Disposition of glycyrrhetic acid after oral administration of Kanzô-tô and Syakuyaku-kanzô-tô in the rat

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Abstract

As a first step toward elucidating the disposition of traditional Chinese formulas which contain licorice, the dispositions of glycyrrhetic acid (GLA) after oral administration of Kanzô-tô (plain licorice) and Syakuyaku-kanzô-tô (combination of licorice and paeoniae) were investigated in the rat. Furthermore, in order to determine the differences between oral administration of Kanzô-tô and intravenous administration of glycyrrhizin (GLZ), the disposition of GLA after intravenous administration of GLA or GLZ was also examined in this study. GLA was measured by an enzyme immuno-antibody technique.

The results obtained from the oral administration of Kanozô-tô and Syakuyaku-kanzô-tô showed that serum concentration of GLA was at significantly high levels in the latter case. In addition, the amount of GLA in fecal excretion was at significantly low levels in cases of Syakuyaku-kanzô-tô administration.

Meanwhile, the serum concentration of GLA after oral administration of Kanzô-tô followed a course of lower levels for a long time in comparison with the cases of intravenously administered GLA or GLZ.

Key words disposition, glycyrrhetic acid, glycyrrhizin, licorice, paeoniae, rat, Kanzô-tô, Syakuyaku-kanzô-tô

Abbreviations GLA, glycyrrhetic acid ; GLZ, glycyrrhizin ; HPLC, high performance liquid chromatography ; Kanzô-tô (Gan-Cao-Tang), 甘草湯 ; Syakuyaku-kanzô-tô (Shao-Yao-Gan-Cao-Tang), 芍薬甘草湯

Introduction

The Chinese formulas of Kanzô-tô (plain licorice) and Syakuyaku-kanzô-tô (combination of licorice and paeoniae) were first described in the ancient textbook of Shang-Han-Lun (about 200 A. D.).[1] Since then, they have been widely used for the purpose of anti-inflammatory, antispasmodic and muscle relaxation effects.

Recently, it has been disclosed that licorice and its effective components possess anti-inflammatory[2] and delta-4-β-reductase suppressing effects.[3] However, no biopharmaceutical study has been performed regarding the decoctions of licorice which are orally administered. Previously, we have reported about the disposition of glycyrrhetic acid (GLA) and its glycosides in humans depending on the oral administration of the decoction of plain licorice.[4] The present

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study was undertaken in an attempt to elucidate the differences of the disposition of GLA between Kanzô-tô and Syakuyaku-kanzô-tô administrations, and to clarify the differences of the disposition of GLA among orally administered plain licorice, intravenously administered GLA and glycyrrhizin (GLZ).

Materials and Methods

Kanzô-tô administration : Five grams of licorice from China (Tochimoto-Tenkaido Co., Ltd., Osaka, Japan) were put in 300 ml of water to make a decoction of 100 ml. The decoction was analyzed to contain 2.67 mg/ml of GLZ by high performance liquid chromatography (HPLC). GLA was not detected in this decoction. The subjects were five male rats (6 weeks old, weighing 190−210 g). After about 15 hours of overnight fasting, decoctions accounting for 5.34 mg/kg of GLZ were administered orally using a metalic stomach tube to the rats.

Syakuyaku-kanzô-tô administration : A mixture of five grams of licorice mentioned above and five grams of Paeoniae Radix from Nara, Japan (Tochimoto Tenkaido Co., Ltd.) were put in 300 ml of water to make a decoction of 100 ml. The decoction contained 2.67 mg/ml of GLZ. The subjects were five male rats (6 weeks old, weighing 190−210 g). After 15 hour of overnight fasting, decoctions amounting to 5.34 mg/kg of glycyrrhizin were administered orally.

Glycyrrhetic acid administration : Two rats (same conditions as described above) were administered 10 mg/kg of GLA (Fluka Co., Ltd., Buchs, Swiss) solution intravenously through a cannulated tube into the jugular vein after 15 hours of overnight fasting.

