Spreading and Retention of Vaginal Formulations in Post-Menopausal Women as Assessed by Gamma Scintigraphy

J. Brown,[1] G. Hooper,[1] C. J. Kenyon,[1] S. Haines,[1] J. Burt,[1] J. M. Humphries,[1] S. P. Newman,[1] S. S. Davis,[2] R. A. Sparrow,[3] and I. R. Wilding[1,4]

Received February 27, 1997; accepted May 15, 1997

Purpose. In this paper we report on the first scintigraphic evaluation of vaginal dosage forms in post-menopausal women. To date, almost nothing is known about the *in vivo* performance of pharmaceutical formulations in the human vagina, which is a major deficiency in the rational design of drug delivery systems for both existing and new indications.

Methods. The vaginal spreading and clearance of a radiolabelled pessary formulation and Replens® (polycarbophil) gel, was assessed in six healthy, post-menopausal female volunteers over a six hour period using the technique of gamma scintigraphy.

Results. In five out of the six subjects studied, clearance of the two formulations exhibited very little intra-subject variation. However, there was considerable inter-subject variability in clearance; in Subject 5 circa 80% of the products were retained whilst in Subject 2 less than 2% was present at the end of the six hour imaging period. Importantly, there was no evidence to suggest that either of the formulations dispersed material beyond the cervix, into the uterus, in any of the subjects studied.

Conclusions. The lack of significant retention of these products in most of the volunteers has obvious implications for the delivery of therapeutic agents. This study shows that gamma scintigraphy is an invaluable technique with which to assess novel formulations aimed at optimising retention in the vagina for topical or systemic drug delivery.

KEY WORDS: vaginal delivery; gamma scintigraphy; bioadhesion; pessary; post-menopausal women.

INTRODUCTION

The development of vaginal dosage forms has recently become of increasing importance with regard not only to local drug therapy, but also to the systemic administration of peptides and proteins (1), and the potential of microbicides for the prevention of heterosexual transmission of human immunodeficiency virus (2). Vaginal delivery systems, such as creams, foams, pessaries and jellies, are considered to reside for relatively short periods of time at the target site due to the self-cleansing action of the vaginal tract (3). However, this is based entirely on anecdotal reports in the literature which suggest that vaginal formulations are prone to leakage (4), and from patient acceptability surveys which suggest that preparations are inconvenient and messy (5). To date, almost nothing is known about the *in vivo* performance of pharmaceutical formulations in the human vagina, which is a major deficiency in the rational design of drug delivery systems for both existing and new indications. For instance, many questions can be raised regarding what happens to a particular formulation inside the vagina, how much it disperses, whether the product is retained and possibly absorbed, or whether it is simply discharged. Likewise, the relative behaviours of different formulations are also unknown.

Gamma scintigraphy has become the method of choice for investigating the fate of pharmaceutical dosage forms in the body (6). We have published on the application of this technique to the evaluation of nasal products (7), rectal formulations, (8) oral delivery systems (9) and inhalation preparations (10). Preliminary feasibility studies have shown that the scintigraphic evaluation of bioadhesive vaginal formulations in the sheep provides an extremely useful and novel tool for evaluating the distribution, spreading and clearance of such formulations in a conscious animal model (4). However, as far as we are aware, no scintigraphic research has been conducted in humans as yet, and questions about physical movement of preparations within the vagina and into the uterus and its appendages remain unanswered. In this paper we report on the first scintigraphic evaluation of vaginal dosage forms in post-menopausal women.

Over recent years the use of bioadhesive polymers to prolong residence time of drug within the vaginal cavity has generated significant interest (3,4,11–13). In particular, bioadhesive formulations, based on polycarbophil, have been developed which are reported to be retained at the site of administration for 3–4 days (3). The primary objective of the study was, therefore, to determine the relative spreading and retention of a putative bioadhesive formulation (Replens®) compared with that of a traditional fatty based pessary. In addition, the study was designed to investigate whether any of the formulation was capable of migrating across the cervix into the upper genital tract; since there are conflicting animal data in the literature of particles passing through the cervix in the rat (14) or being retained entirely in the monkey vagina (15).

