International Journal of Pharmaceutics, 69 (1991) 181–186

ADONIS 037851739100128G

IJP 02337

Visualisation of a fluorinated pharmaceutical formulation in the anaesthetised rat using ^{19}F- and ^{1}H-NMR imaging

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(Received 20 August 1990)
(Modified version received 2 November 1990)
(Accepted 10 November 1990)

Key words: Fluorine; Pharmaceutical formulation; Gastrointestinal tract; NMR imaging; Perfluorocarbon

Summary

The potential applicability of ^{19}F-NMR imaging in observing a fluorinated pharmaceutical in vivo using the rat as a model has been assessed. ^{19}F-NMR imaging was used in parallel with (^{1}H)-NMR imaging to observe and locate a solid dosage form in vivo. In vitro images of a tube phantom containing the fluorinated derivative were acquired initially in order to determine optimal acquisition parameters. Subsequently, in vivo ^{19}F images of the solid dosage form in the gastrointestinal tract of anaesthetised (fasted and nonfasted) rats were obtained and compared. Corresponding ^{1}H images were acquired at the mid-abdomen level of the rat. All images were acquired at a field strength of 4.7 T using a 16-segment copper birdcage resonator as the imaging probe. The ^{19}F images were obtained at the resonant frequency of 188.5 MHz and the proton images at the resonant frequency of 200.3 MHz using a spin-echo pulse sequence. Anatomical proton images showing gross structure of the gastrointestinal tract were acquired in as little as 2 min. Fluorine images were observed in approx. 3 min with a signal-to-noise (SNR) ratio of 7.5 per pixel.

Introduction

There has been an increasing awareness of the value in understanding the behaviour of pharmaceutical formulations in vivo (Anie et al., 1988). Transit times of orally administered pharmaceutical dosage forms through the gastrointestinal tract are of significance as drug absorption is considered to take place primarily from the small intestine. For this reason, the residence time of the dosage form in the stomach and the contact time with the absorption sites of the small intestine will influence the profile of drug absorption.

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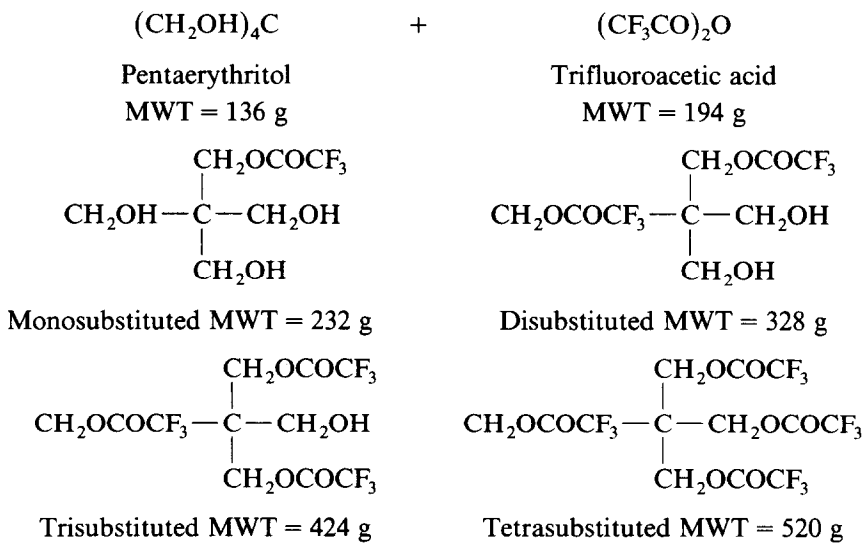
The assessment of the localisation and movement of oral formulations has been examined previously using X-ray (Evans and Roberts, 1981) and radioisotope imaging including gamma scintigraphy (Davis et al., 1984; Fell and Digenis, 1984). To date, NMR imaging has not been used for this application. NMR imaging can, in theory, overcome problems inherent in the previous methods by permitting imaging of a much wider range of materials. Furthermore, ^{19}F-NMR imaging provides an as yet unexplored area for the localisation of formulations in the gastrointestinal tract. ^{19}F is a sensitive nucleus and is chosen for these investigations because of several specific advantages. These are the low intrinsic concentration of fluorine in soft tissue, the high NMR sensitivity of the fluorine nucleus, the 100% natural abundance of



Scheme 1.

the ^{19}F isotope and the large gyromagnetic ratio of the fluorine nucleus, 25.17×10^7 rad T^{-1} s^{-1}, which is close to that of the proton nucleus, 26.75×10^7 rad T^{-1} s^{-1} thereby enabling the observation of ^{19}F with existing ^1H RF components.

