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Abstract

Wound healing is complex and numerous factors overlap perfectly with the goal of wound closure. Among them, we will focus on a large amount of experimental and clinical evidence on the action of GH in wound repair. We will analyze how the physiological rhythm of GH secretion influences this process, and also one of the most important signaling pathways that mediate the effects of GH on tissue regeneration. The role of IGF-1 and the factors that stimulate GH secretion and that have also been shown to improve healing will also be reviewed. In addition, it will be analyzed the cellular senescence process, which plays a key role in nonhealing wounds associated with chronic diseases. The benefit of GH in this last circumstance is especially important. The lesions associated with catabolic states, mainly burns, are considered a delicate situation in which it is extraordinarily difficult to act with growth factors due to the fragile situation of these patients, often children. The positive action of GH in these states will also be described. In summary, we will analyze many evidences about the beneficial effects of GH and its main secretagogues in the healing of wounds.

Keywords: wound healing, growth hormone, tissue regeneration, IGF-1, cellular senescence, chronic diseases, catabolic states, secretagogues

1. Introduction

Wound healing represents a major challenge in medicine due to its complexity and potential severity. It is a sequential process that requires the perfect interaction of many factors and cell types. As is well known, the key aspects in wound healing are the growth of granulation tissue and the proliferation and migration of keratinocytes at the edges of the wound. For this, a series of cytokines and growth factors arriving from blood and others produced locally, act in an autocrine or paracrine manner, orchestrating the communication between cells and
regulating the healing process [1–4]. In the normal repair of a tissue, the resident cells have the mission of producing these cytokines and growth factors that cooperate in their repair function. In fact, it has been discovered that some of them promote cell proliferation, angiogenesis, and synthesis of extracellular matrix (ECM) [5]. Therefore, the direct application in the wound of specific stimulating peptides is expected to increase the healing of chronic ulcers until now considered as incurable.

We currently know that growth hormone (GH) is a pleiotropic factor capable of acting positively in many organs and tissues. For years, the use of GH for wound healing has been investigated [6, 7]. For example, the use of recombinant human GH as an anabolic treatment in burns to accelerate wound healing is already classic [8, 9]. Patients with severe burns who were treated with systemic GH improved both their healing and their survival [10, 11]. More recently, a number of studies have shown that GH is a promising agent in the acceleration of wound healing [12, 13]. In addition to the stimulation of granulation tissue formation, GH increases collagen deposition, and facilitates epithelialization [14, 15]. This effect of GH has been seen in experimental models of undernourished rats, in which the administration of the hormone made the granulation tissue to grow in previously induced wounds [16]. Similar results have been found in GH-transgenic mice models [17]. Although contradictory data can be found in the literature on this particular action of GH, most studies support its benefit, and evidence of its positive effects (Table 1) will be described widely later in the text. It should be noticed that GH activation normally is produced in morbid conditions as catabolic or chronic diseases, and it may have no effect in healthy subjects. For example, when we artificially produce an injury in normal individuals, there are no differences about the speed of wound healing between GH-treated subjects and controls [18]. Furthermore, some data suggest that systemic GH treatment is detrimental for wound healing in healthy individuals [19]. The same is found in healthy people if we try to stimulate the immune system with GH [20]. There is much evidence to support an angiogenic effect of GH in patients with critical limb ischemia that suffer usually from ischemic ulcers; or its benefit in aging or in diabetes mellitus (DM) [21, 22]. The latter data show the specific role that the hormone can play depending on the morbid condition of the patient. Figures 1–3 show the evolution of a patient with critical ischemia of the lower limbs, suffering from an ulcer, before and after 8 weeks of treatment with subcutaneous GH administration (0.4 mg/day).

The regulation of metabolic factors acting on wound healing is well known, albeit some aspects have still to be elucidated. Growth hormone-releasing hormone (GHRH) and Ghrelin are some of the most important factors controlling not only the synthesis and release of GH from the pituitary gland, but also regulating the GH receptor (GHR) and its function [23, 24]. As it will be detailed further, both hormones have also been described as having the ability to improve the healing process. Although this is not the aim of this review, at this point we cannot forget the association between Klotho and the GH/IGF-1 axis [22], especially during aging.

