News Release

Title

Predicting Inchinkoto efficacy, in patients with obstructive jaundice associated with malignant tumors, through pharmacomicrobiomics

Key Points

- To the best of our knowledge, this is the first report to reveal the relationship between the gut microbial profile and activation of a Kampo medicine using samples collected from patients.
- Stool genipin-producing activity varied widely among patients but showed significant correlation with: (1) Bristol stool scale, (2) diversity of intestinal microbiome, (3) abundance of certain microbes especially those of the order *Clostridiales* and *Lactobacillales*, and (4) stool organic acids.
- The stool genipin-producing activity was also correlated to bile flow volume changes observed 2 to 3 days after administration of ICKT.

Summary

Inchinkoto (ICKT) is a popular choleretic and hepatoprotective herbal medicine that is widely used in Japan. Geniposide, a major ingredient of ICKT, is metabolized to genipin by gut microbiota, which exerts a choleretic effect. This study investigates the relationship between stool genipin-producing activity and diversity of the clinical effect of ICKT in patients with malignant obstructive jaundice. Fifty-two patients with malignant obstructive jaundice who underwent external biliary drainage were included. ICKT was administered as three packets per day (7.5 g/day) for three days and 2.5 g on the morning of the fourth day. Stool samples were collected before ICKT administration and bile flow was monitored on a daily basis. The microbiome, genipin-producing activity, and organic acids in stools were analyzed. The Shannon-Wiener (SW) index was calculated to evaluate gut microbiome diversity. The stool genipin-producing activity showed a significant positive correlation with the SW index. Stool genipin-producing activity positively correlated with the order *Clostridia* (obligate anaerobes), but negatively correlated with the order *Lactobacillales* (facultative anaerobes). Moreover, stool genipin-producing activity was positively correlated to the concentration valeric acid, but negatively correlated to the concentration of lactic acid and succinic acid. The change of bile flow at 2 and 3 days after ICKT administration showed significant positive correlation with genipin-producing activity (correlation coefficient, 0.40 and 0.29, respectively, $P<0.05$). An analysis of stool profile, including stool genipin-producing activity, may predict the efficacy of ICKT. Modification of the microbiome may be a target to enhance the therapeutic effect of ICKT.

Research Background

Inchinkoto (ICKT) (TJ-135, Tsumura & Co., Tokyo, Japan) is a popular choleretic and hepatoprotective herbal medicine, which is approved by the Japanese Ministry of Health and Welfare. There is considerable individual diversity in the efficacy of ICKT for patients with obstructive jaundice associated with malignant tumors. Recently, we discovered a biomarker for predicting responders to ICKT using blood metabolites and found a relationship between the profile of gut microbiota and blood levels of genipin, one of the main active ingredients of ICKT. Geniposide, a major component of ICKT, has been shown to be metabolized to genipin by gut microbiota using an animal model. However, the metabolism of geniposide to genipin in human gut microbiota has never been investigated. We hypothesized that the gut microbiota profile could be correlated with the pharmacological action of ICKT, which might explain the observed diversity of patient response to ICKT.

Research Results

ICKT was administered as three packets/day (7.5 g/day) for three days and one packet (2.5 g) on the morning of the fourth day. Stool, serum, and bile samples were collected before administration of ICKT (Figure 1).

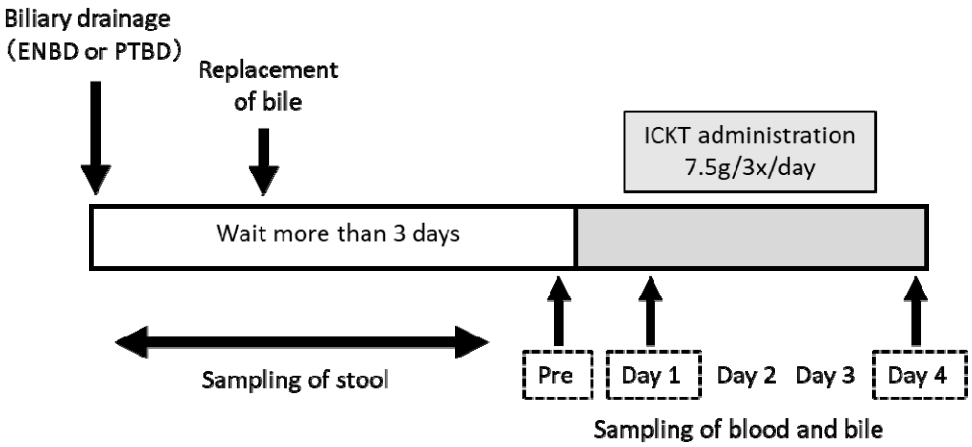


Figure 1. Study design.

