J Med Dent Sci 1997; 44 : 31–35

Original Article

Pharmacokinetic study on acetaminophen: Interaction with a Chinese medicine

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To know the influences of a Chinese traditional medicine (KAKKONTO) on the metabolism of acetaminophen (APAP), we have carried out pharmacokinetic studies on APAP under KAKKONTO coadministration in humans and rats. In humans, the pharmacokinetic parameters were calculated from the blood APAP concentration-time curves of each volunteer. The parameters did not show any significant differences between the KAKKONTO-coadministration group (group K) and the APAP-administration group (group A). KAKKONTO, when given at two different doses, did not show any significant effects on blood APAP level. In rats, the blood APAP level was significantly higher than that of group A ($p<0.01$) only in th 100 mg/kg of group K at 0.25h after APAP administration. There were no other significant differences.

Key words: acetaminophen (APAP), interaction, pharmacokinetics, Chinese traditional drug (KAKKONTO)

Introduction

Acetaminophen (APAP) is one of the most widely used analgesics and antipyretics. Its advantage over aspirin is that it does not cause gastric bleeding or irritation, nor does it depress thromboxane synthesis at therapeutic doses, but it has been shown to induce hepatic injury in man[1] and experimental animals[2] when given in large doses. In recent years, some drugs which are composed of the western medicines and the Chinese traditional medicines such as KAKKONTO are sometimes used in Japan. Although there have been some clinical reports[3] on the usefulness of combining the Chinese traditional medicines and the western medicines, there were very few reports on the effects of the Chinese traditional medicines on the metabolism of the western medicines. In the present study, KAKKONTO was simultaneously administered with APAP to humans and rats, and the time changes of APAP level in their blood were determined. The influences of KAKKONTO on the APAP metabolism were studied.

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Received January 8,: Accepted May 13, 1997

Materials and Methods

Materials

Drugs

Acetaminophen mixture (PL), a Chinese traditional medicine (KAKKONTO), acetaminophen powder (APAP), methanol, perchloric acid, and acetaminophen glucuronide were used.

Each one gram of PL contains acetaminophen 150 mg, salicylamide 270 mg, caffeine 60 mg, and promethazine-methylene-disalicylate 13.5 mg (Shionogi Pharmaceutical Company Ltd., Osaka). KAKKONTO was constituted of *Puerariae Radix, Ephedrae Herba, Zingiberis Rhizoma, Cinnamomi Ramus, Glycyrrhizae Radix, Paeoniae Radix,* and *Zizyphi Fructus,* and one dose contains 3.5 g of KAKKONTO extract

(Tsumura Pharmaceutical Company Ltd., Tokyo). APAP powder was G.P. grade (Yoshida Pharmaceutical Company Ltd., Tokyo). Chromatographic grade methanol was 99.7% pure (Wako Pure Chemical Industries Ltd., Tokyo). Other chemicals were reagent grade. Acetaminophen glucuronide was supplied by Sterling-Winthrop Group Ltd (Guildford Surrey, England).

Methods

Study in healthy volunteers

1) The pharmacokinetic investigations were performed in 6 male healthy volunteers (22–50 Yr, 65–75 kg of body weight) of the laboratary staff, after getting their verbal consent. After refraining from alcohol, tea, coffee and food for one night, they took 12 mg/kg of APAP orally and had venous blood drawn after 0, 0.25, 1, 2, 3, 4, 5, and 7 h. APAP blood levels were determined by high performance liquid chromatography (HPLC). After a one week wash-out period, the same testing was done with the same volunteers, who took the previous dose of APAP together with 5 g of KAKKONTO. We designated the APAP only administration group as group A, and the APAP together with KAKKONTO administration group as group K.

2) Nineteen healthy volunteers (20–50 Yr, 10 male and 9 female, 45–72 kg of body weight) participated in this study after giving us their informed written consent. They were randomly divided into 2 groups: 10subjects in group I and 9 in group II. A cross-over design was applied; that is, a package of PL (containing 150 mg of APAP) was orally given to subjects in one group (group A) and a package of PL together with a package of KAKKONTO (containing 1250 mg of the extract particles) to subjects in the other group (group K). Venous blood was drawn before and at 0.5, 1, 2, 3, and 4 h after the dosage was given. Blood APAP levels and APAP glucuronide levels were determined by HPLC. After a one week wash-out period, the same testing was done in the 2 groups by exchanging the dosed contents.