The key problem is to find the best way for the hormone to be administered, and the best vehicle to carry it out. However, while for GH both systemic and local administration have been demonstrated to be effective in the healing of wounds, in the case of IGF-1, the main mediator of GH actions, systemic, unlike local use, has no effect, probably because GH can have direct actions that are added to its indirect actions stimulating other growth factors involved in
wound healing. It has to be highlighted that the concentration of GH, when applied locally, and the dose, when a systemic administration is chosen, are also of importance and may determine the final effect and/or the appearance of complications. Systemic GH may increase the collagen production and mechanical strength of wounds [15, 25]. It has been reported

A. Inflammation phase

- Stimulates the recruitment of inflammatory cells: monocytes and T-lymphocytes by increasing MCP-1, without changing neutrophil count.
- Diffuse wound occupation of inflammatory cells.

B. Proliferation phase: granulation tissue formation (dose-dependent)

- Diffuse wound occupation of fibroblasts and myofibroblasts.
- Increase fibroblasts proliferation along with total collagen deposition.
- Increase secretion of ECM: scaffold function.
- Increase proliferation and migration of keratinocytes, accelerating epithelization.
- Angiogenesis:
  - Boosts the formation of capillaries.
  - Directly, or indirectly: VEGF, FGF, or SDF-1.
  - Attraction of endothelial cells from the bone marrow.
- It could improve neurogenic response.
- High doses of GH can delay wound closure (overgrowth of granulation tissue).

C. Remodeling phase

- Accelerates the remodeling of the granulation tissue.

Table 1. Key points about evidence of GH and wound healing.

Figure 1. Five minutes reactive hyperemia test. Response to artificially induced ischemia in an affected limb with Chronic limb-threatening ischemia and a nonhealing wound. The limb is compressed until losing the flow for 5 minutes. Results after 8 weeks of systemic GH treatment. RHT: reactive hyperemia test; RHT0: ankle pressure at baseline; RHT30": ankle pressure at 30 seconds; RHT1'-2'-3'-4'-5': ankle pressure at 1, 2, 3, 4 and 5 minutes. y axis: mm Hg (data obtained from the GHAS trial).

wound healing. It has to be highlighted that the concentration of GH, when applied locally, and the dose, when a systemic administration is chosen, are also of importance and may determine the final effect and/or the appearance of complications. Systemic GH may increase the collagen production and mechanical strength of wounds [15, 25]. It has been reported
Figure 2. Evolution in the same patient that in Figure 1 of the ankle-brachial index (ABI), calculated as the rate of the arterial ankle pressure divided by the arterial brachial pressure, and the arterial pressure at the ankle (measured in mmHg). Results show a positive evolution in angiogenesis, parallel to the wound evolution and the 5 minutes RHT (data obtained from the GHAS trial).

Figure 3. Picture of a nonhealing wound in the same patient as in Figure 1 suffering from Chronic limb-threatening ischemia. Evolution after 8 weeks of systemic administration of GH: (A) baseline picture and (B) final picture (data obtained from the GHAS trial).
that systemic GH administration could accelerate the split-thickness skin defect in pigs [7]. However, systemic use of GH may induce side effects that must be considered when using this way. Such collateral effects are dependent on dose and time of administration. Although the topical use of GH seems to be better to reduce the possibility of side effects, unfortunately, this way of administration also present some deficiencies. Nevertheless, GH therapy has also the advantage of its relatively low cost. To produce growth factors for medical use in non-healing wounds is costly, and hence, increasing the production of these factors by local GH administration, could be more cost-effective.

2. Experimental and clinical evidences of GH action on wound healing

GH actions on wound healing have been evaluated in different studies from the macroscopic and microscopic points of view.

During the inflammatory phase of skin wounds in mice, GH stimulated the recruitment of inflammatory cells after 3 days of topical treatment, allowing to improve the degradation of the injured tissue [26]. Monocytes, monocyte chemoattractant protein-1 (MCP-1), and T-lymphocytes play a key role in the control of the healing process. GH is a strong inductor of these cells [27–29] and activates human monocyte chemotaxis and migration [29]. A low dose of exogenous GH administration induces the expression of MCP-1 mRNA up to eight-fold [28]. However, as it will be described further, the stimulation of these immune cells by GH not only benefits inflammatory phase, but also angiogenic and neurogenic responses [19]. After analyzing the areas of wounds in the inflammatory phase when GH is used topically in mice, GH-treated mice increased the number of macrophages by about 15%, and the number of lymphocytes by 50% without changing neutrophil recruitment [26].

