Absorption Characteristics of Novel Compound Calcium Carbonate Granules: Effects of Gastric Acid Deficiency and Exogenous Weak Acids

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Summary: Calcium carbonates are commonly administered as supplements for conditions of calcium deficiency. We report here pharmacokinetic characteristics of a novel formulation, calcium carbonate compound granules (CCCGs), forming complexes of calcium carbonate and calcium citrate in water. CCCGs were compared to a kind of commonly-used calcium carbonate D_3 preparation (CC) in the market in 5-week-old mice that had been treated with omeprazole, to suppress gastric acid secretion, and in untreated control mice. The results showed that: (1) CCCGs had better water solubility than CC *in vitro*; (2) In control mice, calcium absorption rates after CCCGs administration were comparable to those after CC administration; (3) Inhibition of gastric acid secretion did not affect calcium absorption after CCCGs, but moderately decreased it after CC; (4) The presence of phytic acid or tannin did not affect calcium absorption rates after CCCGs but did for CC; and (5) In normal mice, CCCGs did not inhibit gastric emptying and intestinal propulsion, and did not alter the gastrointestinal hormones. The results suggest that CCCGs may be therapeutically advantageous over more commonly used calcium supplement formulations, particularly for adolescents, because of their stable calcium absorption characteristics and their relatively favorable adverse effect profile.
Key words: calcium carbonate; gastric acid inhibition; phytic acid; tannin; calcium absorption

Calcium is the fifth richest element in the body, accounting for about 1.5%–2% of the human body mass, with more than 99% of the composition stored in the bones and teeth[1]. Calcium not only is a necessary structural element for building human body, but also regulates activities as an important physiological regulator of muscle, nerve, and other tissues with the form of ions[2–4]. The Institute of Medicine (IOM) and the National Institutes of Health (NIH) recommend a daily calcium intake of 1200–1500 mg for women and men older than 65[5, 6]. Generally, in view of the recent report that calcium intake from food in many parts of the world including China and other Pacific rim countries and much of South America, is less than 400–500 mg per day[7]. Individuals with special conditions associated with calcium deficiency, such as infants, adolescents, and the elderly[8], or pregnant, lactating, or postmenopausal women[9], may require calcium supplementation[10]. For example, infants and adolescents[8] need more calcium to meet the requirements of rapid growth and development. Adequate levels of calcium and vitamin D are required to maintain bone density and to prevent fractures associated with osteoporosis[11, 12].

Calcium supplements are mainly classified into inorganic, organic, "natural", and amino acid calcium formulations[13]. Long-term use of many of these calcium supplements often causes gastrointestinal symptoms such as bloating, constipation, gas, or a combination of all three[14]. The absorption of calcium is influenced by variables including levels of vitamin D, intra-gastric and intra-intestinal pH[15, 16], and gastrointestinal motility[17–19]. Largely because of its lower pKa, the citrate salt of calcium usually has fewer and less pronounced side effects than the carbonate salt does. However, most or all available calcium formulations are subjected to restricted absorption by forming calcium complexes with anions and other components of gastric and intestinal contents[20].

Recently, we gained a novel formulation, calcium carbonate compound granules (CCCGs), forming a

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complex of calcium carbonate and citric acid in water. In this report, we compared the effects of CCCGs with those of commonly-used calcium carbonate (CC): (1) on rates of calcium absorption in the normal mice or mice with gastric acid having been inhibited by omeprazole; (2) on rates of calcium absorption in the presence of calcium binding agents phytin acid and tannin in foods; (3) on gastric emptying, intestinal propulsion, and levels of the gastrointestinal hormones motilin (MTL) and gastrin (GAS) in control mice.

1 MATERIALS AND METHODS

1.1 Preparation of CCCGs

The CCCGs were prepared according to a patent described (the patent number is ZL201110139889.8 in China). In brief, β-cyclodextrin was combined with vitamin D_3 and dissolved in ethanol-water with an ethanol binder. Then, calcium carbonate was mixed with binder and acid vesicant to form particles in alkaline media. Finally, the two particles were mixed to form the CCCGs. This kind of novel CCCGs contained calcium carbonate, vitamin D, foaming agent and the binder at the ratio of 1:0.3–0.7:1–2:0.01–0.4. Specifically, the foaming agent is selected from one kind or its arbitrary combination in citric acid, apple acid, the tartaric acid and the binder is polyvidone, or hydroxypropyl methyl cellulose or its combination.

1.2 Experimental Animals

Male 5-week-old C57BL/6 mice were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (China). All animal experiments were approved by the Institutional Animal Care and Use Committee of Tongji Medical College, Huazhong University of Science and Technology (China). Experimental colonies were maintained at the Experimental Animal Center of Tongji Medical College (Huazhong University of Science and Technology, China) under specific pathogen-free conditions (SPF). The animals were kept in cages at 23±2°C and fed on the standard laboratory diet and tap water throughout the experiments.

The mice were given calcium-free feed (Beijing Vital River Laboratory Animal Technology Co., Ltd., China) for 1 week (n=8 for each group). To suppress gastric acid secretion, omeprazole (20 mg/kg) was orally administered at a dose of 20 mg/kg daily until accomplishing the experiment, and assessing the calcium absorption. In different groups, solutions of phytic acid (0.5%) (Tokyo Chemical Industry, Japan) or tannin (0.8%) (J&K Chemical Ltd., China) were added. CC (Wyeth Pharmaceuticals Ltd., USA), or calcium citrate (Sinopharm Chemical Reagent Co., Ltd., China) were orally administered at the doses indicated in the text. CCCGs were orally administered at one of three doses (mg Ca/kg body weight): 90, 180, and 360.

1.3 Intragastric pH Values

One after administration of omeprazole (20 mg/kg), the mice were sacrificed and the stomach was removed. The interior was then rinsed with 4 mL physiological saline until thoroughly rinsing the contents of stomach. The pH of the rinsed solution was then determined.

1.4 Sample Preparation

Mice were fed on calcium-free diets for 1 week to metabolize the original residual calcium *in vivo*. Omeprazole (20 mg/kg) was then administered to establish a model of low gastric acid secretion. One h later, CCCGs or CC was administered. Urine samples were then collected at 1, 2, 4, 6, 8, 12 and 24 h post-administration, and volume of excretion was recorded (n=8 for each group). After a week of calcium-free feeding, CCCGs, CC, or calcium citrate were administered continuously for 6 days, and fecal samples were collected daily over the final 3 days.

