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Inhibitory effect of cinnamaldehyde, derived from Cinnamomi cortex, on the growth of influenza A/PR/8 virus *in vitro* and *in vivo*

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Abstract

We have investigated the inhibitory effect of *trans*-cinnamaldehyde (CA), one of the principal constituents of essential oil derived from Cinnamomi cortex, on the growth of influenza A/PR/8 virus *in vitro* and *in vivo*. When 1-h drug treatment was initiated at various times post-infection (p.i.) in Madin–Darby canine kidney cells using a fixed dose of CA (40 μM), the maximum inhibitory effect (29.7% virus yield of control) was obtained when drug treatment was started at 3 h p.i. Under the same treatment schedule, CA inhibited the virus growth in a dose-dependent manner (20–200 μM), and, at 200 μM, the virus yield was reduced to an undetectable level. RT-PCR and SDS-PAGE analyses showed that CA inhibited viral protein synthesis at the post-transcriptional level. In mice infected with the lung-adapted PR-8 virus, inhalation (50 mg/cage/day) and nasal inoculation (250 μg/mouse/day) of CA significantly increased survival rates on the 8 days to 100% and 70%, respectively, in contrast to a survival rate of 20% in the untreated control group. Importantly, inhalation of CA caused virus yield reduction by 1 log in bronchoalveolar lavage fluid on day 6 after infection, compared with that of the untreated control group. These findings might provide further support to the empirical indication of Cinnamomi cortex-containing Kampo medicines for acute respiratory infectious diseases.

Keywords: Cinnamaldehyde; Influenza; Protein synthesis; Survival rate

1. Introduction

Based on the theory of Kampo (traditional Japanese) medicine, several herbs are empirically mixed as a formula for the treatment of various diseases and pathological conditions. For years, several Kampo medicines have been used in the field of infectious respiratory diseases including influenza. To obtain a better understanding of the mechanisms of the therapeutic effect of such Kampo medicines, an *in vitro* examination of each component might be important. Our serial anti-influenza research has revealed that *Ephedra sinica* Stapf (Ephedrae herba) and *Zingiber officinale* Roscoe (Zingiberis siccatum rhizoma), both representative herbs of Kampo medicines used for the treatment of respiratory infections, could inhibit the growth of influenza virus *in vitro*. Interestingly, the former herb could inhibit the growth of influenza A and B viruses by direct addition to the cultures of infected cells owing to the suppression of acidified cellular compartments essential for the uncoating process of influenza virus (Mantani et al., 1999, 2001). In contrast, the latter herb could not inhibit influenza virus growth by direct addition to infected cells, but it could exert an inhibitory effect indirectly via the activation of macrophages, leading to tumor necrosis factor-α production (Imanishi et al., 2006). These findings clearly demonstrated that several herbs in Kampo medicines have the potential to inhibit the growth of influenza virus in various ways.

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Several reports have documented that Cinnamomi cortex (*Cinnamomum cassia* Blume) and its derivative, cinnamaldehyde (CA), exhibit various biological activities *in vitro*, such as antibacterial activity (Chang et al., 2001), induction of apoptosis via reactive oxygen species (Ka et al., 2003), and inhibition of nitric oxide synthesis (Lee et al., 2002). However, little is known about the antiviral activity of CA. This study was therefore undertaken to clarify whether CA could inhibit the growth of influenza virus in Madin-Darby canine kidney (MDCK) cells in a manner different from that of Ephedrae herba and Zingiberis siccatum rhizoma, and to extend the *in vitro* findings to influenza virus-induced pneumonia in mice.

2. Materials and methods

2.1. Drugs

Trans-cinnamaldehyde (CA; Wako Pure Chemicals, Osaka, Japan) was freshly dissolved in dimethyl sulfoxide (DMSO) at 40 mM and then diluted with serum-free Eagle's minimum essential medium (MEM) or Dulbecco's modified MEM (DMEM) to the appropriate concentrations (10–400 μM). These drug solutions were sterilized through a millipore filter (Millipore Corporation, Billerica, MA). DMSO (1%)-containing medium was used as control throughout the experiments *in vitro*. The chemical structure of CA is shown in Fig. 1.

