Metabolism of Kalopanaxsaponin K by Human Intestinal Bacteria and Antirheumatoid Arthritis Activity of Their Metabolites

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When kalopanaxsaponin K (KPK) from Kalopanax pictus was incubated for 24 h at 37 °C with human intestinal microflora, KPK was mainly metabolized to kalopanaxsaponin I (KPI) via kalopanaxsaponin H (KPH) rather than via kalopanaxsaponin J (KPJ), and then transformed to kalopanaxsaponin A (KPA) and hederagenin. Bacteroides sp. and Bifidobacterium sp. and Fusobacterium sp. transformed KPK to KPI and KPA and hederagenin via KPH or KPJ. However, Lactobacillus sp. and Streptococcus sp. transformed KPJ to KPI, KPA, and hederagenin only via KPJ. The metabolite KPA of KPK showed potent antirheumatoid arthritis activity.

Key words kalopanaxsaponin K; antirheumatoid arthritis activity; intestinal bacteria; Kalopanax pictus

The stem bark of Kalopanax pictus belonging to the family Araliaceae has been used in Korea as a tonic, analgesic, antiinflammatory and antidiabetic medicine. From this plant, hederagenin glycosides, syringin, liriodendrin, and conifer-yraldehyde glucosides have been isolated.1–3) Related to biological activities of K. pictus, it was reported that the active components on antidiabetic,4,5) cytotoxic,5,6) and antifungal assays5,6) may be hederagenin monodesmosides. We also reported that kalopanaxsaponin B (KPB) and H (KPH) were easily metabolized by human intestinal bacteria to kalopanaxsaponin A (KPA) and kalopanaxsaponin I (KPI), respectively.7) However, these metabolites were slowly transformed to hederagenin. KPI was slowly transformed to hederagenin via KPA. Among these compounds, the most antidiabetic compound was KPA. However, studies related to the metabolic pathway of KPI to hederagenin in the intestine, the biotransformation of kalopanaxsaponin K (KPK) to KPA, which is an antitumor compound,5) and their biological activities, such as antirheumatoid arthritis, are incomplete.

Therefore we isolated the main components KPB, KPH, and KPK from the stem bark of K. pictus, investigated the metabolic pathway of these kalopanaxsaponins by human intestinal bacteria, and measured the antirheumatoid arthritis activity of the main metabolites. We also screened the biotransforming bacteria of KPK to the bioactive compound KPA from human intestinal bacteria.

MATERIALS AND METHODS

Instrument Melting points were determined on an electrothermal digital melting point apparatus.1H- and 13C-NMR spectra were recorded on a Brucker-AM 500 with tetramethylsilane (TMS) as an internal standard. The TLC chromatogram of metabolites was quantitatively analyzed with a Shimadzu CS-920 TLC-scanner.

Materials Pontamine and histamine were purchased from Sigma Chemical Co. (U.S.A.), Freund’s complete adjuvant (FCA) reagent was from Difco Co. (U.S.A.) and general anaerobic medium (GAM) was from Nissui Pharmaceutical Co., Ltd. (Japan).

Extraction and Isolation of KPK and KPJ from K. pictus K. pictus Nakai was collected in August 1998 in Kangwon province, Korea, and the plant was identified by professor S. Y. Yun (Division of Applied Plant Sciences, Sangji University, Wonju, Korea). A voucher specimen was deposited in the herbarium of Life Science and Natural Resources, Sangji University, Wonju, Korea.

Dried stem bark (4.8 kg) of K. pictus was cut and extracted three times with MeOH under reflux. The extract was filtered and evaporated under reduced pressure to give a viscous mass (630 g) of MeOH extract. This extract was suspended in water and fractionated with ethylacetate and n-butanol.1,3,7) Physicochemical data of isolated KPK and kalopanaxsaponin J (KPJ) were recorded and the values were in good agreement with those of authentic samples.

KPK (1.2 g): Amorphous powder, mp 217—219 °C (dec.), 1H-NMR and 13C-NMR: literature (ref. 7).

KPH (0.8 g): Amorphous powder, mp 217—219 °C (dec.), 1H-NMR and 13C-NMR: literature (ref. 5).

