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Research Papers

Labelling procedure of antacid preparations using ^{99m}Tc-pyrophosphate

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Summary

A new radioisotopic labelling method of two antacid preparations containing magnesium and aluminium ions has been designed. ^{99m}Tc-pyrophosphate was used as tracer agent and, owing to the high labelling efficiency obtained (> 98%), no purification step was necessary. The stability of both labelled antacids was tested as well as their antacid capacity and their protein-binding capacity compared with the unlabelled samples. All these tests were satisfactory enough to consider this ^{99m}Tc labelling method a good tool for preparing labelled antacids that contain Al^{3+} and/or Mg^{2+} and so to evaluate the antacid gastric emptying pattern by means of scintigraphic studies.

Introduction

A large part of antacid preparations are composed mainly of different mixes and structures of magnesium and aluminium salts. In order to evaluate the efficacy of different antacids, a variety of in vitro and in vivo tests are usually performed. One of these in vivo tests is the evaluation of their gastric emptying rate (Fordtran et al., 1973).

To measure this parameter as well as to study its biodistribution in the gastrointestinal tract by means of scintigraphic methods, a reliable, convenient radiolabelled antacid is required. A method of radiolabelling aluminium hydroxide particles with ^{113m}In was described by Jenkins et al. (1983). The labelling method described in that study was laborious and time-consuming since it required six washing steps to remove any traces of ammonia. Moreover, the ^{113m}In is not the appropiate isotope for the Anger gamma cameras used in nuclear medicine. ^{111}In has more suitable physical properties than ^{113m}In and could serve as a tracer using the above-mentioned method, but ^{99m}Tc is preferred because it is the most readily available and proper isotope for in vivo studies.

It is known that ^{99m}Tc(IV) forms a very stable Tc-pyrophosphate (PYP) complex (Steigmand and Richards, 1974). This radiopharmaceutical has

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been used as a bone scintigraphy agent because of its capacity to associate with the calcium atoms on the hydroxyapatite surface to create a stable fixation (Pinkerton et al., 1985). Based on that fact, we designed a method of labelling of antacids containing magnesium and aluminium ions, assuming that Tc-$(PYP)_n$ – (Mg^{2+}) and Tc-$(PYP)_n$-$(Al(OH)^{2+})$ species are formed.

TABLE 1

Labelling efficiencies (%) as a function of the volume of ^{99m}Tc-PyP added to 10 ml of antacid

	Volume of ^{99m}Tc-PyP (ml)				
	0.1	0.25	0.5	1	5
Magaldrate	99.0	99.0	98.9	98.9	98.8
Maalox^R	98.9	98.6	98.6	97.8	90.9

Materials and Methods

Antacids

Commercial antacid dosage forms were used: magaldrate (Boehringer-Mannheim Labs) is a chemical combination of aluminium hydroxide and magnesium hydroxide having a hydrotalcite-like structure, with sulphate as the major interlayer anion and carbonate present in the interlayer space (Serna and White, 1978). 10 ml containing 800 mg of magaldrate has a theoretical neutralization capacity of 26.2 meq./HCl (Guterman et al., 1986). Maalox^R (Rorer Laboratories) is a physical mixture of aluminium hydroxide-magnesium hydroxide gel in suspension dosage form which, per 10 ml, contains 450 mg of aluminium hydroxide and 40 mg of magnesium hydroxide with a theoretical neutralization capacity of 26.0 meq./HCl (Guterman et al., 1986).

Labelling

Antacids were labelled at room temperature as follows:

Preparation of ^{99m}Tc-pyrophosphate (PyP) 1110 MBq of ^{99m}Tc-pertechnetate with a total volume of 5 ml from a just eluted generator were added to a vial containing 15 mg of $Na_4P_2O_7 \cdot 10H_2O$ and 2 mg of $SnCl_2 \cdot 2H_2O$ in lyophilized form (Pyrotec, Sorin). This volume was adjusted with 0.15 M NaCl. After gentle mixing, the resulting solution was allowed to stand for at least 5 min.

Preparation of the ^{99m}Tc-PyP-antacid 10 ml of the antacid, magaldrate or Maalox^R, were put in a glass vial. To ascertain the appropriate volume of ^{99m}Tc-PyP to add to the antacid, tests were performed with 0.1, 0.25, 0.5, 1 and 5 ml. As can be seen in Table 1, acceptable labelling efficiencies were achieved from 0.1 to 1 ml. We chose 0.5 ml in order to equilibrate the labelling eficiency with the radioactive concentration of the tracer; 0.5 ml of the ^{99m}Tc-PyP was added dropwise to the antacid. The resulting suspension was gently mixed and left to stand for 5 min.