1.5 General Procedure for Analysis of Urine and Fecal Samples

Approximately 0.05 g of the fecal sample (n=8) was weighed into a conical flask. 10 mL of HNO_3 and 0.5 mL of perchloric acid (Sinopharm Chemical Reagent Co., Ltd., China) were then added, and the mixture was heated at 280°C until the solution evaporated completely, producing a white solid. After cooling, the processed fecal samples and unprocessed urine samples were diluted with 0.025 mg/mL strontium chloride solution (Sinopharm Chemical Reagent Co., Ltd., China). Calcium levels were determined by atomic absorption spectroscopy.

1.6 Flame Atomic Absorption Spectrometry (FAAS)

FAAS measurements were obtained with a SpectrAA-240FS atomic absorption spectrometer (Agilent, USA). A hollow cathode lamp was used as the radiation source to determine fecal and urinary calcium levels. The lamp was set at 422.7 nm and 10 mA (slit width: 0.5 nm), and the samples were contained in an air-acetylene mixture (acetylene flow rate: 2.00 L/min; oxidant gas flow rate: 13.50 L/min). BGC-D2 was used in the background correction method.

1.7 Calcium Absorption

These fecal samples were collected and then calcium levels were measured by FAAS: Absorption=[(Calcium intake–Fecal calcium)/Calcium intake]×100%, and the apparent calcium absorption rate was calculated in normal mice or the mice with gastric acid secretion inhibition. Urinary calcium was also measured by FAAS, and the mean urinary calcium excretion rate was calculated by the content of calcium excretion/time×100% within the different time ranges of collecting urine. Relative bioavailability (%) of the CCCGs was estimated as the ratio of total sample calcium levels 24 h after CCCGs administration to

those after CC.

1.8 Gastric Emptying

Gastric emptying time was measured by determining the rate of disappearance from the stomach of nutrient semi-solid paste. The paste was prepared stepwise. First, 10 g carboxymethyl cellulose sodium was dissolved in 250 mL distilled water. Then 16 g milk powder, 8 g sugar, 8 g starch, and 2 g activated carbon powder were added sequentially while stirring. This procedure yielded 300 mL of black semi-solid paste. The paste was stored at 4°C.

Either CCCGs or CC was administered for 2 weeks (n=8 for each group). The mice were then deprived of food but allowed free access to water for 16 h. 0.8 mL of semi-solid paste was measured (m) and orally administered. 20 min later, the mice were sacrificed by cervical dislocation, and the stomach was weighed (m_1), washed of the stomach contents, and weighed again (m_2). The gastric emptying rate was calculated as $100\%–[(m_1–m_2)/m\times100\%]$.

1.9 Intestinal Propulsion

The rate of intestinal propulsion was quantified according to a procedure reported previously[21, 22]. As in the procedure for calculating gastric emptying, the mice were given either CCCGs or CC orally for two weeks (n=8 for each group), and then deprived of food but not water for 16 h. Then 10 mL/kg of 10% activated charcoal powder suspended in 0.5% sodium carboxymethyl cellulose was orally administered. Thirty min later, the mice were sacrificed by cervical dislocation and the small intestine was exposed by laparotomy. Intestinal propulsion was then determined as the ratio of the propelling distance of activated charcoal and the whole length of small intestine as a fraction of the total intestinal length.

1.10 Enzyme-linked Immunosorbent Assay (ELISA)

After 2 weeks of continuous CCCGs or CC administration, the mice were sacrificed and the serum of mice was collected by extracting blood from the eye socket. MTL and GAS in the serum of treated and untreated mice were measured by commercial ELISA kits (Nanjing Jiancheng Bio Co., China) (n=8).

1.11 Statistical Analysis

Data are presented as mean±standard deviation (SD). Statistical analysis was performed in GraphPad Prism 5. Differences between multiple groups were examined for statistical significance using one-way analysis of variance (ANOVA) and statistical comparisons between two groups were assessed using the Student's t test. A calculated probability of less than 0.05 was considered to be significant.

2 RESULTS

2.1 Mouse Model of Gastric Acid Inhibition

Gastric acid increases the rate of calcium absorption of calcium carbonate[15, 16]. By increasing gastric pH in a low-pH environment carbonate salts of calcium retard absorption. We used this formulation in order to minimize the variability of calcium absorption. We established a mouse model of suppressed gastric acid secretion by orally administering omeprazole (20 mg/kg) once a day for consecutive 6 days[23]. The effectiveness of omeprazole was confirmed by an average increase in gastric pH of nearly two orders of magnitude in treated mice, from 3.63±0.39 to 5.65±0.34 (fig. 1).

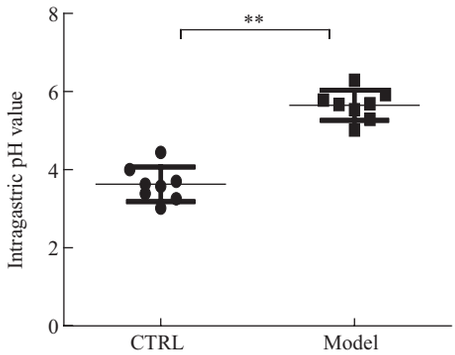

Fig. 1 Intragastric pH of untreated mice (CTRL) and mice that had been treated with omeprazole (20 mg/kg) one h before administration of calcium supplements (Model) (n=8 in each group). Data were represented as mean±SD. $^{**}P$<0.01. The data confirm the effectiveness of omeprazole in suppressing gastric acid secretion. Omeprazole treatment increased intragastric pH by two orders of magnitude, from 3.63±0.39 to 5.65±0.34.

2.2 Rate of Calcium Absorption

In preliminary *in vitro* studies, we found that CCCGs were more water-soluble than CC, when compared in either acidic solutions or water (data not shown). Effects of CC and CCCGs *in vivo*, at the same calcium dose of 180 mg Ca/kg, on calcium absorption in control mice were not different (fig. 2). Notably, suppression of gastric acid production with omeprazole inhibited calcium absorption after CC administration (180 mg Ca/kg), but not after the administration of the same calcium dose of CCCGs. In both experimental groups, calcium absorption rates were the same after administration of CCCGs at 90, 180, or 360 mg Ca/kg. Thus, the calcium absorption of CCCGs was unaffected under the suppression of gastric acid secretion.

