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Rapid Communication

In vitro assessment of skin permeation from a transdermal system for the delivery of oestradiol

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It is important to use in vitro tests in the assessment of the performance of transdermal drug delivery systems. Straightforward dissolution type experiments can be used to show batch to batch conformity and the effects of storage on release profiles. When the system is controlling the delivery of the drug to the systemic circulation it is important to ensure that the release rates are well controlled and that there are no significant changes either between batches or after the storage of a single batch. Data presented by Tymes and co-workers in 1990 showed how the release profiles of an oestradiol containing transdermal patch could be assessed. It was demonstrated that storage of the transdermal system could result in a change in the release profile. After storage of approx. 11 months one batch produced a change in the intrinsic amount released over 96 h which was some 50% greater than originally found. The question arises whether or not this will significantly affect the therapeutic levels of the drug after the patch has been applied in vivo.

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It is possible to produce some information about the potential in vivo consequences by conducting in vitro penetration experiments with the patch in contact with excised human skin mounted in a Franz type diffusion cell. Similar experiments have been conducted for transdermal systems containing nitroglycerin and reasonable correlations found between the predicted in vivo performance and that predicted from in vitro diffusion experiments (Hadgraft et al., 1991). Major discrepancies may be found where the metabolic activity in the skin is an important determinant in the penetration process.

In this communication we have compared the in vitro skin permeation from two batches from each of two oestradiol transdermal systems. One batch had a comparatively new batch date and the other was approx. 5 years old. The release rates across full-thickness human skin were determined and the results compared with the intrinsic release profiles available in the literature (Tymes et al., 1990). The results were also compared to the stated release characteristics, as measured in vivo, to demonstrate the utility of conducting in vitro experiments and show how they can be used in a predictive way. The in vitro release rates across skin have been linked with the clearance kinetics of oestradiol to provide estimates of the

expected plasma levels following transdermal delivery.

The patches evaluated in this study were marketed by Ciba Pharmaceutical Co., Summit, NJ). Four batches were examined: Estraderm 0.05 (1T070056 exp. Oct. 88) [E05.88] and (1F146362 exp. Feb. 93) [E05.93]; Estraderm 0.1 (1T070060 exp. Nov. 88) [E1.88] and (1F140068 exp. Dec. 92) [E1.92]. The delivery rates of 0.05 and 0.1 of these systems are achieved solely by differences in patch size and therefore the release rate per unit area should be identical for both types. The patches were mounted intact in direct contact with human abdominal female skin in all glass Franz type diffusion cells of nominal area 1 cm^2 (accurately measured). Experiments (n = 10) were conducted according to the FDA guidelines (Skelly et al., 1987). The receptor phase, 25% ethanolic phosphate-buffered saline (approx. 3 ml, accurately measured), was thermostatted at 37°C so that the skin surface temperature was maintained at 32 ± 1°C. Oestradiol flux was monitored by periodic sampling (200 μl) over 72 h. Samples were assayed by HPLC using the method described by Tymes et al. (1990) except that the wavelength monitored was 203 nm instead of 191 nm and the mobile phase was water : acetonitrile (50 : 50). The wavelength change was to maximise sensitivity and reduce interference from non specific materials leaching from the skin. The retention time under these conditions was approx. 5 min.

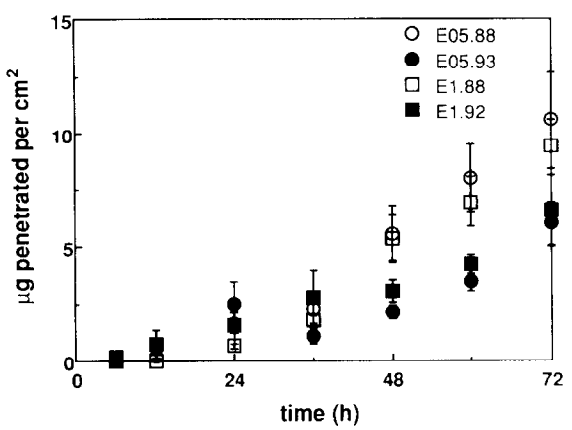

Fig. 1. Comparison between the amounts of oestradiol penetrated through human skin from the four different batches of transdermal systems.

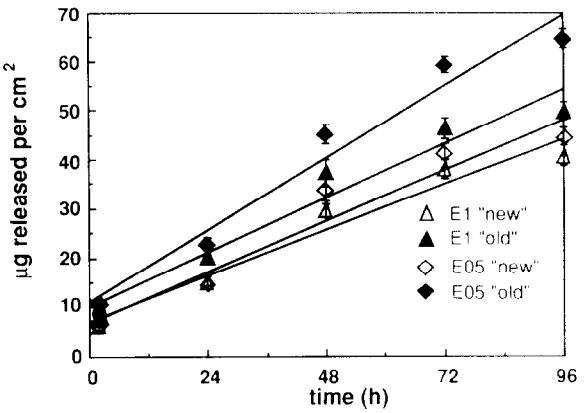

Fig. 2. Comparison between the intrinsic release characteristics of 'new' patches and those stored for 11 months ('old'). Data from Tymes et al. (1990).

