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Short Communication

A sensitive high-performance liquid chromatographic method for the assessment of percutaneous absorption of topical corticosteroids*

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Introduction

Triamcinolone acetonide (TACA) was among the first of the corticosteroids to be assayed by high-performance liquid chromatography (HPLC) [1–5, 7]; halcinonide has also been determined by HPLC [6, 7]. All these reports have been concerned with pharmaceutical preparations which, because of the relatively high steroid content, did not require a particularly sensitive method. Indeed virtually all analytical problems involving corticosteroids in pharmaceutical preparations can be solved by HPLC. Consequently there is now a wealth of literature on the analysis of corticosteroid by HPLC; this is reflected in the inclusion of an HPLC assay for TACA in the US Pharmacopeia [8].

However, far less research has been conducted on HPLC assays for low concentrations of synthetic corticosteroids in biological materials. An HPLC determination of very low concentrations of TACA in human serum was reported in 1981 [9].

Two techniques have been employed in order to gain further insight into the permeation of corticosteroids through the stratum corneum. Firstly, measurement of the steroid released into the receptor phase of a horizontal glass diffusion cell; this technique enables the *in vitro* penetration of the corticosteroid through the stratum corneum to be determined. Secondly, measurement of the steroid present in layers of skin removed by adhesive tape; this technique permits determination of the *in vivo* distribution of the corticosteroid within the stratum corneum.

Since these techniques are concerned with the movement, and hence the measurement, of nanogram (ng) quantities of corticosteroid through and within the stratum corneum, any analytical assay must be relatively sensitive and highly specific.

Experimental

In vitro *penetration studies*

Studies were carried out using cadaver skin and horizontal glass diffusion cells (Fig. 1). From a single piece of skin (epidermis plus dermis) the epidermis was removed by heat separation at 60°C for 30 s. Individual pieces of epidermal membranes of area 5.7 cm^2 were punched out and placed on each cell with the epidermal side uppermost. The exposed surface area for drug application was 2.55 cm^2. The steroid was applied as a 0.1% (m/m) cream (Kenalog containing TACA; Halog E and Halog USP both containing halcinonide) to the upper surface of the stratum corneum. Approximately 4–5 mg of cream was applied to give as even a covering as possible. The

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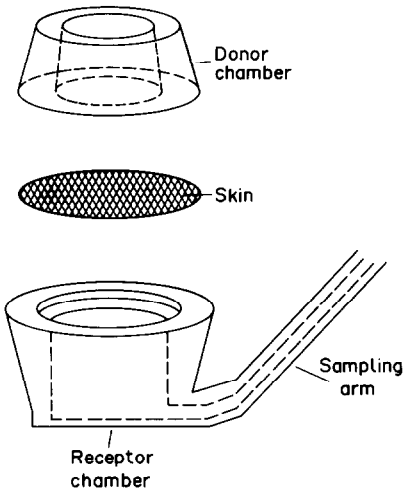

Figure 1
Horizontal glass diffusion cell.

halves of the horizontal glass diffusion cell were clamped together; the receptor chamber was filled with de-ionized water (i.e. the receptor medium) and the whole assembly was placed in a water-bath at 32°C. To aid maintenance of a homogeneous solution within the receptor medium, a micro-magnetic follower was added and the diffusion cells were placed over a multiplace magnetic stirring plate. At pre-determined times, 1 ml of the receptor medium was removed and assayed directly by HPLC. The volume removed was replaced by 1 ml of de-ionized water.

In vivo *permeation studies*

Studies were conducted on the forearms of volunteers. Approximately 4–5 mg of a cream containing 0.1% (m/m) of TACA was applied to a 2 × 2 cm area of the forearm. The sites were then occluded using an Actiderm dermatological patch. At pre-determined times the patch was removed; the site area was dry swabbed then stripped with tape; 10 successive tape-strips were taken. Tape-strip samples were placed in 20-ml glass scintillation vials and stored at 4°C until assayed. To each vial was added 1.0 ml acetonitrile; the contents were mixed and left at ambient temperature for 30 min. Then 0.5 ml of the acetonitrile extract was transferred to an autosampler vial containing 0.5 ml of water. The contents were mixed and assayed directly by HPLC.

HPLC

The HPLC system comprised a Kontron model 420 pump, a Kontron model 460 autosampler and a Hewlett Packard model 3396A integrator. Detection was carried out using a Kratos model Spectroflow 773 UV detector. As well as using a sensitive detector the HPLC system also utilized narrow-bore columns (2 mm dia) in order to further increase the overall sensitivity. Separations were carried out on a 150 × 2 mm column packed with 5-μm Spherisorb ODS1.

Conditions. The eluent was acetonitrile–water (1:1, v/v); the flow rate was 0.16 to 0.30 ml min^{-1}; the injection volume was 10 to 60 μl; and the steroid was detected at 240 nm.

Peak quantification was carried out using peak-height values.

Results and Discussion

TACA gave a linear response over the range 5–100 ng ml^{-1} (regression coefficient = 0.9953; slope = 38.67; intercept = −41.52) and halcinonide gave a linear response over the range 35–350 ng ml^{-1} (regression coefficient = 0.9986; slope = 47.14; intercept = −652.7).

