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Retention and distribution of two ^{99m}Tc-DTPA labelled vaginal dosage forms

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Abstract

To objectively evaluate the performance of new vaginal dosage forms, it is important to determine their time of residence and their distribution. This paper describes the in vivo characteristics of a reference and test product in this situation.

Method: A randomised cross-over study was performed in the same phase of the menstrual cycle in eight pre-menopausal women. The retention and distribution of a commercially available vaginal clotrimazole cream and a test gel product, each "labelled" with ^{99m}Tc-DTPA was assessed by gamma scintigraphy for 24 h after administration of the products. Mass balance analysis was attempted by collecting and counting sanitary napkins worn for the study time. *Results*: Within individuals there was little variation in the clearance of the formulations, but wide variation between individuals with a range between 81 and 1% of the administered doses retained by 24 h. The losses appeared to occur mainly at times of urination with $12 \pm 8\%$ (cream) and $20 \pm 23\%$ (gel) collected on the sanitary napkins, but $46 \pm 34\%$ (cream) and $38 \pm 22\%$ gel activity not accounted for by 24 h. The intravaginal distribution of activity was similar for each product. *Conclusions*: Radioactive tracer methods are useful in assessing and comparing vaginal dosage forms.

Keywords: Vaginal dose-forms; Gamma scintigraphy; Pharmacodynamics

1. Introduction

Local therapy (cream, gel) is used to treat a number of vaginal conditions (infection, hormone deficiency). It has been considered that these therapies may have a relatively short residence time due to self-clearing mechanisms of the vagina, and gravity. Frequent application may be inconvenient, and some formulations are messy. Retention has been difficult to measure quantitatively, it is uncertain if the administered formulations coat the entire surface to be treated.

Nuclear medicine techniques have proved valuable in assessing the distribution and dynamics in the development of dosage forms for many routes of administration of pharmaceuticals. We describe our experience in comparing two vaginal dosage forms.

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2. Materials and methods

2.1. Subjects

Eight (8) healthy, non-pregnant pre-menopausal women (mean age 27, range 20–39) gave informed consent. Following an induction health screen, the subjects were each studied twice, with the test or control formulation administered in random order 28 days apart in order that they be studied in the same phase of their menstrual cycle.

2.2. Preparation of radiolabelled formulations

^{99m}Tc-DTPA was prepared by the addition of 220 MBq of ^{99m}Tc-sodium pertechnetate (Gentech generator, ARI Australia) to a DTPA cold kit (RAH Radiopharmacy). The reference product, Canesten cream (2% clotrimazole, Bayer Australia) was radiolabelled by transferring the cream into a 20 ml syringe, adding 0.1 ml of ^{99m}Tc-DTPA to the top of the cream and then mixing back and forth into a second 20 ml syringe via a fluid dispensing connector over 20 cycles. Homogeneity of the final product was confirmed by scintigraphic imaging of the radiolabelled cream in the 20 ml syringe. The radiolabelled cream was then transferred into the manufacturer supplied applicator (approximately 6 g) so that the final dose contained 4 MBq of activity. For the preparation of the test formulation sufficient ^{99m}Tc-DTPA (1.2 GBq) was added prior to pressurisation and production of the foaming gel so that the administered dose of 0.5 ml contained 4 MBq of activity. Preparations were weighed and counted before and after administration to determine accurately the amount administered. For analysis, all counts were background and decay corrected and expressed as proportion of administered dose.

2.3. Study procedures

The products were administered high in the vagina with the subject supine, the cream was extruded from the applicator, and the foaming gel produced in situ at the tip of the nozzle of the pressurised system. Between imaging, the subjects mostly sat in a chair, but were allowed to walk to simulate typical activity profiles.

Simultaneous anterior and posterior gamma camera images (Picker Prism 2000) were obtained 15 min after dosing, and at 15-min intervals for the first hour. Hourly images were then obtained for 7 h, with a final image at 24 h. Geometric mean counts were recorded for four arbitrary quadrants, the sum of these "whole vagina", and for an extravaginal region. An index of uniformity was calculated from these four quadrants, which represents the sum of the absolute difference in the percentage of the total counts in each region from 25% (i.e. equal distribution in all four quadrants would yield an index of 0, all activity in a single quadrant would yield an index of 150). This index did not take into account activity lost from the region of interest. Grouped results from each preparation were compared at each time point (2-tailed paired *t*-test), and the correlation between retention of the products in each subject were compared. Tomography was obtained at 1, 4 and 7 h. The images were also visually assessed for activity ascending into the uterus, or extra-genital activity.