Glycyrrhizin administration : 10 mg/kg of GLZ (Minophagen Co., Ltd., Tokyo, Japan) were given intravenously to three male rats (same conditions as described above) by using a cannulated tube into the jugular vein after 15 hours of overnight fasting.

Collection of samples : Under light ether anesthesia, the jugular vein was cannulated with polyethylene tubing. Blood samples were drawn through the cannula at appropriate time intervals and then were centrifuged in order to separate the plasma. The total amounts of urine and feces samples within 24 hours after administration were collected by using a metabolic cage.

Enzyme immuno-antibody assay : Measurements of GLA were done according to the second antibody techique by Kanaoka *et al*.[5] In this study, the technique was partly modified as described in our previous report.[4]

Results

Oral administration of Kanzô-tô and Syakuyaku-kanzô-tô

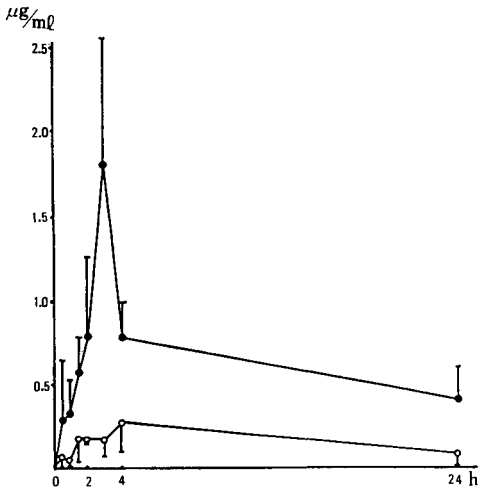

Fig. 1 The changes in glycyrrhetic acid concentration in the serum after oral administration of Kanzô-tô (—○—) and Syakuyaku-kanzô-tô (—●—) administration.

Figure 1 shows the serum concentration of GLA after oral administration of Kanzô-tô and Syakuyaku-kanzô-tô. In spite of the fact that the same dosage of GLZ was used in the decoctions, the serum concentration of GLA was significantly elevated in the case of Syakuyaku-kanzô-tô administration, and this tendency was observed even 24 hours after administration.

Total amounts of fecal excretion of GLA within 24 hours after administraion were listed in Figure 2.

The results show that percentile amounts of GLA in feces to GLA administered are significantly low in cases of Syakuyaku-kanzô-tô.

Concerning the urinary excretion of GLA in both cases, total amounts of GLA within 24 hours after administration were significantly high in cases of Syakuyaku-kanzô-tô administration (Fig. 3).

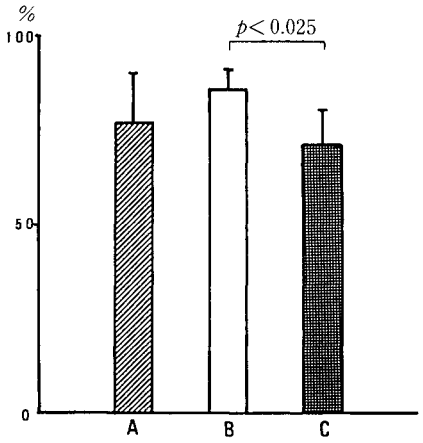

Fig. 2 The total amount of glycyrrhetic acid excretion in feces during 24 hours after administration. A, intravenously administered glycyrrhizin ; B, orally administered Kanzô-tô ; C, orally administered Syakuyaku-kanzô-tô. The amounts are presented by percentiles to dosage of glycyrrhizin administered in each case.

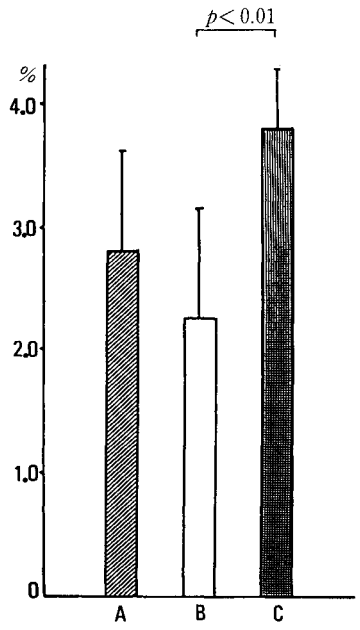

Fig. 3 The total amount of glycyrrhetic acid excretion in urine during 24 hours after administration. A, intravenously administered glycyrrhizin ; B, orally administered Kanzô-tô ; C, orally administered Syakuyaku-kanzô-tô. The amounts are presented by percentiles to dosage of glycyrrhizin administered in each case.