2.2. Virus and cells

Influenza A/PR/8/34 (PR8) virus (H1N1 subtype) was propagated for 3 days at 35 °C in the chorio-allantoic cavities of 10-day-old embryonated hen eggs. The infected allantoic fluids were clarified by centrifugation at 1000 × *g* for 20 min and stored in small portions at −80 °C as a virus stock solution. The allantoic fluids containing A/USSR/92/77 (USSR) (H1N1), A/Aichi/2/68 (Aichi) (H3N2), or B /Lee/40 (Lee) virus were also prepared as above. MDCK cells were cultured as a monolayer in MEM supplemented with 8% fetal bovine serum.

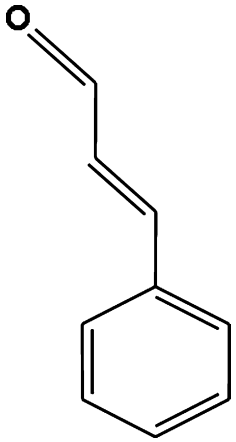

Fig. 1. Chemical structure of CA.

2.3. Cytotoxicity assay

A subconfluent monolayer of MDCK cells in a 96-well plate (Falcon, Becton Dickinson, Franklin Lakes, NJ) was cultured in serum-free MEM with various concentrations of CA at 37 °C for 24 h. Thereafter, the cells were processed for the MTT assay, a sensitive method for monitoring cytotoxicity based on the detection of mitochondrial enzymatic activity reacting with a chromogenic substrate, the color development of which was read at an optical density (OD) of 580/630 nm (Mosmann, 1983). Control cells were cultured in the same manner under drug-free conditions. Triplicate cultures were used so as to perform statistical analysis.

2.4. Virus growth assay

Confluent monolayers of MDCK cells in a 24-well plate (Falcon, Becton Dickinson) were challenged with PR8 virus at a multiplicity of infection (MOI) of 5 plaque-forming units (PFU)/cell under drug-free conditions. After 60-min absorption at room temperature, the infected cells were washed three times with phosphate-buffered saline (PBS) to remove unabsorbed viruses, and were then cultured in serum-free MEM at 37 °C. This time point was designated as 0-h post-infection (p.i.). To evaluate the inhibitory effect of drugs, the infected cells were cultured in the presence of CA, or received 1-h drug treatment initiated at various time points within 6 h p.i. The control-infected cells also received the same treatment with 1% DMSO-containing medium. After 10-h incubation, the cultures were processed through three cycles of freezing and thawing, and clarified by a low speed centrifugation (500 × *g* for 10 min) to determine virus yield in the supernatants by plaque titration on MDCK cells (Tobita, 1975), or hemagglutination (HA) titration as described previously (Ochiai et al., 1995). Triplicate cultures were used so as to perform statistical analysis.

2.5. Reverse transcriptase polymerase chain reaction (RT-PCR)

A confluent monolayer of MDCK cells in a 6-cm dish was infected and washed as above, and incubated in drug-containing MEM. At certain time points p.i., total RNA extracted from the cells (Chomczynski and Sacchi, 1987) was reverse-transcribed with reverse transcriptase (Superscript II, Gibco BRL, Rockville, MD) and oligo dT16 primer. The reverse transcription products served as templates for PCR, consisting of 25 cycles of denaturation (94 °C for 1 min), annealing (55 °C for 2 min), and extension (72 °C for 3 min), using a Thermal Cycler (Perkin-Elmer, Boston, MA) and oligonucleotide primers. The parallel expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was tested under the same PCR conditions as an internal standard. The primer sequences were the same as those of previous reports (Vabret et al., 2000; Prime et al., 1998). For quantification, PCR bands on the gel were scanned using a

computer analysis system (EDAS290 v3.5, Eastman Kodak, New Haven, CT), and the viral mRNA signal was normalized relative to the corresponding GAPDH mRNA signal from the same sample. Data are presented as the mRNA/GAPDH ratio.

2.6. Assay of protein synthesis

A confluent monolayer of MDCK cells in a 24-well plate was infected and washed as above, and cultured in drug-free DMEM. Thereafter, the cells received 1-h drug treatment initiated at 3 h p.i. After incubation for certain periods, the cells were pulse-labeled for 1 h with 10 μCi of 35S-methionine in methionine-deficient DMEM (Kurokawa et al., 1990) and lysed to analyze protein synthesis by electrophoresis on a 12.5% polyacrylamide gel in Tris-glysine-SDS buffer (SDS-PAGE) (Laemmli, 1970). The protein bands on gel fluorography were analyzed using a computer analysis system (BAS 5000, FUJIFILM, Tokyo, Japan).

