Pharmacologically Active Constituents from Plants Used in Traditional Medicine

3-Monoglucuronyl Glycyrrhetinic Acid Is a Possible Marker Compound Related to Licorice-Induced Pseudoaldosteronism

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One of the most common adverse effects of traditional Japanese kampo and traditional Chinese medicine is pseudoaldosteronism caused by licorice. In this review, the authors describe the mechanisms of licorice-induced pseudoaldosteronism by the pharmacokinetics of chemical constituents and its metabolites containing licorice. Glycyrrhizin (GL), the main constituent of licorice, is absorbed as glycyrrhetinic acid (GA), which is a metabolite of GL produced by enterobacteria before its release into the circulation. Circulating GA is metabolized in the liver to become 3-monoglucuronyl-glycyrrhetinic acid (3MGA), which is excreted into the bile via multidrug resistance protein 2 (Mrp2). If Mrp2 function is damaged for some reason, 3MGA is secreted from the liver into the circulation, and excreted into the urine via organic anion transporters expressed at the basolateral side of tubular epithelial cells. Circulating GA cannot be excreted into the urine since GA binds highly to serum albumin and thus does not pass through glomerular filtration and is not a substrate of transporters expressed on tubular epithelial cells. Licorice-induced pseudoaldosteronism develops due to the inhibition of type 2 11β-hydroxysteroid dehydrogenase (11β-HSD2) which results in the accumulation of cortisol in tubular epithelial cells that activate mineral corticoid receptors to stimulate the excretion of potassium that results in hypokalemia. GA, unlike 3MGA, cannot pass through tubular epithelial cells and cannot inhibit the enzyme in the cells. Therefore, 3MGA may be a genuine causative agent for licorice-induced pseudoaldosteronism. When licorice is used, 3MGA in plasma or urine could function as a marker compound to prevent the adverse effects.

Key words licorice; pseudoaldosteronism; 3-monoglucuronyl-glycyrrhetinic acid; organic anion transporter

Licorice is a crude drug prescribed in various herbal formulas in traditional Japanese and Chinese medicines. The origin of licorice was determined to be the root or stolon of Glycyrrhiza uralensis or G. glabra (Leguminosae). In Japan, licorice is present in 109 formulas among 148 ethical kampo extract formulations, and is one of the most frequently used crude drugs in kampo medicine. Licorice is also used worldwide as a natural sweetener for foods.

Licorice contains glycyrrhizin (GL, Fig. 1), which has an anti-inflammatory action and is used not only as an oral agent but also as an injection to improve liver function. GL is a glycoside and contains 2 molecules of glucuronic acid connected to a hydroxyl group at C-3 of glycyrrhetic acid (GA, Fig. 1). When licorice is ingested orally, GL only slightly penetrates through the gastrointestinal tract epithelium due to its highly hydrophilic sugar moiety, and is absorbed as GA after the sugar moiety is hydrolyzed and converted from GL to GA by enterobacteria in the large intestine. Therefore, it is believed that GA is the metabolite responsible for the main pharmacological activity of licorice.

In Europe, it was reported that some patients suffering from hypertension and edema frequently ingested licorice. In 1968, a syndrome with these symptoms was named licorice-induced pseudoaldosteronism and was recognized not only as a simple side effect but as one disease. It is one of the most common adverse effects of traditional Japanese kampo medicine. Aldosterone, which is the origin of the term pseudoaldosteronism, is a hormone that plays a role in regulation of electrolyte levels in the body. Aldosterone binds to the intracytoplasmic mineral corticoid receptor in renal tubule cells, induces expression of the Na+/K+ pump, and then promotes reabsorption of urinary Na+ and secretion of K+. When plasma aldosterone levels increase for some reason, primary aldosteronism occurs with symptoms such as hypertension, edema, hypokalemia, hypernatremia with an increase in the potassium secretional capacity in the renal tubules, metabolic alkalosis, low plasma renin, and muscle ache or numbness due to myopathy. The onset of licorice-induced pseudoaldosteronism depends on the dose and the duration of the licorice dosing period to some extent. However, it has been suggested that there are specific individual factors in the backgrounds of patients suffering licorice-induced pseudoaldosteronism, since some patients do not develop it even if they consume licorice for a long time while other patients develop it with minimum dosage in a short time.