2.3 Rate of Urinary Calcium Excretion

The total 24-h urinary calcium excretions after administration of CCCGs and CC in the control mice were not different: 150.7±4.6 and 150.5±2.4 μg, respectively (fig. 3A). The relative bioavailability of CCCGs was 100%. However, the effects of both compounds in acid-suppressed mice were different. In the gastric acid-suppressed mice, peak urinary excretion after administration of CC appeared earlier

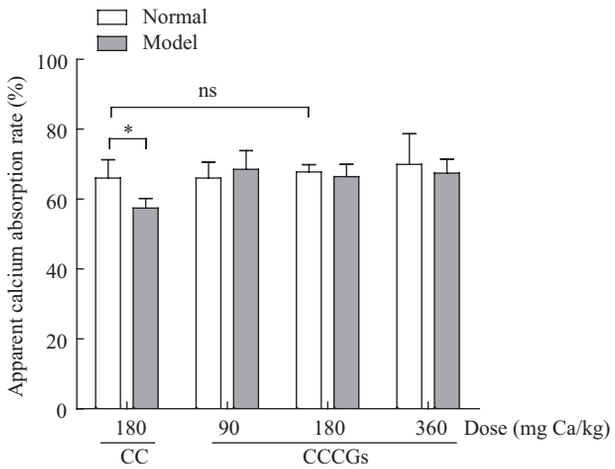

Fig. 2 Effects of CC and CCCGs on calcium absorption in untreated (normal) and omeprazole-treated (model) mice (n=8 in each group). The dose of CC was 180 mg calcium/kg and the three doses of CCCGs were 90, 180, and 360 mg calcium/kg. Data were represented as mean±SD. $^{*}P$<0.05. Inhibition of gastric acid secretion retarded the absorption of calcium from CC but not from the CCCGs.

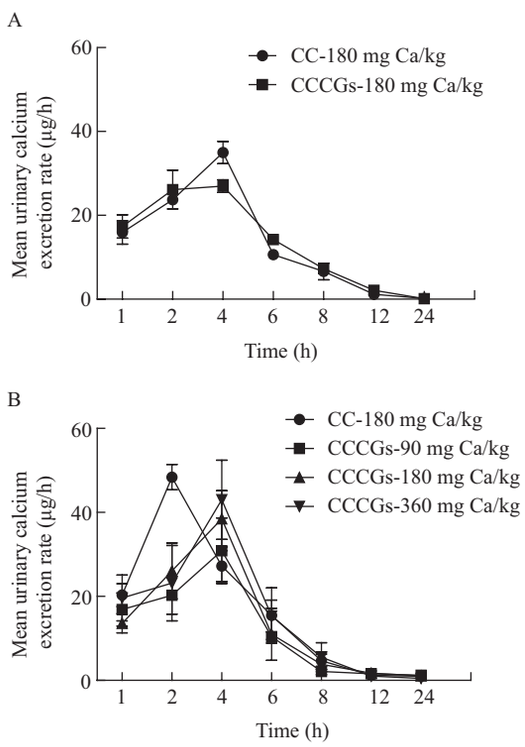

Fig. 3 Comparison of the effects of CCCGs and CC on the rates of urinary calcium excretion
A: Time course of urinary calcium excretion in response to 180 mg calcium/kg CCCGs or CC in untreated control mice (n=8 in each group); B: Time course of urinary calcium excretion in response to CC (180 mg calcium/kg) and CCCGs (90, 180, and 360 mg calcium/kg) in omeprazole-treated mice (n=8 in each group). Peak urinary calcium excretion in mice given CCCGs appeared later, regardless of dose, than that in mice given CC.

than that after CCCGs administration (fig. 3B). The results further supported that CCCGs were more resistant to the variation of gastric pH.

2.4 Effects of Phytic Acid and Tannin on Calcium Absorption

Phytic acid and tannin are common dietary components that can precipitate calcium and inhibit its absorption from the gastrointestinal tract[20, 24]. Here, we tested the effects of phytic acid and tannin administration, as described in Methods, on the subsequent absorption of calcium from CCCGs compared to that of the reference compounds CC and calcium citrate (fig. 4). The presence of either phytic acid or tannin moderately but significantly inhibited the absorption of calcium from CC, but not from either calcium citrate or CCCGs.

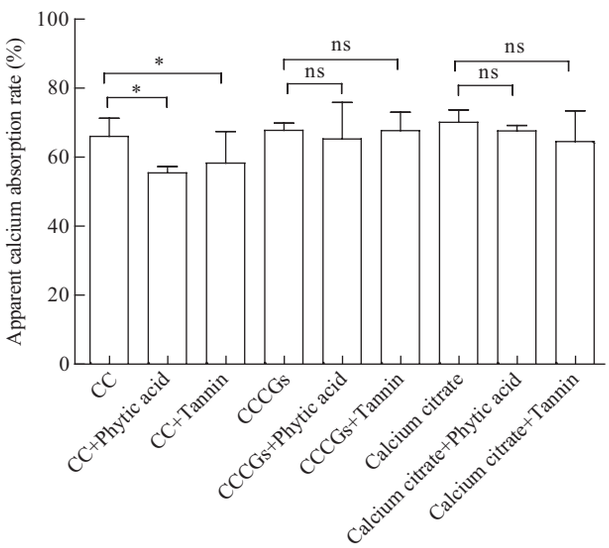

Fig. 4 Effects of the calcium-binding agents phytin acid and tannin on the absorption of calcium from 180 mg calcium/kg CC, calcium citrate, and CCCGs (n=8 in each group). The presence of either phytin acid or tannin inhibited absorption of calcium from CC, but not from calcium citrate or CCCGs. Data were represented as mean±SD. $^{*}P$<0.05; ns: no significant

2.5 Adverse Gastrointestinal Effects

Adverse effects of CCCGs and the reference compound CC on the gastrointestinal tract were determined in normal control mice by focusing on changes in gastric emptying, intestinal propulsion, and serum levels of MTL and GAS. CC slightly but significantly decreased gastric emptying, but CCCGs had no effect on any of the three calcium doses tested (fig. 5A). Similarly, CC, but not CCCGs, inhibited intestinal propulsion (fig. 5B). Neither CC nor CCCGs had any significant effect on levels of MTL or GAS (fig. 5C). Thus, CC, but not CCCGs, decreased gastric emptying and intestinal propulsion, but neither compound altered levels of MTL or GAS.