MATERIALS AND METHODS

Manufacture of Radiolabelled Formulations

A placebo pessary formulation, based on the fatty base, Witepsol, was radiolabelled by the dispersion of ^{99m}Tc-labelled ion exchange resin (Amberlite IRA-410) throughout the preparation. Each pessary consisted of 7.92g of Witepsol and 80mg of labelled resin. The comparator formulation was the putative bioadhesive preparation, Replens®, manufactured by Maropack AG, Switzerland (licence holders Columbia Labs) which consists of purified water BP (78.82%), polycarbophil, mineral oil, glycerine, hydrogenated palm oil glyceride, carbomer 943P, sorbic acid and sodium hydroxide. The Replens® formulation was radiolabelled via the direct incorporation of ^{99m}Tc human serum albumin (^{99m}Tc-HSA) (0.1ml per 2.4g dose of gel) into the formulation such that each dose of gel contained 4MBq of activity at the time of administration. Homogeneity of the radiolabel within the preparation was established prior to the

[1] Pharmaceutical Profiles Limited, 2 Faraday Building, Highfields Science Park, Nottingham NG7 2QP, U.K.
[2] Department of Pharmaceutical Sciences, Nottingham University, University Park, Nottingham NG7 2RD, U.K.
[3] Department of Human Morphology, Queen's Medical Centre, Clifton Boulevard, Nottingham NG7 2UH, U.K.
[4] To whom correspondence should be addressed.

0724-8741/97/0800-1073$12.50/0

study by the scintigraphic imaging of either dispersed gel or sectioned pessary.

Study Design

This was a block randomised crossover study in six healthy, post-menopausal female volunteers (Table 1). For the purposes of the study, the term post-menopausal was defined as either women aged 50 years or over who have been on Hormone Replacement Therapy (HRT) for at least six months, or women who have had no menses for at least 18 months. Prior to entry into the study, the nature of the investigation was explained both verbally and in writing to each subject, and each volunteer provided written consent. Each subject underwent a medical examination, both prior to entering and after completing the study. Approval to administer vaginal preparations, labelled with ^{99m}Tc, to post-menopausal females was obtained from the Department of Health, London, and the Clinical Protocol was approved by the Nottingham University Medical School Ethical Committee.

Study Procedures

The formulations were administered into the posterior fornix, by a nurse, with volunteers in a supine position. Each volunteer received either one 8.0g pessary or one dose (approximately 2.4g) of Replens® gel, on each of two separate occasions, separated by a minimum period of 72 hours. Immediately after administration, a sanitary dressing was applied in order to contain any leakage of the formulations which occurred. This was changed at 1.5, 4.0 and 6.0 hours post-dose.

The volunteers then returned to the sitting or standing position for the remainder of the six hour study period. Dispersion of the formulations within the vagina was monitored over the study day using a gamma camera (General Electric Maxicamera) with a 40cm field of view and fitted with a low energy parallel hole collimator. Anterior and posterior images of 60 seconds duration were acquired at 1, 5, 10, 15 minutes and every 15 minutes thereafter until 4 hours post-dose. Images were then acquired at half hourly intervals until the end of the study day. Additional images were acquired immediately following each change of sanitary dressing.

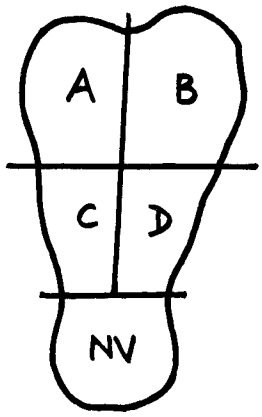

Fig. 1. Vaginal regions of interest.

