. The latter treatment served as a virus control. MEM was used because the exact formulation of the test substance without the active ingredient could not be duplicated.

Compound Toxicity Determination: The test substance was also mixed with an equal volume of MEM with 2% serum without virus treated as described above. This was done to determine the toxicity levels of the residual test substance in the virus titration. The toxicity was assessed by microscopic examination of treated cells.

Results and Discussion

The toxicity (reduction in cell density by about 85%) of the compound was diluted out after the first 10-fold dilution. Thus, any cytopathic effects detected in subsequent dilutions could be probably attributed to virus only. The titer of the virus treated with test substance was $10^{3.5}$ CCID50/ml, while the titer of untreated virus was $10^{5.5}$ CCID50/ml. Hence, the test substance reduced the virus titer by two log_{10} after a 1 h incubation at 37° C. Nevertheless, there remained over three log_{10} of infectious virus, which demonstrates that the compound was not totally effective in reducing viral loads. It remains to be seen whether a two-log drop is sufficient to suggest that the SARS virus is decontaminated in a real clinical situation because no studies have been done to determine the levels of virus that may typically be on inanimate objects and how much virus is required to cause disease. It may be that the test substance will be totally useless as a virucide unless all virus is inactivated.

Additional factors to be considered are the time and temperature of incubation; for practical use, a shorter time period (e. g., 5 or 10 minutes) would be more useful than 1 h, and it

would be important to determine if an equal virucidal effect will occur when the product is incubated with the virus at room temperature rather than at 37° C.

In addition, the active ingredient (personal communication from the submitter), colloidal silver, is probably susceptible to binding by negative ions such as chloride. An object to be decontaminated that is heavily coated, soaked or exposed to salt solutions might not be decontaminated at all if the colloidal silver is bound up by negative ions. This might suggest that the product might not be clinically relevant in such situations.

Conclusions

In a single assay, a colloidal silver-containing test substance demonstrated virucidal activity against SARSCoV when incubated with the virus at 37° C for 1 h. Virus titers were reduced 100-fold, but over 1000 CCID50 units of infectious virus remained despite the 1-hour treatment. It would be important to know if a shorter period of incubation, and incubation at room temperature, would have similar effects. The significance of the present finding for the use of the agent in a real clinical situation remains unclear and will require further study.