The effects of GH on the immune system have been extensively analyzed. In a model of peritonitis, GH reduced bacterial counts in the peritoneal layer and increased the number of exudative neutrophils [30]. Furthermore, GH increases the thymic mass in patients infected with human immunodeficiency virus (HIV), and the number of CD4+ T-lymphocytes [31]. In these cases, GH was able to restore immune function.

A study in male mice in which an incision wound occurred showed that local administration of GH led to increased cellular infiltration in the wound area, mainly occupied by inflammatory cells, fibroblasts, and myofibroblasts, while in the control group (who did not receive the hormone) this type of cellular infiltration was only observed at the edges of the wound. This finding indicates that GH, directly or indirectly, had accelerated the migration and recruitment of cells, such as fibroblasts, to the site of injury [26].

Fibroblasts play a key role in all aspects of this process. In response to early injury signals, fibroblasts proliferate and migrate into the wound. They significantly contribute to the synthesis of the extracellular matrix (ECM), providing a scaffold for cellular ingrowth [32]. In addition, fibroblasts secrete various important cytokines with both autocrine and paracrine effects [33–36]. This concept is schematized in Figure 4.
The role of GH in accelerating the granulation tissue has been described in previous work [37]. The cell recruitment along with collagen deposition was also accelerated in response to GH during the phase of granulation tissue. An increased mitosis and migration of keratinocyte were found after 7 days of the incision in mice treated with the hormone, parallel to the secretion of ECM to give consistency to the aforementioned granulation tissue [26]. In this study, it was observed that GH accelerated the migration and proliferation of these cells already in the first week of treatment [26], but also the analysis of the samples showed that topical treatment with GH, regardless of the concentration used, increased the total collagen deposition after 7 and 14 days of treatment. That is, GH therapy not only accelerated the remodeling of the granulation tissue, but also the epithelization, with a more stratified epidermis. Another study showed that the systemic application of GH stimulated the formation of granulation tissue in wounds of malnourished rats [16].

In vitro studies on plates coated with Matrigel® with endothelial cells have shown that GH produces a mitogen effect, which affects cell morphology, increases ECM and boosts the formation of structures similar to capillaries [38]. Some data supporting the action of GH on collagen deposition have been described in patients with acromegaly, in whom the excess of GH determines severe cardiac damage with fibrosis [22].

The role of GH in fibroblast proliferation is crucial for the wound healing process [39]. In one study, when GH was applied topically, fibroblast proliferation increased significantly, as indicated by a tetrazolium-based colorimetric assay. However, the increase in proliferation differed according to the concentration of GH, being 2.5 IU/L the best dose to stimulate the proliferation of fibroblasts [40].

Angiogenesis plays a key role during the granulation phase and tissue remodeling, as new vessels are required for the progression of wound healing. Endothelial cells express the GHR [41], and the participation of GH in the latter process has been widely demonstrated and reviewed [21, 22, 42]. Moreover, GH-transgenic mice show an increase in blood vessels during tissue repair [19].

Figure 4. Schematic description of the effects of GH on a wound during the early inflammatory process and stages after it. Lastly, GH also induces the acceleration of the granulation tissue and the wound is healed. Blue arrows indicate stimulation.
GH can act directly on endothelial cells through the GHR, or indirectly, by increasing others growth factors such as VEGF, FGF, or SDF-1; in this way, the hormone facilitates the proliferation, migration, and formation of endothelial cell tubes, as well as the attraction of that type of cells from the bone marrow through the CXCR4 receptor for SDF-1 [43].

The formation of blood vessels is already observed 7 days after the local administration of GH in mice. Again, the dose utilized is important, since at $10^{-7}$ M doses of GH a higher number of blood vessels was produced in the granulation tissue, compared to the control group and the group treated with GH $10^{-8}$ M [26]. A similar effect was also observed after 14 days of treatment, indicating that GH maintained its proangiogenic effect during the 2 weeks of application.

