JPP Journal of Pharmacy and Pharmacology

JPP 2001, 53: 441–447

Received September 18, 2000
Accepted November 14, 2000
ISSN 0022-3573

Application of pressure-controlled colon delivery capsule to oral administration of glycyrrhizin in dogs

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Abstract

A colon delivery system has been used to improve the bioavailability of glycyrrhizin, a glycoside of glycyrrhetic acid. The bioavailability of glycyrrhizin is low when administered in conventional oral galenic dosage forms because glycyrrhizin is enzymatically hydrolysed both in the stomach and in the intestine. It was reasoned that if large amounts of glycyrrhizin were directly delivered to the colon, enzymatic activity should be reduced due to saturation so that intact glycyrrhizin could be absorbed into the systemic circulation. Based on this assumption, pressure-controlled colon delivery capsules (PCDCs) were used as a colon delivery system. Eight types of glycyrrhizin solution were prepared and were introduced into PCDCs. After oral administration of the test PCDCs to beagle dogs, blood samples were obtained over 24 h and plasma glycyrrhizin concentrations were measured by an HPLC method. With PCDCs containing aqueous glycyrrhizin and propylene glycol solutions, plasma glycyrrhizin levels were extremely low and the bioavailabilities of glycyrrhizin were 0.6 % and 0.4 %, respectively. When Labrasol was added to both types of glycyrrhizin solution, the bioavailability was improved to 4.6 % for aqueous solution and 3.8 % for propylene glycol solution. When a surfactant, Polysorbate 80, was added in combination with Labrasol, synergistic effects were not obtained. Furthermore, dose-dependent effects of Polysorbate 80 were not obtained. Labrasol, which is a component of self-emulsifying drug delivery systems (SEDDS), has been shown to strongly improve the bioavailability of glycyrrhizin from the colon.

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Introduction

Glycyrrhizin is the main constituent of *Glycyrrhiza glabra* L, and chemically is the glycoside of glycyrrhetic acid. Glycyrrhizin shows anti-allergic, anti-inflammatory, anti-hepatitis and interferon-inducing effects (Van Rossum et al 1998). In addition, glycyrrhizin inhibits the replication of HIV-1 (Abe et al 1982; Hasegawa et al 1994), and clinical evaluation of glycyrrhizin as an anti-AIDS drug has been recently undertaken. Chronic administration of glycyrrhizin is performed in the therapy of both hepatitis and AIDS. For this purpose, oral therapy with glycyrrhizin is preferable. Several studies on the pharmacokinetics of glycyrrhizin after oral administration in both man and experimental animals have been reported. Glycyrrhizin was detectable in the plasma after oral administration of a large dose of glycyrrhizin, 500 mg kg^{-1}, to rats (Wang et al 1995). However, glycyrrhizin was not detectable in human plasma after the oral administration of a therapeutic dose of glycyrrhizin to patients, $1.4 \sim 1.8$ mg kg^{-1} (Nakano et al 1980). The low bioavailability of glycyrrhizin was ascribed to its hydrolysis by gastric juice in the

stomach and by intestinal bacteria or other enzymatic processes in the intestinal wall (Kim et al 1999). On the other hand, its degradation product, glycyrrhetic acid, was detectable at significant concentrations in plasma after oral administration of glycyrrhizin to humans, because glycyrrhizin was converted to glycyrrhetic acid by glycyrrhizin-hydrolysing bacteria in the intestine and the resulting glycyrrhetic acid was absorbed into the systemic circulation (Wang et al 1994).

