Targeted drug delivery in gynaecology: the first uterine pass effect

Carlo Bulletti[1,4], Dominique de Ziegler[2,3], Carlo Flamigni[1], Evaldo Giacomucci[1], Valeria Polli[1], Gianfranco Bolelli[1] and Franca Franceschetti[1]

[1]1st Institute of Obstetrics and Gynecology, University of Bologna, Via Massarenti 13, 40138 Bologna, Italy, [2]Columbia Laboratories, Paris, France and [3]Nyon Medical Center, Nyon, Switzerland

[4]To whom correspondence should be addressed

The objective was to verify the hypothesis of a 'first uterine pass effect' or direct preferential vagina-to-uterus transport, suggested by the evidence of higher than expected uterine tissue concentrations after vaginal administration of progesterone; we used a human ex-vivo uterine perfusion model. A mixture of tritiated (^{3}H) and unlabelled progesterone was applied to the cuff of vaginal tissue remaining attached to the cervix after hysterectomy. At the end of the perfusion period (up to 12 h), ^{3}H and ^{14}C radioactivity was measured in samples of uterine tissue. Tritiated water and [^{14}C]dextran were tested to determine the extent of non-specific vagina-to-uterus transport (leaks). Finally, sections of uterine tissue exposed only to [^{3}H]progesterone were prepared for autoradiography. By 4–5 h after application progesterone had diffused to the entire uterus and had reached a steady state; 4 h after application, progesterone concentrations reached 185 ± 155 and 254 ± 305 ng/100 mg of endometrial and myometrial tissue respectively. Endometrial extraction of progesterone was higher when the experiment was performed on uteri obtained during the luteal phase (280 ± 156 ng/100 mg of endometrial tissue) than those removed during the proliferative phase of the menstrual cycle (74 ± 28 ng/100 mg of endometrial tissue). These data demonstrate that a 'first uterine pass effect' occurs when drugs are delivered vaginally, thereby providing an explanation for the unexpectedly high uterine concentrations relative to the low serum concentration observed after vaginal administration. Hence, the vaginal route permits targeted drug delivery to the uterus, thereby maximizing the desired effects while minimizing the potential for adverse systemic effects.
Key words: endometrium/first uterine pass effect/progesterone/ transvaginal drugs delivery/uterine contractility

Introduction

In recent years targeted drug delivery has become one of the prime objectives in the development of new dosage forms. Targeted delivery is designed to maximize the therapeutic effect while minimizing the potential for systemic side-effects.

The classical approach to targeted drug delivery relies on the proximity of delivery to the active site such as in the use of topical steroids or ophthalmic preparations. Traditionally the vaginal route was used either to provide women with therapy for local disorders or as an alternative route for systemic administration via this highly vascular tissue. This latter use has been seen as particularly advantageous for drugs such as progesterone that are highly metabolized when taken orally and have such poor skin permeability that transdermal administration is impractical. During the course of previous experiments with vaginal progesterone it has become increasingly apparent that uterine effects exceed the response that can reasonably be expected from the circulating levels of progesterone achieved (De Ziegler *et al.*, 1992; Miles *et al.*, 1994). Similar findings have been reported with substances other than progesterone, namely terbutaline (Kullander and Svanberg, 1985) and danazol (Mizutani *et al.*, 1995). These unexpected but markedly similar findings led us to postulate the existence of a preferential delivery to the uterus or a first uterine pass effect of drugs administered vaginally.

To challenge this provocative concept we used a human ex-vivo uterine perfusion model (Bulletti *et al.*, 1986, 1987, 1988a,b). In these experiments we used freshly excised human uteri and applied [^{3}H]progesterone directly over the rim of vaginal tissue removed with the cervix and uterus at the time of surgery. This method was chosen rather than animal studies because of the species-specific differences in the utero-vaginal tract and the advantages of an open circulatory system that allowed us to exclude contaminations inherently linked to the recirculation of radioactive compounds.