Previous work on ^{19}F magnetic resonance spectroscopy and imaging has been largely concerned with various blood substitutes and fluorinated anaesthetics. The blood substitutes which have been studied include perfluorodecalin (Joseph et al., 1985), perfluorotributylamine (FTBA) (Longmaid et al., 1985), perfluorooctylbromide (PFOB) (Ratner et al., 1988) and perfluorophenanthrene (Thomas et al., 1986). There have also been investigations focused primarily on the potential applications of perfluorocarbons (PFCs) as contrast agents for a variety of organs (McFarland et al., 1985). Fluorinated anaesthetics such as halothane have been monitored using ^{19}F-NMR spectroscopy to detect the uptake and elimination from rabbit brain (Wyrwicz et al., 1983). A significant problem, however, in using fluorinated agents, especially at the high fields required for imaging, is the multiplicity of resonances arising from chemically non-equivalent fluorine atoms. These widely spaced resonances (50–150 ppm) introduce chemical shift artifacts in the frequency encoded spatial dimension.

For our investigations, this problem was overcome by synthesising pentaerythritol trifluoroacetates which are symmetrical structures with a single ^{19}F resonant frequency and using them in admixture.

Materials and Methods

Synthesis

Pentaerythritol (2 g) was refluxed with trifluoroacetic anhydride (8 ml) over an oil bath at 35 °C for 4 h (Scheme 1). The colourless liquid obtained was evaporated on a Buchi rotary evaporator to remove any trifluoroacetic acid and purified by a bulb-to-bulb distillation. Spectroscopic analyses was performed, infrared (IR) and NMR (both ^1H and ^{19}F). The product exhibited a predominant resonance line at 0.97 ppm with respect to pure trifluoroacetic acid used as a reference.

Animal studies

The animal studies utilised Sprague-Dawley male rats (200 g). Half the rats were in an 18 h fasted state and the other half had been fed normally. Each rat was anaesthetised (urethane, intraperitoneal; 5% in saline; 0.6 ml/100 g) prior to the oral administration of a pellet and imaging to