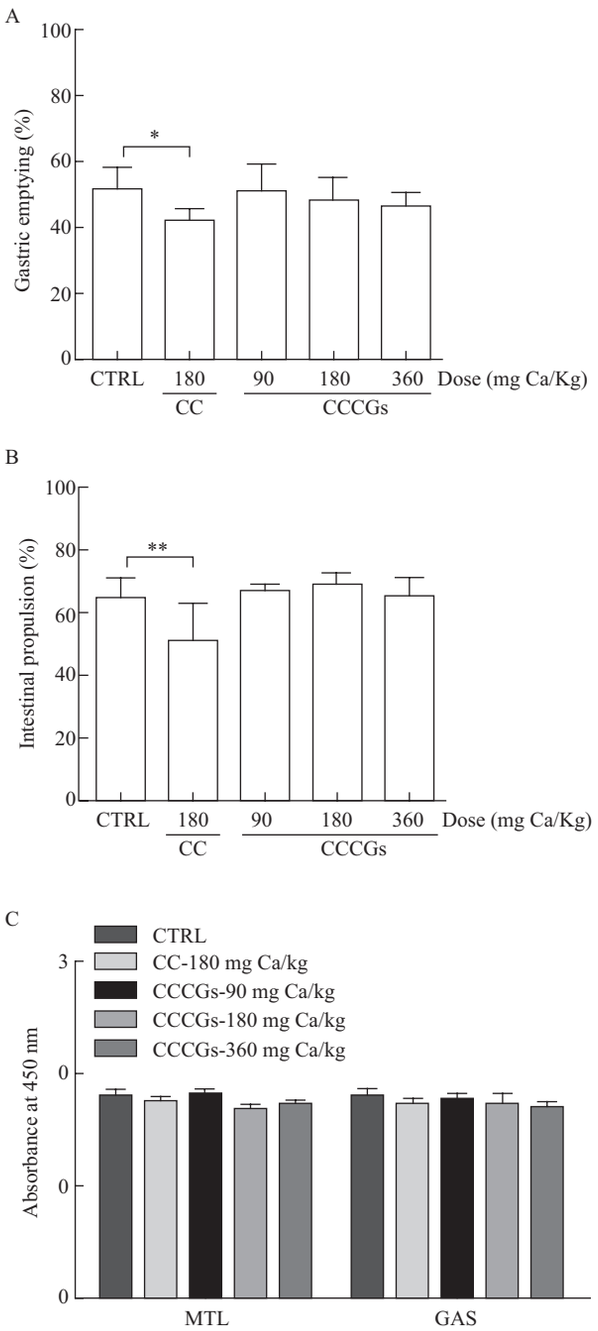

Fig. 5 Effects of CC or CCCGs on gastric emptying (n=8) (A), intestinal propulsion (B), and serum levels of the gastrointestinal hormones MTL and GAS (C)
Data were represented as mean±SD. $^{*}P<0.05$, $^{**}P<0.01$

3 DISCUSSION

Calcium carbonates are widely used as calcium supplements, largely because of their high calcium content and low cost[25]. The rank order of calcium absorption from the three most common calcium salts are calcium citrate > calcium gluconate > calcium carbonate[26]. Limitations of currently-used supplements include adverse gastrointestinal effects, manifested in part by alterations in gastric emptying time and intestinal motility[27–29], and by variable calcium absorption due to fluctuations in gastric pH and the presence of food components that can bind to calcium salts.

For this study, we gained a novel compound of calcium carbonate and calcium citrate to form a granular complex (CCCGs). We then characterized key pharmacokinetic and biological properties of the granules, focusing on solubility, rates of absorption, and actions on gastric and intestinal motility. The effects of CCCGs were compared with those of CC, a commonly used calcium carbonate preparation. The CCCGs granules were formulated to combine citrate and carbonate salts of calcium in an attempt to shield the calcium salts against unwanted effects of gastric acid on calcium absorption, and perhaps to decrease the extent of adverse effects on gastrointestinal motility.

Pharmacokinetic properties of the granules were tested in mice whose gastric acid had been inhibited by omeprazole treatment in an attempt to minimize inhibitory effects of high gastric pH on calcium absorption[20]. Unexpectedly, absorption of calcium after CC administration in this study was actually lower in the omeprazole-treated mice than in the control group (fig. 2). In contrast, calcium absorption after CCCGs administration was not affected by inhibition of gastric acid secretion and the results showed there was no significant difference among different dose groups of CCCGs. *In vitro*, CCCGs were water-soluble in either acidic or water solutions (preliminary observations). Apparently, the unique formulation of the CCCGs may have stabilized calcium absorption under conditions of variable pH *in vivo*. This stabilizing effect seems to be nonspecific. The presence of phytic acid and tannin, two anionic compounds known to precipitate calcium[19, 20], inhibited the absorption of CC but not of CCCGs (fig. 3).

Perhaps the most significant finding of this study was that the CCCGs produced fewer adverse gastrointestinal effects than CC did (fig. 5). CC, as expected, delayed gastric emptying and inhibited intestinal propulsion, but the CCCGs did not. Neither CC nor CCCGs altered serum levels of the gastrointestinal hormones MTL or GAS (fig. 5C), seemingly ruling them out as contributors to the actions of CC on gastrointestinal motility. Instead, the motility-inhibiting actions produced by CC may be attributable at least in part to the dual-salt complex formulation of the CCCGs, resulting in less carbonate per mole of administered calcium.

Presumed benefits of calcium supplementation are somewhat controversial[11]. Concerns have been raised regarding the potential for increasing the risk of adverse cardiovascular events[30]. The prevailing view, however, is that calcium supplements are generally safe when used appropriately[11, 12], and considered to be

important for bone health generally and for conditions of abnormal bone structure or metabolism such as fractures and osteoporosis[13]. The novel calcium formulation described here may be therapeutically advantageous over more commonly used calcium supplement formulations, because of its stable calcium absorption characteristics and its relatively favorable adverse effect profile.

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Conflict of Interest Statement

The authors declare that they have no potential conflicts of interest regarding this work.

