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Stomach outline visualization in gastrointestinal transit studies using scintigraphy

N. J. COURSE, J. M. NEWTON, M. D. SHORT*, *The School of Pharmacy, University of London, Brunswick Square, London WC1N 1AX, UK and* **Department of Medical Physics, University College Hospital, Gower Street, London WC1E 6AU, UK*

Abstract—A major disadvantage of gamma scintigraphy in gastrointestinal transit studies is the inability to provide adequate delineation of anatomical details. As an aid to the important requirement of outlining the stomach to ensure accurate quantification of the time at which material empties from this organ, a new technique is described, using short half-life ^{81m}Kr gas to provide clearer identification of the stomach outline and position.

It is often necessary in research and development, and for regulatory purposes, to have direct information on the position and integrity of a dosage form in-vivo. Gamma scintigraphy is an important method of achieving this whereby a drug or delivery system is labelled with a suitable gamma-emitting radionuclide and the position of the system and its release characteristics are followed using a gamma camera. Unfortunately, such a technique does not provide direct anatomical detail.

Knowledge of gastrointestinal transit time and, particularly, gastric emptying time, is important when formulating a sustained-release and enteric coated preparation since these parameters will determine the absorption profile and bioavailability characteristics of the dosage form.

Gastric emptying studies require that the position of the stomach must be accurately delineated throughout the study period. This has been achieved, with application of a dual energy acquisition system, by giving a ^{99m}Tc DTPA solution with a ^{111}In-labelled dosage form (Feely & Davis 1989) or vice versa. It is usually the drink that is labelled with the lower energy radionuclide ^{99m}Tc, since it will not interfere with the dosage form images and no "down-scatter" count corrections are necessary.

Pellets labelled with ^{99m}Tc have also been administered to outline the stomach for a ^{111}In-labelled single-unit dosage form (Davis et al 1988).

If the operating system requires that the dosage form and the drink need to be labelled with the same radionuclide, e.g. ^{99m}Tc, this may be achieved in single-unit studies by administering the dosage form with a higher activity level of the drink. This would not be suitable for multiple units since quantitative information is required and the drink would interfere with the dosage form counts obtained.

Hardy et al (1985) claimed that administration of pellet systems gives a good outline of the gastro-ileal and the ileo-caecal junctions and therefore it may not be necessary to give a radiolabelled drink. However, Clarke (1989) found that, although pellets gave a good ileo-caecal outline, this was not the case for the stomach region of interest.

Devereux (1987) and Clarke (1989) have administered a ^{99m}Tc DTPA solution at the end of the study period, but found that this was not a true representation of the stomach outline at the time of emptying of the dosage form. The stomach shape and size vary amongst individuals, but also, more importantly, in the same individual at different times of the day.

Maublant et al (1987) administered a radiolabelled solution 24 h after the dosage form so it would not interfere with the gastric image produced. However, it may not be convenient to call subjects back for a second day nor would the data obtained relate to the stomach position at the time of the original study and, while a long-lived radionuclide will continue to give counts from the stomach, emptying of the fluid will not give a total stomach outline.

Some of the problems encountered above may be eliminated by using a short-lived radionuclide to provide stomach delineation without interfering with other radionuclides used in the study. Such a system is provided by the aqueous eluate from ^{81m}Kr gas generator (Medical Research Council, Cyclotron Unit, Hammersmith Hospital, London, UK) (Fig. 1) to outline the stomach. This technique has been investigated as part of a gastrointestinal transit study on single-unit dosage forms using healthy male volunteers, which was approved by the Ethical Committee, University College Hospital, London.

Correspondence: J. M. Newton, The School of Pharmacy, 29–39 Brunswick Square, London WC1N 1AX, UK.

