The Influence of Different Strains and Age on in Vitro Rat Skin Permeability to Water and Mannitol

Ian P. Dick¹ and Robert C. Scott¹,²

Received October 23, 1991; accepted December 18, 1991

Water and mannitol were used as test penetrants to study the effect of age on the skin permeability of the Wistar-derived Alderley Park (AP) rat and Sprague-Dawley (SD) rat. Whole-skin membranes were prepared from rats aged 10 to 120 days, while epidermal membranes were prepared from rats aged 24 to 32 days. The results indicated that the skin permeabilities of the two strains were very similar for either whole-skin or epidermal membranes. The influence of age on skin permeability was found to be negligible for the AP rat, and a small decrease in whole-skin permeability was observed for SD rats above 80 days of age. A statistically derived expression ("the separation efficiency factor") was used to determine the optimum age for preparing intact epidermal membranes; these were 26 days for AP rats and 28 days for SD rats. Histological examination of whole-skin membranes for both strains revealed that the stratum corneum and epidermal thickness did not alter significantly with age (10 to 120 days old). Dermal thickness, hair follicle depth, and, to a lesser extent, the surface area occupied by hair follicles all appeared to be influenced by age, although these changes had no detectable effect on skin permeability.

KEY WORDS: in vitro; percutaneous absorption; age; histology.

INTRODUCTION

Percutaneous absorption studies have helped to improve the therapeutic efficacy and identify the dermal toxicity of topical agents. With the ethical and practical problems associated with human experiments, a wide range of laboratory animal skin alternatives has been used to predict human skin absorption. As a result, the absorption rate for different chemicals has been compared using a common species (1,2) or several species (3,4). However, there has been little consideration of the effect that using different strains of the same species may have on comparisons of absorption data.

Structural changes in skin have been related to age (5–7), most notably between preterm and infant human skin (8,9). However, studies of the effect of age on skin permeability have produced conflicting evidence showing an increase (10–12), a decrease (13–19), or relatively no change (12,13,15,20,21) in permeability with an increase in age for human and animal skin membranes.

In this study, the in vitro absorption of two test penetrants, water and mannitol, was measured through whole-skin and epidermal membranes prepared from Wistar-derived Alderley Park (AP) rats and Sprague-Dawley (SD) rats. By measuring the permeability of rat skin over an extensive age range (10 to 120 days old), both interstrain comparisons and the assessment of the influence of age have been made. Whole-skin samples were also taken for histological examination of the structural development of rat skin for both strains over this age range.

MATERIALS AND METHODS

Chemicals

[¹⁴C]Mannitol (sp act, 60 mCi mmol⁻¹) and [³H]water (sp act, 270 mCi mmol⁻¹) were supplied by Amersham International, Amersham, UK. [¹⁴C]Mannitol was diluted in distilled water to give a final activity of approximately 2.5 μCi ml⁻¹ and made up to a concentration of 1 mg ml⁻¹ with unlabeled mannitol (supplied by Sigma Chemical Co., Poole, UK). Tritiated water was diluted in 0.9% physiological saline to give a final activity of approximately 2.5 μCi ml⁻¹. Optiphase MP scintillation fluid was supplied by LKB and manufactured by FSA Laboratory Supplies (Loughborough, UK).

Membrane Preparation

Alderley Park rats (strain Alp:APfSD) were supplied by the Barri ered Animal Breeding Unit (Alderley Park, Cheshire, UK). Sprague-Dawley rats (strain Crl:CD (SD)BR) were supplied by Charles River UK Ltd. (Margate, Kent, UK). Whole-skin membranes were removed from the dorsal region and epidermal membranes were prepared using a chemical separation technique (22).

In Vitro Percutaneous Absorption Studies

Prepared membranes were mounted on horizontal-membrane static glass diffusion cells (exposure area, 2.54 cm²) and maintained at 30°C in a water bath. A solution of 0.9% physiological saline was used as the receptor fluid. The integrity of the membranes was initially assessed by measuring the permeability of tritiated water (400 μl cm⁻² skin, occluded, Day 1). The absorption of [¹⁴C]mannitol was then studied (200 μl cm⁻² skin, occluded, Days 2–4), and the steady-state absorption rates were calculated for each penetrant. From the absorption rate, a permeability coefficient (cm hr⁻¹) was calculated and this was the final expression of the permeability of each penetrant.