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创新药物复方碳酸钙颗粒的吸收特性：胃酸缺乏和外源性弱酸的影响

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摘要： 碳酸钙D_3作为一种常用的钙补剂剂，用于缺钙乏相关疾病的防治。本文报道了一种创新药物——复方碳酸钙颗粒(CCCGs)的药代动力学特性，其在水中形成可溶解的碳酸钙和柠檬酸钙的络合物。将复方碳酸钙颗粒（CCCGs）和市面上常用的普通碳酸钙D_3（CC）在使用奥美拉唑抑制胃酸分泌的5周龄小鼠与未处理的对照组小鼠进行比较。结果表明：（1）与普通碳酸钙D_3相比，复方碳酸钙颗粒（CCCGs）具有较好的体外水溶性；（2）在对照组小鼠中，复方碳酸钙颗粒（CCCGs）给药后的钙吸收率与普通碳酸钙D_3（CC）相当;（3）抑制胃酸分泌条件下，给予复方碳酸钙颗粒（CCCGs）后，不影响钙的吸收，但给予普通碳酸钙D_3（CC）后，钙的吸收有一定降低；（4）复方碳酸钙颗粒（CCCGs）不受植酸或单宁的影响，其钙的吸收速率稳定，但植酸或单宁对普通碳酸钙D_3（CC）有一定影响；（5）在正常小鼠组，复方碳酸钙颗粒（CCCGs）不抑制胃排空和肠道推进，也不改变胃肠激素。结果表明，相对于其它的钙补充剂，复方碳酸钙颗粒（CCCGs）的疗效可能更有优势，特别是对胃酸缺乏或伴胃肠功能障碍的婴儿、孕产妇和老年人，因为复方碳酸钙颗粒（CCCGs）具有稳定的钙吸收特性和较少的不良反应。

关键字：碳酸钙；胃酸抑制；植酸；单宁；钙的吸收

钙是人体第五丰富的元素，约占人体重量的1.5-2%，其中99%以上的成分储存在骨骼和牙齿中[1]。钙不仅是构建人体骨骼所必需的元素，而且还以钙离子的形式调节肌肉、神经等组织的生理活性[2-4]。美国医学研究所（IOM）和美国国立卫生研究院（NIH）建议女性和65岁以上的男性每天摄入1200毫克至-1500毫克的钙[5,6]。一般来说，世界上许多地方，包括中国和其他环太平洋国家以及南美大部分地区，每天从食物中摄取的钙不足400-500mg[7]。患有与钙缺乏相关疾病的个体，如婴幼儿、青少年和老年人[8]，或孕妇、哺乳期妇女或绝经后妇女[9]，可能需要钙补充剂[10]。例如，婴幼儿和青少年需要更多的钙来满足快速生长发育的需要。维持骨密度和预防骨质疏松性骨折需要足够的钙和维生素D水平[11,12]。

钙补充剂主要分为无机钙、有机钙、“天然钙”和氨基酸钙制剂[13]。长期服用这些钙补充剂常会导致胃肠道症状，如腹胀、便秘、胀气，或三者兼而有之。钙的吸收受多种因素的影响，包括维生素D水平、胃内和肠道内pH值[15,16]和胃肠动力[17-19]。很大程度上由于柠檬酸钙络合物的低解离常数（pKa），它通常比普通碳酸钙D_3有更少的副作用。然而，大多数钙补充剂都受到钙配合物，阴离子或胃和肠道内容物等限制其吸收。[20]

近年来，我们发现了一种创新药——复方碳酸钙颗粒（CCCGs），其在水中形成碳酸钙与柠檬酸的络合物。在这份报告中，我们比较了复方碳酸钙颗粒（CCCGs）和普通碳酸钙D_3（CC）的效果。（1）奥美拉唑抑制胃酸的小鼠和正常小鼠和对钙的吸收率; （2）研究了食物中植酸和单宁对钙离子吸收的影响。（3）对照研究小鼠胃排空、肠推进及胃肠激素胃动素（MTL）、胃泌素（GAS）水平的影响。

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1 材料和方法

1.1 复方碳酸钙颗粒（CCCGs）的制备

复方碳酸钙颗粒（CCCGs）是根据一个专利文件所述进行制备。（中国，专利号为ZL201110139889.8）。简而言之，β-环糊精结合维生素D_3和溶解在乙醇粘结剂包裹。再与碳酸钙粘合形成一组颗粒。另一组用粘合剂和酸性辅料混合，在酸性介质中形成颗粒。最后，两种颗粒混合形成复方碳酸钙颗粒（CCCGs）。这种新型药物含有碳酸钙、维生素D、酸性辅料和粘结剂，配比为1∶0.3-0.7∶1-2∶0.01-0.4。具体地说，酸性辅料是从柠檬酸、苹果酸、酒石酸和粘合剂中的一种或其任意组合中选择的，其组合物为聚维酮或羟丙基甲基纤维素或其组合。

1.2 实验动物

5周龄C57BL/6雄性小鼠从北京维通利华实验动物科技有限公司所购买。所有动物实验均经华中科技大学同济医学院动物保护与使用委员会批准。在特定的无致病性条件下（SPF），同济医学院实验动物中心（华中科技大学）建立了实验菌落。实验过程中，动物被置于23±2℃的笼中，以标准的实验室饲料和自来水喂养。

雄性小鼠（北京维通利华实验动物技术有限公司）被喂入不含钙的食物1周，（每组n=8）。为了抑制胃酸分泌，口服奥美拉唑，每日剂量为20mg/kg，直至实验完成，并评估钙的吸收情况。在不同的实验组中，分别添加植酸溶液（0.5%）（日本东京化学工业）和单宁溶液（0.8%）（中国强生化学有限公司）。口服普通碳酸钙D_3（CC）（美国惠氏制药有限公司）或柠檬酸钙（国药控股化学试剂有限公司），剂量见正文。口服复方碳酸钙颗粒（CCCGs）三种剂量（mg Ca/Kg BW）中的一种：90、180和360mg Ca/Kg。

1.3 胃内pH值

给予奥美拉唑（20mg/kg）1小时后处死小鼠，取胃。然后用4ml生理盐水冲洗胃的内部，直至彻底冲洗胃的内容物。然后测定漂洗液的pH值。

1.4 样品制备

用不含钙的食物喂养小鼠1周，使小鼠体内原有残余钙代谢。用奥美拉唑（20mg/kg）建立低胃酸分泌模型。一小时后，服用复方碳酸钙颗粒（CCCGs）或普通碳酸钙D_3（CC）。分别于给药后1、2、4、6、8、12、24小时采集尿液样本，记录排泄量（每组n=8）。经过一周的无钙喂养。连续给予复方碳酸钙颗粒（CCCGs）、普通碳酸钙D_3（CC）或柠檬酸钙6天，最后3天，每天收集粪便样本。

1.5 尿液和粪便样本分析的一般程序

约0.05g粪便样本（n=8）称重入锥形瓶。加入硝酸HNO_3 10mL，高氯酸0.5mL（国药控股化学试剂有限公司），280℃加热至溶液完全蒸发，形成白色固体。冷却后，用0.025% （m/v）氯化锶溶液（国药控股化学试剂有限公司）稀释粪样和尿样。用原子吸收光谱法测定钙的含量。

