Alterations in the expression of IGF-I isoforms and binding proteins during the wound healing process

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Abstract. There is increasing evidence to indicate that insulin-like growth factor (IGF)-1 plays a crucial role in the regeneration of different tissues following injury. Notably, despite the escalating number of animal studies, studies investigating the role of IGF-1 in the wound healing process in humans are fewer. In this context, the aim of the present study was to evaluate the variations in the expression IGF-1 isoforms (IGF1-Ea, IGF1-Eb and IGF1-Ec), as well as its binding protein and receptor (IGF-BP3 and IGF-1R) during wound healing in patients. The study population comprised of 21 patients presenting with the first episode of sacrococcygeal pilonidal disease. Samples were obtained during surgery, as well as on days 2, 7 and 14 post-operatively. The expression levels of IGF-1 isoforms, as well as that of its binding protein and receptor were evaluated using reverse transcription-quantitative PCR. Statistical analyses were performed using GraphPad Prism software. The Kruskal-Wallis test and Dunn's post hoc test were utilized. The results revealed a statistically significant difference in the expression of IGF-BP3 and IGF-1R during wound healing (P=0.014 and P=0.018, respectively). Specifically, the pairwise post-hoc Dunn test indicated that IGF-BP3 expression was significantly decreased on the 2nd post-operative day compared to the day of surgery, while IGF-1R expression was significantly increased at 14 days post-operatively. The expression of the remaining IGF-1 isoforms was not significantly altered during wound healing. On the whole, as demonstrated herein, IGF-BP3 and IGF-1R appear to play a crucial role during the wound healing process, particularly in patients with large open wounds following pilonidal disease treatment. Further studies are warranted to evaluate the exact role, as well as the possible use of these proteins as enhancers of wound healing.

Introduction

Wound anaplasis is a dynamic, complex process that involves a number of cell types and a variety of biological processes, such as proliferation, differentiation and migration (1). The interactions between these cells are initiated and mediated mostly by growth factors (2-4). One of these factors is insulin-like growth factor (IGF)-1. IGF-1 is a protein with a similar molecular structure to insulin, which has been shown to play a crucial role during growth and exerts several anabolic effects in humans (5). The main secretion of IGF-1 occurs in the liver and is regulated by growth hormone. It is also produced by other organs, such as the skeletal muscle, kidney and brain (6-12). The IGF-1 gene can produce different transcripts through alternative splicing. This phenomenon results in three different IGF-1 transcripts, namely IGF1-Ea, IGF1-Eb and IGF1-Ec, encoding the IGF-1 protein isoforms (6-12).

Approximately 98% of IGF-1 is always bound to one of six binding proteins (IGF-BP). IGF-BP3 accounts for 80% of all IGF binding (13). IGF-1 exerts its effects by binding to specific receptors on the cell surface (10). There is increasing evidence to indicate that IGF-1 plays a main role in the regeneration of different tissues following injury (11,14-17). Of note however, despite the escalating number of animal studies (18-20), there are fewer studies investigating the role of IGF-1 in the wound healing process in humans.

In this context, the present study aimed to evaluate the variations in the expression IGF-1 isoforms (IGF1-Ea, IGF1-Eb and IGF1-Ec), as well as its binding protein and receptor (IGF-BP3 and IGF-1R) during wound healing.

Patients and methods

Patients. A total of 21 patients, presenting with the first episode of sacrococcygeal pilonidal disease were enrolled in the present study, from December, 2017 to December, 2018.
The samples were obtained at the Laiko University Hospital (Athens, Greece). The present study population was selected due to the presence of an open wound and the possibility of sampling on consecutive days. All patients provided their written consent to participate in the study, which followed the 1975 Helsinki guidelines and was approved by the Bioethical Committee of the Medical School of the National and Kapodistrian University of Athens (13-3-2017/Protocol no. 255). Only adult patients were included in the present study and these were patients with a first episode of pilonidal disease. Tissue samples were obtained during surgery (time 0), as well as on day 2, 7 and 14 post-operatively. The days of sampling were selected based on the phases of the healing process, always bearing in mind that different healing phases are not mutually exclusive and tend to overlap.

**Tissue specimens.** The size of the samples was 0.5 cm (depth), consisting of full-thickness biopsies of the wound (skin and subcutaneous tissue). Biopsy samples were immediately transferred in Ambion RNeAlater (Thermo Fisher Scientific Inc.) androzen on site at -80°C.

**RNA isolation and cDNA synthesis.** Once all tissues were collected, RNA extraction was performed. Total RNA was extracted from the tissue samples using the TRIzol protocol (TRItidy G™ reagent, PanReac AppliChem). According to this protocol, the total RNA was obtained in the aqueous phase during the acidic extraction. Following RNA isolation, reverse transcription reaction was performed using the standard protocol of the ProtocScript II First Strand cDNA synthesis kit [ProtocScript® II First Strand cDNA Synthesis kit (#E6560L; New England BioLabs, Inc.)].

