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# STUDIES OF WOUND HEALING IN THE PRESENCE OF AN ANGIOGENESIS FACTOR

Annual Report

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## FOREWORD

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The effects of the angiogenic protein basic fibroblast growth factor was tested in a mouse wound healing assay. Using punch biopsies, the rate of reepithelialization, inflammatory response and degree of vascularization were measured and recombinant FGF were observed. The difference between treated and untreated wounds were small and equivocal.

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During the last quarter, we have conducted a number of wound healing experiments using a mouse skin model and measuring the effects of basic fibroblast growth factor bFGF. This report will describe the experimental protocol, the experiments conducted and the results.

Protocol:

Five week old BalbC mice are used. The mice are anesthetized with pentobarbital diluted 1:17 with sterile saline. Each mouse is injected interpertioneally with 0.2 ml solution. A wound is made using a 6 mm diameter punch biopsy instrument. Each mouse has only one wound. The animals are first shaved and the wound made on the center of the back. The wounds are full thickness wounds. The solution to be tested (20 ul) is applied to the wound using the regimen indicated, animals are sacrificed on the indicated days, the skin removed, and the degree of healing measured after histological

sections are prepared. The parameters measured are, one, wound closure; two, epidermal thickness; three, granuloma thickness; four, degree of inflamatory cells; and five, degree of vascularization. Each parameter is given a score. Wound closure, 0 or 1,1. indicating that the wound was closed. Epidermal thickness, 1 = thin, 2 = normal, and 3 = hyperkeratosis. Granuloma thickness, 1 = thin, 2 = moderate, 3 = thick. Infiltrated cells, 1 = few, 2 = moderate, 3 = high or very cellular. Neovascularization, 1 = few, 2 = moderate, 3 = high. The number of blood vessels is also counted.

The scroes for all mice in any one group are averaged. These scores are presented in the tables accompanying this report. All observations are carried out blind. Recombinant bFGF was supplied by Synergen, Boulder, CO.

Our initial experiments with the closure of incisional wounds were unsuccessful. The wounds rapidly reepithelialized. The degree of reepithilialization was affected by how well the wound

edges were opposed and the amount of hair caught in the wound. No differences were apparent between controls and bFGF treated animals. However, healing was so rapid in the controls (1-2 days), it was not obvious that a stimulation of the rate would have been observable.

For this reason we switched to full thickness punch biopsy wounds which are easier to make and take longer to heal. The following results were obtained.

Expt. 1. Purpose - Compare vehicle alone (Methylcellulose 1.5%) vs bFGF (5 ug) in vehicle. Solutions were applied everyday for four days, animals (4/group) were sacrificed on days 5, 8, and 11. The results (table I) indicate a small early effect in the epidermis which may not be significant and an increase in the dermal parameters at days 8 and 11. These differences appeared to be significant.

Expt. 2. Purpose - This was a repeat of expt. 1 except that

8 mice were used in each group. The results (table II) indicate that there is little difference between control and experimental groups in the epidermal parameters. Differences were found in the dermal parameters although they were not as significant as those observed in expt. 1.

Expt 3. Purpose - to test different concentrations of bFGF in order to establish the most effective concentration. Applications and observations same as in expt. 2. The preparation of the specimens was not good due to poor sectioning. There appeared to be no effect on the epidermis (Table III). In the dermis the vehicle (Methylcellulose) stimulated the dermal parameters. There appeared to be a stimulation of the dermal parameters with bFGF above that observed with the vehicle. However, there was too much of a dose response. Five gave almost the same stimulation at 5,000 nanograms.

Expt. 4. Purpose - will heparin plus recombinant bFGF work better than recombinant bFGF alone. Their appeared to be no difference between the two groups. Therefore, it was concluded that coadministration of heparin plus recombinant bFGF was not necessary. However, the sample numbers were quite small because of poor sectioning.

Expt. 5 Purpose - compare the effects of recombinant bFGF to human placental bFGF. In this experiment, the preparation of the slides by the pathology laboratory was very poor. Therefore, it was impossible to establish unequivocally that the two preparations were the same. From the few samples which were prepared successfully no differences were observed but this was not satistically valid.

Conclusions

The data thus far obtained are equivocal. While we routinely observe increases in healing in the experimental samples when compared to the controls, when multiple samples are scored, the differences are small. We are not certain what the course of this is, but we think this may result from the fact that our grading system is so broad that the effects are obscured. For example, we divide the amount of neovascularization into these groups each of which is assigned a number. They are 1 = 0-5 capillaries per field; 2 = 5-20 capillaries per field; 3 = 20 or more capillaries per field. Thus, two slides which have a score of 2 may differ by four-fold.

Future action steps:

1. Improve the quality of the slide preparation

2. Establish whether cbFGF is equivalent to placental bFGF.

3. Estabilish the minimum dose and number of applications necessary.

4. Test animals with impaired wound healing in order to magnify potential effects of bFGF. Either old or diabetic animals will be used. We will delay studies with pigs until we are certain we have a stimulation of healing in rodents and/or we are certain we understand most of the parameters in the mouse. This will be much more cost and time effective.

**Table I**

Parameters

| Day | Mice | Wound Closure | Epidermal Thickness | Granuloma Thickness | Infiltrated Cells | Degree of Vascularization |
|---|---|---|---|---|---|---|
| 5 | C | 0 | 0 | 1.5 | 1.7 | 1.7 |
| | FGF | 0.5 | 0.5 | 1.7 | 2.5 | 1.7 |
| 8 | C | 0.6 | 1.3 | 1.6 | 2.0 | 2.3 |
| | FGG | .75 | 1.2 | 3.0 | 3.0 | 3.0 |
| 11 | C | 1.0 | 2.0 | 1.5 | 1.7 | 1.7 |
| | FGF | 1.0 | 1.7 | 2.0 | 2.2 | 2.2 |

C = Control Mice

FGF = Experimental Mice

**TableII**

Parameters

| Day | Mice | Wound Closure | Epidermal Thickness | Granuloma Thickness | Infiltrated Cells | Degree of Vascularization |
|---|---|---|---|---|---|---|
| 5 | C | 0.12 | 0.12 | 1.3 | 2.1 | 2.2 |
| | FGF | 0 | 0 | 2.0 | 2.5 | 2.3 |
| 8 | C | 1.0 | 1.5 | 2.0 | 2.1 | 2.1 |
| | FGF | 1.0 | 1.3 | 2.6 | 2.6 | 2.6 |

C = Control Mice

FGF = Experimental Mice

Table III

Parameters

| Sample | Wound Closure | Epidermal Thickness | Granuloma Thickness | Infiltrated Cells | Degree of Vascularization |
|---|---|---|---|---|---|
| PBS | 0.6 | 1.1 | 1.1 | 2.0 | 1.1 |
| Methyl-Cellulose | 0.8 | 1.1 | 1.5 | 2.2 | 1.5 |
| FGF (5 ng) | 0.7 | 1.0 | 1.8 | 2.4 | 1.7 |
| FGF (500 ng) | 0.8 | 0.8 | 1.6 | 2.0 | 1.8 |
| FGF (5,000 ng) | .57 | 0.8 | 1.7 | 2.4 | 1.8 |