Stimulation of Wound Healing by Epidermal Growth Factor

A Dose-dependent Effect

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This work was undertaken to study the effects of various doses of locally applied epidermal growth factor (EGF) on developing granulation tissue in rats. Cylindrical hollow sponge implants were used as an inductive matrix for the growth of granulation tissue. In the test groups, the implants were injected daily with a solution containing 0.2, 1, or 5 μg of EGF in 0.1% albumin while the implants of the control group were treated correspondingly with the carrier solution only. Analyses of granulation tissue in the sponge cylinders were carried out 7 days after implantation. A stimulatory, dose-dependent effect of EGF on granulation tissue formation was observed: cellularity increased, as evidenced by the elevated amounts of nucleic acids, and accumulation of collagen and glycosaminoglycans was enhanced.

EPIDERMAL GROWTH FACTOR (EGF) is a single polypeptide chain of 53 amino acid residues and contains three intramolecular disulfide bonds that are required for biological activity.[1,2] EGF enhances a cascade of cellular events that are part of the mitogenic response produced by EGF, including initiation of DNA synthesis and cell replication, activation of RNA and protein synthesis, and activation of the synthesis of extracellular macromolecules.[3,4]

Although it has been suggested that EGF could be beneficial in wound healing, it has not been extensively used in this respect.[1,2] Local daily application of EGF enhanced accumulation of granulation tissue cells, collagen, and glycosaminoglycans in experimental rat wounds.[5] The present work was undertaken to study the effects of various doses of locally applied EGF on tissue repair in rats using a subcutaneously implanted sponge cylinder as a wound model.

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Methods

Wound Model

A standardized experimental wound model described by Niinikoski, Heughan, and Hunt was used.[6] Viscose cellulose sponge (Säteri Oy, Valkeakoski, Finland) was used as an inductive matrix for repair tissue. The material was cut into cylindrical pieces, 40 mm long and 10 mm in diameter, and a tunnel of 3 mm in diameter was made through the center of the sponge. Silicone rubber discs, 10 mm in diameter and 2 mm thick, were stitched onto both ends of the sponge to create a stable dead space. The cylinders were decontaminated by boiling for 30 minutes in physiological saline and the implantations were performed with strictly aseptic techniques. Male Spraque Dawley rats weighing 230–250 g were anesthetized with ether, and an incision, 4 cm long, was made in the dorsal midline at the caudal portion of the back. Each rat received one sponge cylinder that was implanted longitudinally under the skin, cephalad from the incision. During the experiments, the animals received a normal diet and water *ad libitum* and were housed individually in cages in the animal quarters.

Experimental Protocol

Altogether 24 rats were studied in four groups of six animals. In the control group, the implants were treated immediately after implantation by injecting 0.05 ml of 0.1% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) into the central tunnel of the implant. The implant of the three test groups was injected correspond-

TABLE 1. *Effect of Various Daily Doses of Epidermal Growth Factor (EGF) on Different Components of Developing Granulation Tissue*

			EGF		
		Control	0.2 μg	1 μg	5 μg
DNA	(mg/implant)	6.19 ± 0.83	7.76 ± 0.41 (+25%)	8.19 ± 1.0 (+32%)	10.33 ± 0.63 (+66%)†
RNA-ribose	(mg/implant)	1.53 ± 0.09	1.83 ± 0.04 (+19%)	2.01 ± 0.12 (+31%)†	2.29 ± 0.08 (+49%)‡
RNA-ribose/DNA		0.26 ± 0.03	0.24 ± 0.01 (−10%)	0.26 ± 0.02 (−3%)	0.23 ± 0.02 (−15%)
Nitrogen	(mg/implant)	19.5 ± 1.6	23.0 ± 1.0 (+18%)	25.9 ± 1.4 (+32%)†	28.7 ± 1.0 (+47%)‡
Hydroxyproline	(mg/implant)	1.77 ± 0.18	2.38 ± 0.08 (+34%)*	3.01 ± 0.25 (+70%)†	3.49 ± 0.42 (+97%)†
Hexosamines	(mg/implant)	1.32 ± 0.13	1.53 ± 0.08 (+15%)	1.68 ± 0.11 (+27%)*	1.76 ± 0.08 (+33%)†
Uronic acids	(mg/implant)	1.99 ± 0.13	2.60 ± 0.15 (+30%)*	2.43 ± 0.09 (+22%)	3.16 ± 0.25 (+58%)‡

Means ± SEM are indicated; each group consisted of six rats. The change from control is indicated in the parentheses.

