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Comparative in vitro permeability of human vaginal, small intestinal and colonic mucosa

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Abstract

Our previous experience with a continuous flow-through perfusion system has demonstrated its usefulness for studying diffusion kinetics of drugs across small intestinal mucosa for bioavailability/bioequivalence (BA/BE) studies. During the last decade, delivery of drugs to the colon for systemic absorption as well as for local delivery in certain colonic diseases, has been extensively investigated. For this reason, we sought to assess the in vitro comparative permeability of human vaginal, small intestinal and colonic mucosa using a flow-through perfusion method. It was clear from our studies that human colonic epithelium was statistically significantly ($P < 0.05$) more permeable to water, 17β-estradiol, arecoline and arecaidine than intestinal mucosa. However, both these mucosae were statistically significantly less permeable to the above four permeants than human vaginal mucosa. As previously shown for small intestinal mucosa, the low in vitro permeability of colonic mucosa to drugs with molecular weight >300 Da may necessitate using other epithelial membranes, e.g. vaginal mucosa, as alternative barriers for in vitro BA/BE studies. We also concluded that the flow-through mucosal perfusion system used in our laboratory is therefore also potentially useful for determining the permeability of a therapeutic agent from the colon for registration purposes.

Keywords: Human intestinal mucosa; Permeability studies; Various compounds

1. Introduction

Over the past number of years, several experimental methods have been described to evaluate in vivo/in vitro bioavailability/bioequivalence (BA/BE) of therapeutic agents. In this respect, two regulatory authorities, one in Europe (CMCP) and one in the USA (FDA), have proposed certain guidelines (CPMP Note, 1998; FDA Guidance, 2000). The latter body has suggested that one such in vitro approach to determine the permeability of a drug from the gastrointestinal tract would be to use excised human or animal intestinal tissues. For these purposes, viable or non-viable tissues would be suitable.

Our previous experience with a continuous flow-through perfusion system to determine the diffusion kinetics of a wide variety of therapeutic agents and other chemical compounds across fresh and frozen human vaginal and buccal mucosa, skin, venous tissue and rabbit as well as human corneas, prompted us to use this system to study the permeability characteristics of fresh/frozen small intestinal mucosa (Van der Bijl et al., 1997, 1998a,b,c, 2000a,b, 2001a,b, 2002a,b). In this respect, the diffusion characteristics

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of water, 17β-estradiol, sumatriptan, arecoline, arecaidine, cyclosporine and vasopressin were determined (Van der Bijl and Van Eyk, 2002). No statistically significant differences between the flux values of the compounds tested across fresh and frozen small intestinal tissue were found. Furthermore, the flux rates of these compounds across the intestinal mucosa decreased with increasing molecular size and were low above molecular weights of 300 Da. When compared with flux rates across human vaginal and buccal mucosa, intestinal permeability to these compounds was 36–160% lower. We concluded that the flow-through perfusion system used in our laboratory showed promise as an in vitro method for permeability determination through small intestinal mucosa. However, due to the low permeability of the latter tissue to large compounds we suggested that other human mucosa, e.g. vaginal mucosa, may have to be considered if therapeutic agents having molecular weight >500 Da are evaluated for in vitro BA/BE purposes.

Delivery of drugs to the colon has been extensively investigated during the last decade (Nykanen et al., 2001). Specific targeting of drugs to the colon is recognised to have several therapeutic advantages. Those drugs that are destroyed by gastric acid and/or metabolised by pancreatic enzymes, are only slightly affected in the colon and systemic uptake of drugs formulated specifically for sustained colonic release can be useful in the treatment of nocturnal asthma, angina and arthritis (Kinget et al., 1998). Treatment of colonic diseases, e.g. ulcerative colitis, colorectal cancer and Crohn's disease is more effective when drugs are delivered directly to the affected area and then diffuse through the colonic epithelium. Similarly, it has been shown that colonic delivery of vermicides and colonic diagnostic agents require smaller doses compared to other routes of administration (Kinget et al., 1998). Furthermore, there have been conflicting reports regarding the relative in vitro permeabilities of small intestinal versus colonic epithelium in rats and humans, the order of permeability of these two mucosae not being consistent (Yamamoto et al., 1997; Fagerholm et al., 1997; Oliver et al., 1998).

