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Rapid communication

Investigation of the permeability characteristics of peri-ulcer and whole ischaemic skin tissue

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Abstract

Excessive wound exudate in chronic wounds can cause skin maceration to occur around a wound, which may delay healing and lead to other complications. In order to assess possible treatment options with topical therapy there is a need to characterize the permeability of wound and peri-ulcer tissue. Previously we have reported the permeability of a range of chemicals with differing physicochemical properties in human ulcerated and peri-ulcer ischaemic tissue. The findings suggested that wound tissue and peri-ulcer tissue were not representative of normally functioning skin barriers. In the present work we have investigated the permeability of tritiated water in peri-ulcer and whole skin human tissue obtained from clinically diagnosed diabetic ischaemic patients. Permeability data for peri-ulcer tissue was generally higher and more variable than for normal tissue. Histological examination confirmed the breakdown of the skin tissue in the peri-ulcer area and also in the normal skin tissue samples taken from diabetic ischaemic patients. The impaired skin barrier function both in the peri-ulcer and normal tissue may offer opportunities for dermal and transdermal therapies for management of diabetes-related complications.

Keywords: Wound; Peri-ulcer; Skin; Permeability; Topical delivery; Transdermal delivery

1. Introduction

Wounds may be classified as acute (healing) or chronic (non-healing). Non-healing or chronic wounds present a significant health problem with current health care costs thought to be >US$ 1 billion annually in the United States (Mustoe et al., 2006) and £300–£600 million a year in the United Kingdom (Simon et al., 2004). Examples of chronic skin and soft tissue wounds include the diabetic foot ulcer, the decubitus ulcer, and the venous stasis ulcer. These chronic wounds are characterized by a disruption of the normal wound healing process that occurs in acute wounds because of neuropathy, vascular disease, local ischaemia and hypoxia.

Exudate is generated as part of the normal inflammatory response to a wound with its composition and function being dictated by the nature of the wound. Acute wound exudate has a high protein content, contains essential nutrients for epithelial cells, facilitates the ingress of leukocyte cells and supports cell proliferation (Chen et al., 1992; Ono et al., 1994). In contrast, chronic wound exudate does not support cell proliferation and may contribute to wound chronicity because of its higher enzyme content relative to acute wound exudate (Wysocki et al., 1993; Wysocki, 1996; Yager et al., 1996; Yager and Nwomeh, 1999). Because of the corrosive properties of chronic wound exudate (Rao et al., 1995; Grinnell and Zhu, 1996) it has been described as a wounding agent in its own right (Chen et al., 2004). Furthermore, for acute wounds the volume of exudate produced is usually manageable but exudate volume is generally less predictable for chronic wounds (Cutting, 2003). Failure to manage chronic wound exudate effectively will lead to exposure of the peri-ulcer skin to this liquid with the potential for damage to normal epidermal tissue. This in turn may be followed by maceration of the skin and wound bed which will change the permeability characteristics of this tissue (White and Cutting, 2003).

Topical and transdermal therapies offer a number of advantages for the management of such chronic wounds including localized delivery, no first pass metabolism and limited systemic

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side effects. In order to determine potential drug delivery rates to the wound environment an understanding of the permeability of wound and peri-ulcer tissue is a prerequisite. Previously we reported the findings of *in vitro* percutaneous absorption studies of a range of compounds of differing physicochemical properties in a number of tissues including peri-ulcer and ulcerated tissues from diabetic ischaemic patients (Walker et al., 1997). The data suggested that the barrier integrity of such tissues is compromised relative to healthy skin tissue. In the present paper we report a more extensive investigation of the *in vitro* permeability characteristics of tritiated water in matched normal skin samples and peri-ulcer skin samples from diabetic ischaemic patients. To our knowledge no other quantitative data comparing the permeability of such tissue has previously been published in the literature. The permeability characteristics of the tissue are also compared to the transport characteristics of water in normal tissue samples taken from two other sets of diabetic ischaemic patients with and without skin ulcers in order to investigate the overall barrier properties of the lower limb and to evaluate if ischaemia and/or peripheral vascular disease may influence the barrier properties of lower leg skin.

2. Materials and methods

2.1. Materials

[^{3}H]-Water (purity >99%) was purchased from Amersham Life Science, and a non-aqueous biodegradable counting scintillant (BCS-NA) was purchased from Amersham International.

