Antiviral Chemistry & Chemotherapy 18:???–???

Effect of oral gavage treatment with ZnAL42 and other metallo-ion formulations on influenza A H5N1 and H1N1 virus infections in mice

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Avian influenza H5N1 infections can cause severe, lethal human infections. Whether influenza A virus treatments effectively ameliorate avian influenza H5N1 human infections is uncertain. The research objective was to evaluate the efficacy of novel zinc and other metallo-ion formulations in two influenza A mouse models. Mice infected with influenza A/Duck/MN/1525/81 (H5N1) virus were treated orally 48 h before virus exposure and then twice daily for 13 days with ZnAL42. The optimal dosing regimen for ZnAL42 was achieved at 17.28 mg/kg 48 h prior to virus exposure, twice daily for 7 days. The survival rate was 80% compared with 10% in the untreated control group and a 100% survival rate with ribavirin (75 mg/kg/day, twice a day for 5 days, beginning 4 h before virus exposure). ZnAL42 treatment significantly lessened the decline in arterial oxygen saturation (SaO_2; $P<0.001$). This regimen was also well tolerated by the mice. Manganese and selenium formulations were not inhibitory to virus replication when given therapeutically. Mice were also infected with influenza A/NWS/33 (H1N1) virus and were treated 48 h before virus exposure with three dosages of ZnAL42 (8.64, 1.46 or 0.24 mg/kg/day). Treatment was by oral gavage twice daily for 13 days. The highest dose of ZnAL42 was significantly inhibitory to the virus infection as seen by prevention of deaths and lessening of decline in SaO_2. The data suggest that the prophylactic use of ZnAL42 is effective against avian influenza H5N1 or H1N1 virus infection in mice and should be further explored as an option for treating human influenza virus infections.

Keywords: animal model, avian influenza, H5N1, influenza A, zinc

Introduction

Numerous reports have been published regarding the potential of zinc and zinc salts to inhibit human or experimental viral infections. Viral infections inhibited have included the common cold (Hulisz, 2004), respiratory syncytial virus infections (Suara & Crowe, 2004), cytomegalovirus infections (Li *et al*., 2005) and herpes labialis (Femiano *et al*., 2005). There have been few reports, however, regarding the effect of these materials on influenza virus infections, although it has been shown that the combination of zinc-containing superoxide dismutase and rimantadine hydrochloride appears to significantly protect mice from a challenge with influenza A (H3N2) virus infection (Serkedjieva *et al*., 2003). The full antiviral mechanism of zinc or its salts has not been fully defined, although it has been reported that zinc salts potentiate the antiviral action of the natural cytokine, human interferon (IFN)-α, by 10-fold; no effect was seen on the antiviral action of IFN-β or IFN-γ (Berg *et al*., 2001).

This report describes the results of both toxicity determinations and antiviral experiments designed to demonstrate and optimize the efficacy of selected zinc formulations as well as other metal formulations against moderate and severe infections of avian influenza A (H5N1) infections in mice.

Materials and methods

Animals

Female specific pathogen-free 18–21 g BALB/c mice were obtained from Charles River Laboratories (Wilmington, MA, USA). They were quarantined 5 days prior to use and housed in polycarbonate cages with stainless steel tops. Tap water and standard rodent mouse chow were provided *ad libitum*. The mouse chow contained an undisclosed concentration of zinc oxide.

Virus

Influenza A/Duck/MN/1525/81 (H5N1) was obtained initially from Dr Robert Webster (St Jude Hospital, Memphis, TN, USA). It was passaged through mice until adapted to the point of being capable of inducing pneumonia-associated death in the animals. Influenza A/NWS/33 (H1N1) virus was originally provided by Dr Kenneth Cochran (University of Michigan, Michigan, MI, USA). Pools of each virus were subsequently prepared for animal studies and maintained at -80°C.

