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Research Article

Ginsenoside Compound K Promotes Intestinal Peristalsis and the Pharmacokinetic of Metabolite 20(S)-Protopanaxadiol in Relation to Diarrhea

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Ginsenoside compound K (G-CK) is a rare ginsenoside originating from the traditional herbal medicine ginseng. Recently, G-CK has been found to cause diarrhea in preclinical researches as a candidate drug. This study is aimed at the potential mechanism of G-CK-induced diarrhea. In this study, we found that the treatment of G-CK significantly increased the peristaltic index (PI) with the intragastric administration of charcoal meal suspension at 90 minutes (not 30 min) after the administration of G-CK and had a clear role in promoting defecation. The Ach and 5-HT levels in colon tissue were not affected by G-CK. Additionally, the clinical trial revealed that subjects with diarrhea had lower exposure and higher Vz/*F* of 20(S)-protopanaxadiol (PPD) than nondiarrhea subjects, and there were no statistical differences in the pharmacokinetic parameters of G-CK between diarrhea and nondiarrhea subjects. We therefore concluded that the increased intestinal peristalsis and metabolite 20(S)-PPD were involved in G-CK-induced diarrhea.

1. Introduction

Ginsenoside compound K (G-CK) is a product of the degradation of natural protopanaxadiol- (PPD-) type ginsenosides, including Rb1, Rb2, and Rc [1]. It can be further biotransformed into 20(S)-PPD [2]. G-CK has been identified as an active substance in ginseng with multiple beneficial pharmacological properties [3–5] but regrettably has not been utilized as a clinical medication since its discovery in 1972 [6]. In view of the fact that G-CK exhibits satisfactory anti-inflammatory activity [7], Hisun (Hisun Pharmaceutical Co., Ltd., Taizhou, China) has submitted an Investigational New Drug Application (INDA) to the China Food and Drug Administration (CFDA) and aims to develop G-CK as an antirheumatoid arthritis (RA) candidate drug. Ginseng is a traditional herbal medicine, extensively used in Asia for its beneficial effects [8]. But diarrhea is identified as a common side effect of ginseng [9], with the mechanism remaining unclear. In the preclinical safety evaluation and small-scale clinical trial, we found that G-CK treatment leads to soft stool and loose stool. However, the mechanism of G-CK-induced diarrhea has never been reported.

Drug-induced diarrhea (DID) is a common adverse drug reaction, accounting for about 7% of all adverse drug reactions [10]. Diarrhea can affect the normal physiological function of the intestinal tract, thereby reducing the absorption of drugs and nutrients. It can also cause extreme discomfort to patients and greatly reduce their medication

compliance. Therefore, it is necessary to investigate the mechanism behind diarrhea for candidate drugs and to solve it effectively.

Several potentially overlapping mechanisms have been hypothesized to cause DID. The pathophysiology of DID can be described as involving the following mechanisms: osmotic, inflammatory, motility, and secretory [11].

The cystic fibrosis transmembrane regulator (CFTR) has been reported to be closely related to the pathogenesis of diarrhea. The overactivation of CFTR leads to excessive secretion of fluid from the intestinal wall into the enteral cavity, promoting intestinal peristalsis [12]. In addition, the neurotransmitters such as acetylcholine (Ach) and serotonin (5-HT) act on the smooth muscle of the gastrointestinal tract to promote gastrointestinal motility and contribute to diarrhea finally [13]. We observed that G-CK did not make the intestines in a hypertonic state (Figure S1) in our preliminary experiment. Both results from Liu et al. and our early study suggested that G-CK and 20(S)-PPD may interact with CFTR [14, 15]. Additionally, multiple studies have indicated that ginsenoside Rb1 can facilitate the release of acetylcholine (Ach) from nerve terminals [16–18]. Ginsenoside Rb1 can be metabolized to G-CK, and 20(S)-PPD is the metabolite of G-CK [1]. Therefore, we made a hypothesis that the change of CFTR activity and gastrointestinal motility might be causes of diarrhea induced by G-CK.

To summarize, we selected Ach, 5-HT, and CFTR as the research targets to clarify the mechanism of G-CK-induced diarrhea, which is aimed at providing a theoretical basis for follow-up clinical trials and clinical applications of G-CK.

2. Materials and Methods

2.1. Materials. G-CK (98% purity) and Ginsenoside Compound K Tablet were obtained from Hisun Pharmaceutical Co., Ltd. (Taizhou, Zhejiang, China). The following kits were purchased from the cited commercial sources: Acetylcholine Assay Kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) and Mouse 5-Hydroxytryptamine (5-HT) ELISA Kit (CusAB, Wuhan, China).

