IN-VITRO METHODOLOGY FOR INVESTIGATING QUANTITATIVE RELATIONSHIPS BETWEEN SKIN PERMEATION AND COMPOUND STRUCTURE

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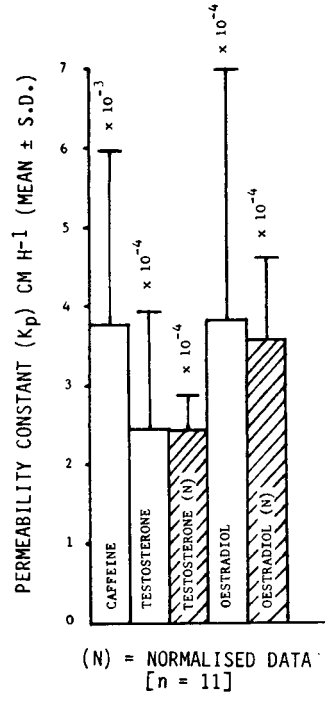
In vitro methods using both human and animal skin are becoming more widely used for attempting to predict the likely percutaneous absorption of drugs and industrial chemicals to which man is exposed (Michaels et al, 1975; Bronaugh et al, 1984). However, wide variations in methodology between workers and large intersubject differences in permeability limit the value of current data for quantitative relationships between permeation and structure to be established.

Our strategy has been to standardise the methodology by applying compounds in suspension at ten times their solubility in light liquid paraffin B.P. (LLP), an inert non-penetrating vehicle. This approach not only maintains the stratum corneum under relatively normal conditions throughout an experiment but also enables a true steady-state penetration rate to be defined. Three radiolabelled compounds, [^{14}C]caffeine (C), [^{14}C]testosterone (T) and [^{3}H]oestradiol (O) were each studied on eleven different donor skins.

Equilibrated suspensions of C, T and O in LLP at concentrations of 10, 40 and 5 mg cm^{-3} respectively were applied ($10^{-3} cm^3 cm^{-2}$) to human abdominal epidermal membranes held in horizontal open-top diffusion cells with phosphate buffered saline (pH 7.4) containing 6% (w/v) PEG-20 oleyl ether as the receptor medium. Cells were maintained in a water bath at 37°C for five days during which time samples were removed from the receptor compartment at regular intervals and assayed for radioactivity. For each cell a permeability constant (Kp, cm h^{-1}) was calculated from the relevant saturated solution concentration (μg cm^{-3}) and the steady-state absorption rate (μg cm^{-2} h^{-1}) determined from the linear portion of the absorption profile by regression analysis.

Kp's for C ranged between 0.9 and 7.1 (cm h^{-1} x 10^{-3}); an eightfold difference between the highest and lowest. Even greater differences were seen for T and O; tenfold and nineteenfold respectively. However, linear regression analysis revealed that an intersample relationship existed between Kp's for C, T and O with correlation coefficients (r) of 0.93. Normalisation of the Kp data on each sample to that of the mean value for C, the most rapid penetrant, resulted in a significant improvement in the coefficients of variance for T from 60.9% to 17.4%, and for O from 82.3% to 31.7%. The range of Kp values was reduced to a factor of 1.7 for T and 2.6 for O. The Figure depicts the Kp's (Mean ± S.D.) for original and normalised data.



Therefore, by using the above method and, for each donor skin in a study including a standard penetrant either in admixture with the test compounds or separately, intersample variations in percutaneous absorption can be reduced to a level where studies can be directly compared. Furthermore, this method may permit realistic predictions of human percutaneous absorption from in vitro studies with animal skin.

Michaels, A.S. et al (1975). Am. Inst. Chem. Eng. 21: 985

Bronaugh, R. et al (1984). J. Pharm. Sci. 73:1255-1258