Compounds

The novel metallo-ion formulations ZnAL42, ZnPC33, MnAL42 and SePC33 were provided by Remedy Research Ltd (London, UK). Each sample was maintained at room temperature. Solutions for animal experiments were prepared in either sterile distilled water or saline at the appropriate concentrations. Oseltamivir (TamiFlu™) was purchased from a local pharmacy and dissolved in sterile saline for use in this study.

Arterial oxygen saturation determinations

Arterial oxygen saturation (SaO_2) was determined using the Ohmeda Biox 3800 pulse oximeter (Ohmeda, Louisville, OH, USA). The ear probe attachment was used with the probe placed on the thigh of the animal. Readings were taken after a 30 s stabilization time on each animal. Use of an earlier Ohmeda model (3740) for measuring effects of influenza virus on SaO_2 in mice has been previously described (Sidwell *et al.*, 1992).

Experimental design

Preliminary toxicity determination. Groups of three mice were treated via oral (p.o.) gavage with each compound to be tested, either alone or in combination using various dosing regimens. Each animal was weighed prior to initial treatment and again 18 h after final treatment and then observed for death or other signs of toxicity for 16 days. After determining the maximum tolerated dose, similar dosage regimens using the maximum tolerated dose, as well as more dilute concentrations, were evaluated in the various prophylactic and therapeutic experiments described below.

Prophylactic and therapeutic experiments. Mice were infected with 60% lethal dose or a 100% lethal dose (LD_{100}) of the H5N1 strain of influenza virus or with an LD_{100} of the H1N1 strain. Infection was achieved by anesthetizing the mice with an intraperitoneal injection of ketamine (100 g/kg) and instilling 90 l of virus solution in the nares. Groups of 10 were each treated p.o. with ZnAL42, ZnPC33, MnAL42 or SePC33 at various dosages ranging from 0.24 mg/kg/day to 17.28 mg/kg/day, depending on the experiment. Animals were treated with compounds either once, twice, three or four times a day. See individual tables for dosage schedules used. As a positive control, oseltamivir or ribavirin was administered p.o.: oseltamivir twice daily for 5 days beginning 4 h post-virus exposure at a dose of 20 mg/kg/day and ribavirin at 75 mg/kg/day with a 4 h pretreatment and subsequently twice daily for 5 days. The animals were observed daily for death for 21 days and SaO_2 levels were measured daily from days 3 to 11, the time when SaO_2 decline normally occurs in influenza virus-infected mice. Three uninfected mice were treated in parallel with each dose of compound and served as toxicity controls. These animals were observed for death and other signs of toxicity for 21 days, and weighed prior to first treatment and again 18 h after the final treatment of zinc. Three normal control mice were held in parallel; these mice were weighed as above and SaO_2 levels determined to provide normal background data.

Statistical analysis

Increases in total survivors were evaluated by χ^2 analysis with Yates' correction for small sample size. Increases in mean day to death and differences in mean SaO_2 values were analysed by *t*-test.

Results

The results from the initial prophylaxis experiment are shown in Table 1. Treatment with ZnAL42, beginning 48 h prior to virus exposure and subsequently twice daily for 13 days, appeared to significantly inhibit the virus infection at the highest dosage used (8.64 mg/kg/day), with 80% of the animals surviving ($P<0.05$); 12 of the 20 placebo control mice died of the infection, the mean day to death being 9.6 ±3.2 days for this group. The SaO_2 levels in the animals treated with high-dose ZnAL42 were significantly higher ($P<0.05$) than in the infected placebo controls, although the mean day to death for both groups was similar. The lower dosages of ZnAL42 did not appear to have a significant effect on the infection. Oseltamivir, the positive control drug used for the study, prevented all the mice from lethal infection, with SaO_2 values being maintained at relatively high levels throughout the experiment. Therapy with ZnPC33 was not considered efficacious, with no significant increase in survivor numbers and in SaO_2 values. Therefore, all further studies focused on the ZnAL42 formulation.

