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Effects of Epidermal Growth Factor Dosage Forms on Mice Full-thickness Skin Wound Zinc Levels and Relation to Wound Strength

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Abstract

Epidermal growth factor (EGF) and zinc promote re-epithelization and reparative tissue strength by enhancing deposition of collagen at the site of the wound. In this study two EGF dosage forms were chosen to assess the effect of zinc levels on wound healing and for comparison with wound tear strengths.

A solution of EGF in 0·9% w/v NaCl and an EGF gel in 0·2% Carbopol 940 polymer (5 μL) were applied to full-thickness skin wounds of mice twice a day for 7 and 15 days. Wound zinc levels were higher on day 7 than on day 15, especially in wounds treated with EGF. The wound zinc levels of the gel + EGF group on day 15 were similar to those of normal control skin.

These results imply that there is a close connection, but no direct relationship, between EGF application in both dosage forms and wound zinc levels during healing.

Wound healing is a complex and dynamic process, and there are many potential sites at which zinc might have a critical function. The early inflammatory response after wounding is followed by proliferation of endothelia, fibroblast and other cells. These cells synthesize a variety of proteins including collagen. The time-span of this process to final maturation of the collagen and the moulding of the repair scar is many weeks.

Zinc accumulates in experimental wounds. However, this accumulation occurred almost immediately and was maximum in the first few days after injury whereas the effect of zinc deficiency, as measured by changes in tensile strength, was not demonstrable until at least ten days after injury (Karcioglu & Sarper 1980). Zinc is required for the structure and activity of metalloenzymes; it participates in RNA and DNA synthesis and in the stabilization of nucleic acids, cell membranes and other cellular organelles (Karcioglu & Sarper 1980). These processes are also involved in wound healing.

It has been reported that epidermal growth factor (EGF) stimulates wound healing in the skin (Pessa et al 1987), cornea (Gönül et al 1992) and gastric mucosa (Poulsen 1993). Many cell types, including dermal fibroblasts, have EGF surface receptors and will, in cell culture, proliferate in response to EGF (Ksander 1989). In addition, it has been reported that EGF induces procollagenase synthesis (Cohen et al 1992). Mammalian collagenase belongs to a family of extracellular metalloproteinases capable of degrading connective tissue components. This family of enzymes is composed of collagenase and require zinc as a cofactor (Cohen et al 1992). Daily intraperitoneal injections of EGF (1 μg) for 10 days elevated serum zinc levels of mice with pressure sores (Gönül et al 1993).

Bioadhesion can be defined as the state in which two materials, at least one of which is biological in nature, are held together for an extended period of time by interfacial forces. Thus, attachment of one biological object to another, e.g. cell attachment, or of a synthetic polymer to a biological substrate, e.g. denture fixative, are examples of bioadhesion. For drug delivery purposes the term bioadhesion implies attachment of a drug carrier system to a specific biological location. The biological surface can be epithelial tissue (Robinson et al 1987). Poly(acrylic acid) was selected as the bioactive component of this system. Controlled-release sys-

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tems containing poly(acrylic acid) have low potential for skin irritation and sensitization at concentrations up to 100% (Industrial Biotest Laboratories 1973).

The aim of this study was to investigate possible interactions of EGF dosage forms during wound healing. Wound zinc levels were studied and compared with those measured during our preliminary investigation of wound strength (Celebi et al 1994).

Materials and Methods

Materials

Epidermal growth factor (EGF) was from Sigma (USA), betadine solution from Kansuk (Turkey), penicillin procaine from Deva (Istanbul, Turkey), physiological saline from Eczacibasi (Istanbul, Turkey) and Carbopol 940 from Biesterfiel (Hamburg, Germany).

Animals

The studies were performed on C57BL-6J-Ola mice of both sexes, $25 \pm 2{\cdot}8$ g. Animals were housed individually in wire cages, and food and tap water were freely available. Fifty-five mice were divided into five equal groups as shown in Table 1.

Dosage forms

The solution dosage form (100 ng mL^{-1}) was prepared in 0·9% NaCl (saline) and in a bioadhesive gel dosage form prepared from polyacrylic acid (Carbopol 940) gel as described previously (Celebi et al 1994).