We have been studying a colon delivery system for a decade and developed an intestinal pressure-controlled colon delivery capsule (PCDC) (Takaya et al 1995; Muraoka et al 1998; Hu et al 2000) which is prepared by coating the inner surface of gelatin capsules with a water-insoluble polymer, ethylcellulose. Drug is introduced into the PCDC with either a suppository base such as polyethylene glycol (PEG 1000) or an oily base like Witepsol or Pharmasol. After oral administration, the gelatin phase dissolves immediately in the stomach. The system behaves like an ethylcellulose balloon containing drug solution. In the upper part of the gastrointestinal tract, the fluidity is such that the ethylcellulose balloon is not directly subjected to intestinal luminal pressures. However, reabsorption of water occurs in the colon and the viscosity of the luminal contents increases (Digenis & Sandefer 1991; Ritschel 1991; Moës 1993). As a result, intestinal pressures due to peristalsis directly affect the ethylcellulose balloon. Since the ethylcellulose balloon cannot tolerate these pressures, it disintegrates in the intestine. With respect to colon-specific drug delivery, a pig model has been developed and validated because the digestive anatomy and physiology of the pig is similar to that of man, with each section of the pig's gastrointestinal tract being comparable anatomically with that of man (Gardner et al 1996; Larsen et al 1989). However, it is difficult for us to orally administer PCDCs to a pig. Therefore, the colon delivery efficiency of PCDCs was at first demonstrated with beagle dogs where 5-aminosalicylic acid, a representative drug for inflammatory bowel disease, was used (Takaya et al 1997). Thereafter, a study in man showed that PCDCs delivered caffeine as a model drug to the ascending colon and disintegrated there by a magnetic source imaging (MSI) method using a superconducting quantum interference device (SQUID) (Hu et al 2000). Based on this background, we proposed that when a large amount of glycyrrhizin was delivered to the colon by PCDCs, the glycyrrhizin-converting enzyme activity of the bacteria in the colon would be saturated and the administered glycyrrhizin could be absorbed into the systemic circulation in an intact form.

Therefore, based on this assumption, we prepared several types of PCDCs containing glycyrrhizin and the bioavailability of glycyrrhizin was studied in beagle dogs.

Materials and Methods

Drugs and animals

Glycyrrhizin disodium salt was purchased from Maruzen Pharmaceutical Co. (Fukuyama, Japan). The α- and β-glycyrrhizins were gifts from Amato Pharmaceutical Co. (Fukuchiyama, Japan). Size-zero gelatin capsules were obtained from Yoshida Co. (Himeji, Japan). Methanol and methylene chloride were obtained from Kanto Chemical Co. (Tokyo, Japan). Propylene glycol was obtained from Nacalai Tesque Inc. (Kyoto, Japan). Ethylcellulose (7G grade, Dow Chemicals) was a gift from Nisshin Chemical Industrial Co. (Osaka, Japan). Polysorbate 80 (TO-10M) was obtained from Nikko Chemicals Co. (Tokyo, Japan). Labrasol (Gattefosé, Genneevilliers Cedex, France) was obtained through CBC Co. (Tokyo, Japan). Male beagle dogs (10.0–13.5 kg) used in this study and standard solid meal of commercial food (Labo D stock) were obtained from Nippon Nousan Co. (Yokohama, Japan). All other materials used were of reagent grade and were used as received.

Preparation of glycyrrhizin solutions

Glycyrrhizin disodium salt (200 mg) was dissolved with 0.2 mL of distilled water or propylene glycol. Where appropriate, surfactants such as Labrasol and Polysorbate 80 were then added to the solution. A total of 8 types of glycyrrhizin solutions were prepared as shown in Table 1. Since the preparation no. 8, containing 200 mg of glycyrrhizin, showed higher viscosity compared with the other preparations, it was diluted with an equal volume of the mixture of propylene glycol and Labrasol to decrease the viscosity.

Preparation of pressure-controlled colon delivery capsule (PCDC) containing glycyrrhizin

Size-zero PCDCs were prepared according to our previously reported method (Takaya et al 1995; Muraoka et al 1998). Briefly, 0.18 mL of 23 % (w/v) ethylcellulose solution dissolved with a mixture of methylene chloride and methanol (4 : 1) was introduced into a size-zero gelatin capsule through the pores at the top and bottom of the capsule, and the inner surface of the gelatin capsule was coated with ethylcellulose by rotating hori-

Table 1 Prepared glycyrrhizin formulations in this study.