Materials and methods

^{81m}Kr is the daughter product of the cyclotron-produced radionuclide ^{81}Rb, which decays with a half-life of 4·6 h. The generator is pre-prepared by loading the parent ^{81}Rb onto a small ion exchange column contained in a radiation shield. The ^{81m}Kr is eluted by passing purified water through the column and the eluate may be mixed with water and orange-juice to make a palatable drink. The single gamma emission from ^{81m}Kr is 190 kev (65% abundance), which is ideal for gamma camera imaging.

It is important for radiation dosimetry that checks are made for absence of the parent radionuclide ^{81}Rb in the elution from the generator. The initial eluate contains a portion of ^{81}Rb. However, after two 50 mL volumes of purified water have been passed through the column, the third eluate contains an insignificant amount of ^{81}Rb (sample counted on a calibrated probe).

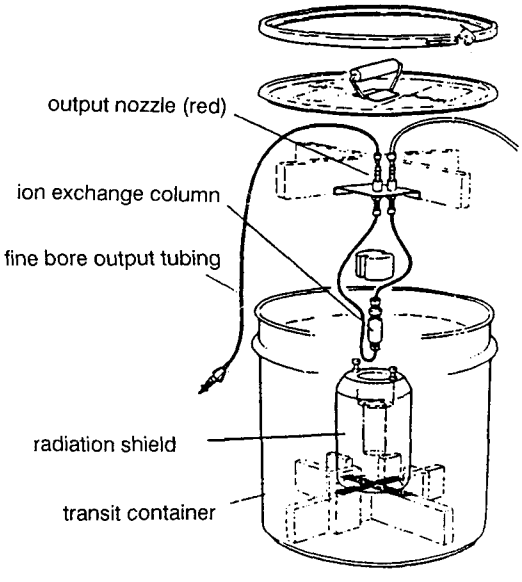

FIG. 1. ^{81m}Kr gas generator.

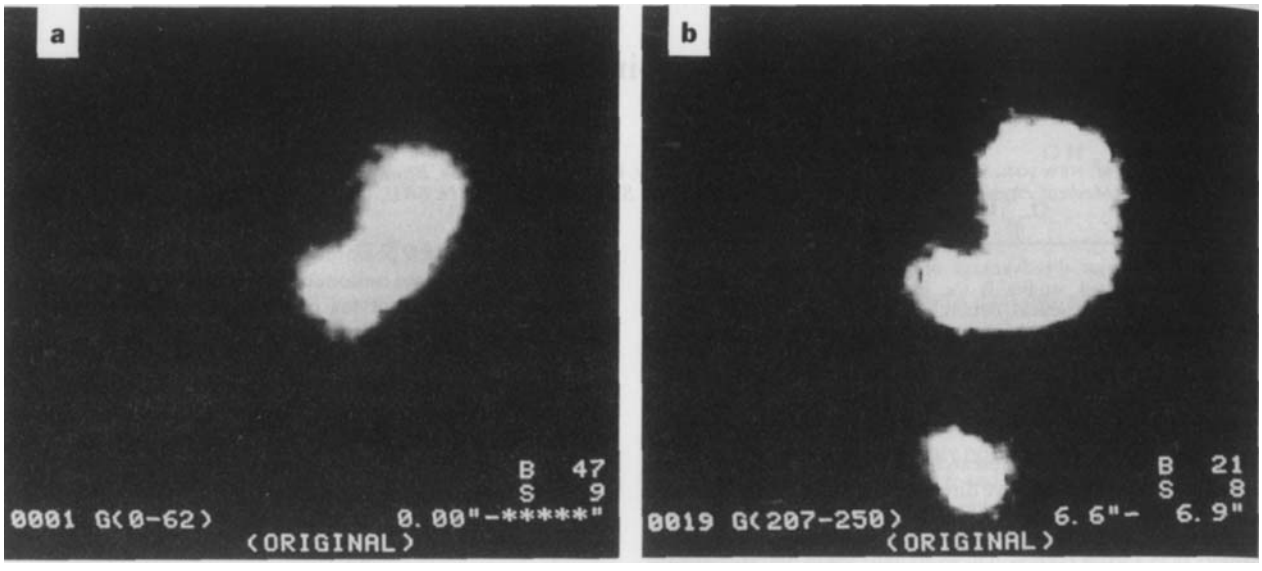

FIG. 2. a. Outline obtained by drinking a 200 mL ^{81m}Kr labelled drink at the start of a gastrointestinal transit study. b. Outline obtained from the same subject after a 879 kCal meal. A ^{99m}Tc tablet that has emptied from the stomach can be observed below the region of interest.

