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Investigation into the potential for iontophoresis facilitated transdermal delivery of acyclovir

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Abstract

The potential for iontophoresis facilitated transdermal transport of acyclovir alone or in combination with a cationic or anionic penetration enhancer (cetrimide or sodium lauryl sulphate respectively) was investigated in the neutral range of the drug (pH 6–8). The amount of drug permeated in vitro across nude mouse skin by means of passive diffusion was low (2.5 $\mu g/cm^2$, 90 min). With iontophoresis treatment the permeation was 3-fold greater than that of passive diffusion when a negative charge was applied to the drug solution, possibly due to some ionisation of the drug. Applying a positive charge increased the iontophoretic permeation slightly over that of passive diffusion due to volume flow. The addition of varying amounts of sodium lauryl sulphate or cetrimide to the donor additionally increased the iontophoretic permeation. A positive donor side was less effective compared with a negative donor mainly due to polarisation during anodal delivery. Cetrimide caused an up to 3-fold higher cathodal permeation of acyclovir compared to sodium lauryl sulphate, largely due to the difference in molecular weight/volume of the permeating ions, but also due to other effects. The increase in permeation with increasing enhancer concentration leveled off at a certain point for both enhancers, possibly due to a change in the zeta potential of the skin.

Key words: Iontophoresis; Electrotransport; Transdermal drug delivery; Penetration enhancer; Sodium lauryl sulfate; Cetrimide; Drug delivery; Acyclovir

1. Introduction

Acyclovir is an acyclic analogue of deoxyguanosine with a molecular weight of 225. It contains two ionisable groups as shown in Fig. 1. At a pH of 7, a condition close to which the experiments reported below were performed, the drug is neutral. The penetration of drugs through skin has been increased by using many different types of penetration enhancers (Aungst et al., 1986; Ranade, 1991; Santus and Baker, 1993). Enhancers reduce the barrier properties of the skin in many different ways (Barry, 1987; Goodman and Barry, 1989), some acting on the lipid bilayer while others act on the keratinised structures. Penetration enhancers have been used to provide succesful topical treatment with acyclovir (White and Jones, 1980). The permeation of ionic

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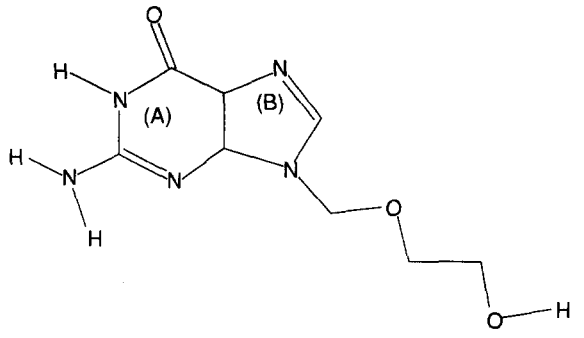

Fig. 1. Structure of acyclovir. The pK_a of 2.52 represents protonation on nitrogen (B), that at 9.35 deprotonation of nitrogen (A).

drugs across the skin has been enhanced by applying an external electric field (Singh and Roberts, 1989; Green et al., 1991a). The delivery of nonionic compounds has also been observed to increase during iontophoresis (Gangarosa et al., 1980; Siddiqui et al., 1985; Burnette and Marrero, 1986; Okabe et al., 1986; Tauber and Gunther, 1989; Roberts et al., 1990; Singh, 1990). Combined iontophoresis of drugs and penetration enhancers has also been reported. Wearley and Chien (1990) investigated the effect of iontophoresis of a neutral compound (azidothymidine) in combination with an organic penetration enhancer (*N*-decylmethyl sulfoxide) and Gay et al. (1992) studied iontophoretic delivery of the negatively charge piroxicam in combination with oleic acid. Both research groups found no additional enhancement was observed with the combination. However, ethanol pretreatment was successfully used by Srinivasan et al. (1990) to enhance iontophoresis of polypeptides. Information on iontophoresis of a neutral drug combined with ionic enhancer is scarce, however, this combination may potentially also provide an efficient transdermal penetration system for acyclovir.

