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Vaginal administration of gentamicin to rats. Pharmaceutical and morphological studies using absorption enhancers

Julie L. Richardson [1], Pawanjit S. Minhas [2], Norman W. Thomas [2] and Lisbeth Illum [1]

[1] *Department of Pharmaceutical Sciences and* [2] *Department of Human Morphology, Queens Medical Centre, University of Nottingham, Nottingham (U.K.)*

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Summary

The vaginal absorption of gentamicin in ovariectomized rats was markedly increased by the coadministration of absorption enhancers. By comparison of the AUCs of the serum concentrations of gentamicin with time profiles, the enhancers investigated could be ranked in terms of efficacy; 1% palmitoylcarnitine > 0.5% lysophosphatidylcholine > 1% laureth-9 > 10% citric acid. The acute effects of solutions of gentamicin and enhancers on the morphology of the vaginal epithelium were quantitated. All enhancers significantly reduced epithelial thickness with laureth-9 and lysophosphatidylcholine causing complete desquamation of approximately 50% of the areas assessed. On the basis of these data, it was possible to rank the severity of the interaction of the drug and enhancer solutions with the vaginal lining; 0.5% lysophosphatidylcholine = 1% laureth-9 > 1% palmitoylcarnitine > 10% citric acid.

Introduction

Many of the novel biogenetically engineered peptide and protein drugs are administered parenterally because of their low bioavailability after oral dosing. This has led to the intensive investigation of alternative means of drug administration including the nasal, buccal, rectal and vaginal routes (Davis, 1986). Generally, peptide absorption is low via these transmucosal routes, but it is enhanced by the coadministration of compounds which increase mucosal permeability (Eppstein and Longenecker, 1988). Many compounds have been employed as absorption enhancers (Lee, 1986), but although they significantly increase drug uptake, some induce unacceptable local irritation.

The vagina has traditionally been used for local treatment but is a potential route for the systemic administration of drugs, particularly those which are susceptible to gut or liver degradation. It is permeable to a wide range of compounds, including peptide drugs (Benziger and Edelson, 1983). Okada et al. (1982), demonstrated in rats that vaginal administration of a luteinising hormone-releasing hormone analogue, leuprolide, showed the greatest potency among non-parenteral routes and absorption was markedly increased by the coadministration of carboxylic acids. A major problem in studies of vaginal absorption in rodent species is the variable structure of the vaginal

Correspondence: J.L Richardson, Department of Pharmaceutical Sciences, University of Nottingham, University Park, Nottingham, NG7 2RD, U.K.

epithelium at different stages of the oestrous cycle (Okada et al., 1983). In order to standardise the thickness of the vaginal epithelium, the animals used in the present study were ovariectomised.

To investigate the effect of enhancers on vaginal absorption, gentamicin was selected as an example of a poorly absorbed, hydrophilic drug (Fix et al., 1986). It was coadministered with enhancer systems of different physicochemical characteristics: amphiphilic compounds, lysophosphatidylcholine, palmitoylcarnitine and laureth-9; and a chelating agent, citric acid. In addition, the histology of the vagina was assessed at the termination of each experiment.

Materials and Methods

Materials

Gentamicin sulphate, citric acid, L-α-lysophosphatidylcholine (LPC), palmitoyl-DL-carnitine chloride (PCC), and polyoxyethylene-9-lauryl ether (laureth-9) were purchased from the Sigma Chemical Company Ltd. (Dorset, U.K.). All other chemicals used were of reagent grade.

The gentamicin was prepared as a solution in isotonic phosphate buffered saline pH 7.4, at a concentration of 30 mg/ml. The 4 absorption enhancers were added separately to the gentamicin solutions at the following concentrations: citric acid, 10% w/v (100 mg/ml); L-α-lysophosphatidylcholine, 0.5% w/v (5 mg/ml); palmitoyl-DL-carnitine chloride, 1% w/v (10 mg/ml); polyoxyethylene-9-lauryl ether, 1% w/v (10 mg/ml). These concentrations were chosen after a review of the literature concerning use of each compound as absorption enhancers vaginally (Okada et al., 1982), nasally (Illum et al., 1988) or in the gastrointestinal tract (Fix et al., 1986).

Vaginal administration of gentamicin and enhancers in rats

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 2 weeks before being used in the absorption studies.

