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Antiviral activity of arbidol against influenza A virus, respiratory syncytial virus, rhinovirus, coxsackie virus and adenovirus *in vitro* and *in vivo*

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Summary

Arbidol, ethyl-6-bromo-4-[(dimethylamino)-methyl]-5-hydroxy-1-methyl-2-[(phenylthio)methyl]-indole-3-carboxylate hydrochloride monohydrate, is an antiviral chemical agent. In this report, we studied the antiviral activity of arbidol against a panel of human respiratory viruses, namely influenza A virus (FLU-A, A/PR/8/34 H1N1), respiratory syncytial virus (RSV), human rhinovirus type 14 (HRV 14), coxsackie virus B3 (CVB3) and adenovirus type 7 (AdV-7) *in vitro* in cell culture. Arbidol was found to present potent inhibitory activity against enveloped and non-enveloped RNA viruses, including FLU-A, RSV, HRV 14 and CVB3 when added before, during, or after viral infection, with 50% inhibitory concentration (IC_{50}) ranging from 2.7 to 13.8 μg/ml. However, arbidol showed selective antiviral activity against AdV-7, a DNA virus, only when added after infection (therapeutic index (TI) = 5.5). Orally administered arbidol at 50 or 100 mg/kg/day beginning 24 h pre-virus exposure for 6 days significantly reduced mean pulmonary virus yields and the rate of mortality in mice infected with FLU-A (A/PR/8/34 H1N1). Our results suggest that arbidol has the ability to elicit protective broad-spectrum antiviral activity against a number of human pathogenic respiratory viruses.

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Introduction

Viral respiratory infections are the most common illnesses experienced by people of all ages. They are also one of the major causes of morbidity and mortality in elderly people and young children throughout the world [19, 24, 29]. Of approximately 200 viral respiratory pathogens, the most important are influenza and respiratory syncytial viruses (RSV). Other important human respiratory viruses include rhinoviruses, parainfluenza viruses, coxsackie viruses, and adenoviruses [3]. Influenza A virus (FLU-A) is an enveloped single negative-strand RNA virus, which is thought to be the cause of upwards of 500,000 deaths globally each year [31]. RSV is the most prevalent infectious agent of acute lower respiratory illness from infants to elderly people [7, 30, 29]. Human rhinovirus (HRV), a non-enveloped single positive-strand RNA virus, is implicated in 50–80% of upper respiratory tract infections and has also been associated with lower respiratory tract disease in high-risk populations, such as patients with asthma or other airway in-

flammation [9, 25]. Coxsackie B viruses are the etiological agents of a wide spectrum of human diseases, including respiratory infection, aseptic meningitis, and fatal myocarditis. Outbreaks of coxsackie B virus infection occur annually throughout the world [26]. Adenovirus, a double-stranded DNA virus lacking an outer membrane, can cause numerous diseases such as respiratory infections, cryptic enteric infection and gastroenteritis [34]. Evidence derived from numerous studies supports a crucial role for respiratory viruses in acute otitis media (AOM) and acute exacerbation of asthma, which are also serious health care problems for children [33]. Several studies have indicated that RSV may be the principal virus leading to the development of AOM, followed by FLU-A and adenovirus [22].

Serious efforts have been put into finding an effective treatment or prevention of respiratory virus infections. However, there are no vaccines available for preventing RSV at this time [29], and the production of a vaccine to prevent HRV infection has not been possible because there are over 100 immunologically non-cross-reactive HRV serotypes [8]. Influenza vaccines are available but induce immune responses of limited duration, limited cross-strain protection, and poor efficacy in frail older adults. Control of these viruses infection remains a public health concern, and treatment by antiviral chemotherapy continues to show promise.

To date, the M_2 ion channel inhibitors, amantadine and rimantadine, have been widely used in prophylaxis of influenza virus infections. However, they inhibit only type A viruses, and their utilization in clinic is further limited by the rapid emergence of resistant virus mutants [12]. Two new neuraminidase inhibitors, zanamivir and oseltamivir, are effective in both prophylaxis and treatment of influenza A and B viruses [11, 13]. The need for an inhaler device and the risk of bronchospasms limits the use of zanamivir. Oseltamivir is being used although the gastrointestinal effects and emergence of resistant variants in some treated populations has limited the use of this drug [14]. Ribavirin is the only antiviral drug approved by the FDA for the treatment of RSV infection, but it is only recommended for use as a small-particle aerosol by RSV-infected children who are at high risk of having serious sequelae. The utilization of ribavirin is limited due to its controversial efficacy and toxicity [32, 15]. Thus, the search for antiviral substances that may elicit broad-spectrum protective efficacy to a panel of respiratory virus pathogens must continue.