2.2. Clinical Trial. Subjects included in the study were screened from two clinical trials under the approval (CDEL20130379) by the China Food and Drug Administration (CFDA). These trials were approved with No.14050 (single-dose trial) and No.14119 (food-effect trial) by the independent ethics committee of the Third Xiangya Hospital affiliated to Central South University and were in accordance with the Declaration of Helsinki and the International Conference of Harmonization guidelines for Good Clinical Practice. The registration numbers were ChiCTR-TRC-14004824 and ChiCTR-IPR-15005787 (http://www.chictr.org.cn/index.aspx). The design of these clinical trials and participant recruitment were described particularly in previous articles [15, 19, 20]. It is especially necessary to mention that all the enrolled subjects had no history of gastrointestinal diseases or gastrointestinal dysfunction.

Blood samples (5 mL) for pharmacokinetic (PK) analysis were collected from each participant. Plasma concentrations of G-CK and 20(S)-PPD were measured using mass spectrometry and liquid chromatography-tandem mass spectrometry (LC-MS/MS, API 4000, ABI Company), in both trials. The plasma samples were stored and analyzed at the chromatography laboratory, Institute of Clinical Pharmacology, Central South University (Changsha, China). Detailed introduction of the collection method of the blood sample and the detection method of plasma concentration were presented in the earlier articles [15, 19, 20].

All the PK parameters were assessed by the WinNonlin version 6.1 (Pharsight Corporation, Mountain View, CA, USA). The maximum concentrations (C_{max}), minimum plasma concentrations at steady state ($C_{min,ss}$), and time to maximum plasma concentration (t_{max}) could be obtained from the plasma concentration or plasma concentration-time data directly. The area under the plasma concentration-time curve is AUC and from time 0 to the last observation AUC_{last}. The AUC for dosing interval was expressed as AU C_τ, where τ is the dosing interval (24 h). The average steady-state drug concentration (C_{avg}) is calculated as AUC_τ/τ. The elimination rate constant (K) was determined by linear regression analysis of the log-linear part of the plasma concentration-time curve. The half-life ($t_{1/2}$) was calculated based on the elimination rate constant, as equal to $(\ln 2)/K$. The apparent clearance (CL/F) and apparent volume of distribution (Vz/F) were also obtained. Additionally, the dose-normalized (to 1 mg of CK) C_{max} (C_{max}/D), $C_{min,ss}$ ($C_{min,ss}/D$), C_{avg} (C_{avg}/D), AUC_{last} (AUC_{last}/D), and AUC_τ (AUC_τ/D) were calculated by dividing each PK result with the homologous dosage of G-CK.

2.3. Cell Experiment

2.3.1. Cell Line. The cell line used throughout this study consisted of FRT cells stably cotransfected with the EYFP-H148Q fluorescence protein and human wild-type CFTR cDNA. FRT cells were cultured in Nutrient F12 coon's medium (Sigma Chemical Co., St. Louis, MO, USA). The media were supplemented with 10% fetal bovine serum (HyClone company, USA), 500 U/ml penicillin, 500 U/ml streptomycin, and 2 mM L-glutamine. The cells were incubated in a 5% CO_2 incubator maintained at 37°C and 90% humidity for 36 hours.

2.3.2. Iodide Influx Fluorescent Assay. FRT cells transfected with CFTR were plated in a black 96-well plate with a clear bottom (Costar, Corning, NY, USA) at a density of 2×10^4 cells/well and incubated until confluent. The cells were washed three times with 300 μl PBS. After that, 50 μl PBS with 0.1 μM FSK and different concentrations of the test compound, G-CK (3.90 μM-1000 μM) or genistein (0.78 μM-200 μM), were added to each well. Ten minutes later, EYFP fluorescence data were recorded using a FLUOstar Galaxy microplate reader (BMG Lab Technologies, Inc.) equipped with HQ500/20X (500 ± 10 nm) excitation, HQ 535/30M (535 ± 15 nm) emission filters (Chroma Technology Corp.), and syringe pumps. Iodide influx rates ($d[I^-]/dt$) were computed as described by Kristidis et al. [21].

Table 1: Demographics of the study participants.

Sample	N^a	Nondiarrhea				Diarrhea			
		Age (years)	Height (m)	Weight (kg)	BMI ($kg \cdot m^{-2}$)	Age (years)	Height (m)	Weight (kg)	BMI ($kg \cdot m^{-2}$)
Sample 1	30	21 ± 3	1.64 ± 0.08	57.64 ± 9.35	21.08 ± 1.51	21 ± 2	1.62 ± 0.10	59.50 ± 10.10	22.59 ± 1.38
Sample 2	24	23 ± 3	1.65 ± 0.07	58.60 ± 7.22	21.53 ± 1.60	24 ± 4	1.68 ± 0.05	56.88 ± 5.33	20.45 ± 1.66
Sample 3	28	24 ± 3	1.66 ± 0.07	59.25 ± 6.73	21.37 ± 1.40	23 ± 3	1.62 ± 0.09	55.00 ± 7.00	20.94 ± 1.18

Sample 1 was pooled from three dose (100, 200, and 400 mg G-CK) groups of the single-dose trial; sample 2 was pooled from the food-effect trial (200 mg of G-CK under the fed condition); sample 3 was pooled from three dose (100, 200, and 400 mg G-CK) groups of the multiple-dose trial. All values are presented as the mean ± SD. SD: standard deviation; G-CK: ginsenoside compound K; BMI: body mass index. [a]Number of participants. Diarrhea *vs.* nondiarrhea: the independent sample *t*-test.