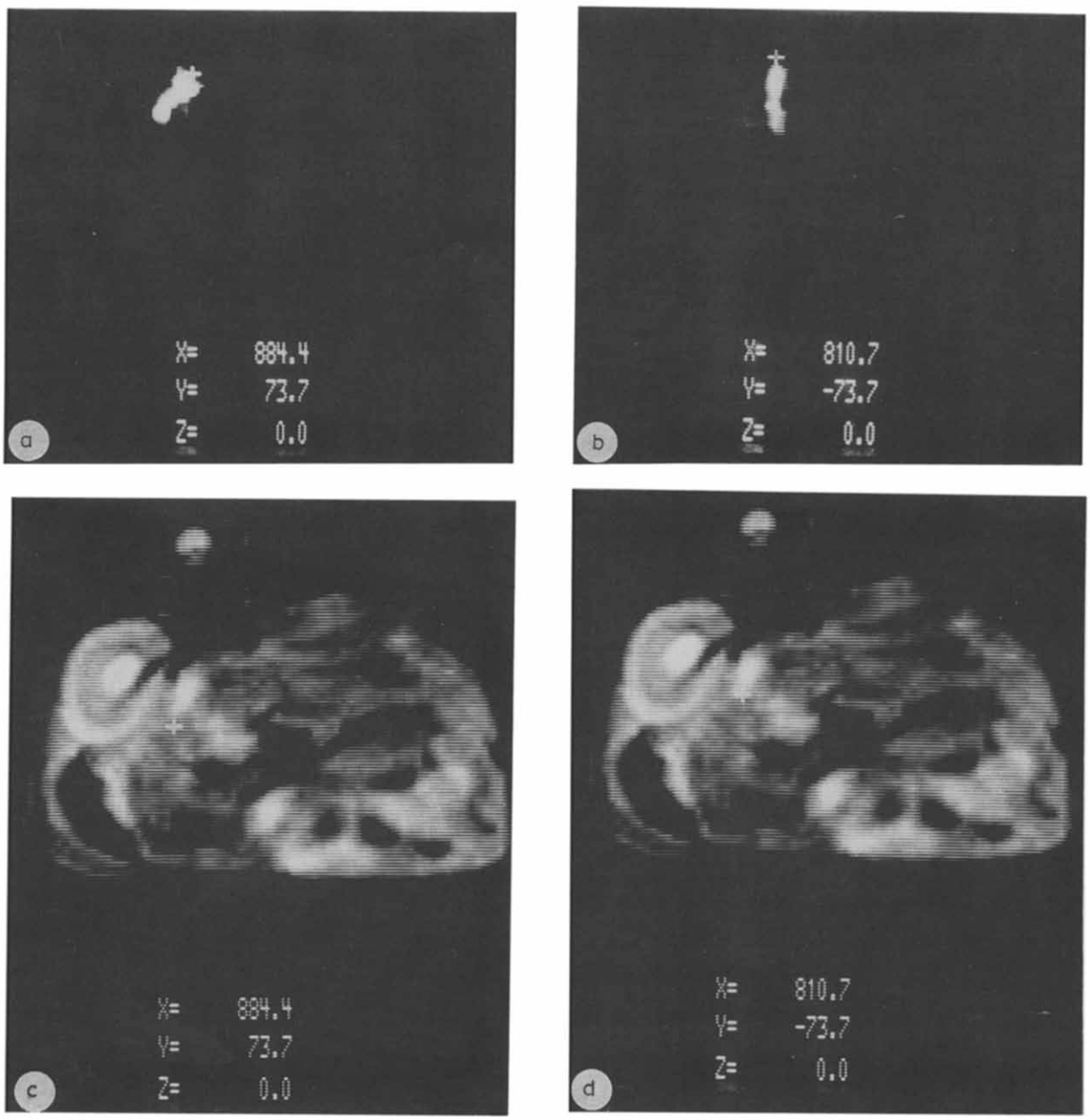


Fig. 1. (a, b) ^{19}F transverse images of a pellet in the gastrointestinal tract of a nonfasted rat. (a) 15 min after dosing; (b) 115 min after dosing. (c, d) Proton images corresponding to above after (c) 15 min and (d) 115 min after dosing.

enable immobilisation of the animal in the magnet. Hollow capsule-shaped pellets made of Nylon-6,6 (bore 2.2 mm, diameter 2.6 mm, length 7.0 mm) were filled with the fluorinated formulation (0.006 g) and sealed to prevent leakage. An administration tube comprising a Luer stainless-steel needle and cone hollowed out to form a cup for the pellet was used during oral dosing of the rat. The pellet was deposited into the distal end of the oesophagus. This procedure was duplicated for

all the studies. The rat was then placed supine in a perspex restraint cage and positioned in the birdcage coil. Each rat was killed (halothane inhalation) and dissected after the experiment to ascertain the position of the pellet.

Instrumentation / imaging protocol

In vivo images were acquired on an ORS/ Bruker biospectrometer (Oxford Research Systems, Abingdon, U.K) operating at a field strength of 4.7 T. A 16-segment copper birdcage resonator was employed as the imaging probe with corresponding 90 and 180° pulse lengths of 190 and 380 μs respectively. The ^{19}F images were obtained at the resonant frequency of 188.5 MHz and the proton images at the resonant frequency of 200.3 MHz. A two-dimensional FT sequence was used to obtain all images. All images consisted of 128 $\times$ 128 independent picture points. Image acquisition was commenced 15 min after the administration of the pellet to the rat. The animals underwent both ^{19}F and ^{1}H scanning in the birdcage coil tuned successively from the ^{19}F frequency to the ^{1}H frequency. There was a time lag of approx. 2 min during which the birdcage was retuned and rematched. ^{19}F imaging was used to locate the pellet in vivo and ^{1}H imaging to assign the pellet to a specific region of the rat. Transverse images were acquired at the level of the abdomen at time intervals to note gross movement of the pellet.

The pulse repetition time for both ^{19}F and ^{1}H imaging, TR, was 1045.2 ms and the echo time, TE, was 35.6 ms. The slice thickness of each image was 4 mm. The proton scans took 2 min to acquire. Due to the low signal-to-noise ratio of the ^{19}F resonance, image acquisition of the fluorine images took approx. 3 min.