1.6 火焰原子吸收光谱法（FAAS）

采用美国安捷伦公司的SpectrAA-240FS原子吸收光谱仪对原子吸收光谱进行了测量。采用空心阴极灯作为辐射源，测定粪便和尿钙水平。灯设为422.7nm和10ma（狭缝宽度0.5nm），样品置于空气-乙炔混合物中（乙炔流量2.00L/min，氧化剂气体流量13.50L/min）。背景校正方法采用BGC-D2。

1.7 钙的吸收

收集这些粪便样本，用FAAS法测定钙水平：吸收=[（钙摄入-粪便钙）/钙摄入量]×100%，计算正常小鼠或胃酸分泌抑制小鼠的表观钙吸收率。采用FAAS测定尿钙，取不同收集尿液时间范围内尿钙排泄量/时间×100%计算尿钙平均排泄率。复方碳酸钙颗粒（CCCGs）CCCG的相对生物利用度（%）的估计为给予复方碳酸钙颗粒（CCCGs）和普通碳酸钙D3（CC）后24小时总样本钙水平的比值。

1.8 胃排空

通过测定营养半固体糊剂从胃中消失的速率，测定胃排空时间。这种浆料是逐步配制的。首先，将10g羧甲基纤维素钠溶于250毫升蒸馏水

中。搅拌时依次加入16g奶粉、8g糖、8g淀粉、2g活性炭粉。这个过程产生了300毫升的黑色半固态糊状物。浆糊在4℃下保存。

喂服复方碳酸钙颗粒（CCCGs）或普通碳酸钙D_3（CC）两周，（每组，n=8）。然后，这些小鼠被剥夺了食物，但被允许在16小时内自由喝水。取0.8 ml半固态糊剂（m），口服。20 min后处死颈椎脱位小鼠，称胃（m1），洗胃内容物，再称胃（m^2）。胃排空率计算为100%-[（m1−m2）/m×100%]。

1.9 肠推进

肠推进率是根据以前报道的程序所量化[20,21]。在计算胃排空的过程中，小鼠喂服复方碳酸钙颗粒（CCCGs）或普通碳酸钙D_3（CC）两周（每组，n=8），禁食禁水16小时。然后口服含0.5%羧甲基纤维素钠的10%活性炭粉10 ml/kg。30分钟后采用颈椎脱位处死小鼠，剖腹探查小肠。确定肠道推进程度为活性炭推进距离与小肠总长度之比，为小肠总长度的一部分。

1.10 酶联免疫吸附实验（ELISA）

连续给予复方碳酸钙颗粒（CCCGs）或普通碳酸钙D_3（CC）两周后，处死小鼠，小鼠眼窝采血，取血清。采用ELISA试剂盒（南京建城生物科技有限公司）对奥美拉唑处理组和未处理组（对照组）小鼠血清胃动素（MTL）或胃泌素（GAS）进行检测。

1.11 统计分析

数据以平均标准差（SD）表示。用GRAPH PAD PRISM 5进行统计分析。采用单因素方差分析（ANOVA）检验多组间差异的统计学意义，采用t检验评价两组间的统计学比较。小于0.05的计算概率被认为是显著的。

2 结果

2.1 小鼠胃酸抑制模型

胃酸增加碳酸钙D_3对钙的吸收速率[15,16]。通过增加胃pH值，在低pH环境中碳酸钙盐的吸收减缓。我们使用这个模型是为了尽量减少钙吸收的可变性。我们建立了连续6天口服奥美拉唑（20 mg/Kg）抑制胃酸分泌的小鼠模型。奥美拉唑的有效性，通过治疗小鼠胃pH值的平均增加近两个数量级得到证实，从3.63±0.39增加到5.65±0.34（图1）。

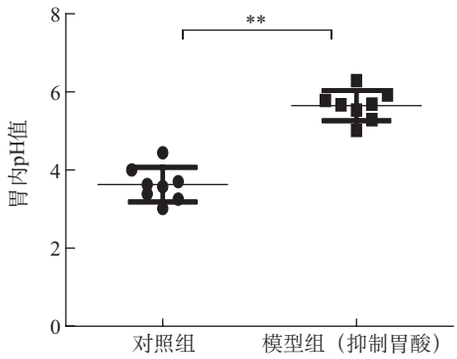

图1 给予钙补充剂前，未使用奥美拉唑组小鼠（对照组）胃内pH和给予奥美拉唑（20mg/kg）小时后的小鼠（模型组）的胃内pH值（各组n=8）。
数据表示为平均标准差SD表示。$^{**}P < 0.01$。这些数据证实了奥美拉唑抑制胃酸分泌的有效性。
奥美拉唑组使胃内pH值由3.63±0.39升高两个数量级，达到5.65±0.34

2.2 钙吸收率

在初步的体外研究中，我们发现，无论是在酸性溶液还是在水中，复方碳酸钙颗粒（CCCGs）都比普通碳酸钙D_3（CC）更容易溶于水。在体内相同剂量180mg Ca/Kg时，复方碳酸钙颗粒（CCCGs）和普通碳酸钙D_3对于对照组（未使用奥美拉唑）小鼠钙吸收的影响没有差异（图2）。值得注意的是，在奥美拉唑抑制胃酸分泌的模型中，服用普通碳酸钙D_3（CC, 180mg Ca/Kg）后的小鼠，出现钙吸收减少，而相同钙剂量的复方碳酸钙颗粒（CCCGs）组则没有出现抑制小鼠钙吸收的情况。在两组实验中，钙吸收率在给予复方碳酸钙颗粒（CCCGs）90、180或360mg Ca/kg时相同。因此，复方碳酸钙颗粒（CCCGs）的体内吸收，不受胃酸分泌抑制的影响。

2.3 尿钙排泄率

服用复方碳酸钙颗粒（CCCGs）和普通碳酸钙D_3（CC）后，两组小鼠的24小时总尿钙排泄没有区别：分别是150.7±4.6,150.5±2.4μg（图3A）。复方碳酸钙颗粒（CCCGs）的相对生物利用度为100%。然而，这两种化合物对胃酸受抑制的小鼠效果是不同的。在胃酸抑制的小鼠中，普通碳酸