Fifty-two patients with obstructive jaundice who underwent external biliary drainage participated in this study. Drained bile was replaced either by intake or through a nasogastric tube. ICKT treatment was started after the drainage had stabilized. The bile and serum samples were collected before (Pre), and 3 hours after administration of ICKT on Day 1 and Day 4. The stool samples were collected before starting ICKT administration.

To clarify the characteristics of extensive and poor genipin producers, we selected the top 10 and bottom 10 subjects according to their stool genipin-producing activities. The Bristol stool scale, diversity of microbiome, and bile flow were compared between these two subgroups. For stool samples collected before ICKT administration (Pre), the Bristol stool scale for the bottom 10 (6 [4.5–6]) was significantly higher compared with the top 10 (3 [3–4], Figure 2A). The SW index in the bottom 10 (2.49 [2.09–2.91]) was significantly lower compared with that for the top 10 (3.75 [3.52-3.86]) (Figure 2B). The change of bile flow at two days after administration of

ICKT (on Day 2) in the top 10 (-40 [-82.5-0] mL) was significantly higher compared to the bottom 10 (85 [27.5-165] mL) (Figure 2C).

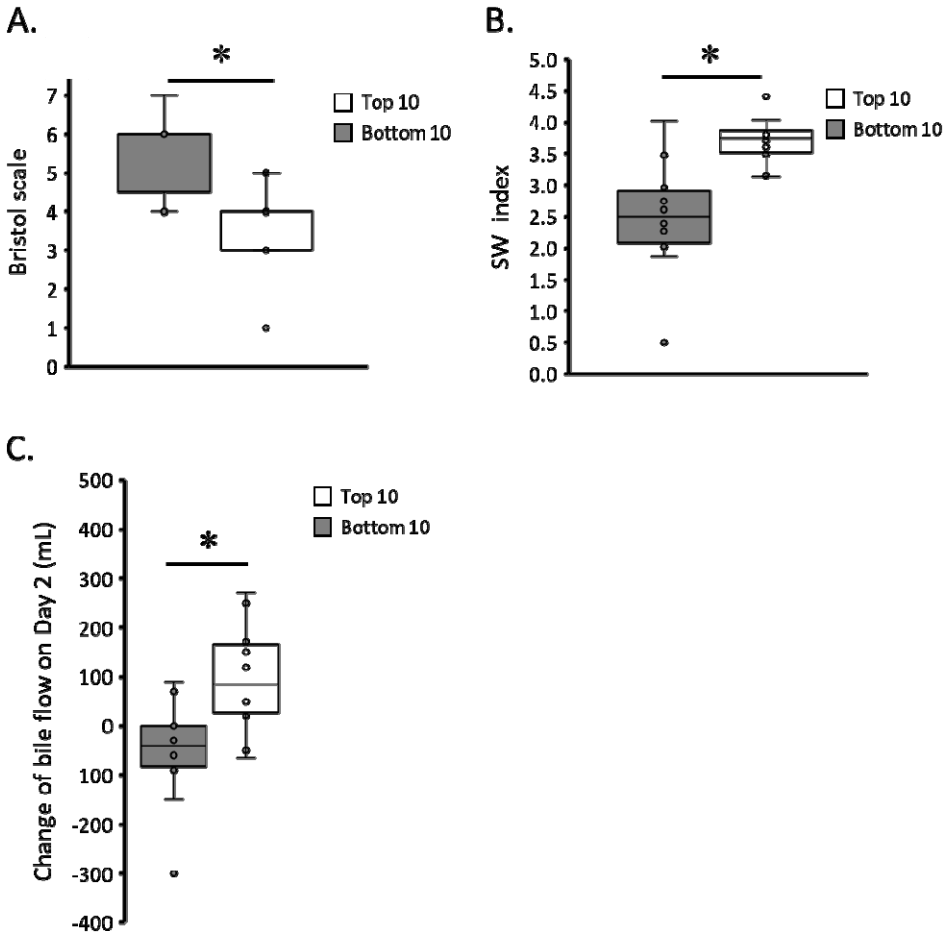


Figure 2. Subgroup analysis of patients with the top 10 and bottom 10 genipin-producing activities.
(A) Bristol stool scale, (B) Shannon-Wiener index (SW index), and (C) bile flow increase on Day 2 were compared between the top 10 and bottom 10 patients based on stool genipin-producing activity. *; $P<0.05$.

