Effect of Gingerol on Cisplatin-Induced Pica Analogous to Emesis Via Modulating Expressions of Dopamine 2 Receptor, Dopamine Transporter and Tyrosine Hydroxylase in the Vomiting Model of Rats

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ABSTRACT

Background Gingerol, the generic term for pungent constituents in ginger, has been used for treating vomiting in China. We are going to investigate the mechanisms of inhibitive effect of gingerol on cisplatin-induced pica behaviour by studying on both peripheral and central levels, and the effects of gingerol on homeostasis of dopamine (DA) transmission: dopamine D2 receptor (D2R), dopamine transporter (DAT) and tyrosine hydroxylase (TH).

Methods The antiemetic effect of gingerol was investigated on a vomiting model in rats induced by cisplatin 3 mg·kg^{-1} intraperitoneal injection (i.p.). Rats were randomly divided into the normal control group (C), simple gingerol control group (CG), cisplatin control group (V), cisplatin + metoclopramide group (M), cisplatin + low-dose gingerol group (GL), cisplatin + middle-dose gingerol group (GM) and cisplatin + high-dose gingerol group (GH). In observation period, rats in Groups C and V were pretreated with sterile saline 3 mL i.g.; rats in Group CG were pretreated with gingerol 40 mg·kg^{-1} i.g.; rats in Group M were pretreated with metoclopramide 2.5 mg·kg^{-1} i.g.; rats in Groups GL, GM and GH were pretreated with gingerol 10, 20 and 40 mg·kg^{-1} i.g. for 3 days, respectively. Cisplatin (3 mg·kg^{-1}, i.p.) was administered one time after each treatment with the antiemetic agent or its vehicle except the Groups C and CG. The distribution of D2R, DAT and TH in the area postrema and ileum were measured by immunohistochemistry and quantitated based on the image analysis, and the expression of DAT and TH in the area postrema and ileum were measured by RT-PCR. The weights of kaolin eaten of the remaining rats were observed in every 6 h continuously for 72 h.

Results The weight of kaolin eaten in rats induced by cisplatin was significantly reduced by pretreatment with gingerol in a dose-dependent manner during the 0–24 h and 24–72 h periods ($P < 0.05$). Gingerol markedly improved gastric emptying induced by cisplatin in a dose-dependent manner ($P < 0.05$), and exhibited effective dose-dependent inhibition on the increase of expression levels of D2R and TH and the decrease of expression levels of DAT in both the ileum and area postrema ($P < 0.05$).

Conclusion Gingerol is effective on cisplatin-induced emesis in rats possibly by inhibiting central or peripheral increase of DA by inhibiting D2R, TH and accelerating DAT.

Key words dopamine 2 receptor; dopamine transport; gingerol; tyrosine hydroxylase; vomiting

It is generally accepted that nausea and vomiting (emesis) are components of protective mechanism by which the human body defends itself against ingested toxins.[1] However, chemotherapy-induced nausea and vomiting (CINV) is a distressing and common adverse event associated with cancer treatment[2] that causes extreme discomfort and seriously impairs quality of patients' life.[3] Despite significant progress in the management of nausea and vomiting associated with chemotherapy, these side effects continue to limit the effectiveness of cancer therapy and weaken the quality of life of its victims.[4] Cisplatin is one of the most effective and currently available chemotherapy agents. But it is also one of the most

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Received 2016 January 15
Accepted 2016 February 5
Abbreviations: 5-HT$_3$, 5-hydroxytryptamine 3; C, normal control group; CG, simple gingerol control group; CINV, chemotherapy-induced nausea and vomiting; D2R, dopamine D2 receptor; DA, dopamine; DAT, dopamine transporter; GH, cisplatin + high-dose gingerol group; GL, cisplatin + low-dose gingerol group; GM, cisplatin + middle-dose gingerol group; i.g., intragastrically; i.p., intraperitoneal injection; M, cisplatin + metoclopramide group; SP, substance P; TH, tyrosine hydroxylase; V, cisplatin control group

reasons on both acute and delayed emesis if anti-emetics are not given.[5, 6] A lot of evidences are proved that cisplatin can lead many adverse events such as CINV, nephrotoxicity and peripheral neuropathy.[7] Therefore, the control of nausea and vomiting is critical for the patient's quality of life and perception of chemotherapy.

Vomiting is caused by noxious stimulation directly or indirectly on the vomiting center such as gastrointestinal tract, vestibular system, chemoreceptor trigger zone, and higher centers in the cortex and thalamus. The nerve impulse takes place when receptors are activated, and transfer to the vomiting center, so emesis is initiated.[8, 9] The chemotherapy-induced emesis is developed via peripheral and central systems, which are independent relatively, and many neurotransmitters have been implicated in the pathogenesis of vomiting.[10, 11] Biochemical signaling of emesis is much more diverse than previously believed.[12] More recently, dopamine (DA) is believed to be a major player in the motivational aspects of nausea and vomiting. The homeostasis of DA transmission is mainly controlled by three regulatory elements: dopamine D2 receptor (D2R), DA transporter (DAT) and tyrosine hydroxylase (TH). D2R, one receptor of DA, is known to activate the chemoreceptor trigger zone in the area postrema of the fourth ventricle floor. This results in efferent impulses to the medullary vomiting centre, which initiates vomiting.[13, 14] DAT, a Na^+/Cl^- dependent plasma membrane protein at nerve endings, can rapidly reuptake DA, and determine the intensity and duration of DA transmission.[15] TH, the rate-limiting enzyme for DA synthesis, participates in the regulation of intra- and extracellular DA levels.[16, 17] However, studies of D2R, DAT and TH were mainly concentrated in Parkinson's disease, obesity and so on.[18–20] And researches of D2R, DAT and TH on vomiting have not been involved.

Rats do not vomit. Instead, anticancer drugs induce kaolin ingestion behavior "pica" in rats, and it is evaluated as an index of nausea/vomiting.[21] Yamamoto et al.[22] have reported that kaolin intake induced by anticancer drugs in rats is related to their clinical emetogenic potential. In the present study, we investigated the effect of gingerol on cisplatin-induced kaolin intake, which is an index of nausea/vomiting in the rat.

