Sequential changes in trace metal, metallothionein and calmodulin concentrations in healing skin wounds

A. B. G. Lansdown, B. Sampson and A. Rowe

Departments of Clinical Chemistry, Imperial College School of Medicine and Charing Cross Hospital, and Clinical Pharmacology, Imperial College School of Medicine, Chelsea and Westminster Hospital, London, UK

(Accepted 4 May 1999)

ABSTRACT

Metalloenzymes have an important role in repair and regenerative processes in skin wounds. Demands for different enzymes vary according to the phase in the healing cascade and constituent events. Sequential changes in the concentrations of calcium, copper, magnesium and zinc were studied in the incisional wound model in the rat over a 10 d period. Copper levels remained low (< 10 µg/g dry weight) throughout, but calcium, magnesium and zinc increased from wounding and peaked at about 5 d at a time of high inflammation, granulation tissue formation and epidermal cell proliferation. Metal concentrations declined to normal by 7 d when inflammation had regressed, re-epithelialisation of the wound site was complete and the ‘normalisation’ phase had commenced. Although the wound was overtly healed by 10 d, the epidermis was still moderately hyperplastic. In view of competitive binding of trace metals at membrane receptors and carrier proteins, the ratios or balance between these trace metals was examined and the significance is discussed. Using immunocytochemistry, we demonstrated increases in metallothionein immunoreactivity as an indication of zinc and copper activity in the papillary dermis and in basal epidermal cells near the wound margin 1–5 d after wounding. This is consistent with metalloenzyme requirements in inflammation and fibrogenesis. Calmodulin, a major cytosolic calcium binding protein was highest in maturing keratinocytes and in sebaceous gland cells of normal skin; it was notably more abundant in the epidermis near the wound margin and in re-epithelialising areas at a time when local calcium levels were highest.

Key words: Skin wounds; repair; trace metalloenzymes; metal ion balance; zinc; calcium; copper; magnesium.

INTRODUCTION

Wound healing in the skin and in other tissues of the human body depends upon the availability of competent cells to carry out repair processes, appropriate activation by hormones, growth factors, cytokines and chemotactic factors, and a microenvironment favouring cell movement, proliferation and functional maturation (Grotendorst, 1992; Hunt & Hussain, 1992). Metal ions and metalloenzymes feature prominently in this wound healing environment and experimental and clinical studies are available to show that deficiencies in zinc, copper, calcium, iron or magnesium are potential causes of abnormal homeostatic mechanisms and impaired wound healing (Moynahan, 1974; Lansdown, 1995). Although attention recently has focused upon the role of metalloproteinases in the degradation of extracellular matrix in the early phases of wound healing (Nwomeh et al. 1998), it is to be expected that the demands for these and other metalloenzymes will vary greatly as the profile of wound healing proceeds. Surprisingly, the action and interaction of trace metals in the skin under normal conditions is not well documented, and sequential changes in the healing wound are not published for any species (Lansdown, 1995).

Much of the trace metal requirement for the body is derived from the diet, but many topical dressings, healing creams and other commercial preparations containing zinc and calcium are now available to improve wound healing. There is a general lack of published data to show how much ‘active’ metal ion
is absorbed percutaneously from these preparations, and we can only speculate on how much additional ion is necessary to advance the wound healing process. Much attention has centred upon the value of supplementary zinc in wound healing; zinc is a constituent of more than 70 enzyme systems, many of which are involved in wound healing (Lansdown, 1993, 1996). However, whereas zinicated creams can be beneficial under some circumstances, excessive zinc may retard healing (Lansdown & Sampson, unpublished studies). This is attributed to the fact that zinc, copper and calcium interact at binding sites on carrier proteins. Excess of one ion is liable to inhibit essential processes modulated by others (Klevay, 1975; Heng et al. 1993). It follows that any xenobiotic ion absorbed into the skin that impairs the availability of zinc or other trace metals, is a potential cause of delayed or nonhealing of wounds (Lansdown, 1996).