The profile of microbiota for the bottom 10 patients, whose stool genipin-producing activity was below the detection limit or extremely low, was dominated by genera *Enterococcus* (0.273 ± 0.277), *Lactobacillus* (0.067 ± 0.111), or *Streptococcus* (0.029 ± 0.052), which are facultative anaerobes. In contrast, the microbiome profile for the top 10 patients were dominated by obligate anaerobes (Figure 3A). The subjects were clustered by stool genipin-producing activity along the nMDS1 axis. No gender difference was found in any clusters. These findings suggested the microbiome profile might affect genipin-producing activity (Figure 3B)

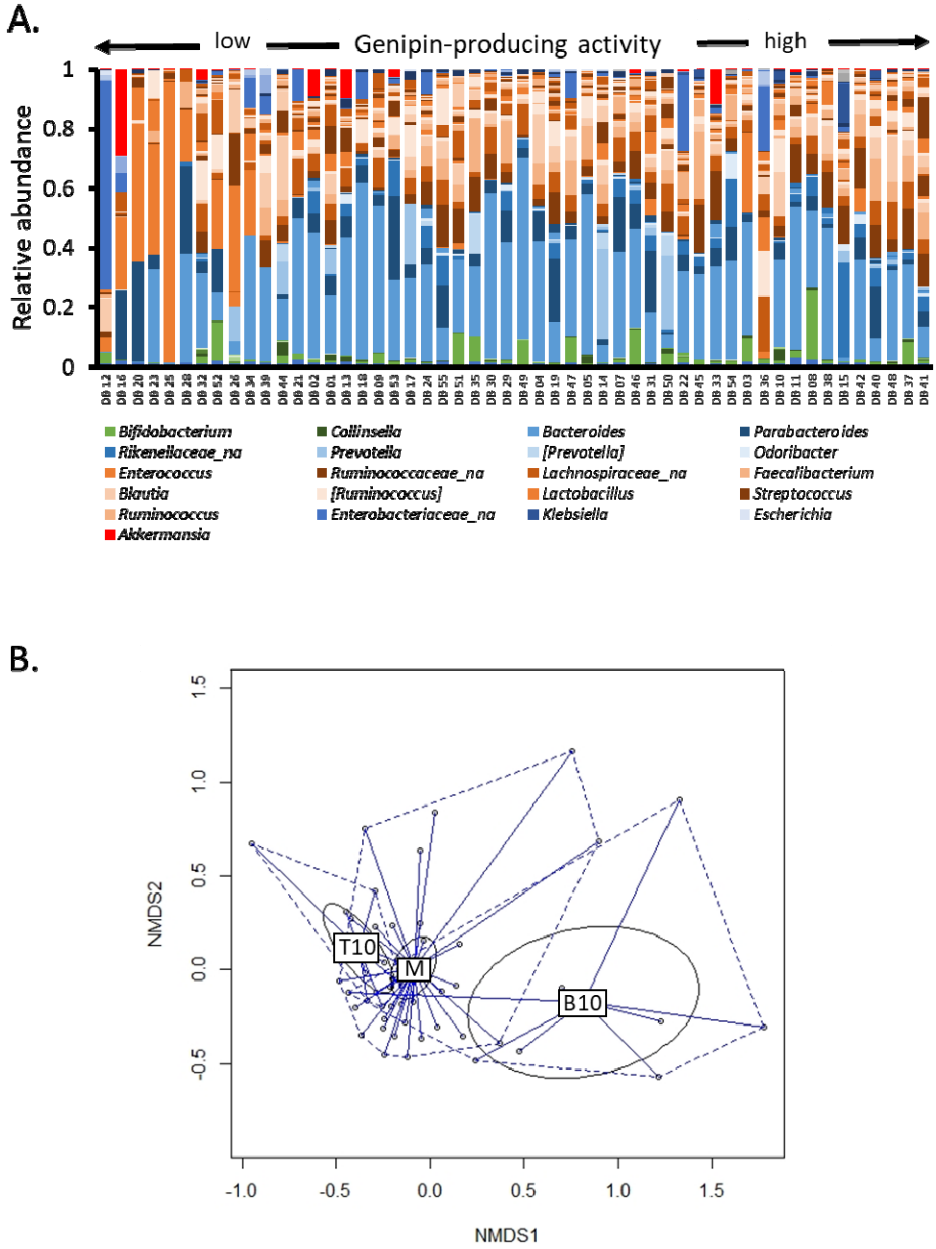


Figure 3. Stool microbiome profile and genipin-producing activity.
(A) Relative abundance of the microbiome at the genus level in stools from each patient listed in order of stool genipin-producing activity. (B) Non-metric multidimensional scaling was performed for the top 10 subgroup (T10), bottom 10 subgroup (B10), and between the top 10 and bottom 10 subgroup (M).