The primary purpose of this study was to explore experimentally the degree to which ionic penetration enhancers could reduce the lag time and/or increase the flux of acyclovir in combination with iontophoresis using a constant current. Sodium lauryl sulphate (NaLS), an anionic surfactant, and cetrimide (CTM), a cationic surfactant, were selected as the model ionic penetration enhancers because of their opposite polarity, their known use as penetration enhancers (Aungst et al., 1986; Mitra and Wirtanen, 1989; Kompaore et al., 1991) and their known effect on the tight junctions in the skin (Rantuccio, 1979; Seaton et al., 1990). Iontophoretic transport across excised full thickness nude mouse skin were carried out using various concentrations of acyclovir, CTM and NaLS. The results were compared with those on the passive diffusion of the drug. The amount of permeated drug was also determined as a function of the current density and polarity of the donor phase.

2. Materials and methods

2.1. Materials

Acyclovir (Wellcome, U.K.), sodium lauryl sulphate (99% pure; Sigma) and cetrimide, sodium chloride, glacial acetic acid and formic acid (all reagent grade from BDH) were used as obtained. All solutions were made using deionised-distilled water.

Ag/AgCl electrodes 12.5 mm in diameter and 1 mm thick (Clark Electromedical Instruments) were employed. These electrodes are nonpolarisable, reversible and, therefore, do not decompose water.

2.2. Skin and preparation of skin

Skin diffusion experiments used full-thickness skin from nude mice (6 weeks old). After killing the mice, the skin was immediately excised, adhering fatty tissue was removed using cotton wool and the skins were frozen at −20°C until use. Before use the skins were thawed and examined for abrasions. All frozen skins were used within 4 months. In-house studies and investigations by Gupta et al. (1991) indicated that the barrier properties of skin had not been lost by short-term freezing. Nude mouse skin differs from human skin, however, due to the increasing danger of human skin being infected with HIV, hepatitis and other human infections, the easy availability of nude mouse skin with similar properties and

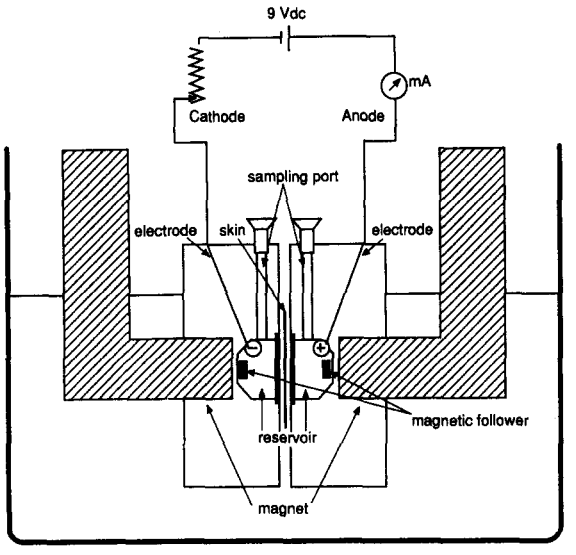

Fig. 2. Schematic diagram of the apparatus used in the passive diffusion and iontophoretic studies.

since nude mouse skin would allow comparison of different delivery protocols, this type of skin was used in the present study.

2.3. Apparatus

A two-compartment perspex diffusion cell was employed (Fig. 2). The volume of each compartment was 5.0 ml, with a contact area of 4.5 cm^2. The electrodes were passed through the side of the compartments and sealed into a permanent position. The distance between the two electrodes at the nearest point was 8 mm. A magnetic follower was placed in the bottom of the compartment. The skin was placed between the two halves of the cell. The cell was immersed in a water bath at 35 ± 0.5°C and a constant stirring rate was maintained in both compartments using external magnets. The constant current required in the iontophoretic studies was generated by a Phoresor Iontophoretic Power Supply module (Ioned Inc., UT).

2.4. Passive and iontophoretic diffusion studies

All transport studies were carried out using 0.9% NaCl in the receptor compartment. During iontophoresis 500 μl aliquots were withdrawn from the receptor compartment at 10 min intervals for 90 min and each replaced with 500 μl fresh normal saline. Passive diffusion studies were continued for approx. 5000 min, samples being taken four times daily. All diffusion studies were carried out at least in triplicate. The pH of the donor solution was measured at the start and at the end of each diffusion study using a Beckman 3560 Digital pH meter fitted with a 5 mm wide Orion 8103 electrode.