Preparation	Glycyrrhizin (mg)	Water (mL)	Propylene glycol (mL)	Labrasol (mL)	Polysorbate 80 (mL)
1	200	0.60	–	–	–
2	200	0.30	–	0.30	–
3	200	0.20	–	0.40	–
4	200	0.30	–	0.30	0.05
5	200	0.30	–	0.30	0.10
6	200	–	0.60	–	–
7	200	–	0.40	0.20	–
8	200	–	0.60	0.60	–

Preparations 1–7 were filled in a pressure-controlled colon delivery capsule, preparation 8 was filled into two pressure-controlled colon delivery capsules.

zontally for 10 h at 40°C. The pore at the bottom of the capsule was sealed with concentrated ethylcellulose solution which served as a glue, and the ethylcellulose-coated capsule was filled with 0.6–0.7 mL of the prepared glycyrrhizin solution. Finally, the pore at the top of the capsule was sealed with an ethylcellulose glue and PCDCs containing glycyrrhizin solutions were obtained. In the case of preparation no. 8, two PCDCs were used to administer this solution, because its volume was twice as large as the other preparations.

Pharmacokinetic study in beagle dogs

Animal experiments were all carried out in accordance with the Guideline for Animal Experimentation in Kyoto Pharmaceutical University. Three adult male beagle dogs were fasted for 24 h before each experiment, while free access to water was allowed. During the course of the experiment, water was not given until 4 h after the test preparation was administered. Each dog received an oral administration of a certain test preparation in all studies. The glycyrrhizin dose was 200 mg/dog. At 4 h after administration, 450 g of the solid meal and water were given. No additional food was given during the study. All the experiments were carried out at the same time of day to exclude influences of circadian rhythm. Drug administration was performed at 1030 h with 20 mL of water. At 30 min before drug administration, a control blood sample (0.5 mL) was taken from the jugular vein. For most studies, each beagle dog received one PCDC containing 200 mg of glycyrrhizin. However, in the case of preparation no. 8, two PCDCs, each containing 100 mg glycyrrhizin, were administered at once. After the oral administration of PCDCs, 0.5-mL blood samples were collected at 1, 2, 3, 4, 5, 6, 8, 10, 12 and 24 h. To determine the bioavailability of oral glycyrrhizin preparations, intravenous administration was carried out in three dogs with 50 mg mL^{-1} body-weight^{-1} of glycyrrhizin dose and blood sample collection over 24 h. The plasma fraction was immediately obtained by centrifuging the blood sample at 12 000 rev min^{-1} for 5 min. These plasma samples were kept in a deep freezer at −80°C until analysed.

Assay of α- and β-glycyrrhizins in dog plasma

The plasma glycyrrhizin concentration in dogs was measured by the HPLC method as we previously reported (Koga et al 2000). The plasma samples for the calibration curves were prepared by spiking with known amounts of drug. A set of six or seven calibration standards was run. The calibration curve was linear over the range of 0.1–25.0 μg mL^{-1} and passed through the origin.

Pharmacokinetic analysis

Pharmacokinetic parameters were determined from the plasma drug concentration–time data. For the plasma disappearance curve of glycyrrhizin, two rate constants, α and β, after intravenous injection of glycyrrhizin solution, were determined by a nonlinear regression analysis using a two-exponential term. The half-lives, $t_{\frac{1}{2}(\alpha)}$ and $t_{\frac{1}{2}(\beta)}$, were determined by dividing ln2 with the α- or β-value (Yoshikawa et al 1995). The time when glycyrrhizin first appeared in the systemic circulation (T_i), the time when the plasma drug concentration reached its maximum concentration (T_{max}), and the peak plasma drug concentration (C_{max}) after oral administration of glycyrrhizin in PCDCs were determined from the authentic plasma concentration–time data.