Recent studies have demonstrated that concentrations of trace metals in normal skin are specific for the region of the body, its keratinisation patterns and contact with the environment (Lansdown, 1985, 1995; Lansdown & Sampson, 1997). Zinc, calcium and magnesium concentrations are highest in areas of pressure keratinisation and parakeratosis, which are normally characterised by high epidermal cell turnover. In the healing wound, the demand for trace metals, mainly in the form of metalloenzyme complexes, can be expected to vary according to the sequential events of haemostasis, inflammation/ granulation tissue formation, cell proliferation and normalisation of the wound site (Lansdown, 1995). Whereas demands for calcium will be high during haemostasis and periods of keratinocyte proliferation and maturation (Menon et al. 1985), zinc metalloenzymes will feature in matrix metalloproteases, RNA and DNA polymerases and enzymes involved in protein synthesis, mitosis, collagenesis and tissue remodelling (Halstead et al. 1974). Copper exhibits a ubiquitous role in cellular metabolism in the skin, but is notable as a component in lysyl oxidase necessary in elastic and collagen fibre cross-linking (Chou et al. 1968).

Present studies were conducted with a view to identifying sequential changes in the mineral and trace metal content of incisional wounds in the rat model, and correlating these with cellular events characterising the major events of the wound healing cascade (Mast, 1992). The rat has been used for many years to study wound healing profiles in the skin and has proved a valuable model in which to evaluate the influence of intrinsic and environmental influences on repair processes (Lansdown & Pate, 1993). In this work, we sought to define our experimental model for use in the evaluation of dietary constituents, topical preparations or medicated dressings which are potentially able to alter trace metal ion uptake and metabolism in the wound environment. From earlier studies, we know that silver can interact with zinc to advance experimental wound healing in the rat (Lansdown et al. 1997). We are unclear at this stage, whether the effect is achieved through applying silver nitrate or silver sulphadiazine throughout the wound healing period, or only during phases of high zinc metalloenzyme activity. In another situation, the xenobiotic metal cadmium was shown to induce a marked increase in ‘bound’ zinc in intact skin (Lansdown & Sampson, 1996). In wounded skin, this would be expected to seriously impair wound healing through inhibiting zinc metalloenzymes involved in matrix degradation, cell proliferation and connective tissue formation.

**MATERIAL AND METHODS**

**Animals**

Adult male Sprague-Dawley rats of the CFY strain (180–200 g body weight) were used in all sections of this study. They were bred under barrier-maintained specified pathogen free conditions at the Charing Cross Campus of Imperial College School of Medicine. They were housed under conditions of 22 ± 2°C, 45–55% relative humidity, and 12 h d/night cycles as specified in the Animals [Scientific Procedures] Act, 1986. The animals were housed in groups of 5 in plastic solid bottomed cages and provided with sterile dust free sawdust as bedding. Pelleted small rodent diet (CRM, Special Diet Services, Witham, Essex)

Fig. 1. Metallothionein distribution in the basal layer of the epidermis of the back skin of a young adult Sprague-Dawley rat. Immunocytochemistry using antimallothionein antibody (DAKO, Copenhagen). ×150.

Fig. 2. Calmodulin distribution in the outer layers of the epidermis of the back skin of a young adult Sprague-Dawley rat. Immunocytochemistry using anticalmodulin polyclonal antibody (Santa Cruz Biotechnology Inc, CA.) ×150.

Fig. 3. Incisional wound site in the rat after 24 h showing the inflammatory cell infiltrate at the wound margin and debris in the wound core. Note lack of inflammation in adjacent tissue. H & E. ×75.

Fig. 4. Narrow tongue of epidermal cells migrating between the acute inflammatory cells and wound debris at deeper aspects of the 48 h incisional wound. H & E. ×150.
Fig. 1. For legend see opposite.
and tap water was provided ad libitum. The mineral content of this diet includes copper (19 mg/kg), zinc (65 mg/kg), magnesium (0.22%), and calcium (0.80%) (Special Diet Services, Information Service, 2.11.98). Other metals found in this diet either as trace metals or as contaminants are not present in sufficiently high levels to significantly influence the metabolism of zinc, calcium, copper or magnesium in wound repair (Lansdown, 1995).