Study in rats

Twenty one *Wistar* rats (8–12 wk old) were divided into 3 groups (5 male and 2 female in each group). KAKKONTO which was suspended in distilled water was orally administered via a stomach tube at 100mg/kg to one group and at 200 mg/kg to another group once a day for 7 days. The third group was used as a control group to which distilled water alone was given in the same manner. The rats were made to fast for one night, and then APAP was orally administered at 10 mg/kg to each of the 3 groups. Blood samples were collected from the tail into heparinized microcapillary tubes at 0.25, 0.5, 1, 2, 3, and 4 h after APAP administration, and blood APAP levels were determined by HPLC.

Determination of APAP and APAP glucuronide

Blood APAP and APAP glucuronide concentrations were determined by HPLC following the methods[4,5] reported previously. In short, the blood samples were deproteinized with 15% of perchloric acid and centrifuged at 5000 rpm for 2 min. The supernatants were filtered through a membrane filter (Millipore, Samplepe 13-GV, 0.22 μm), and then applied onto HPLC. The HPLC apparatus used was a Shimadzu chromatographer consisting of a degasser (model ERC-3312), a liquid chromatograph (model LC-6), an autoinjector (model SIL-6B), a column oven (model CTO-6A), a wavelength variable UV-spectrophotometric detector (model SPD-6A), a system controller (model SCL-6B), and a data processor (Chromatopac: model C-R5A).

The separations were performed on an Ultron S-C18 (25cm×4.6mm) reverse-phase column filled with methanol, and the column was housed in an oven set at 45°C.

APAP was eluted for 13 min, with 15% methanol in water as a mobile phase at a flow rate of 0.7ml/min.

Statistical analysis

The mean value and the standard error were calculated for each group. The variances of the means were tested for homogeneity of distribution by the F-test. When the variances were found to be homogenously distributed, all values were compared by Student's t-test. $p<0.05$ was taken as statistically significant.

Results

The concentration-time curves of APAP in the sera from the six male volunteers are shown in Figure 1. There were no significant differences between group K and group A; although blood APAP levels were somewhat higher in group K at every determination time. The area under the curve (AUC) was calculated by trapezoidal rule. There was no statistical difference between group K and group A in $AUC_{0\rightarrow 7hr}$. No volunteers complained of any abnormal feeling during

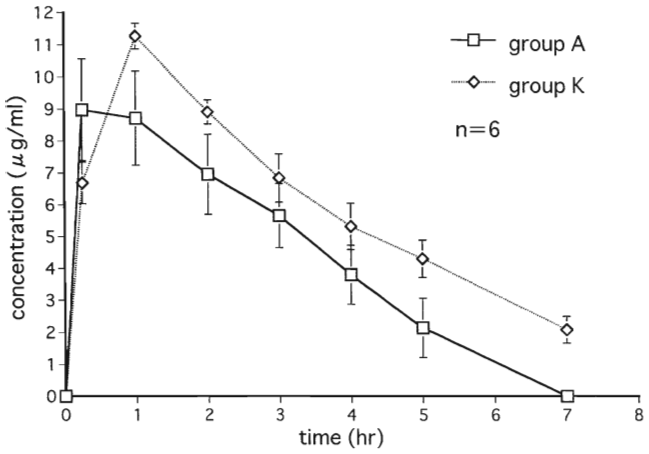

Fig 1. Changes in the acetaminophen concentration of sera from 6 volunteers at the determined times (M±SE)
Dosage: group A: APAP, 12 mg/kg, 1 portion
group K: APAP, 12 mg/kg, 1 portion; the KAKKONTO extract, 5 g/person, 1 portion

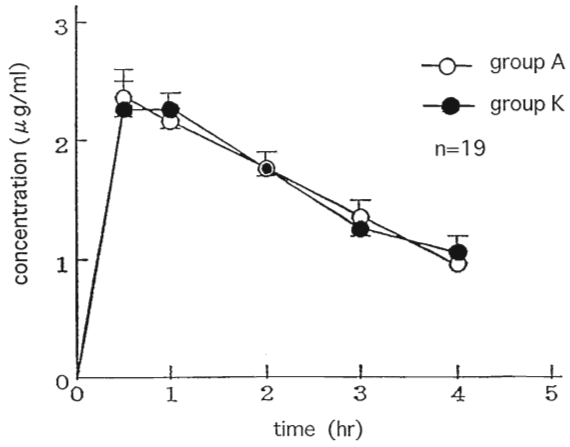

Fig 2. Changes in the acetaminophen concentration of sera from 19 volunteers at the determined times (M±SE)
Dosage: group A: a package of PL containing 150 mg of APAP, 1 portion
group K: a package of PL containing 150 mg of APAP, 1 portion; the KAKKONTO extract, 1.25 g/person, 1 portion

the test.