2.7. Influenza virus-induced pneumonia in mice

An outbred specific-pathogen-free strain of ICR female mice, which were 4 weeks old (body weight, approximately 17 g; Sankyo Laboratory Service Co., Ltd., Japan), was used for infection or mock-infection by intranasal inoculation of a virus suspension containing 5000 PFU/25 μl (four 50% lethal doses of virus) of a mouse lung-adapted PR8 virus (Sakai et al., 2000). The mice, divided into three groups each consisting of 25–30 mice, were kept in independent facilities with independent air-conditioning systems according to the way of drug treatment. In the intranasal administration group, mice received intranasal inoculation of CA (250 μg/mouse/day) once per day during the experiments following the first inoculation at 1 h p.i. In the inhalation group, the drug (50 mg/ml water) in a small container was placed in the cage under a metallic net to prevent its oral intake and left for natural vaporization until daily change. Concentration of the vaporized CA was not determined. The last group, as control, did not receive drug treatment. Five mice per cage were kept at constant room temperature ($20 \pm 2\,^{\circ}C$) in a 12-h light/dark cycle with free access to food and water. In addition to daily observation of survival and body weight, 4–6 mice were killed on day 6 p.i. for preparation of bronchoalveolar lavage fluid (BALF) via tracheal cannula to examine virus yields (Sakai et al., 2000). This experimentation was approved by the Committee on Animal Experimentation, University of Toyama.

2.8. Statistical analysis

Values were presented as mean ± standard deviation (S.D.) of more than three observations. Significance of differences was tested by Student's *t*-test for the comparison of duplicate means and by one-way analysis of variance follow by Bonferroni-correction for the comparison of multiple means. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Cytotoxic activity of CA against MDCK cells

When a subconfluent monolayer of MDCK cells was cultured in the presence of various concentrations of CA for as long as 24 h, the MTT assay showed that the OD value of control cells was 0.386 (mean of triplicate cultures), whereas the values of drug-treated cells at concentrations of 40 μM, 80 μM, 200 μM and 400 μM were 0.337, 0.325, 0.310, and 0.358, respectively. These data indicate that the cytotoxic activity of CA could be ruled out in a dose range of less than 400 μM and, consequently, 200 μM and less was used for the virus growth assay described below.

3.2. Effect of -CA on the growth of influenza A and B viruses in MDCK cells

To test the inhibitory effect of CA on the growth of influenza viruses, the cells were infected with PR8, USSR, Aichi or Lee virus under a drug-free condition and cultured in the presence of various concentrations of CA (10 μM to 40 μM). As shown in Table 1, HA titer at 10 h p.i. was 128 in PR8 virus-infected cells. However, these values decreased to 16, 8 and less than 4 in the presence of 10 μM, 20 μM and 40 μM of CA, respectively. In the case of other virus strains, the similar reduction in HA titers was also observed. These data indicate that CA has potential for the inhibition of influenza virus growth in a dose-dependent fashion without virus strain specificity.

To extend these findings, the cells were infected with PR8 virus under a drug-free condition and received 1-h drug treatment with 40 μM initiated at various times p.i. As shown in Fig. 2, the virus yield of untreated control cells was 5.0×10^4 PFU/ml at 10 h p.i. However, these values tended to decrease with drug treatment initiation up to 3 h p.i. Drug treatment started immediately after infection showed no significant inhibitory effect (78.1% virus yield of the control), but the maximum inhibition (29.7% virus yield of the control) was obtained when drug treatment was started at 3 h p.i. This inhibitory effect then weakened again toward 5 h p.i.

Based on these findings, the dose-related inhibitory effect was examined in a dose range of 20–200 μM when 1-h drug treatment was initiated at 3 h p.i. As shown in Fig. 3, the virus yield at 20 μM was 62.3% of control, and decreased in a dose-dependent fashion (29.7% and 8.3% for 40 μM and 80 μM, respectively).