All these results point out that GH is a member of those molecules that have pleiotropic actions on skin cells, and confirm previous research showing that after an injury to the skin, the process of wound healing is accelerated in GH-transgenic mice overexpressing GH [19]. In the latter study, full-thickness incisional and excisional wounds developed a highly vascularized granulation tissue. However, the bursting strength of these injuries did not increase. In these injured mice, wound closure was even delayed as a result of increased granulation tissue formation, demonstrating that, on one hand, GH can grow this essential tissue for healing, but on the other hand, at high doses, the overgrowth of granulation tissue can even delay wound closure. The authors of this study also support the fact that this action of GH on healing is probably not mediated via IGF-1 [19], in contrast to previous studies that hypothesized a direct role of IGF-1, induced by GH, in healing wounds [44, 45]. Currently, many evidences support the fact that circulating IGF-1 does not affect the wound, but that IGF-1 produced locally by fibroblasts, macrophages, and endothelial cells is the responsible for wound healing [46, 47]. Nevertheless, topically applied GH also increases the concentration of IGF-1 mRNA in the granulation tissue in vivo [48]. In any case, if the effect of IGF-1 on wound healing occurs as a consequence of the local production of IGF-1, induced by GH within the wound, it is equally important the fact that the topical administration of GH can facilitate the healing of wounds.

Furthermore, as it will be described later, some of the hormones related to the control of GH secretion, as GHRH and Ghrelin, have also shown positive effects on wound healing, showing, once again, the strong influence of GH on wound healing.

2.1. Signaling pathways in wound healing

2.1.1. The JAK/STAT signaling pathway

The Janus kinase (JAK) signal transducer and activator of transcription (STAT) pathway is considered one of the most relevant intracellular signaling pathways utilized by hormones, growth factors, and cytokines to carry out their cellular actions [49], and it is also involved in wound healing [50]. Cell proliferation, migration, differentiation, and apoptosis are mediated by this pathway [51]. When its control is altered, this promotes chronic inflammation.

Basically, the regulation of the JAK/STAT pathway is carried out by various mechanisms such as tyrosine phosphatase, internalization-degradation of signaling molecules, receptor antagonists, and inhibitors such as inhibitors of activated STAT proteins (PIAS) or suppressors of cytokine signaling proteins (SOCS) [52].
Indirect examples of the implication of this pathway in wound healing are the relationship of the same with the immune system, the main actor during healing. Inhibitors of the JAK/STAT signaling pathway are currently used to treat autoimmune diseases, including psoriasis and rheumatoid arthritis [53].

GHR is a transmembrane protein belonging to the family of receptors of class I cytokines, which homodimerizes after its binding to the ligand and signals through the family of tyrosine kinases of JAK2 and the recruitment of transcriptional factors of the STAT type, in particular isoforms 3 and 5. Intracellular signaling involves the activation by phosphorylation of different intracellular proteins, including IRS-1, MAPK, and phosphatidylinositol 3-kinase (PI3-K) [54]. Intracellular signaling induced by GH is downregulated by the family of cytokine signaling suppressors (SOCS) [55].

Different members of this pathway have been described, but their main mission is to transmit extracellular information, via specific receptors binding of the ligand and phosphorylation, to the nucleus. To describe the functioning of the latter pathway is out of the scope of this review, and we will focus on those aspects related to wound healing.

**2.1.1. Role of JAK/STAT in wound healing**

Fibroblasts, endothelial cells, keratinocytes, and macrophages, are some of the cells in which cytokines and growth factors, using the JAK/STAT pathway, play a key role during the wound repair process. Pathological conditions can affect the normal functioning of this pathway, delaying the closure of the wound, and leading to the development of a chronic wound [56]. For example, Feng and colleagues studied the gene expression pattern of seven SOCSs members in tissue collected from chronic venous leg ulcer patients; they found significantly higher mRNA levels of SOCS3 and SOCS4 in chronic nonhealing ulcers as compared to healing/healed ulcers [57]. In chronic wounds, it is required that the JAK/STAT pathway be upregulated, especially in cases when its normal functioning has been compromised; more specifically in an environment of senescent cells or DM where there is a reduced growth factor/receptor signaling [53].

As known, GH is one of the growth factors using the JAK/STAT pathway as an intracellular signaling pathway for exerting its actions. In fact, the inhibition of this GH signaling by SOCS members has been defended as a key factor affecting GH effect along with vasoinhibins. Moreover, GH induces the expression of CIS and SOCS1–3, which suggests that these proteins may also play a physiological role in the regulation of GH secretion. SOCS2 seems to act downregulating GH activity [58]. In this sense, pro-inflammatory cytokines such as IL-1β or TNFα, and endotoxins, which are frequently increased in inflammatory states such as DM or in patients suffering from severe peripheral ischemia, may induce SOCS proteins which could lead to a GH insensitivity. Nevertheless, SOCS2 has been found that paradoxically may upregulate GH signaling at high concentrations in mice [58]. Thus, both uncontrolled inflammation and infection at wounds may block the action of growth factors using this pathway, and delay the healing (Figure 5).

