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Rapid Communication

Vaginal administration of propranolol to rats: Absorption and histological effects on the vaginal epithelium

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Summary

Propranolol solution administered vaginally to ovariectomised rats was absorbed rapidly with peak plasma levels attained 2 min after administration. In addition, the acute effects of propranolol and buffer solutions on the morphology of the vaginal epithelium were quantitated. The thickness of the vaginal epithelium was reduced by each treatment and propranolol solution caused significantly more damage than buffer alone.

The vaginal route of administration can be used either to attain a systemic delivery which avoids hepatic first-pass metabolism (Benziger and Edelson, 1983), or in most cases, to treat local conditions within the vagina, including the delivery of contraceptive preparations (Keith et al., 1985). In the latter situation, absorption and systemic distribution of the administered compound are undesirable. Propranolol is an example of a drug which, when given vaginally, exhibits spermicidal activity in both animals (Zipper et al., 1982) and women (Zipper et al., 1983). However, this drug has been shown to be readily absorbed across the mucous membranes of the nasal (Hussain et al., 1980), oral (Duchateau et al., 1986) and vaginal cavities (Patel et al., 1983, Buttar et al., 1988).

In this study, ovariectomised rats were used to investigate whether propranolol would also be significantly absorbed after vaginal administration in this model and whether the drug would induce damage in the vaginal epithelium.

DL-Propranolol hydrochloride was purchased from the Sigma Chemical Company Ltd. (Dorset, U.K.) and prepared as a solution in isotonic phosphate buffered saline (PBS) pH 7.4 at concentrations of 10 and 20 mg/ml. Female Wistar rats (JABU, Sutton Bonington, U.K.), weighing approximately 200 g, were bilaterally ovariectomised under halothane anaesthesia. The operation wounds were closed with Michel clips which were removed 7–10 days later. The animals were allowed to recover for at least two weeks before being used in the vaginal administration studies.

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Six groups of ovariectomised rats (6 rats/group) weighing approximately 220 g, were anaesthatised by intra-peritoneal injection of 60 mg/kg pentobarbitone sodium (60 mg/ml, Sagatal, May and Baker).

One group was used to study the kinetics of the vaginal absorption of propranolol. After tracheotomy and cannulation of the external carotid artery, propranolol solution was instilled into the vaginal tract (1 mg/50 μl/rat) as described previously (Richardson et al., 1989). Blood samples (300 μl) were withdrawn from the carotid artery at intervals over four hours after drug administration. The plasma was separated by centrifugation and stored at −20 C. Thereafter the propranolol concentration was measured spectrofluorimetrically by a modification of a method of Schand et al. (1970).

For the histological study, four groups of rats were dosed vaginally with 100 μl of either PBS or propranolol solution (10 mg/ml) and sacrificed at both 10 and 60 min post treatment by an overdose of pentobarbitone sodium. One group of animals which did not receive an enema was taken as a control. Each vagina was carefully removed and placed in Bouin Hollande fluid and processed by conventional steps for light microscopy. Sections collected at five equidistant sites along the length of the organ were used to quantify epithelial thickness as described previously (Richardson et al., 1989). In brief, the epithelial thickness was assessed from counts of nuclear profiles at ten points on each slide. The data was expressed as a mean of the epithelial thickness for each rat and a frequency distribution for each treatment group. Control and experimental groups were assessed by the application of the Mann-Whitney *U*-test to the mean data.

The vaginal absorption of propranolol was found to be rapid and peak plasma levels were reached two minutes after drug administration (Fig. 1). Plasma propranolol concentrations then rapidly declined and were undetectable after 3 h. These results confirm the work of Buttar et al. (1988) that propranolol is readily absorbed after vaginal administration to rats. It has also been shown that propranolol is absorbed vaginally in women (Patel et al., 1983). In spite of this, propranolol has been considered for use as a vaginal

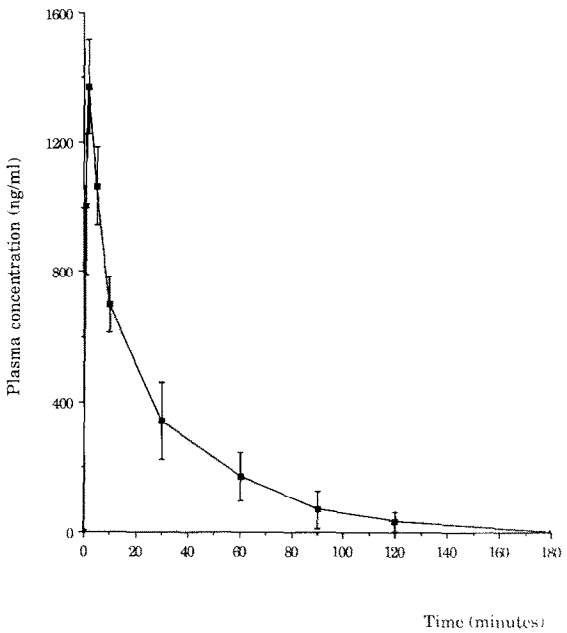

Fig. 1. Plasma propranolol concentration with time following vaginal administration of propranolol solution, 1 mg/50 μl/animal, to ovariectomised rats. Each value represents the mean ± S.E.M. of 6 animals.

spermicide and a preliminary trial was conducted in 198 women (Zipper et al., 1983). Propranolol was found to be an effective spermicide for up to 10 h following vaginal application of an 80 mg tablet. However, nearly 17% of the volunteers were withdrawn from the trial after experiencing local itching or pain.