The toxicity control animals receiving all dosages of all materials evaluated survived, although the animals generally failed to gain weight at the same rate as the normal controls, including the saline-treated animals. This may have been a manifestation of the trauma of oral gavage given twice daily through this experiment, or it may reflect

Table 1. Effect of oral treatment* with ZnAL42 and ZnPC33 on an Influenza A (H5N1) virus infection in mice

		Toxicity controls		Infected, treated mice		
Compound	Dosage, mg/kg/day	Survivors/ Total	Mean host weight change†, g	Survivors/ Total	Mean day to death‡ ±SD	Mean day 11 SaO_2, % ±SD
ZnAL42	8.64	3/3	0.2	8/10§	8.0 ±0.0	83.7 ±4.9§
	1.46	3/3	-0.5	1/10	7.8 ±1.0	76.2
	0.24	3/3	0.0	5/10	7.8 ±0.8	81.3 ±6.8
ZnPC33	8.64	3/3	0.9	3/10	8.0 ±1.3	77.8 ±4.5
	1.46	3/3	0.6	3/10	7.6 ±1.0	78.8 ±6.3
	0.24	3/3	1.0	2/10	8.4 ±1.4	77.5
Oseltamivir	20	3/3	0.6	10/10¶	>16.0 ±0.0#	87.0 ±7.3#
Saline	–	3/3	0.7	8/20	9.6 ±3.2	78.8 ±4.6
Normal controls	–	3/3	1.6	–	>16.0 ±0.0#	90.0 ±2.6

*Treatment schedule: twice daily for 13 days beginning 48 h pre-virus exposure (oseltamivir: twice daily for 5 days beginning 4 h post-virus exposure). †Difference between initial weight and weight 18 h after final treatment. ‡Mean day to death of mice dying prior to day 16. §$P<0.05$; ¶$P<0.01$; #$P<0.001$ compared with saline-treated controls.

a slight lack of tolerance of the animals to the various zinc salts used. It should be noted that oseltamivir-treated animals also gained less weight than the normal controls in this experiment (Table 1).

Another study was carried out to determine if a shorter treatment regimen would be efficacious and ameliorate the potential problems of weight loss due to extended oral gavage treatment (Table 2). ZnAL42 treatment of infected animals, using ZnAL42 at 17.28 mg/kg/day twice daily beginning 48 h prior to virus infection and subsequently for only 7 days after virus exposure, protected 80% of the animals from death compared with the 2/20 animals that survived in the placebo control group ($P<0.001$; Table 2). There was also significant survival in animals treated with the next lowest dose of ZnAL42 (8.64 mg/kg/day) using the same dosing schedule as just described ($P<0.05$). Reducing the frequency and duration of dosing had an inconsistent effect on weight gain. For example, weight gain at the two higher doses of ZnAL42 was not as reduced using the shorter dosage regimen compared with using the longer dosing regimen in the first experiment (See Table 1) or a

Table 2. Effect of the frequency of oral treatment* with ZnAL42 on Influenza A (H5N1) virus infection in mice

			Toxicity controls		Infected, treated mice		
Compound	Dosage, mg/kg/day	Treatment schedule	Survivors/ Total	Mean host weight change†, g	Survivors/ Total	Mean day to death‡ ±SD	Mean day 11 SaO_2, % ±SD
ZnAL42	17.28	qd ×7 beg –48 h	3/3	-0.7	1/10	9.2 ±3.0	75.3 ±0.7
	8.64	qd ×7 beg –48 h	3/3	1.1	3/10	8.4 ±1.1	76.7 ±2.8§
	17.28	bid ×7 beg –48 h	3/3	2.7	8/10#	7.0 ±1.4	81.0 ±3.5#
	8.64	bid ×7 beg –48 h	3/3	0.3	5/10§	10.8 ±3.5¶	78.7 ±2.6¶
	17.28	tid ×7 beg –48 h	3/3	0.5	4/10	9.5 ±1.6¶	76.6 ±2.0§
	8.64	tid ×7 beg –48 h	3/3	1.9	3/10	8.6 ±2.2	76.2 ±1.8
	17.28	qid ×7 beg –48 h	3/3	-0.9	2/10	9.4 ±2.5§	76.0 ±2.0
	8.64	qid ×7 beg –48 h	3/3	-0.5	2/10	7.9 ±1.1	75.4 ±0.8
Ribavirin	75	bid ×5 beg –4 h	3/3	-0.8	10/10#	>21.0 ±0.0#	76.0 ±2.0
Saline	–	qid ×7 beg –48 h	–	–	2/20	9.2 ±3.0	75.4 ±0.8
Normal controls	–	–	5/5	1.9	–	–	86.0 ±1.2#

