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Antipyretic activity of cinnamyl derivatives and related compounds in influenza virus-infected mice

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Abstract

Kakkon-to is composed of seven medicinal herbs and exhibited novel antipyretic activity by suppressing interleukin-1α production responsive to interferon in a murine intranasal influenza virus infection model. Using this model, antipyretic compounds with such novel biological activities were characterized from the herbs. The organic solvent-extractable fractions of *Cinnamomum cassia* among the herbs showed antipyretic activity. We selected six antipyretic compounds from 48 cinnamyl derivatives and related compounds that may be mainly involved in the fractions. Their antipyretic activity was significantly correlated with interleukin-1α regulatory activity. Four of them suppressed interleukin-1α production to a basal level and showed different mode of antipyretic action from that of aspirin in interleukin-1α-injected mice. Structure–bioactivity relationship of the four suggested that an ester bond played an important role for both antipyretic and interleukin-1α regulatory activities. These compounds may be useful in analyzing interleukin-1α-producing cells in fever production and the mechanism of defervescence by suppressing interferon-induced interleukin-1α production. © 1998 Elsevier Science B.V.

Keywords: Medicinal herb; Antipyretic activity; Interleukin-1α; *Cinnamomum cassia*; Kakkon-to; Cinnamyl derivative

1. Introduction

Kakkon-to, a traditional herbal medicine, is composed of seven medicinal herbs (Jiangxu New Medical College, 1978; Nagasaka et al., 1995). This herbal medicine has been used for the treatment of influenza infection and common cold since the ancient time in China. The antipyretic action of Kakkon-to has been recognized as a major benefit in the treatment (Jiangxu New Medical College, 1978; Terasawa, 1993) and it is prescribed more than 20 million doses annually in Japan. We have analyzed the mechanism of fever production in influenza using an influenza virus infection model in *DBA/2 Cr* mouse strain with a high susceptibility for fever in influenza infection and interferon treatment (Kurokawa et al., 1996a). Consequently, we showed the cascade of fever production in influenza infection as follows: influenza virus infection, interferon production, interleukin-1α production, elevated cyclooxygenase activity and prostaglandin E_2 production, fever induction (Kurokawa et al., 1996a). Based on this cascade, Kakkon-to reduces fever production by suppressing the rise of interleukin-1α production subsequent to interferon production caused by influenza virus infection (Kurokawa et al., 1996b). This antipyretic action was different from that of aspirin that shows antipyretic effect by inhibiting cyclooxygenase activity leading to the production of prostaglandin E_2. Thus, Kakkon-to may contain compounds showing such novel antipyretic action.

In this study, we investigated active compounds with antipyretic and interleukin-1α regulatory activities from the components of Kakkon-to by using the murine influenza virus infection model. Four cinnamyl derivatives and related compounds were found to reduce fever production and the rise of interleukin-1α production to the basal level in infected mice significantly. Since cinnamyl derivatives as well as aspirin have been shown to exhibit anti-inflammatory activity (Englberger et al., 1988; Kurup et al., 1989; Murai et al., 1995; Ozaki, 1992; Rampart et al., 1986; Zhang and Ji, 1992), the four compounds were

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consequently suggested to exhibit different mode of anti-inflammatory action from that of aspirin. In fact, we showed that they had different mode of antipyretic action from that of aspirin in interleukin-1α-injected mice. These compounds were suggested to act on cells producing interleukin-1α and reduce the rise of circulating interleukin-1α level to the basal level, resulting in defervescence. Thus, these compounds may be useful in analyzing interleukin-1α-producing cells and the mechanism of defervescence by suppressing interferon-induced interleukin-1α production.

2. Materials and methods

2.1. Preparation of Kakkon-to and its herbal components

Seven dried medicinal herbs in Kakkon-to, *Pueraria pseudo-hirsuta* Tang et Wang (Radix), *Ephedra sinica* Stapf (Cortex), *Zizyphus jujuba* Mill. (Fruit), *Cinnamomum cassia* (Cortex), *Paeonia lactiflora* Pall (Radix), *Glycyrrhiza uralensis* Fisch. (Radix), and *Zingiber officinale* Rosc. (Rhizome), were purchased from Tochimoto Tenkaido, Osaka, Japan. Each hot water-extract of the seven herbs was prepared according to the method as reported previously (Kurokawa et al., 1993; Nagasaka et al., 1995). Briefly, dried herbs (50 g) were boiled in 1000 ml of distilled water for 1 h. The aqueous extract was filtered (No. 2, Toyo Roshi, Japan) and lyophilized. The lyophilized materials were suspended in distilled water at 20 mg ml^{-1}. The suspension of each herbal extract was warmed to 40°C for 15 min and used for oral administration to mice.