In view of the above considerations and the potential usefulness of the flow-through perfusion studies for future in vitro BA/BE determinations, the aim of the current study was to assess the comparative permeability of human vaginal, small intestinal and colonic mucosa to several different chemical compounds.

2. Materials and methods

2.1. Vaginal mucosa

Specimens were obtained from excess tissue removed from 12 post-menopausal patients, mean age 68 ± 17 S.D. (range: 32–76) years, following vaginal hysterectomies at the Louis Leipoldt and Panorama Mediclinic Hospitals, Bellville, South Africa.

2.2. Small intestinal mucosa

Small intestine specimens (mean distance from duodeno-jejunal flexure: 92 ± 57 cm S.D.; range 20–150 cm) were obtained from excess tissue removed from 10 patients (five females, mean age 61 ± 13 S.D. (range: 47–72) years and five males, mean age 59 ± 12 S.D. (range: 43–67) years), following various surgical procedures, from the Department of Surgery, Tygerberg Hospital.

2.3. Colonic mucosa

Two colon specimens, one from the descending region of the colon close to the sigmoid junction (female, 62 years) and the other from the central region of the transverse colon (female, 64 years) were obtained from excess colonic tissue removed from surgical procedures at the Department of Surgery, Tygerberg Hospital.

All above surgical specimens obtained were immediately placed in a transport fluid, prepared as previously described (Van der Bijl et al., 1997, 1998a,b,c, 2000a, 2001a; Van der Bijl and Van Eyk, 2002) and transferred to our laboratory within 1 h. Excess connective and adipose tissue were trimmed away and all specimens were snap-frozen in liquid nitrogen and stored at −85 °C for periods up to 6 months (Van der Bijl et al., 1998d). No specimens were obtained where there was clinical evidence of any disease that might have influenced the permeability characteristics of the different mucosae.

The study was approved by the Ethics Committee of the University of Stellenbosch and the Tygerberg Hospital.

2.4. Permeability experiments

The diffusion kinetics of stable tritium radioisotopes of water, 17β-estradiol, arecoline and arecaidine through vaginal, intestinal and colon mucosae were determined for comparative purposes. The frozen mucosal specimens were equilibrated in PBS (pH 7.4) for 10 min at room temperature prior to the start of each experiment to thaw completely, after which they were carefully cut, so as not to damage the epithelial surfaces, into 4 mm diameter sections. These sections of mucosae were then mounted in flow-through diffusion cells (exposed areas 0.039 cm^2) as previously described (Van der Bijl et al., 1997, 1998a,b,c, 2000a, 2001a; Van der Bijl and Van Eyk, 2002) and permeation studies performed on seven tissue replicates for each patient. Tissue disks were equilibrated for 10 min in PBS (pH 7.4) at 20 °C in both the donor and acceptor compartments of the diffusion cells before each permeability experiment started. This step was followed by the removal of the PBS from the donor-break compartment and the addition of 1.0 ml of PBS containing either 1 μCi ^{3}H-water, 1.4 μCi ^{3}H-17β-estradiol, 0.5 μCi ^{3}H-reduced arecoline or 0.5 μCi ^{3}H-reduced arecaidine. Sigma Chemical Company (St. Louis, MO) supplied the ^{3}H-17β-estradiol and all the other radioisotopes were obtained from Amersham Laboratories (Little Chalfont, Amersham, UK). For determination of donor cell concentration at time zero, 100 μl aliquots were removed within minutes from each of the seven donor compartments. PBS at 20 °C was pumped through the acceptor chambers at a rate of 1.5 ml/h and collected, by means of a fraction collector, at 2-h intervals for 24 h. All permeability studies were performed under sink conditions, i.e. at the completion of each run the concentration of tritiated permeant in the acceptor chamber never reached 10% of that in the donor compartment. To each sample collected, 15 ml scintillation cocktail (Ready Protein+^{TM}; Beckman Instruments, Fullerton, CA) was added and the radioactivity determined using a liquid scintillation counter (Beckman LS 5000TD). The counting of the samples was continued until a 2-s value of 1% was reached. Quenching for each sample was automatically corrected in the counter.