*Treatment schedule: beginning 48 h pre-virus exposure; once daily (qd), twice daily (bid), three times daily (tid) and four times daily (qid). †Difference between initial weight and weight 18 h after final treatment. ‡Mean day to death of mice dying prior to day 21. Experiment duration was 21 days. §$P<0.05$; ¶$P<0.01$; #$P<0.001$ compared with saline-treated controls.

more frequent dosing regimen (Table 2). However, when administered once, three or four times per day, the material was not significantly effective at all doses tested. Administration of the compound four times a day also caused weight loss, reinforcing the concept that the trauma resulting from repeated oral gavage caused animals not to gain weight significantly compared with the normal controls. Ribavirin, a known inhibitor of influenza virus infections in mice, administered as indicated in Table 2, was quite effective in protecting animals against death, with all animals surviving. However, the SaO_2 levels in the ribavirin-treated mice were comparable with those in the saline-treated, infected animals.

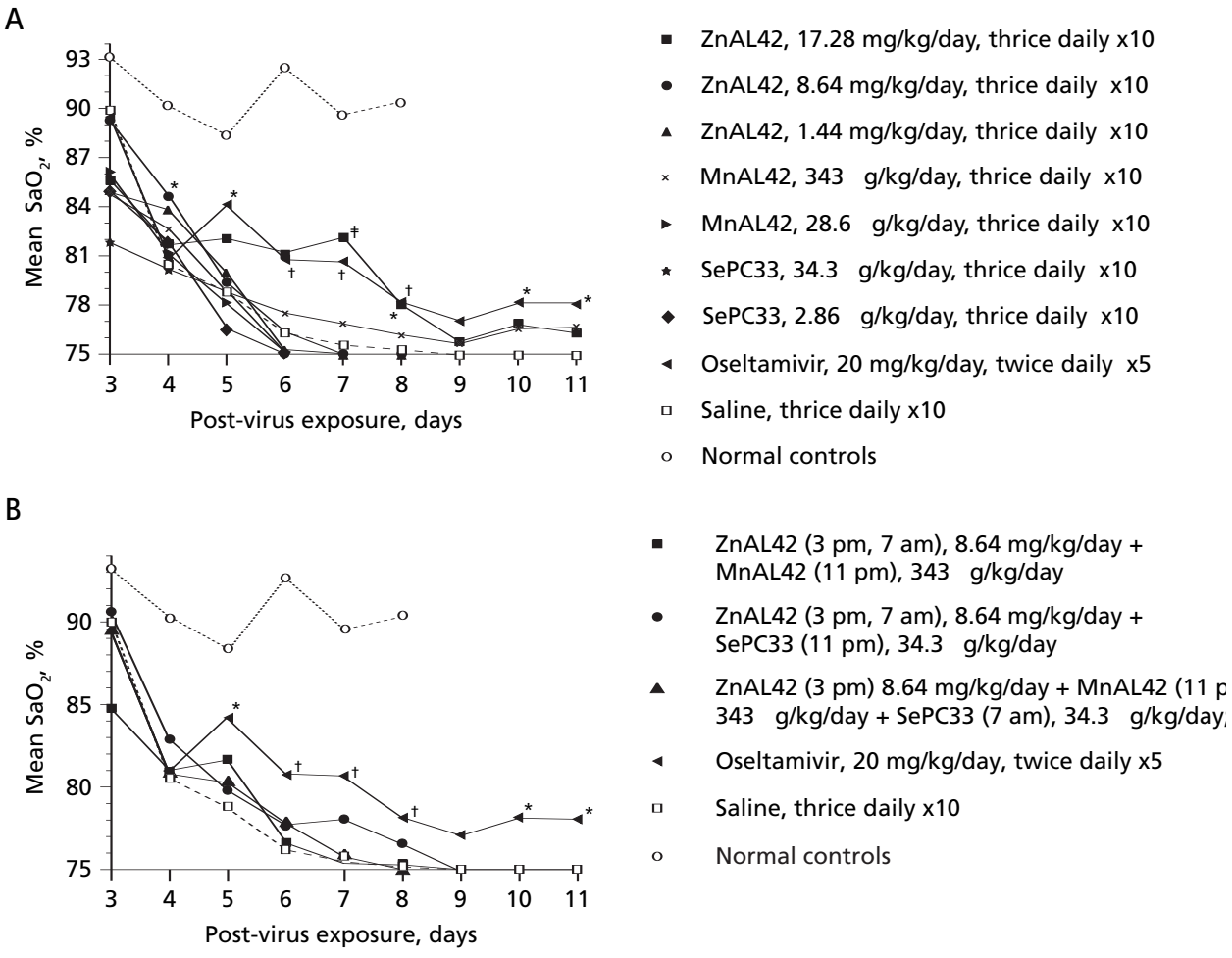
Since ZnAL42 showed some prophylactic efficacy, the compound was also evaluated in a therapeutic experimental regimen. Other metals such as selenium (Deidda *et al.*, 1997; Liu *et al.*, 2004; Wojtowicz *et al.*, 2004) and manganese (Sidwell *et al.*, 1996; Liu *et al.