2.2. Fractionation of C. cassia

Hot water-extract of *C. cassia* was fractionated to identify antipyretic activity. Fifty milliliters of the extract (20 mg ml^{-1}) was sequentially extracted with the equal volume of ether, chloroform and ethylacetate and then each extract was dried under reduced pressure. The remaining aqueous fraction was lyophilized and the dried material was directly extracted sequentially with 20 ml of 99% ethanol and 50% ethanol in water. These extracts were collected and dried under reduced pressure and/or lyophilized. The remainder was dried under reduced pressure and used as an ethanol-insoluble fraction. All fractions separated were dissolved or suspended in 0.5 ml of dimethylsulfoxide (DMSO) and reconstituted to 50 ml final volume by the addition of distilled water, for oral administration to mice.

2.3. Cinnamyl derivatives and related compounds

Forty eight of cinnamyl derivatives and related compounds (Table 1) were supplied from Tsumura, Japan. Many of them have been identified as the components of *C. cassia* (Jiangxu New Medical College, 1978; Sagara et al., 1987; Tsai and Chen, 1984). Each compound was dissolved in 0.4 ml of DMSO and then diluted with distilled water for oral administration to mice.

Table 1
Cinnamyl derivatives and related compounds

(1) benzaldehyde
(2) vanillin
(3) hydrocinnamic acid
(4) 2′-hydroxy acetophenone[a]
(5) benzoic acid methylester
(6) coumarin
(7) eugenol
(8) cuminaldehyde
(9) guaiacol
(10) salicylaldehyde
(11) methylcinnamate
(12) *p*-hydroxycinnamic acid
(13) 2-hydroxy cinnamic acid[a]
(14) 3-hydroxy cinnamic acid
(15) 4-methoxycinnamic acid
(16) 3,4-methoxycinnamic acid
(17) 3,4-dihydroxycinnamic acid
(18) *p*-methylcinnamic acid
(19) 2,5-dimethoxy cinnamic acid
(20) 2,3-dimethoxy cinnamic acid
(21) 3,5-dimethoxy cinnamic acid
(22) 2,4-dimethoxy cinnamic acid
(23) α-cianocinnamic acid
(24) 3-hydroxy-4-methoxycinnamic acid
(25) 4-hydroxy-3,5-dimethoxycinnamic acid
(26) 3,4,5-trimethoxycinnamic acid
(27) 4-formyl cinnamic acid
(28) 3,4-(methylenedioxy) cinnamic acid
(29) *trans*-(3-pyridyl)acryl acid
(30) α-methylcinnamaldehyde
(31)7-hydroxycoumarin[a]
(32) benzylalcohol
(33) benzoic acid
(34) *o*-anisaldehyde
(35) 4-allylanisole[a]
(36) phenethyl acetate
(37) methyleugenol
(38) eugenolacetate
(39) phenylacetaldehyde
(40) furfural
(41) 2-methoxycinnamaldehyde
(42) 1,2-dimethoxy-4-allyl benzene
(43) 3-(2-methoxyphenyl)-propionic acid
(44) geraniol
(45) cinnamic acid ethylester[a]
(46) safrole
(47) acetic acid cinnamyl ester[a]
(48) benzoic acid benzylester

[a]Compounds showing antipyretic activity and suppressive activity of interleukin-1α production.

2.4. Animals

Female *DBA/2 Cr* mice (6-week-old, 17–19 g) were purchased from Sankyo Labo, Japan. The mice were housed

five per cage in a temperature-controlled room, with food and water ad libitum and under a 12 h light/12 h dark diurnal cycle (light at 0700 hours). The temperature in the room was kept at 24 ± 2°C. The mice were acclimated for at least 4 days before starting any experimental procedure. The animal experimentation guidelines of Toyama Medical and Pharmaceutical University were followed in animal studies.