Materials and methods

Surgical specimens

A total of 39 uteri was obtained in the operating room. Each was removed with a rim of 1.5–2 cm of vaginal tissue from women 34–52 years of age undergoing abdominal hysterectomy for early stage cervical carcinoma (CIN) or uterine prolapse. All patients consented freely to participate in the study and to have their uteri used until experiment completion; the study obtained the Institutional Review Board approval. All uteri were then sent to pathology for definitive analysis. The subjects were carefully selected preoperatively as previously described (Bulletti *et al.*, 1986, 1987, 1988a,b). The hysterectomies were programmed to obtain specimens during the proliferative (*n* = 24) and secretory (*n* = 11) phases of normal menstrual cycles. The histological examination was used to confirm the phase of menstrual cycle. Five experiments were performed to establish the non-specific uptake by uterine tissues of membrane-impermeable dextran at different intervals (1, 2, 3, 4, 12 h). Nine experiments were used to determine the accumulation of progesterone

in the endometrium and myometrium 1 (n = 3), 2 (n = 3) and 3 (n= 3) h after the application of progesterone on the cuff of vaginal tissue removed with the hysterectomy. Twelve more uteri were used to confirm the amount of progesterone found in the uterine tissues after 4 h of incubation when it was established that this represented the time required to observe complete uterine impregnation after vaginal administration of progesterone. Nine other uteri were used to establish the amount of progesterone remaining in the uterine tissue or washed out through the uterine veins at 5 (n = 3), 6 (n = 3) and 12 (n = 3) h after its vaginal application, respectively. Finally, four uteri used for autoradiography studies were exposed to a single tracer, [³H]progesterone. These four uteri were not available for histological and pharmacokinetic studies.

Perfusion procedure

Uterine perfusions were performed using a technique extensively reported (Bulletti *et al.*, 1986, 1987, 1988a,b) that allows good preservation of the organ as reflected by oxygenation and responsiveness of tissue samples.

Vaginal administration of test and reference substances: calculation of the tissue extractions

One hour after placing the uterus into the in-vitro perfusion device, the cuff of vaginal tissue removed with the uterus at the time of hysterectomy was covered by a mixture of [³H]progesterone and 100 mg of non-radioactive progesterone in oil. All radiolabelled isotopes were purchased from DuPont NEN (Boston, MA, USA). Transport to the uterus (calculated as whole organ, vaginal, endometrial or myometrial extraction) was measured 1, 2, 3, 4, 5, 6 and 12 h after the vaginal application of the oil containing the mixture of either radioactive (hot) and non-radioactive (cold) test (progesterone) or test radioactive dextran. Radioactive water and butanol were used as freely diffusible reference substances. Venous perfusates were also collected at hourly intervals for radioactivity measurement. The solution applied vaginally consisted of either of [³H]progesterone/ cold progesterone (test substance) and [¹⁴C]butanol (reference substance) for the assessment of specific progesterone extraction, [¹⁴C]dextran (test) and [³H]water (reference) for the assessment of a specific tissue extraction. [³H]Progesterone only was used for the autoradiography. Experiments were interrupted at predetermined time intervals after vaginal application of the mixture of hot and cold progesterone and samples of vagina, myometrium and endometrium were taken with a through-cut biopsy needle (Travenol Laboratories, Deerfield, IL, USA) or a Novak (Chirurgica, Bologna, Italy) curette respectively. These samples were used for histology and determination of ³H and ¹⁴C levels. Representative tissue samples of vagina, endometrium or myometrium (~100 mg) were obtained from each uterus and dissolved in 1 ml of Soluene (Packard Instruments, Chicago, IL, USA) and 15 ml of scintillation fluid (Dimilume 30, Packard Instruments). Moreover, aliquots of the original oil solutions used to administer the mixture of cold and hot progesterone and of venous effluent were similarly prepared for double-isotope liquid scintillation counting in an LKB 1215 Rackbeta 2 scintillation spectrometer (LKB-Produkter AB, Bromma, Sweden). A ratio of at least 2:1 of ³H-labelled test to ¹⁴C-labelled reference radioactivity was used to minimize the error of ¹⁴C radioactivity overlapping into the ³H channel. Isotope counts per min were converted to disintegrations per min making standard quench corrections.