In the past, it was thought that licorice-induced pseudoaldosteronism occurred due to the direct binding of GL or GA to mineral corticoid receptors. However, their affinity to the receptors is markedly lower than that of aldosterone. Therefore, it is now doubted that GL and GA bind directly to a receptor to elicit a cortical action. The affinity of cortisol, which is an adrenal cortical hormone, to mineral corticoid receptors is similar to that of aldosterone. However, under normal conditions, cortisol is degraded into cortisone in the cytoplasm of renal tubular cells by type 2 11β-hydroxysteroid dehydrogenase (11β-HSD2). Since the affinity of cortisone to mineral corticoid receptors is markedly lower than that of aldosterone, the modified cortisol is released into the body. Therefore, 3MGA is secreted from the liver into the circulation, and excreted into the urine via organic anion transporters expressed at the basolateral side of tubular epithelial cells. Circulating GA cannot be excreted into the urine since GA binds highly to serum albumin and thus does not pass through glomerular filtration and is not a substrate of transporters expressed on tubular epithelial cells. Licorice-induced pseudoaldosteronism develops due to the inhibition of type 2 11β-hydroxysteroid dehydrogenase (11β-HSD2) which results in the accumulation of cortisol in tubular epithelial cells that activate mineral corticoid receptors to stimulate the excretion of potassium that results in hypokalemia. GA, unlike 3MGA, cannot pass through tubular epithelial cells and cannot inhibit the enzyme in the cells. Therefore, 3MGA may be a genuine causative agent for licorice-induced pseudoaldosteronism. When licorice is used, 3MGA in plasma or urine could function as a marker compound to prevent the adverse effects.

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eral corticoid receptors is low, activation of the receptor by cortisol is prohibited.\textsuperscript{6} GA and GL have an inhibitory effect on 11\textbeta-HSD2.\textsuperscript{9} It is reported that the inhibitory effect of GA on 11\textbeta-HSD1 was approximately 200 times greater than that of GL.\textsuperscript{10} Furthermore, the compound which appears in blood when we ingest licorice is not GL but rather GA.\textsuperscript{6} Based upon the above, it is thought that licorice-induced pseudoaldosteronism is due to GA inhibiting 11\textbeta-HSD2 in renal tubular cells, resulting in the accumulation of cortisol that activates mineral corticoid receptors.\textsuperscript{11}

However, individual differences in the onset of licorice-induced pseudoaldosteronism cannot be explained from the above findings. In 1995, Kato \textit{et al.} reported the levels of licorice metabolites in the plasma of patients using a kampo prescription containing licorice or using a GL preparation, and they compared those of the patients with or without licorice-induced pseudoaldosteronism.\textsuperscript{12} In their report, 3-monoglucuronyl glycyrrhetinic acid (3MGA), which contains 1 molecule of glucuronic acid connected to a hydroxyl group at C-3 of GA (Fig. 1), was detected in the plasma of the patients who developed licorice-induced pseudoaldosteronism, but was not detected in the plasma of the patients without pseudoaldosteronism. Therefore, it is believed that whether a person has 3MGA as a metabolite of GL might be associated with the onset of the pseudoaldosteronism.

The authors took into consideration individual differences in the blood levels of 3MGA. The authors had bred rats in a choline-deficient diet for 12–13 weeks to make an animal
model for hepatic fibrosis. Using this model, the authors examined the pharmacokinetics of the licorice component and its metabolite.\textsuperscript{13–15} The authors orally administered GL to the hepatic fibrosis model rats or control rats, and compared the profiles of the plasma concentrations of each metabolite between these rats. Higher levels of GL and 3MGA were found in the plasma of hepatic fibrosis model rats than in control rats. However, there were no differences in the plasma concentration profile of GA between these rats. Furthermore, in the hepatic fibrosis model rats, the values of urinary excretion of GL and 3MGA were higher than those in controls (Fig. 2). Urinary excretion of GA in both the hepatic fibrosis model rats and controls was less than the detection limit, and a difference was not observed. These phenomena are reproduced in Eisai hyperbilirubinuria rats (EHBR) which express the loss of function without multi-drug resistance-associated protein 2 (Mrp2).