Groups of 5 ovariectomised rats were anaesthetised by intraperitoneal injection of 60 mg/kg pentobarbitone sodium (60 mg/ml, Sagatal, May and Baker). The rats were tracheotomised and the carotid artery cannulated. A short length of polythene tubing, i.d. 0.58 mm, o.d. 0.96 mm (Portex, U.K.), was attached to a 100 μl syringe and filled with the gentamicin solution, with or without enhancer. The tubing was then gently inserted into the vagina and secured in position with a cyanoacrylate adhesive. The gentamicin solution was instilled into the vaginal tract, 50 μl/250 g rat (6 mg/kg), and the tubing was then clamped to prevent further flux or leakage of drug solution. Blood samples of 300 μl were withdrawn from the carotid artery at 0, 5, 10, 15, 30, 60, 120 and 180 min after drug administration. The serum was separated by centrifugation and stored at −20 °C. The gentamicin concentration was determined by E.M.I.T. (O'Connell et al., 1984), within the next 48 h.

The areas under the curves (AUCs) of serum gentamicin concentration from 0 to 120 min were determined and differences between each treatment group were assessed by the use of the Student *t*-test.

At the end of the vaginal absorption experiments, usually after 2 h, the rats were sacrificed by an overdose of pentobarbitone sodium. The vaginas were then carefully removed and placed in fixative for histology.

In addition, a control group of rats were prepared. They were treated in a similar manner to the experimental groups but did not receive a vaginal enema.

Histological study

The tissues were fixed in Bouin Hollande fluid and processed by conventional steps for light microscopy. Five sections, selected randomly from a group of 9 sections collected through the length of each organ, were used to quantify vaginal epithelial thickness. On each slide, at 10 sites selected randomly using an eye piece graticule, the vaginal epithelial thickness was assessed from counts of nuclear profiles. The data were expressed as mean

epithelial thickness for each rat and frequency distribution for each rat and each treatment group. Control and experimental groups were assessed by the application of the Mann Whitney *U*-test to the mean data.

Results

The vaginal absorption of gentamicin after administration alone or with an enhancer is illustrated in Fig. 1. In the absence of any enhancing agent, low serum levels, less than 2 μg/ml, of gentamicin were detected over 2 h. Each of the enhancers investigated increased the rate and extent of absorption. The coadministration of LPC resulted in a rapid absorption of gentamicin with peak levels of 14 μg/ml. After administration of gentamicin with PCC and the other enhancers, the peak concentrations attained were lower and the absorption profiles broader than with LPC. The areas under the curves (AUCs) of serum gentamicin concentration from 0 to 120 min after each treatment are shown in Table 1. In terms of total absorption, PCC was found to be the most effective enhancer of gentamicin absorption followed by LPC, laureth-9 and citric acid, at these chosen concentrations. By statistical comparison of the AUCs after each treatment, all of the enhancers studied were found to enhance gentamicin absorption significantly (Table 2). In addition, the AUCs after administration of gentamicin with PCC and with LPC were significantly greater than with citric acid. No other differences between the AUCs of each treatment group were statistically significant.

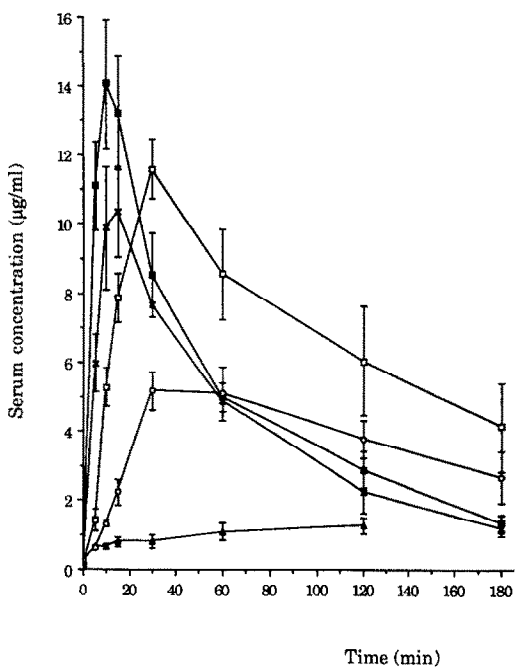

Fig. 1. Serum gentamicin concentration with time following vaginal administration of gentamicin solution, 6 mg/kg, to ovariectomised rats. (▲), Gentamicin solution alone; (□), with 1% palmitoylcarnitine; (■), with 0.5% lysophosphatidylcholine; (×), with 1% laureth-9; (○), with 10% citric acid. Each value represents the mean ± S.E.M. of 3 or 4 animals.