2.5. Mouse influenza virus infection model

Mouse-adapted influenza virus (A/PR/8/34 (H1N1)) was prepared from the lungs of infected mice as described previously (Kurokawa et al., 1990, 1996a). Mice were intranasally infected or mock-infected with 2000–3000 plaque forming units of influenza virus under ether anesthesia. Hot water-extract of each herbal component (5 mg 0.25 ml^{-1} $mouse^{-1}$) or water (0.25 ml) was orally applied by gavage to the mice 3 times daily (approximately 8-h interval) for 4 days starting a day before infection. Since Kakkon-to of 750 mg kg^{-1} day^{-1} for mice corresponds to dose for human use and was effective in reducing fever in infected mice (Kurokawa et al., 1996b), the dose of each herbal component was used as its maximum dose for mice in this experiment. Five to ten mice were used in each group. The rectal temperature was monitored by a thermometer (Sato Keiryoki, Japan) at 42–46 h after infection, at which times fever has been shown to be produced in the murine model previously (Kurokawa et al., 1996a,b). After the measurement of rectal temperature, sera were prepared from four to five mice in each group under ether anesthesia and interleukin-1α concentrations in sera were determined by the enzyme-linked immunosorbent assay (ELISA) using ELISA kits for mouse interleukin-1α (Genzyme, USA) according to the manufacturer's instructions.

The fractions of *C. cassia* (0.25 ml $mouse^{-1}$) and cinnamyl derivatives and related compounds (4.7–14.1 mg 0.25 ml^{-1} kg^{-1}) were orally administered to evaluate their possible antipyretic activity as described above. The doses of compounds for mice were used as dose corresponding to the dose of aspirin which was calculated from doses for human use and exhibited antipyretic activity in mice as described previously (Kurokawa et al., 1996a). The rectal temperature of each mouse was monitored and interleukin-1α concentrations in sera were determined as described above.

2.6. Interleukin-1α-injected mouse model

Antipyretic activity of aspirin, cinnamyl derivatives and related compounds was examined in mice injected intravenously with interleukin-1α to evaluate the mode of their antipyretic action. Recombinant mouse interleukin-1α (6.3×10^7 units mg^{-1}, Genzyme) was dissolved in pyrogen-free saline and intravenously injected to mice at 20 and 150 ng 0.1 ml^{-1} $mouse^{-1}$. Control mice received 0.1 ml of pyrogen-free saline. Aspirin (12.5 mg 0.25 ml^{-1} kg^{-1}), cinnamyl derivatives and related compounds (12.5 mg 0.25 ml^{-1} kg^{-1}) were applied by gavage to the mice 3 times (approximately 8-h interval) in a day before injection. They were further administered to mice 60 min before injection the next morning (at 0900 to 1000 hours). Water (0.25 ml) was used as the control. The dose of compounds for mice was used as dose which showed antipyretic activity in influenza virus infection model in mice. Since this dose of aspirin was effective in reducing rectal temperature of influenza virus-infected mice (Kurokawa et al., 1996a), the similar dosage was adopted in this experiment. The rectal temperature of each mouse was monitored 30 min before injection and 30, 60 and 120 min after injection.

2.7. Statistical analysis

Student's *t*-test was used to evaluate the statistical significance of differences between two groups in rectal temperatures and the concentrations of interleukin-1α in sera. Correlation between antipyretic activity and regulatory activity of interleukin-1α production was statistically evaluated using Pearson's correlation coefficient and the significance was calculated using Fisher's r to z. A P value of less than 0.05 was statistically defined as significant.

3. Results

3.1. Effects of herbal components of Kakkon-to on fever and interleukin-1α production

Seven herbs of Kakkon-to were examined for their antipyretic activity and regulatory activity of interleukin-1α production in a murine influenza virus infection model. Among the seven, *C. cassia*, *Zin. officinale* and *G. uralensis* significantly suppressed fever production compared with infected mice with water-administration and the suppression by *C. cassia* was strongest among the three herbs (Fig. 1a). *C. cassia*, *Zin. officinale*, *E. sinica* and *P. pseudo-hirsuta* suppressed interleukin-1α production significantly compared with infected mice with water-administration. (Fig. 1b). Thus *C. cassia* and *Zin. officinale* were effective to reduce fever production and interleukin-1α production in influenza virus-infected mice.