The authors developed the following hypotheses about the pharmacokinetics of GL and its metabolites after the oral administration of licorice (Fig. 3). When licorice is ingested orally, GL is hydrolyzed into GA by enterobacteria in the large intestine, and then GA is absorbed from the gastrointestinal tract into the circulation. The major component in blood is not GL but GA. GA is not excreted into the urine, but rather is metabolized by glucuronyltransferase expressed in the liver into the conjugate 3MGA. 3MGA is excreted into the bile through Mrp2. Next, some of the 3MGA is re-hydrolyzed into GA by enterobacteria, and is absorbed again indicating the presence of enterohepatic circulation. The remainder that was not absorbed is excreted into the feces. Therefore, if liver function is normal, 3MGA is not present in blood. However, when biliary excretion of 3MGA is prohibited by decrease or dysfunction of Mrp2 in liver, 3MGA would be returned from the liver into blood by transporters such as Mrp3. In other words, when 3MGA is in blood, it is believed that it would interfere with Mrp2 function or expression in the liver.

Next, the authors conducted experiments to study the relationships between pseudoaldosteronism and 3MGA.\textsuperscript{16} Kato et al.\textsuperscript{\textsuperscript{13}} compared the inhibitory effect of 3MGA on rat 11β-HSD2 activity with that of GA using a rat kidney microsome fraction. At the concentration of 1 µM, the inhibitory titers of GA and 3MGA were similar (inhibition %, 96%), and at the concentration of 0.1 µM, that of GA was approximately 1.2 times stronger than that of 3MGA. These results suggest that 3MGA could inhibit 11β-HSD2 \textit{in vitro} to a degree similar to that of GA to cause pseudaldosteronism. The authors measured the dose-dependencies of the inhibition of GL, 3MGA and GA on 11β-HSD2 in a rat kidney microsome fraction and calculated their IC\textsubscript{50}. The values obtained were 2.2 µM for GL, 0.26 µM for 3MGA, and 0.32 µM for GA. Therefore, the 11β-HSD2 antagonisms of GA and 3MGA are approximately 10 times stronger than that of GL, and it was predicted that both GA and 3MGA could become the causative agent for pseudaldosteronism.

The authors noted that GA could not be detected in the urine collected from both liver fibrosis model rats and control rats.\textsuperscript{13} Because the plasma and urinary levels of 3MGA in liver fibrosis model rats were higher than those in controls, we examined the mechanism of the renal excretions of GA and 3MGA. At first, using bovine serum albumin and normal rat serum, the authors examined the albumin-binding rates of 3MGA and GA using an ultrafiltration method and found that both 3MGA and GA were present as forms bound to albumin in ratios exceeding 99.9%. This suggests that almost no 3MGA or GA passes through glomerular filtration to be excreted into the urine. Since the authors could not detect GA but did detect 3MGA in the urine of liver fibrosis model rats, it was concluded that GA could not be excreted through tubular secretion while 3MGA could. The authors prepared rat renal slice segments and measured the uptakes of GA and 3MGA from the media into the specimens. Since 3MGA and GA adhered a lot to the renal tissue surface, kidney slices were incubated in media containing GA and 3MGA at 4°C and 37°C and the active transport was calculated from the dif-

![Image](https://via.placeholder.com/150)

\textbf{Fig. 3. Estimated Metabolic Route of GL in the Body When It Is Orally Administered}
Km values were 50.9 transiently express these transporters. It was found that 3MGA thors conducted uptake experiments using HEK293 cells that organic anion transporting peptide (OATP) 4C1, membre of renal tubule epithelial cells are OAT1, OAT3, and anion transporters (OATs) expressing on the basolateral mem-
Since 3MGA is an anionic organic compound, and the organic
uptakes of 3MGA at 37°C were observed compared with those at 4°C. It is believed the active transport of 3MGA was associated with the transition from the media into the renal tissue. Since 3MGA is an anionic organic compound, and the organic anion transporters (OATs) expressing on the basolateral mem-
ence in uptakes between the temperatures. Differences due to temperature were not observed in the uptake of GA from the media into kidney slices, although significantly higher uptakes of 3MGA at 37°C were observed compared with those at 4°C. It is believed the active transport of 3MGA was associated with the transition from the media into the renal tissue. Since 3MGA is an anionic organic compound, and the organic anion transporters (OATs) expressing on the basolateral mem-

3MGA was the substrate of these transporters and that the apparent Km values were 50.9 µM for OAT1, 21.3 µM for OAT3, and 33.1 µM for OATP4C1. However, differences in the quantity of GA uptake between the mock cells and the cells transiently expressing these transporters were not observed. These results suggest that GA is only very slightly excreted into the urine since GA has high albumin-binding properties and is not the substrate for these transporters and is not excreted through glomerular filtration and tubular secretion.

The inhibitory effects of GA and 3MGA on 11β-HSD2 using a rat kidney microsome fraction in vitro were very simi-
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