The area under the plasma drug concentration vs time curve (AUC) and the area under the first-moment curve (AUMC) after oral administration of the test preparation were calculated using the linear trapezoidal rule up to the last measured plasma drug concentration (Yoshikawa et al 1998). The mean residence time (MRT) after oral administration was calculated by $AUMC_{oral}/AUC_{oral}$. Bioavailability of glycyrrhizin was calculated from the AUC_{oral} and AUC_{iv} by equation 1:

$$BA = (AUC_{oral} \times Dose_{iv})/(AUC_{iv} \times Dose_{oral}) \quad (1)$$

where the mean AUC values of both intravenous and oral dosing groups were used.

Statistics

All values are expressed as their mean $\pm$ s.e. Statistical differences were assumed to be reproducible when $P < 0.05$ (one-sided t-test).

Results and Discussion

According to the report of Guillaume et al (Guillaume et al 1999), α- and β-stereoisomers are present in glycyrrhizin. We initially checked the content of α-glycyrrhizin in our glycyrrhizin powder. By our HPLC method, which can separate α- and β-glycyrrhizins by isocratic HPLC condition, the α-glycyrrhizin content was found to be 2% and the β-glycyrrhizin content was 98%. Therefore, the glycyrrhizin powder was used without further purification.

To determine the bioavailability of glycyrrhizin, the AUC_{iv} of glycyrrhizin was obtained after intravenous injection to dogs as a reference for oral glycyrrhizin preparations. Throughout this study, the possibility of the interconversion of glycyrrhizin stereoisomers was also checked. Figure 1 shows the plasma β-glycyrrhizin concentration-vs-time profiles after intravenous injection of glycyrrhizin solution, 50 mg. After intravenous injection, β-glycyrrhizin disappeared from the systemic circulation with a $t_{\frac{1}{2}(\alpha)}$ of 0.38 ± 0.16 h. Thereafter, the plasma β-glycyrrhizin concentration slowly declined with a $t_{\frac{1}{2}(\beta)}$ of 16.9 ± 4.3 h. The pharmacokinetic parameters determined by a non-compartmental pharmacokinetic analysis are shown in Table 2. The mean AUC_{iv} was 508 ± 27 μg h mL^{-1} and the MRT was 9.26 ± 0.46 h. In this study, the injected glycyrrhizin solution contained 98% β-glycyrrhizin. However, the formation of α-glycyrrhizin was not observed. Also, when α-glycyrrhizin solution was injected intravenously into the dogs, the formation of β-glycyrrhizin was not detected. These results demonstrated that the interconversion of α- and β-glycyrrhizins should not occur in dogs.

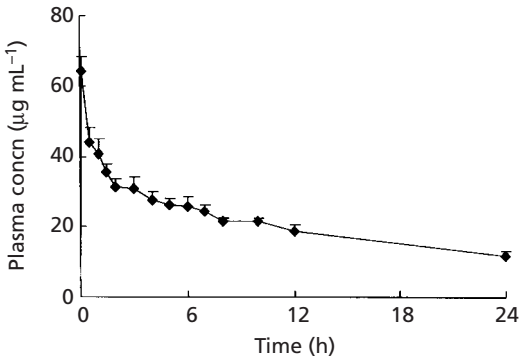

Figure 1 Plasma glycyrrhizin concentration-vs-time profile after intravenous injection of glycyrrhizin solution to dogs. The glycyrrhizin dose was 50 mg mL^{-1}. Results are expressed as the mean $\pm$ s.e. of three experiments.