Analysis of the Scintigraphic Data

The images from each subject were displayed on a colour monitor and the extent of spreading assessed in terms of the anatomical location of the tracer. The computer was used to define regions of interest to allow count rates to be determined for each of four approximately equally sized regions of the vagina, designated A, B, C and D, and a fifth region external to the vagina, designated NV (non-vaginal) (Figure 1). Count rates were then corrected for background activity and decay, and the geometric mean of the anterior and posterior count rates provided a correction for tissue attenuation of the radiation. The corrected counts were expressed as a percentage of the delivered dose.

RESULTS

The results obtained comprised two parts; clearance of the formulations from the vagina, and the extent of spread within the vagina. In five out of the six subjects studied, clearance of the two formulations, within a particular subject, exhibited very little intra-subject variation (Figure 2). However, the behaviour of each formulation between the six subjects varied considerably, particularly with respect to clearance.

Table 1. Gynaecological and Obstetric Profiles for Post-menopausal Women Taking Part in the Study

	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Subject 6
Age	59	57	51	59	44	55
HRT therapy	No	Yes (for at least 8 years)	No	Yes (for at least 3 years)	Yes (for at least 4 years)	Yes (for last 12 years)
Childbirth history	3 children	2 children	None	3 children	2 children	2 children
Gynaecological operations	D&C	None	D&C Tubal ligation	Tubal ligation	D&C	D&C Laparoscopy
Stress incontinence	None	Yes (for at least 5 years)	None	None	None	Yes
Pelvic floor exercise	Only following childbirth	No	No	No	Yes	No
General fitness	Occasional aerobics Swimming	No specific exercise but claims to be reasonably fit	Exercise bike daily	Walking, swimming	2-hour gym session, 3 times a week	Walking, swimming

D&C = Dilatation and cutterage.