2.4. Animal Experiment

2.4.1. Animals. Imprinting Control Region strain male mice (weight 18-22 g, 6-8 weeks old) were supplied by the Hunan SJA Laboratory Animal Co. Ltd. (Changsha, China). The mice were housed at a temperature of 20-22°C and humidity of 70-75%, with a 12-hour light-dark cycle, and food and water administered *ad libitum*. All experimental protocols were endorsed by the Ethics Committee of Hunan Research Center for Drug Safety Evaluation and were in strict accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (NIH Publications No. 80-23, revised 1996). All possible efforts were done to ensure that the animals were comfortable.

2.4.2. Intestinal Transit. Intestinal transit in mice was measured using the charcoal meal test. The mice were assigned randomly to the following three groups: the vehicle control group received 0.5% CMC-Na suspension at 20 ml/kg body weight orally, while two treatment groups received G-CK at 50 and 250 mg/kg body weight orally, respectively. The test animals were fasted for 16 hours prior to the experiment but were allowed free access to water. These subsequently received a single dose of G-CK or vehicle by intragastric gavage. After 30 minutes or 90 minutes of the administration, 0.2 ml of charcoal meal suspension (5% charcoal in 0.5% CMC-Na) was given to each mouse by intragastric gavage. Thirty minutes later, the animals in each group were sacrificed, and the whole small intestines were isolated immediately. The peristaltic index (PI) was calculated for each mouse as the distance traveled by charcoal as a percentage of the total length of the small intestine (pyloric sphincter to caecum) [22].

2.4.3. Measurement of the Frequency of Defecation. In addition to the charcoal meal transit test, the frequency of defecation was also measured to investigate the impact of G-CK on intestinal peristalsis. The mice were included and randomly allotted to three groups: vehicle control group (0.5% CMC-Na, 20 ml/kg) and LCK (50 mg/kg G-CK) and HCK (250 mg/kg G-CK) treatment groups. After the single-dose administration, all animals were put into different cages (one mouse in each cage) with filter paper. The frequency of defecation was measured every hour until 6 h after the administration.

2.4.4. Single-Dose Treatment of G-CK on Mice. In the single-dose treatment experiment, thirty animals were randomly divided into three groups ($n = 10$): vehicle control (0.5% CMC-Na, 20 ml/kg), LCK, and HCK groups. After 16 hours of fasting, each animal was treated with a corresponding treatment of G-CK or vehicle. Three hours later, the animals were sacrificed for the colon tissues which were washed with ice-cold physiological saline and put into a 2 ml cryogenic vial to be stored in liquid nitrogen until use.

2.4.5. Measurement of Colonic Ach and 5-HT Concentrations. Concentrations of colonic Ach and 5-HT were detected using the Acetylcholine Assay Kit, Mouse CREB ELISA Kit, and Mouse 5-HT ELISA Kit, respectively, according to the manufacturers' manuals.

2.5. Statistical Analysis. Values of PK parameters were also represented as the mean ± standard deviation (SD), except for t_{max} which was expressed as the median (range). The independent sample *t*-test was applied on logarithmic transformed C_{max}/D, AUC_{last}/D, $t_{1/2}$, Vz/F, and CL/F, and nonparametric tests were performed on t_{max}, to determine whether there is a significant difference in the PK parameters between diarrhea and nondiarrhea subjects.

All results of the peristaltic index, defecation frequency, colonic Ach, and 5-HT concentrations in colon tissues were expressed as the mean ± standard deviation (SD), and one-way ANOVA was used to compare group differences, followed by Dunnett *t*-tests for multiple comparisons.

Data were analyzed using SPSS v.22.0 (SPSS Inc., USA). Values of $p < 0.05$ were considered statistically significant.

3. Results

3.1. The Relationship between Pharmacokinetics and Diarrhea. A total of 30 subjects from the single-dose trial (sample 1), 24 subjects from the food-effect trial (sample 2), and 28 subjects from the multiple-dose trial (sample 3) were included in this analysis based on the sample size and the evaluation of the dose proportionality [20]. Five, four, and thirteen subjects had diarrhea from the single-dose trial, food-effect trial, and multiple-dose trial, respectively. The baseline demographics including age, height, weight, and body mass index (BMI) are presented as the mean ± SD and provided in Table 1. There were no statistical differences

Table 2: The relationship between pharmacokinetics and diarrhea.