2.2. Skin samples

Ethical approval was obtained from the North East Wales NHS Trust, Wrexham Maelor Hospital for the removal of human skin from elective lower limb surgery with appropriate informed consent. For comparison of normal skin and peri-ulcer skin permeability, tissue was obtained from 10 lower limb amputations of clinically diagnosed diabetic ischaemic patients (6 females and 4 males, mean age 61 ± 18 years). The peri-ulcer skin samples were removed from an area approximately 3–5 cm from the edge of the ulcerated area. Normal tissue was removed from a distance >10 cm away from the ulcer, usually from the calf area. Normal skin samples were also taken from a further 14 diabetic ischaemic patients (4 females and 10 males mean age 66 ± 7 years, six with ulcers and eight without ulcers).

2.3. In vitro percutaneous absorption experiments

All skin permeation experiments were carried out using Franz type horizontal glass diffusion cells as previously described (Dugard et al., 1984). Briefly this method used 1 cm^2 disks of whole skin or human peri-ulcer tissue. The underlying membrane was bathed in stirred distilled water (receptor volume 1.8 ml). A solution of the tritiated water (200 l) was then placed into the donor chamber and the whole cell clamped together and placed into a water bath at 32 °C (±1 °C). Each donor chamber was occluded to prevent evaporation. Experimental run times were 6 h for both tissues.

2.4. Histology

Tissue samples (approximately 5 mm × 5 mm) were placed in neutralised buffered formalin (pH 7) for a minimum of 24 h before paraffin wax embedding. Serial sections were then cut at 5 μm mounted onto glass slides and processed for Haematoxylin and Eosin staining.

2.5. Data analyses

Individual permeability coefficients (k_p) for a given experiment were calculated from the "steady-state" linear portion of a plot of the total amount permeated into the receptor chamber as a function of time using the following relationship:

$$k_p = \frac{J}{[CA]} \quad (1)$$

where J = the measured flux (linear portion) of the graph, C = the applied tritiated water concentration, and A = the area of the membrane available for diffusion (0.64 cm^2).

The means of all data are presented with their standard error of the mean (S.E.M.). Data analysis was performed using an analysis of variance (ANOVA), multiple comparison test, the Newman–Keuls range test ($\alpha = 0.05$).

3. Results and discussion

3.1. In vitro penetration across normal skin versus peri-ulcer skin

The tritiated water permeability coefficient values for normal and peri-ulcer skin for each individual patient are illustrated in Fig. 1. The mean values for normal skin (ranging from $1.09 \pm 0.42 \times 10^{-3}$ cm h^{-1} to $3.59 \pm 2.01 \times 10^{-3}$ cm h^{-1}) are in agreement with previously published data (Walker et al., 1996) evaluating the inter and intra-dermal permeability variations in human lower limb tissue ($2.81 \pm 0.14 \times 10^{-3}$ cm h^{-1}, $n = 125$). These values are at least twice the value that has become generally recognized for human abdominal tissue (Walker et al., 1983), i.e. a k_p value of $\sim 1.0 \times 10^{-3}$ cm h^{-1}.

The trend for permeability values for the peri-ulcer tissue is generally significantly higher for all patients (Fig. 1). Previously the value of tritiated water permeability in peri-ulcer skin has been reported as $11.61 \pm 1.21 \times 10^{-3}$ cm h^{-1} for a smaller sample size ($n = 38$). The results from the present study again show good agreement with a k_p value of $10.79 \pm 1.13 \times 10^{-3}$ cm h^{-1}, ($n = 92$). The higher permeability values for peri-ulcer tissue may be because of internally driven events such as maceration which result in a breakdown of the epidermal/dermal junction which may then lead to a loss of epidermal barrier integrity. While the majority of values for normal skin are consistent with the mean value previously reported for whole ischaemic skin tissue, the peri-ulcer data exhibit much greater variability presumably because of the different effects of exudate levels (Fig. 1).

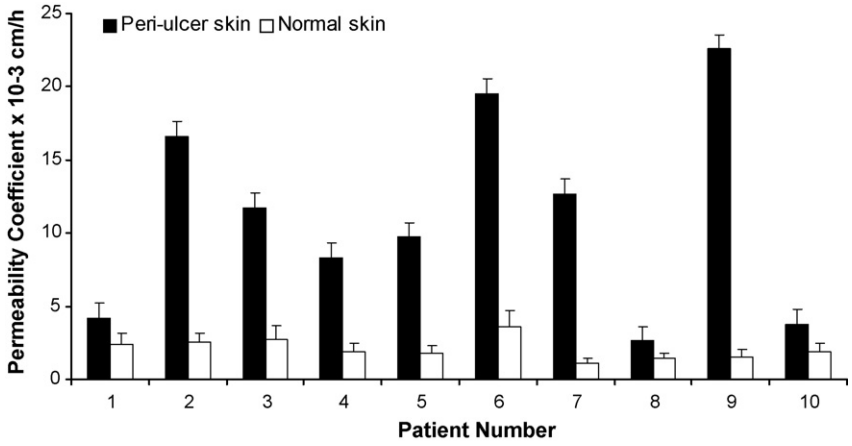

Fig. 1. Tritiated water permeability coefficient values for the 10 patients matched for normal and peri-ulcer skin for each individual patient are illustrated in figure. Each value represents the mean ± S.E.M.