3.2. Antipyretic activity of C. cassia fractions

Since *C. cassia* exhibited the strongest antipyretic activity among the seven herbs (Fig. 1a), this was further fractionated by sequential extractions with organic solvents to evaluate the properties of possible antipyretic com-

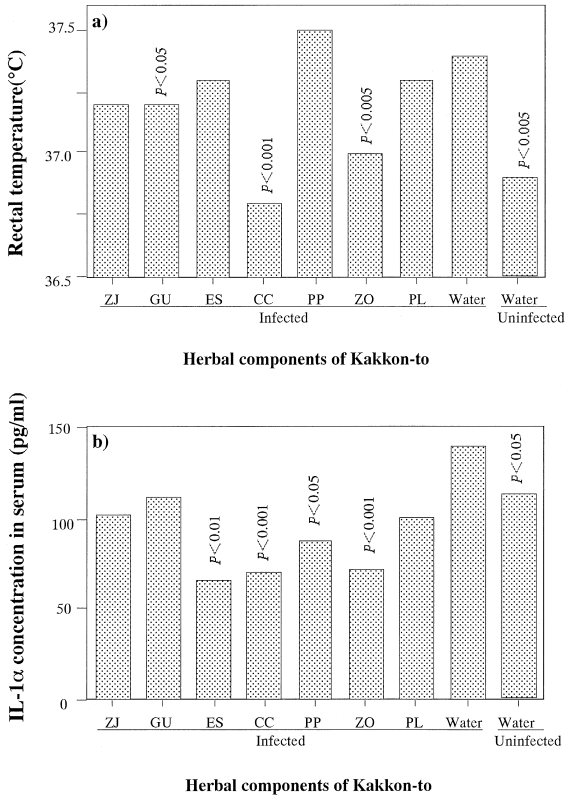

Fig. 1. Effects of seven herbs of Kakkon-to on fever production (a) and on interleukin-1α production (b) in influenza virus-infected mice. *DBA / 2 Cr* mice [10 mice (a) and 5 mice (b) in each group] were intranasally infected or mock-infected (uninfected) with influenza virus. Hot water-extract of each herb or water was administered orally to the mice. The rectal temperature was monitored 42–46 h after infection and interleukin-1α concentrations in sera were determined by ELISA kits as described in the text. ZJ, GU, ES, CC, PP, ZO and PL represent the hot-water extract of *Z. jujuba*, *G. uralensis*, *E. sinica*, *C. cassia*, *P. pseudo-hirsuta*, *Zin. officinale* and *Pae. lactiflora*, respectively. *P* values indicate statistical significance vs. infected mice with water administration by the Student *t*-test.

pounds in *C. cassia*. The fractions separated were examined for their antipyretic activity in the murine infection model. As shown in Fig. 2, fractions extracted with chloroform, 99% ethanol and 50% ethanol significantly reduced rectal temperatures of infected mice compared with infected mice with water-administration. Thus, these organic fractions mainly involved possible antipyretic components in *C. cassia*.

3.3. Antipyretic activity of cinnamyl derivatives and related compounds

Many cinnamyl derivatives and related compounds have been isolated and identified from the organic fractions of *C. cassia* elsewhere (Jiangxu New Medical College, 1978; Price et al., 1979; Sagara et al., 1987; Tsai and Chen, 1984; Wen et al., 1992). Thus, we screened the antipyretic activity of such 48 compounds in infected mice. Among the 48 (Table 1), six compounds (7-hydroxycoumarin, 4-allylanisole, cinnamic acid ethylester, acetic acid cinnamylester, 2′-hydroxyacetophenone and 2-hydroxycinnamic acid, Fig. 5) markedly reduced the rectal temperature of infected mice and the mean temperature levels were similar to or less than that of uninfected mice (data not shown). There was no significant weight loss of mice administered with each of the six compounds for 3 days after infection (data not shown). Hence we selected them as possible antipyretic compounds. Further these compounds were simultaneously examined for correlation between their antipyretic and interleukin-1α regulatory activities. As shown in Fig. 3, the rectal temperatures and interleukin-1α concentrations in infected mice with compound- and water-administration and uninfected mice with water-administration correlated significantly (correlation, 0.5437; P, 0.0003 by Fisher's r to z). All six compounds selected significantly reduced fever production in infected mice and their antipyretic activity was confirmed. Among the 6, four compounds (7-hydroxycoumarin, 4-allylanisole, cinnamic acid ethylester and acetic acid cinnamylester) were significantly effective in suppressing not only the rectal temperature of infected mice but also the rise of interleukin-1α production to the basal level in the serum (Fig. 3). Although the suppression of responsive interleukin-1α production by the other two compounds (2′-hydroxyacetophenone and 2-hydroxycinnamic acid) was not significant, the levels of interleukin-1α production in infected mice treated with them were similar to that in untreated mice (Fig. 3). These two compounds showed weaker interleukin-1α regulatory activity and antipyretic activity than the other four compounds. The six compounds selected, especially four among the six, simultaneously exhibited antipyretic and interleukin-1α regulatory activities in influenza virus-infected mice.

