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Effect of a new chemoprotective agent, 2-(allylthio)pyrazine, on the pharmacokinetics of intravenous theophylline in rats

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Abstract

The effect of 2-(allylthio)pyrazine (2-AP) pretreatment on the pharmacokinetics of theophylline and its metabolites was investigated after 1-min intravenous administration of aminophylline, 5 mg/kg as theophylline, to rats pretreated with three consecutive daily oral administration of 2-AP, 100 mg/kg. The $AUC_{0-2\ h}$ of a metabolite of theophylline, 1,3-dimethyluric acid, 1,3-DMU (62.3 versus 106 μg min/ml), and the percentages of intravenous dose of theophylline excreted in 24-h urine as 1,3-DMU (12.4% versus 20.8%, expressed in terms of theophylline) decreased significantly in 2-AP-pretreated rats when compared with those in control rats. Since CYP1A2 and CYP2E1 are involved in the formation of 1,3-DMU from theophylline, and 2-AP considerably suppressed CYP2E1 and tended to suppress CYP1A2 in rats, decreased formation of 1,3-DMU in 2-AP-pretreated rats could be mainly due to suppression of the CYP2E1 expression by pretreatment with 2-AP. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: 2-(Allylthio)pyrazine; Theophylline; Rats

1. Introduction

Diallylsulfide (a component of *Allium sativum*) has a chemoprotective activity and pyrazine has a binding affinity to cytochrome P4502E1 (inhibits CYP2E1 activity). Therefore, pyrazine was attached to the allylsulfide radical of diallylsulfide to form 2-(allylthio)pyrazine (2-AP, Fig. 1) to increase binding affinity of 2-AP to CYP2E1. In 2-AP-pretreated rats, the percentages of intravenous dose of acetaminophen excreted in both 8-h bile and 24-h urine as acetaminophen-glucuronide conjugate increased significantly, however, the values in 24-h urine as

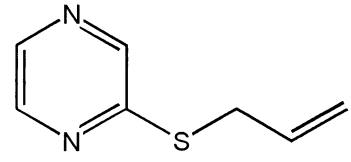

Fig. 1. Chemical structure of 2-AP.

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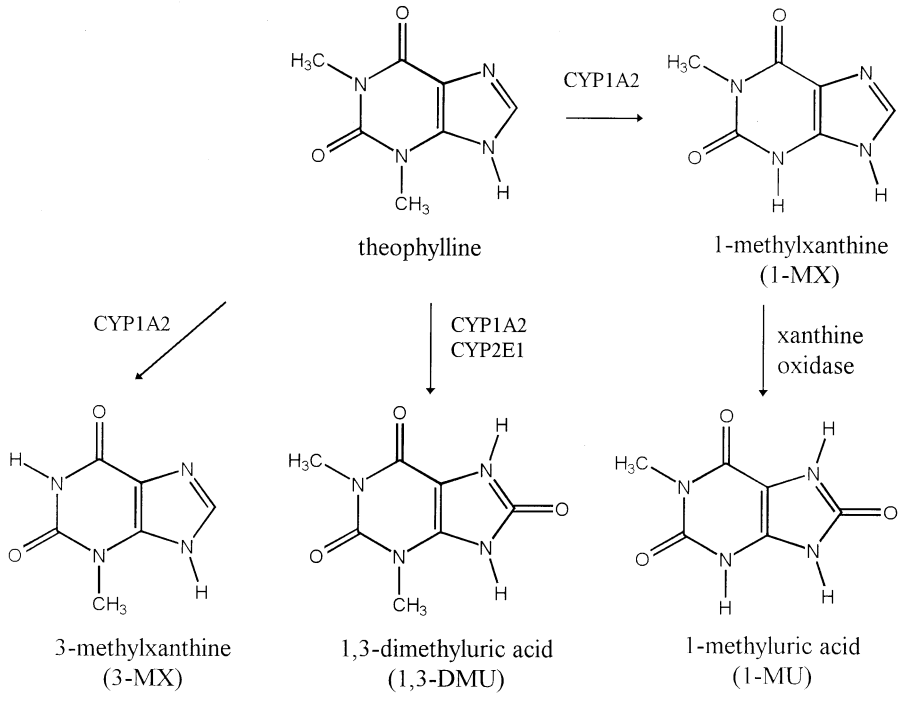