The vaginal, endometrial and myometrial influx indices of [¹⁴C]dextran relative to [³H]water were calculated as previously reported by Pardridge (1981), Pardridge and Mietus (1979), Laufer *et al.* (1983), Verheugen *et al.* (1984) and Steingold *et al.* (1986). In this paradigm, the index for dextran was calculated as (¹⁴C/³H) in endometrium

(vagina or myometrium)/(¹⁴C/³H) mixture here used for the vaginal administration. When [³H]progesterone and [¹⁴C]butanol were used, the index for test progesterone was calculated (³H/¹⁴C) in endometrium (vagina or myometrium)/(³H/¹⁴C) in mixture here used for the vaginal administration.

A mixture containing about 2 μCi [¹⁴C]dextran and 5 μCi [³H]H₂O was applied to the vaginal cuff to determine both the extraction of progesterone from uterine tissues and transit time of this mixture through the organ when a constant flow rate of 25 ml/min was maintained in the arterial perfusion. Because of its membrane impermeability, [¹⁴C]dextran radioactivity was used as a reflector of non-specific transport from the vaginal collar to the uterine tissues. The oil solution applied on the vagina contained ~26 μCi [³H]progesterone and 1.5 μCi of [¹⁴C]butanol. Uterine extraction of isotopes applied vaginally was taken as evidence of direct vagina-to-uterus transport or 'first uterine pass effect' of these substances. Because the experimental uterine perfusion model avoided re-circulation of the perfusate, contamination or venous effluent by residual vascular radioactivity at the second passage through the organ is not possible. [¹⁴C]Butanol served as reference isotope because of its freely diffusable characteristics. Measured radioactivity of [³H]progesterone was converted into nanograms of progesterone assuming identical pharmacokinetics of the labelled and unlabelled progesterone content of the oil. The net vaginal, endometrial and myometrial extractions of the [³H]progesterone and [¹⁴C]butanol were determined for each experiment each concluded at set time intervals after application of the test substance on the vaginal cuff. The extraction of [³H]progesterone and [¹⁴C]butanol increased from the first to third hour and remained constant thereafter for up to 6 h of uterine perfusion. Hence, uterine extraction data of vaginally administered test or reference substances were calculated from specimens analysed ≥4 h after the application of the radioactive of mixture. According to Pardridge (1981), Pardridge and Mietus (1979), Laufer *et al.* (1983), Verheugen *et al.* (1984) and Steingold *et al.* (1986), tissue extraction of test progesterone was obtained from the following formula:

$$E_p = TII \times E_b$$

where E_p and E_b represent the percentage extraction of progesterone and butanol respectively, 4 h after vaginal application of the oil, and TII the tissue influx index. Statistical analysis of the results was performed by the Student's two-tailed *t*-test with two independent means for each individual experiment.

Autoradiography

Autoradiography was performed from thin transverse sections (~5 mm) of the perfused uteri obtained after 1, 2, 3 and 4 h after vaginal application of the standard dose of [³H]progesterone. Uterine sections of the organ featuring the endometrial tissue in its centre were obtained at different distances from the internal os of the cervix in order that the autoradiography data could be used to assess the time dependency of the uterine invasion by vaginally administered progesterone. Tissue sections were positioned on Hyperfilm ³H 18×24 cm (code RPN 535) obtained from Amersham Italia s.r.l. (Milan, Italy) for 28 days at –80°C.

Results

Background dextran (non-specific) accumulation in the endometrium and myometrium was 9 and 6% of the labelled progesterone extracted by these tissues, a value that needed to be subtracted from the amounts of [³H]progesterone counted in tissue samples. Figure 1 shows the endometrial extraction
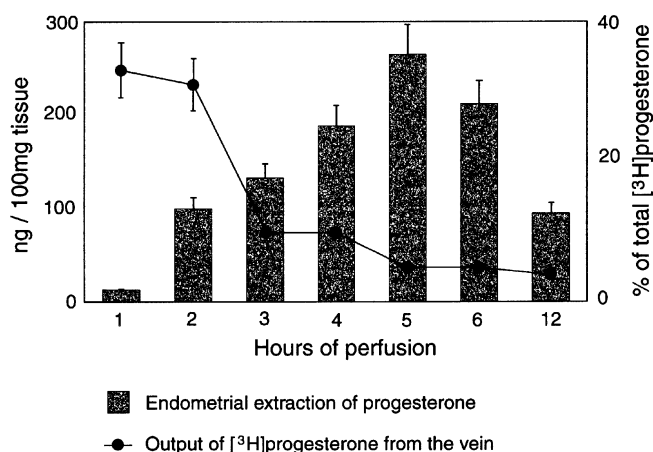
Figure 1. Mean % (± SD) of the total radioactive progesterone that crosses the uterus (*n* = 30 organs) during 12 h following its vaginal application *in vitro* on the vaginal collar (graph), and the amount of its accumulation in the endometrium (bars).