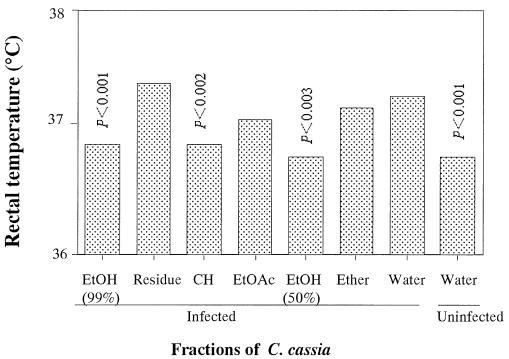

Fig. 2. Effects of the fractions of *C. cassia* on fever production in influenza virus-infected mice. *DBA / 2 Cr* mice (five mice in each group) were intranasally infected or mock-infected (uninfected) with influenza virus. The fractions or water was administered orally to the mice and the rectal temperature was monitored at 42–46 h after infection as described in the text. EtOH and CH represent ethanol and chloroform, respectively. *P* values indicate statistical significance vs. infected mice with water administration by the Student *t*-test.

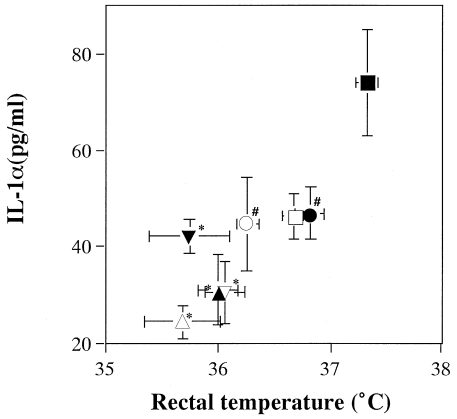

Fig. 3. Correlation between antipyretic and interleukin-1α-suppressive activities of six cinnamyl derivatives and related compounds (▼, 7-hydroxycoumarin; △, 4-allylanisole; ▽, cinnamic acid ethylester; ▲, acetic acid cinnamylester; ○, 2～-hydroxyacetophenone; ●, 2-hydroxycinnamic acid; ■, infected mice with water-administration; □, uninfected mice with water administration). *DBA/2 Cr* mice (five mice in each group) were intranasally infected or mock-infected (uninfected) with influenza virus. The compounds were administered orally to the mice and the rectal temperature was monitored at 42–46 h after infection as described in the text. Interleukin-1α concentrations in sera were determined by ELISA kits as described in the text. $* P < 0.05$ vs. both rectal temperature and interleukin-1α concentration of infected mice with water administration. $^{\#}P < 0.05$ vs. only rectal temperature of infected mice with water administration. Horizontal and vertical bars indicate the range of standard error.

3.4. Comparison of antipyretic activity of aspirin and cinnamyl derivatives and related compounds in interleukin-1α-injected mice

Aspirin and the four compounds (7-hydroxycoumarin, 4-allylanisole, cinnamic acid ethylester and acetic acid cinnamylester) were examined for antipyretic activity in mice with intravenous injection of interleukin-1α to evaluate the mode of their antipyretic action. Mean rectal temperatures significantly increased after interleukin-1α injection at the two different doses (20 and 150 ng) in water-administered mice compared with mice with saline-injection ($P < 0.01$ for mice with 20 ng of interleukin-1α 30 min after injection and $P < 0.05$ for mice with 150 ng of interleukin-1α 60 min after injection). In mice administered with 4-allylanisole, cinnamic acid ethylester and acetic acid cinnamylester, the mean rectal temperatures rose significantly after injection at both doses compared with water-administered mice ($P < 0.01$ or 0.05 for 30 or 60 min after injection). The mean rectal temperature also rose in mice injected with 150 ng of interleukin-1α and administered with 7-hydroxycoumarin ($P < 0.05$ for 30 min after injection), although no significant rise was observed in mice injected with 20 ng of interleukin-1α. However no such higher mean temperature changes were observed in mice administered with aspirin. Aspirin and the four compounds exhibited different activity on fever in interleukin-1α-injected mice.