Ginger, a classical traditional Chinese herbal medicine known as Zingiber officinale Rosce, has been used for treating vomiting for 2000 years in China. It is a common food for cooking for years in East Asia. Some evidences have proved that ginger has effect on vomiting, post-operative nausea and vomiting and CINV.[23–25] Studies with experimental animals (dogs and rats) have shown that the various extracts of ginger and the ginger juice possess anti-emetic effects against CINV. Gingerol is the generic term for pungent constituents in ginger.[26] It has been reported to inhibit contraction of isolated guinea-pig ileum by acting on 5-hydroxytryptamine 3 (5-HT_3) receptors and this is presumed to be related to its antiememtic activity.[27] During our previous research, we have already proved that gingerol has effects on vomiting induced by cisplatin in minks, as well as prevented the high expressions of DA.[28, 29] But, the antiemetic mechanism of gingerol remains unclear, and there are few reports on the study of the homeostasis of DA transmission. Based on our previous studies above, we have guessed that gingerol can adjust the abnormal expression of DA in vomiting by inhibiting synthesis or accelerating degradation of DA.

Therefore, in this study, we are going to investigate the mechanisms of inhibitive effect of gingerol on cisplatin-induced pica behaviour by studying on both peripheral and central levels, and the effects of gingerol on homeostasis of DA transmission: D2R, DAT and TH.

MATERIALS AND METHODS

Animals

Adult male Wistar rats weighing 200 ± 20 g were purchased from Shandong University Laboratory Animal Shelter (Jinan, China). Rats were housed in individual cages under controlled environmental conditions (22 ± 2 °C relative humidity 40–60%, 12 h dark/light cycles, food and water ad libitum). Animals were used in the present study in accordance with the guidelines of the Shandong University Institutional Animal Care and Use Committee. All studies were performed with approval from the committee (2014012-KY).

Reagents

Gingerol (Xi'an Realin Biotechnology, Xi'an, China) was dissolved in 1% tragacanth. Cisplatin (Qilu Pharmaceutical, Jinan, China) was prepared in normal saline at 70 °C followed by gradual cooling to 40 °C and was administered immediately. Metoclopramide (Qilu Pharmaceutical, Jinan, China). Anti-D2R, anti-DAT, anti-TH, and goat anti-rabbit antibody were obtained from Beijing Bioss Biological Technology, Beijing, China.

Kaolin was prepared according to the method of the literature,[30] with minor modifications. Kaolin (Tianjin Kemiou Chemical Reagent, Tianjin, China) was mixed with 7% gum arabic (Tianjin Guangfu Fine Chemical Research Institute, Tianjin, China) in distilled water to form a thick paste. The mixture was placed in a tube and partially dried in a dryer. The mixture was extruded from the tube, cut into a column of the same size as that for normal feed pellets, and dried completely in a dryer.

Animal experiments

105 rats were randomly divided into the following 7 groups: normal control group (C, n = 15), simple gingerol control group (CG, n = 15), cisplatin control group (V, n = 15), cisplatin + metoclopramide group (M, n = 15), cisplatin+ low-dose gingerol group (GL, n = 15), cisplatin+ middle-dose gingerol group (GM, n = 15) and cisplatin + high-dose gingerol group (GH, n = 15). In observation period, rats in Groups C and V were pretreated with sterile saline 3 mL i.g.; rats in Group CG were pretreated with gingerol 40 mg·kg^{-1} i.g.; rats in Group M were pretreated with metoclopramide 2.5 mg·kg^{-1} i.g.; rats in Groups GL, GM and GH were pretreated with gingerol 10, 20 and 40 mg·kg^{-1} i.g. for 3 days, respectively. Gingerol was dissolved with 1% tragacanth. In the second night, rats have a 12 h fast. On the third day, cisplatin (3 mg·kg^{-1}, i.p.) was administered 30 min after each treatment with the antiemetic agent or its vehicle except the Groups C and CG. After administration of cisplatin, rats numbered 1 to 5 in each group were sacrificed to measure the gastric emptying 6 h later.

The weights of kaolin eaten of the remaining rats were observed in every 6 h continuously for 72 h. To measure the kaolin consumption during a 72 h period, the remaining kaolin in the container and kaolin spilled in the cage were collected, dried, and weighed every 6 h.

Dosages of drugs were calculated based on body surface area. Adult patients take metoclopramide, gingerol, cisplatin 30 mg, 200 mg and 35 mg per day, respectively. The body surface area conversion coefficient from humans to rats is 0.018. Because drug metabolism is not exactly the same between human and rat, we utilized 3 different doses of gingerol to evaluate its efficacy. The concentrations of gingerols administered were calculated based on body surface area. Adult patients take gingerols at a dose of 200 mg per day, and the average weight of an adult is 60 kg. The body surface area conversion coefficient from humans to rats is 0.018; thus the normal dose (medium dose) of gingerols administered to rats was 20 mg·kg^{-1}. In order to evaluate the dose-depended effect, we use three different doses group. The high dose is double the medium dose, and the low dose is half the medium dose.

The remaining rats were sacrificed 72 h after administration of cisplatin. Tissues from the area postrema as well as the ileum were removed from rats. The rats were placed in a stereotaxic frame (stoelting, Wood Dale, IL) with their head positioned vertically according to the Rat Brain Atlas by Paxinos and Watson.[31] Stereotaxic coordinates of the area postrema: 0–3 mm lateral to midline, 4.4–4.8 mm posterior to interaural line, 13.68–14.08 mm posterior to bregma.

Measurements of gastric emptying

Gastric emptying was adapted from the literatures with some modifications.[32, 33] Briefly, after a 12 h fast, rats numbered 1 to 5 in each group were given 2 mL of the phenol red solution (0.5 mg/mL) mixed with 1.5% methylcellulose via gavage. Thirty minutes after the feeding, the rat was rapidly sacrificed under pentobarbital anesthesia. The entire stomach was carefully isolated, ligated just above the cardia and below the pylorus, and removed. The amount of phenol red in the stomach was determined by measuring the absorption at 560 nm using a spectrophotometer (Shenhua instrument and meter factory, Shanghai, China). The percentage of gastric emptying was defined as the ratio between the amount of phenol red recovered from the stomach and the amount of phenol red ingested into the stomach (1-the amount of phenol red recovered from the stomach/ the amount of phenol red ingested into the stomach).

