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Inchinko-to

Agent for Liver Fibrosis
Choleretic Agent
Hepatoprotectant

TJ-135

Herbal medicine consisting of *Artemisia capillaris* Spica, Gardenia Fructus and Rhei Rhizoma

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Abstract

Kampo medicine is a Japanese traditional medicine that was developed from traditional herbal medicine originating in ancient China. In Japan, a number of Kampo medicines are now manufactured on a modern industrial scale, whereby the quality and quantity of ingredients are standardized under strict, scientific quality controls. Some of these medicines have demonstrated therapeutic efficacy in multicenter, placebo-controlled, double-blind studies. One such medicine, Inchinko-to, has been recognized as a "magic bullet" for the treatment of jaundice and has long been used in Japan and China as a choleretic and hepatoprotective agent for various types of liver disease. Double-blind, randomized, placebo-controlled clinical trials have not yet been conducted. However, recent experimental studies on the effects and mechanisms of action of this medicine and its ingredients have raised the possibility of developing new pharmacotherapeutic strategies targeting a wide variety of liver diseases. These strategies include: the inhibition of signaling pathways of hepatocyte apoptosis; the suppression of hepatic stellate cell activation and subsequent liver fibrosis; transcriptional activation of bilirubin metabolism via a specific nuclear receptor; and post-translational activation of multidrug resistance-associated protein 2 (MRP2), a multispecific organic anion transporter that plays a critical role in bilirubin clearance and bile salt-independent bile formation.

Introduction

Kampo medicine is a Japanese traditional medicine that was developed from a traditional herbal medicine originating in ancient China (1). The primary mode of Kampo therapy has been via the ingestion of Kampo formulations (*i.e.*, specific combinations of raw herbs) described in classical texts, which are assumed to represent the culmination of empirical knowledge gained over the centuries. In Japan, a number of traditional Kampo medicines are manufactured on a modern industrial scale, whereby the quality and quantity of ingredients are standardized under strict, scientific quality controls. Some of these medicines have demonstrated therapeutic efficacy in multicenter, placebo-controlled, double-blind studies. More than 100 Kampo medicines have been approved as ethical drugs by the Ministry of Health, Welfare and Labor of Japan and are used clinically for the treatment of a wide variety of diseases. A number of physicians who have been educated in Western medicine at Japanese medical schools use Kampo medicines in daily practice (2). Furthermore, more than 1 million patients with various diseases have received long-term treatment with Kampo medicines.

One such medicine, Inchinko-to, has been recognized as a “magic bullet” for the treatment of jaundice and has long been used in Japan and China as a choleretic and hepatoprotective agent for various types of liver disease. Inchinko-to consists of a mixture of three medicinal herbs: *Artemisia capillaris* Spica, Gardenia Fructus and Rhei Rhizoma. Several major ingredients responsible for the beneficial effect of orally administered Inchinko-to and its mechanisms of action have been identified. Genipin, which is an intestinal bacterial metabolite of geniposide (a major ingredient of Gardenia Fructus), has been demonstrated to have potent choleretic and hepatoprotective effects. The choleretic effect is mediated by the rapid enhancement of translocation of a multispecific organic anion transporter, multidrug resistance-associated protein 2 (MRP2), to the canalicular membrane. This results in increased bilirubin and reduced glutathione (GSH)

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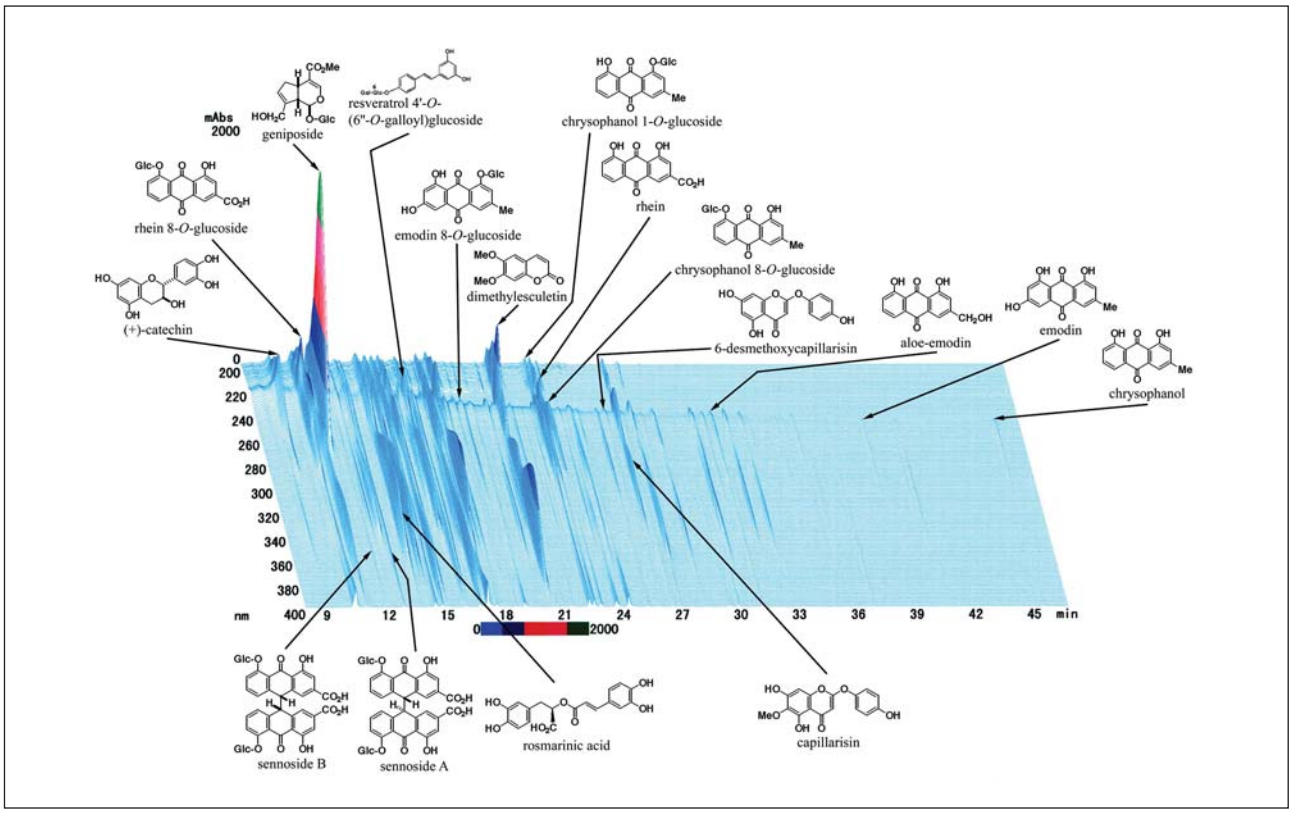

Fig. 1. Three-dimensional (3D) HPLC profile of TJ-135.

excretion into the bile and subsequent bile salt-independent bile formation. The hepatoprotective effect is related to the potent antiapoptotic action of genipin, mediated via interference with apoptotic signaling pathways in mitochondria. 6,7-Dimethylesculetin, a major ingredient of *A. capillaris* Spica, increases the expression of various components involved in bilirubin metabolic pathways. These include sinusoidal and canalicular organic anion transporters, carrier proteins and a conjugating enzyme, which are regulated via constitutive androstane receptor (CAR, NR113) signaling. Although the contribution of emodin and other anthraquinones contained in Rhei Rhizoma to the effect of Inchinko-to has not been fully elucidated, these agents suppress liver fibrosis via blockade of activating signal pathways in hepatic stellate cells.