An outline of the stomach is obtained from an acquired image over 1 min using a Siemens double-headed gamma camera (Rotacamera). Since ^{81m}Kr has a half-life of only 13 s at the end of the 1-min acquisition it has decayed almost completely and therefore does not interfere with the counts from other radionuclides used to label any co-administered dosage form, e.g. ^{99m}Tc, ^{111}In.

However, because of the very short half-life, it is essential that the labelled drink is consumed rapidly after elution to ensure adequate stomach image statistics and hence delineation. If the time between mixing of the eluate with water and orange squash and consumption of the drink was more than about 15 s it was observed that no outline of the stomach was obtained due to the ^{81m}Kr decaying to undetectable levels.

The approximate activity of the ^{81m}Kr eluate was determined by comparing the count obtained on the gamma camera with that obtained for a known activity of ^{99m}Tc (since ^{99m}Tc has similar gamma energy characteristics to ^{81m}Kr). This comparative measurement showed that, at the usual time of the first ^{81m}Kr drink, the radioactivity administered was approximately 37 MBq.

The volume required to provide a good outline of the stomach is about 200 mL. A smaller volume gives only a partly outlined stomach image and conversely, a larger volume would be impractical for the subject to consume within the short time period required. The volume of the stomach of a fasting man is 50 mL or less and it has been shown that the drinking of 225 mL of water raises the pressure within the stomach by less than 2 mm Hg (Davenport 1973). Rees et al (1979) studied the response of the fasted human stomach to the ingestion of liquid meals and showed that 400 mL of 0·15 M saline did not usually disturb the ongoing fasting motor activity. It was, therefore, assumed in our studies that consuming 200 mL of purified water containing 5 mL of orange squash did not alter the initial fasting state of our volunteers.

Results and discussion

Examples of the images obtained are shown in Figs 2, 3. Fig. 2a shows the outline obtained by drinking the 200 mL ^{81m}Kr-labelled drink at the start of a gastrointestinal transit study day (subject fasted from midnight before). Fig. 2b shows the outline obtained in the same subject after a 879 kCal meal. Fig. 3 shows three ^{81m}Kr images from 200 mL radiolabelled drinks consumed at different time intervals. The marker and stomach regions of interest (ROI) were drawn using a lightpen. The stomach ROI was obtained by using the ^{81m}Kr image at t = 0 as a guideline and then viewing the subsequent tablet images. The marker consisted of a point source of ^{99m}Tc taped to the side of the volunteer and the marker ROI was drawn from the first frame of the study. Hence any volunteer movement throughout the study was observed. It can be seen, from the images obtained, how the shape and position of the stomach may vary within a subject depending on the time and their food status. At t = 0, a co-administered ^{99m}Tc tablet is in the stomach. At t = 250 and 445 min the ^{99m}Tc tablet can be seen below the stomach outline (the ^{99m}Tc tablet appears in the ^{81m}Kr image since it has a similar gamma ray energy level). The fourth image in this figure is of a