Arbidol, an anti-influenza therapeutic, was first developed in the Russian Research Chemical-Pharmaceutical Institute. The chemical name of arbidol is ethyl-6-bromo-4-[(dimethylamino)-methyl]-5-hydroxy-1-methyl-2-[(phenylthio)methyl]-indole-3-carboxylate hydrochloride monohydrate. Leneva et al. studied arbidol's effect against influenza virus and found that it showed a pronounced inhibitory effect on influenza virus replication [16]. Fedyakina et al. reported that arbidol exerted a selective inhibiting effect on the replication of highly pathogenic influenza A/H5N1 viruses *in vitro* [6]. Antiviral effects of arbidol have also been reported for hepatitis C virus and hepatitis B virus [2, 5]. With a view to evaluate the antiviral activity of arbidol, we investigate in this report arbidol's effects against a number of human pathogenic respiratory viruses in tissue culture cells and in BALB/c mice.

Materials and methods

Chemicals and reagents

Arbidol was synthesized at Qianjiang Pharmaceutical Co. LTD, Hubei, China. Ribavirin, purchased from Qianjiang Pharmaceutical Co. LTD, was used as positive control compound in antiviral assays. Arbidol was initially dissolved in dimethyl sulfoxide (DMSO) and was further diluted with complete test medium. The final maximum DMSO concentration was 0.05%, which showed no effect on cellular viability or virus replication (data not shown). Therefore, 0.05% DMSO was also added to all no-drug control samples. The efficacy of these preparations did not appear to change upon freezing and short-term storage (1 month at 4 °C).

Cell cultures and viruses

MDCK (Madin-Darby canine kidney) cells were purchased from CDC of Wuhan City, Hubei, China. HEp-2 (human laryngeal carcinoma) cells and HEL (human embryonic lung) cells were maintained in our laboratory. All cell lines were routinely grown in Dulbecco's modified Eagle's medium (DMEM; HyClone) supplemented with 10% heat-inactivated fetal calf serum, 0.1% L-glutamine, 100 U/ml penicillin and 0.1 mg/ml streptomycin. The test medium

used for the cytotoxic assay as well as for antiviral assays contained 2% of the appropriate serum.

FLU-A (A/PR/8/34 H1N1) was propagated in the allantoic cavities of 10-day-old chicken eggs. After 72 h growth at 35 °C and 12 h at 4 °C, the allantoic fluid was harvested and centrifuged at 5000 rpm for 15 min to remove cellular debris, and virus was titered by hemagglutination with guinea pig red blood cells. Sterile filtration was used for additional passages. The virus was passaged three times in embryonated eggs with a hemagglutination titer of 2560. RSV strain Long, coxsackie virus B3 (CVB3), and adenovirus type 7 strain (AdV-7) were maintained in our laboratory and propagated in HEp-2 cells. HRV 14 was also maintained in our laboratory and was propagated in HEL cells. The viruses were stored in small aliquots at −80 °C until use.

Virus titration

Virus titration was performed by the limit dilution method, using a 96-well microtitre plate with 6 wells per dilution. The virus titer was estimated from cytopathogenicity of cells induced by viral infection and expressed as 50% tissue culture infectious doses/ml ($TCID_{50}$/ml) [27].