TABLE 1

AUC of serum gentamicin levels from t = 0–120 min, after vaginal administration of gentamicin solution alone and with enhancers to ovariectomised rats (mean ± S.E.M., n = 3 or 4)

Treatment	AUC in $\mu g \cdot min \cdot ml^{-1}$
Gentamicin solution, 6 mg/kg	121.5 ± 24
With 10% citric acid	492 ± 67
With 1% laureth-9	641 ± 40
With 0.5% lysophosphatidylcholine	762 ± 68.5
With 1% palmitoylcarnitine	940 ± 129

Following ovariectomy, the vaginal epithelium of the control group had a mean thickness of two cell layers (Table 3). The epithelium was irregularly folded with no observable differences within the folds (Fig. 2a). Vaginal administration of gentamicin solution alone or with added enhancers resulted in a reduction of mean epithelial

TABLE 2

Probability values of the differences in the AUC of serum gentamicin concentration with time of each treatment group, assessed using the Student t-test

Treatment	+ Citric acid	+ Laureth-9	+ LPC	+ PCC
Gentamicin solution	0.0059	0.0009	0.0016	0.0055
+ citric acid	–	0.0982	0.0328	0.0181
+ laureth-9	–	–	0.1419	0.0792
+ LPC	–	–	–	0.1968

TABLE 3

Thicknesses of vaginal epithelia of control ovariectomised rats and rats treated with gentamicin solution, alone and with enhancers (mean number of cell layers ± S.D., n = 50 counting sites, 10×5 slides / rat

Rats	Control group	Gentamicin solution	+ citric acid	+ PCC	+ laureth-9	+ LPC
1	2.1 ±0.61	1.86 ± 0.73	1.32 ± 0.62	1.24 ± 0.56	0.24 ± 0.43	0.86 ± 0.67
2	2.06 ± 0.55	2.06 ± 0.59	1.6 ±0.57	1.24 ± 0.56	0.64 ± 0.5	0.36 ± 0.6
3	2.06 ± 0.42	1.82 ± 0.72	1.54 ± 0.73	1.2 ±0.61	1.1 ±0.75	0.42 ± 0.57
4	2.0 ±0.61	1.92 ± 0.53	1.34 ± 0.69	1.06 ± 0.59	0.38 ± 0.49	0.86 ± 0.67
5	2.18 ± 0.48	1.76 ± 0.66	1.34 ± 0.69	1.26 ± 0.63	0.58 ± 0.57	0.38 ± 0.6
Mean epithelial thickness of each group						
	2.08	1.88	1.43	1.2	0.59	0.58

thickness (Table 3, Fig. 2b–d) which was statistically significant when compared to the control group (Table 4). The extent of epithelial cell loss as a result of gentamicin and enhancer treatment is demonstrated by the frequency distribution bar chart of epithelial thicknesses (Fig. 3). Moderate