Initial *in vitro* experiments have shown that the permeation rate of TACA through stratum corneum (approximately 0.2 ng cm^{-2} h^{-1}) was greatly increased by occlusion with an Actiderm dermatological patch (approximately 1.0 ng cm^{-2} h^{-1}). Further initial *in vitro* experiments have shown that the permeation rate of halcinonide through the stratum corneum is influenced by the composition of the cream base. Two different cream preparations, both containing 0.1% (m/m) of halcinonide gave permeation rates of approximately 3.7 ng cm^{-2} h^{-1} (Halog E) and 5.7 ng cm^{-2} h^{-1} (Halog USP). Finally, *in vivo* experiments yielded information upon the distribution of TACA within the stratum corneum. Generally, there was a decrease in the amount of steroid present in successive tape-strip samples; this decrease became less marked with time. At 24 h the first five tape-strip samples contained approximately 88% of the recovered steroid compared with approximately 81% at 48 h and approximately 66% at 72 h. The total amount of TACA did not appear to vary with time, i.e. approximately 350–500 ng of steroid. Typical chromatograms of TACA and halcinonide in the receptor medium (Fig. 2) and TACA in the tape-strip extracts (Fig. 3) are included.

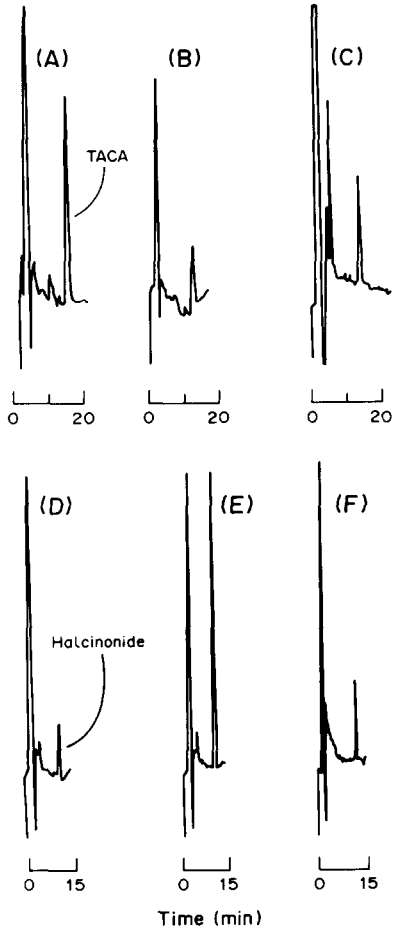

Figure 2
HPLC chromatograms of corticosteroids in diffusion cell receptor medium. (A) TACA standard, 25 ng ml^{-1} (B) TACA standard, 5 ng ml^{-1}; (C) TACA sample, approximately 13 ng ml^{-1}; (D) halcinonide standard, 35 ng ml^{-1}; (E) halcinonide standard, 210 ng ml^{-1}; (F) halcinonide sample, approximately 50 ng ml^{-1}.

The above results, although preliminary in nature, represent the basis for further experiments into the factors influencing the action of topically applied corticosteroids. It has been shown possible to determine not only the *in vitro* permeation rates *per se*, but also how factors such as occlusion and composition of the cream base may affect these rates. The *in vitro* results could give a good guide to *in vivo* permeability of topically applied corticosteroids. Since the 1940s scientists have assumed that the barrier properties in the epidermis of cadaver skin are the same as those *in vivo* [10]. More recently good correlations have been shown between *in vitro* and *in vivo* studies of percutaneous absorption [11, 12]. In conjunction with this approach it is now possible to observe the distribution of corticosteroids within the stratum corneum.

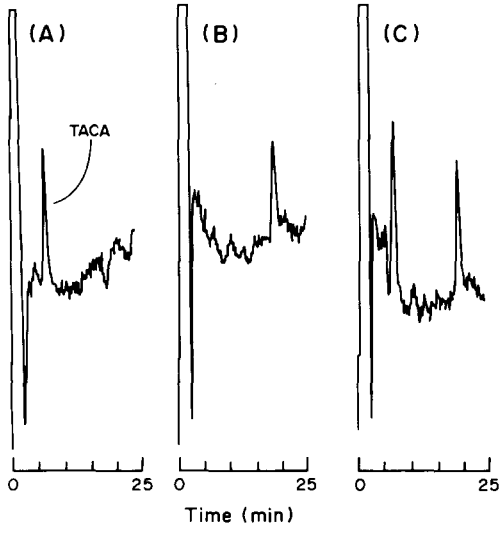

Figure 3
HPLC chromatograms of (A) TACA standard, 25 ng ml^{-1}; (B) extracted control tape-strip sample; (C) TACA sample containing approximately 31 ng ml^{-1}.

Conclusion

The methodology presented above involves a minimal number of sample preparation stages in an attempt to save time and to reduce the cumulative errors associated with the assay of very low concentrations of analytes. Consideration of specificity, sensitivity, and minimal sample preparation resulted in the development of a HPLC procedure for the assay of TCA and halcinonide; the method incorporated a sensitive UV detector and narrow bore (2 mm) analytical columns.

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