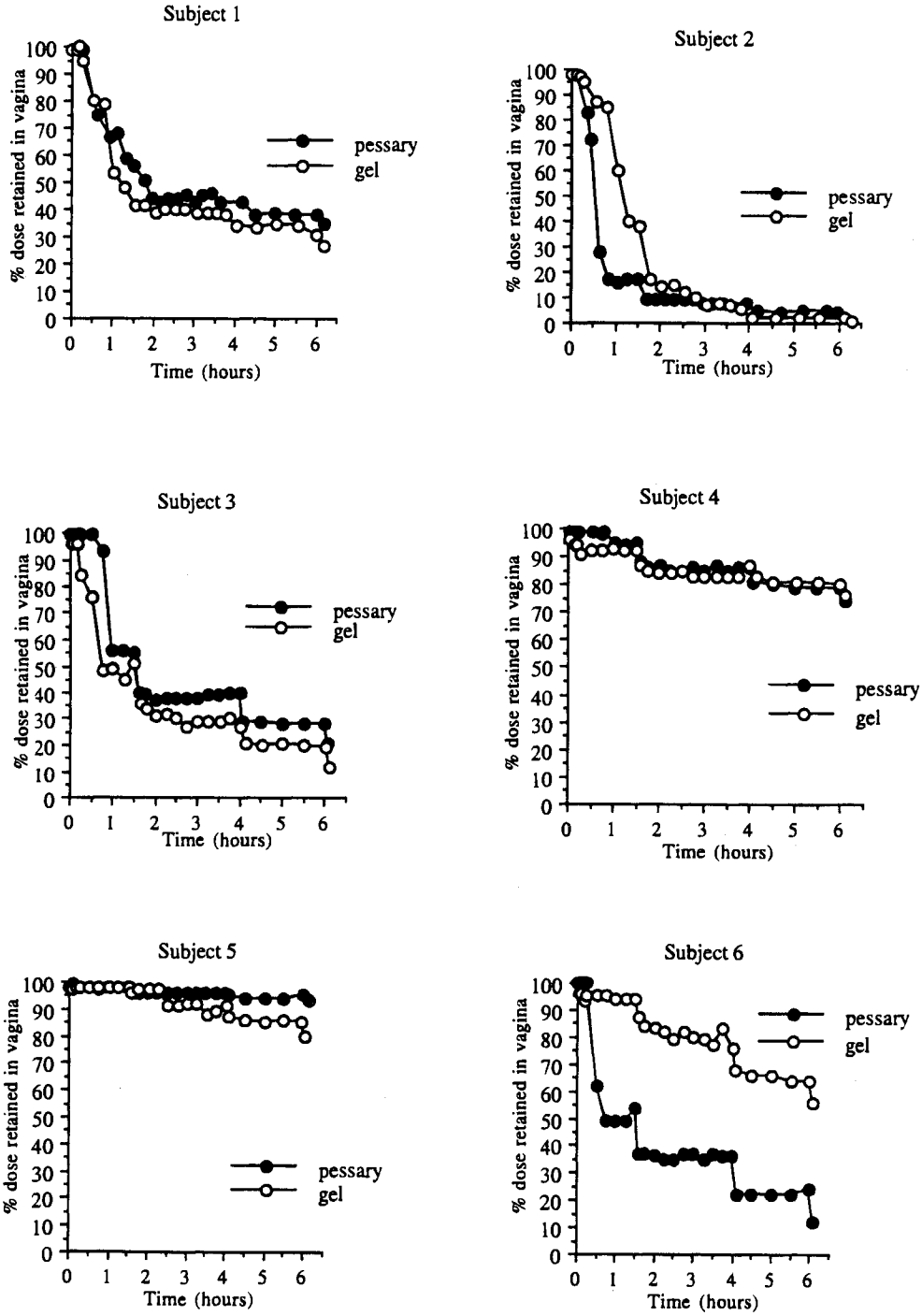

Fig. 2. Retention profiles for the six subjects for gel and pessary formulations.

The percentage of material retained in the vagina was markedly different between subjects. In subjects 4 and 5, both formulations were very well retained. For example, the percentage of the dose present in subject 4, at the end of the imaging period (ie. at approximately six hours post-dose) was 74% for the pessary and 76% for the gel. The formulations were retained to a lesser extent in subjects 1 and 3; in subject 1, the proportion of the dose remaining in the vagina at six hours post-dose was 35% and 27%, for the pessary and gel formulations respectively. In subject 2, both formulations were cleared from the vagina almost completely by the end of the study day, with only 2% and 1% remaining. In only one individual (Subject 6), did the bioadhesive formulation lead to a substantially longer vaginal residence time compared to the conventional preparation; 56% and 12%, respectively, being retained at six hours post-dose.

The extent of spread throughout the four vaginal regions was variable between formulations (Figure 3), and there was some variability between subjects, for each formulation. In general, dispersion of the pessary was confined largely to regions A and B, ie. the upper half of the vagina, in all subjects,

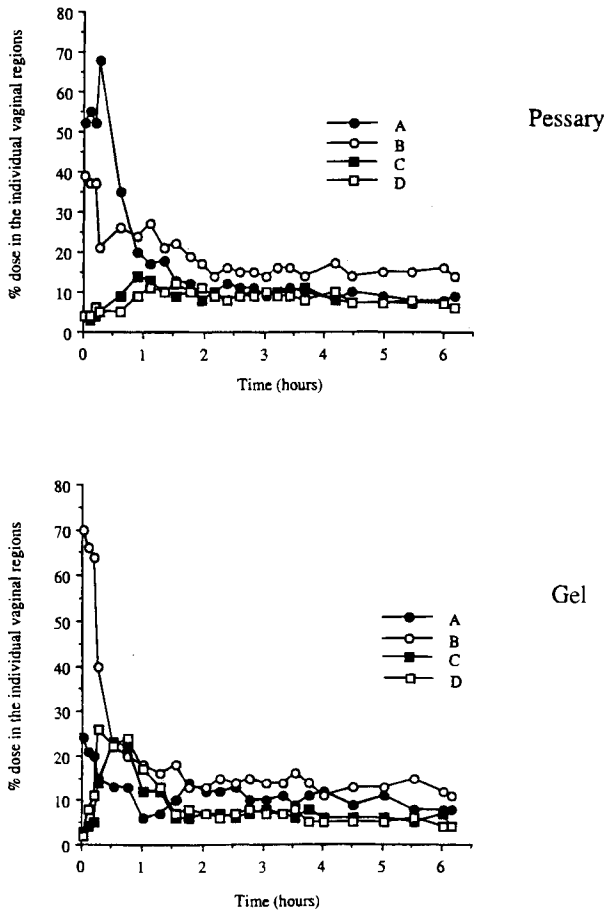

Fig. 3. Representative spreading profiles for gel and pessary in Subject 1.