The vaginal epithelium in the untreated control group was irregularly folded with no observable differences between the folds. It had a consistent appearance between animals and a mean thickness of 2.04 cell layers (Table 1). Vaginal administration of propranolol solution for 10 and 60 min and of PBS for 60 min resulted in a reduction of mean epithelial thickness (Table 1) which was statistically significant when compared to the control group (Table 2). The extent of epithelial cell loss as a result of each treatment is demonstrated by the frequency distribution bar chart of epithelial thicknesses (Fig. 2). After administration of propranolol solution, the vaginal epithelium was thinner in places with some sites of complete desquamation. This epithelial damage was ap-

TABLE 1

Thicknesses of vaginal epithelia of rats treated with buffer alone or containing 1 mg propranolol, for 10 minutes and 60 minutes (mean number of cell layers ± S.D., n = 10 counting sites × 5 slides per rat, 6 rats per group)

Control group	Buffer solution		Propranolol solution	
	10	60	10	60 (min)
2.10 ± 0.61	2.10 ± 0.54	1.88 ± 0.69	1.68 ± 0.71	1.54 ± 0.68
2.06 ± 0.55	1.92 ± 0.88	1.88 ± 0.56	1.58 ± 0.64	1.10 ± 0.51
2.06 ± 0.42	2.02 ± 0.51	1.94 ± 0.62	1.66 ± 0.66	1.26 ± 0.69
2.0 ± 0.61	1.92 ± 0.49	1.90 ± 0.61	1.64 ± 0.63	1.46 ± 0.61
2.18 ± 0.48	2.58 ± 0.70	1.92 ± 0.56	1.18 ± 0.52	1.32 ± 0.62
1.82 ± 0.52	1.86 ± 0.57	1.92 ± 0.53	1.38 ± 0.53	1.32 ± 0.59
Mean epithelial thickness of each group				
2.04	2.07	1.91	1.52	1.33

parent after 10 minutes and more pronounced after 60 min, with mean epithelial thicknesses of 1.52 and 1.33 cell layers, respectively. After administration of PBS alone, slight damage to the vaginal epithelium was detected after 60 min but this was significantly less than after treatment with propranolol solution for the same length of time. The damage seen after each treatment was known to be related to the presence of the vaginal enemas as care was taken to ensure that tissue sections were sampled from above the inserted cannula.

These results support the findings of Wheatley et al. (1988) that after exposure of the nasal mucosa to propranolol in vitro, extensive damage was observed. However, similar studies utilising the buccal mucosa did not show any major damage after application of propranolol for several hours (Le Brun et al., 1989). This may reflect the structural differences between the nasal and buccal mucosae and hence the differing sensitivities to drug-induced damage.

Clearly, the ovariectomised rat is a model system in which the vaginal absorption of drugs and

TABLE 2

Probability values of the differences in mean vaginal epithelial thicknesses of each treatment group, assessed using the Mann-Whitney U-test

Treatment	PBS		Propranolol	
	10 min	60 min	10 min	60 min
Control group	0.19	0.016	0.0005	0.0005
PBS (10 min)	–	0.06	0.0005	0.0005
PBS (60 min)	–	–	0.0005	0.0005
Propranolol (10 min)	–	–	–	0.025

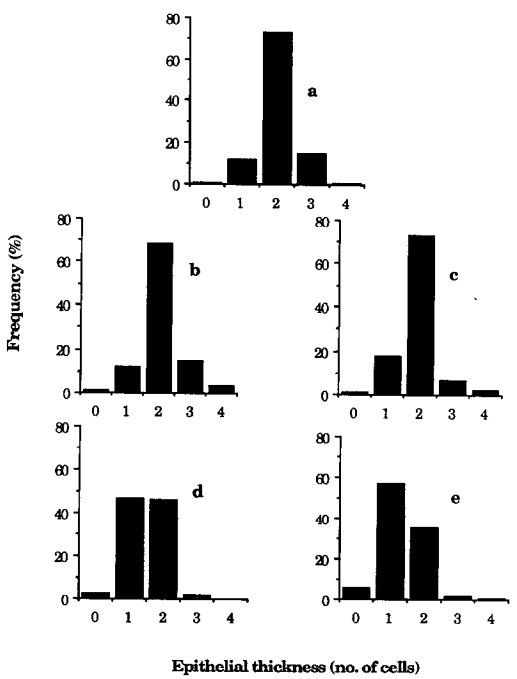

Fig. 2. Bar chart illustrating the frequency of sample sites with epithelial thicknesses between 0 (completely denuded) and 4 cell layers. (a), control group; (b), buffer solution for 10 min and (c), 60 min; (d), propranolol solution for 10 min and (e), 60 minutes.

their effects on the vaginal epithelium may be greater than in the normal rat or in women, in which the epithelial thickness is significantly greater. Studies on the bioelectric activity of the rat vaginal epithelium have shown that the effects of spermicides were highly dependent on the oestrous cycle, with the thick epithelium at pro-oestrus presenting a significantly greater barrier to drug entry than the thinner epithelium at metoestrus or dioestrus (Levin, 1987). Hence, the vagina of the ovariectomised rat was subsequently chosen as a standardised and sensitive model for the assessment of the local action of these drugs (Levin and Parker, 1987). In spite of these differences in sensitivity of the vaginal epithelia, our results suggest that the local irritation reported after vaginal use of propranolol in women may be due to epithelial damage. This, coupled with the ready vaginal absorption of propranolol resulting in depleted concentrations in the vaginal tract, may caution the use of propranolol as a vaginal contraceptive.

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