Effects of kale ingestion on pharmacokinetics of acetaminophen in rats

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ABSTRACT

Kale is a cruciferous vegetable (Brassicaceae) that contains a large amount of health-promoting phytochemicals. The chronic ingestion of cabbage of the same family is known to accelerate conjugating acetaminophen (AA) and decrease the plasma AA level. Therefore, we examined to clarify the effects of kale on the pharmacokinetics of AA, its glucuronide (AA-G) and sulfate (AA-S). AA was orally administered to rats pre-treated with kale or cabbage (2000 mg/kg/day) for one week. Blood samples were collected from the jugular vein, and the concentrations of AA, AA-G and AA-S were determined. In results, kale ingestion induced an increase in the area under the concentration-time curve (AUC) and a decrease in the clearance of AA, whereas cabbage had almost no influence. In addition, there were significant differences in the AUC of AA-G between the control and kale groups. mRNA expression levels of UDP-glucuronosyltransferases, the enzymes involved in glucuronidation, in the kale group were significantly higher than those in the control group. In conclusion, kale ingestion increased the plasma concentrations of both AA and AA-G. The results suggest that kale ingestion accelerates the glucuronidation of AA, but an increase of plasma AA levels has a different cause than the cause of glucuronidation.

Kale (Brassica oleracea L. var acephala DC) is a leafy green vegetable belonging to the cabbage family (Brassicaceae) which has a high nutritional content because it is rich in phytochemicals, and contains high concentrations of minerals and vitamins, carotenoid, dietary fiber, and antioxidant compounds, including polyphenols and phenolic acids (9, 18). Some epidemiological studies have provided sound scientific evidence that the regular consumption of Brassicaceae, including cabbage, broccoli, cauliflower and brussel sprouts, may be highly effective in reducing cancer risk (1, 22, 24, 30). Brassicaceae plants contain a high amount of specific phytochemicals known as glucosinolates, a large group of sulfur-containing glucosides. Upon the disruption of plant tissues (food processing, chewing, etc.), glucosinolates come into contact with plant myrosinase, and then myrosinase catalyzes the conversion of glucosinolates to isothiocyanates (ITCs). There are various kinds of ITCs, phenethyl ITC (cabbage, watercress), benzyl ITC (papaya), allyl ITC (black mustard), and sulforaphane (broccoli), which have pungent components and a strong flavor that stimulates people’s appetite. It has been known that ITCs contribute to the chemoprevention function of Brassicaceae by inducing phase 2 detoxication enzymes (e.g., UDP-glucuronosyltransferases (UGTs) and sulfotransferases (SULTs)), and they have attracted intense research interest (2, 5, 8, 13, 21, 28). Whereas, there is possibility that Brassicaceae plants promote the conjugation of some drugs, and then decrease effects of drugs. Previous study showed that a diet containing cabbage induced the conjugation of acetaminophen (AA),

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and decreased the plasma AA level (16). Thus, it is possible that the ingestion of kale of the same family accelerates conjugating the glucuronidation and sulfation of some drugs, and then decreases effects of drugs.

The aim of this study was to clarify the effects of the ingestion of kale on the pharmacokinetics of AA, its glucuronidation and sulfation, and mRNA expressions of UGTs and SULTs.

MATERIALS AND METHODS

Materials. Acetaminophen (AA) and caffeine acid as an internal standard were purchased from Wako Pure Chemical Industries, Ltd (Tokyo, Japan). Acetaminophen glucuronide (AA-G) and its sulfate (AA-S) were obtained from Nacalai Tesque, Ltd (Kyoto, Japan).

Freeze-dried Super Aojiru, a Japanese vegetable drink made of kale and formulated with twintose (FANCL Co., Yokohama, Japan) was used for kale. Cabbage free of agricultural chemicals was obtained from Tohken Co. (Nagoya, Japan), liquidized by a food mixer and freeze-dried.

Animals and ethical considerations. This study was approved by the animal ethics committee of the FANCL Co. Specific pathogen-free male Sprague-Dawley rats were obtained from Japan SLC (Hamamatsu, Japan) at 6 weeks of age (weighing 160–180 g) and acclimatized for a week before the experiment. The animals were randomized and housed two per cage with free access to food and water, and maintained on a 12-h light/dark cycle in strictly controlled conditions of 24 ± 3°C and 55 ± 20% relative humidity.

Animal treatments. The animals were divided into 3 groups (control, kale, and cabbage group; 5–6 rats for each group). For co-treatment, the animals received one oral dose of either 2000 mg/kg kale or cabbage dissolved into carboxymethylcellulose sodium salt solution once a day for 7 days. Nine hours after the last treatment with kale or cabbage, access to the diet was removed and only water was provided.