for approximately one hour post-dose. After this time, the formulation was fairly evenly distributed throughout regions A–D in 3 out of 6 subjects (1, 2 and 5). In subjects 3 and 4, a larger proportion of the dose resided in regions A and B than in C and D, for the remainder of the 6 hour imaging period, whilst in subject 6, the reverse was the case. However, the gel formulation was observed to be uniformly distributed in all four regions, immediately following administration, in all subjects. The actual percentages present at any particular time were obviously dependent on the degree of clearance that had already occurred.

A series of representative scintigraphic images showing the clearance and spreading of the formulations in one subject are provided in Figure 4. Importantly, there was no evidence to suggest that either of the formulations dispersed material beyond the cervix, into the uterus, in any of the subjects studied.

DISCUSSION

To the best of our knowledge this is the first study to use gamma scintigraphy to assess the behaviour of vaginal dosage forms in human subjects. The lack of reliable information regarding the likelihood of intra-uterine spread and the consequent uncertainty in the calculation of radiation dosages required that all subjects be post-menopausal. Changes to the vagina following the menopause include a contraction in both length and width, loss of rugae, epithelial atrophy and a decrease in the depth of the fornices; these are likely to be at least partly reversed by hormone replacement therapy (HRT) which four volunteers were taking at the time of the study.

Gamma scintigraphy allows the movement of the dosage form to be followed and quantified. A major finding is that following placement in the posterior fornix no spread into the cavity of the uterus or beyond was observed in any of the volunteers for either the pessary or the gel formulations. If material from the vagina is to enter the uterus it must first traverse the cervical canal. Atrophic post-menopausal changes greatly diminish the cervical production of mucous and the external os can, as the cervix shrinks, come to be level with the roof of the vaginal vault. It is not clear to what extent these changes are affected by HRT nor how they might affect the movement of materials into the uterus from the vagina. The stenosis of the cervical canal which sometimes occurs postmenopausally would however clearly reduce the likelihood of vaginal contents entering the uterus. Conversely, a variable but often substantial quantity of the dosage form was lost from the vagina via the introitus. Even as early as approximately two hours post-dosing, retention within the vagina varied from 97% to 9%; at about six hours these figures were 93% and 1%. In the majority of cases the profile was of early and heavy loss of the formulation followed by a relative plateau.

In five of the six volunteers the pessary and gel formulations behaved similarly for a particular volunteer but displayed great differences between volunteers; in a single volunteer the gel was relatively well retained whilst the pessary was not. Thus, once the pessary had melted its retention behaviour was generally indistinguishable from that of the gel. It is therefore interesting to try and speculate as to the reasons for a lack of *in vivo* bioadhesion for the gel formulation. The Replens® formulation consists of approximately 78% water along with polycarbophil (1–3%) and other humectants and lipid lubricants. The presentation of the product is that of a thick emulsion gel of approximately 60,000 to 80,000 cps viscosity (3). Whilst there is evidence that polycarbophil can adhere to mucosal tissue in its dry state this is principally due to its ability to imbibe water. The vaginal product contains the polymer in a heavily hydrated form which presumably significantly reduces the potential for *in vivo* adhesion. It is therefore likely that the vaginal spreading and retention characteristics of the formulation reflect those of a thick gel or solution rather than a bioadhesive product. Further research to evaluate gel formulations of similar viscosity, with and without polycarbophil, would be useful to further investigate this issue.