MTT assay

The cytotoxicity and antiviral activity of the compound were determined using quantitative colorimetric MTT [(3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide)] assay [21, 23, 8, 18]. Briefly, MDCK, HEp-2, and HEL cells were seeded at 2×10^4 cells per well in 96-well plates and grown at subconfluence. After removal of the growth medium, serial two-fold dilutions of the compound in 200 µl test medium were added. At each concentration, four wells were infected with 100 $TCID_{50}$/0.1 ml of virus while four wells were left uninfected for toxicity determination. Cells were fed with arbidol daily since its half-life in cultured cells is about 18 h [10]. Plates were incubated at 37 °C (for CVB3 and AdV-7) or at 35 °C (for FLU-A, RSV, and HRV 14) and the development of cytopathic effect (CPE) was monitored daily by light microscopy until the virus-infected, untreated cells showed CPE up to 80%. At this time point, the culture medium was removed and 25 µl of the MTT solution (5 mg/ml in phosphate buffered saline, PBS) was added to each well. The plate was further incubated for 4 h to allow MTT formazan formation. After removal of supernatant, 50 µl of DMSO was added for solubilization of formazan crystals and these were homogenized on a microplate shaker for 15 min. The optical densities (OD) were then read using a microplate spectrophotometer at double wavelengths of 540 and 690 nm. Results were expressed as a percentage of OD value of treated cell cultures with respect to untreated ones. All data were analyzed with SPSS 11.5, and the 50% cytotoxic (CC_{50}) and 50% inhibitory (IC_{50}) concentrations of the agent for the different cell lines were determined. Thus, the therapeutic index (TI) for each compound was also determined from CC_{50}/IC_{50}.

Antiviral activity in vitro

Drug treatment before virus infection

Serial two-fold dilutions of the test compound were dissolved in DMEM and incubated with cells for 24 h at 37 °C (for CVB3 and AdV-7) or at 35 °C (for FLU-A, RSV, and HRV 14) in 5% CO_2 atmosphere. After removal of the compound, the cells were washed twice with PBS and challenged with 100 $TCID_{50}$/0.1 ml of FLU-A, RSV, HRV 14, CVB3 or AdV-7, corresponding to a multiplicity of infection (MOI) of 0.1, 1.0, 1.0, 1.0, and 0.01, respectively. After 1 h incubation for virus adsorption, the monolayers were rinsed twice with PBS and were further incubated with test medium until typical CPE was visible (2-day incubation with FLU-A, HRV 14, CVB3 and AdV-7; 5-day incubation with RSV). The inhibition of the virus-induced CPE was scored by light microscopy and measured by the MTT assay. Four untreated virus controls and four uninfected, untreated cell controls were included in all assays. The IC_{50s} were determined as described above. All data presented are results of experiments performed in triplicate.

Virucidal assay

Viral suspensions containing 100 $TCID_{50}$/0.1 ml of viruses were incubated with an equal volume of medium with or without the test compound for 1 h at 35 °C (for FLU-A, RSV, and HRV 14) or at 37 °C (for CVB3 and AdV-7). One hundred microliters of mixed suspension was then added to subconfluent monolayers of cells. After an incubation time of 1 h, the solutions containing both compound and viruses were removed; the cell monolayers were rinsed carefully with PBS and further incubated with 200 µl test medium. After incubation for 2 days (with FLU-A, HRV 14, CVB3 and AdV-7) or 5 days (with RSV), the virucidal effect was determined using the MTT assay following the protocol described above (Drug treatment before virus infection Section).

Drug treatment after virus infection

The experiment was carried out as stated above with the following difference: monolayers were challenged with 100 $TCID_{50}$/0.1 ml viruses for 1 h. The cell sheets were washed with PBS and overlaid with different doses of the compound in 200 µl test medium.

In vivo toxicity determinations

Arbidol and ribavirin were each evaluated for the dose considered lethally toxic to mice. The doses studied for each compound were 500, 250, 125, 62.5 and 31.3 mg/kg/day.

The mice ($n = 8$) were treated with each compound by oral gavage for 6 days. The animal weights were determined prior to the first treatment and again 18 h after the final treatment. They were observed for death daily for 21 days.