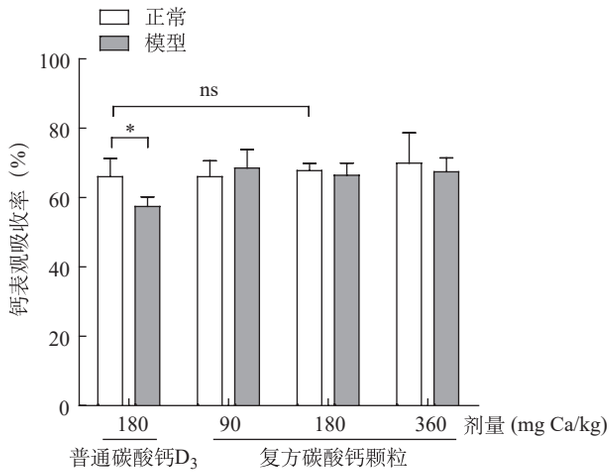

图2 复方碳酸钙颗粒（CCCGs）和普通碳酸钙D_3（CC）对未处理（正常）和奥美拉唑处理后（模型）小鼠钙吸收的影响（每组n=8）。普通碳酸钙D_3（CC）剂量为180 mg Ca /kg和360 mg Ca /kg。数据用平均标准差SD表示。$^*P$ <0.05。胃酸分泌的抑制情况下，普通碳酸钙D_3（CC）的吸收受影响，然而复方碳酸钙颗粒（CCCGs）的体内吸收，并不受胃酸分泌抑制的影响。

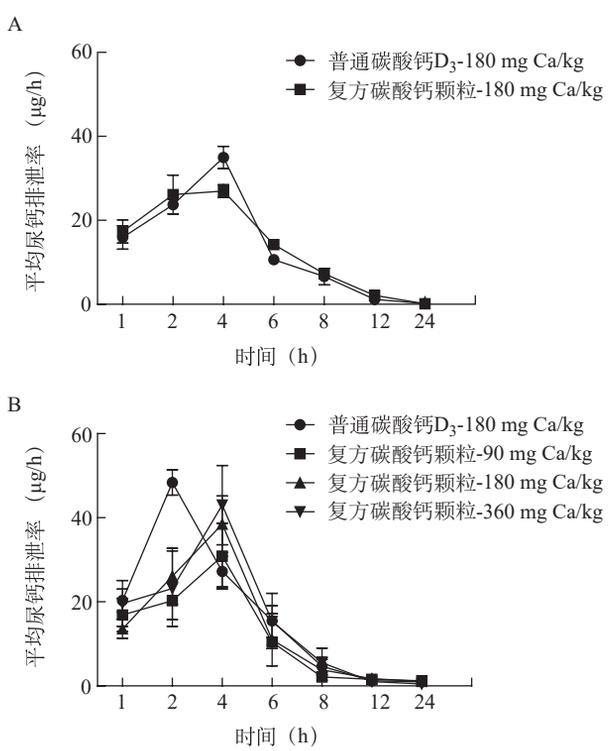

图3 比较复方碳酸钙颗粒（CCCGs）和普通碳酸钙D_3（CC）对尿钙排泄率的影响
A：未治疗对照组小鼠的尿钙排泄对使用钙浓度为180mg /kg 复方碳酸钙颗粒（CCCGs）或普通碳酸钙D_3（CC）的反应时间（每组N=8）；
B：奥美拉唑组小鼠（每组n=8）在普通碳酸钙D_3（180mg钙/kg）和复方碳酸钙颗粒CCCGs（90、180和360mg Ca/kg）作用下尿钙排泄的时间进程。无论剂量大小，给予复方碳酸钙颗粒（CCCGs）的小鼠尿钙排泄峰值出现的时间较给予普通碳酸钙D_3（CC）的小鼠晚。

钙D_3（CC）给药组的排尿高峰出现时间早于复方碳酸钙颗粒（CCCGs）组 （图3B）。结果进一步证明，复方碳酸钙颗粒（CCCGs）对胃pH值的变化具有较强的耐受性。

2.4 植酸和单宁对钙吸收的影响

植酸和单宁是可与钙形成沉淀并抑制钙从胃肠道吸收的常见膳食成分[20,24]。在此，我们测试了方法中所述的植酸和单宁给药对复方碳酸钙颗粒（CCCGs）中钙后续吸收的影响，并与对照药物普通碳酸钙D_3（CC）和柠檬酸钙进行了比较（图4）。结果有些出人意料：植酸或单宁存在的条件下，小鼠对于普通碳酸钙D_3（CC）的吸收有一定的抑制作用，但对柠檬酸钙或复方碳酸钙颗粒（CCCGs）的吸收无明显抑制作用。

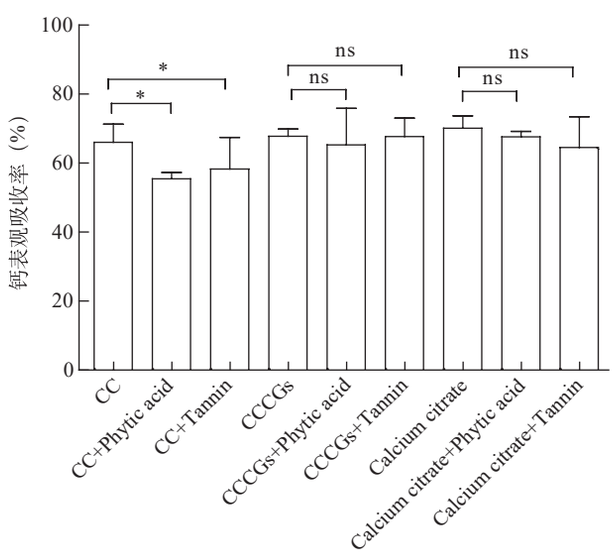

图4 植酸钙和单宁对于给予180mg Ca/Kg普通碳酸钙D_3（CC），柠檬酸钙，复方碳酸钙颗粒（CCCGs）小鼠的钙吸收影响，每组（n=8）。植酸或单宁均对普通碳酸钙D_3（CC）有抑制钙吸收作用，但不影响柠檬酸钙或复方碳酸钙颗粒（CCCGs）。数据表示为平均标准差（SD）。$^*p$<0.05; ns:无统计学意义。

2.5 胃肠道副反应

通过观察正常模型中对照组小鼠胃排空、肠道推进、血清胃动素（MTL）和胃泌素（GAS）水平的变化，确定了复方碳酸钙颗粒（CCCGs）和对照品普通碳酸钙D_3（CC）对胃肠道的不良影响。普通碳酸钙D_3（CC）轻微但显著减少胃排空。然而，复方碳酸钙颗粒（CCCGs）在三种钙剂量测试中的任何一种，都没有减少胃排空的

副反应（图5a）。同样，普通碳酸钙D_3（CC）还抑制肠道推进（图5b），然而复方碳酸钙颗粒（CCCGs）没有此类作用。普通碳酸钙D_3（CC）和复方碳酸钙颗粒（CCCGs）对血清胃动素（MTL）或胃泌素（GAS）水平均无显著影响（图5c）。因此，普通碳酸钙D_3（CC）能减少胃排空和肠推进，存在潜在的腹胀、便秘风险。