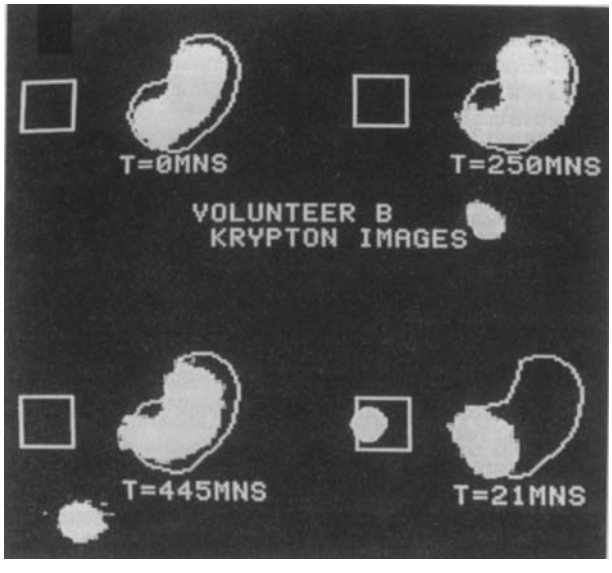

FIG. 3. Outlines from three ^{81m}Kr images at t = 0, 250 and 445 min after commencement of the study. The fourth image is of a co-administered ^{99m}Tc tablet and a corresponding ^{99m}Tc marker.

co-administered ^{99m}Tc tablet, which is located in the stomach at t=21 min. The ^{99m}Tc marker can also be seen in this image.

The radiation dose to the stomach wall from one administration of 37 MBq ^{81m}Kr drink is 0·007 mGy (Medical Internal Radiation Dose Dosimetry System, Society of Nuclear Medicine, USA). Because of the low doses involved, a number of radiolabelled drinks may be given throughout a gastrointestinal transit study day to determine the position of the stomach. This will be particularly useful for multiple unit dosage forms where quantitative data for the percentage of the dosage form remaining in the stomach with time are required and hence a precise knowledge of stomach delineation and location is available throughout the study.

References

Clarke, G. M. (1989) Gastrointestinal Transit of Spherical Granules of Differing Size and Density. Ph.D. Thesis, University of London

Davenport, H. W. (1973) In: Physiology of the Digestive Tract. 3rd edn, Year Book Medical Press, Chicago, pp 45, 49

Davis, S. S., Nornig-Christensen, F., Khosla, R., Feely, L. C. (1988) Gastric emptying of large single unit dosage forms. J. Pharm. Pharmacol. 40: 205–207

Devereux, J. E. (1987) Gastrointestinal Transit of Multiple Unit Dosage Forms. Ph.D. Thesis, University of London

Feely, L. C., Davis, S. S. (1989) Correlation of phenylpropanolamine bioavailability with gastrointestinal transit by scintigraphic monitoring of ^{111}In-labelled hydroxypropyl methylcellulose matrices. Pharm. Res. 6: 274–278

Hardy, J. G., Wilson, C. G., Wood, E. (1985) Drug delivery to the proximal colon. J. Pharm. Pharmacol. 37: 874–877

Maublant, J. C., Sournac, M., Aiache, J. M., Veyre, A. (1987) Dissolution rate and transit times of 99m Tc DTPA labelled tablets. Eur. J. Nucl. Med. 15: 143–145

Rees, W. D. W., Go, V. L. W. Malageleda, J.-R. (1979) Simultaneous measurement of antroduodenal motility, gastric emptying and duodenogastric reflux in man. Gut 20: 963–970

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Contractions induced by phenylephrine and noradrenaline are differently affected by endothelium-dependent relaxation in rat aorta

M. AUGUET, R. TRICOCHE*, P. BRAQUET, *Institut Henri Beaufour Research Labs, ZA de Courtaboeuf, 1 ave des Tropiques, 91952 Les Ulis Cedex, France, * CNRS UA 290, Faculté des Sciences, 40 avenue du Recteur Pineau, 86022 Poitiers, France*