Protective efficacy in mice

Specific-pathogen-free female BALB/c mice, 5–7 weeks old (17–19 g), obtained from Animal Center of Wuhan University, were used in all experiments. Mice were anesthetized by aether (Shanghai Chemicals Inc, China) and infected intranasally with 50 µl viral suspension containing approximately 10^5 $TCID_{50}$ of influenza A virus. The mice were divided into five groups; and arbidol at a dose of 25, 50, or 100 mg/kg/day, ribavirin at a dose of 68 mg/kg/day or a placebo was orally administered to the mice three times daily (at 8-h intervals) for 6 days beginning 24 h pre-virus exposure. The placebo controls received 0.5% methylcellulose solution instead of the drug. In the survival rate study ($n = 10$) the mice were observed for mortality daily for 21 days after infection. The protection was estimated by the reduction of the rate of mortality and prolongation of mean day to death (MDD). In the lung virus yield study ($n = 8$), the mice were sacrificed by cervical dislocation on the 5th day after viral exposure. The body weights of the mice were recorded daily until the animals were killed. The lungs were harvested, weighed, and subsequently homogenized to ~10% (w/v) suspensions in test medium. The homogenates were frozen and thawed twice to release the virus and centrifuged at 3000 rpm for 10 min. Virus titration was determined as described above. The lung index was expressed as the ratio of mean lung weights to mean body weights was also determined [28].

Statistical analysis

The data were analyzed by SPSS 11.5 software. Differences in mean day to death, mean body weights, lung virus yields and lung indexes compared with the control values were evaluated by Student's *t*-test. The log rank test was used to evaluate differences in the survival rates of the mice. A P value of < 0.05 was considered statistically significant.

Results

Cytotoxicities of arbidol and ribavirin

The cytotoxicities of arbidol and ribavirin for MDCK, HEL, and HEp-2 cells were evaluated. The results are summarized in Table 1. Subconfluent monolayers treated with arbidol at concentrations of 1–16 µg/ml did not show any visible changes in cell morphology or cell density, whereas 32 µg/ml of arbidol caused microscopically detectable alterations. Arbidol exhibited cytotoxicity against MDCK, HEL, and HEp-2 cells, with CC_{50} of 69.4, 72.5, and 85.4 µg/ml, respectively. Thus, the maximal concentration of 16 µg/ml of arbidol was adopted in the mode of action study to ensure that its antiviral effect was not due to cytotoxicity.

Table 1. Cytotoxicities of arbidol and ribavirin

Compound	Cell line/CC_{50}(µg/ml)[a]		
	MDCK	HEp-2	HEL
Arbidol	69.4 ± 8.5	85.4 ± 6.6	72.5 ± 3.2
Ribavirin	232.4 ± 11.6	256.6 ± 10.2	189.3 ± 6.8

[a] Mean ± S.D. values are shown from three independent experiments.
CC_{50} is the cytotoxic concentration required to reduce the number of viable cells by 50%.

Antiviral activity in vitro

Drug treatment before infection

Cells were treated with arbidol or ribavirin prior to viral infection as described in Materials and Methods. As seen in Table 2, arbidol showed significant inhibitory activity against FLU-A and RSV, with IC_{50s} of 2.7 and 8.7 µg/ml, resulting in TIs of 25.7 and 9.8, respectively. Infected MDCK cells treated with arbidol at concentrations up to 4 µg/ml did not show any visible CPE in comparison with the virus-control wells, which showed typical CPE. In contrast, ribavirin could not inhibit FLU-A or RSV replication in this assay. Arbidol showed relatively weaker activity against HRV 14 and CVB3, with IC_{50s} being greater than that for FLU-A, resulting in lower TIs of 5.4 and 6.7. It is interesting to note that arbidol lacked inhibitory activity against AdV-7, a non-enveloped, double-stranded DNA virus.

Virucidal activity

To investigate the direct inactivating effect of arbidol, viruses were treated for 1 h with concentrations of arbidol ranging from 1 to 16 µg/ml. As shown in Table 2, arbidol was virucidal with FLU-A, RSV, HRV 14, and CVB3, with IC_{50s} of 4.3, 10.4, 13.8, and 13.1 µg/ml, respectively. Concen-

Table 2. Antiviral activity of arbidol against different viruses

	IC_{50s}[a] (μg/ml)				
	FLU-A	RSV	HRV 14	CVB3	AdV-7
Drug added before infection	2.7 ± 1.0	8.7 ± 1.4	13.4 ± 1.3	12.7 ± 0.4	NR
Virucidal assay	4.3 ± 0.7	10.4 ± 1.1	13.8 ± 0.4	13.1 ± 0.6	NR
Drug added after infection	9.6 ± 1.0	11.5 ± 1.2	12.5 ± 1.7	9.5 ± 0.6	15.4 ± 0.3

[a] Mean ± S.D. values are shown from three independent experiments.
NR: IC_{50} not reached.
IC_{50} is the inhibitory concentration required to reduce viral replication by 50%.

trations of arbidol >8 μg/ml completely abolished the biological activity of FLU-A virus. Arbidol inhibited the CPE of FLU-A on MDCK cells, RSV on HEp-2 cells, HRV 14 on HEL cells, and CVB3 on HEp-2 cells, with TIs of 16.1, 8.2, 5.3, and 6.5, respectively.