A number of other ingredients have also been reported to have choleretic, hepatoprotective, analgesic, antiinflammatory and other pleiotropic effects that are potentially beneficial in preventing various liver diseases. However, the characterization of these effects and the mechanisms of action remain to be determined. Relevant toxicological and pharmacokinetic studies have generally not been carried out. Furthermore, no well-controlled clinical studies have been conducted. However, experimental investigations and several case reports demonstrating possible beneficial effects of Inchinko-to in severe liver disorders, many of which are currently untreatable, encourage future research to establish its effectiveness in the treatment of liver diseases.

Preparation

Inchinko-to is typically prepared as follows. A mixture of 5.0 g of the spike of *A. capillaris* Thunberg, 4.0 g of the fruit of *Gardenia jasminoides* Ellis and 1.0 g of the rhizome of certain *Rheum* species (*Rheum palmatum* Linne, *Rheum tanguticum* Maximowicz, *Rheum officilane* Baillon or *Rheum coreanum* Nakai) is boiled in 10 times its weight in water for 60 min and filtered. The liquid extract is then spray- or freeze-dried to obtain the extracted powder. Kampo medicines such as Inchinko-to are presently manufactured according to the Ethical Kampo Medicine Drug GMP regulations and the self-imposed regulations of the Japan Kampo-Medicine Manufacturers Association.

Inchinko-to contains a large number of constituents derived from various classes of compounds. All manufacturers of Kampo medicines as ethical drugs in Japan ensure that their extracts contain two or more of the major compounds characterizing the particular medicine group. Chemical analyses of the constituent compounds in Kampo medicines, including Inchinko-to and its constituent herbs, have generally been performed by high-performance liquid chromatography (HPLC) combined

with photodiode array (PDA) and evaporative light scattering (ELS) detection to obtain an overall view of as many compounds in the Kampo formulation as possible. The ultraviolet-visible light (UV-VIS) profile of three-dimensional (3D) HPLC analysis of the most widely used Inchinko-to product (TJ-135; Tsumura & Co.) is shown in Figure 1. The representative Kampo medicine supplier Tsumura & Co. has been constructing a database that includes retention times and UV-VIS profiles for approximately 1,000 compounds purified from various herbs, and more than 100 fingerprint patterns of Kampo medicines and constituent herbs made by 3D HPLC analysis. Such a database will enable us to provide a convenient method of assigning constituent compounds contained in herbs and Kampo medicines. It will also provide the means to control extract quality from a more comprehensive and global viewpoint, by providing the "fingerprint" for each medicine and herb. A number of routine quality controls have been carried out: these include microbiological specifications, determination of uniformity of content and disintegration time, accelerated and long-term stability studies, stability tests of active ingredients and other characteristic ingredients. In all cases, high batch-to-batch reproducibility is obtained.

Pharmacological Actions

In the medical treatment book "Shang han za bing lun: medical text by Zhang Zhong jing, comprising the Shang han lun and the Jin kui yao lue", written in China in the beginning of the third century A.D., Inchinko-to (Yin-Chen-Hao-Tang in Chinese) had already been described as an effective drug for the treatment of jaundice. Modern phytochemistry has identified various choleretic compounds present in Inchinko-to and its constituent herbs since the third decade of the 20th century. Among these, capillarisin and 6,7-dimethylesculetin from *A. capillaris* Spica and geniposide from Gardenia Fructus have been identified as major ingredients. The choleretic effect of Inchinko-to and its ingredients has been examined in experimental animals since the mid-1970s by several investigators in Japan. These studies suggested that this effect is largely bile salt-independent, and is due to actions by multiple ingredients, including capillarisin, 6,7-dimethylesculetin and geniposide, via distinct mechanisms. However, the detailed mechanisms by which Inchinko-to and its ingredients exert choleretic effects had not been clarified until recently. Advances in research on bile formation and hepatocellular transporters have