of progesterone and the corresponding venous outflow of this compound during the 12 h uterine perfusions that followed vaginal application of labelled and unlabelled progesterone on 30 organs. The [³H]progesterone started to be recovered in uterine effluent during the first hour of perfusion (33 ± 9% of total dose, mean ± SD) and persisted to efflux from the uterus during the second hour (31 ± 12% of total dose). Thereafter, progesterone outflow decreased during the third and fourth hours of perfusion (10 ± 5% and 10 ± 11% respectively) and remained constant at 5 ± 3%, 5 ± 2% and 4 ± 4% during the fifth, sixth and 12th hours of perfusion respectively. The mean ± SD extractions of progesterone from 100 mg of endometrium collected at timed intervals after vaginal application of the [³H]- and cold progesterone loads were: 12 ± 10 ng (*n* = 3), 99 ± 19 ng (*n* = 3), 132 ± 37 ng (*n* = 3), 188 ± 155 ng (*n* = 12), 265 ± 64 ng (*n* = 3), 212 ± 46 ng (*n* = 3) and 95±34 ng (*n* = 3) after 1, 2, 3, 4, 5, 6 and 12 h respectively. The mean ± SD extractions of progesterone from 100 mg of myometrium (data not shown) collected at the same timed intervals after vaginal application of progesterone were: 0 ng (*n* = 3), 31 ± 5 ng (*n* = 3), 267 ± 84 ng (*n* = 3), 254 ± 305 ng (*n* = 12), 299 ± 87 ng (*n* = 3), 223 ± 98 ng (*n* = 3), 77 ± 23 ng (*n* = 3). The endometrial and myometrial extractions of progesterone 4 h after vaginal application on 12 uteri are reported in Figure 2. For comparison simultaneous extraction from vaginal tissue was also reported (6.5 + 9.7 µg/100 mg of tissue) and depicted in Figure 2.

After 4 h of perfusion of four organs, autoradiography of uterine sections showed uniform capture of radioactivity by the endometrium in all the sections obtained as illustrated in Figure 3 (top left and right). In the myometrium, however, autoradiography showed an apparent accumulation of radioactive tracer in, or near, vascular casts. Figure 3 (bottom left and right) shows the progression of the front of progesterone and
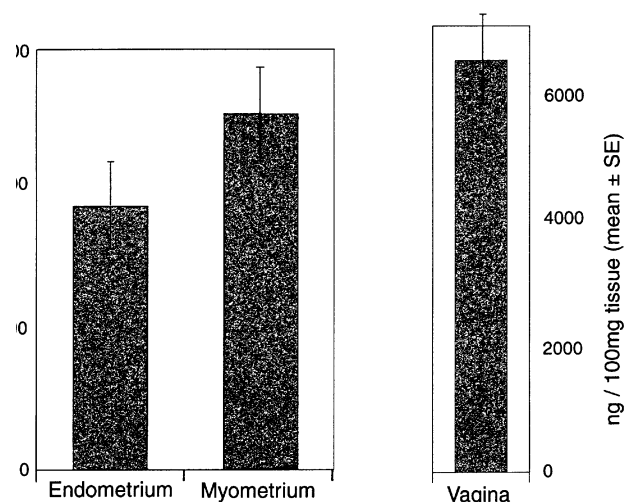

'e 2. Steady state of the accumulations (mean ± SE) of ‥sterone (ng/100 mg) in the endometrium, myometrium and vagina 4 h after its vaginal application on 12 uteri *in vitro*.

butanol migration into the uterine organ as a function of time as established from all the reported experiments (extraction data and autoradiography).

