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Efficiency of herbal medicine Dai-kenchu-to on portal blood flow in rat models

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HIGHLIGHTS

- Effect of DKT to PBF.
- Normal and cirrhotic liver.
- Continuous observation of PBF.

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ABSTRACT

Introduction: To clarify the influence of Dai-Kenchu-To (DKT) on portal blood flow (PBF), PBF was continuously measured with Doppler ultrasound.
Methods: Normal liver rats were divided into a DKT 90 mg/kg, DKT 270 mg/kg administered group, and control, while cirrhotic liver rats were divided into a DKT-LC 90 mg/kg administered group and Control-LC. The PBF was measured after the administration of either DKT or water for 60 min by laser Doppler flowmetry system.
Results: The PBF in the DKT 90 increased approximately 10 min after DKT was administrated, and elevated levels were maintained for approximately 10 min. A comparison of the increase in PBF by the calculating the area under the curve (AUC) revealed that flow was significantly higher in the DKT 90 compared to either the control or the DKT 270 ($p < 0.05$). The cirrhotic liver group showed stable PBF in both the DKT-LC and Control-LC. The AUC, revealed no significant difference between the DKT-LC and Control-LC.
Discussion: DKT induced an increase in PBF in normal livers; however, its effects were insufficient to increase PBF in the cirrhotic livers. No increase in the portal blood flow in the cirrhotic liver rats was probably the result of the cirrhotic liver, which had fibrotic change, and, therefore, may not have had sufficient compliance to accept the increasing blood flow volume from the intestinal tract.
Conclusion: We suggested DKT has the potential to protect the liver by increasing PBF when the liver has either normal or mild to moderate dysfunction.

1. Introduction

Dai-kenchu-to (DKT) is a traditional herbal medicine that has clinical effects on intestinal obstruction subsequent to laparotomy [1] and is used widely in the gastroenterological field. DKT is composed of 4 crude compounds, including dried ginger rhizome, ginseng root, rice gluten, and Zanthoxylum fruit [2]. The effect of each element of DKT on blood flow and mobility in the gastrointestinal tract is examined [3,4]. Ogasawara et al. reported that DKT increases portal blood flow (PBF) in the early phase after oral administration in humans [5]. A previous report described that an increase of the PBF leads to accelerated liver regeneration [6]. However, there have been no studies that continuously observed the dynamic change of PBF volume in response to DKT administration using animal models. The current study continuously measured the PBF using Doppler

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ultrasound in both a normal and cirrhotic liver rat model to clarify the influence of DKT on PBF.

2. Methods

Male Fisher rats (200–250 g weight, 6–8 weeks old) were purchased from Charles River Inc. (Kanagawa, Japan), and the animal protocol was approved by the Animal Experimentation Committee of Nagasaki University. The animals were free of all pathogens and were housed under standard conditions (room temperature 24 °C, humidity 50 ± 5%, 12 h/12 h light–dark cycle).

A commercially available kampo medicine, DKT, was purchased from Tumura Co. Ltd (Tokyo, Japan). It was prepared as a dried extract powder of Ginseng radix, Zanthoxyli fructus, and Zingiberis siccaatum rhizome in the ratio 3:2:5, respectively [7].

The normal liver rats were divided into three groups: the DKT 90 group (n = 5) received intragastric administration of 90 mg/kg DKT; the DKT 270 group (n = 4) received intragastric administration of 270 mg/kg DKT; and the control group (n = 4) received intragastric administration of 10 ml/kg of drinking water.

Liver cirrhosis was induced by intraperitoneal injections of 10 mg/kg of dimethylnitrosamine (DMN) (Sigma, AT. Louis, MO) dissolved in saline to obtain a 1% solution, administered 3 consecutive days a week for 3 weeks [8]. Liver was cirrhotic both macroscopically and microscopically (Fig. 1-a). The rats were divided into two groups: the DKT-LC group (n = 3) received intragastric administration of 90 mg/kg DKT; and the Control-LC group (n = 3) received intragastric administration of 10 ml/kg drinking water.

The rats were placed under pentobarbital (64.8 mg/ml, 1 ml/kg) anesthesia by intraperitoneal injection, laparotomized, and gastrostomy performed. Either DKT or water was administrated throughout the gastrostomy when the PBF became stable after laparotomy, and the PBF was measured for 60 min by laser Doppler flowmetry system (Toshiba Medical Systems Co. Tochigi, Japan) (Fig. 1-B). A pencil probe (Toshiba Medical Systems Co. Tochigi, Japan) was placed over the mid-lobe of the liver, and the position of the probe was not altered during the course of the experiments. The penetration depth of the laser Doppler flowmetry system used was 4–5 mm. The liver is supplied blood flow from both hepatic artery and portal vein, however the supply from portal vein is 70–80% of all blood supply, that is why we determined that the flow measured by the Doppler ultrasound reflect the portal blood flow in this study. Blood flow was recorded on a computer and analyzed using Power Labo (ADInstruments Japan Inc. Tokyo, Japan).