**Experimental details**

Incisional full thickness skin wounds (15 mm long) were made surgically with a scalpel in the closely shaved middorsal skin of fully anaesthetised rats. A single middorsal wound was made in each animal. Skin sites were cleansed with 70% ethyl alcohol and animals anaesthetised with a single intraperitoneal injection of Hypnorm (fentanyl/fluanisone) (Janssen Animal Health) and midazolam combination anaesthetic (0.27 ml/100 g body weight). This provided anaesthesia for > 30 min with good muscle relaxation (Flecknall, 1987). Wounds were sutured at 3 equidistant sites using polyamide monofibre (Ethicon, Edinburgh, Scotland). To standardise wounding, all procedures were conducted during the period 09.00–10.30 h.

Groups of 10 rats received wounds as above and were euthanised by carbon dioxide asphyxiation after 1, 2, 3, 5, 7 and 10 d. Earlier studies have demonstrated that rat wounds heal well within 10 d (Lansdown & Pate, 1993). At autopsy, wound sites were excised, with normal tissue to within 1.5 mm of the incision line and samples taken for trace metal analysis, histology and immunocytochemistry. Wounds from 5 animals for each time period, were cryopreserved at −10.30 h.

In the sectioned at 6 µm and immunocytochemistry performed (Rowe et al. 1997) using Vectastain ABC Elite reagents (Vector Laboratories, Burlingham, CA, USA) according to the supplier’s protocol. Rat-adsorbed biotinylated antimouse IgG (Vector Laboratories) was used for the detection of IgG primary antibodies. The chromagen employed was 3,3’-diaminobenzene (Vector Laboratories) with Harris’s haematoxylin counterstain. Monoclonal mouse antimetallothionein antibody was kindly donated by DAKO Laboratories (Copenhagen, Denmark). It was supplied in liquid form in 0.05 m Tris HCl, 15 mm NaN3, pH 7.5, 1% bovine serum albumin (BSA). DAKO-metallothionein antibody is inhibited specifically and equally well by glutaraldehyde polymerised human, horse, sheep or rat metallothioneins (MT-I and MT-II) suggesting that its is directed against a single and highly conserved epitope (DAKO Information Sheet). It was diluted 1:150. Anticalmodulin goat polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) was diluted 1:100. Negative control sections were incubated with no primary antibody.

**Metal analysis**

Tissue samples were dried to a constant weight at 120 °C and then dissolved in concentrated nitric acid (Aristar Grade, BDH, Poole, Dorset, UK) by heating overnight at 90 °C. The resulting solutions were diluted to an appropriate volume using ultrapure water. Appropriate reference standards were analysed at the same time (see Lansdown & Sampson, 1997). Calcium, magnesium and zinc concentrations were measured by flame atomic spectrophotometry (Unicam 939, ATI Unicam) after dilution with lanthanum chloride (for calcium and magnesium) or 6% butanol-ol (for zinc). Copper was measured by electrothermal atomisation flame atomic spectrophotometry (Unicam 939 with GF90 electrothermal atomiser).

**RESULTS**

The skin on the back of an adult rat is densely haired and is devoid of sweat glands, although sebaceous glands are prominent in association with the numerous hair follicles. The epidermis is 4–6 cells thick and comprises characteristic layers of basal, spinous,
granular and keratinised cells. Under normal conditions, basal cells express cytoplasmic metallothionein and low levels of calmodulin (Figs 1, 2). Minimal amounts of metallothionein are present in more superficial layers of the epidermis, but calmodulin is more noticeable in maturing keratinocytes. The dermis comprises collagen-rich connective tissue interspersed with a modest vascular supply and nervous tissue. Under normal conditions this tissue displays low levels calmodulin and metallothionein. Macrophages, fibroblasts and the panicus carnosus muscle (PCM) in the deeper aspects of the zona reticularis exhibit immunoreactivity for both proteins.

**Healing profiles in the skin wound**

**Haemostatic and early post wound period (0–12 h)**

All animals recovered well from anesthesia and exhibited no signs of ill health at any time. No fatalities occurred. Following surgery, there was a minimal haemostatic phase (< 30 min). Wounds dried quickly and showed no appreciable adverse response to the suture material used. Histological examination of 12 h wounds showed local oedema in the region of the incision line but minimal evidence of inflammatory cell infiltration in marginal areas. The epidermis at the wound margin was necrotic. Aggregations of red blood cells were evident at all levels from the surface of the skin to the hypodermis.