Parameters	Sample 1		Sample 2		Sample 3	
	Nondiarrhea ($n = 25$)	Diarrhea ($n = 5$)	Nondiarrhea ($n = 20$)	Diarrhea ($n = 4$)	Nondiarrhea ($n = 15$)	Diarrhea ($n = 13$)
G-CK						
C_{max}/D ($ng \cdot ml^{-1}$)	3.57 ± 2.45	3.51 ± 2.21	8.16 ± 3.08	6.30 ± 1.51	24.15 ± 11.71	16.18 ± 7.38
$C_{min,ss}/D$ ($ng \cdot ml^{-1}$)	—	—	—	—	7.79 ± 3.79	5.38 ± 2.10
C_{avg}/D ($ng \cdot ml^{-1}$)	—	—	—	—	1.54 ± 0.68	1.19 ± 0.42
AUC_{last}/D ($h \cdot ng \cdot ml^{-1}$)	29.43 ± 23.40	28.22 ± 17.03	63.33 ± 20.91	61.32 ± 21.12	239.48 ± 116.80	168.36 ± 62.58
AUC_{τ}/D ($h \cdot ng \cdot ml^{-1}$)	—	—	—	—	187.04 ± 90.93	129.19 ± 50.37
Vz/F (l)	1434.96 ± 637.65	1200.00 ± 588.26	650.37 ± 406.46	661.29 ± 262.49	572.59 ± 202.16	510.41 ± 232.53
CL/F ($l \cdot h^{-1}$)	47.82 ± 24.23	43.08 ± 18.34	18.33 ± 10.49	17.49 ± 6.18	11.97 ± 4.15	9.89 ± 2.55
$t_{1/2}$ (h)	22.21 ± 4.54	20.60 ± 6.88	24.51 ± 2.71	26.08 ± 4.58	34.49 ± 9.66	34.86 ± 11.03
t_{max} (h)	3.00 (2.00-6.00)	3.00 (2.50-6.00)	2.25 (1.50-5.00)	2.50 (1.50-3.50)	3.50 (1.50-5.00)	3.50 (1.50-5.00)
20(S)-PPD						
C_{max}/D ($ng \cdot ml^{-1}$)[a]	0.035 ± 0.035	0.008 ± 0.003**	0.021 ± 0.015	0.003 ± 0.002**	0.048 ± 0.030	0.024 ± 0.021*
$C_{min,ss}/D$ ($ng \cdot ml^{-1}$)[a]	—	—	—	—	0.020 ± 0.016	0.009 ± 0.010*
C_{avg}/D ($ng \cdot ml^{-1}$)[a]	—	—	—	—	0.033 ± 0.022	0.014 ± 0.013**
AUC_{last}/D ($h \cdot ng \cdot ml^{-1}$)	0.99 ± 0.97	0.25 ± 0.15	0.63 ± 0.48	0.10 ± 0.05*	1.58 ± 1.04	0.80 ± 0.94*
AUC_{τ}/D ($h \cdot ng \cdot ml^{-1}$)	—	—	—	—	0.79 ± 0.54	0.34 ± 0.30**
Vz/F (l)	79354.83 ± 113809.94	121513.00 ± 9200.87[b]	53043.11 ± 48210.92	—[c]	136169.43 ± 361901.90	373907.03 ± 566133.57**
CL/F ($l \cdot h^{-1}$)	2546.82 ± 3692.48	2509.85 ± 626.72[b]	1558.00 ± 793.29	—[c]	2356.27 ± 2894.14	5736.78 ± 4136.67**
$t_{1/2}$ (h)	37.29 ± 90.42	34.30 ± 6.08	21.24 ± 9.74	—[c]	22.33 ± 17.07	43.38 ± 43.45
t_{max} (h)	24.00 (5.00-48.00)	24.00 (12.00-36.00)	24.00 (24.00-36.00)	24.00 (24.00-24.00)	6.00 (0.25-24.00)	6.00 (0.25-24.00)

C_{max}/D: the maximum plasma concentration normalized to doses of G-CK; $C_{min,ss}/D$: the minimum concentrations normalized to doses of G-CK; C_{avg}/D: the average concentration normalized to doses of G-CK; AUC_{last}/D: the area under the plasma concentration-time curve from zero to the time of the last quantifiable concentration normalized to doses of G-CK; AUC_{τ}/D: the area under the plasma concentration-time curve from zero to 24 h normalized to doses of G-CK; Vz/F: apparent volume of distribution after extravascular administration; CL/F: the apparent plasma clearance of the drug after extravascular administration; $t_{1/2}$: terminal half-life; t_{max}: time to maximum plasma concentration. Sample 1 was pooled from three dose (100, 200, and 400 mg G-CK) groups of the single-dose trial; sample 2.