Table 1
Inhibitory effect of CA on the growth of influenza A and B viruses in MDCK cells

Virus	Subtype	HA titers at 10 h p.i. in culture supernatants in the presence of the indicated CA concentrations (μM)			
		40	20	10	(–)
A/PR/8	H1N1	<4	8	16	128
A/USSR	H1N1	<4	8	16	128
A/Aichi	H3N2	<4	16	64	256
B/Lee		<4	8	16	64

The representative data from three experiments are shown.

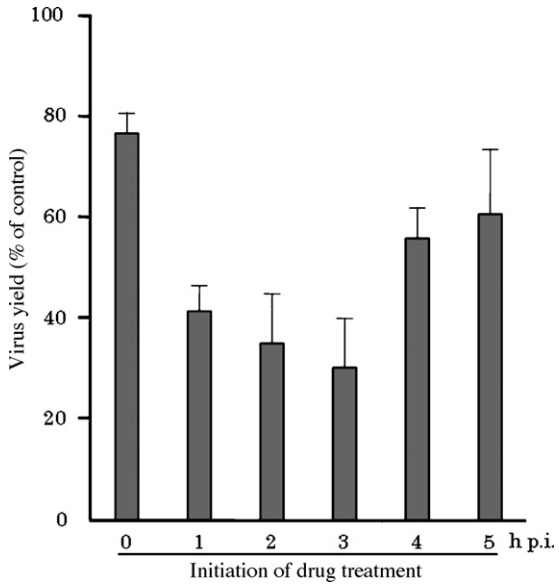

Fig. 2. Time-related inhibitory effect of CA on the growth of influenza PR8 virus in MDCK cells. A confluent monolayer of MDCK cells in a 24-well plate was infected with PR8 virus at a multiplicity of infection (MOI) of 5 PFU/cell. At the indicated times p.i., infected cells received 1-h treatment with CA (40 μM). After 10-h incubation, culture supernatants were collected to examine virus yields by plaque assay. Data are expressed as the percent of virus yield in control-infected cells receiving 1-h treatment with a drug-free medium. Data are expressed as mean (column) ± S.D. (thin bar) of triplicate cultures.

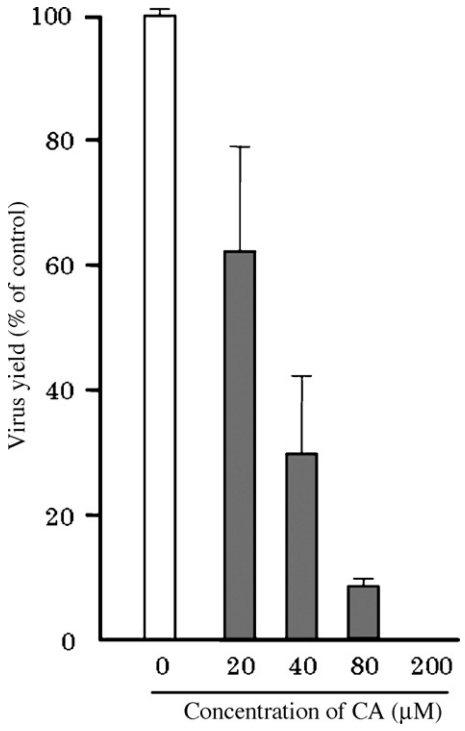

Fig. 3. Dose-related inhibitory effect of CA on the growth of influenza PR8 virus in MDCK cells. A confluent monolayer of MDCK cells in a 24-well plate was infected with PR8 virus at an MOI of 5 PFU/cell under drug-free conditions. At 3 h p.i., the infected cells received 1-h treatment with various concentrations of CA and then were further cultured in drug-free medium. After 10-h incubation, culture supernatants were collected to determine virus yield. Data are expressed as mean (column) ± S.D. (thin bar) of triplicate cultures.

Finally, virus growth was not observed at all at 200 μM. These findings suggest that CA has the potential to inhibit virus growth in a unique manner, targeting a mid-stage of the virus growth cycle.

3.3. Effect of CA on the synthesis of viral mRNAs and proteins

As viral mRNA, as well as viral proteins, are synthesized in mid-stage, their syntheses were compared between drug-treated and untreated infected cells. As shown in Fig. 4A, when the cells were infected with PR8 virus in the absence of drug and cultured in the presence or absence of drug (40 μM) until the cells were processed for RT-PCR, virus mRNAs could be detected in the drug-treated cells at almost the same level as in the drug-untreated cells. The normalization of hemagglutinin protein and matrix protein mRNA signals to the corresponding GAPDH mRNA signals from the same sample revealed no significant difference between the drug-untreated and -treated cells (Fig. 4B). These findings indicate that CA does not affect viral mRNA synthesis.