*, 2004) in various formulations have been reported to have antiviral activity. Therefore, these two metals, similarly formulated to the ZnAL42, were also included in the therapeutic experiment. In addition, various combinations of the metallo-ion formulations were also tested for inhibition of virus infection in mice. For this efficacy study (Table 3), an LD_{100} dose of virus was used. Groups of 10 were each treated p.o. with ZnAL42 at dosages of 17.28, 8.64 or 1.44 mg/kg/day, with MnAL42 at dosages of 343 or 28.6 g/kg/day or with SePC33 at dosages of 34.3 or 2.986 g/kg/day every 8 h for 10 days, beginning 24 h after virus exposure. In addition to the above, the following combinations were also studied: ZnAL42 (8.64 mg/kg/day administered in a divided dose at 7 am and 3 pm) + MnAL42 (343 g/kg given at 11 pm); ZnAL42 (8.64 mg/kg administered in a divided dose at 7 am and 3 pm) + SePC33 (34.3 g/kg given at 11 pm); ZnAL42 (8.64 mg/kg given at 3 pm) + MnAL42 (343 g/kg given at 11 pm) + SePC33 (34.3 g/kg given at 7 am). In this experiment, 20/20 of the placebo control mice died of the infection, the mean day to death being 6.2 ±2.3 days (Table 3). The animals were also observed daily for death for 21 days, and SaO_2 levels measured daily from days 3 to 11, the time period when SaO_2 decline normally occurs in influenza virus-infected mice (Figure 1A,B). Oseltamivir, the positive control drug used for the study, prevented death in only 2/10 of the infected mice, although the mean day to death of the treated animals that died was 8.4 ±2.0 days, a highly significant delay in death. This more potent virus challenge may have been somewhat overwhelming for the oseltamivir dosing regimen used. However, the SaO_2 values in this treatment group were maintained at signifi-