The mean ± SD extractions of progesterone from specimens obtained 4 h after vaginal application during the proliferative phase were 74 ± 28 ng (*n* = 5) and 141 ± 161 ng (*n* = 5)/100 mg of endometrium and myometrium respectively, compared with 280 ± 156 ng (*n* = 7) (*P* < 0.01) and 346 ± 367 ng (*n* = 7) (not significantly different)/100 mg of endometrium and myometrium respectively in organs obtained during the secretory phase of the menstrual cycle (Figure 4a,b).

Discussion

The study demonstrated that in this human ex-vivo uterine perfusion model radioactive progesterone applied to the vaginal cuff, remaining after hysterectomy, progressively migrates into the uterus and reaches high concentration in both the endometrium and the myometrium. Hence, vaginal drug administration results in high uterine concentration (Miles *et al.*, 1994; Mizutani *et al.*, 1995) associated with a direct transport from the vagina to the uterus — the first uterine pass effect. In an elegant study conducted on agonadal women preparing for embryo donation Miles *et al.* (1994) observed that vaginal administration of micronized progesterone enhanced progesterone delivery to the uterus by ~10-fold when compared to i.m. injections despite the markedly higher (~7-fold greater) circulating concentrations achieved with i.m. administration. Remarkably, in this and other studies, vaginal progesterone reliably induced a synchronous secretory transformation of the endometrium (Miles *et al.*, 1994). A similar uterotrophic paradox was found when serum and uterine concentrations of danazol were compared following either vaginal delivery of 100 mg/day or oral administration of 400 mg/day (Mizutani *et al.*, 1995). Consistent with this theory of uterine selectivity after vaginal administration is the observation of Kullander and Svanberg (1985) that vaginal administration of the β-agonist terbutaline in women just prior to hysterectomy