A sanitary pad was changed after each imaging event, and counted to determine the activity recovered. The difference between the sum of the activity seen on the gamma camera, and counted on the sanitary pad, and that administered was the activity "not accounted for". Times of urination were recorded, but neither urine nor toilet tissues were collected.

The worst-case effective radiation dose for the subjects (assuming total retention of each administered dose) was calculated at 0.14 mSv for each study.

The study was approved by the Human Research Ethics Committee of the Royal Adelaide Hospital.

3. Results

All subjects tolerated the procedures well, with no significant adverse events.

Satisfactory images of the vagina were obtained with both the reference and the test product (Fig. 1). These objectively showed little difference between the reference and test product. Fig. 2 shows the arbitrary quadrants analysed for uniformity of distribution. Total vaginal retention was regarded as the sum of quadrants A–D, and individual and grouped results are shown in Figs. 3 and 4. The retention of the two products was not significantly different at any time point, with a trend toward slightly more retention of the

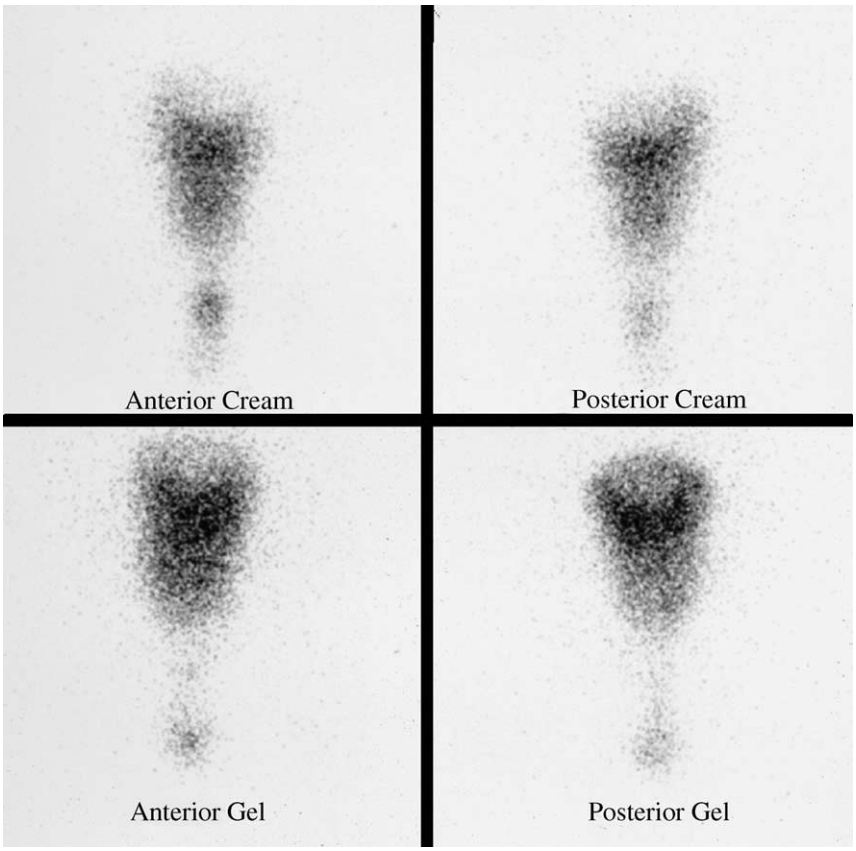

Fig. 1. Anterior and posterior gamma camera images of reference and test product obtained 1 h after administration of the reference (upper) and test (lower) products in the same individual 28 days apart. The distribution is very similar.

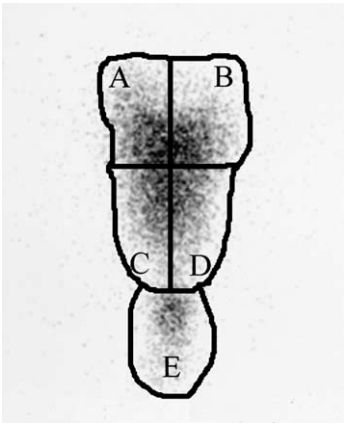

Fig. 2. Arbitrary regions of interest, total ($\sum$ A–D), quadrants (A–D) and extravaginal region (E) used in distribution analysis.

reference than the test product before 3 h (Fig. 4). There was a highly significant correlation between the retention of each product in individuals at all time points ($r = 0.90$ at 24 h). The between subject differences (S.D. at 24 h 27%) were greater than the between product (S.D. at 24 h 12%) differences $P < 0.05\%$ (i.e. the tendency for retention of products was more dependant on the individual rather than the product).