Table 1. The effect of EGF dosage forms on full-thickness incision wound zinc levels ($\mu g\, g^{-1}$ dry weight) in mice.

Treatment	Day 7	Day 15
Untreated	145 ± 36	53 ± 7†,††
Physiological saline	166 ± 25	38 ± 13†,††
Gel	177 ± 12	43 ± 13†,††
Physiological saline + EGF	202 ± 53	155 ± 17*
Gel + EGF	234 ± 50*††	126 ± 18*†

The dermal tissue zinc level was $138 \pm 59\, \mu g\, g^{-1}$ dry weight. Saline treatment was with 0·9% NaCl solution (5 μL) twice a day for 15 days. Saline + EGF treatment was 100 ng EGF mL^{-1} saline (5 μL) twice a day for 15 days. Gel treatment was with Carbopol 940 (0·2%w/w, 5 μL) twice a day for 15 days. Gel + EGF was treatment with 100 ng EGF mL^{-1} gel (5 μL) twice a day for 15 days. *$P < 0{\cdot}05$, significant difference between results from untreated controls and from treated groups. †$P < 0{\cdot}05$, significant difference between results on the 7th and 15th days. ††$P < 0{\cdot}05$, significant difference between results from normal skin and wound tissue; $n = 5$ for each group.

Wound model

Full-thickness skin wounds were inflicted under diethyl ether anaesthesia after clipping the backs of the animals. The skin was scrubbed with iodine swab sticks. Two linear surgical wounds 1 cm long were produced on each mouse by cutting the skin downwards perpendicular to the lumbar spine (Dincer et al 1996). The wounds were closed immediately with 5/0 atraumatic silk sutures at mid-incision. After the operation each animal received a single intraperitoneal dose of 400 int. units penicillin procaine. The sutures were removed on day 7 and the two EGF forms and the vehicles (5 μL) were applied on both sides of the wounds of each animal, twice a day for 7 and 15 days. Five wounded but untreated animals were used as controls on days 7 and 15.

Animals were killed with an overdose of anaesthetic on days 7 and 15 after the operation. Tissue specimens from the right wounds were removed, weighed, rinsed in saline, blotted dry and frozen at −40 °C for zinc content. Specimens from the left side were used to test wound tear strength.

Measurement of wound zinc levels

Wound zinc levels were measured by atomic absorption spectrophotometry (AAS) at 213·9 nm using a Perkin-Elmer Model 3030 spectrophotometer. All stages of tissue preparation and mineral analysis were performed in glassware acid-washed in nitric acid and extensively rinsed with deionized water. Wound tissue (100 mg) was dried overnight at 100 °C, weighed and digested in 1·0 mL ultrapure HNO_3–H_2SO_4 (3 : 1) for 2 h at 80 °C. Before zinc analysis by AAS the samples were diluted 1 : 10 with deionized water (Etzel et al 1988).

Statistics

All data are expressed as means ± s.d. The significance of the difference between results from different treatments was assessed by one-way analysis of variance; differences between results from treatment groups and control were analysed by the Mann–Whitney U-test. $P < 0{\cdot}05$ was considered to be significant.

Results

The level of zinc in normal skin tissue was $138 \pm 59\, \mu g\, g^{-1}$ dry weight. Zinc levels in untreated wounds were lower on day 15 ($53 \pm 7\, \mu g\, g^{-1}$) than day 7 ($145 \pm 36\, \mu g\, g^{-1}$). The differences between wound zinc levels on these two days were statistically significant in untreated groups and in groups treated with physiological saline, gel and EGF + gel. Treatment of the incision

wounds with gel + EGF increased wound zinc levels significantly ($P < 0.05$) on day 7 compared with normal skin and control wounds. There was also an increase in the level of zinc in wound tissue of groups treated with vehicle and with EGF in saline solution, but this difference was not statistically significant. The zinc levels in wound tissue of vehicle-treated and untreated groups were significantly lower on day 15 than on the day 7. On day 15 the levels of zinc in the wounds of the EGF-treated groups were higher than in untreated wounds ($P < 0.05$) but they were similar to zinc levels in normal dermal tissue.