**Inflammatory and proliferative phase (1–7 d)**

Crusting and scab formation occurred along the wound suture line within 24 h and this persisted for approximately 5 d. After this time, we noted a progressive loss of sutures and prominent hair growth. Where sutures had been lost, insertion marks remained.

By 24 h the wounds exhibited a prominent central core of cell debris with haemolysed erythrocytes extending through the epidermis and dermis into the hypodermis. In the dermis, this mass was separated from mildly oedematous but otherwise histologically normal tissue by a zone of acute inflammatory cells (mainly neutrophils). Initially, this infiltrate was more pronounced in the reticular zone than in the papillary area (Fig. 3). The epidermis at the wound margin consisted of a cap of fragmented and necrotic tissue adjacent to histologically normal keratinocytes. Mitotic activity was not noted at this stage in histologically normal-looking keratinocytes adjacent to the wound margin. In the deeper reticular dermis, the severed PCM was associated with infiltrates of neutrophils, monocytes and a few eosinophils. At this stage, epidermal concentrations of calmodulin near the wound margin had increased appreciably and were present in basal cells, which in intact skin are usually low in this protein.

Metallothionein levels in the epidermis and dermal fibroblasts near the wound margin after 24 h exhibited a marginal increase.

After 48 h, there was a pronounced increase in the dermal inflammatory cell infiltrate in the wound margin. Importantly, by this stage mitotic activity was present in the epidermis at the wound margin and tongues of epithelial cells had begun to migrate downward with the effect of separating the central core of tissue debris and inflammatory cells from the ‘peripheral’ zone of histologically normal tissue (Fig. 4). There was minimal evidence of an epidermal basement membrane in the early stages of re-epithelialisation. A diffuse inflammatory cell infiltrate and some oedema persisted in the dermis near the wound margin, this being more obvious in the deeper reticular region, where vascular dilation was noted. By 48 h postwounding, dermal inflammatory cell infiltrates included monocytes, some fibroblasts and macrophages. Minimal evidence of collagenesis was seen until after 72 h when the fibroblast population of the wound site had markedly increased both in the reticular and papillary regions. We noted a follicular cell hyperplasia in the region of the wound, such that follicles cut during surgery were contributing to the re-epithelialisation process.

The re-epithelialisation process continued until approximately 5 d after surgery, when the wound site resembled a crater lined by epithelial cells and containing a central core of wound debris and inflammatory cells. In deeper aspects of the crater the epidermis was not well defined and lacked a clear basement membrane (Fig. 5). A few chronic inflammatory cells were present. Except in these deeper layers, keratohyalin granules were prominent in the reformed epidermis and in places extended over up to 60% of the epidermal thickness. In the 5 d wound, the reformed epidermis was up to 3 times the normal thickness and mitoses were numerous in the basal layer, both in the new epidermis and in adjacent areas. The stratum corneum was identifiable as a thin strand in the early re-epithelialisation process but by 5 d extended across the wound site. Some parakeratosis was present in the deeper regions of the wound crater.

At this time, the re-epithelialised epidermis showed minimal evidence of the columnar epidermal cell organisation although more superficial cells were flattened parallel with the skin surface.
Fig. 5. Reepithelialised incisional skin wound in the rat after 5 d. Base of the crater to demonstrate the residual inflammatory cell infiltrate (mainly lymphocytes), fibroblasts and early fibrogenesis. Note the lack of basement membrane at the epidermal-dermal interface. Masson’s trichrome method. x150.

Fig. 6. Metallothionein distribution in the epidermis and papillary dermis of the incisional skin wound after 24 h. × 150.
Fig. 9. Rat incisional wound site after 10 d demonstrating the fibrous scar tissue, minimal inflammatory cell infiltration and modestly hyperplastic epidermis. H & E. × 75.
Fig. 10. Increased metallothionein concentrations in macrophages in the hypodermis under an incisional wound site in the rat 10 d following surgery. Immunocytochemistry using DAKO antibody. × 75.