4. Discussion

We examined the antipyretic and interleukin-1α regulatory activities of medicinal herbs using an influenza virus infection model in mice. Among the herbs, *C. cassia* exhibited the strongest antipyretic activity and also suppressed the rise of interleukin-1α production to the basal level in infected mice significantly (Fig. 1). Its organic solvent-extractable fractions showed significant antipyretic activity (Fig. 2). This herb has been shown to contain cinnamyl derivatives and related compounds which can be extracted with organic solvents such as ethanol, chloroform, etc. and which are representative of the biological activity of *C. cassia* (Jiangxu New Medical College, 1978; Sagara et al., 1987; Tanaka et al., 1987; Terasawa, 1993; Tsai and Chen, 1984). We screened possible antipyretic compounds from the cinnamyl derivatives and related compounds. When fever was induced in influenza virus-infected mice, the level of interleukin-1α rose markedly in serum but those of interferon-γ and tumor necrosis factor-a were not affected (Kurokawa et al., 1996a). Kakkon-to suppressed only interleukin-1α production and reduced fever, although interferon activity and the levels of interferon-γ and tumor necrosis factor-α in serum were not affected (Kurokawa et al., 1996b). Therefore, we have focused on the level of interleukin-1α in serum and evaluated the relationship between fever and the level of interleukin-1α by cinnamyl derivatives and related compounds in influenza virus-infected mice.

We found that six cinnamyl derivatives and related components had significant antipyretic activity and that four of them also exhibited interleukin-1α regulatory activity significantly in this study. Cinnamyl derivatives and related compounds as well as aspirin have been shown to exhibit anti-inflammatory activity (Englberger et al., 1988; Kurup et al., 1989; Murai et al., 1995; Ozaki, 1992; Rampart et al., 1986; Zhang and Ji, 1992). However aspirin did not reduce the level of interleukin-1α production in infected mice significantly (Kurokawa et al., 1996a). Since there was a correlation between antipyretic and interleukin-1α regulatory activities of the six compounds (Fig. 3), their antipyretic activity may be due to the suppression of responsive interleukin-1α production. Thus, such mode of anti-inflammatory action by these compounds may be characteristically different from that of aspirin which inhibits cyclooxygenase activity but does not suppress interleukin-1α production.

Intranasal influenza infection in mice causes cellular infiltration of the respiratory tract (Hennet et al., 1992; Hurd and Heath, 1975; Sullivan et al., 1976). In our previous study, the levels of interleukin-1α rose markedly in the bronchoalveolar lavage fluid of lungs of mice after influenza virus infection (Kurokawa et al., 1996a), indicating that strong inflammation might occur locally in the lungs of infected mice. The rise of interleukin-1α level, as an endogenous pyrogen, was also observed in the serum of