Next, we investigated correlation analysis using all 52 samples. The characteristics of patients with a variety of stool genipin-producing activities were analyzed using Spearman's correlation coefficient. Of the 36 positively correlated genera, 21 genera belonged to the order *Clostridia,* e.g., genera *Ruminococcus* (0.013 ± 0.020), *Oscillospira* (0.006 ± 0.005), unknown genus in family *Ruminococcaceae* (0.055 ± 0.051) (Spearman's correlation coefficient; 0.68 [P<0.001], 0.68 [P<0.001], and 0.58 [P<0.001], respectively). By contrast, 4 out of 10 negatively correlated genera belonged to the order *Lactobacillales* e.g., genus *Enterococcus* (0.062 ± 0.161,

Spearman's correlation coefficient; 0.43 [P=0.002]) (Figure 4A). Moreover, stool genipin-producing activity was positively correlated with the concentration of stool valeric acid (Spearman's correlation coefficient; 0.40 [P=0.003]), and negatively correlated with the concentration of stool lactic acid and succinic acid (Spearman's correlation coefficient; -0.28 [P=0.047] and -0.32 [P=0.019], respectively) (Figure 4B). The change of bile flow at 2 and 3 days after administration of ICKT showed significant positive correlation with genipin-producing activity (Spearman's correlation coefficient; 0.40 [P=0.004] and 0.29 [P=0.038], respectively) (Figure 4C). White blood cell (WBC) count and Bristol stool scale before administration of ICKT was also correlated with the stool genipin-producing activity (Spearman's correlation coefficient; -0.31 [P=0.027] and -0.41 [P=0.003], respectively) (Figure 4C).