* $p < 0.05$; † $p < 0.01$; ‡ $p < 0.001$.

ingly with 0.05 ml of a solution containing 0.2, 1, or 5 μg of EGF in 0.1% BSA. Human EGF was obtained as a donation from Chiron Corporation (Emeryville, CA). Injections of all groups were repeated daily under strictly aseptic conditions. Bacteriological examinations of wound fluid were performed in each group 7 days after implantation, and no infections were observed. After collection of the wound fluid samples, the rats were anesthetized with ether and killed. The implants were dissected free from the surrounding tissues, and the silicone rubber discs were removed. Nucleic acids were extracted from the implants according to the method of Schmidt and Thannhauser.[7] DNA was determined by the diphenylamine reaction,[8] and RNA was assayed as RNA-ribose by the method of Ceriotti.[9] Aliquots were taken for the determination of nitrogen,[10] hydroxyproline,[11] hexosamines,[12] and uronic acids.[13]

Statistical Analysis

Results are expressed as means ± standard error. The significance of differences in means between the control and the experimental groups was tested by a one-way analysis of variance, where the factor used was the mode of treatment. The pairwise comparisons between daily mean values were made using Student's t-test with Bonferroni correction after discovering by the one-way analysis of variance that there was an overall variation between these mean values. Statistical processing was carried out using a BMDP computer program library.[14]

Results

Data of the effects of EGF on various wound healing parameters are shown in Table 1. After daily application of 5 μg of EGF, statistically significant increases were observed in the accumulation of DNA (+66%) and RNA (+49%) as well as in the amounts of tissue nitrogen (+47%), collagen hydroxyproline (+97%), hexosamines (+33%), and uronic acids (+58%). Similar but less profound effects on granulation tissue parameters were observed after daily application of 1 μg of EGF. The lowest dose of EGF had no marked effects on the amounts of nucleic acids, tissue nitrogen, or hexosamines, but even in this group the amounts of hydroxyproline and uronic acids rose significantly above the control level. The RNA-ribose/DNA ratios in the different treatment groups remained close to the control value.

Discussion

In earlier studies, extracts of the submaxillary gland of the mouse, when injected into newborn animals, induced precocious eyelid opening and incisor eruption due to a direct stimulation of epidermal growth and keratinization.[15] The factor responsible for these effects was isolated and found to be a low molecular weight, heat stable, nondialyzable polypeptide that accounted for approximately 0.5% of the protein content of the submaxillary gland. A homologous polypeptide, human epidermal growth factor (urogastrone), was detected and isolated from human urine.[1,2] Since then, human EGF has been produced through recombinant DNA techniques in quantities large enough for experimentation.[16]

Experimental studies in mice have suggested that delivery of saliva to a wounded area may have implications as a physiological response to injury, mediated, at least in part, by EGF. Removal of submaxillary and sublingual glands from mice was shown to delay closure in full-thickness skin wounds. Duct ligation of the submaxillary gland retarded wound closure significantly, but not to the same extent as gland ablation.[17] Evidence has also been presented that topical application of EGF to a standardized open back wound in mice enhances wound closure.[18] Topical EGF increased the rate of epithelialization of split-thickness skin wounds in pigs.[19] Local application of an ointment containing EGF to rabbit ear wounds increased the thickness and cellularity of epithelium, inhibited wound contracture, and enhanced connective tissue maturation.[20]

In a preliminary work,[5] daily local application of 5 μg of EGF resulted in elevated amounts of nucleic acids and enhanced accumulation of collagen and glycosaminoglycans in subcutaneous sponge cylinders 10 days after implantation. However, single application of EGF, given immediately after wounding, had no effect on the developing granulation tissue.

The present study demonstrates a stimulatory, dose-dependent effect of daily local application of EGF on several granulation tissue parameters, namely the cellularity and accumulation of collagen and glycosaminoglycans. The fact that the RNA-ribose/DNA ratio remained unchanged suggests that the structural synthetic capability of the cells was not influenced significantly. The favorable effect of the EGF treatment on collagen accumulation could be caused by an increase in the number of collagen-producing cells and/or direct stimulation of protein synthesis.

The beneficial effect of EGF on wound healing can also be explained by earlier observations according to which EGF enhances a cascade of cellular events that are part of the mitogenic response produced by this polypeptide.[1,2] This results in an augmented cellular response to injury and enhanced accumulation of extracellular macromolecules, especially collagen and glycosaminoglycans, in repair tissue.

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