2.5. Calculation of flux values

The flux values (J) across the membranes were calculated by means of the following relationship

$$J = \frac{Q}{A \times t} \quad (\text{dpm cm}^{-2}\,\text{min}^{-1})$$

where Q is the quantity of substance crossing membrane (in dpm), A is the membrane area exposed (in cm^2) and t is the time of exposure (in min).

2.6. Steady-state kinetics

The reaching of a steady state (equilibrium kinetics) was assumed for a particular specimen and tritiated permeant, when no statistically significant differences ($P < 0.05$; ANOVA and Duncan's multiple range test) between flux values were obtained over at least two consecutive time intervals.

2.7. Statistical analysis

Non-linear regression analyses were performed using a SigmaPlot 2001 Version 7.101 computer programme (SPSS Inc.). An F-test was used to compare entire curves (Motulski, 1995). A significance level of 5% was used for all tests and comparisons.

3. Results

The overall mean flux values of water across human vaginal, colonic and small intestinal mucosa versus time are shown in Fig. 1. For all three tissues, steady-state fluxes were obtained after approximately 5 h. Significant differences, at the 5% level, could be demonstrated between the steady-state flux values across small intestinal and vaginal mucosa ($P = 0.0015$), between small intestinal and colonic mucosa ($P = 0.008$) and also between colonic and vaginal mucosa ($P = 0.0001$) over the entire course of the experiment. Steady-state flux values for water across colonic mucosa were on average between 16 and 22% higher than those across small intestinal mucosa, and between 29 and 55% higher across vaginal than across colonic mucosa.

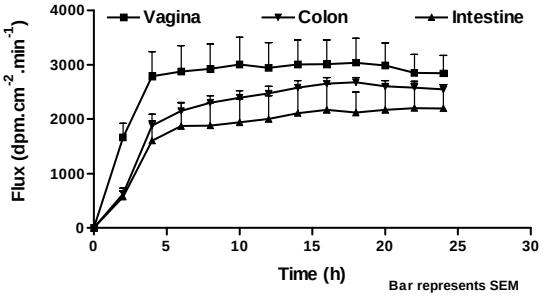

Fig. 1. Overall mean flux values of water across human vaginal, colonic and small intestinal mucosa.

For 17β-estradiol flux rates across the above three mucosae, steady-state conditions were not reached over the 24-h time course over which the experiment was conducted (Fig. 2). However, for comparative purposes, steady-state flux values were estimated by averaging flux rates at 20, 22 and 24 h. Flux rates of 17β-estradiol were approximately 19–20% higher on average across colonic than small intestinal mucosa over the entire duration of the permeability experiment, these differences being statistically significantly different ($P = 0.0003$). Flux rates across vaginal mucosa were approximately 50–152% higher as compared to small intestinal mucosa and found to be statistically significantly different ($P = 1 \times 10^{-12}$). Statistically significant differences ($P = 2 \times 10^{-9}$) were also found for flux rates of 17β-estradiol across vaginal and colonic mucosa.