There was no significant difference between the distributions of the products as estimated by the index of distribution during the studies (Fig. 5). Single Photon Tomographic (Fig. 6) imaging shows the volumetric distribution of the test product. Although impressive images representing this distribution could be obtained, they were of little use in the analysis of the data, due to the arbitrary display threshold

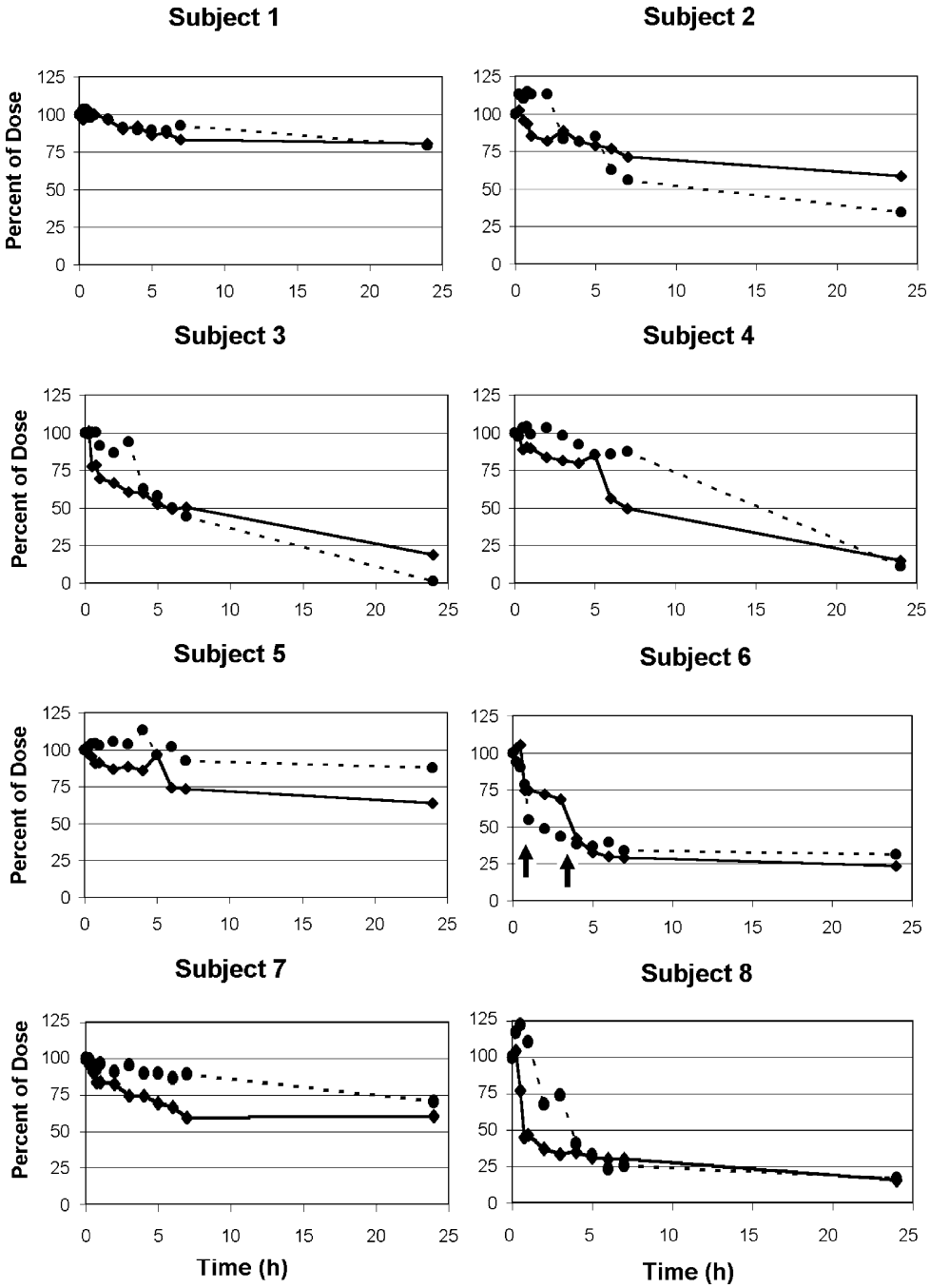

Fig. 3. Individual paired data for retention of reference and test product indicating that comparison within individual is more marked than with type of product. (Solid: cream, broken: gel). Examples of clearance at urination are demonstrated by the abrupt fall in counts of cream at 1 and 3 h, and gel at 1 h in subject 6.