2.5. Effect of iontophoresis

Two studies of the effect of iontophoresis on the transdermal penetration of acyclovir were carried out, one with the donor side made cathodic and another with the donor side made anodic by appropriate connection to the Phoresor. A direct current of 3 mA (0.67 mA/cm^2) was applied. These two studies were compared with the passive diffusion of acyclovir. The donor solution for all three experiments contained 1.5 g/l of acyclovir at a pH of 7 ± 1. No buffer was used in the donor medium to avoid the additional ions this would cause.

2.6. Effect of ionic enhancers

The effect of the presence of ionic penetration enhancers on the skin permeation of acyclovir was studied using NaLS and CTM. The concentration of NaLS and CTM in the donor solution was 2 g/l, the donor concentration of acyclovir being 1.5 g/l.

2.7. Effect of iontophoresis in combination with ionic enhancers

Iontophoretic transdermal permeation of acyclovir in the presence of ionic enhancers NaLS and CTM was studied, with 1.5 g/l acyclovir added to the donor phase and with either 2.0 g/l NaLS or 2.0 g/l CTM. The donor side was made both cathodal and anodal. A direct current of 3 mA was applied.

2.8. Effect of increasing acyclovir concentration with iontophoresis and ionic enhancer

In order to study the effect of increasing drug concentration on the transdermal permeation of acyclovir with iontophoresis and in the presence of ionic penetration enhancers 0.5, 1.5, or 5.0 g/l acyclovir was added to the donor solution together with 2.0 g/l NaLS or CTM. The donor side was made cathodal and a constant current of 3 mA was applied.

2.9. Effect of increasing concentration of ionic enhancers with iontophoresis

In order to examine the effect of increasing surfactant concentration on the transdermal permeation of acyclovir with iontophoresis, 1.5 g/l acyclovir was added to the donor solution together with 0.5, 2.0 or 10 g/l NaLS or CTM. The donor phase was made cathodal and a constant current of 3 mA was applied.

2.10. Effect of increasing current density with iontophoresis and ionic enhancers

A direct current of 1, 3 and 5 mA (current densities 0.22, 0.67 and 1.11 mA/cm^2, respectively) from a cathodal donor solution containing 1.5 g/l acyclovir together with 2.0 g/l NaLS or CTM was applied in order to determine the effect of increasing current density on the transdermal permeation of acyclovir in the presence of ionic enhancers.

2.11. Analysis of acyclovir

The amount of acyclovir in the receptor solutions was quantitated by HPLC. A ConstaMetric 3000 pump (LDS), an autoinjector (Shimadzu), a variable-wavelength Spectromonitor 3100 detector (LDS) and a CI 4100 integrator (LDC) were used for the analyses. A Hichrom Partisil 10 ODS column (500 mm × 4 mm) with a precolumn of the same material and a mobile phase of glacial acetic acid, formic acid and deionised water in the ratio 5 : 5 : 1000 were employed. At a flow rate of 3 ml/min, the retention time of acyclovir was approx. 10 min. Detection was performed at 254 nm.

2.12. Treatment of data

The amount of acyclovir permeated with time is given per cm^2. The flux at a given time point was calculated from the amount of acyclovir permeated per cm^2 of skin from one time point to the next.

3. Results and discussion

3.1. Effect of iontophoresis

The cumulative amounts of acyclovir transported across the skin during the first 90 min of passive diffusion and anodal and cathodal iontophoresis are listed in Table 1. The permeation during cathodal iontophoresis was 3-fold greater than that during passive diffusion, whereas permeation during anodal iontophoresis was only marginally greater than that during passive diffusion. The pK_a values for acyclovir are 2.52 and 9.35. Depending upon the pH, therefore, the drug can exist in cationic, zwitterionic or anionic form. According to the protonation equation for bases:

$$\% \text{ ionised} = 100(1 + 10^{(\text{pH} - \text{p}K_a)})^{-1} \quad (1)$$

Table 1
Cumulative delivery (μg/cm^2, 90 min) of acyclovir (mean ± SD), from a donor solution containing 1.5 g/l acyclovir alone or together with 2.0 g/l NaLS or CTM