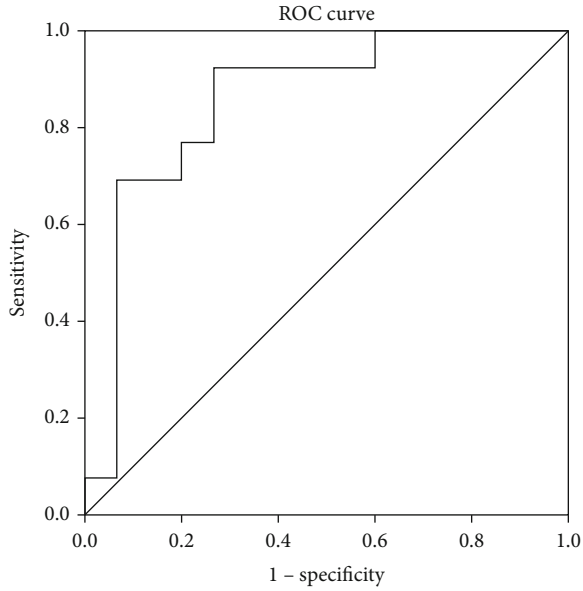

Figure 1: The ROC curve for Vz/F of 20(S)-PPD. The areas under the ROC curve for Vz/F was 0.856 (SE 0.075, 95% C.I. 0.709-1.000, $p = 0.001$). Vz/F: apparent volume of distribution after extravascular administration; 20(S)-PPD: 20(S)-protopanoxadiol; SE: standard error; C.I.: confidence intervals of the areas under the ROC curve.

in age, height, weight, or BMI between diarrhea and nondiarrhea subjects in sample 1, sample 2, or sample 3.

The PK parameters of G-CK and 20(S)-PPD in diarrhea and nondiarrhea subjects are summarized in Table 2. The PK parameters including C_{max}/D, $C_{min,ss}/D$, C_{avg}/D, AUC_{last}/D, AUC_{τ}/D, Vz/F, CL/F, $t_{1/2}$, and t_{max} were used in these comparative analyses. The results showed that there were no statistical differences in all the PK parameters of G-CK analyzed in this study and $t_{1/2}$ and t_{max} of 20(S)-PPD between diarrhea and nondiarrhea subjects (Table 1). Caught by surprise, Vz/F and CL/F in diarrhea subjects were significantly higher than that of nondiarrhea subjects. In diarrhea subjects, the exposure of 20(S)-PPD characterised by the values of C_{max}/D, $C_{min,ss}/D$, C_{avg}/D, AUC_{last}/D, and AUC_{τ}/D were obviously lower than that of nondiarrhea subjects. In a binary logistic regression model, only the Vz/F (beta = 1.120, $p = 0.015$) showed a significant correlation with diarrhea in sample 3. The ROC curves of Vz/F are shown in Figure 1. The areas under the ROC curve for Vz/F in sample 3 were 0.856 (SE 0.075, 95% C.I. 0.709-1.000, $p = 0.001$). The cut-off value was defined as the corresponding value of the parameter, when the value of the sensitivity minus (1-specificity) was maxima. The sensitivity and specificity for the cut-off point of Vz/$F \geq 56980.31$ l were 92.31% and 73.33%, respectively.

3.2. Effects of G-CK on CFTR Chloride Channel Activity. The effect of G-CK on CFTR chloride channel activity was evaluated using a cell-based fluorescence assay. Genistein was used as a positive control and PBS as a negative control. The Fischer rat thyroid epithelial (FRT) cells were incubated with different concentrations of G-CK for 10 min, and I^- was then pumped into each well in the presence of 0.1 μM forskolin (FSK). The EC_{50} of G-CK was 224.7 μM, which suggested that G-CK has a negligible effect on CFTR chloride channel activity (Figure 2).

3.3. Effects of G-CK on Peristaltic Index and Defecation Frequency. To investigate the effects of G-CK on the PI in mice, 0.2 ml of charcoal meal suspension (5% charcoal in 0.5% CMC-Na) was administered to each animal 30 or 90 minutes after treatment with G-CK. The results revealed that 90 minutes after the administration of G-CK, both low dosage (LCK; 50 mg/kg G-CK) and high dosage (HCK; 250 mg/kg G-CK) observably stimulated intestinal transit in mice when compared to the control group (Figure 3). This phenomenon had not been observed under the condition that charcoal meal suspension was given 30 minutes after the administration of G-CK. HCK significantly increased the frequency of defecation compared with the control and LCK groups every hour from 1 h to 4 h after treatment (Figure 3). We also observed that treatment with G-CK caused the mice to produce soft stools and watery stools; the latter was observed only in one mouse from the HCK group.

3.4. Effects of G-CK on Ach and 5-HT Levels in Colon Tissues. For the exact facilitating role of G-CK in gastrointestinal motility, the detection of the neurotransmitter level was preferred. To examine the potential contribution of Ach and 5-HT to G-CK-induced diarrhea, we used the corresponding test kits to evaluate their concentrations in colon tissues. These results indicated that LCK and HCK had no effect on the levels of Ach and 5-HT. All of the above results are presented in Figure 4.

4. Discussion

Ginseng is generally well tolerated in adults and is "generally recognized as safe" by the U.S. Food and Drug Administration. Diarrhea is a common side effect of ginseng and G-CK. In this study, it was proved that G-CK did induce diarrhea both in healthy volunteers and mice, and we first investigated the potential mechanism of diarrhea induced by G-CK. The outline of this study is shown in Figure 5.