Fig. 2. Theophylline biotransformation (based on Teunissen et al., 1985).

acetaminophen-glutathione and acetaminophen-cysteine conjugates decreased significantly compared with those in control rats (Kwak et al., 1998). This might be due to an increase in uridine diphosphoglucuronyl transferase activities and suppression of CYP2E1 expression by pretreatment with 2-AP (Kwak et al., 1998). 2-AP showed hepatoprotective effect by inhibiting CYP2E1 expression (Kim et al., 1997), and hepatoprotective and radioprotective effects by enhancing expression of detoxifying enzymes such as hepatic microsomal epoxide hydrolase and cytosolic glutathione *S*-transferase A3 (Nam, 1998). 2-AP also showed chemopreventive effect on vinyl carbamate- or vinyl carbamate epoxide-induced hepatotoxicity, mutagenicity, and tumorigenicity (Surh et al., 1997), and hepatoprotective effect on retinoyl palmitate- and pyridine-potentiated carbon tetrachloride-induced hepatotoxicity (Kim et al., 1996). 2-AP is under preclinical study as a chemoprotective agent to prevent the development of cancer.

Parenteral theophylline has been considered the treatment of choice for the management of acute bronchospasm associated with asthma or chronic obstructive pulmonary disease. Theophylline has poor relationship between intravenous dose and plasma (serum) concentration because of great intersubject variations in clearance. A clear and definitive relationship between plasma (serum) concentrations and the severity of toxic effects may not be well defined. Theophylline toxicity can result in serious morbidity and mortality. Therefore, it is desirable to monitor the plasma (serum) concentrations of theophylline to increase desirable effects and to decrease side (toxic) effects.

In humans, theophylline is almost entirely (90%) metabolized in the liver by the hepatic mixed-function oxidase system (Ogilvie, 1978) to form 3-methylxanthine (3-MX) by CYP1A2, 1-methylxanthine (1-MX) by CYP1A2, and 1,3-dimethyluric acid (1,3-DMU) by CYP1A2 and CYP2E1 (Fig. 2). 1-MX undergoes further oxidation by xanthine oxidase to form 1-methyluric acid (1-MU; Fig. 2). After intravenous adminis-

tration of theophylline, 1.3–20 mg/whole body weight, to rats, the major metabolites excreted in 24-h urine were 1,3-DMU (14.4–30.2% of theophylline dose) and 1-MU (11.8–26.4% of theophylline dose; Teunissen et al., 1985). Theophylline was reported (McManus et al., 1988) to be metabolized by multiple forms of cytochrome P450s in human, rabbit, and rat liver microsomes. It was reported (Rasmussen et al., 1995) that CYP1A2 and CYP2E1 were equally important isoforms for the formation of 1,3-DMU from theophylline in human liver microsomes. It was also reported (Sarkar et al., 1992) that the formation of 1-MX and 3-MX from theophylline correlated with CYP1A2 in human microsomes. 2-AP considerably suppressed CYP2E1 expression (Kim et al., 1997) and tended to suppress CYP1A2 in rats (unpublished data). Therefore, pretreatment with 2-AP could change the formation of 1,3-DMU due to inhibition of CYP2E1 after intravenous administration of theophylline to rats.

The purpose of this paper is to report the effect of 2-AP pretreatment on the pharmacokinetics of theophylline and its metabolites after intravenous administration of aminophylline to rats.

