Decalcification of Calcium Polycarbophil in Rats

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The in vivo decalcification of calcium polycarbophil was examined. The decalcification ratio of [45Ca]calcium polycarbophil in the stomach after oral dosing to rats was more than 70% at each designated time and quite closely followed the in vitro decalcification curve, indicating that the greater part of the calcium ion is released from calcium polycarbophil under normal gastric acidic conditions. The residual radioactivity in rat gastrointestinal was nearly equal to that after oral administration of either [44Ca]calcium chloride or [45Ca]calcium chloride–polycarbophil. The serum level of radioactivity was nearly equal to that after oral dosing of [44Ca]calcium lactate. These results indicate that the greater part of orally administered calcium polycarbophil released calcium ions to produce polycarbophil in vivo.

Key words calcium polycarbophil; polycarbophil; decalcification; calcium absorption; rat

Calcium polycarbophil is the calcium salt of polycarbophil, a high-molecular water-absorbing polymer. In our previous in vitro study, we demonstrated that calcium polycarbophil released calcium ions under acidic conditions, and the resultant polycarbophil had the ability to absorb at least 70 times its original weight of water under neutral conditions. On the basis of these physicochemical properties, as well as its safety and effectiveness, this compound has been used for the treatment of both constipation and diarrhea. Though decalcification of calcium polycarbophil is necessary for the appearance of its pharmacological effects, it has not yet been examined in vivo.

The purpose of the present study was to clarify the decalcification of calcium polycarbophil in vivo. We evaluated the decalcification of calcium polycarbophil in the rat stomach using the 45Ca-labelled compound ([45Ca]calcium polycarbophil) and measured the residual radioactivity in the rat gastrointestinal tract. In addition, the serum radioactivity levels after oral administration of [45Ca]calcium polycarbophil in rats were examined in comparison with those after oral dosing of [45Ca]calcium lactate, a calcium supplement which is completely dissolved under acidic conditions.

MATERIALS AND METHODS

Materials Polycarbophil was prepared by Hokuriku Seiyaku Co., Ltd. from calcium polycarbophil (B. I. Chemicals, U.S.A.). [45Ca]Calcium polycarbophil was synthesized in our laboratory from [44Ca]calcium chloride (sp. act 37 MBq/0.8 ml, Du Pont NEN) and polycarbophil. [45Ca]Calcium lactate was also synthesized in our laboratory from [45Ca]calcium chloride. All other reagents were of analytical grade.

Animals Male Wistar rats weighing 200 to 300 g were used. Animals were acclimatized to the breeding environment for at least 1 week and were starved for at least 20 h prior to use.

Synthesis of [45Ca]Calcium Polycarbophil and [44Ca]-Calcium Lactate Initially [45Ca]calcium chloride was diluted with calcium chloride and dissolved in water. This solution was stirred using a magnetic stirrer and a two-fold molar excess of sodium carbonate solution was added dropwise. Stirring was continued for 1 h, then the produced [45Ca]calcium carbonate was collected by filtration.

[45Ca]Calcium polycarbophil was synthesized as follows: Polycarbophil and water were placed in a three-necked flask and the polymer was allowed to swell at room temperature. A labo-stirrer (LR-51B, Yamato Scientific Co., Ltd., Japan) was fitted to the flask, and [44Ca]calcium carbonate suspension was added dropwise with stirring at 60 °C in an oil bath. Stirring was continued overnight, then the product was collected by filtration on a filter cloth and dried at 105 °C overnight. It was ground to a powder with a mortar and pestle and dried at 200 °C for 1 h. The specific activity of synthesized [45Ca]calcium polycarbophil was 10.4 kBq/mg and the calcium content was 20.3%.

[45Ca]Calcium lactate was synthesized as follows: Lactic acid and water were placed into a three-necked flask equipped with a condenser. [45Ca]Calcium carbonate suspension was added dropwise to the flask with stirring at 60 °C. After stirring for 1 h, the reaction mixture was passed through filter paper and the filtrate was freeze-dried. The product was dried at 80 °C for 1 h and at 120 °C for 4 h, then ground to a powder. The specific activity of synthesized [45Ca]calcium lactate was 14.3 kBq/mg and the calcium content was 15.1%.

Dose Preparation and Administration [45Ca]Calcium polycarbophil and polycarbophil were emulsified with physiological saline, and [45Ca]calcium chloride and [45Ca]calcium lactate were dissolved in physiological saline. Each compound containing 45Ca was administered orally to rats at a dose of 20 mg calcium equivalent/5 ml/kg. As calcium polycarbophil comprised 20% calcium and 80% polycarbophil, an oral dose of polycarbophil was 80 mg/kg. In the residual radioactivity test and the in vivo decalcification rate test, ileocecal ligature with a silk suture was carried out prior to the dosing.