Although not properly known, the JAK/STAT signaling is regulated by SOCS proteins, having an influence on the action of cytokines and growth factors, as well as on the cells involved in the wound repair process [52]. SOCS have been related to inflammatory diseases,
since in the absence of SOCS3, IL-6 acts decreasing tumor necrosis factor alpha (TNFα), by inhibiting the STAT3 signaling. It has to be highlighted that immune response, crucial in wound healing, is modulated by IL6 [59]. SOCS4 and SOCS5 have also been linked to EGF signaling, regulating its receptor (EGFR), and affecting its signaling capacity in senescent fibroblast cells [53].

The effects of the JAK/STAT pathway seem to be time-dependent: positive and protective in early phases, while negative and inhibitory in the later chronic phase [60].

Upregulation of the STAT genes and activation of the STAT proteins have been directly linked to wound healing in intestinal epithelial cells [61].

Further investigations into cellular and molecular mechanisms and signaling pathways involved in wound healing, and methods of activating senescent cells through various treatments will add possible benefits on this process in the future; therefore GH, the anti-aging factor for excellence, could be one of them.

2.2. GH, circadian rhythm disorders, and wound healing

All organisms have an adaptive mechanism, and several of their functions are synchronized to environmental factors and possess biological clocks that endogenously estimate the time. Consequently, functions such as the sleep-wake cycle and secretion of various hormones
exhibit a rhythm with a characteristic period of ~24 hours (the so-called circadian rhythms). There is a relationship between feeding, the organs involved in food intake, metabolic networks, and circadian physiology. One of the most important endocrine axes involved in circadian rhythm is the axis Ghrelin-GH-IGF-1. The coordinating role of these hormones lies in regulating appetite, behavior, growth, and cell proliferation, with a clear influence in the metabolic regulation of nutrients and all those processes dependent of them, as it is wound healing. Some hormones have been implied in the regulation of circadian GH production, as cortisol, thyrotropin (TSH), and insulin, in addition to some important neurotransmitters [62].

Although GH is mainly released by the anterior pituitary gland, there is a peripheral GH production in practically all the organism, highly dependent on developmental stages, at the level of tissues as nervous system, or the immune, cardiovascular, gonadal, and musculoskeletal system. This peripheral GH plays an autocrine and paracrine role [63]. In humans, plasma GH shows a circadian pattern of secretion, different according to sex and age; during puberty, the hormone reaches its highest plasma values, but once puberty ends, the secretion of the hormone begins to decline until being practically undetectable in elder people [64].

The circadian pattern of GH is affected by nutritional status (caloric intake), age, stress, sex, physical exercise, and lack of sleep. Nutritional status is a key determinant in the regulation of GH secretion; thus, while fasting increases the frequency of GH secretion pulses, while IGF-1 levels decrease, in obesity the opposite occurs, at least during childhood [65]. During fasting, GHR are downregulated [66, 67]. Evidence for a circadian effect on the reduction of human GH gene expression has been demonstrated in response to excess caloric intake [68], and obesity has been associated to the suppression of circulating GH [69].

The highest pulse amplitude of GH secretion is observed during the REM phase of the sleep, while sleep deprivation leads to a strong inhibition of nocturnal GH secretion [70, 71].

Several studies have shown that prolonged sleep deprivation, with the subsequent stress, leads to a reduction in body mass, elevated energy metabolism, changes in circulating hormones, and loss of immune system integrity [72]. Stress mediators act on immune cells to modulate the production of key regulatory cytokines [73, 74]. Thus, circadian rhythm disorders affect the levels of IL-1, IL-2, IL-6, TNFα, natural killer cells, adrenocorticotropic hormone (ACTH), cortisol, GH, and melatonin, all of them playing a key role in wound healing [75–77]. Melatonin has potential effects on the immune system, as inhibition of pineal melatonin synthesis with propranolol or pinealectomy results in immunosuppression and negative effects on wound healing [78]. Yet another study found that melatonin improved wound healing when given at night, coinciding with its normal circadian period of secretion [79].