The effect of CA on the synthesis of viral proteins was then examined. As shown in Fig. 5A, in drug-untreated infected cells, synthesis of viral proteins could be detected at 4 h p.i. and became stronger towards 6 h p.i. On the other hand, when infected cells received 1-h drug treatment (40 μM) begun at 3 h p.i., faint bands on the gel could be detected, but they did not become stronger towards later times, in contrast to what happened in drug-untreated cells (Fig. 5B). Fig. 5C shows that the synthesis of viral proteins was inhibited in a dose-dependent fashion.

3.4. Therapeutic efficacy of CA in influenza virus-induced pneumonia in mice

To expand the *in vitro* findings, the therapeutic efficacy of the drug was evaluated in influenza virus-induced pneumonia in mice. As shown in Fig. 6A, control mice began to die on day 4, and finally the survival rate decreased to 20% on day 10. In contrast, mice in the intranasal inoculation group (250 μg/mouse/day) did not begin to die until day 5, and then a significantly higher value (70%, $P < 0.05$) was noted on day 8. However, during the last 2 days, this value decreased sharply to 30%. When a lower dose (100 μg/mouse/day) was used for the intranasal inoculation, the survival rate was similar to that of untreated control group (data not shown). On the other hand, all mice in the inhalation group were still alive on day 8 ($P < 0.05$) followed by a slight decrease (80%) on day 10. The beneficial effect of CA treatment was more clearly shown if monitored by body weight loss (Fig. 6B). In contrast to the mock-infected group whose body weight continued to increase, the gain in body weight ceased and actually began to decrease from 4 days p.i. in the drug-untreated group. However, both intranasal inoculation and inhalation of CA prevented a significant decline ($P < 0.05$) in body weight during the observation period.

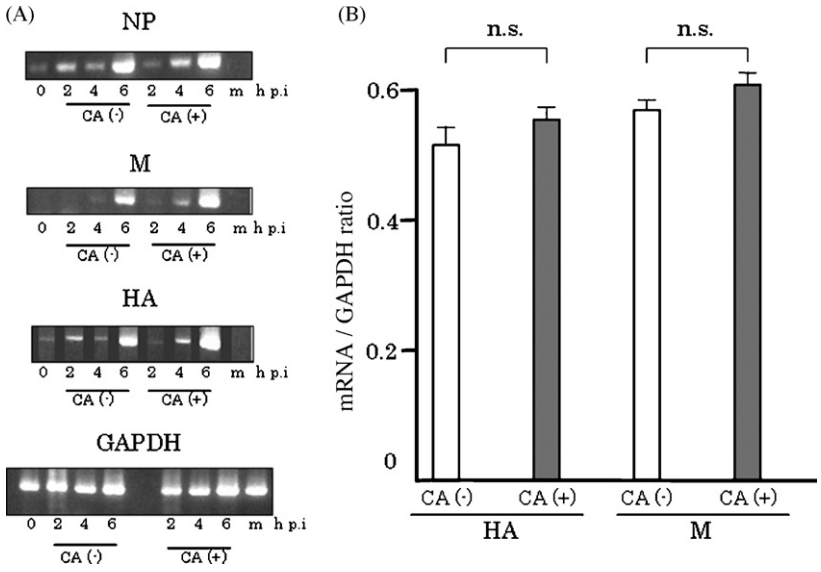

Fig. 4. Effect of CA on the synthesis of viral mRNAs in MDCK cells. A confluent monolayer of MDCK cells in a 6-cm dish was infected with PR8 virus at an MOI of 5 PFU/cell for 1 h, and then cultured in the presence or absence of 40 μM CA. At the indicated times p.i., total RNA was extracted from the cells. Mock-infected cells (m) were also processed in the same manner as infected cells. The extracted RNA was subjected to RT-PCR analysis using oligo (dT) 16 primers. Thereafter, PCR-amplified cDNAs derived from these RNAs were applied to agarose gel electrophoresis. The mRNA levels on the gel photograph at 6 h p.i. in panel A were quantified by densitometric analysis and expressed as mRNA/GAPDH ratio by mean ± S.D. of three independent experiments in panel B. Individual RNAs are indicated by the following abbreviations: HA, hemagglutinin; NP, nucleoprotein; M, matrix protein. "n.s." indicates statistical non-significance between control and CA-treated cells.