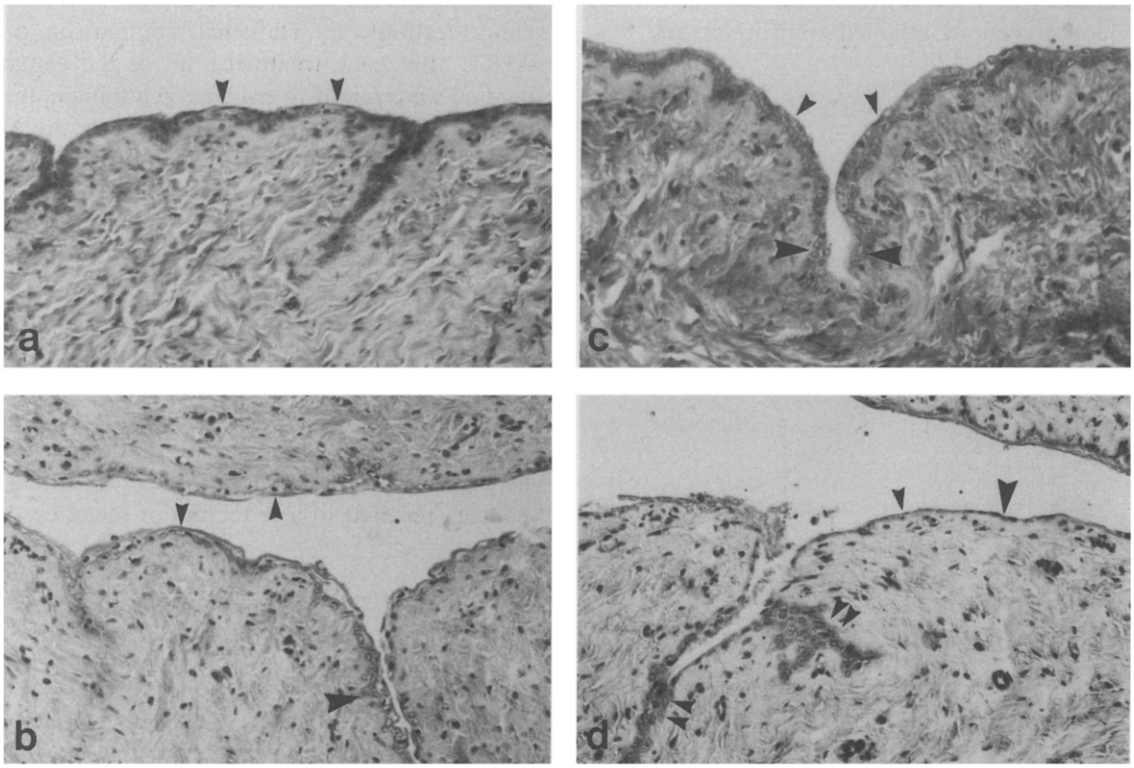

Fig. 2. Photomicrographs of vaginal mucosa, ×200. a: untreated ovariectomised tissue showing a regular surface epithelium (arrows). b: gentamicin plus citric acid treated tissue. The epithelium lining the mucosal folds (large arrow) is slightly thicker than that on the ridges (small arrows). The latter shows clear evidence of cell detachment. c: gentamicin treated tissue. The epithelium is regular and of similar thickness on the surface of the ridges (small arrows) and in the mucosal folds (large arrows). d: gentamicin plus laureth-9 treated tissue. The epithelium lining the mucosal folds (double arrow) has a normal appearance, but over the ridges are sites covered either by a squamous epithelium with flattened nuclei (large arrow) or devoid of nuclei (small arrow).

TABLE 4

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

Treatment	Gentamicin solution	+ Citric acid	+ PCC	+ Laureth-9	+ LPC
Control group	0.027	0.002	0.002	0.002	0.002
Gentamicin alone	–	0.002	0.002	0.002	0.002
+ Citric acid	–	–	0.005	0.005	0.005
+ PCC	–	–	–	0.005	0.005
+ Laureth-9	–	–	–	–	0.53

epithelial damage was evident after treatment with gentamicin in combination with citric acid and with PCC, with mean epithelial thickness of 1.43 and 1.2, respectively. Severe damage to the vaginal epithelium occurred after administration of gentamicin with LPC and laureth-9. Extensive cell loss was observed with approximately 50% of points assessed being stripped of epithelium. Areas of one or two cell layers were mainly confined to the folds of the epithelium where penetration by the drug and enhancer solution may have been restricted.

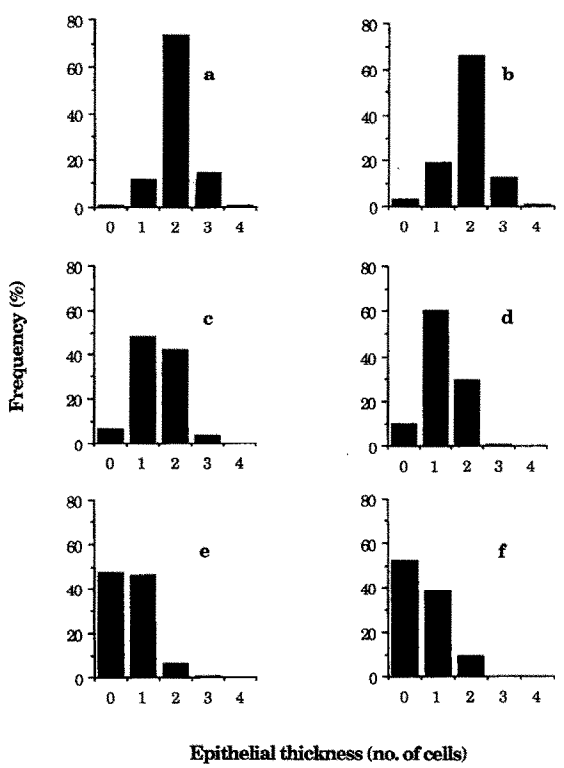

Fig. 3. Bar chart illustrating the frequency of sample sites with epithelial thicknesses between 0 (completely denuded) and 4 cell layers. a: control group; b: gentamicin solution alone; c: with 10% citric acid; d: with 1% palmitoylcarnitine; e: with 1% laureth-9; f: with 0.5% lysophosphatidylcholine.