Extraction and Isolation of Metabolites To obtain the metabolites of KPK by human intestinal bacteria, the reaction mixture contained 0.4 g KPK (or KPJ) and 2 g fresh stool specimen of human (male, twenty) in a final volume of 500 ml of an anaerobic dilution medium.8) The mixture was incubated at 37 °C for 24 h. The reaction mixture was extracted three times with ethylacetate. EtOAc soluble portion of KPK-reacted mixture was evaporated under reduced pressure and the metabolites were isolated by silica gel column chromatography with the solvent of CHCl3/MeOH/H2O (7:3:1, lower phase) according to the previous method.7) Physicochemical data of KPJ (3 mg), KPA (35 mg), KPH (3 mg), HA (hederagenin 3-O-α-L-arabinopyranoside, 0.2 mg) and hederagenin (1.2 mg) were taken and their values were in good agreement with authentic specimens, respectively.

Hederagenin: Amorphous powder, mp 318—320 °C. 1H- and 13C-NMR: literature (ref. 7).

HA: Colorless needles from MeOH, mp 228—230 °C (dec.). 1H-NMR: literature (ref. 7).

KPA: Colorless needles from MeOH, mp 265—268 °C (dec.). 1H- and 13C-NMR: literature (ref. 7).

KPJ: Amorphous powder, mp 217—219 °C (dec.), 1H- and
The assay mixture contained 4 ml of KPK (or KPJ) 0.6 mm and 0.1 g fresh fecal suspension (human intestinal bacteria fraction) in a final volume of 20 ml of an anaerobic dilution medium. The mixture was incubated at 37 °C for 24 h and an aliquot (1 ml) of the reaction mixture was periodically extracted twice with 5 ml of ethylacetate. The ethylacetate extract was analyzed by TLC.

**Screening of Intestinal Bacterial Strains Metabolizing KPK**

According to our previous method, a suspension of the fresh feces of a healthy male volunteer was diluted 10^5 to 10^6-fold with GAM broth. An aliquot (200 μl) of the 10^6-diluted human feces was inoculated on a GAM agar plate, which was anaerobically incubated at 37 °C for 4 d. Colonies were isolated and each colony was incubated in 5 ml of GAM broth containing 0.4% KPK for 1 d. The cultured medium was extracted with 5 ml of ethylacetate. After evaporating the ethylacetate fraction, it was analyzed for KPJ, KPI, KPA, and hederagenin by TLC. Twenty bacterial strains containing previously isolated bacteria were isolated. Identification of the isolated bacteria was performed according to Bergey's manual.

**Animals**

Male Sprague–Dawley (SD) rats (200–220 g) and male ICR mice (20–25 g) were supplied from Daehan Experimental Animal Breeding Center. All rats and mice were housed in wire cages, fed with standard laboratory chow (Samyang Feed Production Co.) and allowed water ad libitum.

Anti-inflammatory activity was measured according to the method of Saito et al. For the induction of rheumatoid arthritis, 0.05 ml of FCA reagent was injected to right footpads of the rats. The induction of rheumatoid arthritis in rats was confirmed 2 weeks after FCA treatment. KPA and KPI (5, 10, 20 mg/kg/d) were dissolved in saline and intraperitoneally administered daily for 3, 5, 7, and 10 d. The effect was taken by plethysmometer (Ugo Basile, Italy). The inhibitory effect was calculated as follows:

\[
\text{inhibition of edema (\%)} = \frac{\text{volume of control group} - \text{volume of treated group}}{\text{volume of control group}} \times 100
\]

The vascular permeability test was performed according to Whittle's method. Pontamine sky blue saline solution 4% (0.1 ml/10 g mouse body weight) was injected into the tail vein 30 min after the intraperitoneal administration of KPA or KPI. Histamine 0.5% (0.1 ml/10 g mouse body weight) was injected into the abdominal cavity 15 min after injection of pontamine sky blue saline solution. The mice were killed 20 min after the final injection, and then the pigment exuded into abdominal cavity was washed with 10 ml of distilled water. This washed solution was centrifuged (3000 rpm, 10 min) and the absorbance of the resulting supernatant was measured at 570 nm.