Drug treatment after infection

Subconfluent cells were infected with the various viruses and then incubated with the drugs as described in Materials and Methods. Arbidol was broadly inhibitory for the five viruses when added after infection. The rank order of virus sensitivity to arbidol was CVB3, RSV, FLU-A, HRV 14, and AdV-7. The IC_{50} values ranged from 9.5 to 15.4 μg/ml.

Toxicities in vivo

Oral gavage treatment with arbidol and ribavirin for 6 days indicated the approximate 50% lethal dose (LD_{50}) of rivavirin to be 213 mg/kg/day, whereas arbidol was better tolerated, the LD_{50} dose being approximately 314 mg/kg/day (Table 3). It should be noted that no obvious weight loss was seen at dosages below the LD_{50} dose. No attempt was made to determine the cause of death in the mice in this range-finding study.

Protective efficacy of arbidol in mice

By Day 3, after viral exposure, clinical signs of murine influenza pneumonia were observed in some mice, especially in the placebo controls. Changes

Table 3. Comparison of toxicity of oral gavage treatment[a] with arbidol and ribavirin in mice

Compound	Dosage (mg/kg/day)	Survived/ total	MDD[b] ± S.D.	Mean weight change[c] (g)	Estimated LD_{50}[d] (mg/kg/day)
Arbidol	500	0/8	7.2 ± 1.2	−1.7	314
	250	7/8	8.0 ± 0.0	−1.2	
	125	8/8	>21.0	0.4	
	62.5	8/8	>21.0	0.7	
	31.3	8/8	>21.0	0.5	
Ribavirin	500	0/8	6.3 ± 0.7	−2.9	213
	250	3/8	7.4 ± 0.8	−2.3	
	125	6/8	7.9 ± 1.3	0.3	
	62.5	8/8	>21.0	0.7	
	31.3	8/8	>21.0	0.8	
Normal controls	–	6/6	>21.0	0.5	

[a] Treated by oral gavage for 6 days beginning 24 h pre-virus infection.
[b] Mean day to death of mice dying prior to day 21.
[c] Difference between weight prior to start of treatment and weight 18 h after end of therapy.
[d] Determined by line of regression.

Table 4. Effect of oral treatment with arbidol in mouse influenza model

Compound	Dosage (mg/kg/day)	Dead/ total	MDD[a] ± S.D.
Arbidol	100	0/10**	>21.0 ± 0.0**
	50	3/10*	9.4 ± 2.9*
	25	5/10	8.6 ± 1.8*
Ribavirin	68	1/10**	12.0 ± 0.0**
Controls[b]	–	8/10	6.4 ± 1.2

[a] Mean day to death of mice dying prior to day 21.
[b] The placebo controls received 0.5% methylcellulose solution instead of the drug.
*$P<0.05$ vs. placebo-treated controls; **$P<0.01$ vs. placebo-treated controls.

in behavior, such as tendencies to huddle, diminished vitality, and ruffled fur were also observed. Parameters for determining the protective efficacy of arbidol against influenza virus A/PR/8/34-infected mice included prevention of death through 21 days and lessening of lung virus titer and lung index at day 5 postinfection.

Most of the mice infected with influenza virus A/PR/8/34 died within 21 days if they were treated with only placebo (Survival rate = 20%) (Table 4). Orally administered arbidol prevented influenza-virus-induced death in a dose-dependent manner. For the groups treated with arbidol at a dose of 50 or 25 mg/kg/day, the survival rates were 70 and 50%, respectively. In this experiment, 100 mg/kg/day of arbidol demonstrated relatively greater effect to the mice than 68 mg/kg/day of ribavirin, approximately one-third of the LD_{50} dose of each compound, in terms of the better survival rate and the higher length of MDD ($P<0.05$).