The "Separation Efficiency Factor"

This statistically derived formula was used to quantify the efficiency of preparing "intact" epidermal membranes from different aged rats. The separation efficiency factor (SEF value) was calculated at each age using the following expression:

SEF = \frac{\text{mean of No. of "intact" membranes per skin}}{\text{No. of membranes prepared per skin}} \times 100\%

¹ Central Toxicology Laboratory, ICI PLC, Alderley Park, Nr Macclesfield, Cheshire, U.K.
² To whom correspondence should be addressed.
An epidermal membrane was deemed intact if it displayed a tritiated water permeability similar to that obtained using whole skin (<2.5 × 10⁻³ cm hr⁻¹) (22).

**Histology**

Histology was performed on whole-skin samples over the age range 10 to 120 days old. This included the examination of stratum corneum thickness, viable epidermal thickness, dermal thickness, hair follicle depth, and the skin surface area occupied by hair follicles.

**RESULTS**

**Influence of Strain**

Graphical comparisons of the permeability of AP rat and SD rat whole skins are presented in Fig. 1 for the two test penetrants over the age range 10 to 120 days old. The mean permeability coefficients (K_p) obtained for each strain of rat were compared at each age, treating each penetrant independently (two-sided Student’s t test: P < 0.05 indicated by asterisks). The skin permeabilities of both strains of rat were very similar, and where significant differences were recorded, they were comparable to the magnitude of the variation in skin permeability found between rats of the same strain.

The overall mean K_p values (±SE) for whole-skin membranes calculated over the entire age range were also similar for both strains for water [AP rat = 1.43 (±0.05) × 10⁻³ cm hr⁻¹, n = 178; SD rat = 1.53 (±0.06) × 10⁻³ cm hr⁻¹, n = 150] and mannitol [AP rat = 3.23 (±0.17) × 10⁻⁴ cm hr⁻¹, n = 178; SD rat = 2.89 (±0.17) × 10⁻⁴ cm hr⁻¹, n = 150]. Interstrain similarities were also indicated by the overall mean K_p values obtained for epidermal membranes for water [AP rat = 1.16 (±0.08) × 10⁻³ cm hr⁻¹, n = 30; SD rat = 1.41 (±0.08) × 10⁻³ cm hr⁻¹, n = 22] and, to a lesser extent, mannitol [AP rat = 2.30 (±0.27) × 10⁻⁴ cm hr⁻¹, n = 30; SD rat = 0.89 (±0.15) × 10⁻⁴ cm hr⁻¹, n = 22].

**Influence of Age**

The mean K_p value at each age was statistically compared with the overall mean K_p value for the entire age range for each penetrant; any significant differences were highlighted by asterisks (two-sided Student’s t test: *P < 0.05, **P < 0.01). Generally, age did not affect the skin permeability of either strain of rat for the test penetrants. For instance, where significant differences were recorded for AP rat skin permeabilities, the difference between the mean K_p at these ages and the overall mean K_p was less than a factor of two. However, for SD rats, there was an indication of a decrease in whole-skin permeability for 100- and 120-day-old animals, where the mean K_p's at these ages were significantly less than the overall mean K_p value for both penetrants.

The effect of age (24–32 days only) on the permeability of the test penetrants (mean K_p) through AP rat and SD rat epidermal membranes was also examined (Fig. 2). The mean K_p values did not differ significantly from the overall mean K_p value, although the permeability of water through 30-day-old AP rat epidermis was significantly higher (P < 0.05).

A ‘‘separation efficiency factor’’ (SEF value) for epidermal membrane preparation from AP rats and SD rats was calculated at each age. For AP rat epidermal membranes, the SEF value was greatest for 26-day-old rats, decreasing for older animals. A similar relationship between the SEF value and age was exhibited for SD rat epidermal membranes, the maximum SEF value occurring at 28 days of age.

**Histology**

Changes in the structure of skin as determined by histological examination were studied over the age 10–120 days. For both strains of rat, there was very little variation with age for stratum corneum thickness (range, 16.3–24.8 μm) and viable epidermal thickness (range, 14.0–20.1 μm). Age did influence the dermal thickness (range, 264 μm at 26 days for AP and 796 μm at 60 days for AP rats), minimum hair follicle depth (range, 144 μm for AP at 24 days and 734 μm

---

**Fig. 1.** The effect of age on the mean permeability coefficient (K_p) ± SE (7 < n < 18) for water and mannitol through AP rat whole skin. Two-sided Student’s t test between the mean K_p at each age and the overall mean K_p: (*) P < 0.05; (**) P < 0.01. (▲) AP/water; (▲) SD/water; (□) AP/mannitol; (△) SD/mannitol.

**Fig. 2.** An interstrain comparison of the mean permeability coefficient for water and mannitol through AP rat and SD rat epidermal membranes at each age. Two-sided Student’s t test: (*) P < 0.05; (**) P < 0.01. (▲) AP/water; (▲) SD/water; (□) AP/mannitol; (△) SD/mannitol.