Immunohistochemistry of D2R, DAT and TH

Tissues from the area postrema as well as the ileum were removed from rats for immunohistochemical analysis of D2R, DAT and TH. The ileum was excised at a distance of 20 cm from the pylorus. The samples were fixed in 10% buffered formalin at 4 °C for 24–48 h. This was followed by cryoprotection for 12–18 h at 4 °C using 30% sucrose or until the samples equilibrated. The samples were sectioned (4 μm thick) using a freezing microtome (ASpZr35) and standard immunohistochemical procedures were used to visualize D2R, DAT and TH protein. Sections were washed 4 × 10 min in PBS (pH 7.4) at room temperature and were blocked with 10% normal goat serum. Sections were then incubated with anti-D2R, anti-TH or anti-DAT (Beijing Bioss Biological Technology, Beijing, China) overnight on a shaker at room temperature. Following washing (4 × 10 min) with PBS, sections were incubated for 2 h with goat anti-rabbit antibody. Finally, sections were washed (3 × 10 min) in PBS, transferred onto gelatin-coated slides. Tissue processed without the primary D2R antibody, DAT antibody of TH antibody served as a negative control. Adjacent sections were mounted onto plus-coated slides and stained using Neutral Red (0.5%), washed through a series of increasing alcohol concentrations, cleared with xylene and coverslipped with Neutral Blasam.

Five fields from every section were randomly selected for examination using an Olympus CKX41-32PH microscope equipped with an imaging system (Olympus Optical, Japan). The distribution and staining of positive expression of the area postrema and ileum tissue were observed under 200 light microscopes. The optical densities were measured by Image-Pro Plus v 6.0 software (Media Cybernetics, Rockville, MD).

RT-PCR of DAT and TH

To measure mRNA expressions of DAT and TH in the area postrema and ileum tissue, the tissues in rats were quickly immersed in RNAlater and stored at –80 °C. The total RNA was sequentially extracted using TRIZOL Reagent (Sigma-Aldrich, St. Louis, MO) according to the manufacturer's instruction. The RNA was treated with DNase (DNAfree, Ambion, Austin, TX) in order to remove contaminating genomic DNA, followed by phenol, chloroform extraction and ethanol precipitation. Total RNA was assessed for purity using the NanoDrop system. Total RNA was performed in a two-step procedure as described by Power SYBR Green PCR Master Mix kits (Applied Biosystems). Briefly, in the first step, cDNA was prepared from 500 ng RNA by reverse transcription in a final volume of 20 μL in a thermal cycler (Tgradient 96, Whatman Biometra, Niedersachsen, Germany). The samples were incubated at 37 °C for 60 min and 95 °C for 5 min. The cDNA was stored at –20 °C. The rat-specific primers for the genes of PPT, NEP and beta-actin were designed using Primer Premier 5.0 (Premier Biosoft, Palo Alto, CA). The primer sequences are shown in Table 1. Primers were synthesized by BioAsia Corp. (Shanghai, China). In the second step, quantitative real-time PCR was performed on the LightCycler apparatus (Roche Diag Diagnostics, Mannheim, Germany) using Power SYBR Green. PCR Master Mix kits (Applied Biosystems). The reaction was conducted with an initial denaturing at 95 °C for 10s, and then involved 40 cycles of 55 °C 10 s, 72 °C 15 s, and terminated by a cooling step 30 s at 40 °C. A melting-curve analysis was performed to confirm the absence of primer dimmers in specific PCR products. The efficiency of RT-PCR was assessed with serial dilutions of a sample of cDNA from the normal control group. Each experiment was performed in duplicate and the data were analyzed using LightCycler Software 4.0 (Roche Diagnostics). Beta-actin mRNA was used as the housekeeping gene, and all data are represented relative to its expression as fold change from control group.

Statistical analysis

All statistical analyses were performed using SPSS 17.0 software (SPSS, Chicago, IL). Data were expressed as mean ± S.E. Analysis of variance (ANOVA) was performed when more than two groups were compared. Values of $P < 0.05$ were considered statistically significant.

Table 1. The sequence of each PCR primer

Gene	Forward	Reverse
Beta-actin	5'- GAGGGAAATCGTGCGTGAC -3'	5'- GGACTCATCGTACTCCTGCTTG -3'
TH	5'- GTGTTTCAATGCACCCAGTAT -3'	5'- GTCCAATGTCCTGGGAGA -3'
DAT	5'- CTGCTCTACTTCAGCCTAT -3'	5'- GCATGGTAGCTGTGATCC -3'

DAT, dopamine transporter; PCR, polymerase chain reaction; TH, tyrosine hydroxylase.

RESULTS

Pica induced by cisplatin

As shown in Fig. 1, rats dosed with vehicle followed by cisplatin had two distinct periods of pica behaviour. There was an initial acute phase in 24 h after administration with cisplatin. After this period there was a delayed phase last near 48 h in Group V. Metoclopramide significantly inhibited the pica response during 24 h following cisplatin injection ($P < 0.05$). However, there was no significant decrease of kaolin eaten during 24–72 h. Gingerol dose-dependently decreased the weight of kaolin eaten induced by cisplatin during the 72 h observation period with significant decreases on each of the three test days ($P < 0.05$). And simple gingerol did not significantly induce the pica behavior, there was no significant difference in the weight of kaolin eaten with Group C.

Gastric emptying

Gastric emptying was significantly delayed after administration with cisplatin. As shown in Fig. 2, the percentage of gastric emptying was significantly decreased in Group V compared with Groups C and CG ($P < 0.05$), and the delayed gastric emptying was significantly improved after the treatment with metoclopramide and gingerol in comparison with Group V ($P < 0.05$). Metoclopramide had better effect than low-dose and middle-dose gingerol ($P < 0.05$). Gingerol improved gastric empting in a dose dependent manner ($P < 0.05$).