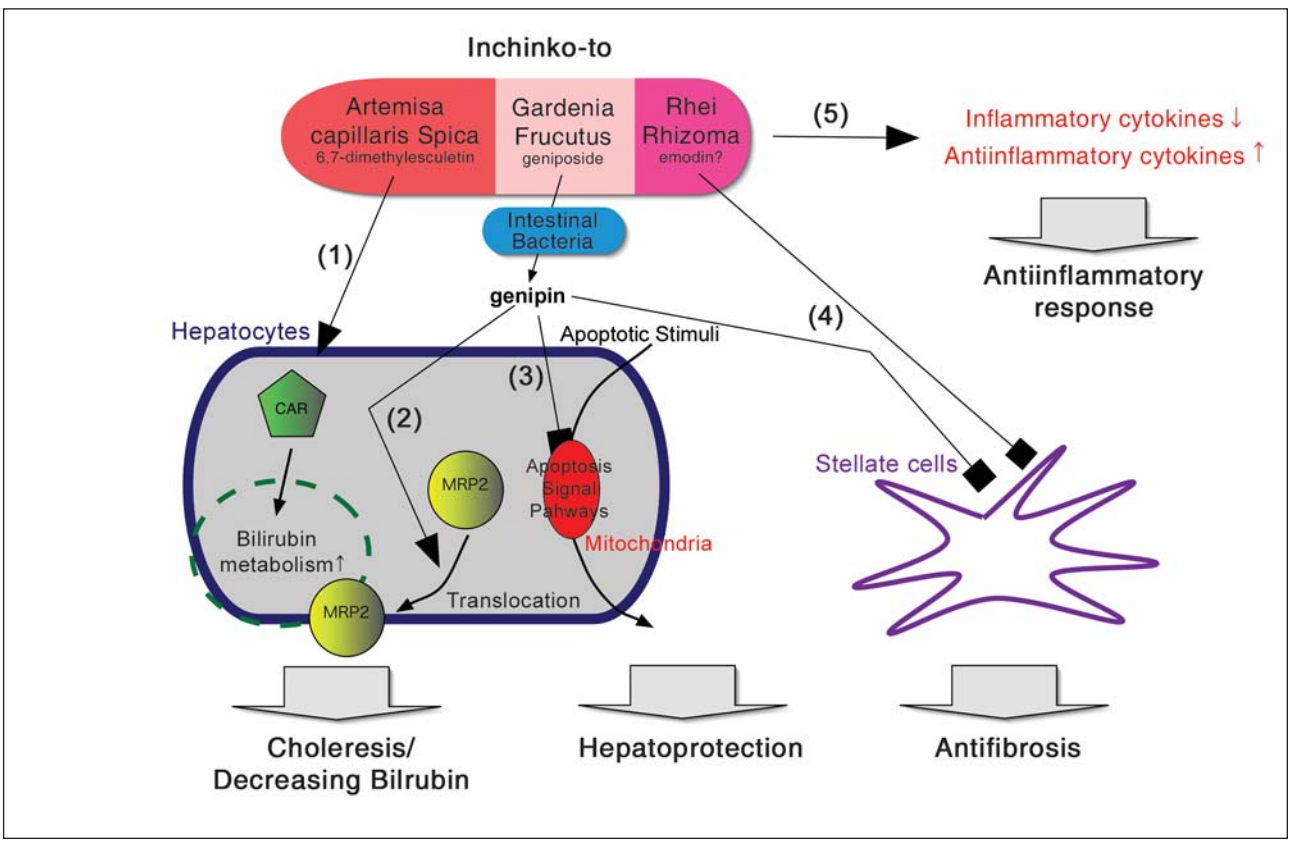

Fig. 2. Modes of action of Inchinko-to and its ingredients. (1) 6,7-Dimethylesculetin activates bilirubin metabolism via a constitutive androstane receptor (CAR). (2) Genipin enhances translocation of multidrug transporter-associated protein 2 (MRP2) into the canalicular membrane. (3) Genipin interferes with mitochondrial apoptotic signal pathways. (4) Anthraquinones inhibit the activation of hepatic stellate cells. (5) Inchinko-to modulates cytokine responses *in vivo*.

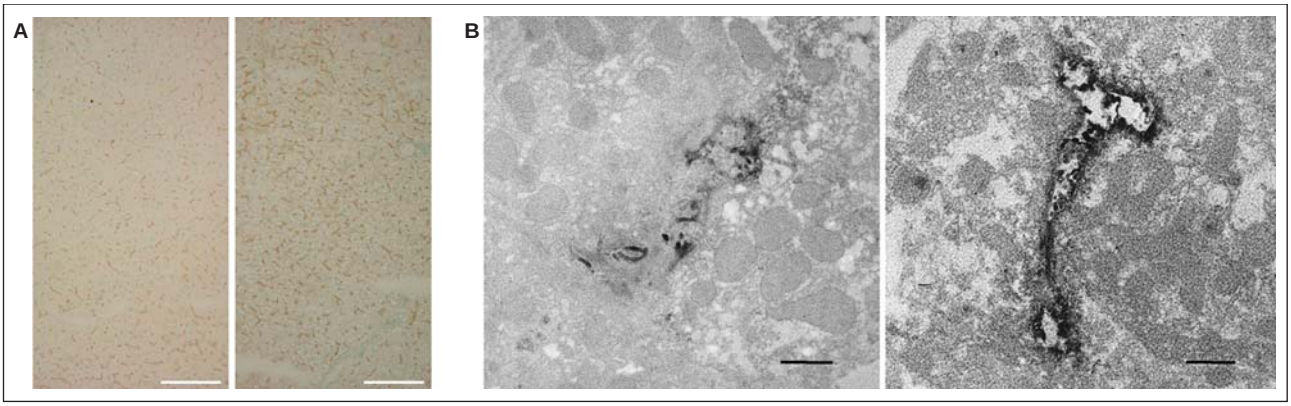

Fig. 3. Immunohistochemical localization of MRP2 protein in the rat liver by light (A) and electron (B) microscopy. Left panels indicate saline-treated livers and right panels indicate genipin-treated livers. Bar = 1.0 μm.

opened the way to an understanding of the mechanisms of action of the agents.

Geniposide, a major ingredient of Gardenia Fructus, is an iridoid compound comprising about 5% (3) to 15% (unpublished observations) of Inchinko-to by weight. Upon oral administration, geniposide is converted to its aglycone genipin by intestinal bacteria. Takeda and colleagues have investigated the choleretic effect of genipin and related iridoid compounds (4) and the relationships between structure and choleretic activity (5). They demonstrated that the effect is mediated by bile salt-independent canalicular bile formation and that the hemiacetal moiety of iridoid compounds plays an important role.

Recently, Shoda *et al.* (6) reported that the enhancement of bile acid-independent bile formation by genipin is mediated by rapid stimulation of exocytosis and insertion of the multispecific organic anion transporter multidrug resistance-associated protein 2 (MRP2, Abcc1) into the bile canaliculi (Fig. 2). Infusion of genipin rapidly increased bile flow by 230%. Genipin also enhanced biliary secretion of bilirubin conjugates (513%) and reduced glutathione (GSH; 336%), both of which are transported mainly by MRP2. Excretion of bile acid was not changed following genipin infusion. Kinetic analysis of canalicular membrane vesicles prepared from genipin-treated rat liver established that the enhanced transport of various MRP2-specific substrates (200%) is due to the increased amount of MRP2 protein in the canalicular membrane. These effects of genipin were completely abrogated in MRP2-deficient Eisai hyperbilirubinemic rats. The effect of genipin is rapid (within 30 min) and is not accompanied by increases in MRP2 mRNA or protein expression in whole cells, indicating that it enhances the redistribution of MRP2 into the canalicular membrane. This is supported by immunohistochemical studies of MRP2 using light and electron microscopy (Fig. 3).

One-week oral administration of genipin, geniposide and Inchinko-to showed similar enhancement of bile flow, biliary GSH secretion and MRP2 translocation. Thus, the potent enhancement of bilirubin secretion and bile formation by genipin may at least partly explain the well-known beneficial effect of Inchinko-to on jaundice and choleostasis. Confirmation of the effect of genipin and Inchinko-to on human MRP2 is now in progress in an experimental study using chimeric mice with a humanized liver, and in a clinical study in patients undergoing biliary drainage for obstructive jaundice.

The mechanism of action of 6,7-dimethylesculetin (scoparone or 6,7-dimethoxycoumarin), a major ingredient of *A. capillaris* Spica, has been elucidated in recent studies by Huang *et al.* using Yin Zhi Huang, a Chinese herbal drug closely related to Inchinko-to (7). Yin Zhi Huang contains extracts from four different plants: *A. capillaris, G. jasminoides* Ellis, *R. officinale* Baillon and *Scutellaria baicalensis* Georgi. Three of these four constituent herbs are also present in Inchinko-to. Yin Zhi Huang, the decoction of *A. capillaris* and 6,7-dimethylesculetin, accelerated the clearance of exogenously injected bilirubin. The effect of these agents was suggested to be mediated by the constitutive androstane receptor (CAR, NR113), a key regulator of bilirubin clearance in the liver, because the effect was completely abrogated in CAR knockout mice (Fig. 2). In primary hepatocytes from both wild-type mice and mice expressing only human CAR, 6,7-dimethylesculetin activated CAR and also accelerated bilirubin clearance *in vivo*. These effects were accompanied by an increase in the transcription of various components involved in bilirubin metabolic pathways: a sinusoidal organic anion transporter (OATP2), ligandin carrier proteins (glutathione *S*-transferase A1 and/or A2), the conjugating enzyme UDP-glucuronosyltransferase 1A1 (UGT1A1) and MRP2. While the possible stimulatory effect of Inchinko-to on CAR has not been investigated directly, the well-known contribution of 6,7-dimethylesculetin to the beneficial effect of Inchinko-to suggests the drug has a similar action.