Notwithstanding all these data, some studies disagree with the concept that sleep exerts a predominant influence on GH release and its effects whatever the conditions be, as it seems to occur compensatory mechanisms promoting GH pulses during wakefulness [80].

Thus, GH influences on wound healing progression. Physiologic circadian rhythm, with higher levels of the hormone during the night, will make a faster healing of wounds during the night, and the alteration of this pattern by different factors might exert a deleterious effect on wound healing via GH and others hormones related to it, although compensatory mechanisms have been described in the long-term.
IGF-1 and wound healing

IGF-1 is considered as the main mediator of GH actions, and it has been considered as the “authentic” GH, at least for growing, although GH exerts many actions directly without the participation of IGF-1 [27].

IGF-1 is a polypeptide structural and functionally similar to insulin. It is produced in the liver and practically all extrahepatic tissues, and its production depends not only of GH, but also is strongly influenced by the nutritional status of the organism, at least in the liver. The local production of IGF-1 has been shown to regulate many physiological and pathophysiological states such as fetal development, atherosclerosis, and tissue repair. During tissue repair, IGF-1 is secreted by platelets, macrophages, and fibroblasts of the wound [5].

In wounds, IGF-1 increases protein production and cell proliferation and migration, which are crucial in the healing process [81, 82]. IGF-1 expression is enhanced in subcutaneous [5], and incisional [83] wounds, and in postburn injuries [84]. Some studies have shown that the administration of exogenous IGF-I enhanced protein synthesis in severely burned experimental animals [85].

Moreover, the levels of this growth factor are reduced in the wound environment of diabetic patients. Wound-related parameters as proteins, DNA, hydroxyproline, and macrophages have been shown to be decreased as a consequence of diabetes. After 14 days of treatment with IGF-1 in rats with diabetes produced by streptozotocin, it was observed that the total values of hydroxyproline, DNA, proteins, and macrophages increased by 48, 52, 31, and 40%, respectively [5]. These data support the fact that the suppression of IGF-1 and the macrophage function impairment within the wound environment by the diabetic state are responsible, at least in part, for the delay of wound healing in this disease.

In this context, the relationship between the IGF-1 receptor (IGF-1R) and the estrogen receptor (ER) is of interest. Locally administered IGF-1 promotes wound repair in an estrogen-deprived animal model, the ovariectomized (Ovx) mouse, mainly by dampening the local inflammatory response and promoting re-epithelialization. Using specific IGF-1R and ER antagonists it has been shown how IGF-1-mediated effects on re-epithelialization were directly mediated by IGF-1R [86]. In contrast, the anti-inflammatory effects of IGF-1 were predominantly mediated by ERs, in particular ERα (Figure 6). When ERa-null mice were used, IGF-1 could not promote healing and local inflammation increased [86]. These findings illustrate the great complexity of interactions between growth factors at the cutaneous level.

Recent data on the systemic administration of IGF-1 have shown an apparent lack of effect on wound healing. Therefore, perhaps only the IGF-1 produced locally by fibroblasts and macrophages contributes to the regulation of wound healing [46, 47], although it is also possible that the dose used and the type of administration do not have been the most appropriate in this case. If the systemic IGF-1 is ineffective in wound healing, topical administration of IGF-1 could be considered, as other growth factors such as EGF, TGFβ, or the own GH. In addition, IGF-1 systemic administration produces mild complications as hypoglycemia and hypotension. These limit its clinical usefulness.
Figure 6. Local expression of IGF-1 in a wound. This expression may be induced by GH, but also IGF-1 may proceed from platelets, macrophages, and fibroblasts. Local IGF-1 induces protein synthesis and cell proliferation by interacting with its receptor IGF-1R, and also has anti-inflammatory effects, although in this case IGF-1 seems to act via the estrogen receptor α (ERα).

4. Analogs of growth hormone-releasing hormone (GHRH) and wound healing

The complexity of GH regulation seems to be related to the multiple roles that GH plays in the human body, very far than those classically thought [27]. Interestingly, some of these roles are played in conjunction with GH-stimulating factors.