The quantities (μg) of oestradiol penetrating through each cm^2 of the skin are shown in Fig. 1.

It is instructive to compare the new data with those generated by Tymes et al. The intrinsic release patterns of the devices are given in Fig. 2. Since the patches are known to have a reservoir with a rate-controlling membrane, the release was expected to show a burst of drug (that contained in the adhesive and the membrane) followed by a linear steady state region. These characteristics can be clearly seen in Fig. 2. Linear regression analysis of the data points in Fig. 2 gave the steady-state flux (slope) and an indication of the amount of drug contained in the adhesive and membrane (intercept) (Table 2).

Analysis of variance for the 24, 48, 60 and 72 h data points was carried out using Anovar software on an Apple Macintosh with the Fisher's

TABLE 1

Comparison of p values at various time points for the data from the four batches

h	E05.88:E05.93	E1.88:E1.92	E05.88:E1.88	E05.93:E1.92
24	0.434	0.372	0.332	0.391
48	0.007	0.064	0.851	0.452
60	0.002	0.051	0.460	0.553
72	0.046	0.209	0.592	0.803

TABLE 2

An analysis of the intrinsic release rates for the fresh ('new') and stored 11 months ('old') patches using data from Tymes et al. (1990)

Patch	Steady-state release rate ($\mu g/cm^2$ per h)	Intercept ($\mu g/cm^2$)	Correlation coefficient
0.1 new	0.39	7.32	0.975
0.05 new	0.43	6.96	0.965
0.1 old	0.46	10.21	0.976
0.05 old	0.61	10.97	0.982

LSD post-hoc. Table 1 shows the p values obtained at various time points on comparison of the batches of different ages and dose levels.

It was apparent that there was little or no difference between batches of similar age (cf. E05.88:E1.88 and E05.93:E1.92) in the amount of oestradiol penetrated at any of these time points. It therefore appeared that the manufacturing processes for producing the two different dose systems are similar and reproducible and that the dose is directly related to the patch area. On the other hand, there were clear differences between penetration from patches of the same dose level but different age from 48 h (cf. E05.88:E05.93 and E1.88:E1.92). The data indicate that after extended storage a change occurs in the formulation such that a higher amount of oestradiol is able to penetrate the skin. The reason for this change is not known but it is possible that the drug accumulates in the adhesive, whilst at the same time loss of the volatile ethanol in the patch would produce a supersaturated concentration of oestradiol. At this higher activity state a larger flux of drug would be expected. These findings are in agreement with those of Tymes et al. (1990) who also showed that stored patches released greater amounts of the drug.

The intercepts showed an accumulation of the drug on storage which may account for the higher fluxes through the skin. However, the data also indicated that the steady-state release rate for the 0.05 patch after 11 months storage was higher than the other systems. This may have been a result of some membrane breakdown as the product exceeded its expiry date.

The steady-state diffusion rates across human skin can be calculated from the data in Fig. 1 and the fluxes obtained are provided in Table 3.

The release rates are substantially slower than the intrinsic release properties given in Table 2 and demonstrate that the skin is the principal controlling feature in the delivery of the drug into the systemic circulation. This can be quantified using the equations given by Guy and Hadgraft (1992) if it is assumed that the old and new batches used in the current study have similar intrinsic release characteristics to those described by Tymes et al. (1990). The fractional control provided by the device and the skin is given in Table 4.

One potential problem in the above analysis is that the in vitro penetration through the skin does not provide an exact match with the quoted in vivo dose per day (see Table 2). There may be several reasons for this. Firstly, the skin used for the in vitro measurements was full thickness and may have overestimated the barrier properties of the skin. This was chosen deliberately to minimise formulation effects and to investigate whether or not the differences found in the intrinsic release rate experiments were likely to be

TABLE 3

Comparison of observed and claimed delivery rates of oestradiol

Patch	Steady-state absorption rate ($\mu g/cm^2$ per h)	Nominal amount delivered per day in vitro (mg)	Claimed amount delivered per day (mg)	Correlation coefficient
E1.92	0.09	0.040	0.10	0.976
E05.93	0.07	0.017	0.05	0.885
E1.88	0.15	0.070	0.10	0.970
E05.88	0.16	0.038	0.05	0.977

TABLE 4

Fractional control provided by the device and the skin in the delivery of oestradiol

Patch	Fractional control by device	Fractional control by skin	Estimated steady-state plasma levels (pg/ml)
E1.92	0.23	0.77	27
E05.93	0.16	0.84	10
E1.88	0.33	0.67	45
E05.88	0.26	0.74	24

of significance when the full barrier properties of the skin were additionally imposed. Secondly, static diffusion cells were used, whereas normal skin is perfused by the local circulation. These two factors would tend to underestimate the potential total amount of bioavailable drug.

The input into the body can be equated with the clearance kinetics to provide an estimate of the steady state plasma levels. Using data from a previous analysis (Guy and Hadgraft, 1986) and standard pharmacokinetic procedures the plasma levels obtained in Table 4 were derived. These are of the same order of magnitude as those found therapeutically demonstrating the value of in vitro experiments in the prediction of in vivo blood levels.

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