Results and Discussion

Fig. 1a and b shows ^{19}F transverse images of a pellet in the gastrointestinal tract of a nonfasted rat, acquired 15 and 115 min, respectively, after dosing. The cursor position shown by the coordinates indicates the position of the pellet, and is different in Fig. 1a as compared with Fig. 1b. The pellet appears to be changing in position and is moving in the anticlockwise direction. Proton images corresponding to the ^{19}F images acquired 15 and 115 min after dosing are shown in Fig. 1c and d, respectively. These depict structures such as the spinal canal, muscle, stomach, kidneys, small and large intestines, bowel gas and body wall. The cursor positions on the proton images mark the location of the pellet with time. For the proton images, the cursor indicates the pellet is in the stomach of the rat and remains in the stomach even after 265 min. On account of the varying cursor coordinates on the ^{19}F images, one may conclude that the pellet is moving in vivo. The minor changes in pellet position could be attributed to the fact that the experiment was performed with a nonfasted rat, therefore the stomach contained ingested food before the experiment was commenced. The pellet mixes with food in the stomach and its movement is restricted. Furthermore, the rat was anaesthetised prior to imaging and it is likely that urethane interferes with the normal peristaltic waves of the stomach thus inhibiting gastric emptying of the pellet. On dissection, the pellet was located in the forestomach close to the limiting ridge and imbedded in food remnants within the stomach of the rat.

Fig. 2a and b are ^{19}F transverse images of a pellet in vivo acquired with a fasted rat. The movement of the pellet as indicated by the cursor coordinates appears to be more pronounced in this case as compared with the nonfasted state. In Fig. 2a, acquired 15 min after dosing, the fluorine image of the pellet appears vertical. However, 265 min after dosing (see Fig. 2b) there appears to be an anticlockwise tilt of the pellet from its previous position. Selected proton images corresponding to fluorine images obtained 15 and 265 min after dosing are provided in Fig. 2c and d, respectively. The proton scans differ and reveal that the pellet has moved significantly during the experiment as compared with the results obtained with the nonfasted state. On dissection, the pellet was located in the pylorus at the junction of the duodenum. In the absence of urethane, used as an anaesthetic to immobilise the animals during these experiments, motility of the pellet could be enhanced resulting in the observation of the pellet in the intestinal processes of the rat. However, it is essential that