2. Materials and methods

2.1. Chemicals

Aminophylline intravenous solution (250 mg/10 ml ample) was a product of Daewon (Seoul, South Korea). 1,3-DMU, 1-MU, 3-MX, and β-hydroxyethyltheophylline (the internal standard of high-performance liquid chromatography (HPLC) assay) were purchased from Sigma (St. Louis, MO, USA). 2-AP was kindly donated by Bukwang (Seoul, South Korea). Other chemicals were of reagent grade or HPLC grade, and used without further purification.

2.2. Pretreatment of rats

Male Sprague–Dawley rats, 250–290 g, were purchased from Charles River Company (Atsugi, Japan). The rats were randomly divided in two groups; the control group and 2-AP-pretreated group. 2-AP (dissolved in 40% polyethylene glycol 400), 100 mg/kg, was administered orally (total oral volume was approximately 1 ml) for consecutive 3 days for 2-AP-pretreated rats. For the control rats, an equal volume of 40% polyethylene glycol was administered orally. In the morning of the fourth day, the carotid artery and the jugular vein were cannulated with polyethylene tube (Clay Adams, Parsippany, NJ, USA) for blood sampling and drug administration, respectively, under light ether anesthesia. Both cannulae were exteriorized to the dorsal side of the neck and connected individually with long Silastic tube (Dow Corning, Midland, MI, USA). Both Silastic tubes were covered with a wire to allow free movement of the rat. The exposed areas were surgically sutured. Each rat was housed individually in a rat metabolic cage (Daejong, Seoul, South Korea) and allowed 4–5 h to recover from anesthesia before the commencement of the experiment. They were not restrained at any time during the study. Heparinized 0.9% NaCl injectable solution (20 U/ml), 0.3 ml, was used to flush each cannula to prevent blood clotting.

2.3. Intravenous infusion study

Aminophylline, 5 mg/kg as theophylline, was intravenously infused over 1 min via the jugular vein to the control ($n = 8$) and 2-AP-pretreated ($n = 8$) rats. Total infusion volume was 1 ml. Blood samples (0.12 ml) were collected via the carotid artery at 0 (to serve as a control), 1 (at the end of the infusion), 5, 15, 30, 45, 60, 90, 120, 180, 240, 300, and 360 min after intravenous administration. Each blood sample was centrifuged immediately and a 50-μl aliquot of each plasma sample was stored in the −20°C freezer until HPLC analysis of theophylline and its metabolites (Kwaskatsu et al., 1989). At the end of 24 h, each rat was exsanguinated and sacrificed by cervical dislocation. At the same time, the metabolic cage was rinsed with 10 ml of distilled water. The rinsings were combined with the 24-h urine sample. After measuring the exact volume of the combined urine sample, an aliquot of the combined urine sample was frozen until HPLC analy-

sis of theophylline and its metabolites (Kwaskatsu et al., 1989).

2.4. HPLC analysis of theophylline

The concentrations of theophylline and its metabolites in the biological samples above were analyzed by the reported HPLC method (Kwaskatsu et al., 1989); a 300-μl aliquot of acetonitrile (containing 2 μg/ml of the internal standard, β-hydroxyethyl theophylline) was added to a 50-μl aliquot of plasma and/or urine sample. After vortex-mixing and centrifugation, a 300-μl aliquot of supernatant was evaporated under N_2 gas. The residue was reconstituted with a 100-μl aliquot of the mobile phase and a 50-μl aliquot of the supernatant was injected directly onto the HPLC column. The mobile phase, 10 mM acetate buffer (pH 5.0):acetonitrile:tetrahydrofuran (94:5:1, v/v/v) was run at a flow rate of 1.0 ml/min and the column effluent was monitored by a UV detector set at 280 nm. The detection limits for theophylline, 1,3-DMU, and 1-MU in both rat plasma and urine were 100, 100 and 200 ng/ml, respectively. The coefficients of variation of the assay (within- and between-day) were generally low (below 8.90%).

