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Effects of epidermal growth factor dosage forms on dermal wound strength in mice

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Abstract—The effect of topically administered epidermal growth factor (EGF) dosage forms was investigated on skin wound healing in mice. Two EGF dosage forms were prepared containing 100 ng mL^{-1} EGF. The solution dosage form was prepared in 0·9% w/v NaCl. A bioadhesive gel form was prepared in 0·2% Carbopol 940 polymer. The two dosage forms were applied on the skin incision wounds of mice at the rate of 5 μL twice a day for 7 and 15 days. The wound tear strength was tested for skin wound healing at the 7th and 15th days of treatment and compared with controls. The results indicate that the wound tear strength of mice were significantly higher at the 15th day of treatment in the gel-treated group compared with the solution-treated mice and controls ($P < 0·001$).

Epidermal growth factor (EGF) is a 53 amino acid mitogenic polypeptide present in many mammalian species, in a variety of tissues and body fluids. It is one of a number of growth factors being investigated for their potential to expedite the wound healing process (Dibiase & Rhodes 1991). In addition, EGF has been shown to stimulate keratinocyte division in-vitro and epidermal regeneration in-vivo (Brown et al 1989). It also has been shown to have an effect on mesenchymal cells by producing marked proliferation of the dermis in partial-thickness wounds and by increasing the tensile strength of surgical incision (Brown et al 1988).

The objective of the present study was to develop a dosage form which releases EGF slowly and to compare its effect on the skin wound healing with a solution dosage form.

Materials and methods

Materials. Epidermal growth factor (EGF, Sigma, USA), betadine solution (Kansuk, Turkey), penicillin procaine (Deva, Turkey), physiological saline (Eczacibaşi, Istanbul, Turkey) and Carbopol 940 (Biesterfield, Hamburg, Germany) were from the manufacturers indicated.

Methods. The solution dosage form (100 ng mL^{-1}) was prepared in 0·9% NaCl (saline).

The bioadhesive gel dosage form was prepared in a polyacrylic acid (Carbopol 940) gel (CP 940). Polyacrylic acid is a bioadhesive polymer and adheres to mucosa and tissues, exhibiting many advantages for topical therapy, including good flow and thickening properties, non-irritancy, and potential for a suitable release rate of the incorporated drug (Park & Robinson 1984; Hui & Robinson 1985).

Preparation of the aqueous solution of CP 940 (0·20%) was carried out as follows. A weighed amount of polymer was carefully added to the required amount of water while mixing with the aid of a mechanical stirrer until the polymer was completed dispersed. It was then stored at room temperature overnight. Sodium hydroxide solution (10%) was added dropwise to the dispersion when a light blue colour was obtained, at neutralization. The pH of the gel was adjusted to 6·8–7·0 and EGF (100 ng mL^{-1}) was added. These dosage forms were divided into portions and stored separately at $< 4°C$ for daily applications.

Adult male and female mice, 25 + 2·8 g, were studied in groups of 15. During the experiments, the animals received a normal diet and had full access to water and were housed individually in cages. Mice were anaesthetized with ether. Two linear surgical wounds 1 cm long were produced in each mouse by cutting the skin perpendicular to the lumbar spine downwards. The full thickness skin wounds were closed immediately with discontinuous silk-sutured at 0·5 cm intervals. Each animal received one injection of penicillin procaine (400 int. units) (Yue et al 1987). In the test groups, two EGF dosage forms (5 μL) were applied on the skin incision wounds of mice, twice a day for 7 and 15 days. The wound tear strength was tested at the 7th and 15th days.

In the control group, vehicle (0·9% NaCl and CP 940 gel) was applied under the same conditions. At the 7th and 15th days mice were killed by ether anaesthesia. The skin flaps were dissected (5 × 5 mm portions) and the wound tear strength tested with a polygraph (grass model 7 force displacement transducer FT 03). Statistical analysis was carried out using Student's *t*-test.

Results and discussion

Table 1 shows the wound tear strength of mice was significantly higher at the 15th day of treatment in the gel-treated group compared with the solution-treated mice and controls ($P < 0·001$).

The effects of growth factors, especially EGF, on wound healing is well established. The delivery methods and formulation criteria will become critical issues for the commercial development of this growth factor. Bandages, gels and lotions are currently being used for topical applications of growth factors to the area of soft tissue wounds (Ksander 1989) and on corneal wounds (Gönül et al 1992).

It has been reported that EGF in saline solution was ineffective in certain wound healing models. EGF in slow-release formulations, such as multilamellar liposomes or collagen gels, has been shown to enhance the re-epithelization and tensile strength of wounds. Moreover, collagen gels are biodegradable and may also enhance the stability of the growth factor (Brown et al 1988; Dijke & Iwata 1989). Our findings are consistent with these reports.

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Table 1. Effects of EGF dosage forms on the wound tear strength.