Verification that ^{99m}Tc-PyP forms stable complexes with Mg and Al cations was established as follows: 1 ml of 2 N NaOH was added to three polystyrene tubes containing 0.1 ml of ^{99m}Tc-PyP each plus 2 ml of $MgCl_2$ saturated solution, 2 ml of 0.3 M $AlCl_3$ and 2 ml of 0.15 M NaCl. The three tubes were then shaken and centrifuged ($1000 \times g$ for 10 min). The liquid supernatant and the pellet formed were separated and counted in a gamma-counter. The stability of the labelled antacids at room temperature at 1, 2, 3, 4, 5, 6, and 24 h was tested as follows: 2 ml of 0.15 M NaCl were added to 0.5 ml of the labelled antacid, then mixed and centrifuged at $1000 \times g$ for 10 min. The supernatant containing the ^{99m}Tc not bound to the antacid was separated from the ^{99m}Tc-PyP-antacid precipitate and both phases counted in a gamma-counter 24–48 h later.

The stability of the labelled compounds in contact with gastric juice was tested as follows: 0.2 ml of labelled antacid were added to 2 ml of gastric juice adjusted to range from pH 1.5 to 7, mixed and incubated at 37 °C for 10, 30, 60 and 120 min. After incubation, the unaltered ^{99m}Tc-PyP-antacid was measured by centrifuging at $1000 \times g$ for 10 min and separating and counting both phases as above.

Antacid capacity of the labelled antacids was compared with that of the unlabelled antacids as follows: 2 ml of antacid (unlabelled or labelled) was added under continuous stirring to a glass recipient containing 10 ml of 0.4 M HCl and 10 ml of 0.15 M NaCl. The pH was measured and the

time necessary to reach pH 3.4, 3.5 and 3.6 was recorded. In order to ascertain whether the labelling of the antacids would change the binding to proteins 10 μl of ^{125}I-HSA (2 μg human serum albumin) was added to 0.5 ml of antacid (unlabelled or labelled), incubated at 37 °C for 5 min, 2 ml of 0.15 M NaCl was added and then centrifuged at 1000 × *g* for 5 min. The activity of the solid and the liquid phase was measured.

To assess whether the labelling procedure might induce changes in the size of the colloidal particles and the formation of macroaggregates of the antacids, dark-field microscopy of the labelled and unlabelled antacids was performed.

Results

Table 2 shows that ^{99m}Tc-PYP forms insoluble complexes with Mg^{2+} and Al^{3+} or $Al(OH)^{2+}$. Labelling efficiency in 35 experiments was always more than 98%.

Both ^{99m}Tc-PYP-antacids remained practically unaltered for at least 6 h after preparation: more than 97% of the radioactivity was found to be bound to the precipitated antacid. At 24 h the labelling stability was still more than 90%. The labelling stability of the ^{99m}Tc-PYP-antacids in contact with gastric juice is shown in Tables 3 and 4. The radioactivity bound to magaldrate was similar in all pH media and the average values ($n = 3$) were equal to 96% or more. Labelled Maalox^R seems slightly less stable than magaldrate, especially at pH 1.5 at 60 min after labelling.

Antacid capacity of the labelled antacids as compared to the unlabelled forms is shown in Table 5. No significant differences were observed in the time needed to achieve a fixed pH between labelled and unlabelled antacids.

TABLE 2

Percent of total radioactivity in the precipitate formed after the addition of 2 ml of the salt solutions with 0.1 ml of ^{99m}Tc pyrophosphates and 1 ml of 2 N NaOH

$MgCl_2$ saturated	99.0% cpm in the precipitate
0.3 M $AlCl_3$	65.3% cpm in the precipitate
0.15 M NaCl	0.4% cpm in the test tube

TABLE 3

Percent of radioactivity bound to magaldrate in contact with gastric juices at pH 1.5, 2, 3 and 7 at different times (n = 3, mean ± SD)

Time (min)	pH 1.5	pH 2	pH 3	pH 7
10	97.7±0.1	97.5±0.2	97.4±0.2	98.4±0.1
30	97.7±0.2	97.2±0.2	97.2±0.2	98.3±0.1
60	97.5±0.2	97.0±0.4	97.1±0.3	98.5±0.4
120	96.8±0.5	97.0±0.4	97.0±0.2	98.5±0.4

Table 6 lists the percentages of ^{125}I-HSA bound to the antacids in the five experiments performed. Significant differences in the protein binding capacity between the unlabelled and the ^{99m}Tc-labelled antacids were not found. Dark-field microscopy observations did not disclose the presence of any macroaggregates, in either labelled or unlabelled antacids, and the particulate size was about 0.5 μm for Maalox^R and between 2 and 7 μm for magaldrate.