Overall mean flux rates of arecoline across vaginal, colonic and small intestinal mucosa versus time are shown in Fig. 3. Steady-state fluxes were reached after approximately 12 h. As for 17β-estradiol, flux rates

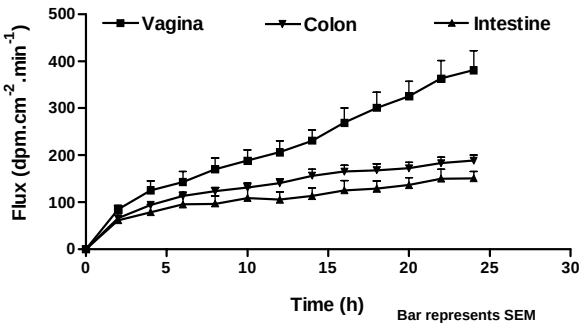

Fig. 2. Overall mean flux values of 17β-estradiol across human vaginal, colonic and small intestinal mucosa.

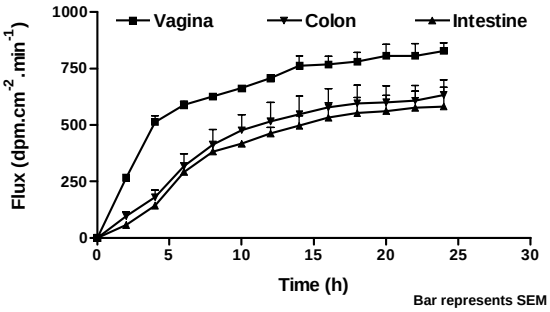

Fig. 3. Overall mean flux values of arecoline across human vaginal, colonic and small intestinal mucosa.

of arecoline across vaginal mucosa were between 30 and 185% higher than those across small intestinal mucosa and were found to be significantly different ($P = 1 \times 10^{-10}$). Flux rates across vaginal and colonic mucosa were also statistically significantly different ($P = 0.0004$). No significant differences were found between arecoline flux rates across colonic and small intestinal mucosa ($P = 0.4$). The flux rates found for arecaidine across the above three mucosae can be seen in Fig. 4. Steady-state conditions were reached after approximately 12 h. Flux rates of arecaidine across vaginal mucosa were between 30 and 192% higher when compared to small intestinal mucosa over the entire experiment and statistically significant differences were again found ($P = 0.0006$). After 15 h, flux rates across colonic mucosa were slightly higher than those across vaginal mucosa, but over the entire time course of the experiment no significant differences

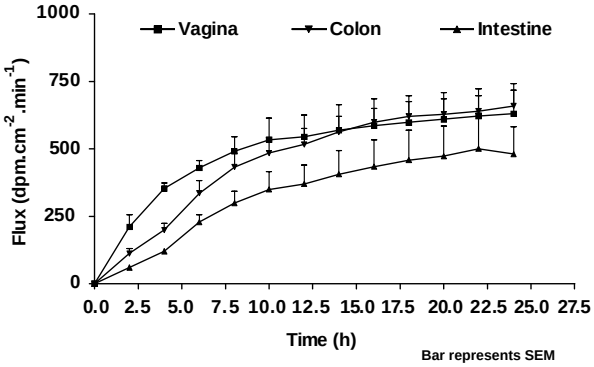

Fig. 4. Overall mean flux values of arecaidine across human vaginal, colonic and small intestinal mucosa.

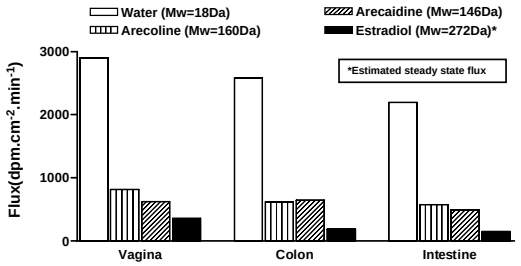

Fig. 5. True and estimated steady-state (20–24 h) flux values for various permeants across human vaginal, colonic and small intestinal mucosa.

were observed ($P = 0.5$) between these two mucosa. For the flux rates of arecaidine across colonic and small intestinal mucosa, significant differences were found ($P = 0.005$) over the course of the experiment. True and estimated steady-state flux values for water, 17β-estradiol, arecoline and arecaidine across vaginal, colonic and small intestinal mucosa are shown in Fig. 5.