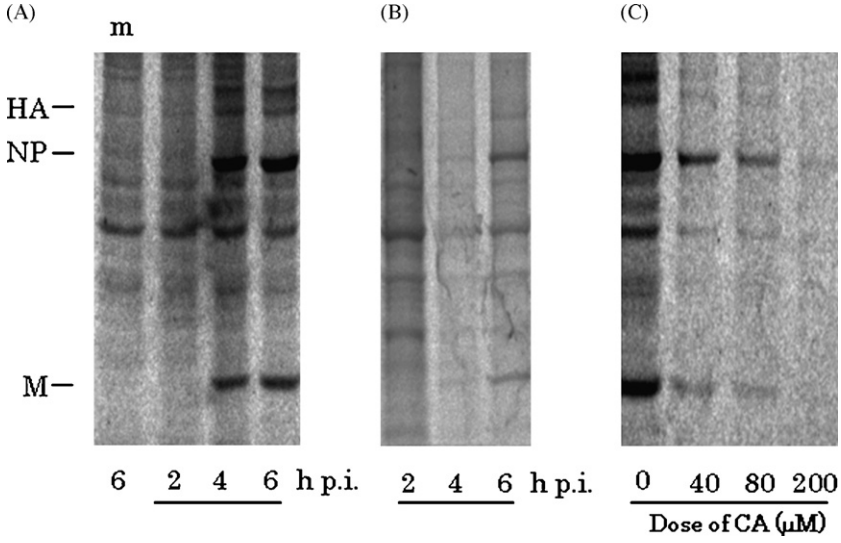

Fig. 5. Inhibitory effect of CA on the virus protein synthesis in MDCK cells. A confluent monolayer of MDCK cells in a 24-well plate was infected with PR8 virus at an MOI of 5 PFU/cell for 1 h, and cultured under drug-free conditions. At 3 h p.i., the infected cells received 1-h treatment with (B) or without (A) 40 μM of CA. Alternatively, the cells received 1-h treatment with various concentrations of CA initiated at 3 h p.i. (C). These infected cells (A–C) and mock-infected cells (m) were labeled at the indicated times after infection for 1 h with 10 μCi of 35S-methionine. Thereafter, the cells were lysated to analyze by SDS-PAGE with a aid of computer analysis system. For viral protein abbreviations, see the legend for Fig. 4.

Furthermore, to evaluate virus growth in response to drug inhalation, the virus titers in BALF were compared between the drug-untreated and -inhalation groups. Virus yields in BALF obtained from the untreated group were surprisingly high, i.e., 1.9×10^7 PFU/mouse on day 6 p.i. In contrast, those in the inhalation group were almost 1 log lower at 1.6×10^6 PFU/mouse (Fig. 7). The difference in virus yields of the two groups was significant ($P < 0.05$).

4. Discussion

The present study demonstrated that CA inhibits the growth of influenza PR8 virus in MDCK cells in a concentration-dependent fashion (20–200 μM) without cytotoxicity, as confirmed by the MTT assay. Importantly, time-related studies showed that the inhibitory effect of CA depends on the initiation time of drug treatment. CA exhibited the most powerful