**Reverse transcription-quantitative polymerase chain reaction (RT-qPCR).** In total, five sets of primers (Table I) were used to amplify five different target mRNAs, IGF1-Ea, IGF1-Eb, IGF1-Ec, IGF-BP3 and IGF-1R. 18S ribosomal RNA (rRNA) was used as the housekeeping gene. The primers used to amplify five different target mRNAs, IGF1-Ea, IGF1-Eb, IGF1-Ec, IGF-BP3 and IGF-1R. 18S ribosomal RNA (rRNA) was used as the housekeeping gene. The primers used to amplify five different target mRNAs, IGF1-Ea, IGF1-Eb, IGF1-Ec.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequence (5′-3′)</th>
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<tbody>
<tr>
<td>IGF1-Ea</td>
<td>GTGGAGACAGGGGTTTTTATTTC</td>
</tr>
<tr>
<td>IGF1-Ea</td>
<td>CTTGTTCCTGCCACTCCCTCTACT</td>
</tr>
<tr>
<td>IGF1-Eb</td>
<td>ATGGCCCTCTGCATCTCTC</td>
</tr>
<tr>
<td>IGF1-Eb</td>
<td>CCTCCTCTGTTCCCTCCTC</td>
</tr>
<tr>
<td>IGF1-Ec</td>
<td>CGAAAGTCAGAAGAAGGAGAG</td>
</tr>
<tr>
<td>IGF1-Ec</td>
<td>ACAGGTAATCTGTCGAGCAGGC</td>
</tr>
<tr>
<td>IGF-1R</td>
<td>ACCTCCTCCCAAACCTCAC</td>
</tr>
<tr>
<td>IGF-1R</td>
<td>CAGGCAGCAACAGACACACAGAC</td>
</tr>
<tr>
<td>IGF-BP3</td>
<td>AGTGAAGCTGGAGGAGAAGCCGA</td>
</tr>
<tr>
<td>IGF-BP3</td>
<td>CCTTGGTGTTAGCCCTTGGAGA</td>
</tr>
</tbody>
</table>

RT-qPCR, reverse transcription-quantitative PCR; IGF, insulin-like growth factor; F, forward; R, reverse.

**Statistical analysis.** The statistical analysis of the results was performed using GraphPad Prism software (GraphPad Prism version 8.0.0 for Windows, GraphPad Software, Inc.; www.graphpad.com). Statistical analysis of relative quantification data (ΔΔCq) included a non-parametric Kruskal-Wallis test with multiple comparisons of the three timepoints post-surgery compared with baseline (day of surgery) and Dunn's method as a post-hoc test. A P-value <0.05 was considered to indicate a statistically significant difference.

**Results**

The median age of the study population was 25 years old (range, 18-33 years), with almost 80% (17/21) being males. No patient had received corticosteroids or any other immunosuppressive medication. All patients had an ASA (American Society of Anesthesiologists) (22) score of I. No antibiotics or other medications were prescribed post-operatively apart from paracetamol. The wound care was performed on a daily basis-with mechanical irrigation with normal saline and simple gauze dressings by the patient or their immediate family members except for the days that they participated in the protocol.

**IGF-1 isoforms (IGF1-Ea, IGF1-Eb, IGF1-Ec).** The expression of IGF-1 isoforms (IGF-1Ea, IGF-1Eb and IGF-1Ec) was not significantly altered during the process of wound healing in the present study population. All wounds were clean and healing in an expected manner; despite that, no marked differences were noted in the expression of these growth factors (Fig. I).

**IGF-1-BP3.** The expression of IGF-BP3 was significantly increased during the post-operative period (P=0.014) compared
with the baseline. A pairwise post-hoc Dunn test indicated that it was significantly decreased on day 2 post-operatively compared to the day of surgery (Fig. 1).

IGF-1R. The expression of IGF-1R was also increased post-operatively (P=0.018), with a significant increase observed on the 14th post-operative day compared to the day of surgery (Fig. 1).

Discussion

Wound healing is a complex process that occurs in several phases and involves several factors. IGF-1 is a hormone that plays a crucial role during growth and development and is expressed in several tissues in humans where it exerts several anabolic effects (23,24).

Several animal studies have been conducted to assess the role of IGF-1 in the process of wound anaplasis, using both local as well as systemic administration. Lynch et al (3) studied the application of recombinant IGF-I and platelet-derived growth factor-2 in partial thickness wounds, which were surgically induced in the back and thoracic areas of young white Yorkshire pigs. They reported a 132% increase in the dermal thickness and a 300% increase in the number of connective tissue cells within the wound site as well as in the collagen content and maturity, following (3). Moreover, a placebo-controlled trial demonstrated that IGF-1 depletion in hypophysectomized rats resulted in a 50% reduction in wound protein levels and hydroxyproline content, and that when IGF-1 was administered the levels of these variables returned to normal levels (25).

Of note, the present study has several limitations. Firstly, there was no control group, and the intended sample size (n=30) was not reached. In addition, there was no standardized method of estimating the healing process of the wound, which severely restricted the translation of the results. Furthermore, additional experiments, such as western blot analysis and immunohistochemistry were not employed.

In conclusion, IGF-1 is a hormone with profound anabolic activities and a crucial role in wound anaplasis. The IGF-1-induced stimulation of wound healing has been demonstrated in several animal studies. A recent systematic review (30) demonstrated a potentially promising, evidence-based practice favoring the use of IGF-1 in addressing patients with large burn wounds, chronic diabetic ulcers, and patients with impaired wound healing. Studying the variations of IGF-1 expression may help in the wound healing process by detecting the solely responsible binding protein and receptor on the cell surface, resulting in most targeted future therapies. Thus, further consistent clinical trials are warranted, focusing on the medical use of recombinant IGF-1 in patients whose healing process has been compromised.
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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors’ contributions

ZG contributed to the experiments and the drafting of the manuscript. APa and APh contributed to the statistical analysis. APa, DV and EK contributed to the experimental process. APh, GT, GK and DM were involved in the conception and design of the study. ZG and DM confirm the authenticity of all raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

All patients provided their written consent to participate in the study, which followed the 1975 Helsinki guidelines and was approved by the Bioethical Committee of the Medical School of the National and Kapodistrian University of Athens (13-3-2017/Protocol no. 255).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References


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