Blood samples were collected by heparinized syringes from the jugular vein at 15, 30, 60, 90 and 120 min after oral administration of 25 mg/kg AA. The samples were centrifuged immediately at 3000 rpm for 10 min, and stored at −30°C until analysis. The rats were anesthetized with diethyl ether and exsanguinations from the abdominal aorta immediate after the last sampling, and the liver was then collected and preserved with RNA later (QIAGEN, Tokyo, Japan).

Determinations of plasma AA, AA-G and AA-S. Plasma AA, AA-G and AA-S were determined by ultra performance liquid chromatography (UPLC; ACQUITY UPLC® System, Waters, Tokyo, Japan). Twenty μL of plasma with caffeine acid at a final concentration of 10 μg/mg were deproteinized with 6% of perchloric acid and centrifuged at 5000 rpm for 5 min. The supernatants were filtered and UPLC analysis was then performed. UPLC was performed using a BEH C18, 2.1 × 50 mm column (Waters) connected with a guard column (ACQUITY BEH C18 VanGuard Pre-column, 1.7 μm, 2.1 × 5 mm; Waters) at 40°C. Mobile phase A consisted of water containing 0.05% trifluoroacetic acid, while mobile phase B consisted of methanol (0.1% trifluoroacetic acid). The solvents were delivered (Solvent MGR: BSM, Waters) at the flow rate of 0.35 mL/min with linear gradient A/B = 99/1 to 99/1 (0–2.5 min), 99/1 to 58/42 (2.5–5.2 min), 58/42 to 0/100 (5.2–5.3 min), 0/100 to 0/100 (5.3–6 min), and 0/100 to 99/1 (6–6.1 min). AA, AA-G, and AA-S were detected by using a photo diode array detector (PDA detector; UPD, Waters) with UV 240 nm for AA, AA-G and AA-S, 320 nm for caffeine acid.

Determination of mRNA Levels. Total RNA was prepared from the liver using an RNeasy Mini Kit (QIAGEN). The samples were quantitated by spectrophotometry, and 1 μg of total RNA was used to generate cDNA by reverse transcription using a Prime Script reverse transcription reagent kit (Takara, Ohtsu, Japan) according to the manufacturer’s protocol. cDNA synthesized from 50 ng of total RNA was subjected to quantitative real-time polymerase chain reaction with a Light Cycler 480 system (Roche Diagnostics, Tokyo, Japan) using SYBR® Premix Ex Taq™ II reagent (Takara) according to the manufacturer’s specifications. The primers used for ugt1a1 (Accession No. NM_012683), ugt1a6 (Accession No. NM_001039691), ugt2b1 (Accession No. NM_173295), sult1a1 (Accession No. NM_031834), sult2b1 (Accession No. NM_001039665) and sult1c2 (Accession No. NM_133547) are shown in Table 1. PCR amplification was as follows: an initial step for 2 min at 50°C, then 10 min at 95°C, followed by 45 cycles at 95°C for 15 s and 60°C for 1 min. β-2-microglobulin (B2m) was used as a housekeeping gene for data analysis. All mRNA expression levels were normalized to B2m mRNA in the same preparation.
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Pharmacokinetic parameters analysis. The peak plasma concentration (C_{max}) of AA, AA-G and AA-S were obtained from the actual data recorded after the oral administration of AA. Other pharmacokinetic parameters, half-life (t_{1/2}), the area under the concentration-time curve (AUC_{0-120}), the mean residence time (MRT_{0-120}), clearance, and apparent volume of distribution (Vd) were calculated by moment analysis software (Microsoft, Tokyo, Japan).

Statistical analysis. Values are presented as the means ± SEM. Differences were considered significant at P < 0.05 for all analyses. The variances of means were tested for homogeneity of distribution by the F-test. When the variances were found to be homogeneity distributed, all values were assessed using Dunnett’s test, otherwise, Steel’s test was used.

RESULTS

The concentration-time curves of AA in plasma after the administration of 25 mg/kg AA, with or without 2000 mg/kg kale or cabbage pretreatment, are shown in Fig. 1. There were significant differences in AA level at 15 min between the control and cabbage groups. The blood AA level was significantly higher in the kale group than that in the control only at 30 min. Table 2 summarizes the pharmacokinetic parameters of AA following oral administration of AA. The AUC_{0-120} of AA increased significantly when AA was administered after pretreatment with kale, showing 9.95 ± 0.39 μg/mL and 11.65 ± 0.51 μg/mL for the control and kale groups, respectively. In addition, there were significant differences in MRT_{0-120} and t_{1/2} of AA between the control and kale groups. The clearance of AA decreased significantly with kale pretreatment. On the other hand, none of the mean pharmacokinetics parameters, including the AUC_{0-120} and clearance of AA, were significantly changed by pretreatment with cabbage.