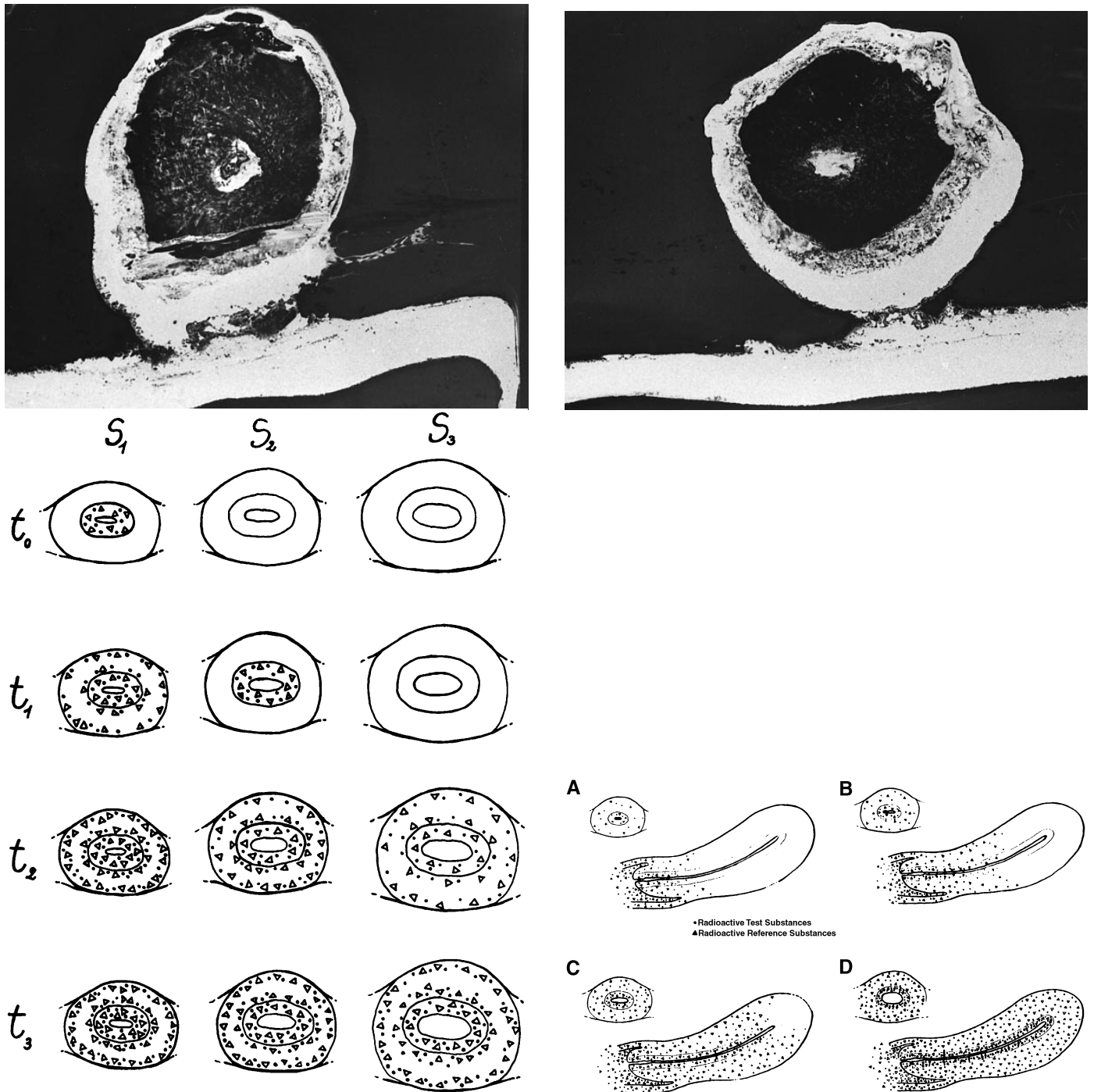
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Figure 3. Autoradiography of two representative layers (**top left** and **right**) of human uterus obtained 4 h after vaginal application of radioactive progesterone and uterine perfusion of four organs *in vitro*. Progesterone accumulated in the endometrium (in the middle of the radiographs), around the organ, in the external third of the muscle, and, apparently, around the vessels. The endometrial uptake of radioactive progesterone (in the centre) was obtained by cutting transverse sections of the organ at different distances from the internal os of the cervix, in order to collect autoradiography data enabling an assessment of time dependency of the uterine invasion by vaginally administered progesterone. The front of migration of progesterone is reported in the diagrams in (**bottom left**) and (**bottom right**) as a function of time after its application during the extracorporeal perfusion of the uterus ($n = 4$). Radioactive test substances were [³H]progesterone and [³H]water, and the corresponding reference (freely diffusible) substances were [¹⁴C]butanol and [¹⁴C]dextran respectively. S_1, S_2 and S_3 are three transverse sections of the perfused uteri (**c**) collected after 1 (t_0), 2 (t_1), 3 (t_2) and 4 (t_3) h from vaginal application of radioactive progesterone under uterine extracorporeal perfusion at body temperature. The schematic views (**bottom right**) of the front of migration of the radioactive test and reference substances in longitudinal section after their vaginal application show their progressive transit through the organ over time (**A–D**).
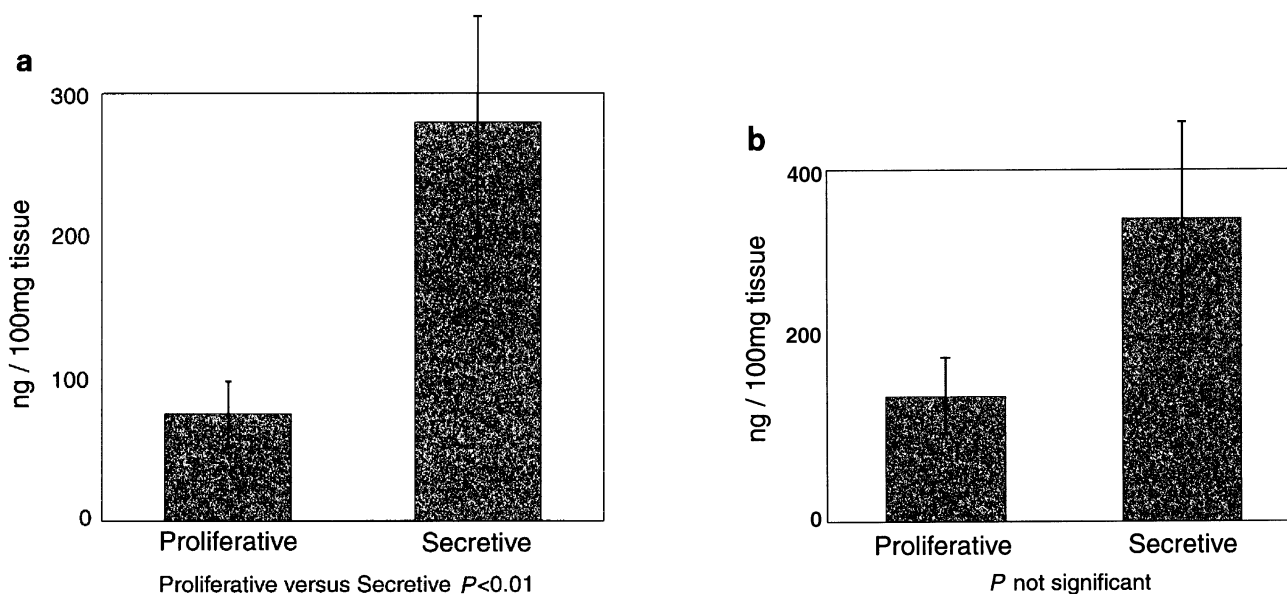
Figure 4. Accumulation of progesterone (mean ± SD) in the endometrium (**a**) and myometrium (**b**) after extraction, in both the proliferative and secretory phases of the menstrual cycle.