Experimental	Cumulative delivery of acyclovir (μg/cm^2, 90 min)		
	Acyclovir alone	Acyclovir + NaLS	Acyclovir + CTM
Passive	2.5 ± 1.0 (10)	4.1 ± 3.6 (3)	2.5 ± 0.9 (3)
Donor positive	3.4 ± 0.4 (3)	6.7 ± 0.3 (3)	13.3 ± 2.4 (3)
Donor negative	7.6 ± 1.2 (3)	16.7 ± 2.4 (3)	45.7 ± 3.5 (3)

The donor side was made cathodic or anodic with a current density of 0.67 mA/cm^2, alternatively no current was applied to the donor side. The number in parentheses indicates the number of replicates.

at a pH of 7 less than 1% of the acyclovir will be ionised and at a pH of 8 this will have increased to 4%. At the start of the cathodal diffusion experiments the pH of the donor solutions ranged from pH 6.5 to 6.8. At the end of the experiment the pH of the donor solutions ranged from pH 7.1 to 7.7. The resulting increase in deprotonated acyclovir may, therefore, explain the greater permeation of acyclovir during cathodal iontophoresis compared with that of passive diffusion. The pH of all the donor solutions at the end of anodal iontophoresis was above 6.2 but below 7.0, and protonation of acyclovir would therefore not have occured during anodal iontophoresis. The keratin in the stratum corneum has been shown to have an isoelectric point of pH 3–4 (Rosendahl, 1942; Harris, 1967; Wearley and Chien, 1990). The stratum corneum will therefore be negatively charged at a pH around 7. The result of this negative charge is that the skin is permselective (Rein, 1924; Burnette and Ongpipattanakul, 1987; Wearley et al., 1989a; Pikal, 1992). During iontophoresis this permselectivity causes the positive counterions to the fixed negative charge to move in the direction of the negative electrode; these ions will carry a layer of solvent due to frictional forces (Wearley and Chien, 1990; Pikal, 1992). This flow is called the volume flow. When the donor side is made positive water molecules will, therefore, move by means of this current induced volume flow into the receptor side (Pikal, 1990; Pikal and Shah, 1990a,b; Petelenz, 1992), which would provide an additional mechanism of transport of acyclovir compared to passive diffusion and may explain why anodal delivery of acyclovir is greater than passive diffusion. The overall increase is low, consistent with the findings by Tauber and Gunther (1989), and is probably due to the passive diffusion component being large enough to almost mask the convective flow.

3.2. Effect of some enhancers

The cumulative amounts of acyclovir permeated the first 90 min of passive diffusion are shown in Table 1. During the first 90 min of passive diffusion NaLS and CTM did not appreciably enhance the passive delivery of acyclovir.

Table 2
Diffusion parameters for acyclovir from donor solutions containing 1.5 g/l acyclovir alone or together with 2.0 g/l NaLS or CTM (mean ± SD)

Donor	Flux (μg/cm^2 per h)	Lag time (h)
Acyclovir	41.3 ± 11.9 (10)	7.2 ± 1.7 (10)
Acyclovir + NaLS	72.3 ± 19.2 (3)	6.0 ± 2.8 (3)
Acyclovir + CTM	113.5 ± 25.7 (3)	5.7 ± 3.1 (3)

The number in parentheses indicates the number of replicates.

However, other studies (Aungst et al., 1986; Ashton et al., 1987; Kompaore et al., 1991; Kushla and Zatz, 1991), as well as our investigations extended to around 80 h indicated that drug transport across the skin was significantly increased when the data were analysed beyond 90 min. The lag time and flux for the 80 h diffusion studies are shown in Table 2. Table 2 shows that the lag time for acyclovir was more than 5 h. According to Barry (1983), the lag time represents mainly penetration through hair follicles, and sebaceous and sweat glands. During the first 90 min of passive diffusion the enhancer-skin interactions may therefore not be so pronounced, and this may explain the results.