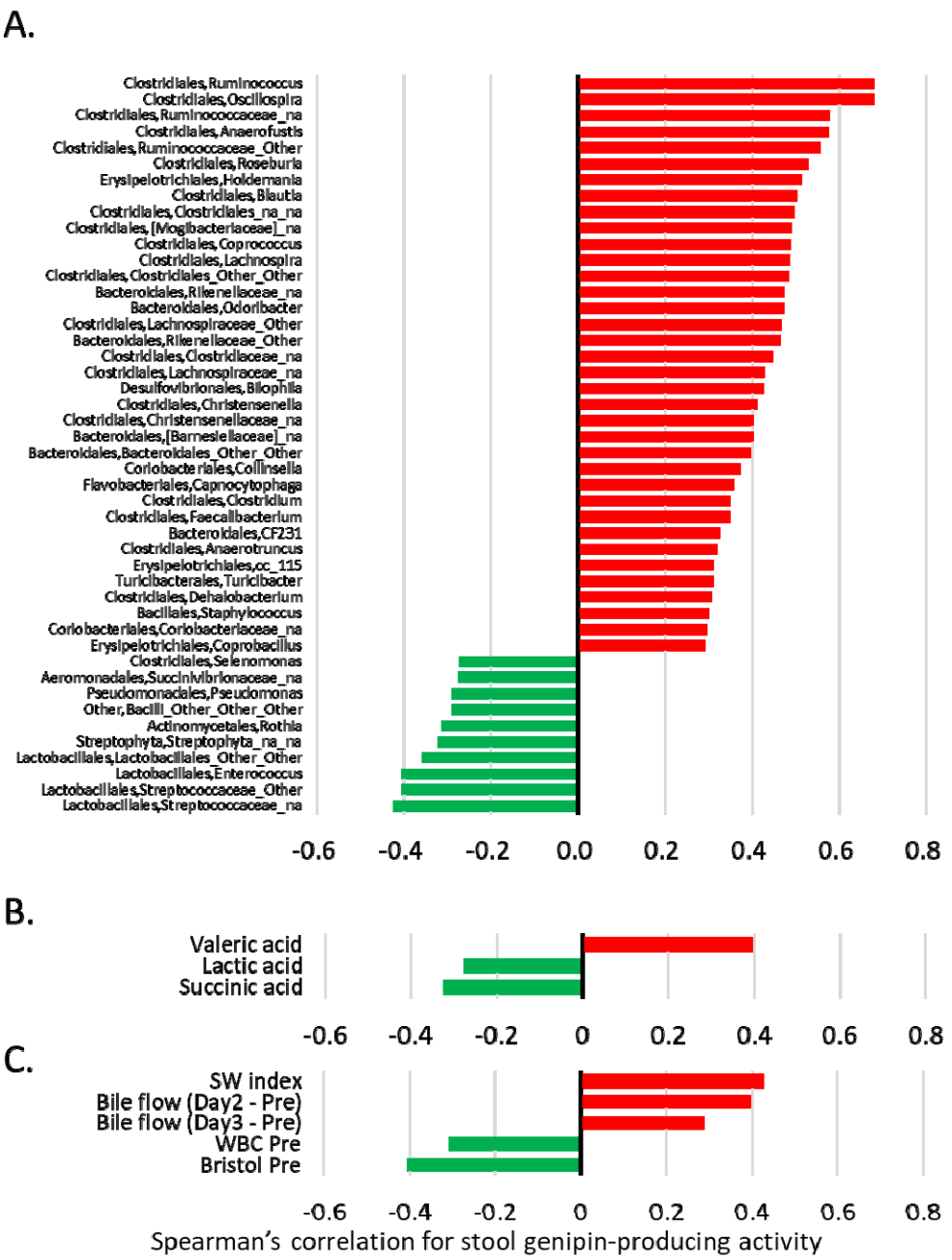
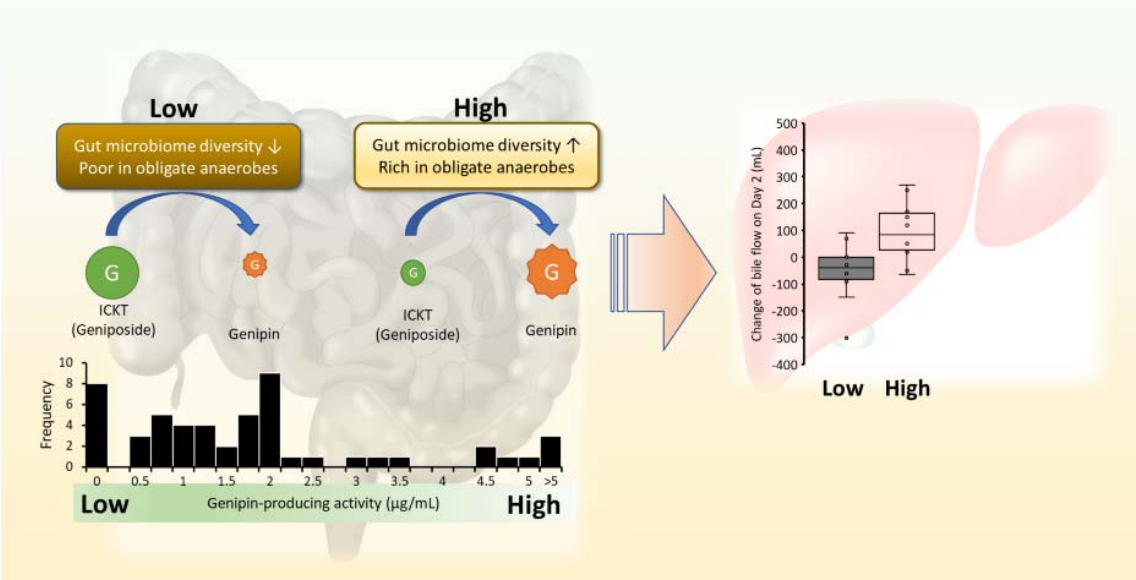


Figure 4. Correlation analysis for stool genipin-producing activity

Correlation analyses between stool genipin-producing activity and (A) relative abundance of microbiome, (B) stool organic acids concentrations, and (C) clinical data were performed using Spearman's correlation analysis. Only values that gave a significant correlation ($P<0.05$ by uncorrelated test) are shown. Red bar; positive correlation. Green bar; negative correlation.

Research Summary and Future Perspective

In this study, we identified a relationship between stool genipin-producing activity and choleretic activity in patients who were administered ICKT. The stool genipin-producing activity was correlated with stool profile. The analysis of stool profiles, including microbiome diversity and organic acid concentrations, may be used to predict the pharmacological action of ICKT. Modification of the stool profile may be a therapeutic target to enhance the pharmacological action of ICKT.



Publication

Journal: Pharmacological Research

Title: Predicting Inchinkoto efficacy, in patients with obstructive jaundice associated with malignant tumors, through pharmacomicrobiomics

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DOI : https://doi.org/10.1016/j.phrs.2021.105981

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https://www.med.nagoya-u.ac.jp/medical_J/research/pdf/Pha_Res_2021117.pdf