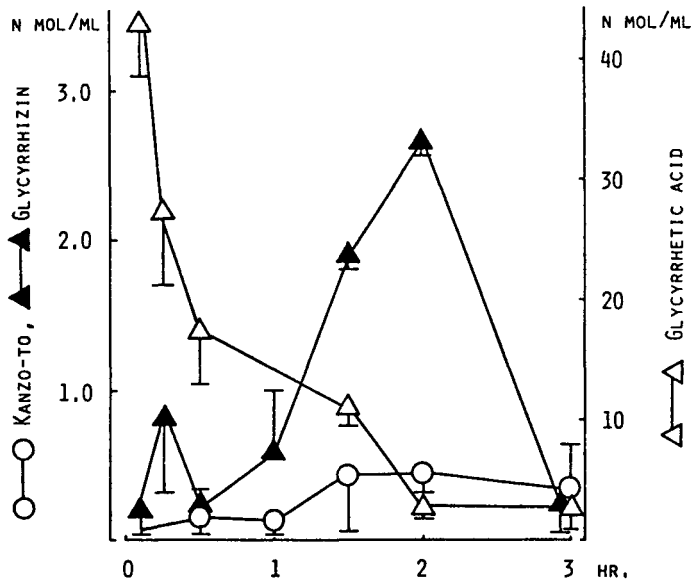

Fig. 4 The changes in glycyrrhetic acid concentration in the serum.
Open triangles, intravenously administered glycyrrhetic acid (GLA) ; closed triangles, intravenously administered glycyrrhizin(GLZ) ; open circles, orally administered Kanzô-tô.

Comparison of oral administration of Kanzô-tô and intravenously administered GLA and GLZ

Figure 4 shows the serum concentration of GLA after oral administration of Kanzô-tô and the intravenously administered GLA and GLZ. As mentioned above, the decoction of Kanzô-tô does not contain GLA but GLZ and its analogs. Therefore, this result indicates that there are significant differences in serum concentration of GLA between orally administered GLZ and intravenously given GLZ. Meanwhile, in cases of intravenous administration of GLA, the serum concentraion of GLA decreases at a constant rate.

The total amount of fecal excretion of intravenously given GLZ within 24 hours is about 80 %, an amout not significantly different from that of orally administered Kanzô-tô (Fig. 2). Concerning the total amounts of urinary excretion of GLA after GLZ and Kanzô-tô administration within 24 hours, no significant differences were observed between the two cases (Fig. 3), in spite of the considerable differences seen in AUC values of GLA in the serum.

Discussion

Since 1976, when formulas of traditional Chinese medicine were introduced into the Medical Insurance Scheme by the Ministry of Welfare of Japan, about 80 kinds of medicines containing licorice have been widely used in daily practice in our country. As an adverse effect of these medicines, it has been known that licorice causes pseudoaldosteronism.[6] However, no biopharmaceutical study had as yet been carried out on orally taken decoctions with licorice until the previous investigation regarding the disposition of GLA in humans.[4]

The present study has elucidated that the disposition of plain licorice (Kanzô-tô) and Syakuyaku-kanzô-tô (combination of licorice and paeoniae) is significantly different. That is, the serum concentration of GLA after oral administration is significantly influenced by the pre-

sence or absence of paeoniae.

Tomimori and Yoshimoto[7] have reported that the extracted amount of GLZ in Syakuyaku-kanzô-tô is reduced to 61 % of the amount of plain licorice. In the present study, however, the same amounts of GLZ were obtained from both formulas. The conditions of decoction employed in our study are those seen in daily practice, whereas the preparation of Tomimori and Yoshimoto[7] is made in a different manner, i.e., powdered materials decocted by small amounts of water. Anyhow, in the present study, any differences in the amounts of administered GLZ between the two cases need not be taken into consideration.