D2R, TH and DAT immunostaining expression in area postrema and ileum

D2R (Fig. 3A), TH (Fig. 4A) and DAT (Fig. 5A) staining intensities (Red-brown deposits indicate positive staining) were mainly situated in the mucosa and submucosa

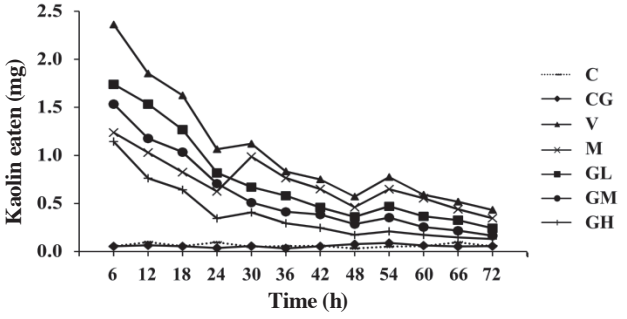

Fig. 1. Pica induced by cisplatin.
Rats had two distinct periods of eating kaolin in the cisplatin groups, and Ginger significantly decreased the weight of kaolin eaten induced by cisplatin during the 72 h observation period. Metoclopramide significantly decreased the weight of kaolin eaten during 24 h following cisplatin injection, but there was no significant decrease in the pica during 24–72 h. C: normal control group, pretreated with sterile saline 3 mL every day (n = 10); CG: simple gingerol control group, pretreated with gingerol 40 mg·kg^{-1} i.g. every day (n = 10); V: cisplatin control group, pretreated with sterile saline 3 mL every day (n = 10); M: cisplatin + metoclopramide group, pretreated with metoclopramide 2.5 mg·kg^{-1} i.g. every day (n = 10); GL: cisplatin + low-dose gingerol group, pretreated with gingerol 10 mg·kg^{-1} i.g. every day (n = 10); GM: cisplatin + middle-dose gingerol group, pretreated with gingerol 20 mg·kg^{-1} i.g. every day (n = 10); GH: cisplatin + high-dose gingerol group, pretreated with gingerol 40 mg·kg^{-1} i.g. every day (n = 10). i.g., intragastrically.

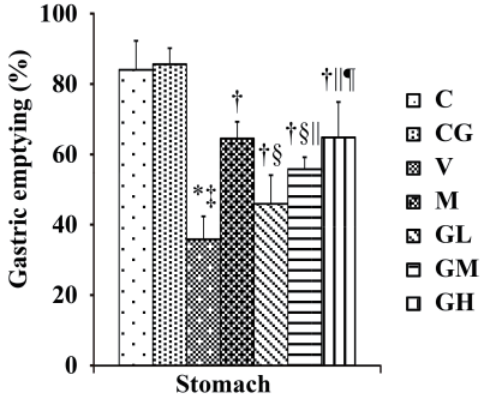

Fig. 2. Gastric emptying.
C: normal control group, pretreated with sterile saline 3 mL every day (n = 5); CG: simple gingerol control group, pretreated with gingerol 40 mg·kg^{-1} i.g every day (n = 5); V: cisplatin control group, pretreated with sterile saline 3 mL every day (n = 5); M: cisplatin + metoclopramide group, pretreated with metoclopramide 2.5 mg·kg^{-1} i.g. every day (n = 5); GL: cisplatin + low-dose gingerol group, pretreated with gingerol 10 mg·kg^{-1} i.g. every day (n = 5); GM: cisplatin + middle-dose gingerol group, pretreated with gingerol 20 mg·kg^{-1} i.g. every day (n = 5); GH: cisplatin + high-dose gingerol group, pretreated with gingerol 40 mg·kg^{-1} i.g. every day (n = 5). *P < 0.05 vs. Group C, ‡P < 0.05 vs. Group CG, †P < 0.05 vs. Group V, §P < 0.05 vs. Group M, ‖P < 0.05 vs. Group GL, ¶P < 0.05 vs. Group GM. i.g., intragastrically.

of the ileum as well as in the neurons of the area postrema. Compared with Group C and CG, there were more D2R, TH and less DAT immunoexpressions detected in Group V. Metoclopramide and gingerol had significant effect on the expression of D2R, TH and DAT in both the area postrema and ileum. Gingerol markedly suppressed the amount of D2R and TH detected and increased the amount of DAT detedted in a dose-dependent manner (× 200).

Mean optical density values of D2R, TH and DAT are showed in Figs. 3B, 4B and 5B, respectively. The photographs generated were quantitatively analyzed the optical density of D2R, TH and DAT by Image-Pro Plus. D2R and TH expressions significantly increased after treated with cisplatin while DAT expressions decreased (P < 0.05). Metoclopramide and gingerol had significant reducing effect on the elevated expressions of D2R, TH and elevation effect on the increased expressions of DAT in both the area postrema and ileum (P < 0.05). The increase of D2R, TH expressions and decrease of DAT expression in the area postrema and ileum induced by cisplatin was significantly inhibited by gingerol in a dose dependent manner (P < 0.05). Metoclopramide had better effect than low-dose and middle-dose gingerol on the expression of D2R (P < 0.05); had better effect than low-dose gingerol and weaker effect than high-dose gingerol on the expression of TH (P < 0.05); had weaker effect than high-dose gingerol on the expression of DAT (P < 0.05) both in the area postrema and ileum.

mRNA expression of TH and DAT in area postrema and ileum

As shown in Fig. 6, TH mRNA expression in both area postrema and ileum in Group V was significantly increased while DAT decreased compared to that in other groups (P < 0.05). Metoclopramide and gingerol could decrease TH mRNA expression (P < 0.05) and increase DAT mRNA expression (P < 0.05). Metoclopramide had better effect than low-dose gingerol (P < 0.05). Gingerol decrease TH mRNA expression and increase DAT mRNA expression in a dose dependent manner in both area postrema and ileum (P < 0.05).