Next, the usefulness of the PCDC as an oral dosage form for glycyrrhizin was examined. Figure 2 shows the plasma glycyrrhizin concentration-vs-time profiles after PCDCs with different liquid formulations were administered to beagle dogs. In the case of preparation no. 1, the plasma glycyrrhizin level reached its maximum concentration, C_{max}, at approximately 8 h after oral administration. Pharmacokinetic analysis was performed and the obtained parameters are shown in Table 3. The mean C_{max} was 1.06 ± 0.58 μg mL^{-1}. The T_i, when glycyrrhizin first appeared into the systemic circulation, was 3.33 ± 1.76 h. The colon arrival time (CAT) of oral preparations in our beagle dogs was estimated to be 3.5 ± 0.3 h by a sulfasalazine test (Hu et al 1999). Hence, because the T_i obtained was equivalent to CAT in the dog, PCDC preparation no. 1 delivered glycyrrhizin to the colon and released it there. The bioavailability of glycyrrhizin without additives was calculated to be 0.6%. When solubilizers such as Labrasol and Polysorbate 80 were added to the glycyrrhizin solution, the bioavailability was improved (preparation nos 2–5). By formulating with 0.3 and 0.4 mL Labrasol (preparation nos 2 and 3, respectively), the bioavailability of glycyrrhizin increased by approximately 6 and 3 fold that obtained with the aqueous glycyrrhizin solution (preparation no. 1). However, the effect of Labrasol was not dose dependent. Preparation no. 3 contained 1.5-times the amount of Labrasol in preparation no. 2. However, both C_{max} and AUC_{oral} were lower with preparation no. 3 than those obtained with preparation no. 2. Furthermore, another surfactant, Polysorbate 80, was formulated together with Labrasol (preparation nos 4 and 5)

Table 2 Pharmacokinetic parameters of β- glycyrrhizin after iv injection (500 mg glycyrrhizin) to beagle dogs.

α (h^{-1})	$t_{\frac{1}{2}(\alpha)}$ (h)	β (h^{-1})	$t_{\frac{1}{2}(\beta)}$ (h)	$AUC_{(0-24)}$ ($\mu g\ h\ mL^{-1}$)	MRT (h)
2.47 ± 0.75	0.38 ± 0.16	0.05 ± 0.01	16.9 ± 4.3	508 ± 27	9.26 ± 0.46

α and β are rate constants at the α- and β- phases. $t_{\frac{1}{2}(\alpha)}$and $t_{\frac{1}{2}(\beta)}$ are the half-lives at the two phases. $AUC_{(0-24)}$, area under the plasma glycyrrhizin concentration vs time curve from time zero to 24 h; MRT, mean residence time. Each value represents the mean ± s.e.m., n = 3.

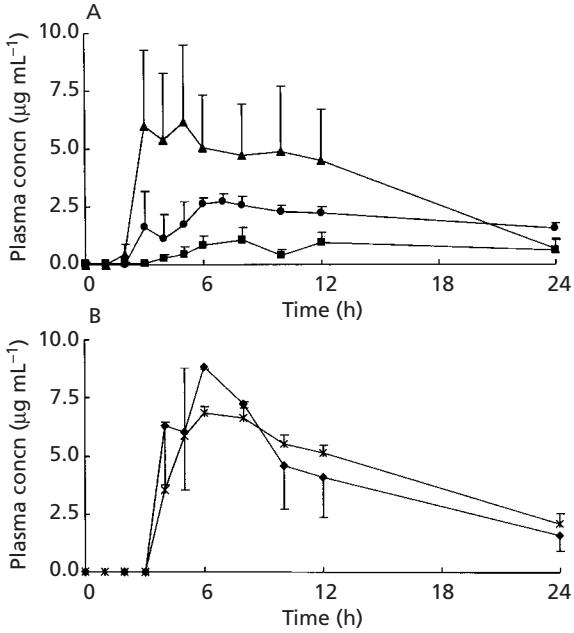

Figure 2 Plasma glycyrrhizin concentration-vs-time profiles after oral administration of pressure-controlled colon delivery capsules containing glycyrrhizin solutions to dogs. The dose of glycyrrhizin was 200 mg. Results are expressed as the mean ± s.e. of three or four experiments. A. ■, Preparation no. 1; ▲, preparation no. 2; ●, preparation no. 3. B. ×, Preparation no. 4; ◆, preparation no. 5.

and the obtained plasma glycyrrhizin level-vs-time profiles were compared (Figure 2B). However, C_{max} and AUC_{oral} did not further increase, since the bioavailabilities were 4.6 and 4.3% for preparations no. 4 and no. 5, respectively. Therefore, a synergistic effect of these surfactants was not obtained.