Results from clinical trials indicated that G-CK caused diarrhea [19, 20]. It has been reported that some substrates or inhibitors of multidrug resistance-associated protein 4 (MRP4) could activate CFTR-mediated chloride flow by inhibiting MRP4-mediated cAMP efflux [12, 23]. Overactivation of the CFTR channel leads to excessive secretion of fluid from the intestinal wall into the enteral cavity, resulting in secretory diarrhea [12]. In our previous research, we found that G-CK might be the substrate of multidrug resistance protein 4 (MRP4) [15]. In addition, the effect of 20(S)-PPD (the metabolite of G-CK) on CFTR activity has been reported in the literature [14]. Therefore, we analyzed the correlation between diarrhea and pharmacokinetic parameters of G-CK and 20(S)-PPD. Results in the present study indicated that there was no correlation between pharmacokinetic parameters of G-CK and diarrhea, and the diarrhea subjects had a

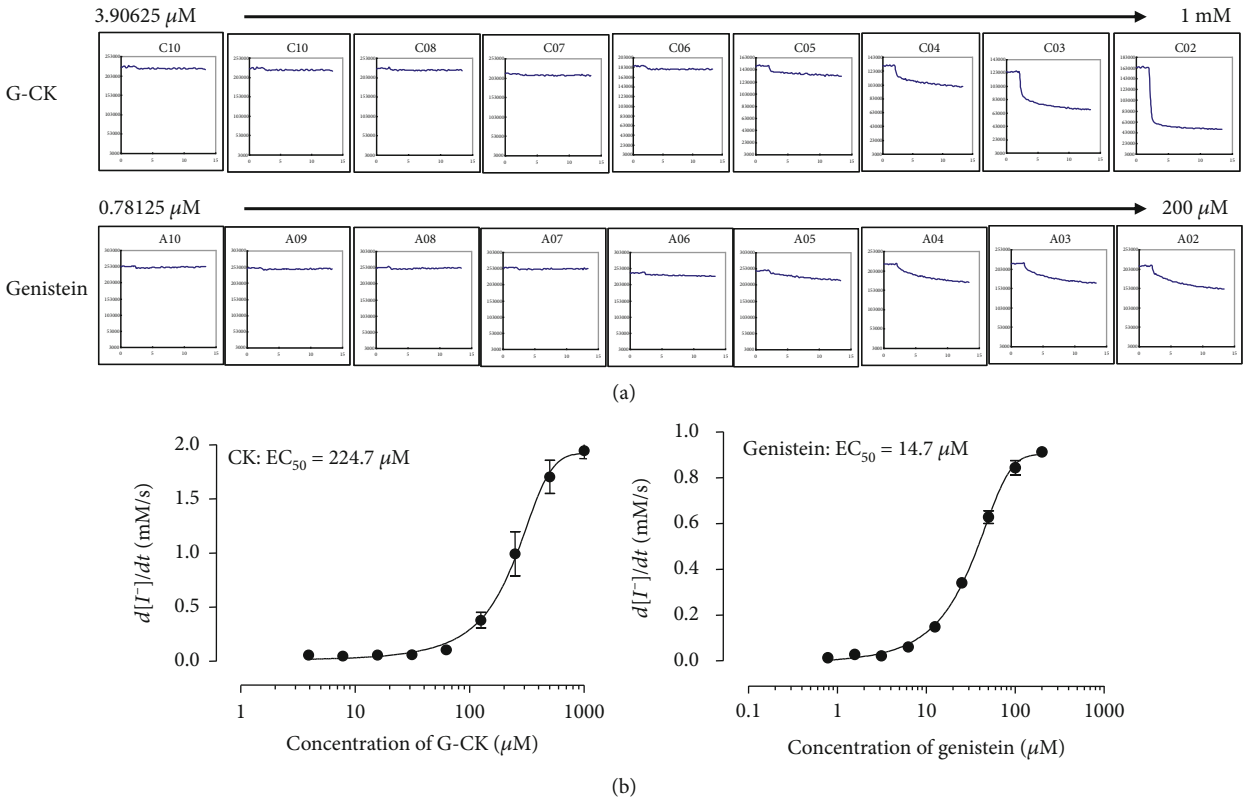

Figure 2: Effects of G-CK on CFTR chloride channel activity: (a) concentration-response curve for G-CK; (b) concentration-response curve for the genistein positive control. G-CK: ginsenoside compound K; CFTR: cystic fibrosis transmembrane conductance regulator.