4. Discussion

Previous studies have shown that vaginal and small intestinal mucosal specimens can be frozen and banked without their permeability properties to a number of different permeants being changed (Van der Bijl et al., 1998d; Van der Bijl and Van Eyk, 2002). In view of the foregoing, as well as preliminary results from studies on fresh and frozen colonic mucosal specimens conducted in our laboratory, the assumption of using frozen/thawed colonic tissue for the current permeability study was considered to be a reasonable one.

From the results obtained in the present study, it is clear that the order of permeability of the three mucosae studied was small intestinal mucosa < colonic mucosa < vaginal mucosa, for all four permeants studied (Figs. 1–4). Steady-state flux rates were obtained for most of the permeants that diffused across the three mucosae. As observed in our previous study, 17β-estradiol did not reach a steady state across vaginal, colonic and small intestinal mucosa, but the flux rates gradually increased over the time course of the experiment. However, these increases were more pronounced for vaginal mucosa than for the other two tissues. These results once more concurred with our previous findings on the passage of this lipophilic hormone across human buccal, vaginal and small intestinal mucosa (Van der Bijl et al., 1998a; Van der Bijl and Van Eyk, 2002). In accordance with our previous study on the permeability of small intestinal mucosa, the flux rates of arecoline (MW 160 Da), the methyl ester of arecaidine, were found to be marginally higher than those of arecaidine (MW 146 Da) itself for vaginal and small intestinal mucosa over the entire course of the experiment (Van der Bijl and Van Eyk, 2002). Although, after 15 h, the flux rate of arecaidine across colonic mucosa was marginally higher than that across vaginal tissue, no significant differences between these two mucosae could be demonstrated over the entire time course of the experiment. On the basis of molecular size alone, one would expect arecaidine to have higher flux rates across membranes than arecoline. This compound is, however, a weak organic acid which can ionize and this alters its partitioning into biological membranes from solution. The net effect of these complex interactions between arecaidine and the particular mucosal membrane being used is therefore difficult to predict.

While there have been reports on the relative differences between the permeability of colonic versus small intestinal mucosa in rats and humans, these findings have been equivocal (Yamamoto et al., 1997; Fagerholm et al., 1997; Oliver et al., 1998). Using isolated intestinal membranes from rats in a modified Ussing chamber and a mathematical model for rat intestine and human jejunum, the ranking order of permeability in both studies was found to be jejunum > ileum > colon (Yamamoto et al., 1997; Oliver et al., 1998). On the other hand, some experiments have demonstrated rat colon to be the region of the intestine having the highest absorptive capacity (Fagerholm et al., 1997). However, it must be kept in mind that the many morphological and functional differences between animal and human intestinal mucosa may make direct comparison of their relative permeabilities to various chemical entities difficult.

It is clear from our studies that human colonic epithelium was statistically significantly ($P < 0.05$) more permeable than small intestinal mucosa to all four permeants tested. Furthermore, as previously reported for small intestinal mucosa, a similar molecular weight dependent flux relationship for the various

permeants across colonic mucosa, was observed (Van der Bijl and Van Eyk, 2002). It was also evident that for colonic mucosa, as for small intestinal mucosa, flux rates decreased sharply for compounds having molecular weight >300 Da (Fig. 5).

In conclusion, we have demonstrated that human colonic mucosa was consistently more permeable in vitro to water, 17β-estradiol, arecoline and arecaidine than small intestine. However, both mucosae were less permeable to the above four permeants evaluated than human vaginal mucosa. The flow-through mucosal perfusion system used in our laboratory is therefore also potentially useful as a method for determining the permeability of therapeutic agents from the colon for drug registration purposes, as suggested by two prominent drug regulatory authorities (CPMP Note, 1998; FDA Guidance, 2000). As found for small intestinal mucosa, the low permeability of colonic mucosa to drugs with weights >300 Da may necessitate using other epithelial membranes, e.g. vaginal mucosa, as alternative barriers for in vitro BA/BE studies.

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