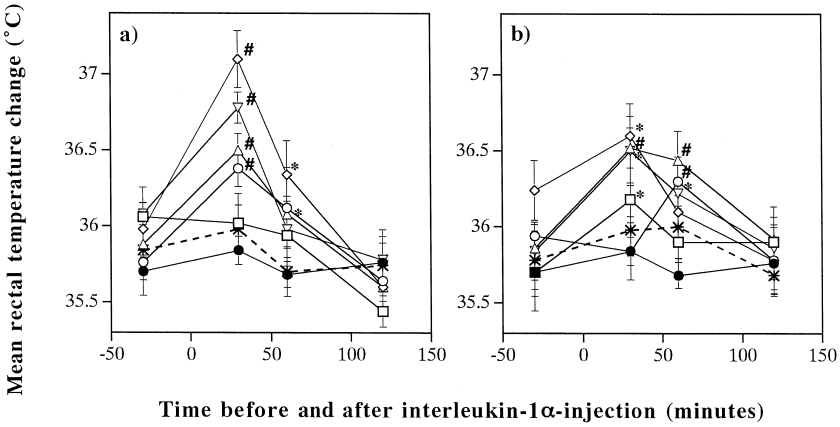

Fig. 4. Antipyretic activity of aspirin and cinnamyl derivatives and related compounds in mice injected intravenously with interleukin-1α: (a) 20 ng mouse^{-1}; (b) 150 ng mouse^{-1}). Aspirin (−) and the four compounds (□, 7-hydroxycoumarin; ◇, 4-allylanisole; △, cinnamic acid ethylester; ▽, acetic acid cinnamylester) were administered orally to *DBA / 2 Cr* mice (five mice in each group) and interleukin-1α was intravenously injected to the mice as described in the text. In water-administered mice, interleukin-1α (○) and saline (●) were injected. The rectal temperature was monitored at 30 min before and 30, 60 and 120 min after injection. $* P < 0.05$ vs. water-administered mice with saline-injection. $^{\#}P < 0.01$ vs. water-administered mice with saline-injection. Horizontal bars indicate the range of standard error.

infected mice. Since circulating endogenous pyrogens are suggested to interact with sensory elements in the brain and induce febrile response (Blatteis, 1992; Hashimoto et al., 1991; Saper and Breder, 1992), interleukin-1α could be a possible endogenous pyrogen that produces fever in influenza infection. As shown in Fig. 4, aspirin showed antipyretic activity in interleukin-1α-injected mice but four cinnamyl derivatives and related compounds selected did not as expected. This indicates that the compounds have different mode of antipyretic action from that of aspirin. Thus the four compounds might act on the cells producing interleukin-1α and reduce the level of circulating interleukin-1α, resulting in defervescence.

We have demonstrated the structure–bioactivity relationship of cinnamyl derivatives and related compounds (Fig. 5), indicating the important structural requirement of these compounds for antipyretic and interleukin-1α regulatory activities in influenza infection. Among the four compounds with both antipyretic and interleukin-1α regulatory activities, acetic acid cinnamylester and cinnamic acid ethylester are ester compounds, but any other cinnamyl derivatives and related compounds examined in this study are not. 7-Hydroxycoumarin among the four is also a compound that involves possible ester bond in the ring. The ester bond may play a significant role in the interaction with cells producing interleukin-1α to suppress responsive interleukin-1α production in serum. Thus the six cinnamyl derivatives and related compounds, especially four among them, selected may be useful to analyze interleukin-1α-producing cells and the mechanism of defervescence by suppressing interferon-induced interleukin-1α production.

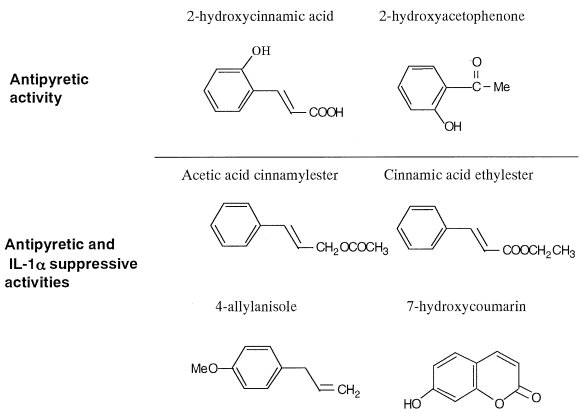

Fig. 5. Relationship between structure and bioactivity of cinnamyl derivatives and related compounds.

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References

Blatteis, C.M., 1992. Role of the OVLT in the febrile response to circulating pyrogens. Prog. Brain Res. 91, 409–412.

Englberger, W., Hadding, U., Etschenberg, E., Graf, E., Leyck, S., Winkelmann, J., Parnham, M.J., 1988. Rosmarinic acid: a new inhibitor of complement C3-convertase with anti-inflammatory activity. Int. J. Immunopharmacol. 10, 729–737.

Hashimoto, M., Ishikawa, Y., Yokota, S., Goto, F., Bando, T., Sakakibara, Y., Iriki, M., 1991. Action site of circulating interleukin-1 on the rabbit brain. Brain Res. 540, 217–223.

Hennet, T., Ziltener, H.J., Frei, K., Peterhans, E., 1992. A kinetic study of immune mediators in the lungs of mice infected with influenza A virus. J. Immunol. 149, 932–939.

Hurd, J., Heath, R.B., 1975. Effect of cyclophosphamide on infections in mice caused by virulent and avirulent strains of influenza virus. Infect. Immun. 11, 886–889.