The reason for the variation between volunteers is not yet clear. The 'retainers' (volunteers 4 & 5) did not appear to differ from the other subjects with respect to their obstetric and gynaecological history. Once the pessary has melted it is tempting to speculate that its loss and that of the gel will be in part determined by the inclination of the vagina to the vertical. It was not possible, for technical reasons, to obtain images in a coronal plane which would have permitted some quantification of the alignment of the vagina and hence of the variation between volunteers. It is also tempting to propose

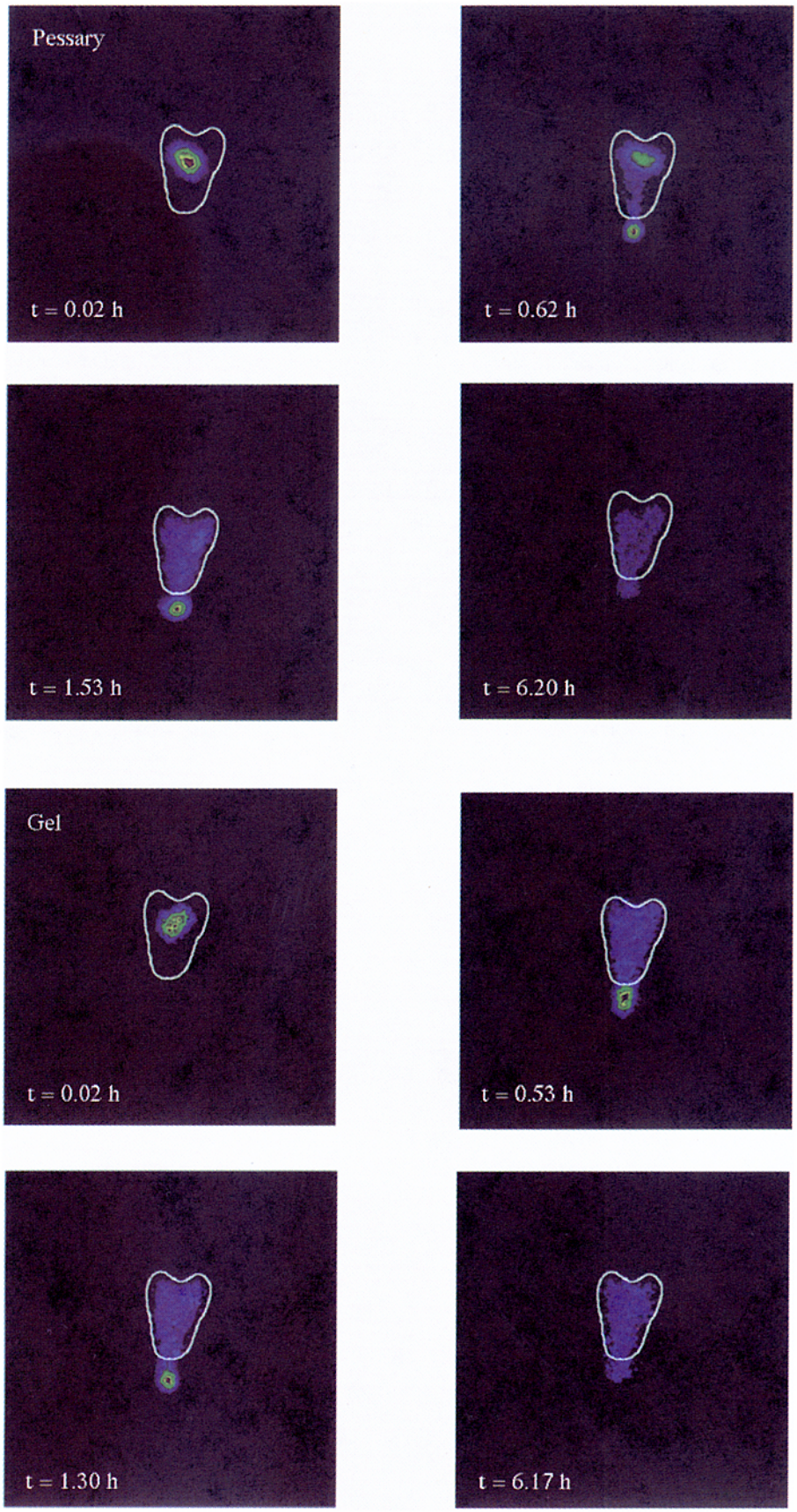

Fig. 4. Vaginal spreading and clearance of pessary and gel formulations in Subject 1.