	Wound tear strength (N cm^{-1})	
	7th day	15th day
Untreated (n = 5)	0·75 ± 0·25	1·4 ± 0·5
Physiological solution (n = 5)	0·75 ± 0·5	1·4 ± 0·5
Gel (n = 5)	0·5 ± 0·25	1·2 ± 0·25
Physiological solution + EGF (n = 15)	1·25 ± 0·2	1·9 ± 0·2
Gel + EGF (n = 15)	1·4 ± 0·2	3·95 ± 0·75

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Involvement of nitric oxide in the response to 5-hydroxytryptamine in the rat in-vivo

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Abstract—The involvement of nitric oxide (NO) in the effects of 5-HT on intestinal secretion and cardiovascular function in anaesthetized rats was investigated using N^G-nitro-L-arginine methyl ester (L-NAME), a specific NO-synthase antagonist, and its optical isomer D-NAME. L-NAME significantly reduced the prolonged hypotensive response to 5-HT. It also caused a small rightward shift in the colonic 5-HT dose-response curve. This suggests that NO plays a significant role in the prolonged hypotensive response to 5-HT, and may make a small contribution to the secretory response of the colon, but not that of the jejunum, in the rat in-vivo.

5-Hydroxytryptamine (5-HT) is known to induce intestinal secretion (Hardcastle et al 1981), but the mechanisms responsible remain unclear, with evidence for both a direct action of 5-HT on the enterocyte (Hirose & Chang 1988) and indirect effects mediated by the enteric nervous system (Franks et al 1993). It has recently been reported that 5-HT induces a relaxation of gastrointestinal smooth muscle which is mediated by nitric oxide (NO) (Bogers et al 1991; Allescher et al 1992), an agent that has now been implicated in a range of physiological processes throughout the body (see Moncada et al (1991) for review).

The involvement of NO in 5-HT-induced intestinal secretion was investigated in-vivo using the specific inhibitor of NO synthase, N^G-nitro-L-arginine methyl ester (L-NAME) (Moncada et al 1991). The preparation used provided an opportunity to examine not only the intestinal effects of 5-HT, but also the changes in cardiovascular function that follow intravenous administration of the amine.

Materials and methods

Male Wistar rats, 230–250 g, from the Sheffield Field Laboratories, with free access to food and water, were anaesthetized by intraperitoneal injection of 70 mg kg^{-1} sodium pentobarbitone. Following tracheotomy, 5 cm segments of proximal jejunum and colon were isolated by tying off at the distal end and inserting a cannula into the proximal end. The contents were washed out and the loops filled with warm 154 mM NaCl. The potential difference (PD) across each loop was measured between a salt bridge electrode in contact with the luminal fluid and a common reference electrode in contact with the peritoneal fluid by means of a wick electrode. Each pair of electrodes was connected via calomel half-cells to a differential input electrometer. Blood pressure was measured via the femoral artery using a Druck pressure transducer (type 3389). Heart rate was calculated from the pulse pressure by a Lectromed rate meter (model 5250). Jejunal and colonic PD values, blood pressure and heart rate were all recorded on computer using CED Chart software. All drugs were administered via a cannula in the femoral vein.

Non-cumulative dose-response curves to 5-HT were constructed in the absence and presence of either L-NAME or D-NAME (1·3 followed by 13 μmol kg^{-1}). Data were analysed using CED Spike2 software and Student's unpaired t-test (except where otherwise stated) was used for statistical analysis.

Acetylcholine chloride, 5-hydroxytryptamine creatinine sulphate (5-HT), N^G-nitro-L-arginine methyl ester (L-NAME), N^G-nitro-D-arginine methyl ester (D-NAME) and atropine methyl nitrate were obtained from Sigma Chemical Co., Poole, UK.

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Results

Intestinal responses. Basal PD values in the jejunum and colon were 6·4 ± 0·3 and 14·5 ± 0·6 mV, respectively (n = 10), the serosa being positive with respect to the mucosa.

5-HT induced a dose-dependent rise in transintestinal PD in both the jejunum and the colon with maximum responses of 4·4 ± 0·3 and 7·9 ± 0·7 mV, and EC50 values of 27 ± 2·3 and 42 ± 5·6 nmol kg^{-1}, respectively (n = 10). These values were not significantly affected by D-NAME (Table 1). L-NAME (neither 1·3 nor 13 μmol kg^{-1}) had no effect on the 5-HT-induced maximum rise in transintestinal PD (PD_{max}) in either of the regions investigated when compared with values obtained in the presence of equimolar D-NAME. It did, however, increase the colonic EC50 value from 42 ± 5 to 69 ± 10 nmol kg^{-1} with 1·3 μmol kg^{-1} and to 87 ± 20 nmol kg^{-1} with 13 μmol kg^{-1} ($P < 0·05$