The concentration-time curves of APAP and APAP glucuronide in sera from the 19 volunteers are shown in Figures 2 and 3, respectively. The pharmacokinetic parameters for each individual were calculated by using a multiexponential computer program (HP-9845). The profile of the concentration-time curves of APAP was best described by a one-compartment open model, because the blood APAP level declined exponentially after attaining the peak level. The following pharmacokinetic parameters were estimated:

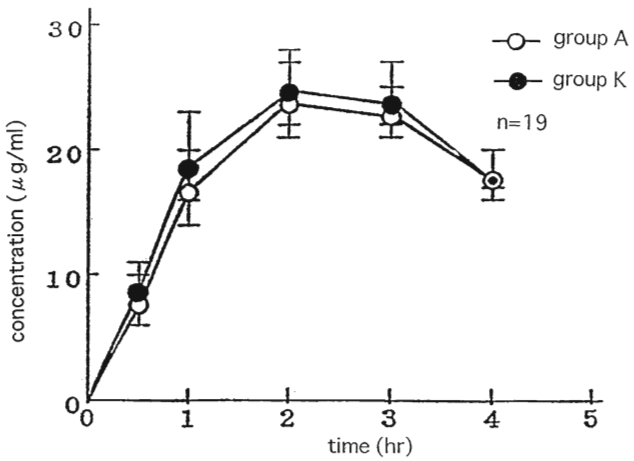

Fig 3. Changes in the acetaminophen glucuronide concentration of sera from 19 volunteers at the determined times (M±SE)
Dosage: group A: a package of PL containing 150 mg of APAP, 1 portion
group K: a package of PL containing 150 mg of APAP, 1 portion; the KAKKONTO extract, 1.25 g/person, 1 portion

the peak concentration (C_{max}), the time to peak concentration (t_{max}), the elimination half-life ($t_{1/2}$) and the area under the serum concentration-time curves ($AUC_{0\rightarrow4hr}$) for the experimental period (Table 1). The profile of the concentration-time curves of the APAP glucuronide levels could not be fitted to any pharmacokinetic model, and its $AUC_{0\rightarrow4hr}$ was calculated by a trapezoidal rule. There were no significant differences between group A and group K in all of the pharmacokinetic parameters. There were no signs of any adverse reaction of the drugs in volunteers during the test.

The concentration-time curves of APAP in rat sera are shown in Figure 4. The blood APAP level was significantly higher ($p<0.01$) in the 100 mg/kg part of group K than that in group A only at 0.25 h after APAP administration. There was no significant difference between group K and group A in $AUC_{0\rightarrow4hr}$.

Discussion

To Know the effects of KAKKONTO on APAP metabolism, we have performed 2 series of studies in humans. At first, we investigated the effects of comparatively large doses of APAP and KAKKONTO in a small number of volunteers. The profiles of the concentration-time curves of APAP in the individuals gave us an impression that KAKKONTO might increase blood APAP levels as shown in Figure 1.

Table 1. Comparison of the Pharmacokinetic Parameters Obtained from Concentration of APAP and Its Glucuronide in Blood from 19 Volunteers (M±SE)
Dosage: group A: a package of PL containing 150 mg of APAP, 1 portion
group K: a package of PL containing 150 mg of APAP, 1 portion; the KAKKONTO extract, 1.25 g/person, 1 portion

pharmacokinetic	parameters	C_{max} (μg/ml)	t_{max} (hr)	$t_{1/2}$ (hr)	$AUC_{0\rightarrow4\ hr}$ (μg.hr/ml)
APAP	group A	2.61±0.18	0.54±0.10	2.52±0.16	6.60±0.35
	group K	2.65±0.17	0.56±0.11	2.58±0.24	6.55±0.37
APAP-G	group A	25.6±2.64	2.37±0.17	4.62±1.02	74.0±8.38
	group K	26.7±3.27	2.26±0.20	4.98±0.90	75.8±9.32

APAP-G: APAP glucuronide
(n=19)