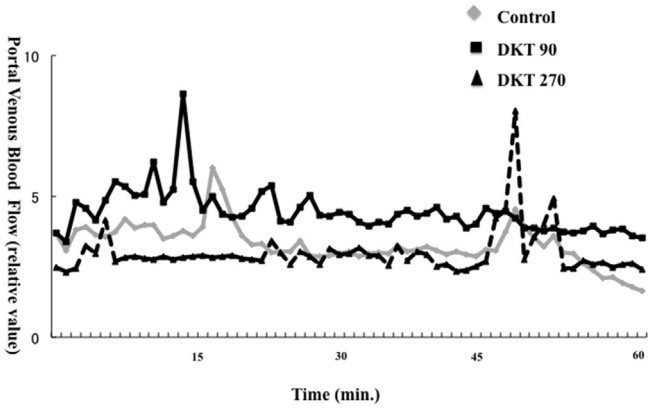

Fig. 2. Change in portal blood flow (relative value) after administration of either DKT or water in normal rat livers. Flow increased approximately 10 min after DKT administration and continued for approximately 10 min in the DKT 90 group.

Because the previous study reported that DKT administration didn't give any influence to the mean arterial blood flow pressure in rat model [9], we didn't measure the systemic blood pressure in this study.

3. Statistical analysis

The Mann–Whitney *u*-test was used for the statistical analysis. p values < 0.05 were regarded to be statistically significant.

4. Results

The portal blood flow in the DKT 90 group increased started approximately 10 min after DKT was administrated, and this condition was maintained for approximately 10 min. On the other hand, no significant increasing of portal blood flow was observed in the DKT 270 group and the control group (Fig. 2). A comparison of the increase in portal blood flow by the calculating the area under the curve (AUC) revealed that the portal blood flow was significantly higher in the DKT 90 group in comparison to both the control group and the DKT 270 group (Fig. 3; $p < 0.05$).

The PBF was almost stable for 60 min after administration of the medicine in both the DKT-LC and Control-LC groups (Fig. 4). The AUC revealed no significant difference between the DKT-LC and Control-LC groups (Fig. 5). No elevation of the PBF was observed when the liver had cirrhotic change.

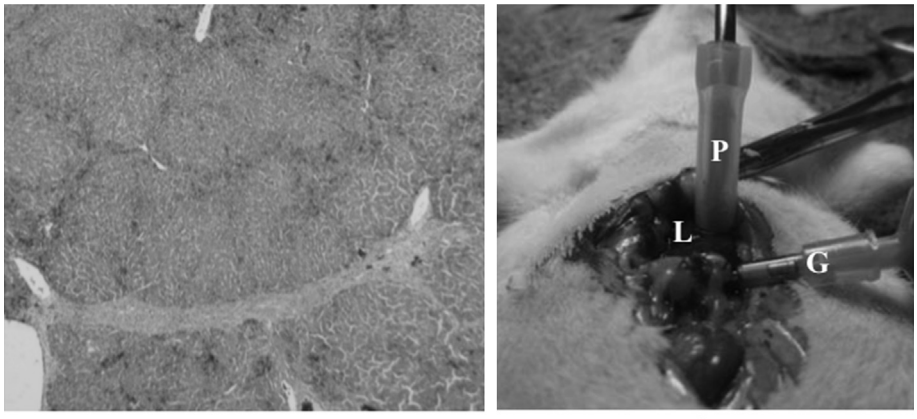

Fig. 1. **a** Microscopic findings of DKT-LC group rat liver (H&E, x40). The regenerative nodules of hepatocytes are surrounded by fibrous connective tissue that bridges between portal tract. **b** Portal blood flow measurement using laser Doppler flowmetry system. P; probe, L; liver, G; gastrostomy.

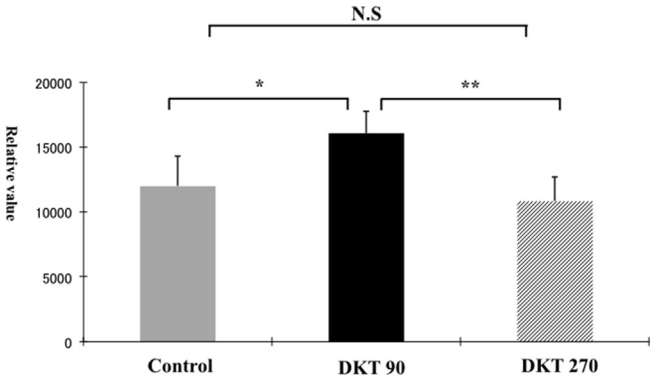

Fig. 3. AUC of portal blood flow in normal liver rats. The increase in the portal blood flow in the DKT 90 group is significantly higher than that in the other two groups. $^{*}p < 0.05$, $^{**}p < 0.05$.