3.3. Effects of iontophoresis in combination with ionic enhancers

Data on the anodal and cathodal iontophoresis in the presence of NaLS or CTM are also shown in Table 1. With the addition of NaLS and CTM during iontophoresis the skin permeation of acyclovir is observed to increase, as shown in Table 1. CTM was 2–3-fold more effective compared to NaLS, and cathodal iontophoresis in combination with ionic enhancers was found to be 2.5–3.5-fold more effective than anodal iontophoresis in combination with ionic enhancers. According to Wearley and Chien (1990), Hill (1984) and Miyamoto et al. (1989), positive and negative ions can move through uncharged narrow pores in the presence of an electric field, pushing neutral molecules in their path; this is called convective flow and the process will occur both when the donor phase is made cathodal and anodal. How-

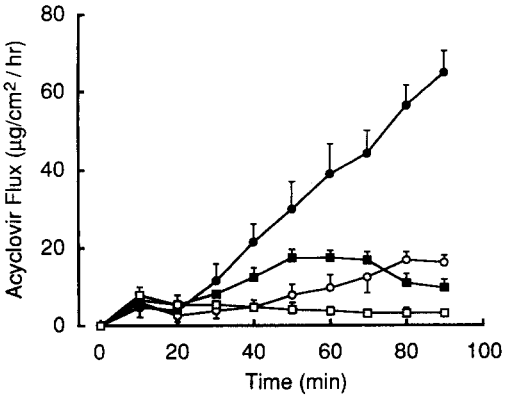

Fig. 3. Flux of acyclovir delivered from a 1.5 g/l solution of acyclovir also containing 2.0 g/l CTM following 90 min iontophoresis at 0.67 mA/cm^2 donor side positive (○) and donor side negative (●) as well as the flux from an aqueous solution containing 1.5 g/l acyclovir and 2.0 g/l NaLS following 90 min iontophoresis at 0.67 mA/cm^2 donor side positive (□) and donor side negative (■). Results are the average of at least three experiments ± SD.

ever, because of the skin's net negative charge at neutral pH the transport of cations should be favoured over anions. This is in good agreement with the findings of many authors (Burnette and Ongpipattanakul, 1987; Srinivasan et al., 1989; Wearley et al., 1989a; Pikal and Shah, 1990a,b; Sims and Higuchi, 1990; Wearley and Chien, 1990). Table 1 suggests that cathodal delivery in combination with NaLS or CTM gave the best results. The flux profiles for anodal and cathodal delivery of acyclovir in combination with NaLS or CTM are shown in Fig. 3. Fig. 3 shows for the solutions containing NaLS that an apparent maximum is reached relatively quickly, particularly when the donor phase is anodal, after which there is a gradual reduction in the flux. Towards the end of anodal delivery with CTM this phenomenon is also seen. This observation has also been described by Gay et al. (1992) and Green et al. (1991a,b) and may be explained by the changes in ion concentrations that take place during iontophoresis. During anodal delivery with NaLS providing the ions, Na^+ will be driven through the skin, at the same time negative ions in the receptor chamber (Cl^-) being moved in the opposite direction. The Na^+ ion is more mobile than the Cl^- ion (Gangarosa et al., 1978), and during iontophoresis the Cl^- concentration in the anodal chamber will increase, eventually causing electrochemical polarisation decreasing the magnitude of effective current (Boxtel, 1977). Direct current iontophoresis will always eventually develop a skin polarisation potential (Chien et al., 1989). The permselectivity of the skin would slow this process down for cathodal delivery. This effect alone does not explain the results, since no polarisation was seen for cathodal iontophoresis with CTM. Application of an electric field increases the movement of water into the skin. This increase in hydration may increase the permeation rate (Wearley and Chien (1990). Hydration may, however, cause the routes through appendages to swell shut (Burette and Ongpipattanakul, 1988). Thus, if the major pathway for the flux of ions is the appendacal route this would decrease the permeation. All the experiments shown in Fig. 3 demonstrate a peak around 10 min and a reduction in permeation rate at 20 min which may be due to hydration of the skin. At later time points the skin is likely to have completely hydrated. Hydration is therefore likely only to have played a small part in the results. Cathodal delivery caused the pH of the donor phase to increase slightly and the percentage of ionised acyclovir was therefore increased as described earlier. The fraction of acyclovir ions compared with the total concentration of ions having the same charge in the donor solution is likely to be small. Co-ion competition for the current means that only a small fraction of the acyclovir ions would be carried by the total current, as described by several authors (Bellatone et al., 1986; Kasting and Keister, 1989; Wearley et al., 1989a; Phipps and Gyory, 1992). This effect may only provide a minor contribution to the results. The increase could also have been caused by a direct effect on the skin. The presence of an electric field may provide sufficient heat to make conformational changes in the lipid bilayer in the skin increasing the fluidity or causing polarisation of the proteins in the skin (Bellantone et al., 1986; Burnette and Ongpipattanakul, 1988; Srinivasan et al., 1989; Wearley et al., 1989b). Surfactants can lower the resistance of the stratum corneum and has also been described (Aungst et