The pharmacokinetic parameters of AA-G and AA-S are shown in Tables 3 and 4, respectively. When the rats were pretreated with kale, the plasma AA-G level and the AUC_{0-120} of AA-G were significantly increased, and clearance of AA-G was significantly reduced compared with the vehicle control. Regarding AA-S, neither kale nor cabbage ingestion had an effect on the plasma level or pharmacokinetic parameters.

There were significant differences in mRNA levels of ugt1a1 and ugt1a6 in the liver between the control and kale groups (Fig. 2). No significant differences in other mRNA expressions of ugt2b1, sult1a1, sult2b1, or sult2c1 between the control group and the kale or cabbage group were confirmed (Figs. 2 and 3).

DISCUSSION

AA is mainly glucuronidated and sulfated by UGT and SULT, and AA glucuronide and sulfate are the main urinary excretion products of AA. It has also been established that cytochrome P450 enzymes (CYP) are responsible for the bioactivation of AA in the liver (11, 19) and that the CYP-dependent oxidation of AA results in the formation of the toxic N-acetyl-p-benzoquinoneimine, causing hepatocellu-

**Table 1** Primer sequences used for real-time PCR quantification

<table>
<thead>
<tr>
<th>Isoforms</th>
<th>Primers</th>
</tr>
</thead>
<tbody>
<tr>
<td>ugt1a1</td>
<td>Forward: ATCATGCCCAACATGGTTTT</td>
</tr>
<tr>
<td>ugt1a6</td>
<td>Forward: AATTCAGATGGTGCTGAGG</td>
</tr>
<tr>
<td>ugt2b1</td>
<td>Forward: TGCAAGATCAACCGGATAACA</td>
</tr>
<tr>
<td>sult1a1</td>
<td>Forward: TACACAACCATCCCCACTGA</td>
</tr>
<tr>
<td>sult2b1</td>
<td>Forward: CAACCGGTAAGCTTCTTCAG</td>
</tr>
<tr>
<td>sult1c2</td>
<td>Forward: TCAGTGTTGGAGCCAGTGGAG</td>
</tr>
<tr>
<td>B2m</td>
<td>Forward: TGACCGTGATCTTCTGGTG</td>
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</tbody>
</table>
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The possibility that the ingestion of kale influences the pharmacokinetics on AA. We will conduct an investigation to confirm this, and we also plan to examine the effects of kale intake on CYP metabolism. The interpretation of epidemiological data and the exploitation of Brassica vegetables for human health require an understanding of glucosinolate chemistry and metabolism across the whole food chain, from production and processing to consumption. Many researchers have investigated the ITC attributes of Brassicaceae, which contribute to its chemoprevention function (2, 5, 8, 13, 22, 28). It has also been found that ITCs modulate UGT activity (3, 25). The main aim of the present study was to clarify the effects of kale intake on the glucuronidation and sulfation of AA. The results showed a change in the glucuronidation of AA by kale pretreatment. We believe the reason for this is that kale ingestion influences the pharmacokinetics on AA. We will conduct an investigation to confirm this, and we also plan to examine the effects of kale intake on CYP metabolism.

Each value represents the mean ± S.E. of 5–6 animals. AA was administrated orally after pretreatment with 1-week kale or cabbage. C_{max}: the peak plasma concentration, t_{1/2}: half-life, AUC: the area under the concentration-time curve, MRT: the mean residence time, CL: clearance, Vd: apparent volume of distribution * The asterisks indicate the values significantly different from the control (P < 0.05).

Table 2  Pharmacokinetic parameters of AA following oral administration of AA

<table>
<thead>
<tr>
<th></th>
<th>C_{max} (μg/mL)</th>
<th>AUC_{0–120} (μg/mL × h)</th>
<th>MRT_{0–120} (h)</th>
<th>T_{1/2} (h)</th>
<th>Vd (L/kg)</th>
<th>CL (L/h/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12.14 ± 0.26</td>
<td>9.95 ± 0.39</td>
<td>0.40 ± 0.03</td>
<td>0.30 ± 0.02</td>
<td>1.00 ± 0.06</td>
<td>2.53 ± 0.11</td>
</tr>
<tr>
<td>Kale</td>
<td>12.24 ± 0.83</td>
<td>11.65 ± 0.51*</td>
<td>0.54 ± 0.05*</td>
<td>0.41 ± 0.04*</td>
<td>1.17 ± 0.09</td>
<td>2.16 ± 0.09*</td>
</tr>
<tr>
<td>Cabbage</td>
<td>9.94 ± 0.42*</td>
<td>8.92 ± 0.27</td>
<td>0.44 ± 0.06</td>
<td>0.32 ± 0.04</td>
<td>1.24 ± 0.15</td>
<td>2.81 ± 0.08</td>
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</table>