The dermal inflammatory cell population after 5 d, was predominantly of fibroblasts and lymphocytes with macrophages, eosinophils and a few mast cells present at deeper layers and in the region of the PCM. Collagenesis was active beneath the wound and in the papillary epidermis adjacent to the wound site. Granulation tissue with prominent vascular dilatation was notable, particularly in the deep dermis and hypodermis.

The inflammatory and proliferative phases of wound healing were marked by an increase in metallothionein in epidermal cells at the wound margin and in regenerating tissue (Fig. 6). Interestingly, we noted a pronounced increase in metallothionein in fibroblasts and macrophages of the papillary epidermis in wounds examined up to 5 d.

Calmodulin in contrast was essentially epidermal in distribution. It increased markedly over the first 4 d of re-epithelialisation in the tongues of migrating keratinocytes (Fig. 7). Increased epidermal calmodulin persisted in the re-epithelialised epidermis well into the normalisation phase.

Normalisation phase (7–10 + d)

Healing in the rat wound from 5 d was associated with a pronounced decline in the inflammatory cell infiltrate in and near the wound margin, and a progressive reduction in the crater formation with loss of the wound debris and a realignment of epidermal cells. Thus after 7 d, the wound site was characterised...
Fig. 11. Sequential changes in zinc, calcium, copper and magnesium in the healing wound site.

by a pit-like configuration and lined by a hyperplastic epidermis with a prominent keratohyalin granulation and hyperkeratinisation (Fig. 8). We noted a pronounced formation of rete pegs at the epidermal–dermal interface of the new epidermis, which was up to 3 times the normal thickness. There was no evidence of repair in the PCM but collagen deposition/scar tissue formation was evident at this site. After 10 d, the normalisation process in the rat wound was well advanced (Fig. 9), but the epidermis was still marginally thicker than normal and cellular realignment incomplete. Epidermal mitoses were appreciably less obvious than during the 3–5 d period but some follicular cell hyperplasia persisted. Dermal cellularity was greatly reduced, and blood vessels appreciably less obvious than at earlier stages. Scar tissue was prominent.

From 5 d after wounding, metallothionein levels declined in the epidermis, papillary dermis and follicular epithelium to normal levels. A slightly increased level of immunoreactivity was seen in the region of the PCM and in macrophages in the hypodermis (Fig. 10). Calmodulin concentrations were appreciably higher than normal in the hyperplastic epidermis in maturing keratinocytes but not in most basal cells.

Trace metal analyses

The mean concentrations of zinc, copper, calcium and magnesium in uninjured shaved back skin of the young adult Sprague Dawley rat are illustrated in Figure 11. In the skin wound site, we noted a progressive increase in zinc, calcium and magnesium up 5 d postwounding and then a decline to normal values by 7 d. Zinc concentrations were marginally higher than normal 10 d after wounding. Copper concentrations were very low at all stages in the wound healing profile, but did show a marginal increase in the first 2 d.

Because certain metals compete for binding sites on carrier proteins, it was of interest to examine changes in the ratio of metal concentrations in the period of study (Fig. 12). Thus, there was a progressive decline in the zinc:calcium ratio over first 5 d after wounding. The zinc:calcium ratio had normalised by 7 d but had increased further by 10 d. In contrast, the relative concentration of calcium to magnesium increased to 5 d after wounding but had declined to near normal levels by 7 d. Although copper levels were low for the wound healing period studied, the ratio of zinc to
copper appeared to decline in the early postwound period (~ 1–2 d). They were increased at later stages.