**RESULTS AND DISCUSSION**

**Metabolites of KPK by Human Intestinal Microflora**

To investigate the metabolites of KPK broken down by human intestinal bacteria, KPK was anaerobically incubated for 24 h with a bacterial mixture from human feces. Then the metabolites were extracted with ethylacetate and analyzed by TLC. Five metabolites, three main metabolites and two minor ones, were observed by TLC. By analysis of 1H- and 13C-NMR spectra, the three main metabolites were identified as KPI, KPA and hederagenin, and the two minor ones were identified as KPJ and HA. When KPJ was anaerobically incubated for 24 h with a bacterial mixture from human feces, its main metabolites were KPI, KPA, and hederagenin.

When KPK was anaerobically incubated for 24 h with 20 bacterial strains isolated from human feces, *Bacteroides* sp., *Bifidobacterium* sp. and *Fusobacterium* sp. transformed KPK to KPI and/or KPA and hederagenin via KPH or KPJ (Table 1). However, *Lactobacillus* sp. and *Streptococcus* sp. transformed KPK to KPI, KPA, and hederagenin only via KPJ.

**Time Course of Metabolism of KPK and KPJ by Human Intestinal Microflora**

The time course of metabolism of KPK by human intestinal microflora is shown in Fig. 1. When KPK was incubated with human intestinal microflora, KPH, KPJ, and KPI were produced 4 h after incubation. The main metabolites were KPH and KPI (KPH was dramatically decreased and KPI was increased 9 h after the start of incubation). KPA, KPI, and hederagenin were also observed from 9 to 24 h after incubation, but these compounds were minor metabolites. After 15 h of incubation, KPI was found to be a main metabolite while KPA, HA, and hederagenin were detected as the minor metabolites. KPI and hederagenin were the main metabolites 24 h after incubation.

When KPJ was incubated with human intestinal microflora, KPI was observed after 4 h of incubation. However, the transforming activity of KPJ to KPI was weak. Therefore the transformation of KPJ to hederagenin was also slow during 24 h of incubation. The main metabolic pathway of KPK by human intestinal microflora proceeds to KPI via KPH rather than KPJ.
When KPK was incubated with Bacteroides JY-6, its metabolic time course was similar to that of human intestinal bacteria (data not shown). However, when KPK was incubated with Eubacterium A-44, KPI was a main metabolite 4 h after incubation. KPH, KPB, and KPJ were observed as metabolites 9 h after incubation. Another metabolite, KPA, was observed 20 h after incubation as a main metabolite. This KPA was slowly metabolized to hederagenin.

Based on these findings, the time course of KPK metabolism by human intestinal bacteria was as follows: in the early stage, KPK began to be quickly converted to KPH and slowly to KPJ. Then these compounds were metabolized to KPI, and then slowly metabolized to KPA, HA, and hederagenin (Chart 1).

**Antiinflammatory Effect of the Main Metabolites KPI and KPA**

The inhibitory effects of KPI and KPA, which were the main metabolites of KPK produced by human intestinal bacteria, were measured in a FCA reagent-induced rat rheumatoid arthritis model (Table 2). After the induction of rheumatoid arthritis, KPA or KPI (5, 10, 20 mg/kg) were intraperitoneally administered for 10 d, respectively. The edema was significantly reduced 5 d after the administration of KPA at the two higher doses. The administration of KPI 5 mg/kg did not inhibit the edema, and the 10 mg/kg dose showed weak inhibitory effects. Both KPA and KPI quickly showed significant effects when their doses were increased. The prolongation of KPA and KPI administration exhibited more potent antiedema effects.

To confirm the anti-edema effects, the inhibitory activity of KPA and KPI on vascular permeability was also measured (Table 3). KPA and KPI reduced the vascular permeability, and KPA was more effective than KPI.