DISCUSSION

Nausea and vomiting are complex protective mechanisms. However, when these symptoms recur frequently, they can significantly reduce the quality of life and can also be detrimental to health.[34] Chemotherapy can be a life-prolonging treatment for many cancer patients, but it is often associated with profound nausea and vomiting that is so distressing.[35] Despite advances in anti-emetic therapy, chemotherapy-induced nausea and vomiting

still poses a significant burden to patients undergoing chemotherapy.[36] Metoclopramide, as a clinical drug of D2R antagonists, has effects on vomiting and gastrointestinal diseases.[37] It is reported that metoclopramide, given in doses of 10 mg, has been shown to an effective prophlaxis against vomiting.[38] However, a recent meta-analysis showed no statistically significant amount of extrapyramidal symptoms, dizziness, headache, or sedation with this dose of metaclopramide1.[39] Traditional Chinese medicines have attracted attention due to their distinctive biological activities without toxicity and/or side-effects.[40] Gingerol has been shown to be effective antiemetics on both acute and delayed emesis in our previous studies.[41, 42] In this study, the amount of consumption of kaolin was significantly reduced after administration with gingerol compared with the control group during the whole 72 h. And gingerol has a dose-dependent effect. Therefore, we had investigated the antiemetic efficacy of gingerol on cisplatin-induced vomiting model of rats. In this study, gastric emptying was significantly delayed after administration with cisplatin and the delayed gastric emptying was significantly improved after the treatment with metoclopramide and gingerol. Gingerol improved gastric empting in a dose dependent manner.

Dopamine receptors, specifically D_2 and D_3, are known to play a role in nausea and emesis, most likely through inhibition of adenylate cyclase,[43] which alters the amount of cAMP within neurons located in the area postrema.[44] D2R, one receptor of DA, is known

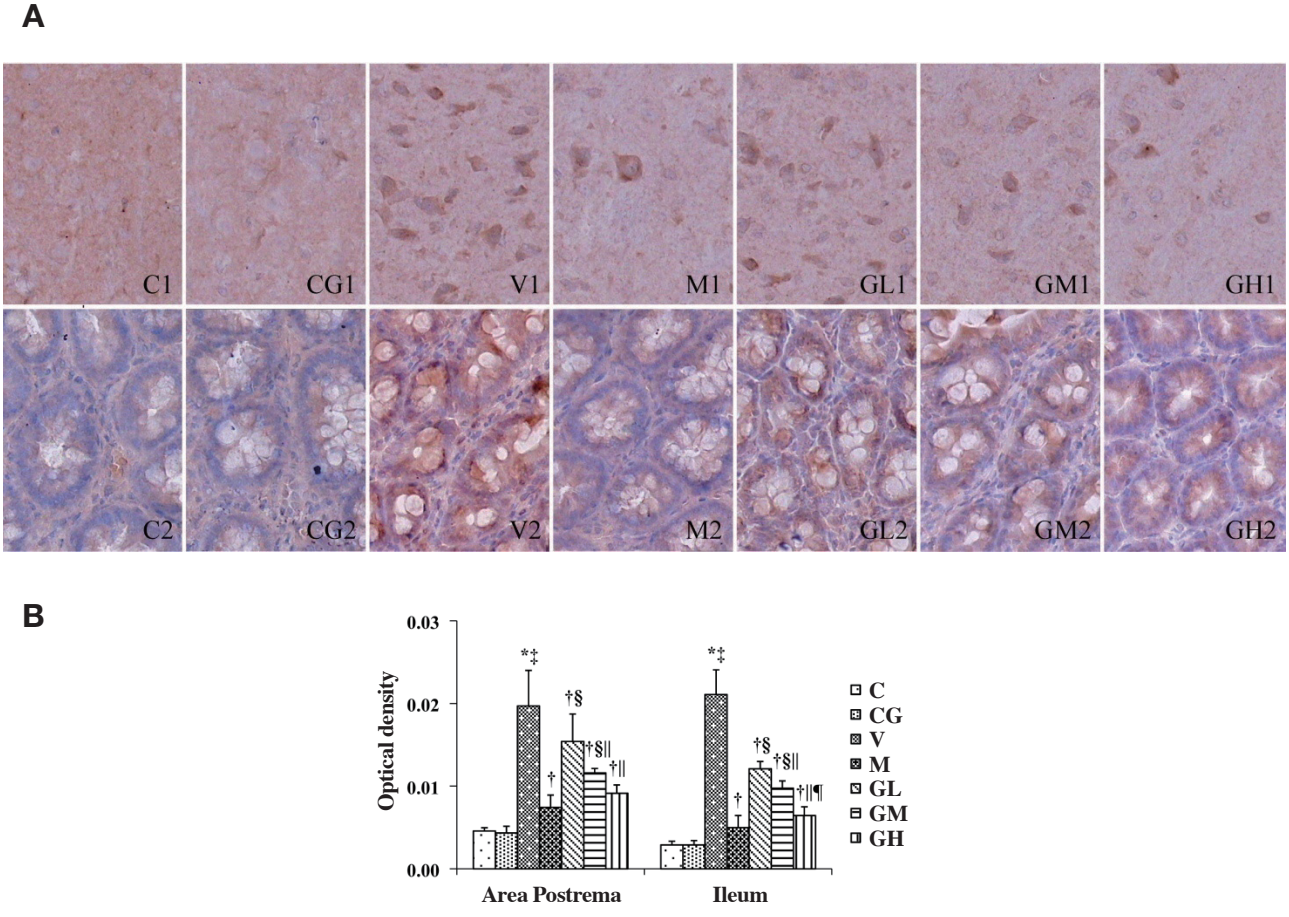

Fig. 3. D2R immunostaining expression in area postrema and ileum.
A: Immunohistochemistry expression of D2R in area postrema and ileum. The figures above (C1, CG1, V1, M1, GL1, GM1, GH1) show the expression in area postrema. The figures below (C2, CG2, V2, M2, GL2, GM2, GH2) show the expression in ileum. **B:** Mean optical density values of D2R. The photographs generated were quantitatively analyzed the optical density of D2R with Image-Pro Plus. C: normal control group, pretreated with sterile saline 3 mL every day (n = 10); CG: simple gingerol control group, pretreated with gingerol 40 mg·kg^{-1} i.g. every day (n = 10); V: cisplatin control group, pretreated with sterile saline 3 mL every day (n = 10); M: cisplatin + metoclopramide group, pretreated with metoclopramide 2.5 mg·kg^{-1} i.g. every day (n = 10); GL: cisplatin + low-dose gingerol group, pretreated with gingerol 10 mg·kg^{-1} i.g. every day (n = 10); GM: cisplatin + middle-dose gingerol group, pretreated with gingerol 20 mg·kg^{-1} i.g. every day (n = 10); GH: cisplatin + high-dose gingerol group, pretreated with gingerol 40 mg·kg^{-1} i.g. every day (n = 10). *P < 0.05 vs. Group C, ‡P < 0.05 vs. Group CG, †P < 0.05 vs. Group V, §P < 0.05 vs. Group M, ‖P < 0.05 vs. Group GL, ¶P < 0.05 vs. Group GM. D2R, dopamine D2 receptor; i.g., intragastrically.