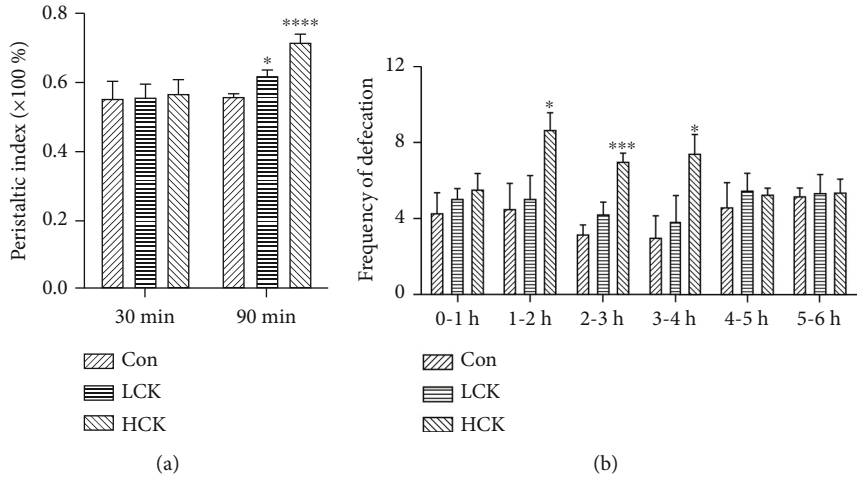

Figure 3: Effects of G-CK on the peristaltic index and frequency of defecation in mice. (a) Effects of G-CK on the peristaltic index when the charcoal meal suspension was given at 30 minutes and 90 minutes after the administration of G-CK; (b) effects of G-CK on the frequency of defecation per hour. Con: 0.5% CMC-Na; LCK: low dose of G-CK (50 mg/kg G-CK); HCK: high dose of G-CK (250 mg/kg G-CK). Values are expressed as the mean ± SD, $n = 10$ per group. SD: standard deviation. $^{*}p < 0.05$, $^{***}p < 0.001$, and $^{****}p < 0.0001$ *vs.* control group (one-way ANOVA followed by Dunnett *t*-tests).

higher Vz/*F* and lower exposure of 20(S)-PPD than nondiarrhea subjects. ROC analysis showed that the high Vz/*F* of 20(S)-protopanaxadiol (PPD) predicted diarrhea in healthy volunteers. Some studies showed that G-CK and 20(S)-PPD in the circulation were mostly excreted into the bile [24, 25]; the higher Vz/*F* in diarrhea subjects suggested a possibility that there were more 20(S)-PPD enriched in the liver and excreted into the bile. Thus, we suspected that G-CK might not affect the activity of CFTR. In order to verify this hypothesis, we explored the impact of G-CK on the CFTR activity *in vitro*. The result indicated that G-CK did not affect the CFTR activity.

Then, we put forward another hypothesis; diarrhea caused by G-CK cannot be separated from absorption; thus,

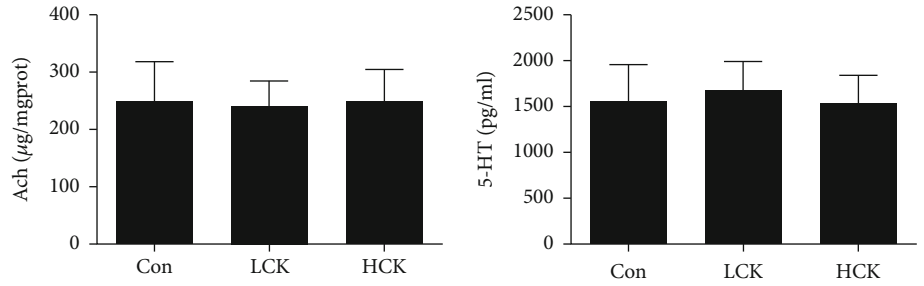

Figure 4: Effects of G-CK on Ach and 5-HT levels in colon tissues. Con: 0.5% CMC-Na; LCK: low dose of G-CK (50 mg/kg G-CK); HCK: high dose of G-CK (250 mg/kg G-CK). Values are expressed as the mean ± SD, $n = 10$ per group. SD: standard deviation (one-way ANOVA followed by Dunnett t-tests).