Capillarisin, isolated from *A. capillaris*, has been described as a more potent choleretic agent than 6,7-dimethylesculetin (8). However, the content of capillarisin in Inchinko-to is rather small (9-12), and for this reason, a possible contribution of capillarisin to the choleretic effect of Inchinko-to remains to be determined (13). The

detailed mechanism by which capillarisin exerts choleretic effects also remains to be clarified. There are preliminary reports of choleretic activity of compounds such as *p*-hydrocyacetophenone, capillartemisin A, B and B1, artepillin A and C, scopoletin, isoscopoletin and capillene from *A. capillaris* (14, 15), but further investigations have not been carried out.

The second most frequent indication for Inchinko-to is in the treatment of acute hepatitis. Various studies have demonstrated hepatoprotective actions of Inchinko-to and the ingredients capillarisin, arcapillin, and especially 6,7-dimethylesculetin and genipin, including *in vivo* hepatic injury models using hepatotoxins such as carbon tetrachloride (CCl_4) (16), galactosamine (17, 18), α-naphthylisothiocyanate (19, 20), transforming growth factor-β1 (TGF-β1) (21), concanavalin A (ConA) (22) and Fas stimulation (23). Capillarisin has been reported to protect primary cultured rat hepatocytes from *tert*-butylhydroperoxide-induced oxidative damage by stabilizing the GSH system and quenching free radicals (24). 6,7-Dimethylesculetin has been observed to inhibit the release of inflammatory mediators in RAW 264.7 cells upon stimulation by interferon gamma plus lipopolysaccharide (LPS) (25). Upregulation of antiinflammatory cytokines such as IL-10 by Inchinko-to has been also reported in the ConA-induced hepatitis model (Fig. 2). However, further study is necessary to clarify the mechanisms by which these ingredients exert hepatoprotective activity *in vivo*. Among these ingredients, detailed analysis of the active compound genipin and its mechanism of action has been performed in the Fas-dependent lethal hepatic injury model.

The Fas receptor is constitutively expressed on the surface of hepatocytes and is thought to be involved in both physiological and pathological hepatocyte death processes. It can be shown experimentally that Fas stimulation induces rapid and massive hepatocyte apoptosis and subsequent fulminant liver injury. Furthermore, oral administration of Inchinko-to has been shown to rescue mice from Fas-mediated lethal liver injury (23). The effect of Inchinko-to has been attributed, at least in part, to the action of genipin. Oral administration of Gardenia Fructus extract or geniposide in amounts equivalent to those contained in Inchinko-to afforded similar protection against liver apoptosis, while other constituent herbs provided no protection. Furthermore, intravenous injection of genipin, but not geniposide, is effective. Pretreatment with antibiotics that reduce levels of intestinal bacteria responsible for the conversion of geniposide to genipin abrogated the effects of orally administered geniposide and Inchinko-to. In contrast, the effect of orally administered genipin was not altered by antibiotic treatment. It is therefore proposed that Inchinko-to exerts its hepatoprotective effect in the Fas-induced liver apoptosis model via genipin generated *in vivo* from geniposide. Oral administration of genipin displayed no effect on the expression and Fas ligand-binding activity of Fas on hepatocytes, or the expression of Bcl-2 family proteins, which are well-known intracellular regulators of apoptosis. Genipin had no inhibitory effect on the proteolytic activity of caspases, specific proteases which are signal mediators and/or executers of apoptosis, *in vitro*. Mitochondrial permeability transition and decay of membrane potential, which are both representative apoptotic signaling events in mitochondria, were potently attenuated by genipin treatment in the presence or absence of Fas stimulation. These data suggest that genipin suppresses hepatocyte apoptosis by interfering with the apoptotic signal pathway in mitochondria (Fig. 2).

Liver cirrhosis, the result of a wide variety of liver diseases, is a worldwide health problem. Therefore, the question as to whether Inchinko-to has antifibrotic effects has generated much interest among researchers. Three groups have reported an antifibrotic effect for Inchinko-to in several liver fibrosis models. Pretreatment with Inchinko-to had potent protective effects against liver fibrosis in choline-deficient diet-induced (26), CCl_4-induced, porcine serum-induced (27) and thioacetamide-induced (28) rat fibrosis models.

Although genipin has some antifibrotic properties, two groups have given more attention to the constituent herb Rhei Rhizoma and its ingredient emodin. An antifibrotic effect for emodin has also been reported independently (29). Inchinko-to has been shown to inhibit increases in collagen expression, hydroxyproline content, the number and proliferation of activated stellate cells, the expression of smooth muscle α-actin (a marker of stellate cell activation) and the area of preneoplastic foci (Fig. 2). Emodin inhibited the activation of mitogen-activated protein (MAP) kinases such as ERK (extracellular signal-regulated kinase) and JNK (*c*-JUN NH_2-terminal protein kinase) (26) and platelet-derived growth factor receptor β (PDGFRβ) signaling (28) in primary cultured or immortalized stellate cells.

6,7-Dimethylesculetin is known to have vasodilating (30-33), analgesic and antiinflammatory effects (13). Capillarisin, geniposide and their derivatives have been reported to have antitumor effects (34-40). Genipin is also known to have purgative (41) and hypolipidemic (42) effects and to modulate gastric function (43). *In vitro* and *in vivo* studies have addressed the possible beneficial effects of geniposide on memory impairment (44, 45). Extracts of *Artemisia* species and its ingredients caffeic acid and chlorogenic acid have been observed to inhibit lipid peroxidation in serum (46). Numerous papers have been published concerning the biological activities of anthraquinones (rhein, aloe-emodin, emodin, sennosides, etc.), phenolic compounds (catechin, epicathechin, epicatechingallate, lindleyin, etc.) and other ingredients contained in Rhei Rhizoma; therefore, we will not discuss this topic in the current review.

Pharmacokinetics and Metabolism

Inchinko-to has a great variety of ingredients present in large amounts, but only a small fraction of ingredients responsible for its beneficial effects have so far been identified. As yet, insufficient and preliminary data are

available for a limited number of ingredients. Furthermore, in common with other Kampo medicines, the metabolism of ingredients, not only by human metabolic pathways but also by intestinal bacterial flora, is critically important for the beneficial (and detrimental) effects of Inchinko-to. At present, therefore, it is almost impossible to review the pharmacokinetic, metabolic and toxicological profiles of Inchinko-to. In this section we summarize reports concerning not only Inchinko-to, but also its constituent herbs and major ingredients.