Oral administration of arbidol beginning 24 h pre-virus infection significantly decreased the virus titers of mice lung homogenates. In the groups treated with arbidol at 25, 50, or 100 mg/kg/day, the mean virus yields were reduced to 3.2, 2.4 and 2.0 ($P<0.01$) Log_{10} $TCID_{50}$/lung, respectively, whereas the yields in placebo controls were 4.9 Log_{10} $TCID_{50}$/lung (Table 5). Based on the decelerated loss of the body weight, the beneficial effects of arbidol treatment at 50 and 100 mg/kg/day were noticeable as early as on day 3 post challenge (Fig. 1). At day 5, the maximum mean weight loss

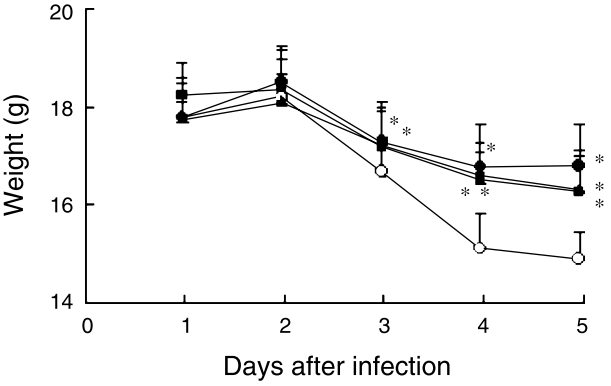

Fig. 1. Effects of orally administered arbidol on weight loss in influenza-virus-infected mice (5–7 weeks old). Mice were infected with influenza virus A/PR/8/34 as described in Materials and Methods. Mice were treated with an oral dose of arbidol of 25 (■), 50 (▲), or 100 (●) mg/kg/day or with 0.5% methylcellulose solution as a control (○) for 6 days beginning 24 h before infection. *$P<0.05$ vs. placebo-treated controls (Student's *t*-test)

in the 25, 50, and 100 mg/kg/day arbidol-treated groups were 1.97, 1.44, and 0.7 g ($P<0.05$), respectively, while the maximum mean weight loss in the placebo-control group was 2.89 g. Additionally, the i.n. infection with FLU-A virus led to an increase in mean lung weight, which was detectable on day 5 after viral exposure (Table 5). However, lung weights of mice treated with arbidol at 50 and

Table 5. Effect of oral treatment[a] with arbidol on lung virus yield in mouse influenza model

Group ($n = 8$)	Mean lung parameters[b]	
	Weight (mg ± S.D.)	Virus titer (Log_{10}/lung ± S.D.)
Arbidol at 100 mg/kg/day	122 ± 9**	2.0 ± 0.3**
Arbidol at 50 mg/kg/day	119 ± 8**	2.4 ± 0.2**
Arbidol at 25 mg/kg/day	136 ± 18*	3.2 ± 0.3**
Controls	158 ± 22	4.9 ± 0.1

[a] Treated by oral gavage for 6 days beginning 24 h pre-virus infection.
[b] Mean ± S.D. values are obtained from a single representative experiment.
*$P<0.05$ vs. placebo-treated controls; **$P<0.01$ vs. placebo-treated controls.

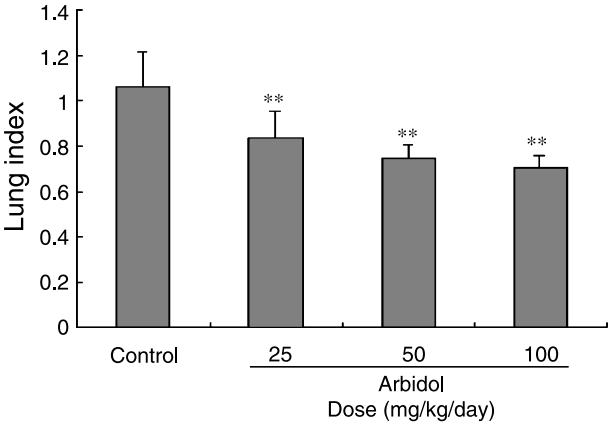

Fig. 2. Effect of oral administration of arbidol on prevention of lung index increase in influenza-virus-infected mice. Mice were infected with influenza virus A/PR/8/34 at 10^5 $TCID_{50}$/mouse, and the lung index was determined as described in Materials and Methods. Mice were treated with an oral dose of arbidol of 25, 50, or 100 mg/kg/day or with 0.5% methylcellulose solution as a control for 6 days beginning 24 h before infection. $^{**}P < 0.01$ compared to the results for placebo-treated controls (Student's *t*-test)