Similar to the results obtained in the previous report in humans,[4] GLA is not detected in the decoctions of Kanzô-tô and Syakuyaku-kanzô-tô, but it is in the serum. This indicates that GLZ is changed to GLA by gastric juices, intestinal bacterias, and/or by other enzymatic processes in the digestive system and liver. Therefore, the different serum GLA concentrations observed in the two formulas suggest the possibility that paeoniae exerts significant influence on the metabolic processes converting GLZ to GLA in the digestive system and/or in the liver.

The present study has revealed that the amount of fecal excretion of GLA is reduced in the presence of paeoniae. This fact suggests two possibilities : one is that paeoniae added to licorice accelerates the converting process from GLZ to GLA and retains it in the serum or intestine by hepato-intestinal circulation, and the other is that paeoniae does not act on the gastro-intestinal system but solely in the liver. The amount of GLZ in feces was not examined in this study, leaving the true role of paeoniae in GLZ disposition a subject for further study.

The present investigation has also revealed that the amount of GLA in urinary excretion is markedly elevated in Syakuyaku-kanzô-tô in comparison with Kanzô-tô. However, in both cases the percentile amount of GLA to dosage administered is only a few percentage points and therefore no perceptible influence on the serum concentration of GLA is seen. As a result, it can be supposed that the differences observed in urinary excretion of GLA in both cases are reflected in the different levels of GLA in the serum. In any event, from the point of view of the clinical application of Syakuyaku-kanzô-tô,[8] if GLA has specific pharmacological activities as an antispasmodic in the urinary tracts, this formulation yields a higher concentration of GLA in urine than that of Kanzô-tô.

The results from the comparison between orally administered Kanzô-tô and intravenously given GLA or GLZ have shown that serum GLA levels after Kanzô-tô administration are significantly different from those of intravenous GLA or GLZ. In other words, the serum GLA concentration decreases constantly with time, whereas in cases of GLZ administration, serum GLA values increased for about 2 hours, after which they reduced to zero. These results are essentially similar to the data reported by Ichikawa *et al.* for GLA[9] and Nakano *et al.* for GLZ;[10] which means that i.v. GLZ converts to GLA in the liver and is eliminated from the serum.

The time course of the serum GLA concentration varies greatly from both intravenously administered GLZ and GLA. This indicates two possibilities : one is that it takes about 90 minutes to absorb GLZ and/or its analogs from the gastro-intestinal system, and to convert to GLA, and the other is that it takes about 90 minutes to absorb GLA from the gastro-intestinal system. As the authors reported previously, GLA-glycosides were detected in the serum prior to GLA after oral administration of decocted plain licorice in humans.[4] This seems to indicate that the former possibility is the more likely.

In daily practice in clinics, there are two types of GLZ administration : one is the intravenous method and the other is per os. The findings from our study suggest that as far as Kanzô-tô and Syakuyaku-kanzô-tô are concerned, they yield significant serum levels of GLA. However, whether GLZ is actually absorbed intact form the gastro-intestinal system has still not been confirmed, and further examinations regarding this problem are called for.

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和文抄録

甘草配剤の伝統的中国医学の方剤の体内動態を明らかにする第一段階として，甘草湯と芍薬甘草湯を経口投与し，血中のグリチルレチン酸（GLA）の挙動をラットを用いて検討した。さらに経口投与した甘草煎液と静脈内投与したグリチルリチン（GLZ）とGLAとの相違をみるために，GLAとGLZの静脈内投与も試みた。

GLAは酵素免疫抗体法を用いて測定した。甘草湯と芍薬甘草湯の経口投与後の血中GLA濃度は後者で有意に高かった。さらに芍薬甘草湯投与群では糞便中のGLA濃度は甘草湯投与群に比べ，有意に低下していることも明らかになった。

一方，甘草湯投与後の血中GLA濃度は静脈内投与したGLZやGLAの場合に比して，長時間持続する傾向を示した。

Refereces

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