Geniposide is resistant to hydrolysis by gastric acid. Conversion of geniposide to genipin appears to require β-D-glucosidase activity present in intestinal bacteria, because rat liver homogenates, which have β-D-glucosidase and esterase activity, do not metabolize geniposide by hydrolysis. In addition, a wide range of intestinal bacteria have the ability to hydrolyze geniposide (47-49). Furthermore, as described below, the ability of antibiotic pretreatment to abrogate Inchinko-to and/or geniposide efficacy/toxicity has been proposed on the basis of both experimental and clinical evidence. Genipin has been suggested to be further converted to the nitrogen-containing compound genipinine (50) and blue pigments (51-53) in the presence of ammonia and amino acids, respectively. Genipin reacts selectively with the sulfhydryl groups of GSH and cysteine, resulting in transient reduction of GSH content in the liver. At higher concentrations, it is possible that cross-linking of certain proteins by genipin occurs (54). Based on previous studies and our unpublished observations, it can be speculated that when Inchinko-to is administered orally, its main ingredient geniposide presumably follows the following course: 1) a proportion of geniposide is converted to genipin by intestinal bacteria; 2) a proportion of genipin is further converted to several compounds, including cross-linked proteins; 3) geniposide, genipin, and perhaps some metabolites, are absorbed into the portal vein and transported to the liver; 4) genipin is conjugated mainly as the glucuronide in the liver; 5) a proportion of unconjugated and conjugated genipin is secreted into the bile by the MRP2 transporter; and 6) genipin and its metabolites in part circulate in the enterohepatic cycle, in part are excreted into the feces and in part into the urine. It must be noted that, due to its physicochemical properties, genipin may bind various amino acids, proteins and therefore tissues and organs. Furthermore, *de novo* synthesized genipin, if present in excessive amounts, may damage intestinal bacteria and cause diarrhea, which in turn reduces genipin generation (23). Thus, the efficacy and toxicity of geniposide are considered to be rate-limited by intestinal bacteria.

Experimental pharmacokinetic studies of genipin have not been performed. However, it has been reported that when 1200 mg/kg of an extract of Gardenia Fructus (equivalent to 214.6 mg/kg of geniposide) was orally administered to male ddY mice, plasma geniposide levels reached a peak of approximately 103.1 μg/ml at 30 min and then gradually decreased. Very low concentrations of genipin (0.07 μg/ml), peaking at 60 min, were detected in the plasma (55).

A preliminary study reported that oral administration of 25, 50 and 100 mg/kg of 6,7-dimethylesculetin gave plasma concentrations of approximately 5, 5 and 30 μg/ml, respectively, at 60 min (56). Inchinko-to at a dose of 6.5 g/kg (corresponding to a dose of 6,7-dimethylesculetin of 25 mg/kg) gave a plasma concentration of approximately 2 μg/ml at 60-120 min.

Toxicity

The toxicological evaluation of Inchinko-to has included general and specific studies aimed at determining the potential risks associated with its oral use in humans. These have included acute toxicity and repeated-dose subchronic studies performed in rodents. In addition, studies investigating the cytotoxicity and mutagenic potential of Inchinko-to have been performed. Acute oral toxicity studies of TJ-135 (the representative Inchinko-to product made by Tsumura & Co.) in SD rats (57) recorded mortality, daily clinical observations, food consumption and body weight gain for 14 days after dosing. Also, in some studies, necropsy and histopathological examinations were performed on the survivors upon completion of the study. The doses of TJ-135 used for the acute toxicity studies ranged from 4 to 16 g/kg. The dose used in humans is 1.5 g/day, which corresponds to 21.4 mg/kg assuming a mean body weight of 70 kg. Hence, experiments investigating the toxicity of TJ-135 administered at up to 16 g/kg have included doses 747 times higher than the dose used in humans. The estimated lethal doses of orally administered TJ-135 were > 16 g/kg for male and 12 g/kg for female SD rats, respectively. Decreases in body weight gain, chromaturia, loose stools, diarrhea and sedation were observed at doses > 4 g/kg. Chromatism in liver and kidney was observed at a dose of 16 g/kg in male rats and at > 12 g/kg in female rats. Histopathological examination did not show differences between control and treated animals (58). The ingredients responsible for the observed change in body weight gain and for the clinical observations, together with the mechanism by which these changes were induced, remain to be clarified. However, there have been some reports on toxicity of constituent herbs and ingredients, as follows: 1) anthraquinones of Rhei Rhizoma cause chromaturia; 2) genipin reacts with amino acids and produces blue pigments; 3) oral administration of geniposide and genipin causes chromatism in liver, kidney, spleen, urine and feces; 4) prolonged feeding of high doses of crocin dyes (ingredients of Gardenia Fructus) to rats induces a reversible hepatic black pigmentation (59); and 5) Rhei rhizoma, Gardenia Fructus and genipin have potent cathartic effects.

A study investigating the toxicity of repeated oral doses of TJ-135 was conducted in SD rats. Animals were randomly distributed to 4 experimental groups (12 animals/sex/group): a control and 3 groups treated orally with different doses of TJ-135 (250, 1000 and 4000 mg/kg) for 28 days. Analysis of mortality, weight gain, clinical observations, ophthalmological examination,

urinalysis, hematological examination, biochemical examination, necropsy, organ weight analysis and histopathological examination were performed upon completion of the study. Increases in reticulocyte count and slight histopathological changes including chromatism were observed in males and females in the 1000 mg/kg group. One male rat treated with 4000 mg/kg TJ-135 died. Surviving animals receiving the same range of doses showed a decrease in body weight gain, loose stools, anemia, increases in leukocyte count, inflammatory changes in the large intestine and histopathological changes in the liver, spleen and kidneys. The no-observed-adverse-effect (NOAE) level of TJ-135 in the study was therefore assumed to be 250 mg/kg for both males and females, more than 10 times larger than the dose used in humans (60).

The possible genotoxic potential of Inchinko-to was investigated using a bacterial reverse mutation test (the Ames test), an *in vivo* micronucleus test in mouse bone marrow cells and an unscheduled DNA synthesis test in rat hepatocytes (61). Inchinko-to showed no mutagenic potential either *in vitro* or *in vivo*.

The acute toxicity of genipin was investigated in male ddY mice and male Wistar rats (43). The LD_{50} values at 72 h following i.v. and p.o. administration of genipin were 153 and 237 mg/kg, respectively, while geniposide at a dose of 3 g/kg i.v. and p.o. showed no lethality. In rats, oral administration of 1-80 mg/kg genipin was reported to be devoid of toxic effects, while i.p. administration of 80 mg/kg genipin resulted in significant elevations in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities (62). This hepatotoxic effect at 24 h after i.p. administration was similar to that of orally administered geniposide at a dose of 320 mg/kg. The hepatoxicity of orally administered geniposide could be abrogated by pretreatment with antibiotics or cysteine and could be enhanced by pretreatment with the GSH-depleting agent buthionine sulfoximine. These data suggest that the hepatotoxicity of geniposide requires intestinal bacteria and is related to hepatic GSH levels. The profound effects produced by genipin on hepatic GSH levels probably reflect its potent enhancement of MRP2 function and marked reactivity with the sulfydryl group of GSH. However, the effect of geniposide/genipin on hepatic GSH may be more complex. We have observed that oral administration of geniposide or genipin resulted in long-lasting elevation in GSH levels following a sharp but transient decrease (unpublished observations). Furthermore, Kang *et al.* suggested that orally administered geniposide has the ability to inhibit a cytochrome P-450 3A monooxygenase and to increase GSH content in rat liver (63).

In vitro evaluation has suggested that genipin is genotoxic at concentrations above 50 ppm (64, 65).