100 mg/kg/day remained relatively normal compared to the placebo controls ($P < 0.01$) (Table 5). Therefore, arbidol treatment at 25, 50, or 100 mg/kg/day dramatically prevented lung index increases compared to the placebo controls ($P < 0.01$) (Fig. 2). These results suggest that arbidol may be effective for prevention of influenza virus infection.

Discussion

We have demonstrated the broad-spectrum antiviral activity of arbidol *in vitro*. First, we used a simple and rapid staining method (MTT assay) to identify the mode of action of arbidol against a series of respiratory viruses. Arbidol was found to present antiviral activity against enveloped and non-enveloped RNA viruses, namely FLU-A, RSV, HRV 14, and CVB3 when added before, during, or after infection. Besides, arbidol showed weak activity against AdV-7, a DNA virus when added after infection. The high *in vitro* inhibitory activity obtained for arbidol against influenza virus in these studies and others [16, 17] was reflected in the *in vivo* (BALB/c mice) studies where significant anti-FLU-A activity was also observed. Orally administered arbidol at 50 or 100 mg/kg/day 24 h before infection with influenza virus A/PR/8/34 for 6 days significantly reduced mean pulmonary virus yields in mice and the rate of mortality. Our results suggest that arbidol, a potent non-specific, broad-spectrum antiviral agent, should deserve our attention in future [3].

In our study, enveloped viruses were found to be more sensitive to arbidol than non-enveloped viruses. The results of pre-treatment assay and virucidal assay showed that arbidol exhibited significant inhibitory activity against FLU-A and RSV, two enveloped viruses, while it showed weak activity or no activity against HRV 14, CVB3, or AdV-7, three non-enveloped viruses. These results, taken together, are in agreement with previous studies that showed that the mechanism of arbidol action against influenza viruses is connected to inhibition of the process of membrane fusion [17, 1]. In addition, Boriskin et al. reported recently that the antiviral activity of arbidol towards hepatitis C virus is due to a direct effect of arbidol on virus-cell membrane interactions [2]. However, the exact antiviral mechanism of arbidol is an interesting subject for further studies.

Based on its chemical structure, which contains a carboxylic acid ester moiety, arbidol may be a substrate for hydrolysis *in vivo*, leading to the intracellular accumulation [2]. The fact that arbidol displayed prophylactic activity when administered 24 h before infection might indicate a prerequisite for arbidol accumulation in intracellular compartments before antiviral activity is observed. Clearly, additional studies of arbidol and various chemical derivatives are warranted.

It has been reported that the nucleoside analogue ribavirin inhibits both DNA and RNA viruses [20, 29], and in our study, ribavirin inhibited the replication of some RNA viruses, FLU-A and RSV (data not shown), but not the DNA virus, AdV-7. Besides, ribavirin could not inhibit RSV when added before infection but could inhibit RSV replication when added after infection [18]. In contrast, cells pretreated with arbidol were resistant to subsequent infection with FLU-A, RSV, HRV 14, and CVB3. In addition, arbidol showed an inhibitory effect against AdV-7 when added after infection. In our experiment, 100 mg/kg/day of arbidol

demonstrated a relatively greater effect in mice than 68 mg/kg/day of ribavirin, approximately one-third of the LD_{50} dose of each compound, in terms of the better survival rate and longer MDD ($P < 0.05$). Accordingly, arbidol may be a better candidate than ribavirin in treating respiratory virus infections.

In view of the *in vitro* and *in vivo* data, we conclude that arbidol has the ability to elicit protective broad-spectrum antiviral activity against a number of respiratory viruses. Arbidol may play a significant role in medical countermeasures against respiratory virus infections.

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