VIRUCIDAL EFFECT OF A NEWLY DEVELOPED NICKEL ALLOY ON MOUSE CORONAVIRUS

Norio Hirano and Takenori Nakayama*

1. INTRODUCTION

A newly recognized disease, severe acute respiratory syndrome (SARS), was first reported in China in February 2003. A few months after the first outbreak of SARS, the disease was transmitted worldwide in more than 20 countries of Asia, Europe, and North America. A novel coronavirus (CoV) was detected in patients with SARS and identified as causative agent. Civet cats have been suspected as natural host of SARS CoV, which infects human beings by oral or intranasal route; the infected hosts sheds the virus into air through respiratory route and/or feces from intestinal tract. The routes of entry and shedding of SARS CoV is similar to those of mouse hepatitis virus (MHV; mouse CoV), which causes a variety of diseases such as diarrhea, hepatitis, encephalitis, and wasting syndrome of nude mice.

To control SARS CoV infection, several disinfectants and tools were examined. Recently, He *et al*.[1] reported an inactivation of SARS CoV by silver alloy. Sagripanti *et al*.[2] reported virucidal effect of copper and iron ions on herpes simplex virus and bacteriophages.

Recently, Yamada *et al*.[3] demonstrated that a newly developed nickel-alloy (Ni-alloy) metal showed bacteriocidal effect, suggesting that Ni-alloy might be a useful antibacterial agent. However, there was no attempt to confirm the virucidal properties of this alloy. We attempt to define the virucidal effect of Ni-alloy on MHV, as a model for controling SARS CoV infection. For this study, we selected MHV strain NuU[4] as model virus among MHV strains. The virus has low virulence in mice but is the most heat-stable strain among 9 MHV strains examined.[5]

2. MATERIALS AND METHODS

MHV-U strain was grown and assayed in DBT cells, as described previously. DBT cells were grown in Eagle's minimum essential medium (MEM) containing 10% newborn calf serum (NCS) and 10% tryptose phosphate broth at 37°C.

* N. Hirano Iwate University, Morioka 020-8550, Japan. T. Nakayama, Kobe Steel Ltd. Kobe, 651-2271, Japan.

After virus inoculation, the serum content was reduced to 5%. The culture fluid harvested from infected DBT cell cultures after incubation at 37°C for 24 hr was clarified by centrifugation and stored as virus material at – 70°C until use.

An infectivity assay of MHV was performed by inoculating DBT cells prepared in 24-well culture dishes in 5% CO_2 incubator at 37°C. The cultures were once washed with MEM and inoculated with 0.2 ml amounts of virus samples appropriately diluted with MEM. The inoculated cultures, after virus adsorption at 37°C for 60 minutes, were fed with maintenance medium.

The infectious titers were expressed in log $TCID_{50}$/0.2 ml by Reed and Muench method.

3. RESULTS

Virucidal effect at 37°C: To examine the virucidal effect of the metal, 5 ml of virus material was introduced onto a stainless steel dish coated (50 mm in diameter and 10 mm in depth) with or without Ni-alloy metal and was incubated in a 5% CO_2 incubator at 37°C for 12 and 24 hr. As a control, a plastic dish (55 mm in diameter) was filled with 5 ml of virus material as the same manner. After incubation for 12 and 24 hr, the sample from Ni-alloy coated dish showed a titer decrease of 2.8 and more than 5 log units, respectively. However, samples from the stainless steel or plastic dish showed a decrease of 0.8 and 3.2 log units or 0.8 and 2.8 log units.

Table 1. MHV-U in dishes incubated at 26°C for 0 to 72 hr.

Incubation (hr)	Infectivity titer of virus material from dish[1)]		
	Stainless steel	Ni-alloy-coated	Plastic
0	5.5[1]	5.5	5.5
12	4.7 (0.8)	3.7 (1.8)	5.2 (0.3)
24	4.2 (1.3)	2.7 (2.8)	4.7 (0.8)
48	3.7 (1.8)	<0.5 (>5.0)	4.5 (1.0)
72	2.7 (2.8)	<0.5 (>5.0)	3.7 (1.8)

[1] Infectivity (decreased) log $TCID_{50}$ /0.2 ml.

Virucidal effect at 26°C as room temperature: The same experiments were carried out at 26°C for 12, 24, 48, and 72 hr. After incubation for 48 hr, the samples from Ni-alloy coated dishes showed a decrease of more than 5 log units, as shown in Table 1. For the same time of incubation, samples from stainless steel and plastic dishes showed a small decrease of 1.8 and 1.0 log units, respectively. Even for 72 hr of incubation, the decrease of infectivity of samples from stainless and plastic dishes were 2.8 and 1.8 log units. A cytotoxic effect on DBT cells was only found in the nondiluted samples from Ni-alloy coated dish incubated for 24 or more hours.

Virucidal effect of Ni-alloy powder: To define the direct virucidal effect of Ni-alloy metal on MHV-U, 5 ml amount of the virus was mixed with 1 g of Ni-alloy powder in test tube and incubated at 37°C for 2 hr. The virus mixed with the powder at 37°C for 2 hr showed a remarkable decrease of 3 log units in titer. However, after incubation, the virus material without alloy powder showed no detectable decrease in titer. MEM incubated with powder for 2 hr did not show a cytotoxic effect on DBT cells. MEM incubated with Ni-alloy for 7 days did not show virucidal effect on MHV-U when mixed with virus material. However, such MEM showed a strong cytotoxic effect on DBT cells.

4. CONCLUSIONS

The present study demonstrated that newly developed Ni-alloy showed a virucidal effect on MHV-U at different temperatures. These findings suggest that Ni-alloy might be useful for inactivating SARS CoV He *et al*.[1] reported inactivation of SARS CoV by silver ions, and Sagripanti *et al.*[2] showed a virucidal effect of copper and iron ions on herpes simplex virus. In this study, MEM incubated with Ni-alloy powder for 7 days showed a cytotoxic effect on DBT cells but not a virucidal effect on MHV-U when MEM was mixed with virus. These findings suggest that Ni-alloy ions do not directly inactivate MHV-U. However, the mechanisms of virucidal effect of Ni-alloy are still unknown. Further studies of virucidal actions of Ni-alloy on other CoV and viruses are underway.

5. REFERENCES

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