The acute toxicity of oral 6,7-dimethylesculetin was investigated in male ddY mice and the LD50 at day 7 was found to be 940 mg/kg (13).

Clinical Studies

Unfortunately, large-scale well-controlled clinical studies have not been conducted, although a considerable number of clinical reports have been published. Much of the published work reporting the beneficial effects of Inchinko-to for the treatment of acute liver failure, viral hepatitis, jaundice, cholestasis, postoperative liver injury, primary biliary cirrhosis, biliary artesia and sclerosing cholangitis has been written in Japanese. At present, only four papers in English are available. Onji *et al.* (66) reported that the combined use of ursodeoxycholic acid and Inchinko-to in 3 jaundiced patients with primary biliary cirrhosis resulted in clinical and biochemical improvement, including a decrease in bilirubin levels in all patients. Kobayashi *et al.* (67) reported a beneficial effect for Inchinko-to in postoperative biliary artesia patients. Eighteen such patients aged 3-23 years, with elevated ALT and γ-glutamyltranspeptidase (γ-GTP) but normal serum total bilirubin levels, were treated with Inchinko-to for 2 years. All patients had been receiving ursodeoxycholic acid for at least 1 year without improvement before Inchinko-to treatment. All subjects tolerated the drugs well and completed the study without difficulty. Measurements were made before and after treatment of AST, ALT, γ-GTP, total bile acid and serum total bilirubin as markers of liver failure, and of hyaluronic acid, prolyl-hydroxylase, procollagen type III and type IV collagen as markers of liver fibrosis. The percentage of subjects who improved (defined by a > 25% decrease in the parameter for each patient) after treatment was 72% for ALT, γ-GTP and total bile acid and 67% for hyaluronic acid. The mean values for all serum markers significantly decreased after Inchinko-to treatment.

Another study evaluated the efficacy of Inchinko-to in the treatment of liver fibrosis in 6 patients with biliary artesia without jaundice (68). The authors reported that 5 of the 6 patients showed significant decreases in AST, ALT and γ-GTP after 1-3 years of Inchinko-to treatment. In a separate study, Arai *et al.* (69) reported a case of severe acute hepatitis of unknown etiology treated with Inchinko-to. The patient had grade I hepatic encephalopathy, liver atrophy and massive ascites, and was treated with transfusions of fresh frozen plasma, glucagon-insulin therapy to improve vital function, and lacturose and kanamycin to prevent encephalopathy. While AST and ALT decreased after these treatments, prothrombin time remained unimproved and serum total bilirubin continued to increase. Additional treatment with glycyrrhizin, ursodeoxycholic acid and Inchinko-to showed no apparent beneficial effect. However, after stopping kanamycin treatment, serum total bilirubin and prothrombin time began to improve. An abdominal CT study at 3 months showed that the liver had increased in size. Fresh frozen plasma was stopped at day 99, and after 4 months the patient was discharged from the hospital.

While the studies described above did not establish the efficacy and safety of Inchinko-to in the treatment of liver diseases, they encourage further extensive research

which may open the way to the development of new pharmacotherapeutic strategies aimed at a wide variety of liver diseases.

Conclusions

Kampo medicines are now manufactured in Japan on a modern industrial scale whereby the quality and quantity of ingredients are standardized under strict, scientific quality controls. Inchinko-to, one of these Kampo medicines, has been approved as an ethical drug and is used for the treatment of liver diseases in Japan by physicians who have been educated in Western medicine. Recent experimental studies concerning the effects and the mechanism of action of the drug and its ingredients have addressed the possibility of developing new pharmacotherapeutic strategies aimed at a wide variety of liver diseases. Although no well-controlled clinical studies have been conducted, several case studies have demonstrated possible beneficial effects of the drug for severe liver disorders, many of which are currently untreatable. Appropriate toxicological and pharmacokinetic studies have generally not been carried out. However, in addition to empirical knowledge about safety gained over the centuries, this drug is now used ethically in Japan under the extensive surveillance system of drug safety covering all medical institutions, pharmacies and pharmaceutical manufacturers. This will encourage future research to establish the efficacy of Inchinko-to in the treatment of liver diseases.

Sources

Ethical Inchinko-to is supplied by Tsumura & Co., Kanebo Pharmaceuticals Co., Ltd., and several other Kampo medicine manufacturers in Japan.

References

1. Yamamura, Y. *Preface*. In: Recent Advances in the Pharmacology of Kampo Japanese Herbal) Medicines, International Congress Series 854. E. Hosoya and Y. Yamamura (Eds). Excerpta Medica, 1988, 3.

2. Watanabe, K. *What is Kampo?* http://web.sc.itc.keio.ac.jp/kampo/vc/index.html

3. Taguchi, H., Endo, T., Nakajima, K., Aburada, M. [*Studies on the constituents and evaluation of Gardenia Fructus*] [abstract in English]. Proc Symp Wakan-Yaku 1975, 9: 85-91.

4. Takeda, S., Endo, T., Aburada, M. *Pharmacological studies on iridoid compounds. III. The choleretic mechanism of iridoid compounds*. J Pharmacobiodyn 1981, 4: 612-23.

5. Takeda, S., Yuasa, K., Endo, T., Aburada, M. *Pharmacological studies on iridoid compounds. II. Relationship between structures and choleretic actions of iridoid compound*. J Pharmacobiodyn 1980, 3: 485-92.

6. Shoda, J., Miura, T., Utsunomiya, H. et al. *Genipin enhances Mrp2 (Abcc2)-mediated bile formation and organic anion transport in rat liver*. Hepatology 2004, 39: 167-78.

7. Huang, W., Zhang, J., Moore, D.D. *A traditional herbal medicine enhances bilirubin clearance by activating the nuclear receptor CAR*. J Clin Invest 2004, 113: 137-43.

8. Komiya, T., Tsukui, M., Oshio, H. [*Studies on "Inchinko". I. Capillarisin, a new choleretic substance*] [abstract in English]. Yakugaku Zasshi 1976, 96: 841-54.

9. Kagawa, K., Okuno, I., Noro, Y. *Colorimetric determination of capillarisin*. Shoyakugaku Zasshi 1984, 38: 133-7.

10. Okuno, I., Kagawa, K., Noro, Y., Namba, T. *Pharmacological studies on the crude drug "Inchinko" in Japan (VI). Seasonal variation in chemical constituents of Artemisia capillaris THUNB*. Shoyakugaku Zasshi 1983, 37: 199-203.

11. Akahori, A., Kagawa, K., Okuno, I. [*Determination of scoparone, capillarin and capllin in the crude drug "Inchinko"*] [abstract in English]. Shoyakugaku Zasshi 1978, 32: 177-84.

12. Wang, X., Saito, K., Kano, Y. *On the evaluation of the preparation of Chinese medicinal prescriptions (VII). HPLC analysis of the components in "Inchinko-to" and "Inchin-gorei-san"*. Shoyakugaku Zasshi 1993, 47: 243-8.