The overall normal skin permeability value for all patients, i.e. the 10 patients selected for investigation of peri-ulcer skin permeability and the additional fourteen patients is $2.65 \pm 0.17 \times 10^{-3}$ cm h^{-1} ($n = 242$).

3.2. Comparison of normal skin permeability data with total population permeability data

Fig. 2 illustrates the skin permeability data for normal skin for the remaining 14 patients and includes the normal skin data for group selected for the peri-ulcer skin studies for direct comparison. These results showed that the group of 6 patients with ulcers present showed a much higher incidence of increased permeability for normal skin (e.g. 55.17% of samples with permeability values above the overall normal skin permeability value of $2.65 \pm 0.17 \times 10^{-3}$ cm h^{-1}, $n = 242$). This value was markedly higher than the corresponding value for normal skin in the original group of 10 patients selected to study normal and peri-ulcer skin permeability (17.76% of values higher than overall mean value). In the non-ulcerated patients 48.05% presented values above the overall mean again suggesting greater variability than that seen in the peri-ulcer study group. The mean range for normal skin permeability for the ulcerated group is $1.14 \pm 0.13 \times 10^{-3}$ cm h^{-1} to $5.76 \pm 0.34 \times 10^{-3}$ cm h^{-1} and for the non-ulcerated group the mean range is $1.27 \pm 0.42 \times 10^{-3}$ cm h^{-1} to $4.27 \pm 1.67 \times 10^{-3}$ cm h^{-1}. These data suggests that such skin is not necessarily "normal" but may be subject to skin breakdown. If the barrier of such tissue is compromised further opportunities for topical and transdermal therapy may exist at these sites as well as at peri-wound tissue.

3.3. Histological examination of peri-ulcer and normal skin tissue

Under histopathological examination, the skin sections taken from peri-ulcer tissue (Fig. 3a and b) showed generalized acanthosis and hyperkeratosis present in the epidermal layers. In the dermis there was generalized elastosis in the papillary dermis with enlargement of blood vessels and vasculitis present in the

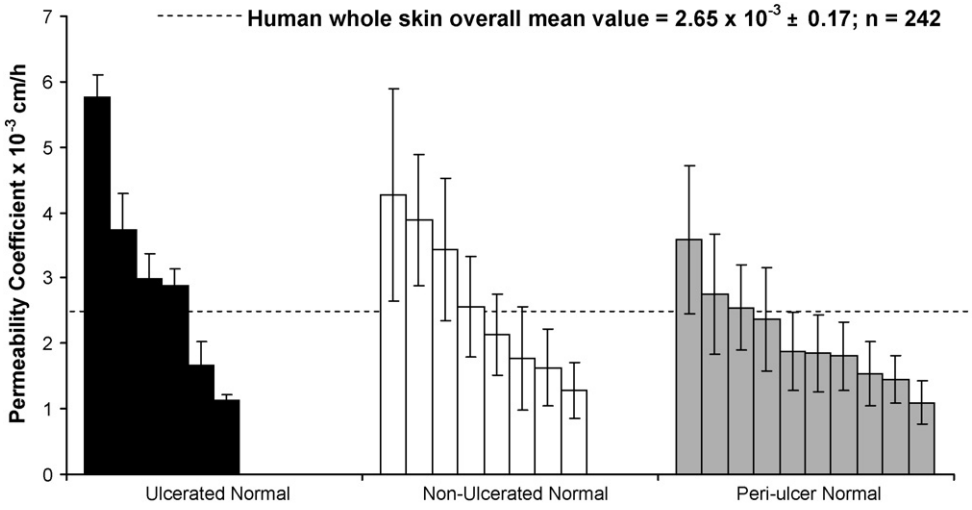

Fig. 2. Skin permeability data for normal skin for the remaining fourteen patients and includes the normal skin data for the 10 patients selected for the peri-ulcer skin studies for direct comparison. Each value represents the mean ± S.E.M.