2.5. Pharmacokinetic analysis

The total area under the plasma concentration-time curve from time zero to time infinity (AUC, for theophylline) or to the last measured time in plasma ($AUC_{0-2\ h}$, for 1,3-DMU) was calculated by the trapezoidal rule method (Kim et al., 1993); this method employed the logarithmic trapezoidal rule (Chiou, 1978) for the calculation of the area during the declining plasma-level phase, and the linear trapezoidal rule for the rising plasma-level phase. The area from the last data point to time infinity (in the calculation of AUC) was estimated by dividing the last measured plasma concentration by the terminal rate constant.

A standard method (Gibaldi and Perrier, 1982) was used to calculate the following pharmacokinetic parameters; the time-averaged total body clearance (CL), the area under the first moment of the plasma concentration-time curve (AUMC), the mean residence time (MRT), the apparent volume of distribution at steady state (V_{SS}), and the time-averaged renal (CL_R) and non-renal (CL_{NR}) clearances (Kim et al., 1993).

The harmonic mean method was employed for the calculation of the mean values of terminal half-life (Eatman et al., 1977), each clearance (Chiou, 1980), and V_{SS} (Chiou, 1979).

2.6. Statistical analysis

A P value of less than 0.05 was considered to be statistically significant using unpaired t-test. All data are expressed as mean ± standard deviation.

3. Results and discussion

Many investigators have reported the dose-dependent metabolic disposition of theophylline in humans (Lesko, 1979; Birkett et al., 1984; Massey et al., 1984) and in rats (Teunissen et al., 1985). Therefore, theophylline, 5 mg/kg, which has been reported to be in the range of linear pharmacokinetics in rats (Teunissen et al., 1985), was administered intravenously to rats. The mean arterial plasma concentration-time profiles of theophylline and 1,3-DMU after intravenous administration of theophylline, 5 mg/kg, to control ($n = 8$) and 2-AP-pretreated ($n = 8$) rats are shown in Fig. 3, and the relevant pharmacokinetic parameters are listed in Table 1. After intravenous administration, the plasma concentrations of theophylline declined in a polyexponential fashion for both groups of rats (Fig. 3) with mean terminal half-lives of 84.2 min for the control rats and 61.4 min for the 2-AP-pretreated rats (Table 1).

In 2-AP-pretreated rats, the plasma concentrations of 1,3-DMU were significantly lower from 60 min after intravenous administration of theophylline than those in control rats (Fig. 3). This resulted in a significant decrease in $AUC_{0-2\ h}$ of 1,3-DMU (62.3 versus 106 μg min/ml) and the percentages of intravenous dose of theophylline excreted in 24-h urine as 1,3-DMU (12.4 versus 20.8%, expressed in terms of theophylline) suggesting that the formation of 1,3-DMU from

theophylline decreased significantly in 2-AP-pretreated rats. Since CYP1A2 and CYP2E1 are equally important isoforms for the formation of 1,3-DMU from theophylline (Rasmussen et al., 1995), and 2-AP considerably suppressed CYP-2E1 expression and tended to suppress CYP1A2 in rats (the IC_{50} values were 12 μM and 15 mM for CYP2E1 and CYP1A2, respectively), decreased formation of 1,3-DMU in 2-AP-pretreated rats could be mainly due to suppression of CYP2E1 expression by pretreatment with 2-AP (Kim et al., 1997).

The V_{SS} of theophylline decreased significantly (370 versus 517 ml/kg) in 2-AP-pretreated rats, and this resulted in a significant decrease in both terminal half-life (61.4 versus 84.2 min) and MRT (78.2 versus 116 min; Table 1). Studies previously conducted in rats, 2-AP had a high affinity to rat tissues; the V_{SS} of 2-AP was considerably high, 4580–5890 ml/kg after intravenous administration of 2-AP, 50–100 mg/kg to rats (Han and Lee, 1999). A decrease in the V_{SS} of theophylline in 2-AP-pretreated rats could be a result of the ability of 2-AP to decrease the tissue binding of theophylline. Similar results were also reported with 8-methoxysoralen (Apseloff et al., 1990) and chloroquine (Motti and Ashton, 1998).