13. Yamahara, J., Matsuda, H., Sawada, T., Mibu, H., Fujimura, H. [*Biologically active principles of crude drugs. Pharmacological evaluation of Artemisiae capillaris FLOS*] [abstract in English]. Yakugaku Zasshi 1982, 102: 285-91.

14. Okuno, I., Uchida, K., Nakamura, M., Sakurai, K. *Studies on choleretic constituents in Artemisia capillaris THUNB*. Chem Pharm Bull (Tokyo) 1988, 36: 769-75.

15. Kitagawa, I., Fukuda, Y., Yoshihara, M., Yamahara, J., Yoshikawa, M. *Capillartemisin A and B, two new choleretic principles from Artemisiae capillaris herba*. Chem Pharm Bull (Tokyo) 1983, 31: 352-5.

16. Kiso, Y., Suzuki, Y., Konno, C., Hikino, H., Hashimoto, I., Yagi, Y. *Application of carbon tetrachloride-induced liver lesion in mice for screening of liver protective crude drugs*. Shoyakugaku Zasshi 1982, 36: 238-44.

17. Kiso, Y., Ogasawara, S., Hirota, K. et al. *Antihepatotoxic principles of Artemisia capillaris buds*. Planta Med 1984, 81-5.

18. Ihara, N., Arichi, S. [*Protective effect of oriental crude drugs and Kampo medicament on a fulminant type of galactosamine-induced hepatitis*] [abstract in English]. Proc Symp Wakan-Yaku 1981, 14: 45-55.

19. Takeda, S., Iizuka, A., Funo, S. et al. [*Effect of Chinese prescription "Inchinko-to" on experimental hepatobiliary injury induced by alpha-naphthylisothiocianate*] [abstract in English]. J Med Pharm Soc Wakan-Yaku 1984, 1: 230-4.

20. Kumazawa, N., Ohta, S., Tu, S.-H. et al. [*Protective effects of various methanol extracts of crude drugs on experimental hepatic injury induced by alpha-naphthylisothiocyanate in rats*] [abstract in English]. Yakugaku Zasshi 1991, 111: 199-204.

21. Yamamoto, M., Ogawa, K., Morita, M., Fukuda, K., Komatsu, Y. *The herbal medicine Inchin-ko-to inhibits liver cell apoptosis induced by transforming growth factor beta1*. Hepatology 1996, 23: 552-9.

22. Yamashiki, M., Mase, A., Arai, I. et al. *Effects of the Japanese herbal medicine 'Inchinko-to' (TJ-135) on concanavalin A-induced hepatitis in mice*. Clin Sci (Lond) 2000, 99: 421-31.

23. Yamamoto, M., Miura, N., Ohtake, N. et al. *Genipin, a metabolite derived from the herbal medicine Inchin-ko-to, and suppression of Fas-induced lethal liver apoptosis in mice*. Gastroenterology 2000, 118: 380-9.

24. Chu, C.Y., Tseng, T.H., Hwang, J.M., Chou, F.P., Wang, C.J. *Protective effects of capillarisin on tert-butylhydroperoxide-induced oxidative damage in rat primary hepatocytes*. Arch Toxicol 1999, 73: 263-8.

25. Jang, S., 2nd, Kim, Y.J., Lee, W.Y. et al. *Scoparone from Artemisia capillaris inhibits the release of inflammatory mediators in RAW 264.7 cells upon stimulation cells by interferon-gamma Plus LPS*. Arch Pharm Res 2005, 28: 203-8.

26. Sakaida, I., Tsuchiya, M., Kawaguchi, K., Kimura, T., Terai, S., Okita, K. *Herbal medicine Inchin-ko-to (TJ-135) prevents liver fibrosis and enzyme-altered lesions in rat liver cirrhosis induced by a choline-deficient L-amino acid-defined diet*. J Hepatol 2003, 38: 762-9.

27. Inao, M., Mochida, S., Matsui, A. et al. *Japanese herbal medicine Inchin-ko-to as a therapeutic drug for liver fibrosis*. J Hepatol 2004, 41: 584-91.

28. Imanishi, Y., Maeda, N., Otogawa, K. et al. *Herb medicine Inchin-ko-to (TJ-135) regulates PDGF-BB-dependent signaling pathways of hepatic stellate cells in primary culture and attenuates development of liver fibrosis induced by thioacetamide administration in rats*. J Hepatol 2004, 41: 242-50.

29. Zhan, Y., Li, D., Wei, H. et al. *Emodin on hepatic fibrosis in rats*. Chin Med J (Engl) 2000, 113: 599-601.

30. Yamahara, J., Kobayashi, G., Matsuda, H., Katayama, T., Fujimura, H. *Vascular dilatory action of Artemisia capillaris bud extracts and their active constituent*. J Ethnopharmacol 1989, 26: 129-36.

31. Yamahara, J., Kobayashi, G., Matsuda, H., Iwamoto, M., Fujimura, H. *Vascular dilatory action of the Chinese crude drug. II. Effects of scoparone on calcium mobilization*. Chem Pharm Bull (Tokyo) 1989, 37: 485-9.

32. Yamahara, J., Kobayashi, G., Matsuda, H., Katayama, T., Fujimura, H. *The effect of scoparone, a coumarin derivative isolated from the Chinese crude drug Artemisiae capillaris flos, on the heart*. Chem Pharm Bull (Tokyo) 1989, 37: 1297-9.

33. Huang, H.C., Lee, C.R., Weng, Y.I., Lee, M.C., Lee, Y.T. *Vasodilator effect of scoparone (6,7-dimethoxycoumarin) from a Chinese herb*. Eur J Pharmacol 1992, 218: 123-8.

34. Xu, Q., Mori, H., Sakamoto, O. et al. [*Antitumor principle of Artemisiae capillaris HERBA and its related compound*] [abstract in English]. J Med Pharm Soc Wakan-Yaku 1989, 6: 1-7.

35. Wang, S.W., Lai, C.Y., Wang, C.J. *Inhibitory effect of geniposide on aflatoxin B1-induced DNA repair synthesis in primary cultured rat hepatocytes*. Cancer Lett 1992, 65: 133-7.

36. Tseng, T.H., Chu, C.Y., Wang, C.J. *Inhibition of penta-acetyl geniposide on AFB1-induced genotoxicity in C3H10T1/2 cells*. Cancer Lett 1992, 62: 233-42.

37. Kapadia, G.J., Sharma, S.C., Tokuda, H., Nishino, H., Ueda, S. *Inhibitory effect of iridoids on Epstein-Barr virus activation by a short-term in vitro assay for anti-tumor promoters*. Cancer Lett 1996, 102: 223-6.

38. Hsu, H.Y., Yang, J.J., Lin, S.Y., Lin, C.C. *Comparisons of geniposidic acid and geniposide on antitumor and radioprotection after sublethal irradiation*. Cancer Lett 1997, 113: 31-7.

39. Wang, C.J., Tseng, T.H., Lin, J.K. *Penta-acetyl geniposide: Isolation, identification and primary effect on C6 glioma cells in vitro*. Anticancer Res 1992, 12: 911-5.