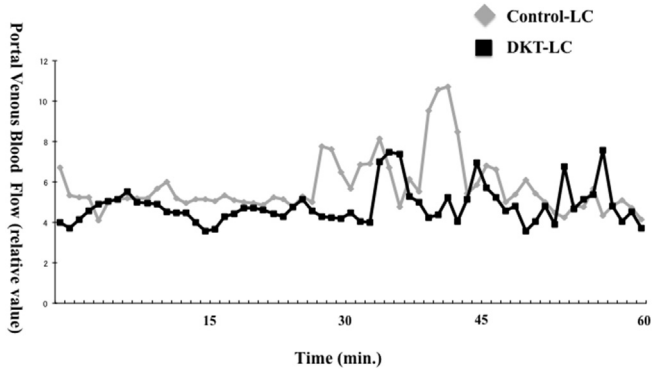

Fig. 4. Changing of portal blood flow (relative value) after the administration of either DKT or water in cirrhotic liver rats. The flow was mostly stable in both groups.

5. Discussion

DKT induces a dose-dependent increase in the intestinal blood flow [10], and the active ingredient in DKT most responsible for this effect is dried ginger rhizome [9]. The current study demonstrated an increase in the portal PBF following intragastric administration of DKT in the normal liver rat model. Logically, then, increasing the intestinal blood flow volume should increase the volume of PBF. No increase in the PBF in the cirrhotic liver rats was probably the result of the cirrhotic liver, which had fibrotic change, and, therefore, may not have had sufficient compliance to accept the increasing blood flow volume from the intestinal tract. We are considering the possibility that there is an optimum density for DKT to affect PBF, however it is still unclear why DKT increased small intestinal blood flow in a dose-related manner but not PBF. We are continuing the investigation.

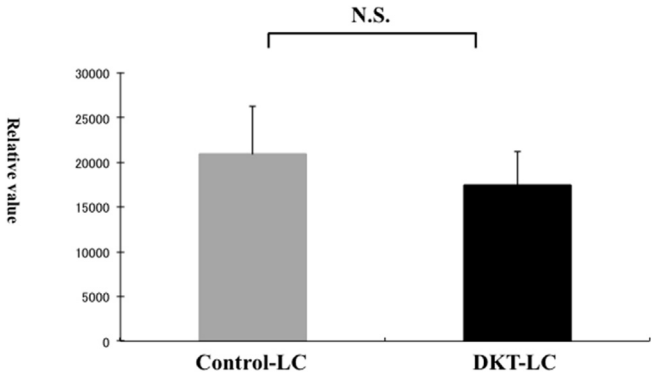

Fig. 5. AUC of portal blood flow on cirrhotic liver rats. There was no significant difference between the DKT-LC and Control-LC groups.

Kaiho et al. [11] reported that DKT significantly decreases postoperative serum ammonia levels in hepatectomized patients. Although the mechanisms of decreasing serum ammonia levels are unclear, the decrease is certain to be associated with remnant liver protection after hepatectomy.

Nishi et al. [12] demonstrated that DKT suppressed inflammatory reaction, stimulated bowel movement and improved oral intake after hepatic resection, which may decrease serious morbidity after hepatic resection.

Concerning these previous reports and these results (which demonstrated the efficacy of DKT to the liver), we hypothesized that DKT may accelerate liver regeneration after hepatectomy. A 70% hepatectomy rat model was established according to the classical Higgins-Anderson procedure [13], and the model was then gavaged with DKT every 12 h from 12 h before the laparotomy to 72 h after the laparotomy. The liver/body weight ratio and number of Ki67 positive cells were compared between the DKT group and control group. However, there was no significant difference between the 2 groups (data not shown). The effectiveness of DKT on PBF may only last for 10–20 min after administration, and PBF elevation for 10–20 min twice a day was insufficient to accelerate liver regeneration, especially since the liver was normal and, thus, already considered to have a good potential for regeneration.

In conclusion, administration of DKT increased PBF in normal liver rats, but failed to cause any observed reaction in the cirrhotic liver group. Although further investigation is needed, we suggested DKT has the potential to protect the liver by increasing PBF when the liver has either normal or mild to moderate dysfunction.

Conflicts of interest

None.

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