to activate the chemoreceptor trigger zone in the area postrema of the fourth ventricle floor. This results in efferent impulses to the medullary vomiting centre, which initiates vomiting.[13, 14] Metoclopramide is a potent D2Receptor antagonist and binds to D_2 receptors in the area postrema.[43] We have already proved that gingerol can reduce the expression of DA in minks.[29] In this study, the expressions of D2R was increased after inject with cisplatin in both the mucosa and submucosa of the ileum as well as in the neurons of the area postrema. Metoclopramide and gingerol had significant effect on the expression of D2R in both the area postrema and ileum. Gingerol markedly suppressed the amount of D2R detected in a dose-dependent manner. We have got the consistent conclusion with our previous study that gingerol can significantly inhibit cisplatin-induced vomiting by improve the increased D2R expression on both area postrema and ileum.

DAT, a Na^+/Cl^- dependent plasma membrane protein at nerve endings, can rapidly reuptake DA, and determine the intensity and duration of DA transmission.[15] It is extensively distributed in peripheral and central nervous system and participated in many physiological processes. K Lemmer has proved that DAT can up-regulate the expression of DA in neuroendocrine gastrointestinal tumor cells.[45] The expression of DAT is associated with some clinical diseases, such as Parkinson's disease,[46] and is known to play an important role in gastrointestinal physiology.[47] But it has not been investigated in CINV so far. In this study, we have measured expression