40. Lee, M.J., Hsu, J.D., Wang, C.J. *Inhibition of 12-O-tetradecanoylphorbol-13-acetate-caused tumor promotion in benzo[a]pyrene-initiated CD-1 mouse skin by geniposide*. Anticancer Res 1995, 15: 411-6.

41. Yamauchi, K., Fujimoto, N., Kuwano, S., Inouye, H., Inoue, K. *The mechanism of purgative action of geniposide, an iridoid glucoside of the fruit of Gardenia, in mice*. Planta Med 1976, 30: 39-47.

42. Miura, T., Nishiyama, Y., Ichimaru, M., Moriyasu, M., Kato, A. *Hypoglycemic activity and structure-activity relationship of iridoidal glycosides*. Biol Pharm Bull 1996, 19: 160-1.

43. Harada, M., Tenmyo, N., Aburada, M., Endo, T. [*Pharmacological studies of Gardeniae Fructus. I. Effect of geniposide and genipin on the biliary excretion, the gastric juice secretion, and the gastric contraction, and other pharmacological actions*] [abstract in English]. Yakugaku Zasshi 1974, 94: 157-62.

44. Imakura, K., Yamazaki, M., Mohri, T. *Counteraction of genipin to the trauma- and scopolamine-induced impairment of memory in mice*. J Trad Med 1996, 13: 173-9.

45. Yamazaki, M., Chiba, K., Mohri, T. *Neuritogenic effect of natural iridoid compounds on PC12h cells and its possible relation to signaling protein kinases*. Biol Pharm Bull 1996, 19: 791-5.

46. Kimura, Y., Okuda, H., Okuda, T., Hatano, T., Agata, I., Arichi, S. *Studies on the activities of tannins and related compounds from medicinal plants and drugs. VII. Effects of extracts of leaves of Artemisia species, and caffeic acid and chlorogenic acid on lipid metabolic injury in rats fed peroxidized oil*. Chem Pharm Bull (Tokyo) 1985, 33: 2028-34.

47. Akao, T., Kobashi, K., Aburada, M. *Enzymic studies on the animal and intestinal bacterial metabolism of geniposide*. Biol Pharm Bull 1994, 17: 1573-6.

48. Yang, L., Akao, T., Kobashi, K. *Purification and characterization of a geniposide-hydrolyzing beta-glucosidase from Eubacterium sp. A-44, a strict anaerobe from human feces*. Biol Pharm Bull 1995, 18: 1175-8.

49. Miyagoshi, M., Amagaya, S., Ogihara, Y. *Structural transformation of geniposide, gardenoside, and related compounds in rat gastrointestinal contents*. Planta Med 1988, 54: 556-7.

50. Kawata, Y., Hattori, M., Akao, T., Kobashi, K., Namba, T. *Formation of nitrogen-containing metabolites from geniposide and gardenoside by human intestinal bacteria*. Planta Med 1991, 57: 536-42.

51. Touyama, R., Takeda, Y., Inoue, K. et al. *Studies on the blue pigments produced from genipin and methylamine. I. Structure of the brownish-red pigments, intermediates leading to the blue pigments*. Chem Pharm Bull (Tokyo) 1994, 42: 668-73.

52. Fujikawa, S., Fukui, Y., Koga, K. *Structure of genipocyanin G1, a spontaneous reaction product between genipin and glycine*. Tetrahedron Lett 1987, 28: 4699-700.

53. Fujikawa, S., Fukui, Y., Koga, K., Kumada, J. *Brilliant skyblue pigment formation from gardenia fruits*. J Ferment Technol 1987, 65: 419-24.

54. Sung, H.W., Huang, R.N., Huang, L.L., Tsai, C.C., Chiu, C.T. *Feasibility study of a natural crosslinking reagent for biological tissue fixation*. J Biomed Mater Res 1998, 42: 560-7.

55. Ueno, K., Takeda, Y., Iwasaki, Y., Yoshizaki, F. *Simultaneous estimation of genposide and genipin in mouse plasma using high-performance liquid chromatography*. Anal Sci 2001, 17: 1237-9.

56. Yamahara, J., Fukuda, Y., Sawada, T., Fujimura, H. [*The effectiveness of Chinese prescription. The pharmacokinetic study on the blood concentration after the administration of capillaris Flos and 6,7-dimethylesculetin*] [abstract in English]. Shoyakugaku Zasshi 1981, 35: 108-14.

57. Product Profile of "TSUMURA Inchinko-to Extract Granules for Ethical Use" (in Japanese) 2004.

58. Company Data, Tsumura & Co., 2001.

59. Wang, C.J., Hwang, L.S., Lin, J.K. *Reversible hepatic black pigmentation and enzyme alteration induced by prolonged feeding of high dose of crocin dyes in rats*. Proc Natl Sci Counc Repub China B 1984, 8: 246-53.

60. Company Data, Tsumura & Co., 2003.

61. Katami, M., Kuboniwa, H., Maemura, S., Yanagisawa, T. *Genotoxicity of extracts of Japanese traditional herbal medicines (Kampo)*. Environ Mutagen Res 2002, 24: 1-15.

62. Yamano, T., Tsujimoto, Y., Noda, T. et al. *Hepatotoxicity of geniposide in rats*. Food Chem Toxicol 1990, 28: 515-9.

63. Kang, J.J., Wang, H.W., Liu, T.Y., Chen, Y.C., Ueng, T.H. *Modulation of cytochrome P-450-dependent monooxygenases, glutathione and glutathione S-transferase in rat liver by geniposide from Gardenia jasminoides*. Food Chem Toxicol 1997, 35: 957-65.

64. Ozaki, A., Kitano, M., Furusawa, N., Yamaguchi, H., Kuroda, K., Endo, G.. *Genotoxicity of gardenia yellow and its components*. Food Chem Toxicol 2002, 40: 1603-10.

65. Tsai, C.C., Huang, R.N., Sung, H.W., Liang, H.C. *In vitro evaluation of the genotoxicity of a naturally occurring crosslinking agent (genipin) for biologic tissue fixation*. J Biomed Mater Res 2000, 52: 58-65.

66. Onji, M., Kikuchi, T., Michikata, K., Yamashita, Y., Ohta, Y. *Combined use of ursodeoxycholic acid and Inchin-ko-to in jaundiced patients with primary biliary cirrhosis*. J Med Pharm Soc Wakan-Yaku 1990, 7: 161-7.

67. Kobayashi, H., Horikoshi, K., Yamataka, A., Lane, G.J., Yamamoto, M., Miyano, T. *Beneficial effect of a traditional herbal medicine (Inchin-ko-to) in postoperative biliary atresia patients*. Pediatr Surg Int 2001, 17: 386-9.

68. Iinuma, Y., Kubota, M., Yagi, M., Kanada, S., Yamazaki, S., Kinoshita, Y. *Effects of the herbal medicine Inchinko-to on liver function in postoperative patients with biliary atresia – A pilot study*. J Pediatr Surg 2003, 38: 1607-11.

69. Arai, M., Yokosuka, O., Fukai, K. et al. *A case of severe acute hepatitis of unknown etiology treated with the Chinese herbal medicine Inchinko-to*. Hepatol Res 2004, 28: 161-5.