![Chart 1. Proposed Metabolic Pathway of Kalopanaxsaponin K by Human Intestinal Microflora](image)

### Table 1. KPK and KPJ Transforming Activity of Intestinal Bacteria Isolated from Human Feces

<table>
<thead>
<tr>
<th>Microbe</th>
<th>KPH</th>
<th>KPB</th>
<th>KPJ</th>
<th>KPI</th>
<th>KPA</th>
<th>HA</th>
<th>H</th>
<th>Transformed product$^a$ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteroides HJ15</td>
<td>0</td>
<td>2.13</td>
<td>1.43</td>
<td>0.78</td>
<td>0.68</td>
<td>0</td>
<td>5.03</td>
<td>KPI 0.86 0.47 0.93 7.0</td>
</tr>
<tr>
<td>Bacteroides JY6</td>
<td>32.30</td>
<td>7.70</td>
<td>&lt;0.05</td>
<td>25.31</td>
<td>7.39</td>
<td>0</td>
<td>6.16</td>
<td>KPI 78.58 8.70 0 6.16</td>
</tr>
<tr>
<td>Bifidobacterium K103</td>
<td>2.79</td>
<td>1.31</td>
<td>0.96</td>
<td>1.37</td>
<td>1.62</td>
<td>0</td>
<td>3.04</td>
<td>KPI 6.86 2.42 0 4.46</td>
</tr>
<tr>
<td>Bifidobacterium K110</td>
<td>2.79</td>
<td>2.02</td>
<td>0.90</td>
<td>0.78</td>
<td>1.37</td>
<td>0</td>
<td>0</td>
<td>KPA &lt;0.05 0.81 0 0</td>
</tr>
<tr>
<td>Eubacterium A44</td>
<td>2.79</td>
<td>4.15</td>
<td>4.28</td>
<td>0</td>
<td>0.99</td>
<td>0</td>
<td>0.21</td>
<td>KPA &lt;0.05 0.578 0 0.93</td>
</tr>
<tr>
<td>Eubacterium L8</td>
<td>0</td>
<td>0</td>
<td>&lt;0.05</td>
<td>2.29</td>
<td>1.24</td>
<td>0</td>
<td>3.33</td>
<td>KPA &lt;0.05 0 0 11.54</td>
</tr>
<tr>
<td>Fusobacterium K60</td>
<td>2.79</td>
<td>0.82</td>
<td>0.83</td>
<td>0</td>
<td>1.55</td>
<td>0.81</td>
<td>4.46</td>
<td>KPA &lt;0.05 0.99 0.75 4.74</td>
</tr>
<tr>
<td>Lactobacillus L2</td>
<td>0</td>
<td>0</td>
<td>&lt;0.05</td>
<td>2.10</td>
<td>7.02</td>
<td>0.75</td>
<td>0</td>
<td>KPA &lt;0.05 2.42 1.12 0</td>
</tr>
<tr>
<td>Streptococcus S2</td>
<td>0</td>
<td>0</td>
<td>0.83</td>
<td>3.07</td>
<td>6.77</td>
<td>1.18</td>
<td>0</td>
<td>KPA 11.69 0.81 0 0</td>
</tr>
<tr>
<td>Streptococcus S10</td>
<td>0</td>
<td>0</td>
<td>&lt;0.05</td>
<td>1.61</td>
<td>2.80</td>
<td>0</td>
<td>0</td>
<td>KPA &lt;0.05 0.99 0 0</td>
</tr>
</tbody>
</table>

*a* To assay transformed products, the reaction mixture was incubated at 37 °C for 20 h with a 25 mg pellet of each intestinal bacterium.
When the biological activities of kalopanaxsaponins were investigated in hyperglycemia, cytotoxicity, mutagenicity, and hepatotoxicity model, KPA was found to be the most active compound. In this FCA-induced rat rheumatoid arthritis model, KPA was also effective.

In Korea, the stem bark of *K. pictus* belonging to the family Araliaceae has been used as a tonic, analgesic, anti-inflammatory and anti-diabetic agent. Its main components were kalopanaxsaponins, such as KPH, KPB, and KPK. However, it is thought that these compounds are not absorbed from the intestine. Therefore these compounds come into contact with intestinal bacteria after oral administration and are transformed into KPI, KPA, and hederagenin. Particularly if *Enterobacterium* A-44 is involved in the metabolism of these kalopanaxsaponins, the main metabolite should be KPA. This biotransformation should be catalyzed in the human intestine by intestinal bacteria and then the metabolites KPI and KPA could play an important role in the anti-rheumatic activity of these compounds. Finally, we believe that KPK, KPB and KPH in *K. pictus* are natural prodrugs for the anti-rheumatic compound KPA.

**Acknowledgements** This work was supported by the BK 21 grant from the Ministry of Education and Kyung Hee University.

**REFERENCES**