A non-aqueous solvent, propylene glycol, was also used to solubilize glycyrrhizin, and PCDCs (preparation no. 6) were administered to dogs. Although the viscosity of propylene glycol was slightly higher than that of water, the PCDCs disintegrated in the colon and glycyrrhizin was thought to be delivered there, because the plasma glycyrrhizin concentration started to increase at approximately 4 h after oral administration (Figure 3). The T_i obtained with this preparation was 4.25 ± 0.25 h. However, both the C_{max} and AUC_{oral} values were lower than those observed with aqueous preparation no. 1. The bioavailability of glycyrrhizin for preparation no. 6 was 0.4%. When Labrasol was added to propylene glycol glycyrrhizin solution, the bioavailability of glycyrrhizin increased to 3.8% and 3.7% for preparations nos 7 and 8, respectively. The AUC_{oral} values of these preparations were similar. Hence, in both aqueous and propylene glycol systems, it appears that there are optimum amounts of Labrasol to improve the oral bioavailability of glycyrrhizin.

Glycyrrhizin has been used over the past 20 years in the treatment of chronic hepatitis in Japan. Intravenous glycyrrhizin has also been used for allergic diseases, mainly in the dermatological field (Van Rossum et al 1998). Glycyrrhizin has been also used in Europe and China for the treatment of gastritis, especially chronic gastritis (Van Rossum et al 1998). Oral therapy is not recommended, because of low bioavailability of glycyrrhizin. Instead, intravenous therapy has been used. However, Fujioka et al (1992) developed a glycyrrhizin suppository and nonparenteral therapy was being tested by his group. According to their clinical pharmacological studies, both the serum glutamic oxaloacetic transaminase and glutamic pyruvic transaminase levels decreased after 200-mg chronic rectal dosing with glycyrrhizin. Although the therapeutic window of glycyrrhizin has not been established yet, a relatively higher plasma level of around 3–5 μg mL^{-1} may be needed for the treatment of hepatitis (Dr Fujioka, personal communication). To determine the bioavailability of glycyrrhizin itself, we administered 200 mg of glycyrrhizin in plain gelatin capsules to dogs. However, insufficient glycyrrhizin concentrations were obtained in the dog plasma for 24 h, because of rapid hydrolysis in both the stomach and intestine. Therefore, in this study, solubilizers like propylene glycol, Polysorbate 80 and

Table 3 Pharmacokinetic parameters of glycyrrhizin from the test preparations in this study.

Preparation	T_i (h)	C_{max} (μg mL^{-1})	T_{max} (h)	$AUC_{(0-24)}$ (μg h mL^{-1})	MRT (h)	BA (%)
1	3.33±1.76	1.06±0.58	4.33±2.33	14.18±8.05	8.43±4.44	0.6
2	2.67±0.33	6.47±3.12	6.00±1.00	80.27±36.46	6.54±3.32	4.0
3	4.66±0.33	3.20±0.79	5.67±1.45	42.54±8.47	8.28±4.16	2.1
4	4.00±0	8.37±0.92	5.67±1.20	92.35±10.36	11.42±0.39	4.6
5	4.66±0.67	9.66±8.68	4.67±0.67	87.50±30.75	6.01±3.32	4.3
6	4.25±0.25	0.67±0.14	8.50±1.71	7.16±1.84	11.45±1.77	0.4
7	3.20±0.20	5.84±1.65	5.00±1.51	76.42±18.29	11.97±0.74	3.8
8	4.00±0	7.12±0.39	4.33±0.33	75.81±11.67	11.53±0.79	3.7

T_i, time when glycyrrhizin first appeared in the systemic circulation after oral administration in pressure-controlled colon delivery capsules; C_{max}, peak plasma glycyrrhizin concentration; T_{max}, time when peak plasma glycyrrhizin appeared; BA, bioavailability.