al., 1986; Ashton et al., 1987; Kushla and Zatz, 1991). The almost 3-fold difference in cathodal permeation of acyclovir with NaLS and CTM may largely be explained by the difference in molecular weight/volume of the permeating ions (Yoshida and Roberts, 1993).

3.4. Effect of increasing acyclovir concentration with iontophoresis and ionic enhancers

The cathodal transport of acyclovir in the presence of NaLS or CTM increased as the acyclovir concentration increased until the saturation limit had been reached (the aqueous solubility of acyclovir at 35°C is 2.2 g/l) following which no further increase was obtained, as shown in Fig. 4. There was a linear relationship between the amount permeated and the acyclovir concentration below the saturation concentration. Similar results have been obtained by many others (Bellantone et al., 1986; Wearley et al., 1989a; Thysman et al., 1992; Ahn et al., 1993) and confirm that for optimum penetration the donor should be saturated with the drug.

3.5. Effect of increasing concentration of ionic enhancers with iontophoresis

On both the addition of increasing concentrations of NaLS or CTM from cathodal delivery, the skin permeation of acyclovir was observed to increase. The results shown in Fig. 5 indicate that the transport of acyclovir did not increase proportionally as the NaLS or CTM concentration in the donor solution increased. At the highest concentration the increase appeared to level off. Fig. 6 depicts the flux of acyclovir delivered from the cathode with increasing concentration of NaLS or CTM. Fig. 6 shows that polarisation only influenced results following 50 min delivery with NaLS.

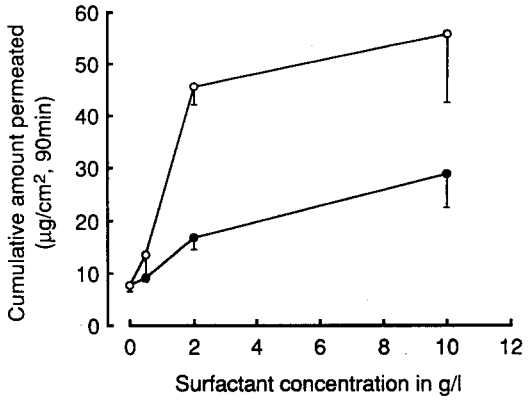

Fig. 5. Effect of increasing NaLS or CTM concentration on the cumulative penetration of acyclovir ($\mu g/cm^2$, 90 min) from a cathodal donor solution containing 1.0 g/l acyclovir and 0.5, 2 or 10 g/l NaLS (●) or CTM (○). The applied current density was 0.67 mA/cm^2. Each data point is the mean of at least three experiments ± SD.

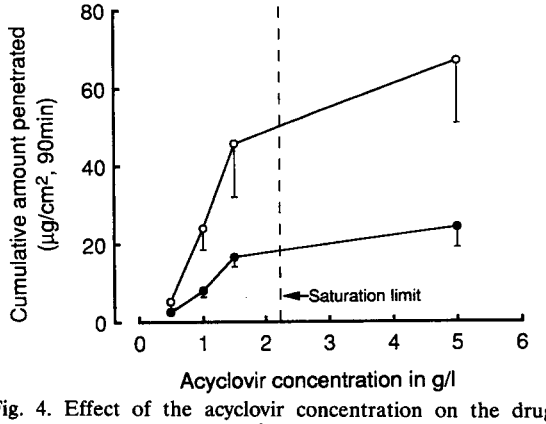

Fig. 4. Effect of the acyclovir concentration on the drugs cumulative delivery ($\mu g/cm^2$, 90 min) from cathodal donor solutions containing 0.5, 1.0, 1.5 or 5.0 g/l acyclovir and 2.0 g/l NaLS (●) or 2.0 g/l CTM (○). The applied current density was 0.67 mA/cm^2. Each data point is the mean of at least three experiments ± SD.