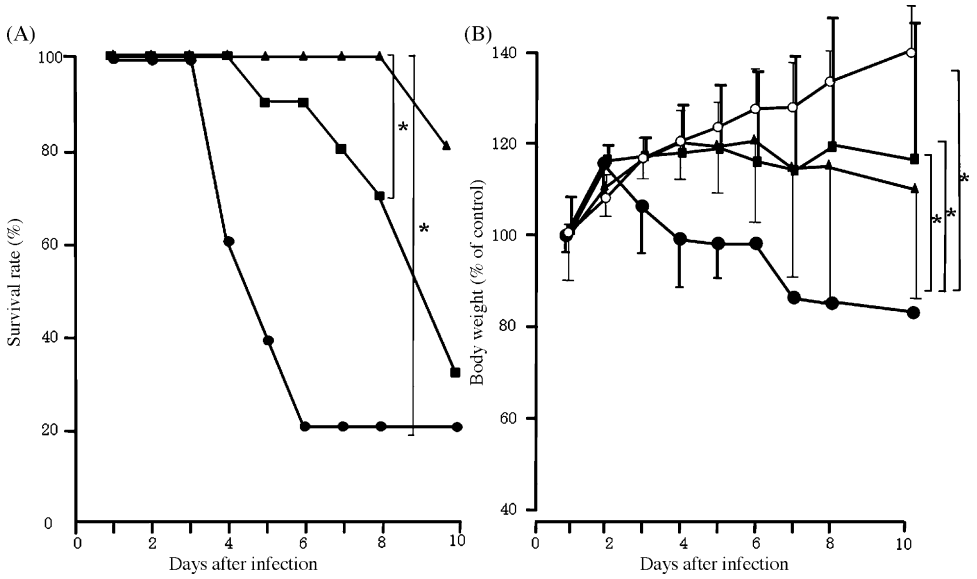

Fig. 6. Effect of CA administration on survival rate and body weight loss in influenza virus-induced pneumonia in mice. Infected mice daily received single intranasal inoculation (250 μg/mouse: closed squares), inhalation (50 mg/cage: closed triangles) of CA, or no drug-treatment (closed circles). Each cage contained 5 mice. Survival rate (A) and body weight (B) of mice were monitored daily for comparison among experimental groups including the mock-infected group (open circles in panel B). Each point shows mean ± S.D. of 10–13 mice in each experimental group. Asterisk indicates statistical significant ($P < 0.05$) difference from the control.

inhibitory effect when 1-h drug treatment was started at 3 h p.i., indicating that CA targets the mid-stage of virus growth, in sharp contrast to Ephedrae herba, which targets the early stage of virus growth (Mantani et al., 1999, 2001). It was also shown that this manner of drug treatment could decrease virus yields in a dose-dependent manner (20–200 μM), and especially, treatment with 200 μM reduced the yield to an undetectable level.

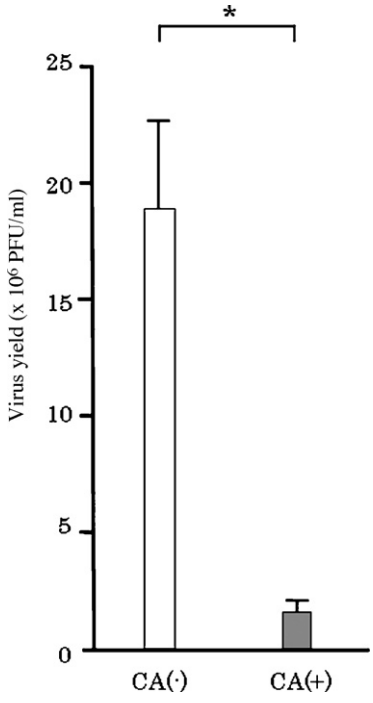

Fig. 7. Effect of inhalation of CA on the virus yields in BALF of infected mice. BALFs were collected from five mice of CA inhalation group (CA) and drug-untreated group (control) on day 6 to examine virus yields. Infection and drug-treatment conditions are the same as described in the legend for Fig. 6. Data are expressed as mean (column) ± S.D. (thin bar). Asterisk indicates statistical significance ($P < 0.05$) from the control.

In the growth cycle of the influenza virus, the mid-stage is responsible for the synthesis of various virus materials such as viral RNAs and proteins necessary for progeny viruses. Therefore, it was examined whether CA inhibits RNA and/or protein synthesis. RT-PCR and SDS-PAGE assays clearly demonstrated that CA inhibits viral protein synthesis at the post-transcription level, based on the fact that mRNA is synthesized in drug-treated cells at almost the same level as in drug-untreated cells. A previous report showed that CA selectively inhibits the growth of tumor cells by retarding protein synthesis via the trapping of sulfhydryl-containing amino acid (i.e. cysteine) residues in cells (Moon and Pack, 1983), suggesting that the trapping of sulfhydryl residue in amino acids might occur unselectively in both viral and cellular proteins in the presence of CA. Indeed, we observed that 1-h drug treatment also inhibits cellular protein synthesis in mock-infected cells in a dose-dependent fashion. In the case of influenza virus infection, it is well known that in virus-infected cells viral protein synthesis ceases if the synthesis of cellular proteins ceases (Hey, 1998). This shut-off phenomenon could be observed by the band densities becoming gradually faint with the lapse of time p.i. (see Fig. 5). Taking this unique phenomenon in influenza virus-infected cells together with our findings, the CA-mediated inhibition of viral proteins might be generated based on the selective synthesis of viral proteins in infected cells, leading to the inhibition of virus growth. However, the possibility remains that CA induces inactivation or degradation of functional viral proteins. In this connection, when three viruses (PR8, Aichi and Lee) with HA titers of 64 to 128 were incubated with various concentrations of CA for 1 h at 37 °C, these values did not change after the treatment with as much as 200 μM of CA for either virus (data not shown). It is likely