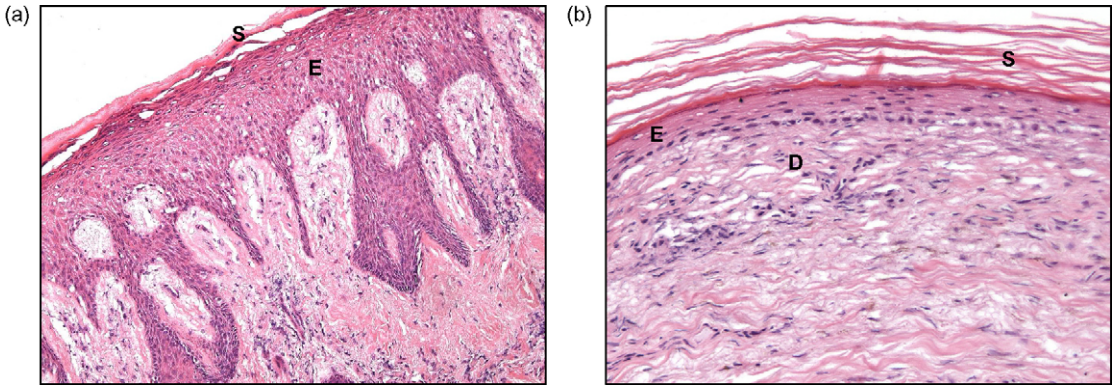

Fig. 3. (a and b) H&E stained peri-ulcer skin samples from individual patient; S = stratum corneum, E = epidermis, D = dermis.

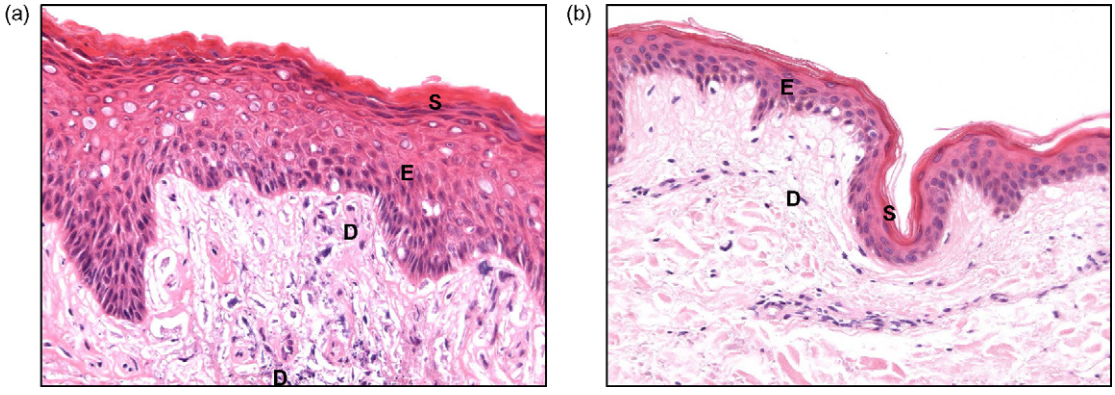

Fig. 4. (a and b) H&E stained normal skin samples from individual patients; S = stratum corneum, E = epidermis, D = dermis.

deep dermis. Fig. 3a and b highlight the differences that may occur between individual patients. In Fig. 3a there appears to be some possible epidermal dermal separation and the dermis shows marked ridges indicative of highly inflamed tissue. The deeper dermis also showed loosely arranged collagen with variable areas of thick peri-follicular elastic fibres. In contrast in Fig. 3b the epidermis is much thinner with loose connective dermal tissue and marked basophilia of focally thick dermal elastic fibres. Similarly the normal tissue sections shown in Fig. 4a and b showed differences in the appearance of the epidermis in particular. Both appeared to have a thin intact stratum corneum layer, but there were marked differences in the thickness of the epidermis between Fig. 4a and b. There was evidence of reasonable dermal structure in Fig. 4a, with some symmetrical collagen present, whereas the collagen was much more loosely arranged in Fig. 4b.

4. Conclusions

Despite the considerable number of percutaneous absorption papers reported in the literature few studies have investigated the permeation characteristics of ulcerated and peri-ulcer tissue. These data confirm that as well as the wound itself, the peri-wound or peripheral skin barrier is likely to be compromised and is therefore not truly representative of normally functioning skin.