**Discussion**

Wound healing in acute skin wounds is a well defined sequence of events involving cell–cell contact and cell–macromolecular interactions leading to haemostasis, inflammation (granulation tissue formation), fibroplasia, newvascularisation, contraction, and re-epithelialisation of the wound site (Cai et al. 1991). Repair processes and their associated biology have traditionally involved the use of in vitro systems and laboratory animal models which allow the constituent processes to be studied under controlled conditions with the avoidance of such variables as genetic configuration, nutrition and environmental factors (Mulder, 1991). With new technology and the availability of specific antisera, it now possible to investigate more closely the role of cytokines, growth factors and other modulators in cellular morphogenesis, extracellular matrix protein synthesis, and membrane receptor activity (Mast, 1992; Nickoloff et al. 1991; Kilcullen et al. 1998). Although the participation of metalloenzymes is appreciated, the present work is the only evaluation to our knowledge, where sequential changes in trace metal concentrations have been correlated with cytological events in the healing cascade. We assume here that largest concentrations have been correlated with cytological events between this phase and the period of epithelial cell proliferation (Mulder, 1991) achieves haemostasis. Calcium performs a key role in this haemostatic process, probably as a primary catalyst in platelet aggregation and in the production of clotting factors VIII, IX and X (Born & Cross, 1964; Blair et al. 1990). Calcium is also necessary in neutrophil activation and epidermal cell proliferation at slightly later stages. In the rat wound model, haemostasis is accomplished within 30 min but after 12 h minimal evidence is seen histologically of impending repair mechanisms. Nevertheless, the importance of calcium at these early stages in wound healing is recognised by the 100% increase in local concentrations of the metal in the 24 h after wounding. The importance of calmodulin as a major calcium binding protein in keratinocyte maturation is demonstrated in this study by heavy deposits in the regenerating epidermis at and near the wound margin.

Inflammation and granulation tissue formation are promoted largely by secretions released from activated platelets (Wahl & Wahl, 1992). In the rat, this phase has commenced by 24 h after wounding and persists until at least 5 d. There is considerable overlap between this phase and the period of epithelial cell proliferation and fibrogenesis, which also seems to peak at about 5 d postwounding. By this time, local concentrations of calcium, zinc and magnesium have increased greatly over prewounding levels. Although zinc metalloenzymes play a major role in immune and inflammatory responses and are essential in tissues subject to proliferation and metabolic activity (Landsdown, 1996), our analyses show that demands for calcium are notably higher at all stages in the wound healing profile. Whereas calcium is required for the activation of many intercellular reactions, zinc tends to act as an inhibitor (Chvapil et al. 1975; Brewer et al. 1979). Zinc is now known to regulate calcium uptake and its contribution to the calmodulin-cAMP pathway. Zinc injected intradermally has been shown to reduce calmodulin and calcium concentrations in the skin, and raise cAMP levels (Heng et al. 1993). We can appreciate that the critical ratio of zinc:calcium will change as the inflammation/granulation tissue formation progresses, and proliferative activity gathers momentum in the epidermis at the wound margin. Thus the zinc:calcium ratio declined from the time of wounding until approximately 5 d. Thereafter,

---

**Table. Trace metal concentrations in the middorsal skin of a young adult Sprague–Dawley rat**

<table>
<thead>
<tr>
<th>Trace Metal</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zinc</td>
<td>63.94 ± 11.73 µg/g dry tissue weight</td>
</tr>
<tr>
<td>Copper</td>
<td>5.13 ± 1.06 µg/g</td>
</tr>
<tr>
<td>Calcium</td>
<td>0.59 ± 0.01 mg/g</td>
</tr>
<tr>
<td>Magnesium</td>
<td>0.46 ± 0.03 mg/g</td>
</tr>
</tbody>
</table>

---
metal concentrations and ratios of zinc:calcium tended to return to prewounding levels by 7 d.

The final phase in the wound healing cascade is characterised by a marked decline in the cellularity of the wound site with reduction in the inflammatory cell infiltrate and granulation tissue stage, and consolidation of the fibrous scar tissue. The re-epithelialisation process is complete and the collagen IV-rich basement membrane reformed. Once this is achieved, the epidermal ‘normalisation’ process involves a realignment of postmitotic cells from a lateral or semilateral migration pathway (as seen in the re-epithelialisation process) to the vertical movement (Pinkus, 1970). In the rat, this occurs over the 7–10 d postwounding period. But, even after 10 d the wound exhibited an epidermis 2 to 3 times thicker than normal. By 10 d, we saw a fractionally higher zinc concentration and increased zinc:calcium ratio. It is conceivable that in this situation, zinc ions are involved in suppressing calcium motivated epidermal cell proliferation and maturation (Hennings et al. 1980; Yuspa et al. 1989).