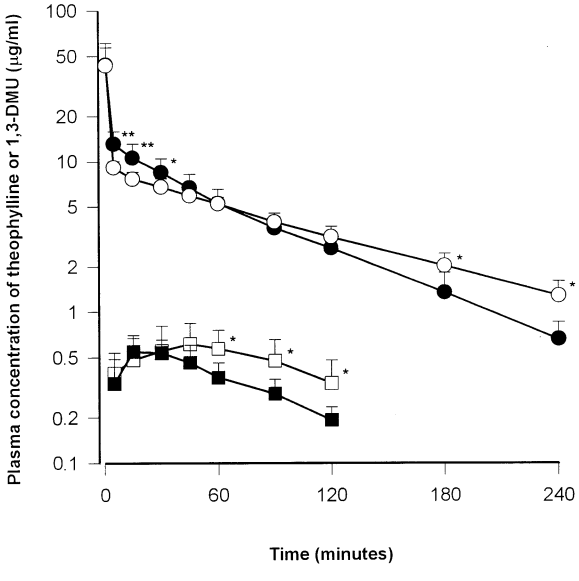

Fig. 3. Mean arterial plasma concentration-time profiles of theophylline (circle) or 1,3-DMU (rectangle) after 1-min intravenous infusion of theophylline, 5 mg/kg, to control (open symbol, $n = 8$) and 2-AP-pretreated (closed symbol, $n = 8$) rats. Bars represent standard deviation. $^{*}P < 0.05$ and $^{**}P < 0.01$.

Table 1
Pharmacokinetic parameters of theophylline and its metabolites after 1-min intravenous infusion of aminophylline, 5 mg/kg as theophylline, to control and 2-AP-pretreated rats[a]

Parameters	Control rats	2-AP-pretreated rats
Theophylline		
Terminal half-life (min)	84.2 ± 11.4	61.4 ± 10.7**
AUC (μg min/ml)	1120 ± 185	1040 ± 234
MRT (min)	116 ± 15.8	78.2 ± 10.8***
CL (ml/min per kg)	4.45 ± 0.815	4.79 ± 0.991
CL_R (ml/min per kg)	0.674 ± 0.302	1.20 ± 0.538*
CL_{NR} (ml/min per kg)	3.08 ± 0.462	3.38 ± 1.01
V_{SS} (ml/kg)	517 ± 44.0	370 ± 92.6**
$X_{U,\ 0\text{–}24\ h}$[b] (% of dose)	27.8 ± 13.0	28.0 ± 8.72
1,3-DMU		
$AUC_{0\text{–}2\ h}$ (μg min/ml)	106 ± 46.4	62.3 ± 12.3*
$X_{U,\ 0\text{–}24\ h}$ (% of dose)[c]	20.8 ± 5.30	12.4 ± 2.72*
1-MU		
$X_{U,\ 0\text{–}24\ h}$ (% of dose)[c]	18.3 ± 6.31	15.7 ± 2.60

[a] Each value represents the mean ± S.D. ($n = 8$, each).
[b] Total amount excreted in 24-h urine.
[c] Expressed in terms of theophylline.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

The CL_R values of theophylline in 2-AP-pretreated rats were significantly faster (1.20 versus 0.674 ml/min per kg) than those in control rats, however, CL and CL_{NR} of theophylline were not significantly different between two groups of rats (Table 1). The percentages of intravenous dose of theophylline excreted in 24-h urine as 1-MU were not significantly different between two groups of rats (Table 1). 3-MX was not detected in plasma and urine as previously reported (Teunissen et al., 1985).

In conclusion, after intravenous administration of theophylline, 5 mg/kg, to rats, the percentages of intravenous dose of theophylline excreted in 24-h urine as 1,3-DMU (expressed in terms of theophylline) decreased significantly in 2-AP-pretreated rats. This could be due to sup-

pression of the CYP2E1 expression by pretreatment with 2-AP.

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