The variability in the water permeability values for peri-ulcer tissue together with the histological images confirms that the presence of an impaired skin barrier in such tissue is likely to be due to the effects of wound exudate on the tissue. Interestingly, the permeability of "normal" skin tissue in such patients is also quite variable. The three sets of normal skin permeability data presented in these studies suggest the likelihood of the presence of an impaired skin permeability barrier even in what appears to be intact tissue. Therefore even where there is no obvious of skin damage in the form of an ulcer there is evidence of breakdown of the composite layers presumably due to a limited vascular supply and possible tissue hypoxia. Without a fully differentiated stratum corneum barrier present many of the problems associated with transdermal delivery across intact skin might be expected to be reduced. It should therefore be possible to administer active agents to the wound periphery which will permit an overall more efficient delivery to the rear aspect of the wound. In addition, the results suggest that opportunities exist for more efficient transdermal delivery of other agents (e.g. analgesics or drugs related to the management of diabetes) through possibly compromised peripheral skin areas in diabetic ischaemic patients.

References

Chen, W.Y., Rogers, A.A., Lydon, M.J., 1992. Characterization of biologic properties of wound fluid collected during early stages of wound healing. J. Invest. Dermatol. 99, 559–564.

Chen, W.Y.J., Rogers, A.A., Walker, M., Waring, M., Bowler, P.G., Bishop, S.M., 2004. A rethink of the complexity of chronic wounds—implications for treatment. *European Tissue Repair Society*, Bulletin 10.2–10.3, pp. 65–69.

Cutting, K.F., 2003. Wound exudate: composition and functions. Br. J. Community Nurs. 8, 4–9.

Dugard, P.H., Walker, M., Mawdlsey, S.J., Scott, R.C., 1984. Absorption of some glycol ethers through human skin in vitro. Environ. Health. Persp. 57, 193–197.

Grinnell, F., Zhu, M., 1996. Fibronectin degradation in chronic wounds depends on the relative levels of elastase, alpha1-proteinase inhibitor, and alpha2-macroglobulin. J. Invest. Dermatol. 106, 335–341.

Mustoe, T.A., O'Shaughessy, Kloeters, O., 2006. Chronic wound pathogenesis and current treatment strategies: a unifying hypothesis. Plast. Reconstr. Surg. 117, 35S–41S.

Ono, I., Gunji, H., Suda, K., Iwatsuki, K., Kaneko, F., 1994. Evaluation of cytokines in donor site wound fluids. Scand. J. Plast. Reconstr. Surg. Hand Surg. 28, 269–273.

Rao, C.N., Ladin, D.A., Liu, Y.Y., Chilukuri, K., Hou, Z.Z., Woodley, D.T., 1995. Alpha 1-antitrypsin is degraded and non-functional in chronic wounds but intact and functional in acute wounds: the inhibitor protects fibronectin from degradation by chronic wound fluid enzymes. J. Invest. Dermatol. 105, 572–578.

Simon, D.A., Dix, F.P., McCollum, C.N., 2004. Management of venous leg ulcers. BMJ 328, 1358–1362.

Walker, M., Dugard, P.H., Scott, R.C., 1983. In vitro percutaneous absorption studies: a comparison of human and laboratory species. Human. Toxicol. 2, 561–562.

Walker, M., Patrick, K., Lian, P., Scriven, M., 1996. The European Tissue Repair Society Focused Meeting on the Chronic Wound, Cardiff, UK.

Walker, M., Hulme, T.A., Rippon, M.G., Walmsle, R.S., Gunnigle, S., Lewin, M., Winsey, S., 1997. In vitro model(s) for the percutaneous delivery of active tissue repair agents. J. Pharm. Sci. 86, 1379–1384.

White, R.J., Cutting, K.F., 2003. Interventions to avoid maceration of the skin and wound bed. Br. J. Nurs. 12, 1186–1201.

Wysocki, A.B., Staiano-Coico, L., Grinnell, F., 1993. Wound fluid from chronic leg ulcers contains elevated levels of metalloproteinases MMP-2 and MMP-9. J. Invest. Dermatol. 101, 64–68.

Wysocki, A.B., 1996. Wound fluids and the pathogenesis of chronic wounds. J. Wound Ostomy Continence Nurs. 23, 283–290.

Yager, D.R., Nwomeh, B.C., 1999. The proteolytic environment of chronic wounds. Wound Repair Regen. 7, 433–441.

Yager, D.R., Zhang, L.Y., Liang, H.X., Diegelmann, R.F., Cohen, I.K., 1996. Wound fluids from human pressure ulcers contain elevated matrix metalloproteinase levels and activity compared to surgical wound fluids. J. Invest. Dermatol. 107, 743–748.