Discussion

Radionuclide imaging techniques are well established for the monitoring of gastric emptying (Calabuig et al., 1988; Carrió et al., 1989). Some studies have been undertaken using ^{113m}In-labelled antacids by means of gamma camera imaging (Bennet et al., 1984; May et al., 1984). The labeling method used in those studies involves a coprecipitation of indium hydroxide with aluminium hydroxide in ammonia medium with six washing steps in order to eliminate any traces of ammonia

TABLE 4

Percent of radioactivity bound to Maalox in contact with gastric juices at pH 1.5, 2, 3 and 7 at different times (n = 3, mean ± SD)

Time (min)	pH 1.5	pH 2	pH 3	pH 7
10	93.1±0.1	96.5±0.1	96.6±0.1	95.6±0.1
30	92.2±0.2	94.9±0.1	96.0±0.3	95.2±0.2
60	90.0±0.2	94.5±0.1	95.3±0.3	94.8±0.2
120	85.2±0.4	91.8±0.9	94.8±0.3	94.1±0.2

TABLE 5

Antacid capacity of the labelled antacids [time (in s) to reach fixed pH was recorded (mean ± SD); see details in text]

	pH 3.4 ($n = 5$)	pH 3.5 ($n = 5$)	pH 3.6 ($n = 5$)
Unlabeled magaldrate	13 ± 1.1	25 ± 1.4	40 ± 2.7
^{99m}Tc-magaldrate	13 ± 1.0	25 ± 1.5	39 ± 2.8
Unlabelled Maalox F	56 ± 3.0	61 ± 3.6	68 ± 4.3
^{99m}Tc-Maalox F	57 ± 2.9	59 ± 3.6	65 ± 4.5

in the labeled antacid. Moreover, the physical properties of ^{113m}In are not optimal for scintigraphics purposes.

Ideally, the antacid should be radiolabeled with an isotope of one of its components, but none of the elements has a readily available suitable radioisotope for use in humans.

In this study, we have developed a new method of labeling antacids using ^{99m}Tc as a tracer. This available isotope has better physical properties than ^{113m}In and forms a stable ^{99m}Tc-pyrophosphate complex that is able to bind Mg^{2+} and $Al(OH)^{2+}$ that are present in most antacid formulations.

The ^{99m}Tc-PYP-magaldrate complex has satisfactory stability even in contact with gastric juices at acidic pH and has the same antacid capacity compared with the unlabelled forms. ^{99m}Tc-PYP-Maalox has good stability until pH 2 at 60 min. However, at pH 1.5 the ^{99m}Tc bound to the antacid falls to near 90% at 60 min after labelling. Nevertheless, the overall stability results can be considered satisfactory for both antacids.

Dark-field microscopy demonstrated that this labelling method did not lead to the formation of macroaggregates which might show an emptying pattern different from that of the unlabelled antacids. The results obtained from HSA binding suggest that this labelling method did not change its binding capacity to the proteins. These unchanged antacid properties could be explained by the fact that only 1 atom of ^{99m}Tc was estimated to be in contact for $1-1.5 \times 10^7$ atoms of Mg and $5-5.5 \times 10^6$ atoms of Al. We used ^{99m}Tc-magaldrate and ^{99m}Tc-Maalox^R labelled as described here in order to evaluate the gastric emptying of both antacids (Monés et al., 1990).

TABLE 6

Percent of ^{125}I-HSA bound to magaldrate and Maalox ($n = 5$, mean ± SD) (see details in text)

	Magaldrate	Maalox
Unlabelled	99.1 ± 0.1	78.9 ± 0.7
^{99m}Tc-labelled	98.6 ± 0.1	78.5 ± 0.8

The ^{99m}Tc-PYP labelling method described in the present study is easier and faster than that using indium isotopes, since it does not involve centrifugation or washing. Furthermore, ^{99m}Tc has advantages such as better physical properties for accurate detection, less radiation delivered to patients and lower cost. Therefore, it can be performed in any nuclear medicine department without any special requirements.

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