Table 3  Pharmacokinetic parameters of AA-G following the oral administration of AA

<table>
<thead>
<tr>
<th></th>
<th>C_{max} (μg/mL)</th>
<th>AUC_{0–120} (μg/mL × h)</th>
<th>MRT_{0–120} (h)</th>
<th>T_{1/2} (h)</th>
<th>Vd (L/kg)</th>
<th>Clearance (L/h/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.51 ± 0.67</td>
<td>6.52 ± 0.75</td>
<td>0.68 ± 0.07</td>
<td>0.50 ± 0.04</td>
<td>2.65 ± 0.22</td>
<td>4.05 ± 0.45</td>
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<td>Kale</td>
<td>7.09 ± 1.00</td>
<td>8.89 ± 0.78*</td>
<td>0.71 ± 0.09</td>
<td>0.57 ± 0.09</td>
<td>2.30 ± 0.41</td>
<td>2.90 ± 0.24*</td>
</tr>
<tr>
<td>Cabbage</td>
<td>4.67 ± 0.72</td>
<td>6.66 ± 0.49</td>
<td>0.79 ± 0.15</td>
<td>0.58 ± 0.10</td>
<td>2.83 ± 0.39</td>
<td>3.86 ± 0.33</td>
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Table 4  Pharmacokinetic parameters of AA-S following oral administration of AA

<table>
<thead>
<tr>
<th></th>
<th>C_{max} (mg/mL)</th>
<th>AUC_{0–120} (μg/mL × h)</th>
<th>MRT_{0–120} (h)</th>
<th>T_{1/2} (h)</th>
<th>Vd (L/kg)</th>
<th>Clearance (L/h/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>44.55 ± 2.31</td>
<td>54.67 ± 2.95</td>
<td>0.54 ± 0.04</td>
<td>0.39 ± 0.02</td>
<td>0.25 ± 0.03</td>
<td>0.46 ± 0.03</td>
</tr>
<tr>
<td>Kale</td>
<td>48.20 ± 2.50</td>
<td>66.31 ± 6.13</td>
<td>0.59 ± 0.05</td>
<td>0.42 ± 0.02</td>
<td>0.24 ± 0.04</td>
<td>0.39 ± 0.03</td>
</tr>
<tr>
<td>Cabbage</td>
<td>46.51 ± 2.88</td>
<td>55.72 ± 2.18</td>
<td>0.55 ± 0.02</td>
<td>0.39 ± 0.03</td>
<td>0.25 ± 0.01</td>
<td>0.45 ± 0.02</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E. of 5–6 animals. AA was administrated orally after pretreatment with 1-week kale or cabbage. C_{max}: the peak plasma concentration, t_{1/2}: half-life, AUC: the area under the concentration-time curve, MRT: the mean residence time, CL: clearance, Vd: apparent volume of distribution * The asterisks indicate the values significantly different from the control (P < 0.05).
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ences the glucuronidation of drugs, such as AA. On the other hand, although the amounts of kale administered to rats in this experiment (2000 mg/kg) were 15-fold higher than the amount of human intake in a day, the AUC0–120 of AA became only 1.2-fold higher following kale intake, and the clearance of AA became just 0.9-fold higher compared with the vehicle intake. Based on these results, we are uncertain whether the amount of human intake in a day would influence the glucuronidation and sulfation of AA.

Pantuck et al. showed that a diet containing cabbage induced the glucuronidation and sulfation of AA, and decreased the plasma AA level in humans (16); however, in the present study, cabbage ingestion had no affect on the pharmacokinetics of AA. One cause of this difference was that only cabbage was given in this study, while both brussels sprouts and cabbage were given in the previous study by Pantuck et al. It was suggested that there were differences in total ITCs contents between cabbage given in this study and previous study. Therefore, there is possibility that the differences of those ITCs contents led to the different results.

We will make another attempt to clarify the interaction between kale and drugs in the future, in particular the effects of kale ingestion on CYP metabolism.

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REFERENCES