resulted in uterine vein drug concentrations that exceeded those in the general circulation. The practical applications of direct vagina-to-uterus transport have already been utilized in clinical trials. Hausknecht (1995) showed that the vaginal administration of misoprostol tablets after the parenteral administration of methotrexate induced a reliable termination of early pregnancies, thereby confirming the greater efficiency of vaginal versus oral misoprostol in inducing uterine contractions (El-Refaey *et al.*, 1995).

The prompt migration of progesterone into the uterine tissue in a human in-vitro extracorporeal perfusion non-recycling system provides the documentary evidence of direct vagina-to-uterus transport. Since progesterone administered vaginally accumulates in the uterus disproportionately compared with the situation after oral ingestion, a vagina-to-uterus diffusion system permitting the concentration of hormone independent of its vascular effluent levels can be postulated. The phenomenon demonstrated by our experiment with the in-vitro uterine perfusions shows that a fraction of the vaginally administered progesterone reaches the uterus before being transported elsewhere in the body which is equivalent to a functional analogue of an anatomical portal system.

The current study is an adaptation of a model originally developed to delineate the transport of steroids from the vascular compartment to the uterine tissue (Bulletti *et al.*, 1988a) in order to assess the response to steroid hormones *ex vivo* (Bulletti *et al.*, 1987) and the electromechanical events induced (Bulletti *et al.*, 1993). Moreover, the physiological nature of this ex-vivo model was demonstrated in a previous study which showed that the early steps of embryo implantation occur when the uterus is maintained in this perfusion system (Bulletti *et al.*, 1988b).

After 4 h of uterine perfusion, a steady state level in tissue was achieved. Tissue concentrations after vaginal administration of [³H]progesterone and the radioactivity recovered from the venous effluent also reached steady state. The quantity of

³H recovered from the venous effluent was a reflection of progesterone exiting from the organ. Chromatographic analysis of the perfusate should determine whether local progesterone metabolism occurred in the uterus and this will be the subject of an additional study. The quantity of [¹⁴C]dextran that accumulated in the uterine tissue 4 h after vaginal application represents an aspecific vagina-to-uterus transport because dextran is a membrane-impermeable compound.

The accumulation of radioactive tracer observed by autoradiography appears homogeneous in the endometrial tissue but myometrial uptake shows a less uniform dispersion. There is a notable accumulation of [³H]progesterone around vascular casts (Figure 3 top left and right). This observation may be seen as supporting the hypothesis of a counter arterial-to-venous perfusion. Alternatively, passive diffusion possibly facilitated by rhythmic peristaltic-like uterine contractions may be followed by vessel capture of steroids leading to radioactivity accumulation in the film. Yet despite autoradiography showing a lower accumulation of radioactive progesterone in the myometrium, higher tissue concentrations were found in the myometrium than in the endometrium. It is possible that radioactive progesterone accumulates in the walls of vessels, thereby mapping their topography on the autoradiographic film, whereas radioactivity in the extravascular system, which is more diffused in the myometrium than endometrium, is not detected on the film. Since the ratio of vessels to extravascular tissue is higher in the endometrium than the myometrium, this could explain why there is a more homogeneous appearance of the endometrium with autoradiography.

