charles river
accelerating drug development. exactly.

Pfizer

The Assessment of the Barrier Function of Cow Skin Membranes *in vitro*

C Roper[1], S Madden[1] and S Marsh[2]
[1]Charles River Preclinical Services, Edinburgh, EH33 2NE, UK
[2]Pfizer Animal Health, Veterinary Medicine Pharmaceuticals R & D, Kalamazoo, MI 49001, USA

Introduction

In order to perform a study involving the application of a topical drug to cow skin, the barrier function of dermatomed membranes from cow skin needed to be assessed. Since there was no information in the literature, a study was designed to assess the barrier function of full-thickness (FT) and split-thickness (ST) cow skin membranes *in vitro* using a tritiated water barrier integrity test.

Cow Skin

Full thickness skin membranes were obtained from four cows that had been slaughtered at a local abattoir for meat purposes and the skin was excess to requirements. Details of the age, sex and breed of the cow or the site from which the skin was obtained were not supplied by the abattoir. Split-thickness skin membranes (*ca* 200 to 1000 μm) were prepared using a dermatome. The thickness of each sample was measured using a micrometer (Table 1).

Nominal Thickness	Actual Thickness (μm)
Full-thickness	3875±833
Split thickness (1000 μm)	1033±54
Split thickness (800 μm)	808±10
Split thickness (600 μm)	583±24
Split thickness (400 μm)	388±5
Split thickness (200 μm)	240±35*

*It was not possible to prepare the 200 μm membrane for Cow 3.

Table 1. Thickness of cow skin membranes

Materials

Tritium-3 labelled water ([^{3}H]-water) was obtained from GE Healthcare. This had a specific activity and radiochemical purity of 185 MBq/mL and >99%, respectively. Reverse osmosis water (18.2 MΩ) was prepared at Charles River. All other materials were obtained from commercial suppliers.

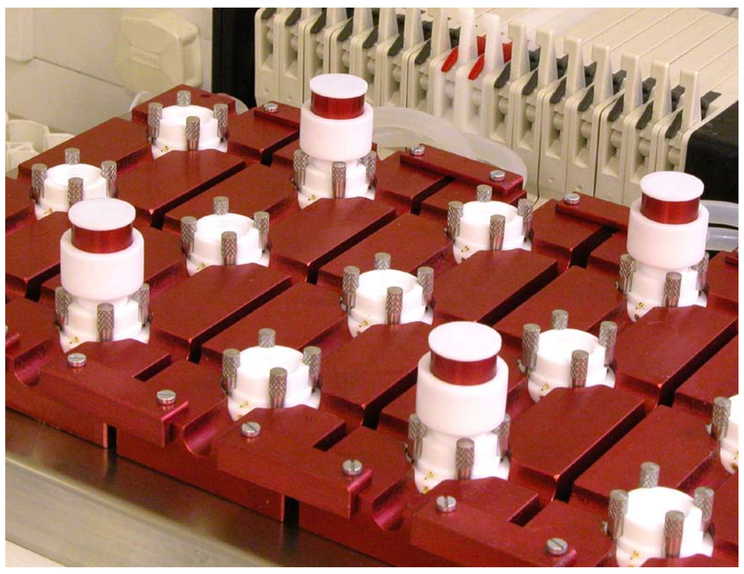


Figure 1. Flow-through diffusion cells

Methods

Full and split-thickness cow skin membranes were mounted in flow through diffusion cells (Scott/ Dick, University of Newcastle upon Tyne, UK) (Figure 1). Receptor fluid, tissue culture medium containing bovine serum albumin (*ca* 5%, w/v), glucose (*ca* 1%, w/v), Streptomycin (*ca* 0.1 mg/mL) and Penicillin G (*ca* 100 units/mL), was pumped underneath the skin at a flow rate of *ca* 1.5 mL/h. Carbon dioxide (5%) in air was continuously bubbled over the headspace in the receptor fluid reservoir. A [^{3}H]-water barrier integrity test was performed over a 2 h period. [^{3}H]-Water (radiodiluted to 412,270 dpm/mL, 0.25 mL) was applied to the stratum corneum surface of the cow skin (0.64 cm^2) following a predose receptor fluid collection. Receptor fluid was then collected in two hourly fractions. The skin was then rinsed with water. Radioactivity was determined in the samples by liquid scintillation counting in a Packard 2100TR liquid scintillation analyser.

Results

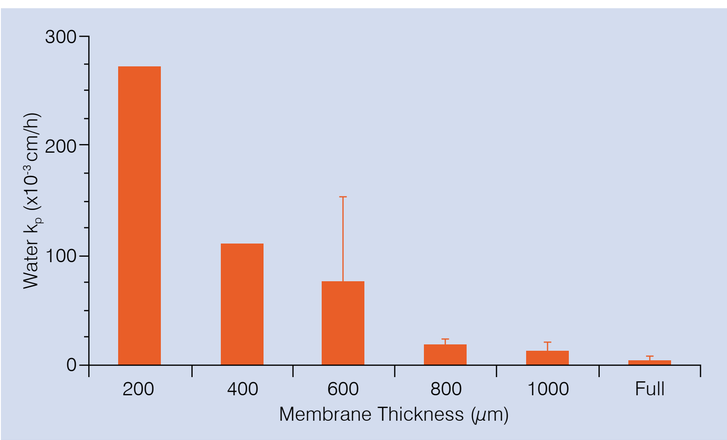
The results are shown in the Table 2 and Graph 1. Results are presented as mean + SD.

The full thickness skin had the lowest mean [^{3}H]-water k_p (4.74 x 10^{-3} cm/h). However, the lower dermis fat did block the receptor chamber for 2 samples. There was no statistical (Students paired t-test) difference (p=0.074) between the water k_p for the 1000 μm (13.1 x 10^{-3} cm/h) and 800 μm (17.6 x 10^{-3} cm/h) split-thickness membranes. All 800 μm and 1000 μm membranes that were dosed with [^{3}H]-water generated useable data and were, therefore, considered to be intact. In the 800 and 1000 μm thickness groups, the highest value measured (27.9 x 10^{-3} cm/h) was for an 800 μm thickness skin sample. For the 600 μm split-thickness skin membrane, only 1 sample was damaged, but the barrier function was greatly reduced with a mean [^{3}H]-water k_p of 76.3 x 10^{-3} cm/h. For the 200 μm and 400 μm split-thickness membranes, only 2 samples each generated useable data (272 and 110 x 10^{-3} cm/h, respectively). Most samples leaked as soon as they were placed into the diffusion cells.

Nominal thickness (μm)	Actual thickness (μm)	Water k_p (x 10^{-3} cm/h)	No. of usable samples	No. of samples dosed
200 ST	240 ± 35	272*	2	6
400 ST	388 ± 5	110*	2	8
600 ST	83 ± 24	76.3± 77.8	7	8
800 ST	808 ± 10	17.6 ± 6.38	9	9
1000 ST	1033 ± 54	13.1 ± 7.70	9	9
FT	3875 ± 833	4.74 ± 2.52	6	8

* less than 3 replicates

Table 2. [^{3}H]-Water barrier integrity for full and split-thickness cow skin membranes



Graph 1. A bar graph of water barrier integrity for full and split-thickness cow skin membranes

Conclusion

In conclusion, split-thickness cow skin membranes can be prepared and used between 800 and 1000 μm. It is recommended that a maximum acceptable [^{3}H]-water permeability coefficient of 30 x 10^{-3} cm/h should be used for cow split-thickness skin membranes.

Skin Forum, London
06/2008
www.criver.com