SUMMARY
Zinc oxide is a common ingredient in bandages for wound management. Experimental studies have demonstrated beneficial effects of topical zinc oxide on the restoration of epithelium during wound repair by as yet unknown mechanisms of action. In this study, zinc oxide was found to up-regulate one growth factor (insulin-like growth factor-1) and matrix metalloproteinase activity several fold in standardised porcine wounds. These findings indicate that zinc oxide promotes epithelialisation by enhancing endogenous growth factors and enzymes important for epithelial proliferation and migration.

INTRODUCTION
Zinc is an essential trace element for development and growth. More than 300 enzymes are dependent on zinc for activity such as matrix metalloproteinases; (MMPs), and DNA and RNA polymerases.1-2. Zinc fingers belong to an even larger group of zinc-containing proteins.1-3 These zinc protrusions are found predominately in transcription factors that interact with the promoter region of DNA before a segment is transcribed into RNA coding for growth factors.3 The crucial role of zinc in these biological and biochemical systems can explain the retarded wound repair response seen in zinc deficient patients and normalization of the wound healing mechanisms with zinc therapy.4 However, there is limited evidence for using zinc enterally unless the patient is truly zinc deficient.5 Zinc is more commonly used as zinc oxide in various topical preparations to treat skin lesions.5 In contrast to zinc given orally, zinc administered topically appears to be beneficial regardless of zinc status.6 The increased demand for zinc during wound repair is satisfied for prolonged periods by zinc oxide administered to the wound site.6,7 When applied locally, zinc oxide is solubilized slowly and supraphysiological concentrations of ionic zinc (an elevation of about 4-5 times) are achieved at the wound site over an extended period.8 We have demonstrated a stimulatory action of zinc oxide on the healing of leg ulcers compared with placebo in a double-blind, randomized controlled clinical trial.9 Beneficial healing effects of topical zinc oxide have also been confirmed repeatedly in skin wounds of various depths in zinc-sufficient pigs. Specifically, zinc oxide accelerates re-epithelialisation by yet unknown mechanisms.6 The polypeptide growth factor IGF-I is crucial for epidermis homeostasis and the zinc-dependent MMPs are required for optimal epithelial migration.10-12 Our aims were to examine the effect of zinc oxide on endogenous IGF-I levels and MMP activation in wounds in domestic pigs on zinc-sufficient diets.

MATERIALS AND METHODS
Animal wound model and treatments
Twelve full-thickness cutaneous wounds (4.4 cm x 2.2 cm, 4-6 mm deep) were made on anaesthesised domestic pigs. On each pig the six wounds on the right side were treated with zinc oxide and the six wounds on the left side with the control dressing. Zinc oxide (0.3 mg zinc/cm² or 17 mg zinc/g) was bound to a 100% cotton gauze dressing with polyvinylpyrrolidone.5 The control dressings contained polyvinylpyrrolidone only. The dressings were covered with separate adhesive polyurethane film dressings (Tegaderm, 3M, St. Paul, MN, USA). Sterile saline (1.5 ml) was injected into the dressings with a needle on a syringe. The dressings were changed every two to three days. The investigator was unaware of which group the samples came from when measuring wound areas and IGF-I mRNA.

WOUND AREA MEASUREMENTS
The outline of the epithelial front was drawn on a sterile plastic sheet and the non-epithelialised area measured by planimetry.

IGF mRNA assay
Granulation tissue for analysis of IGF-I mRNA concentration was obtained with a 6-mm punch biopsy from the centre of the wounds (Figure 1). The extraction of nucleic acids and hybridisation RNAase protection/solution reactions were carried out as described earlier by Tarnow et al.10

Activation of endogenous MMPs
An assay that utilises the present collagen in wound tissue as substrate for the endogenous MMPs was applied.13 Briefly, homogenates of control-treated wound tissue were incubated ex vivo with zinc oxide (4 mg/ml) or without, and the amount of collagen degradation products released into the incubation medium measured indirectly as hydroxyproline.

Statistical analyses
Student's t-test was applied to the data, given as mean sem. p < 0.05 was chosen as level significance.

Zinc Oxide
Augments endogenous expression of insulin-like growth factor-I (IGF-I) and activates matrix metalloproteinases (MMPs) in wounds

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RESULTS

Wound healing

The changes in wound area for zinc-treated and vehicle-treated wounds are depicted in Figure 2. A beneficial effect on wound closure of topical zinc oxide became apparent after the wound cavities were filled with granulation tissue to the level of surrounding normal skin on day 7 enabling epithelialisation from wound edges. The non-epithelialized area of the wounds, expressed as percentage of initial wound area, treated with zinc oxide (22.7 ± 1.3 %, mean ± sem) was significantly (p < 0.01) smaller than control-treated wounds (42.9 ± 5.0 %) day 11 (Figure 2).

IGF-I mRNA levels

Elevated levels (p < 0.05) of IGF-I mRNA were found in zinc oxide-treated (1.7 ± 0.2 amol/µmol DNA) compared with control-treated (1.0 ± 0.1 amol/µmol DNA) wounds on day 4 but no significant differences between the two groups were found from day 4 onwards.

Activation of MMPs

Zinc oxide added to wound tissue homogenates increased (P < 0.05) the activity of both active and latent MMPs about 5-fold compared with control-treated wound tissue homogenates (Figure 3).

DISCUSSION

In the present experimental study, locally applied zinc oxide promoted epithelialisation of standardized full-thickness skin wounds confirming previous results in a partial-thickness wound model in pigs. Apart from zinc's moderate anti-bacterial, anti-inflammatory and cytoprotective activities, our biochemical and cellular findings can possibly explain zinc's mechanisms of action on epithelialisation of cutaneous wounds. Zinc oxide activated endogenous zinc-dependent matrix metalloproteinases, which may facilitate keratinocyte migration. Furthermore, zinc oxide augmented endogenous expression of one growth factor insulin-like growth factor-I (IGF-I) in granulation tissue. Recent in vitro work, where zinc enhanced epithelial migration due to up-regulation of IGF-I specifically in fibroblasts, supports the hypothesis that zinc promotes wound healing by increasing endogenous growth factors. In addition, other cell culture studies showed synergistic effects of zinc and IGF-I in NIH 3T3 fibroblasts. Our study indicates that apart from being an essential trace element, zinc exerts beneficial pharmacological actions on wound healing when applied locally as zinc oxide. Oral zinc merely corrects a nutritional deficit. Increased endogenous expression of IGF-I and activation of MMPs may explain the stimulatory action of zinc oxide on resurfacing of wounds.

Topical zinc also appeared to promote healing of small and acute skin wounds in humans. Work in our laboratory is in progress to elucidate the action of topical zinc oxide on acute wounds healing by secondary intention in humans.
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