# THE INFLUENCE OF INSULIN-LIKE GROWTH FACTOR I AND ITS ANALOGUES ON FIBROBLASTS AND DERMAL WOUND HEALING

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# SYNOPSIS

A comprehension of the wound repair process is central to all surgical practice. The science of wound healing has progressed dramatically in recent years to facilitate this understanding, particularly in the area of growth factor biology and offers exciting possibilities for the manipulation of this process.

One cytokine that has shown particular promise has been insulin-like growth factor-I (IGF-I). As the second messenger for growth hormone (GH), it is postulated to mediate the majority of anabolic functions associated with GH. Of clinical importance is the associated reversal of previously depressed IGF-I levels and reduced protein catabolism when GH is administered to patients with severe burns. The potential role of IGF-I is further supported by the finding that IGF-I is found locally at the wound site as well as in measurable concentrations systemically. It appears, also, to have important vulnerary effects upon fibroblasts and keratinocytes including cell migration and protein synthesis. Six carrier proteins, known as IGF binding proteins (IGFBPs), have been identified. Although little is known of how they modulate IGF biology within a wound it appears that IGFBP-1 and -3 can potentiate IGF-I activity.

With the development of recombinant DNA techniques, a range of IGF variants (analogues) are now commercially available. The value of these peptides lies in their apparent increased biological activity *in vitro* and to a more limited degree *in vivo*. Primarily, the greater potency of the analogues is related to decreased affinity for IGFBPs and consequently increased IGF bioavailability. Therefore they can be used to examine indirectly the influence that IGFBPs have in wound repair.

In light of this information, the objectives of this study were to investigate:

- the levels of IGFs and the presence of binding proteins in human wound fluid;

- the potency of IGF-I and two analogues in several *in vitro* models of fibroblast activity;
- the effect of IGF-I and one of the analogues upon healing in both normal and diabetic rodent wounds.

Characterisation of IGFs and IGFBPs in human wound fluid and plasma was achieved by obtaining time matched wound fluid samples (from split skin graft donor sites) and plasma. These were subjected to acid gel permeation chromatography (acid gel HPLC) or acid-ethanol extraction to separate the binding proteins from the IGFs and then performing RIAs to quantify the IGFs. The identity of IGFBPs found in the samples was determined using Western ligand and immunoblotting techniques.

Established models of cell growth (methylene blue dye absorbance by cultured monolayers and radiolabelled thymidine incorporation) were used to examine the potency of a variety of growth factors. Specifically these included recombinant human IGF-I (rhIGF-I); two IGF-I analogues (des(1-3) IGF-I and long [$Arg^3$] IGF-I ($LR^3$ IGF-I)), IGF-II, GH and transforming growth factor β (TGFβ). Experiments examining protein synthesis (proline incorporation) and the functional activity of fibroblasts (fibroblast populated collagen lattice contraction, FPCL) were also preformed.

The efficacies of IGF-I, IGFBP-2, IGF-II, GH and the analogue, $LR^3$ IGF-I were tested using a rodent incisional model of wound repair. Compromised repair was examined using streptozotocin-induced diabetes in the same model.

Healing by secondary intention was examined using an established model of excisional wound repair modified to explore an IGF-I analogue's ability to augment repair in both normal and diabetic wounds.

Several important findings were documented. These were:

- Western ligand and immunoblots of human wound fluid samples revealed the presence of IGFBPs -2, -3 and -4. The intensity of these bands was less than those obtained for time matched samples of plasma. Specifically, wound fluid contained low molecular weight IGFBP-3 fragments.

- IGF-I and IGF-II concentrations as determined by RIA following either acid gel HPLC or acid-ethanol extraction were correlated for each procedure using linear regression. Good correlation for the extraction techniques was observed for IGF-I analysis, in contrast poor correlation was seen for IGF-II. This was particularly evident in the wound fluid samples.
- FPCL assays and cell growth experiments demonstrated a clearly greater response to IGF-I compared to GH and importantly a lack of synergism for a combination of GH and IGF-I. In addition, greater potency was demonstrated by the analogues, $LR^3$ IGF-I and des (1-3) IGF-I, when applied to the FPCL model. The studies of protein synthesis ($^3$H-proline incorporation) confirmed the relative potency of the IGF-I analogues compared to rhIGF-I as well as IGF-II.

- Topically applied GH, IGF-I or the two in combination (concentration of 100μg/ml), failed to produce significant increases in strength in rodent wounds as compared to the vehicle treated control wounds. However when this was increased to 1mg/ml of peptide, IGF-I treated wounds were up to 40% stronger than their paired vehicle treated wounds ($p=0.008$).
- Diabetic animals demonstrated weaker wounds than the normal animals and this reduced wound strength was not restored by rhIGF-I nor $LR^3$ IGF-I although there appeared to be a trend in favour of $LR^3$ IGF-I

Having identified that IGFBP-2 is found in human wound fluid, this binding protein was applied in combination with IGF-I to incisional wounds using our rodent model. The results obtained in this model indicated that it did not enhance the effect of rhIGF.

Wound strength data gathered from the excisional wound study showed that wounds on normal animals treated with 10μg of $LR^3$ IGF-1 per treatment were significantly weaker than control wounds however rhIGF-I and $LR^3$ IGF-I at a dose of 100μg did not alter strength significantly. In conjunction, no treatment altered the rate of wound contraction however diabetes slowed the rate of wound closure.

Although IGFs are well documented as mitogens for cell lines involved in wound repair, little success has been reported demonstrating a similar potency using animal models except where the peptide is coupled with an IGFBP or in studies of compromised wound healing. The human wound fluid study confirmed the existence of IGFBPs and the presence of IGFBP fragments in the acute wound environment.

Despite clearly demonstrated increases in activity of the analogues using tissue culture models, this potency could not be translated to enhancement of dermal wound repair. The failure of *in vitro* success to translate to animal models raises clinically relevant points. Products that may be suitable as topical wound healing agent should be tested *in vivo* as well as *in vitro*. Secondly these studies suggest that a single agent is unlikely to be effective when used alone. The timing of treatment application and a myriad of other factors within the wound, including IGFBPs may be important variables in determining the role of IGF-I in wound repair.

In conclusion, the studies outlined in this thesis provide evidence that:

- IGF-I, IGF-II and their IGFBPs are present in exudate produced by a partial thickness cutaneous wound.
- IGFBPs negatively modulate the activity of IGFs *in vitro*.
- In contrast IGFs do not necessarily exhibit enhanced activity *in vivo* at the wound site if their IGFBP affinity is decreased. Possible roles of IGFBPs in wound repair are discussed.