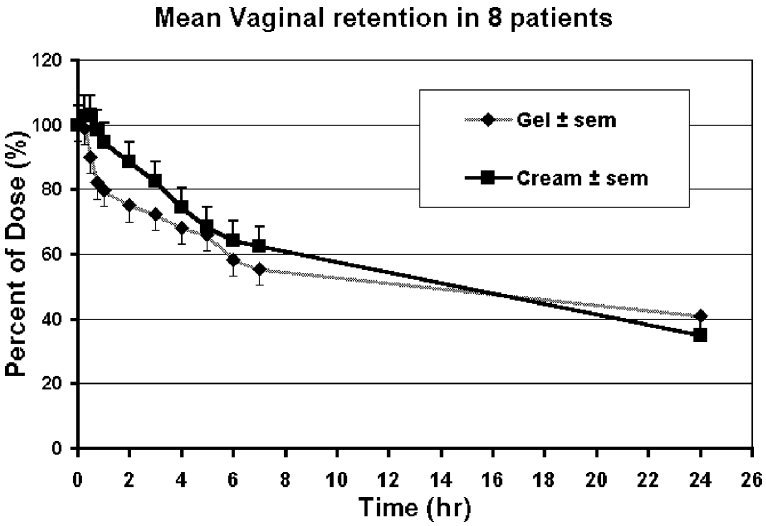

Fig. 4. Grouped data demonstrating total vaginal retention of products over 24 h.

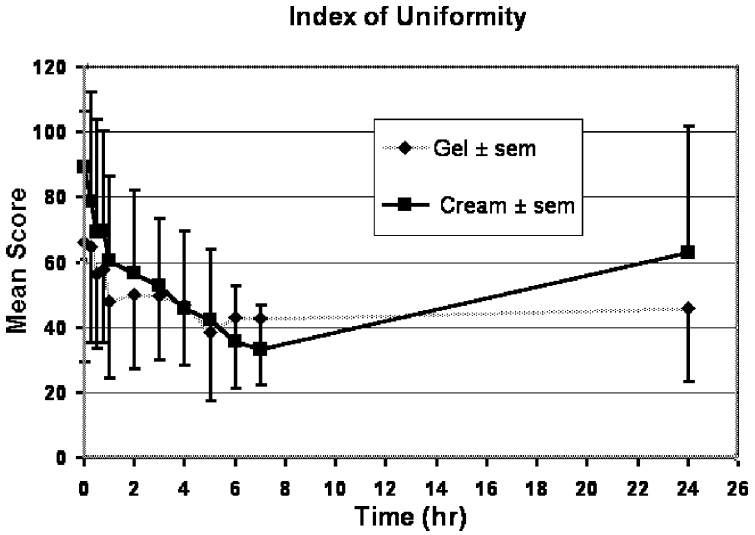

Fig. 5. Grouped data showing "index of distribution", showing similar uniformity of distribution in vaginal segments between products.

changing the apparent distribution, and the difficulty in quantifying the information.

The quantity of material not accounted for gradually increased from nil in the first hour to 46±34% (cream) and 38 ± 22% (gel) at 24 h. This was presumably lost from the vestibule at the time of urination. In many of the time-activity curves, there was a significant fall in the retained counts after some episodes of urination. Cumulatively 12 ± 8% (cream) 20 ± 23% (gel) was found on the sanitary napkin.

4. Discussion

Gamma scintigraphy has proven useful in in vivo evaluation of dosage forms administered by parenteral rectal, buccal, nasal, pulmonary and ophthalmic routes (Meseguer et al., 1994), but only one paper to our knowledge has addressed vaginal formulations using similar methodology (Brown et al., 1997). Previous studies have used colposcopy to assess vaginal irritation of products (Elias et al., 1997), radiolabelled