Table 3. Effect of oral therapeutic treatment* with ZnAL42, MnAL42, SePC33 and the combination of ZnAL42 + MnAL42, ZnAL42 + SePC33, and ZnAL42 + MnAL42 + SePC33 on a lethal Influenza A (H5N1) virus infection in mice

		Toxicity controls		Infected, treated mice		
Compound	Dosage, /kg/day	Survivors/ Total	Mean host weight change†, g	Survivors/ Total	Mean day to death‡ ±SD	Mean day 11 SaO_2, % ±SD
ZnAL42	17.28 mg	3/3	-1.3	1/10	8.1 ±1.2#	76.3 ±4.1
	8.64 mg	3/3	-1.1	0/10	5.9 ±0.7	75.0 ±0.0
	1.44 mg	3/3	0.1	0/10	6.1 ±1.0	75.0 ±0.0
MnAL42	343 g	3/3	-0.1	1/10	6.4 ±1.9	76.7 ±4.2
	28.6 g	3/3	-0.3	0/10	5.6 ±0.8	75.0 ±0.0
SePC33	34.3 g	3/3	-0.1	0/10	5.7 ±0.7	75.0 ±0.0
	2.86 g	3/3	-0.2	0/10	5.5 ±0.5	75.0 ±0.0
ZnAL42 (3 pm, 7 am) + MnAL42 (11 pm)	8.64 mg 343 g	3/3	-1.0	0/10	7.0 ±1.2	75.0 ±0.0
ZnAL42 (3 pm, 7 am) + SePC33 (11 pm)	8.64 mg 34.3 g	3/3	-0.9	0/10	6.6 ±1.7	75.0 ±0.0
ZnAL42 (3 pm) + MnAL42 (11 pm) + SePC33 (7 am)	8.64 mg 343 g 34.3 g	3/3	-1.0	0/10	6.3 ±1.3	75.0 ±0.0
Oseltamivir	20 mg	3/3	0.3	2/10	8.4 ±2.0¶	78.1 ±5.8§
Saline	–	–	–	0/20	6.2 ±2.3	75.0 ±0.0
Normal controls	–	5/5	0.8	–	–	90.4 ±2.3

*Treatment schedule for metal compounds: three times daily in groups of 10 beginning 24 h post-virus exposure; oseltamivir: twice daily in groups of 5 beginning 24 h post-virus exposure. †Difference between initial weight and weight 18 h after final treatment. ‡Mean day to death of mice dying prior to day 21. §$P<0.05$; ¶$P<0.01$; #$P<0.001$ compared with saline-treated controls.

Figure 1. Effect of oral therapeutic treatments on SaO_2 decline in influenza A (H5N1) virus-infected mice



(A) Effect of p.o. therapeutic treatment with ZnAL42, MnAL42, SePC33 and oseltamivir on arterial oxygen saturation (SaO_2) decline in influenza A (H5N1) virus-infected mice. **P*<0.05, †*P*<0.01, ‡*P*<0.001. **(B)** Effect of p.o. therapeutic treatment with ZnAL42 + MnAL42, ZnAL42 + SePC33, ZnAL42 + MnAL42 + SePC33, and oseltamivir on SaO_2 decline in influenza A (H5N1) virus-infected mice. **P*<0.05, †*P*<0.01, ‡*P*<0.001. bid, twice daily; p.o., orally; tid, three times daily.

cantly higher levels throughout the experiment than seen in the placebo controls (Figure 1A,B).

Treatment with the highest dose (17.28 mg/kg/day) of ZnAL42 used alone prevented death in only one of the 10 infected, treated mice, although the mean day to death was prolonged to 8.1 ±1.2 days, which was highly significant (*P*<0.001). In addition, the SaO_2 decline was significantly lessened (Figure 1A). The lower dosages of this compound were not significantly inhibitory to the infection.