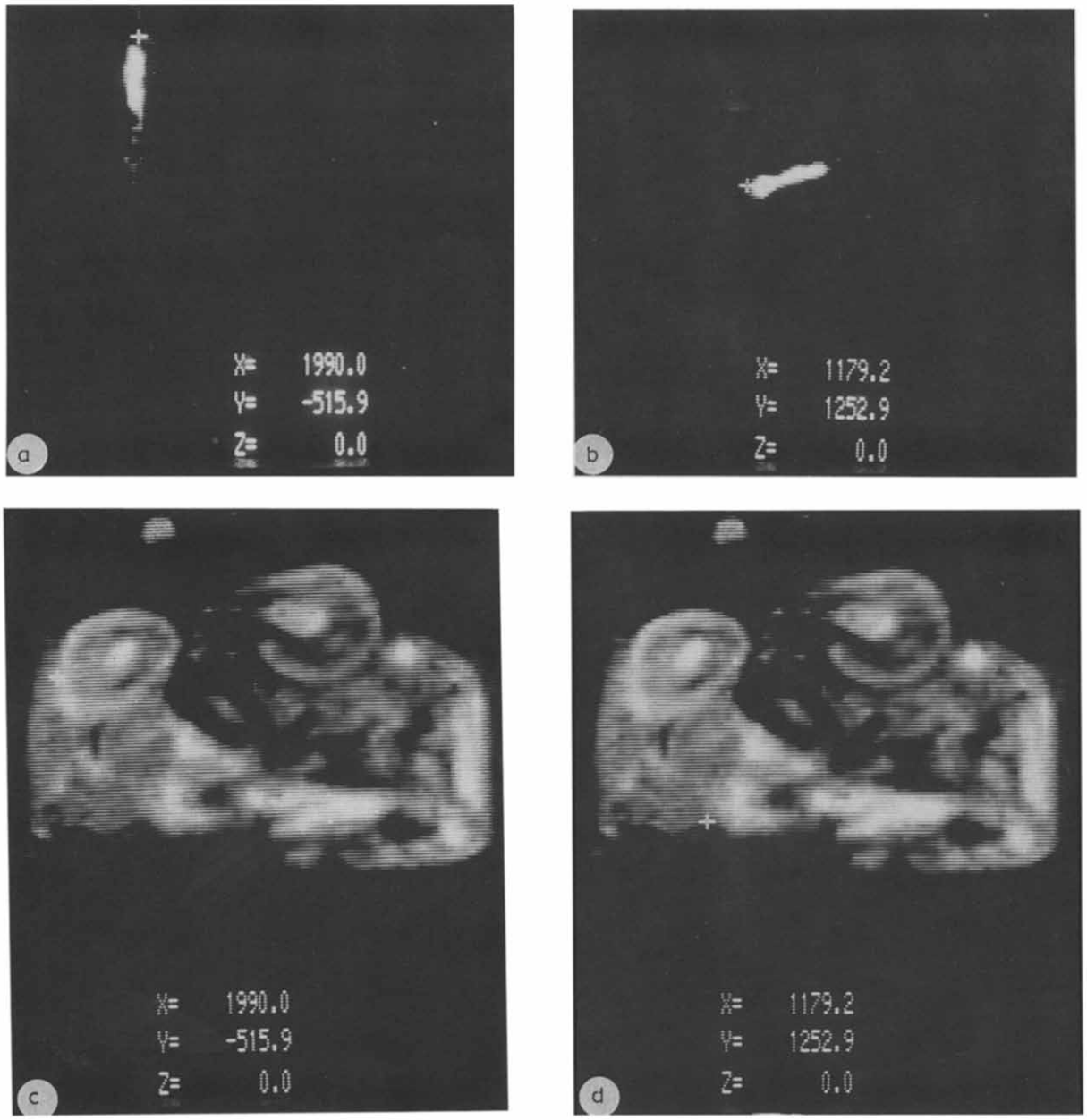


Fig. 2. (a, b) ^{19}F transverse images of a pellet in the gastrointestinal tract of a fasted rat. (a) 15 min after dosing; (b) 265 min after dosing. (c, d) Proton images corresponding to above after (c) 15 min and (d) 265 min after dosing.

movements of the animal are prevented as far as possible to improve image quality and to reduce motional artifacts on the image, which are already prevalent due to normal heart function and respiratory motion of the animal. Perhaps the use of a sedative such as diazepam would alleviate this problem. Overall greater movement of the pellet occurs in the fasted state as deduced from these experiments using ^{19}F- and ^{1}H-NMR imaging.

These images represent initial results on the use

of ^{19}F- and ^{1}H-NMR imaging to observe and localise a solid dosage form in the gastrointestinal tract. In these studies advantage is taken of the fact that there are no background signals, originating from the animal, so that signals observed are exclusively from the pellet containing the fluorinated derivative. The initial aim was to select a product containing a high proportional mass of fluorine and a single line in its NMR spectrum, with convenient handling characteristics. Another important feature of these studies was the accurate spatial correlation that was achieved between the fluorine and proton images. This was possible due to the selection of the birdcage resonator as the imaging probe. The birdcage resonator may be tuned and matched to the appropriate frequency between successive ^{19}F and ^{1}H imaging without moving the animal or resonator because the respective resonant frequencies of ^{19}F and ^{1}H are close, i.e 188.5 and 200.3 MHz, respectively. The reliability of the administration procedure means that provided the administration tube reaches the distal end of the oesophagus, pellets deposited reach the stomach within 10 min.

The relative sensitivity of fluorine is 94% to that of hydrogen at constant frequency and therefore, fluorine and hydrogen produce similar signal strengths as observed in our images. The acquisition time associated with the ^{19}F images, 3 min, shows an improvement on previously published data. The geometry of the birdcage imaging probe is such that the restraint cage containing the rat fills the volume of the probe, so that detection sensitivity is maximised and a better SNR is realised.

Further studies would involve the use of non-anaesthetised rats or sedated rats to enable greater movement of the pellet in vivo. Other ^{19}F imaging agents could be formulated having the characteristics previously mentioned. These could be potentially administered in dosage forms and the entire movement through the GI tract observed. We conclude that the method is sufficiently fast and sensitive to obtain data regarding transit times.

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