Jiangxu New Medical College, 1978. Dictionary of Chinese Medicinal Materials. Shanghai Science and Technology Press, Shanghai, China (in Chinese).

Kurokawa, M., Ochiai, H., Nakajima, K., Niwayama, S., 1990. Inhibitory effect of protein kinase C inhibitor on the replication of influenza type A virus. J. Gen. Virol. 71, 2149–2155.

Kurokawa, M., Ochiai, H., Nagasaka, K., Neki, M., Xu, H., Kadota, S., Sutardjo, S., Matsumoto, T., Namba, T., Shiraki, K., 1993. Antiviral traditional medicines against herpes simplex virus (HSV-1), poliovirus, and measles virus in vitro and their therapeutic efficacies for HSV-1 infection in mice. Antiviral Res. 22, 175–188.

Kurokawa, M., Imakita, M., Kumeda, C.A., Shiraki, K., 1996a. Cascade of fever production in mice infected with influenza virus. J. Med. Virol. 50, 152–158.

Kurokawa, M., Imakita, M., Kumeda, C.A., Yukawa, T.A., Shiraki, K., 1996b. Kakkon-to suppressed interleukin-1α production responsive to interferon and alleviated influenza infection in mice. J. Tradition. Med. 13, 201–209.

Kurup, A., Kumar, A.V., Rao, M.N., 1989. Anti-inflammatory activity of cinnamic acids. Pharmazie 44, 870.

Murai, M., Tamayama, Y., Nishibe, S., 1995. Phenylethanoids in the herb *Plantago lanceolata* and inhibitory effect on arachidonic acid-induced mouse ear edema. Planta Med. 61, 479–480.

Nagasaka, K., Kurokawa, M., Imakita, M., Shiraki, K., 1995. Efficacy of Kakkon-to, a traditional herb medicine, in herpes simplex virus type 1 infection in mice. J. Med. Virol. 46, 28–34.

Ozaki, Y., 1992. Anti-inflammatory effect of tetramethylpyrazine and ferulic acid. Chem. Pharm. Bull. 40, 954–956.

Price, P.W. Jr., Edens, R., Hendrix, D.L., Deming, S.N., 1979. Optimized reverse-phase high-performance liquid chromatographic separation of cinnamic acids and related compounds. Anal. Biochem. 93, 227–233.

Rampart, M., Beetens, J.R., Bult, H., Herman, A.G., Parnham, M.J., Winkelmann, J., 1986. Complement-dependent stimulation of prostacyclin biosynthesis: inhibition by rosmarinic acid. Biochem. Pharmacol. 35, 1397–1400.

Segara, K., Oshima, T., Yoshida, T., Tong, Y.Y., Zhang, G., Chen, Y.H., 1987. Determination of cinnamomi cortex by high-performance liquid chromatography. J. Chromatogr. 409, 365–370.

Saper, C.B., Breder, C.D., 1992. Endogenous pyrogens in the CNS: role in the febrile response. Prog. Brain Res. 93, 419–429.

Sullivan, J.L., Mayner, R.E., Barry, D.W., Ennis, F.A., 1976. Influenza virus infection in nude mice. J. Infect. Dis. 133, 91–94.

Tanaka, S., Yoon, Y.H., Fukui, H., Tabata, M., Akira, T., Okano, K., Iwai, M., Iga, Y., Yokoyama, K., 1987. Antiulcerogenic compounds isolated from Chinese cinnamon. Planta Med. 55, 245–248.

Terasawa, K., 1993. KAMPO, K.K. Standard McIntyre, Tokyo, pp. 101–110.

Tsai, S.Y., Chen, S.C., 1984. A fluorometric assay of *trans*-cinnamaldehyde in cinnamon. J. Nat. Prod. 47, 536–538.

Wen, K.C., Huang, C.Y., Liu, F.S., 1992. Determination of cinnamic acid and paeoniflorin in traditional chinese medicinal preparations by high-performance liquid chromatography. J. Chromatogr. 593, 191–199.

Zhang, L.P., Ji, Z.Z., 1992. Synthesis, anti-inflammatory and anticancer activity of cinnamic acids, their derivatives and analogues. Yao Hsueh Hsueh Oao (Acta Pharm. Sinica) 27, 817–823.