The modulation of trace metal ions in normal mammalian systems is not well documented but is likely to involve carrier proteins, growth factors and cytokines (Hembry et al. 1985; Winge & Nielson, 1985). In the present study, we have examined only calmodulin and metallothionein as markers. Metallothionein occurs as 2 major isoforms, MT-I and MT-II, which may be expressed in a tissue-dependant manner (Pauwels et al. 1994; Yang et al. 1994). There are also 2 other isoforms, MT-III which occurs primarily in neural tissue (Palmiter et al. 1992), and MT-IV which has been identified in squamous epithelial tissue (Quaife et al. 1994). The antibody used in the present study is known to react with MT-I and MT-II (DAKO, Copenhagen), but it is unclear if it reacts with MT-IV also. Although we noted that calmodulin was present in most tissues, it was highest in differentiating keratinocytes reflecting the importance of calcium in this process. Metallothioneins on the other hand, related well to zinc and possibly copper involvement in dermal fibrogenesis and scar tissue formation. Thus we saw increases in metallothionein reaction product in the papillary dermis at about the time at a time of early inflammatory cell infiltration and fibrogenesis (collagenesis) with a decline at later stages in wound healing. These changes are consistent with the demand for zinc dependant RNA and DNA-polymerases in essential repair processes (Lindeman & Mills, 1980). Metallothioneins are induced by and bind copper, such that changes in their distribution are likely to reflect areas of increased copper metabolism and the requirement for copper in lysyl oxidase in collagenesis and elastic tissue formation (Chou et al. 1968; Madden & Peacock, 1968).

Therapeutically, advantage is taken of the need for additional calcium in wound healing in the development of calcium alginate products (Jarvis et al. 1987; Blair et al. 1990; Lansdown & Payne, 1994). Thus, when calcium alginate fibres contact the blood, calcium ions exchange for sodium in the wound exudate and are released into the wound environment to be available for haemostatic and other calcium dependant processes. Zinc-containing products have been developed variously for treating diaper rash and other minor skin lesions (Lansdown, 1993, 1996). Experimental studies have demonstrated that high concentrations of zinc applied to the skin in an amphiphilic cream to aid absorption, can be detrimental in wound healing, presumably because the excess zinc absorbed is inhibiting calcium dependant pathways (Lansdown & Sampson, unpublished). Although copper may have a role in stabilising epidermal cells and as Solcoderm is claimed to be successful in treating epidermal cysts (Ronnan et al. 1993), excess copper is contraindicated in wound healing (Barranco, 1972; Sucvi et al. 1981; Fisher, 1986). Our studies show that demands for copper are particularly low in normal wound healing in the rat, suggesting that excess concentrations are likely to prove toxic through upsetting critical balances with other divalent trace metal ions.

The skin in the rat is overtly different from human skin but the constituent tissues are of a similar origin. They will undergo comparable morphogenetic patterns and be subject to the influence of similar chemotactic systems, growth regulators, cytokines, and nutritional factors. In the presence of injury, we can expect cellular responses and homeostatic mechanisms to respond in a similar fashion in rats and humans, differences being more in the rate and magnitude of reaction, than of type. In mechanistic studies the rat wound model is presented as an initial screen in which to evaluate the potential value of a dietary or therapeutic means of advancing tissue repair.

Toxicologically, we can predict that any situation liable to impair the uptake or metabolism of trace metals, or alter the critical ratios of one trace metal to another will be detrimental in wound healing in the skin. This will include the application of medicated dressings and topical creams designed to deliver increased levels of zinc or calcium. More importantly, percutaneous absorption of environmental pollutants containing lead, cadmium or other xenobiotic min-
erals which are known to interact with zinc or other essential trace element are likely to be detrimental in wound healing (Lansdown, 1995). On the other hand, silver which is known to induce metallothionein and through preferential binding releases zinc, has been shown to advance wound healing in the skin. (Lansdown et al. 1997).

ACKNOWLEDGEMENTS

We are grateful to Mr Obah Ojarikre for his assistance with statistics and preparation of the tables used in this paper.

REFERENCES