The other metal formulations used did not protect against death when used alone or in combination. MnAL42 used at 343 g/kg/day prevented death of only one of 10 infected mice, and did not significantly prolong the mean day to death nor lessen SaO_2 decline (Figure 1A,B). Treatment with SePC33 was also not considered inhibitory to the influenza infection in this experiment at either dosage used. The combinations of the various products used were also uniformly not significantly inhibitory to this rather severe virus infection.

No deaths were observed in any of the toxicity control mice treated in this study, although mild weight loss of up to 1.3 g was observed in most of the treated groups. Only at the lowest dose of ZnAL42 was weight gain seen, as well as in the oseltamivir-treated mice and in the normal controls.

To determine if zinc metallo-ions inhibited other influenza A virus strains, mice were infected with influenza A/NWS/33 (H1N1) virus and treated with ZnAL42. Mice

Table 4. Effect of prophylactic oral gavage treatment with ZnAL42 on an Influenza A (H1N1) virus infection in mice

		Toxicity controls		Infected, treated mice		
Compound	Dosage, mg/kg/day	Survivors/ Total	Mean host weight change*, g	Survivors/ Total	Mean day to death† ±SD	Mean day 11 SaO_2, % ±SD
ZnAL42	8.64	3/3	0.8	3/10‡	11.0 ±0.8‡	80.4 ±5.1¶
	1.46	3/3	1.1	0/10	10.2 ±1.3	76.1 ±2.0
	0.24	3/3	1.0	4/10§	9.3 ±1.5	76.7 ±2.2
Oseltamivir	20	3/3	0.6	10/10¶	>21.0 ±0.0¶	86.2 ±3.3¶
Saline	–	–	–	0/20	10.0 ±1.7	75.5 ±1.0
Normal controls	–	5/5	1.6	–	–	85.6 ±3.2

*Difference between initial weight and weight 18 h after final treatment. †Mean day to death of mice dying prior to day 21. ‡$P<0.05$; §$P<0.01$; ¶$P<0.001$ compared with saline-treated control.

infected with this strain of virus were treated beginning 48 h before virus exposure with three dosages of ZnAL42, the dosages being 8.64, 1.46 or 0.24 mg/kg/day. Treatment was by oral gavage twice daily for 13 days. In this experiment, all of the placebo control mice died of the infection, the mean day to death being 10.0 ±1.7 days. The highest and lowest doses of ZnAL42 were significantly inhibitory to the virus infection as seen by prevention of deaths and lessening of decline in SaO_2 (Table 4). Oseltamivir, run in parallel as a positive control, totally prevented deaths of the mice and maintained SaO_2 levels at significantly higher levels than seen in placebo-treated animals. Treatment with this drug was twice daily for 5 days beginning 4 h post-virus exposure at a dose of 20 mg/kg/day. All the compounds used in the study were well tolerated by the toxicity control mice.

Discussion

Mice infected with influenza A/Duck/MN/1525/81 (H5N1) virus were treated beginning 48 h before virus exposure with three dosages of ZnAL42 or ZnPC33 by oral gavage twice daily for 13 days. The highest dose of ZnAL42 was significantly inhibitory to the virus infection as seen by prevention of deaths and lessening of decline in SaO_2. All doses tested appeared to be well tolerated, as was the higher dose of 8.64 mg/kg/day used in the prophylactic study. A similar experiment was carried out in which the frequency of the dosing was varied and the duration of treatment was shortened because of concerns that the route of administration may have been responsible for the lack of weight gain. Oral gavage has the potential to cause physical trauma to the mouth and oesophagus of the animal because of the specialized needle used to deliver the drug to the oesophagus in a multiple dosing regimen. In addition, the metal compounds could have also reduced appetite due to the taste of zinc. In this experiment, the ZnAL2 was again shown to be effective in reducing mortality and extending mean day to death compared with the placebo control animals, although only when administered twice a day for 7 days. This probably achieves the equivalent efficacious dosage within the blood and tissues of the body that a lesser dose given more frequently and for a longer period of time may achieve. Interestingly, the negative effects on weight gain that occurred in the experiments with more frequent and prolonged dosing seemed to be ameliorated using a higher dose, but implementing a less frequent and less prolonged dosage regimen.