Abstract—In rings of rat aorta precontracted with phenylephrine (10 μM) or noradrenaline (10 μM), addition of carbachol (10 μM) produced an endothelium-dependent relaxation. However, regardless of the concentration of agonist tested, both the intensity and duration of the relaxation were significantly less when noradrenaline, rather than phenylephrine, was used as the precontracting agent. The different responses observed do not appear to be related to destruction of endothelium-derived relaxing factor by autoxidation of noradrenaline since neither EDTA (30 μM) nor superoxide dismutase (30 units mL^{-1}) improved the relaxation to carbachol. In addition, in endothelium-free rings, the noradrenaline (1 μM)-induced contraction was less sensitive than the phenylephrine (1 μM)-induced contraction to sodium nitroprusside (0·1 μM) or to 8-Br-cGMP (300 μM). With phenylephrine-, but not noradrenaline-, induced contraction, the relaxation triggered by carbachol was significantly reduced by pretreatment of the aortic rings with chloroethylclonidine (50 μM), which inactivates a subpopulation of α_1-adrenoceptors. Thus, the results confirm that both alkylation sensitive and resistant α_1-adrenoceptors exist in rat aorta and indicate that EDRF may discriminate between these two α_1-adrenoceptor subtypes which are differently affected by phenylephrine and noradrenaline.

Since the original experiments described by Furchgott & Zawadzki (1980), the addition of a cholinergic agonist to preconstricted preparations enables the demonstration of a functional endothelium. Once stimulated by the muscarinic agonist, the endothelial cells release an endothelium derived relaxing factor (EDRF), identified as nitric oxide (Palmer et al 1987), which induces the relaxation of the underlying smooth muscle through activation of soluble guanylate cyclase. However, the nature of the contractile mechanism elicited by the precontracting agent may influence the vasodilatory properties of EDRF (Furchgott et al 1981). Thus, in rabbit aorta, the acetylcholine-induced relaxation was diminished when K^+, instead of noradrenaline, was used as precontracting agent. In rat aorta, endothelium removal differently affected the contractile response to various α-adrenoceptor agonists such as noradrenaline and clonidine (Egleme et al 1984; Lues & Schümann 1984; Martin et al 1986). In this artery, it was also demonstrated that among the different contractile events elicited by adrenoceptor agonists such as intracellular Ca^{2+} release and extracellular Ca^{2+} influx through receptor-dependent channels and potential-dependent channels. This latter process was less sensitive to EDRF (Auguet et al 1989a, b).

More recent studies have indicated that the α-adrenoceptor receptors of rat aorta do not constitute a homogeneous population (Han et al 1990; Oriowo & Bevan 1990; Piascik et al 1990). This has been experimentally demonstrated by use of chloroethylclonidine, an alkylating agent, which inactivates a subpopulation of α_1-adrenoceptors (Johnson & Minneman 1987). From these results it has been postulated that α-adrenoceptors of rat aorta are composed of α_{1A}-subtypes, insensitive to alkylation by chloroethylclonidine, and α_{1B}-subtypes, inactivated by chloroethylclonidine, according to the classification of Morrow & Creese (1986). The contractile processes elicited by α_{1A}-adrenoceptor activation are largely dependent on Ca^{2+} influx through potential-dependent channels (Han et al 1987, 1990).

In this present study, we investigated the effect of pretreatment with chloroethylclonidine on endothelium-dependent relaxation in rat aorta. For this purpose, two full α_1-adrenoceptors agonists (noradrenaline and phenylephrine) were used.

Materials and methods

Thoracic aortae were excised from male Sprague-Dawley rats killed by cervical dislocation (270–360 g, Charles River, Paris) and cleaned of surrounding tissue. Rings 2 mm wide were cut under a dissecting lens and were suspended in organ baths containing 10 mL of physiological solution (for composition see below) under a tension of 2 g at 37°C and gassed with 95% O_2–5% CO_2. Contractile responses were measured by using force-displacement transducers (Statham UC_2) coupled to a Gould

Correspondence: M. Auguet, Institut Henri Beaufour Research Labs, ZA de Courtaboeuf, 1 avenue des Tropiques, 91952 Les Ulis Cedex, France.