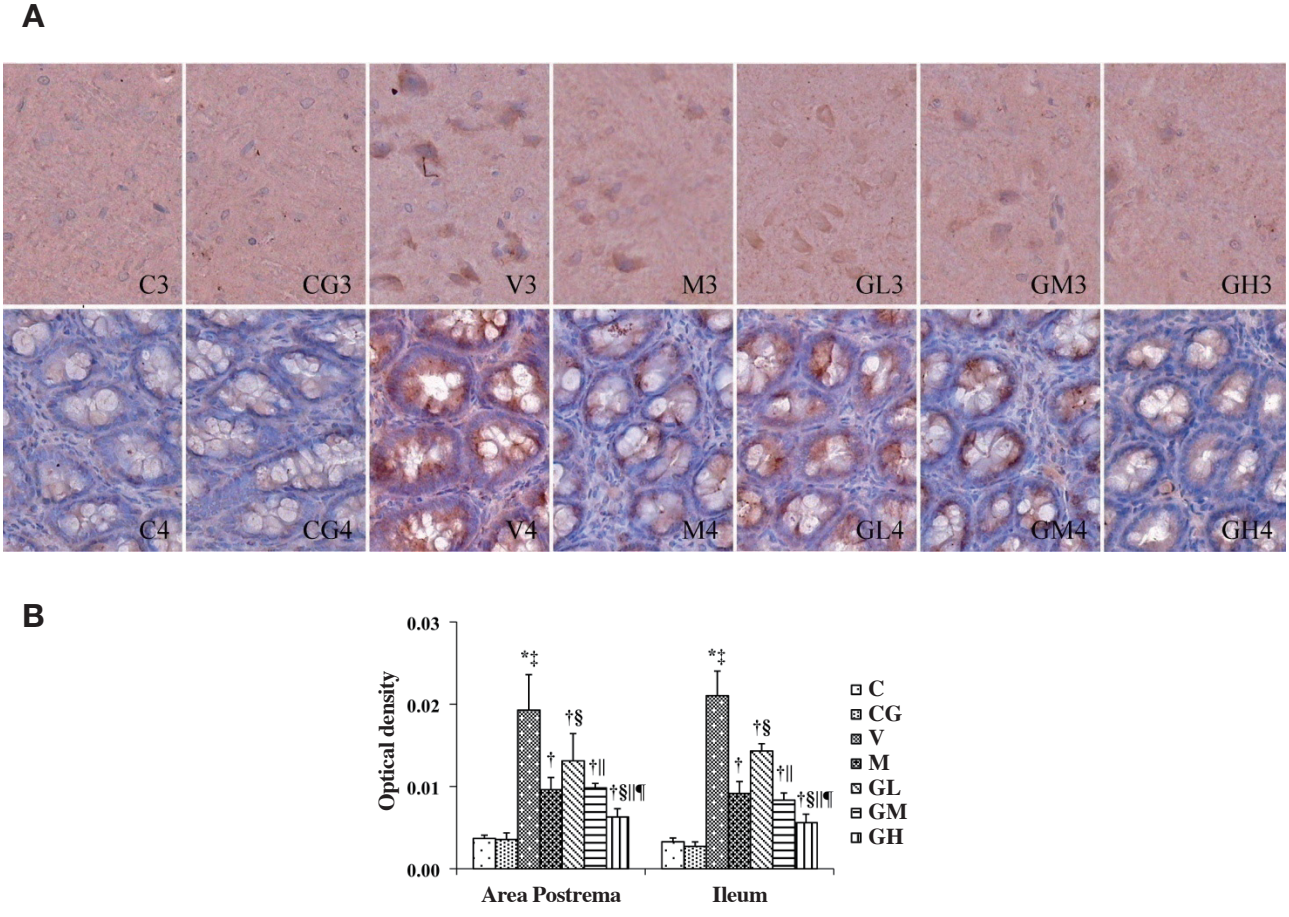

Fig. 4. TH immunostaining expression in area postrema and ileum.
A: Immunohistochemistry expression of TH in area postrema and ileum. The figures above (C3, CG3, V3, M3, GL3, GM3, GH3) show the expression in area postrema. The figures below (C4, CG4, V4, M4, GL4, GM4, GH4) show the expression in ileum. **B:** Mean optical density values of TH. The photographs generated were quantitatively analyzed the optical density of TH with Image-Pro Plus. C: normal control group, pretreated with sterile saline 3 mL every day (n = 10); CG: simple gingerol control group, pretreated with gingerol 40 mg·kg^{-1} i.g. every day (n = 10); V: cisplatin control group, pretreated with sterile saline 3 mL every day (n = 10); M: cisplatin + metoclopramide group, pretreated with metoclopramide 2.5 mg·kg^{-1} i.g. every day (n = 10); GL: cisplatin + low-dose gingerol group, pretreated with gingerol 10 mg·kg^{-1} i.g. every day (n = 10); GM: cisplatin + middle-dose gingerol group, pretreated with gingerol 20 mg·kg^{-1} i.g. every day (n = 10); GH: cisplatin + high-dose gingerol group, pretreated with gingerol 40 mg·kg^{-1} i.g. every day (n = 10). *P < 0.05 vs. Group C, ‡P < 0.05 vs. Group CG, †P < 0.05 vs. Group V, §P < 0.05 vs. Group M, ‖P < 0.05 vs. Group GL, ¶P < 0.05 vs. Group GM. i.g., intragastrically; TH, tyrosine hydroxylase.

of DAT to evaluate the mechanism why gingerol can improve vomiting by regulating the abnormal expression of DA. DAT mRNA expression in both area postrema and ileum in Group V was significantly decreased compared to that in other groups. Metoclopramide and gingerol could increase it. Gingerol increase DAT mRNA expression in a dose dependent manner in both area postrema and ileum. And the expression of DAT has positive correlation with DA.

Except D2R and DAT, we have also analyzed TH to explore the mechanism of DA and vomiting. A lot of enzymes are involved in the metabolism of the DA. TH, the rate-limiting enzyme for DA synthesis, participates in the regulation of intra- and extracellular DA levels.[16, 17] Some evidences reveal that TH and DA have negative correlation on Parkinson disease[48] and gastroparesis.[49] In the present research, we measure the expression of TH by both immunohistochemistry and RT-PCR. The results illustrate that metoclopramide and gingerol could decrease TH immunostaining expression and mRNA expression. By considering the similar changes of D2R and TH, we have inferred that gingerol has effect on D2R, TH and DAT to regulate the abnormal expression of DA on the treatment of vomiting induced by cisplatin in rats.

In present findings, gingerol has showed efficacy effects on vomiting induced by cisplatin. Gingerol could efficiently reduce DA expression by inhibiting synthesis and accelerating decomposition by adjusting mRNA associated with DA, such as D2R, DAT and TH. On