that variations in retention are related to some sphincteric effect in the lower vagina. However, a consequence of such an effect would presumably be pooling of material proximal to the sphincter and there was no evidence from the scintigraphic images that this occurred. Indeed the intra-vaginal distribution of the formulations failed to show any particular pattern other than that of spread throughout the vagina soon after administration.

The walls of the vagina are normally in contact and it may be that the subjects are retaining only enough material to coat these surfaces. If this is the case then either the coated area varies substantially, (even if we consider only the 'non-retainers'), or the thickness of the coating varies greatly between volunteers. However, even in the 'retainers' there was no scintigraphic evidence of localised pooling.

The lack of retention of these products in most of the volunteers has obvious implications for the delivery of therapeutic agents. If these are intended to be locally active it is difficult to imagine an administered dose which would both be effective in the early, high loss cases and yet avoid excessive exposure in the 'retainers'. In addition, the vagina offers a further alternative route for the systemic administration of drugs. To use it for this purpose would almost certainly require much better control over the residence time of any formulation which was to be so used, so that the dose received by the patient would be within a defined limit.

This study demonstrates that gamma scintigraphy is a valuable method with which to assess novel formulations aimed at optimising retention in the vagina for topical or systemic drug delivery.

REFERENCES

1. J. L. Richardson and L. Illum. *Adv. Drug Del. Rev.* **8**:341–366 (1992)
2. C. J. Elias and L. Heise. *Population Council Working Papers* **6**:1–105 (1993)
3. J. R. Robinson and W. J. Bologna. *J. Cont. Rel.* **28**:87–94 (1994)
4. J. L. Richardson, J. Whetstone, A. N. Fisher, P. Watts, N. F. Farraj, M. Hinchcliffe, L. Benedetti and L. Illum. *J. Cont. Rel* **42**:133–142 (1996)
5. A. Joglekar, C. T. Rhodes and M. Danish. *Drug Dev. Ind. Pharm.* **17**:2103–2113 (1991)
6. G. Meseuguer, R. Gurny and P. Buri. *J. Drug Target.* **2**:269–288 (1994)
7. S. P. Newman, K. P. Steed, J. G. Hardy, I. R. Wilding, G. Hooper and R. A. Sparrow. *J. Pharm. Pharmacol.* **46**:657–660 (1994)
8. I. R. Wilding, C. J. Kenyon, G. Hooper, S. Marshall, J. S. McCracken, S. Chauhan, H. J. Staab and J. Armbrecht. *Aliment. Pharmacol. Therapeut.* **9**:161–166 (1995)
9. I. R. Wilding, A. J. Coupe and S. S. Davis. *Adv. Drug Del. Rev.* **7**:87–117 (1991)
10. S. P. Newman. *Critical Reviews in Therapeutic Drug Carrier Systems* **10**:65–109 (1993)
11. J. L. Richardson, P. A. Ramires, M. R. Miglietta, M. Rochira, L. Bacelle, L. Callegaro and L. Benedetti. *Int. J. Pharm.* **115**:9–15 (1995)
12. P. Britton, P. Flanagan, W. P. Hart and D. Linkin. *United States Patent No. 5,458,884*
13. P. Putteman, M. K. J. François and E. C. L. Snoeck. *PCT Patent No. WO 95/31178*
14. W. J. Henderson, T. C. Hamilton, M. S. Baylis, C. G. Pierrepoint and K. Griffeths. *Environ. Res.* **40**:247–250 (1986)
15. A. P. Wehner and R. E. Weller. *Food Chem. Toxicol.* **24**:329–338 (1986)