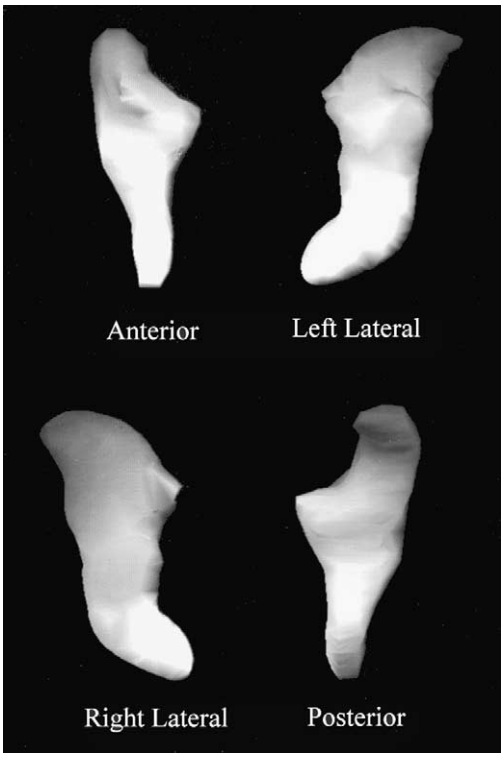

Fig. 6. Four views of typical 3D reconstruction of tomographic data 1 h after administration of test product.

formulations to estimate absorption (John et al., 1998), or repeat swabs to assess qualitative presence/absence of pharmaceutical after administration of different dosage forms (Weinstein et al., 1994). The distribution of material in the vagina has been assessed by scintigraphy (Brown et al., 1997) and MRI (Barnhart et al., 2001), and transport (sperm) through the genital tract by scintigraphy (Kunz et al., 1996). Release of pharmaceutical from intravaginal dosage forms in rabbits using radionuclide methods has been described (Asikoglu et al., 1995).

^{99m}Tc-Pertechnetate is slowly absorbed across the vaginal mucosa and there may be a "portal" transport of activity preferentially to the uterus (Cicinelli et al., 2001). ^{99m}Tc-DTPA is only slightly absorbed (<1%) across intestinal epithelium (Chaudhuri, 1974) and permeability to water across buccal and vaginal epithelium are similar (van der Bijl et al., 1997) but the permeability of vaginal epithelium for ^{99m}Tc-DTPA is not known. For the purposes of this study, it was assumed that it is not significantly absorbed, and that the radiopharmaceutical monitored the behaviour of the vehicle in which it was applied. The molecular components of the vehicle were not labelled as the intention of the study was to determine if the vehicles would lead to differential retention of a potentially included therapeutic product. Over a period of time it is likely that the label would dissociate from the vehicle as the latter changed texture, but this would also be true of potential therapeutic agents. Specific binding of ^{99m}Tc-DTPA to the components of the vehicles was not assessed directly. It is a very tightly chelated compound (dissociation constant $< 10^{-21}$) and most unlikely to bind to any other material and as a nuclear medicine tracer it regarded as essentially biologically inert. Free DTPA in the kit may chelate with metals in the vehicles. The content was <0.1 mg DTPA per application.

The distribution and retention of material in the vagina has not been extensively studied because of methodological difficulties, nevertheless there is a usual assumption that the vagina has a "self clearing" mechanism, which is relatively effective. This may reduce the effectiveness of topically applied medication, and distribution is assisted by ambulation (Barnhart et al., 2001). The majority of the vagina has opposed walls during most activities, and therefore it represents a potential space with a small volume. Formulations which aid local retention by muco-adhesion or other mechanisms are theoretically attractive.

This study has shown that the retention of activity in the vagina of two formulations is similar in individuals, but there is great variability between individuals. The findings correspond with those of Brown et al. (1997), who was using two formulations different to those in this study. This study showed that retention of product at 24 h ranged between 81 and 1% of administered dose. There was a tendency for the retained counts to fall after urination, and much of the product appears to be lost at this time, rather than more steadily appearing on the sanitary napkins. The distribution of activity is more difficult to assess, as the subregions we used are arbitrary, and the potential total surface of the vagina to be coated for the formulations is not necessarily displayed on the scan. The early falls in the index of uniformity corresponded to more distal passage of the activity (quadrants C and D) from the application sites high (quadrants A and B) in the vagina.

Nevertheless, neither visual nor semi-quantitative assessment of the distribution of the two formulations showed any significant differences. There was no evidence of ascension of activity into the uterus, nor of activity in the urinary bladder to suggest absorption and subsequent urinary excretion of the radiopharmaceutical.

5. Conclusion

This paper describes a technique for assessing the retention and distribution of topical vaginal dosage forms. The results demonstrated essential equivalence between the reference and test products.

Acknowledgements

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