that CA might affect the protein synthesis process, but not cause degradation of the preexisting or synthesized proteins.

To expand on the findings obtained in the *in vitro* assay, we further studied whether the drug exerts sufficient therapeutic efficacy in an infected mouse model. Considering the short half-life of CA in rat blood and lungs after oral administration by enzymatic degradation (Yuan et al., 1992; Sapienza et al., 1993; Cheung et al., 2003), we attempted direct delivery routes via the air ways of mice, *i.e.*, intranasal inoculation and inhalation of the vapor. Using these drug administration protocols, the survival rates and loss in body weight, sensitive indicators of the severity of pneumonia (Sakai et al., 2000), were monitored. In contrast to the drug-untreated control group with remarkable loss of body weight and mortality rate as high as 80% on day 10, both nasal inoculation and, especially, inhalation induced beneficial effects on these indicators. Although a simple comparison between these rates should be avoided because the doses used for these administrations were different and the actual concentration reaching the lung after vaporization of CA was not determined, at least it can be stated that inhalation is an effective and safe delivery routes for CA to improve lethal influenza virus-induced pneumonia. Comparing with considerable improvement in these indicators by inhalation, virus growth in the lungs on day 6 was restricted to 1 log reduction. It should be further studied whether this beneficial effect of CA inhalation is due a direct inhibition of virus growth or mediated by host defense. Following a previous report (Akaike et al., 1996), we are presently studying this issue focusing on the nitric oxide in influenza virus-induced pneumonia in mice.

CA is one of major components in the essential oil of Cinnamomi cortex. At the same time, CA has long been known as a flavor compound and the Food and Drug Administration of the United States has confirmed its safe oral intake in rats and mice (National Toxicology Program, 2004), but neither the toxicity of inhaled CA nor its influence on the mucosal epithelium has been clarified. Inhaling vapors of essential oils leads to various biological effects such as a decrease in ACTH level, an increase in plasma gonadotropin levels (Yamada et al., 2005), and anticonvulsive effects (Koo et al., 2004). Our study demonstrated that inhalation of CA did not induce any adverse reactions, but rather showed a beneficial effect on influenza virus-induced pneumonia in mice. At the present time, Kampo medicines are administered only by the oral route. Therefore, this administration might represent a novel approach, with the potential of preventing human influenza.

Antiviral research, in most cases, targets the functions of virus proteins or enzymes. This is also the case with anti-influenza virus drugs. Amantadine is an ion channel inhibitor targeting the M2 protein of influenza A viruses, and oseltamivir is a selective neuraminidase inhibitor for both A and B influenza viruses. These drugs offer powerful inhibition, but their inhibitory effects are exhibited only in sensitive strains and may be inevitably compounded by drug resistance issues. Therefore, further research should also focus on the cellular events responsible for virus growth. We have observed that CA inhibits the growth of influenza strains such as A/USSR/92/77 (H1N1), A/Aichi/2/68, A/Urdon/77 (H3N2), and B (Lee/40 and Singapore/64) by a 1-h treatment initiated at the mid-stage of the growth cycle, without differences in inhibitory efficacy among the strains (data not shown).

In summary, this study has clearly demonstrated that a 1-h treatment with CA could induce complete or partial reduction in virus growth by the inhibition of viral protein synthesis at the mid-stage of the growth cycle *in vitro*. Moreover, the direct application of CA in airways of infected mice led to a significant rescue from lethal influenza virus-induced pneumonia accompanied by a considerable reduction in virus yields in the lungs.

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