In Vivo Decalcification Test [45Ca]Calcium poly-
carbophil was administered orally to rats. At the designated time (0.5, 1, 2, 6 h), the stomach was isolated and the pH of its content was measured. The content was immediately washed out with a Britton-Robinson buffer of the measured pH, and then centrifuged. The resulting supernatant was used to determine the quantity of calcium ion released from $[^{45}\text{Ca}]$calcium polycarbophil. Then, the pellet was extracted with 0.1 N hydrochloric acid (HCl) solution, and the supernatant after centrifugation was used to determine the residual calcium, representing non-decalcified $[^{45}\text{Ca}]$calcium polycarbophil.

**Residual Radioactivity Test** The gastrointestine, from gullet to ileum, was isolated at a designated time after dosing of $[^{45}\text{Ca}]$calcium polycarbophil, $[^{45}\text{Ca}]$calcium chloride or $[^{45}\text{Ca}]$calcium chloride + polycarbophil to rats. The gastrointestine was opened, washed internally with 0.1 N HCl and extracted with 0.1 N HCl. The combined washing and extract was centrifuged and an aliquot of the supernatant was taken to determine the radioactivity.

**Serum Level Study** Blood was taken at the designated time from the tail vein after dosing of $[^{45}\text{Ca}]$calcium polycarbophil or $[^{45}\text{Ca}]$calcium lactate to rats, and was allowed to stand at room temperature for 1 h, then centrifuged at 2000 $\times$ g for 10 min to obtain serum.

**Analysis** Radioactivity was measured using a liquid scintillation counter (LSC-1000, Aloka). Data are given as the mean ± standard error of the mean (S.E.) for 4 to 5 animals. The data obtained in the residual radioactivity test were analyzed using Scheffe's analysis of variance and data from the serum level study, with the unpaired t-test. The criterion for a statistically significant difference was $p<0.05$.

**RESULTS**

**In Vivo Decalcification Rate** Figure 1 shows the decalcification ratio in the stomach after oral administration of $[^{45}\text{Ca}]$calcium polycarbophil to rats. Decalcification ratios were more than 70% at all times and were very similar to the in vitro decalcification ratios reported previously.

**Residual Radioactivity in the Gastrointestinal** To examine the absorption of calcium ions derived from $[^{45}\text{Ca}]$calcium polycarbophil, the time-course of residual radioactivity in the gastrointestinal after oral administration of $[^{45}\text{Ca}]$calcium polycarbophil was determined. As shown in Fig. 2, the residual radioactivity decreased in a time-dependent manner.

Figure 3 shows the percentages of residual radioactivity in the gastrointestinal after oral administration of $[^{45}\text{Ca}]$ labelled compounds to rats. The residual percentage after oral administration of $[^{45}\text{Ca}]$calcium polycarbophil was not significantly different from that after $[^{45}\text{Ca}]$calcium chloride dosing or after concomitant dosing of $[^{45}\text{Ca}]$calcium chloride and polycarbophil (Scheffe's analysis of variance).

**Serum Levels of Calcium Derived from Calcium Polycarbophil** In order to clarify the extent of in vivo decalcification, serum radioactivity levels in rats after oral administration of $[^{45}\text{Ca}]$calcium polycarbophil were compared to those after oral dosing of $[^{45}\text{Ca}]$calcium lactate. As shown in Fig. 4, serum radioactivity levels after...
the same as that in vitro, indicating that the greater part of the calcium ion is released from calcium polycarboPhil under normal gastric acidic conditions.

As calcium is absorbed from the intestine in an ionized form, we considered that the absorption of calcium could be used as an indicator of the decalcification of calcium polycarboPhil. The residual radioactivity in the gastrointestinal after oral administration of [\(^{45}\)Ca]calcium polycarboPhil to rats decreased in a time-dependent manner, indicating that calcium released from calcium polycarboPhil was absorbed from the rat intestine. The residual radioactivity in rat gastrointestinal after oral administration of [\(^{45}\)Ca]calcium polycarboPhil was comparable both to that after oral dosing of [\(^{45}\)Ca]calcium chloride and to that after concomitantly oral dosing of [\(^{45}\)Ca]calcium chloride and polycarboPhil.

Calcium lactate is soluble in water, and in a buffer adjusted to pH 2.0—5.6 with hydrochloric acid and containing inorganic matter from gastric juice. Consequently, [\(^{45}\)Ca]calcium lactate is expected to be completely dissolved in the stomach after oral administration. The area under the curve (AUC) values of serum radioactivity levels after oral dosing of [\(^{45}\)Ca]calcium polycarboPhil were not significantly different from those after oral dosing of [\(^{45}\)Ca]calcium lactate, indicating that the greater part of [\(^{45}\)Ca]calcium polycarboPhil is decalcified in vivo, as calcium lactate dissolves completely under acidic conditions.

**DISCUSSION**

As the decalcification of calcium polycarboPhil is necessary for the appearance of its pharmacological effects, we tested it in vivo using a \(^{45}\)Ca-labelled compound. The decalcification ratios of calcium polycarboPhil in rat stomach were more than 70% at all times and essentially

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**REFERENCES**