It may be significant that effective therapy with ZnAL42 was achieved when treatment started 2 days before virus exposure. This suggests that pretreatment may have sufficiently prevented the establishment of a virus infection allowing the immune systems of the mice to successfully prevent a lethal infection. Thus, the ZnAL42 formulation may have not directly inhibited virus replication. This is supported by the finding that mice infected with virus and treated beginning 24 h after virus exposure with any dosage of ZnAL42, or in combinations with MnAL42 or SePC33, by oral gavage were not significantly protected against lethal virus infection nor were they protected against the negative impact of the infection on lung function as measured by SaO_2 measurements.

The results demonstrated that treatment with ZnAL42 significantly inhibited an infection in mice induced by influenza A (H5N1) virus, but treatments with other similar metal formulations were not effective. To our knowledge, this is the first report of zinc metallo-ions having a positive effect when used alone in an influenza virus infection.

The data also suggest that treatment with ZnAL42 was significantly inhibitory to an infection in mice induced by influenza A (H1N1) virus at both high and low doses. However, the lower dosages did not appear to have a significant effect on the decline of SaO_2 levels. This latter obser-

vation, coupled with the protection of the mice at the lowest dose but not at the middle dose of ZnAL42 used, is puzzling. However, the differences seen could simply be due to experimental variability.

The mechanism whereby ZnAL42 inhibited the influenza virus infection of mice in these studies may simply be one of coating the virion with zinc to prevent attachment and penetration, as has been shown with herpes simplex virus (Kumel *et al*., 1991) or to reduce virus neuraminidase activity, as been shown in kinetic studies with purified influenza neuraminidase (Johansson & Brett, 2003).

It has been reported that the combination of zinc-containing superoxide dismutase and a normally ineffective dose of rimantadine was significantly protective to mice infected with an influenza A (H3N2) virus (Serkedjieva *et al*., 2003). Evidence is increasing that the oxidoreductive (redox) balance of cells is involved in viral infections, particularly influenza virus infections; it has been proposed that reactive oxygen species are produced in the lung as a result of an influenza virus infection, and these oxygen free radicals increase local inflammation and thus contribute to pulmonary tissue damage (Blake *et al*., 1983; Tate & Repine, 1983). In addition, in cystic fibrosis (CSF) patients with acute influenza-induced encephalopathy, nitric oxide levels have been shown to be high, an indication of oxidative stress, and concomitantly, serum zinc levels were greatly reduced compared with CSF patients without influenza-induced encephalopathy (Kawashima *et al*., 2005). It has also been shown that superoxide free radical generation by alveolar phagocytic cells was significantly increased after influenza virus infection and remained at high levels throughout the infection (Oda *et al*., 1989). In addition, it has been shown that infection with a number of viruses can result in a depletion of the antioxidant glutathione in host cells (Palamara *et al*., 2005). Based on such observations, a number of antioxidants have thus demonstrated efficacy against experimentally induced influenza virus infections. In keeping with the above observation, we have previously demonstrated that manganese superoxide dismutase was significantly inhibitory to such infections (Sidwell *et al*., 1996). Therefore, it may be possible that the ZnAL42 formulation used in this study, when administered prior to infection, could have caused the systemic zinc balance to be maintained despite the perturbation of the redox balance induced by the influenza virus infection (Blake *et al*., 1983; Tate & Repine, 1983), leading to a protection of lung cells from the destructive oxidative effects induced by influenza virus infection.

In conclusion, the data suggest that the prophylactic use of ZnAL42 is effective against avian influenza H5N1 virus infection in mice and should be further explored as an option for treating human influenza virus infections.

Acknowledgements

This work was supported, in part, by contract NO1-AI-15345 from the Virology Branch, NIAID, National Institutes of Health, Bethesda, MD, USA.

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Received 7 December 2006, accepted 21 February 2007