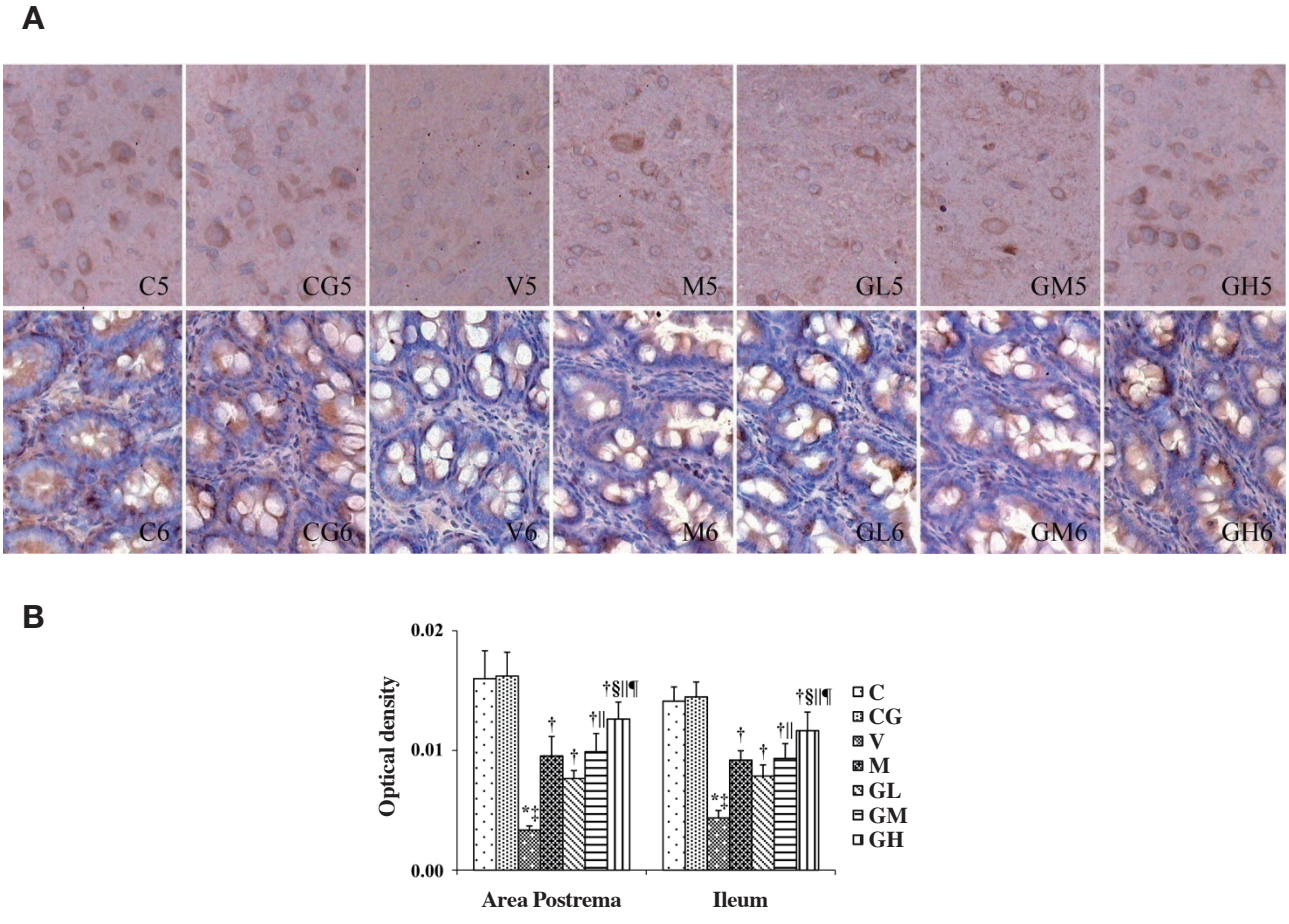

Fig. 5. DAT immunostaining expression in area postrema and ileum.
A: Immunohistochemistry expression of DAT in area postrema and ileum. The figures above (C5, CG5, V5, M5, GL5, GM5, GH5) show the expression in area postrema. The figures below (C6, CG6, V6, M6, GL6, GM6, GH6) show the expression in ileum. **B:** Mean optical density values of DAT. The photographs generated were quantitatively analyzed the optical density of DAT with Image-Pro Plus. C: normal control group, pretreated with sterile saline 3 mL every day (n = 10); CG: simple gingerol control group, pretreated with gingerol 40 mg·kg^{-1} i.g. every day (n = 10); V: cisplatin control group, pretreated with sterile saline 3 mL every day (n = 10); M: cisplatin + metoclopramide group, pretreated with metoclopramide 2.5 mg·kg^{-1} i.g. every day (n = 10); GL: cisplatin + low-dose gingerol group, pretreated with gingerol 10 mg·kg^{-1} i.g. every day (n = 10); GM: cisplatin + middle-dose gingerol group, pretreated with gingerol 20 mg·kg^{-1} i.g. every day (n = 10); GH: cisplatin + high-dose gingerol group, pretreated with gingerol 40 mg·kg^{-1} i.g. every day (n = 10). *P < 0.05 vs. Group C, ‡P < 0.05 vs. Group CG, †P < 0.05 vs. Group V, §P < 0.05 vs. Group M, ‖P < 0.05 vs. Group GL, ¶P < 0.05 vs. Group GM. DAT, dopamine transporter; i.g., intragastrically.

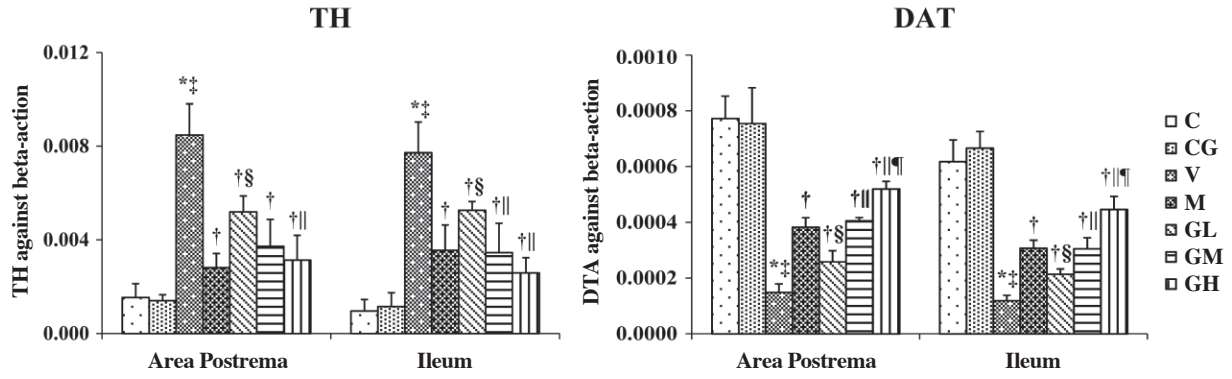

Fig. 6. Changes of mRNA expression of TH and DAT in area postrema and ileum.
Levels of TH and DAT against beta-actin mRNA expression are shown in the above histogram. The left-side graphs show the expression in area postrema. The right-side graphs show the expression in ileum. C: normal control group, pretreated with sterile saline 3 mL every day (n = 10); CG: simple gingerol control group, pretreated with gingerol 40 mg·kg^{-1} i.g. every day (n = 10); V: cisplatin control group, pretreated with sterile saline 3 mL every day (n = 10); M: cisplatin + metoclopramide group, pretreated with metoclopramide 2.5 mg·kg^{-1} i.g. every day (n = 10); GL: cisplatin + low-dose gingerol group, pretreated with gingerol 10 mg·kg^{-1} i.g. every day (n = 10); GM: cisplatin + middle-dose gingerol group, pretreated with gingerol 20 mg·kg^{-1} i.g. every day (n = 10); GH: cisplatin + high-dose gingerol group, pretreated with gingerol 40 mg·kg^{-1} i.g. every day (n = 10). *P < 0.05 vs. Group C, ‡P < 0.05 vs. Group CG, †P < 0.05 vs. Group V, §P < 0.05 vs. Group M, ‖P < 0.05 vs. Group GL, ¶P < 0.05 vs. Group GM. DAT, dopamine transporter; i.g., intragastrically; TH, tyrosine hydroxylase.

the other hand, the effect of gingerol showed stronger effects than metaclopramide on expressions of DAT and TH when using at the higher dosage. According to that metaclopramide is a clinical drug of D2R antagonists on emesis,[37] as well as amount of side effects,[39] we speculate that gingerol has more comprehensive effects than metaclopramide on emesis and vomiting, while gingerol can regulate the abnormal expression of DA by inhibiting synthesis and accelerating decomposition by adjusting mRNA or enzymes associated with DA, such as D2R, DAT and TH.

Nevertheless, the reason why gingerol can modulate expression of D2R, DAT and TH not only in the ileum but also area postrema remains unclear. Because the mRNA levels were changed, it might be modulated in the transcriptional step or others. This research might be partly proved the effect on cisplatin-induced pica analogous to emesis via modulating expressions of D2R, DAT and TH in the vomiting model of rats. For the further more study of underlying molecular biological mechanisms, we will use the Western blot method combine with RT-PCR method in the next experiment.

Acknowledgments: This work was supported by National Natural Science Foundation of China (81273702).

The authors declare no conflict of interest.

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