Table 2. Effects of EGF dosage-forms on wound tear-strength (Celebi et al 1994).

	Wound tear strength ($N\,cm^{-1}$)	
	Day 7	Day 15
Untreated (n = 5)	0·75 ± 0·25	1·4 ± 0·5
Physiological saline (n = 5)	0·75 ± 0·5	1·4 ± 0·5
Gel (n = 5)	0·5 ± 0·25	1·2 ± 0·25
Physiological saline + EGF (n = 15)	1·25 ± 0·2	1·9 ± 0·2
Gel + EGF (n = 15)	1·4 ± 0·2	3·95 ± 0·75*

* $P < 0.001$, significant difference between results from control and treated groups.

Discussion

The effect of EGF on epidermal cells in-vivo, the primary mechanism of enhanced wound healing induced by EGF, is most probably a result of increased proliferation of epidermal cells (Brown et al 1986). Local daily application of EGF solution enhanced epithelization and accumulation of granulation tissue cells, collagen and glycosaminoglycans in experimental wounds (Laato et al 1986; Schultz et al 1987). Topical administration of EGF accelerates epidermal regeneration of partial thickness or split-thickness burns in-vivo (Schultz et al 1987). Most recently attention has been focused on endogenous substances such as EGF and collagen (Mian et al 1992). The complexity of the factors involved adds uncertainty to the choice of appropriate parameters such as re-epithelization, angiogenesis, wound contraction, wound strengths, and extent of inflammation. Previous studies which failed to show acceleration of healing of partial-thickness epidermal wounds by EGF (Brown et al 1986) might have used conditions that did not provide sufficient continuous exposure of residual epithelial cells to EGF.

It is generally recognized that the in-situ tensile strength of the healing wound reflects the rate of repair (Kahlson et al 1960; Kilic et al 1994). The wound tensile strength must be greater when the healing process is complete. The effect of EGF dosage forms on wound tear strength has been reported elsewhere (Celebi et al 1994). Wound tensile strength in mice was significantly ($P < 0.05$) higher on day 15 of treatment in the group treated with EGF + gel (Table 2).

The results are indicative of similar wound healing patterns in the first seven days in terms of increased tensile strength and control of wound zinc-level after application of EGF in bioadhesive polymer.

Sustained release of EGF from the bioadhesive polymer dosage form during seven days supported the accumulation of zinc in the wound tissue. As a result DNA and protein synthesis, mitosis and cell proliferation in wound tissue are affected by the level of zinc. Collagen synthesis is one of the most important processes in wound repair. EGF and zinc have been reported to increase re-epithelization and reparative tissue strength by inducing enhanced deposition of collagen at the site of the wound (Cohen et al 1992; Babül et al 1996). The accumulation of zinc in wounds is at its highest in the first 2–3 days after injury; the zinc accumulates over several days in excess of that in adjacent, uninjured tissue and then slowly returns to levels similar to those in uninjured tissue after approximately two weeks (Karcioglu & Sarper 1980).

Zinc is essential for the structure and activity of more than 100 metalloenzymes; it participates in RNA and DNA synthesis and in the stabilization of nucleic acids, cell membranes and other cellular organelles (Karcioglu & Sarper 1980). Excess zinc can interfere with copper metabolism and consequently with wound healing because of the special role of copper as a constituent of lysyl oxidase. The function of hydroxylation of lysyl residues is to provide substrates for glycosylation and for the formation of certain covalent intermolecular cross-links, an important determinant of collagen fibril tensile strength (Cohen et al 1992). Collagenases require zinc as a cofactor. Mammalian collagenase belongs to a family of extracellular metalloproteinases that are capable of degrading connective tissue components (Cohen et al 1992). Therefore wound zinc levels have an important role in both collagen cross-linking and degradation.

On the basis of these results and those from our preliminary study it can be speculated that in contrast with the similar zinc levels of the wounds, the effect of EGF on wound tear strength is time- and dosage form-dependent.

From the results from this study we can conclude that although there is a close connection between EGF application, in both dosage forms, and zinc levels of wounds during healing, there is no direct relationship between skin wound zinc level and application to dermal wounds of EGF either in solution or as bioadhesive gel.

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