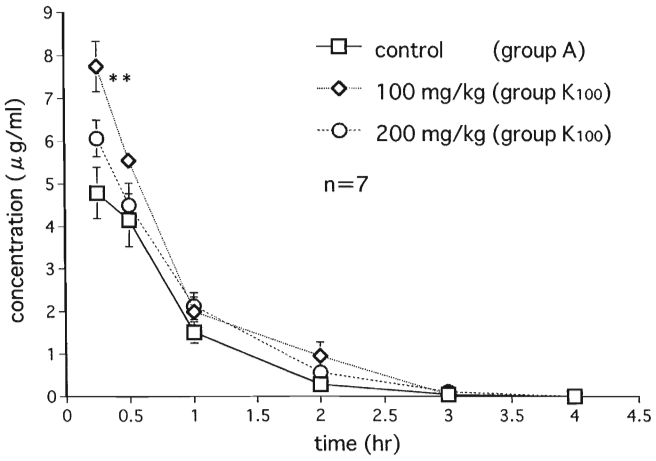

Fig 4. Changes in the acetaminophen concentration of sera from rats at the determined times (M±SE)(**: $p<0.01$)
Dosage: group A: distilled water administered once a day for 7 days followed by APAP, 10 mg/kg, 1 portion
group K_{100}: The KAKKONTO extract, 100 mg/kg, once a day for 7 days followed by APAP, 10 mg/kg, 1 portion
group k_{200}: the KAKKONTO extract, 200 mg/kg, once a day for 7 days followed by APAP, 10 mg/kg, 1 portion

Thinking of the results and the clinical usage of APAP and KAKKONTO, we made another study in a larger number of volunteers using the clinical dosages of APAP and KAKKONTO. This time we measured blood levels of APAP and APAP glucuronide, which is one of the major metabolites of APAP. However, as shown in Figure 2, the profiles of the concentration-time curves of APAP were very similar in the 2 groups. As shown in Table 1, the differences in the pharmacokinetic parameters such as C_{max}, t_{max}, $t_{1/2}$, and $AUC_{0\rightarrow4hr}$ between the two groups were not significant. These results indicate that KAKKONTO did not affect the metabolism of APAP under the conditions of the present studies.

The profiles of Figure 1 and 2 were not identical, and the reason may be that different dosages of KAKKONTO and APAP and different blood sampling times were used. The KAKKONTO dose was 5 g per person in the 1st study and 1.25 g per person in the 2nd study; the APAP dose was 12 mg/kg in the 1st study and 50 mg/person in the 2nd study. In the 2nd study, we had to discontinue the blood sampling at 4 h after the drug administration because of the conditions of the volunteers. The dosages given were chosen based on the report which shows the therapeutic doses of APAP are 325-600 mg/person every 4 h[6].

The APAP used was not identical in the 2 studies. In the 1st study, APAP powder was used; in the 2nd study, we used APAP complex (PL). This was because APAP is often used in the form of a mixture. In the case of PL, it is known that blood APAP levels are not affected by the other components in the mixture[7].

It is generally accepted that APAP is metabolized in the liver mainly by conjugation to the glucuronide and sulfate, and then the conjugates are eliminated by the kidney. A small proportion of acetaminophen is metabolized by liver cytochrome P-450-mediated mixed function oxidases to yield reactive metabolites[8]. Although the results of the 2nd study seem to indicate that KAKKONTO does not affect the metabolism of APAP, this time we measured only APAP glucuronide, and other metabolites such as sulfate, cysteine and mercapturic acid conjugates should be measured before we can come to an accurate conclusion.

In the results of the rat experiment as shown in Figure 4, the blood APAP level for the 100 mg/kg of group K was significantly higher than that for the 200 mg/kg of group k only at 0.25 h after APAP

administration. Further investigations are necessary to clarify the unusual dose response reaction of the Chinese traditional medicine. Although this finding must be confirmed under various conditions, considering these changes of blood APAP level, there still seems to be a possibility that KAKKONTO may exert some effects on the metabolism of APAP in rats.

The finding that we could see a significant difference in rats, but not in humans, may imply some meaning other than species difference. It is thought that Chinese traditional medicines take a relatively long time to exert their effects. In the present study we gave KAKKONTO only once to humans, but continued administering it for 7 days to rats. Taking all this information into account, it would seem necessary to carry out further studies, which we should try to use KAKKONTO in humans longer, for example for 7 days or more, to understand the veiled nature of the Chinese traditional drug.

In conclusion, although we could not demonstrate that KAKKONTO affects the metabolism of APAP in humans in the present study, a clearer understanding of the kinetics of APAP in the condition of coadministration with KAKKONTO is of prime importance for the establishment of safe and effective dose regimes for both acute and long term therapy.

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