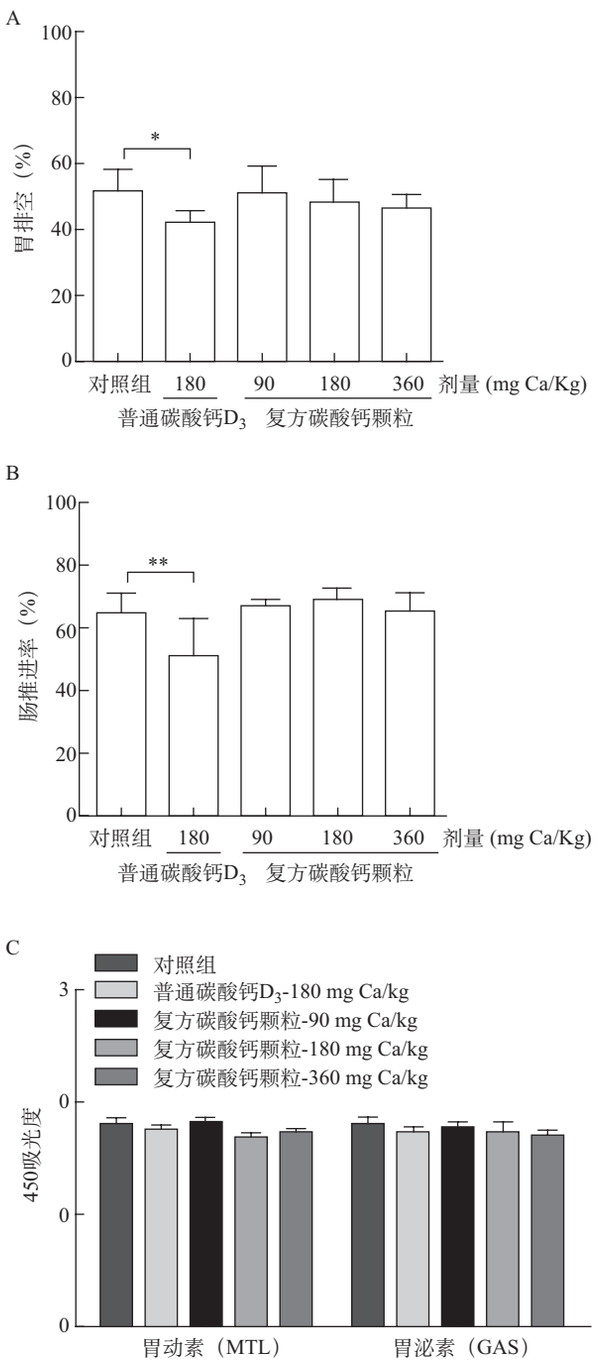

图5 普通碳酸钙D3（CC）或复方碳酸钙颗粒（CCCGs）对胃排空（n=8）A、肠推进（B）及血清胃动素（MTL）、胃泌素（GAS）水平的影响（C）。数据表示为平均标准差（SD）。$^{*}p<0.05$，$^{**}p<0.01$

3 讨论

碳酸钙D_3作为钙补充剂，因其钙含量高、成本低而被广泛用。最常见的三种钙补充剂的钙吸收率：柠檬酸钙>葡萄糖酸钙>碳酸钙[26]。目前常用的钙补充剂的局限性包括胃肠道不良反应，部分表现为胃排空时间和肠道运动的改变[27-29]，以及由于胃pH值的波动和可与钙离子结合的食物成分存在，从而引起钙吸收的变化。在本研究中，我们对创新药物——复方碳酸钙颗粒的主要药代动力学和生物学特性进行了研究，重点研究其溶解度、吸收率以及对胃肠动力的影响。比较了复方碳酸钙颗粒（CCCGs）与普通碳酸钙D_3的效果。复方碳酸钙颗粒（CCCGs）的创新是为了在体外将不溶于水的碳酸钙转化成易溶于水的柠檬酸钙络合物且体外释放CO_2，摆脱胃酸的依赖并且还可能减少胃肠动力等因素对钙吸收的不良影响程度。

以奥美拉唑进行胃酸抑制的小鼠为实验对象，研究了复方碳酸钙颗粒（CCCGs）的药代动力学特性，发现：复方碳酸钙颗粒（CCCGs）的体内吸收，并不受胃酸分泌抑制的影响[20]。出乎意料的是，与对照组相比，给予普通碳酸钙D_3（CC）后，奥美拉唑模型的小鼠对钙的实际吸收更低（图2）。而给予复方碳酸钙颗粒（CCCGs）组小鼠，出现钙的吸收不受胃酸分泌的影响，且结果还显示给予不同剂量组的复方碳酸钙颗粒（CCCGs）组小鼠之间，其钙的吸收无显著差异。在体外，复方碳酸钙颗粒（CCCGs）在酸性或水溶液中都是水溶性的。显然，其独特的配方和工艺，使其不受体内pH值变化的影响，钙的吸收依然稳定。这种稳定效应似乎不是特异性的，植酸和单宁是已知的两种可以沉淀钙的阴离子化合物[19,20]，它们的存在抑制了普通碳酸钙D_3的吸收，但没有抑制复方碳酸钙颗粒（CCCGs）的吸收（图3）。

也许本研究最重要的发现是复方碳酸钙颗粒（CCCGs）的胃肠道不良反应比普通碳酸钙D_3（CC）少（图5）。与预期一样，普通碳酸钙D_3（CC）延迟胃排空，抑制肠道推进，但复方碳酸钙颗粒（CCCGs）没有这些副作用。两者都没有改变血清中胃肠激素-胃动素（MTL）或胃泌素

GAS的水平（图5C），似乎排除了MTL和GAS是普通碳酸钙D_3（CC）对胃肠动力作用的因素。

假设钙的补充益处是有争议的[11]。人们已经开始关注增加心血管不良事件风险的可能性。然而，目前流行的观点是，如果适当使用钙补充剂，一般来说是安全的[11,12]，并被认为对骨骼健康以及骨结构或代谢异常的情况（如骨折和骨质疏松症[13]）是非常重要的。本文所述的创新药物复方碳酸钙颗粒（CCCGs）由于其稳定的钙吸收特性和较少的不良反应特性，在治疗上可能优于那些常用的钙补充剂，如普通碳酸钙D_3（CC）。

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