The vaginal tissues of the control group were of consistent appearance and epithelial thickness. This confirmed the success of the ovariectomisation procedure and provided a standard model for the investigation of drug and enhancer effects. Although care was taken to minimize epithelial damage on dosing, tissue sections were taken above the level of the inserted polythene tube. Thus any epithelial damage seen was known to be caused by the solutions administered.

Discussion

The results obtained demonstrate that greater epithelial damage and enhanced absorption of gentamicin was caused by the 3 enhancers laureth-9, LPC and PCC, which all have a detergent-like activity. In spite of the higher concentration employed, compared to the other 3 enhancers, both absorption enhancement and epithelial damage were reduced with citric acid, a chelating agent with no surface-active properties.

The mechanism for absorption enhancement by surface-active compounds is unclear, but it may involve solubilisation of cell membrane components resulting in increased permeability. The enhancement of nasal absorption of insulin by

laureth-9 has been linked with its ability to release protein from the nasal mucosa (Hirai et al., 1981). In addition, significant changes in the structure of microvilli on the nasal mucosa were demonstrated after treatment with laureth-9. LPC is a lysophospholipid which markedly enhanced the nasal absorption of gentamicin in rats and in sheep (Illum et al., 1988). Lysophospholipids are surface-active amphiphiles generated naturally in biological membranes. They are active in low concentrations and are converted within the membrane to normal cell components (Stafford and Dennis, 1988). The influence of 10 mM LPC, a concentration equivalent to that used in the present study, on the morphology and permeability of the rat stomach has been investigated (Karlqvist et al., 1986). Their findings suggested that LPC may damage the gastric mucosa with a resultant increase in permeability.

In this study, the vaginal tissues were removed from the rats 2 h after drug and enhancer administration while peak gentamicin serum levels were generally attained between 10 and 30 min. Thus the state of the vaginal epithelium at the time of maximum absorption is not known. However, in another study, the effect of propranolol on the vaginal epithelium of the ovariectomised rat was investigated after 10 min and 1 h (Richardson et al., in preparation). A reduction in epithelial thickness was apparent 10 min after drug administration and epithelial cell loss was only slightly increased after exposure to propranolol for 1 h. These results infer that epithelial cell loss seen after exposure to gentamicin and enhancer solutions for 2 h may also be apparent soon after administration. From our results, it is possible that the enhanced absorption of gentamicin in the presence of LPC and laureth-9 may be due to the partial removal of the vaginal epithelium and therefore a reduction in the barrier to drug diffusion. PCC was found to be the most effective absorption enhancer investigated whilst only causing moderate epithelial damage. This reflects the results from a study of the enhancing effects of PCC in the G.I. tract of rats (Fix et al., 1986). PCC, at a concentration of 10 mg/ml, significantly enhanced the rectal absorption of cefoxitin whilst only causing minor tissue alterations. Although the mechanism of absorption enhancement is unknown, our results suggest that epithelial damage may offer a partial explanation.

Citric acid is a carboxylic acid with chelating activity and it is thought that the mechanism of absorption enhancement may be through an action on calcium ions. Slight damage to the vaginal epithelium did occur but this may prove to be readily reversible. When citric acid was used vaginally in rats at dioestrus, changes in the vaginal epithelium were described as slight and recovery of the epithelium as relatively rapid (Okada et al., 1982).

The vaginal epithelium of the ovariectomised rat may be more susceptible to drug-induced damage than that of the normal rat. When laureth-9 was investigated as a potential enhancer of vaginal absorption in dioestrus rats, very little effect was noted (Okada et al., 1982). It was thought that the stratified vaginal epithelium may be resistant to desquamation and therefore drug absorption was not improved. This provides a note of caution as to the extrapolation of these results to other animal models or to women. The vaginal epithelium of the ovariectomised rat appears to be very sensitive to drug and enhancer insults and thus both enhancement and epithelial damage may be exaggerated.

In conclusion, the surfactant-type absorption enhancers investigated appear to be effective enhancers of the vaginal absorption of gentamicin in ovariectomised rats. Their action may be linked with moderate to severe epithelial damage. Citric acid was found to enhance absorption whilst causing more minor epithelial damage.

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