Moreover, the cyclical development of a complex network of microvessels in the endometrium that culminates during the luteal phase of the menstrual cycle may explain the differences in progesterone concentrations observed between phases of the cycle in which the uteri were obtained. Two other mechanisms could also help explain the higher accumulation of

progesterone in the endometrium during the luteal phase. First, a higher concentration of progesterone receptors might account for higher accumulation during the early luteal phase; however, progesterone receptors are already present in the late follicular phase and disappear from the endometrial gland during the second half of the luteal phase. Second, the different patterns of uterine contractility that have been observed in the two phases of the menstrual cycle (Bulletti *et al.*, 1993) could also provide an explanation for the different degrees of radioactive capture, possibly by an enhanced facilitation of passive transportation at given times in the menstrual cycle.

Targeting drug delivery through vaginal administration is particularly appealing for substances destined to exert their primary action on the uterus itself. This includes progesterone that is needed to antagonize the proliferative effects of oestrogen replacement therapy on the endometrium, to prepare the endometrium for embryo implantation in assisted reproductive techniques and for luteal phase support in infertility treatments and early pregnancy. Among the benefits of administering progesterone vaginally are: (i) the avoidance of local pain and possibly abscess from i.m. injections, particularly when prolonged progesterone treatment is warranted as for recipients of oocyte donation; (ii) the avoidance of extensive first-pass hepatic metabolism of progesterone that is seen after oral administration (Maxson and Hargrove, 1985) and the undesirable and possibly dangerous hypnotic effects (e.g. during motor vehicle operation) of progesterone metabolites (Arafat *et al.*, 1988); and (iii) the possibility of a truly physiological, non-oral, hormonal replacement when combined with transdermal oestradiol in cases where a high risk of cardiovascular disease precludes the use of oral or transdermal synthetic progestins such as in the secondary prevention of cardiovascular disease.

Our demonstration that vaginal administration of progesterone is associated with a first uterine pass effect opens new therapeutic options for the administration of compounds whose primary site of action is the uterus. The frequency and intensity of uterine peristaltic contractions were found to change during each phase of the menstrual cycle (Kunz *et al.*, 1996) and they may provide an important biological function in the human, such as gamete/embryo transportation; abnormal uterine contractility may thus cause sterility and infertility through disturbances in gamete/embryo transportation and embryo nidation. Abnormal uterine contractions may also be related to ectopic pregnancy and dysmenorrhoea, and the use of substances which participate in the regulation of those contractions may have an important role in the future management of several gynaecological diseases. Other smooth muscle-based organs show abnormal contractility (e.g. irritable bowel disease or flutter/ fibrillation of the heart). Primary candidates for targeted vaginal-to-uterine delivery are those agents that either antagonize or stimulate uterine contractions. The utero-relaxants most commonly used to prevent preterm labour are the β-adrenergic agonists such as terbutaline. Further studies should determine if there is a benefit in the administration of terbutaline vaginally and the achievement of a first uterine pass effect. Presumably, such administration would maximize the relaxant effect on the uterus while avoiding the dangerous effects on heart rate and

glucose metabolism which limit oral and parenteral use of these agents.

Another group of potentially useful utero-relaxants, the nitric oxide donors such as nitroglycerine, may also benefit from targeted delivery to the uterus. The clinical advantage of administering utero-contractant drugs such as misoprostol vaginally has already been demonstrated (Hausknecht, 1995). An added advantage of the vaginal route of administration is the potential to sustain the release by admixing the drug with a bioadhesive gel (De Ziegler *et al.*, 1995) thereby combining the advantages of non-oral targeted administration with those of controlled and sustained release. Finally, our demonstration of a first uterine pass effect offers a fascinating new perspective for the development of vaginal contragestive therapy, which can alter endometrial receptivity without changing the cyclical production of sex hormones.

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