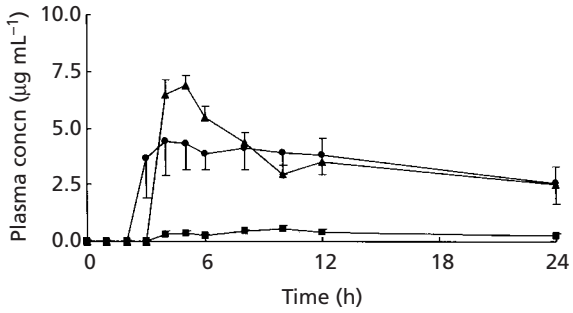

Figure 3 Plasma glycyrrhizin concentration-vs-time profiles after oral administrations of pressure-controlled colon delivery capsules containing glycyrrhizin solutions to dogs. ■, Preparation no. 6; ▲, preparation no. 7; ●, preparation no. 8. The dose of glycyrrhizin was 200 mg. Results are expressed as the mean±s.e. of three or four experiments.

Labrasol were used to prepare PCDCs containing the glycyrrhizin. Labrasol is a saturated polyglycolysed C_8–C_{10} glyceride and has been used as a main component of self-emulsifying drug delivery systems (SEDDS) for DMP 323, a poorly water-soluble HIV-1 protease inhibitor, to improve its bioavailability (Aungst et al 1997). A typical SEDDS formulation is composed of an appropriate liquid, surfactant and co-surfactant. Ideally, these formulations facilitate the subsequent assimilation of drug to relevant bile salt micellar structures, which would be expected to improve the extent and consistency of drug absorption (Constantinides 1995). With respect to this point, further studies must be performed. The advantage of our PCDC is that glycyrrhizin can be formulated as a liquid preparation. After glycyrrhizin is delivered to the colon by PCDC, glycyrrhizin solution is squeezed from the PCDC by colon luminal pressure, resulting in a high concentration. Therefore, the hydrolytic enzyme activity of the intestinal flora is thought to be saturated, and a considerable amount of glycyrrhizin absorbed into the systemic circulation.

Recently Hebden et al (1999) reported that the most important factor determining colonic drug absorption was the water content of the stool. We also reported that the bioavailability of poorly-water-soluble drugs was well correlated with the solubility of drugs in the colon (Yoshikawa et al 1999). Therefore, glycyrrhizin was formulated in PCDCs as a solution. As the free form of glycyrrhizin is not soluble in water, the bioavailability of glycyrrhizin tablet is low (Wang et al 1995; Takeda et al 1996). Yamamura et al (1995) administered glycyrrhizin through an intraperitoneal route and obtained a dramatically increased bioavailability of glycyrrhizin. On the other hand, Fujioka et al (1992) succeeded in increasing the bioavailability of glycyrrhizin by formulating glycyrrhizin disodium salt into a suppository. Therefore, we used glycyrrhizin disodium salt in this study. By formulating glycyrrhizin disodium salt as a solution with Labrasol in PCDCs, the bioavailability of glycyrrhizin was greatly increased. As the hydrophile–lipophile balance of Labrasol is 14, it should act as a surfactant and was thought to enhance the absorption of glycyrrhizin, because surfactants have been used as an absorption enhancer. The precise absorption-enhancing mechanism on Labrasol is now under investigation using an in-vitro membrane permeability system.

In summary, the bioavailability of glycyrrhizin was improved by formulating glycyrrhizin disodium salt as a solution in colon delivery capsules. In particular, when Labrasol was added to both aqueous and non-aqueous glycyrrhizin solutions, the bioavailability was considerably increased. After oral administration of 200 mg of

glycyrrhizin disodium salt in PCDCs to beagle dogs, the peak glycyrrhizin levels were around 7.0 μg mL^{-1}, which is equivalent to the plasma glycyrrhizin levels in hepatitis patients given the same dose of glycyrrhizin rectally. These results strongly support the feasibility of oral glycyrrhizin therapy for chronic hepatitis patients.

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