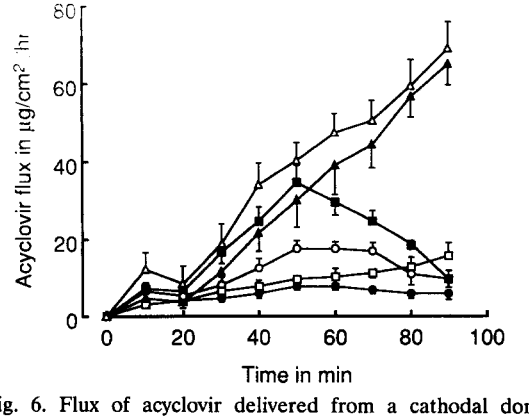

Fig. 6. Flux of acyclovir delivered from a cathodal donor solution containing 1.5 g/l acyclovir following up to 90 min iontophoresis at 0.67 mA/cm^2. The donor solution also contained 0.5 g/l NaLS (●), 2.0 g/l NaLS (○), 10.0 g/l NaLS (■), 0.5 g/l CTM (□), 2.0 g/l CTM (▲) and 10.0 g/l CTM (△). Results are the mean of at least three experiments ± SD.

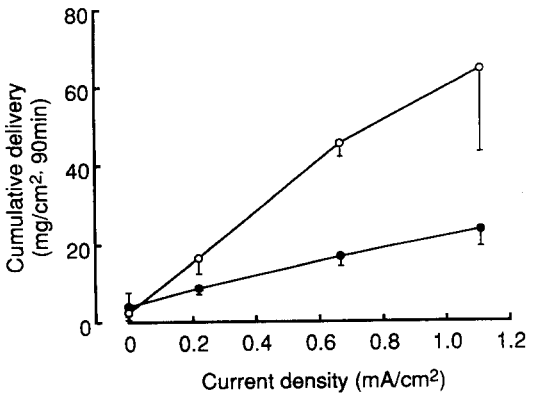

Fig. 7. Effect of applied current densities on the cumulative delivery ($\mu g/cm^2$, 90 min) of acyclovir from donor solutions containing 1.5 g/l acyclovir and 2.0 g/l NaLS (●) or 2.0 g/l CTM (○). The donor side was made negative. Applied current densities 0.22, 0.67 and 1.11 mA/cm^2. Each data point is the mean of at least three experiments ± SD.

The plateau in acyclovir permeation may, therefore, not be explained by polarisation. Wearley and Chien (1990) suggested that the plateau effect could be attributed to the effects of ions on the zeta potential of the skin.

3.6. Effect of increasing current density with iontophoresis and ionic enhancers

The effect of current density on cathodal iontophoresis of acyclovir in presence of NaLS or CTM is shown in Fig. 7. The amount penetrated increased with increasing current density. A linear relationship for NaLS and for CTM was observed between the amount of acyclovir penetrated and the current density with a slope of 17.6 when NaLS had been included and 56.8 when CTM had been included. Similar results have been reported by others (Bellantone et al., 1986; Burnette and Marrero, 1986; Sanderson et al., 1987). The linearity of permeation with increasing current suggests that the barrier properties of the skin were similar for current densities up to 1.11 mA/cm^2.

In conclusion, this study showed that with a continuous (DC) current cathodal iontophoresis of acyclovir produced the highest permeation rate, probably due to some ionisation of the drug. In the presence of ionic enhancers cathodal iontophoresis also gave best results, probably due to polarisation during anodal delivery. CTM was found to enhance cathodal iontophoresis more than NaLS; this is likely to be due to differences in the molecular weight/volume of the permeating ions. Other factors such as hydration, vehicle-skin/current-skin interactions and ionisation may also influence the results. The study shows that it is possible to achieve higher flux values for acyclovir in minutes by using iontophoresis together with an ionic enhancer than could be obtained following hours of passive diffusion.

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