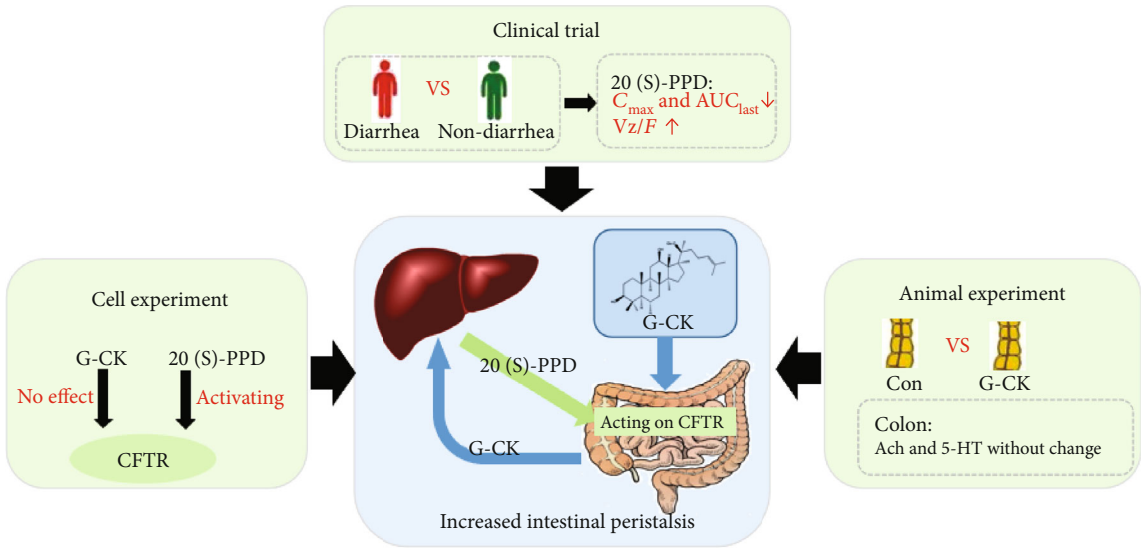

Figure 5: Outline of the present study. G-CK: ginsenoside compound K; 20(S)-PPD: 20(S)-protopanoxadiol; CFTR: cystic fibrosis transmembrane conductance regulator.

the effect of G-CK on the peristaltic index and frequency of defecation were detected. The charcoal meal transit test and the frequency of defecation are classical methods used to assess gastrointestinal motility [26–29]. In this study, the charcoal meal was administered at thirty minutes and 90 minutes (for t_{max} of G-CK is 1.5 h to 3 h in mice) after the G-CK treatment. This test manifested that the charcoal meal forward was urged with the pretreatment of G-CK for 90 min, but not 30 min. In addition, HCK obviously boosted the frequency of defecation in 1-4 h and did not affect that of 0-1 h. This phenomenon further suggested that the absorption was the basis for G-CK to cause diarrhea.

Except CFTR, we also investigated the role of Ach and 5-HT in diarrhea of G-CK. Ach and 5-HT are important neurotransmitters, which play a critical role in the stimulation of the gastrointestinal smooth muscle. Ach is released into the synaptic gap and causes various physiological changes, when the nerve impulse reaches the nerve endings and causes presynaptic membrane depolarization. Once separated from the receptor, Ach is rapidly hydrolyzed by acetylcholinesterase into choline and acetic acid. 90% of 5-HT molecules in the body are synthesized, secreted, and exerted by enterochromaffin cells (EC) in the intestine. In the present study, the G-CK did not disturb the content of Ach and 5-HT in the colon tissues.

5. Conclusions

In this study, it was proved that G-CK did induce diarrhea in the animal model and healthy volunteers, and we first investigated the potential mechanism of diarrhea induced by G-CK. The main results include a clear correlation between higher Vz/F of 20(S)-PPD and diarrhea, no activation of G-CK on CFTR, time-dependent promotional effect of G-CK on PI, and no effect of G-CK on colonic Ach and 5-HT contents, which suggested that the metabolite 20(S)-PPD was the critical factor of diarrhea induced by G-CK. Further studies are needed to elucidate the precise relationship between 20(S)-PPD and diarrhea elicited by G-CK treatment.

Abbreviations

5-HT: Serotonin
Ach: Acetylcholine

AUC: The area under the plasma concentration-time curve
AUC_{last}: The area under the plasma concentration-time curve from time 0 to the last observation
BMI: Body mass index
CFDA: China Food and Drug Administration
CFTR: Cystic fibrosis transmembrane conductance regulator
C_{max}: Maximum concentrations
CL/*F*: The apparent clearance
DID: Drug-induced diarrhea
EC: Enterochromaffin cell
FSK: Forskolin
FRT: Fischer rat thyroid epithelial
G-CK: Ginsenoside compound K
HCK: High dose of G-CK
INDA: Investigational New Drug Application
LCK: Low dose of G-CK
MRP4: Multidrug resistance protein 4
PK: Pharmacokinetic
PPD: Protopanaxadiol
PI: Peristaltic index
PVDF: Polyvinylidene difluoride
RA: Rheumatoid arthritis
t_{max}: Time to maximum plasma concentration
Vz/*F*: The apparent volume of distribution.

Data Availability

As G-CK is a drug candidate, detailed preclinical and clinical data are temporarily classified and not suitable for public disclosure.

Conflicts of Interest

The authors confirm that there are no conflicts of interest.

Authors' Contributions

LC and LZ were responsible for the study design, data collection, statistical analysis, and manuscript writing. XZ helped to carry out animal experiments. DO, GY, and JL were involved in the implementation of trials. ZT participated in the chromatographic analysis. DO and ZL guided the design and implementation of the whole research. All authors reviewed the results and approved the final version of the manuscript.

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Supplementary Materials

Figure S1: effect of G-CK on the intestinal osmotic pressure: A: control (physiologic saline); B: magnesium sulfate; C: 0.5% CMC-Na; D: 400 mg/kg G-CK. G-CK: ginsenoside compound K. Compared with the control group, there was a hypertonic state in the intestinal cavity of the magnesium sulfate group. The intestinal osmotic pressure of the 0.5% CMC-Na and G-CK groups was normal. *(Supplementary Materials)*

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