As described, growth hormone-releasing hormone (GHRH) is an important neuroendocrine peptide secreted by the hypothalamus, regulating the synthesis and release of GH [87]. Classically, it was thought that the role of GHRH simply was the regulation of the synthesis and secretion of GH [88, 89]. However, the detection of GHRH and its receptors, as well as the expression of GHRH gene in several extra-hypothalamic tissues, including placenta, ovary, testis, digestive tract, and tumors [90, 91], suggests that GHRH plays a wider role than simply acting on the regulation of pituitary GH secretion; in fact, it seems to be particularly involved in conditions aimed at tissue regeneration and repair. The presence of the peptide in peripheral tissues highlights the possibility that locally produced GHRH might act as an autocrine growth factor playing a role in cell proliferation. In addition to its own actions in various tissues, several GHRH agonists have been developed showing that the effects of this neuropeptide could include direct actions on wound healing. For example, a pioneer work demonstrated that the GHRH agonist JI-38 stimulates the proliferation and migration of mouse embryonic fibroblasts (MEF) [92]. The upregulation of GHRH receptor (GHRH-R) and its splicing variant 1 (SV1) in GHRH-R negative 3T3 fibroblasts has been shown to promote its proliferation when GHRH and its analogs are given [93, 94]. Despite it is logical to think
that fibroblast stimulation by GHRH agonist could be mediated by GH/IGF-1. Some authors have found that using MR-409 and MR-502 GHRH agonists, there was a promotion of wound healing by stimulating the proliferation and survival of dermal fibroblast through phosphorylation of the ERK1/2 and AKT pathways, although neither GH nor IGF-1 was found to be significantly increased in fibroblasts after 4 hours exposure to these agonists. Moreover, none of the agonists showed an effect on the expression levels of either IGF-1 receptor (IGF1-R) or its phosphorylated isoform. Thus, these findings imply direct effects of GHRH and its agonists on extra-pituitary cells and tissues [95].

GHRH affects the proliferation of fibroblasts as well as their migration and the expression of smooth muscle actin α (α-SMA) [92], which is organized into stress fibers and exerts contractile forces on the extracellular matrix [96]. Therefore, it seems that GHRH can regulate, simultaneously, both the kinetic profile and the differentiation of fibroblasts in myofibroblasts (Figure 7).

The suppression of growth of fibroblasts in not healing-wound environment is partially due to the decreased sensitivity of resident cells and rapid degradation of growth factors used in different therapies by proteases released from inflammatory cells and bacteria [97, 98]. Therefore, it would be necessary to have a factor that exerted a strong mitogen action on the fibroblasts, while being resistant to proteolytic degradation. In this sense, unlike the natural GHRH [95], the above mentioned MR agonists seem to have an increased resistance to degradation by proteases, because many of the coded amino acids in the peptide chain have been replaced with synthetic non-natural and/or non-coded amino acids which are much less susceptible to such degradation [99]. Consequently, these analogs have demonstrated a greatly prolonged half-life in vivo, making them promising agents for use in wound healing, where an environment rich in proteases is often found. Even more, it was found that MR class agonists do not stimulate tumor growth or neoplastic transformation [95].

Another factor supporting the use of GHRH agonists has been found in human dermal microvascular endothelial cells (HDMEC), that seems to express both pituitary GHRH-R and its splicing variant 1 (SV1). HDMEC is responsible for angiogenesis, a critical event for granulation tissue formation [95].

The endogenous GHRH produced by fibroblasts regulates its own activity, and the role that GHRH signaling may play in physiological maintenance of wound healing could improve with some GHRH agonists.

The high concentration of glucose in diabetic patients inhibits the proliferation of fibroblasts and favors resistance to growth factors, decreasing wound healing. Interestingly, MR-409 enhances the survival of transplanted pancreatic islets and helps to lower blood glucose in diabetic SCID mice [100]; therefore, it would be interesting to investigate whether it might benefit diabetic wounds which are hard to cure, partially because of the special adverse bacterial environment. However, some other aspects of diabetic injuries should also be addressed; such is the case of the affectation of the neuropathic response, the true conductor in this process.

Despite these data, the precise physiologic and biochemical mechanism for GHRH accelerating wound healing remains unclear. Besides, the production of GHRH in dermal wounds still seems not to be clear. Moreover, given its short lifetime, it is unlikely that plasma GHRH
may reach adequate levels to contribute to wound healing. A possibility, not explored, is that some GHRH agonists produced in dermal wounds during healing might be responsible for the activity of GHRH on wound healing.

Whether this apparently novel function of GHRH is operational in a different kind of healing or it is indicative of the activity of a structurally related peptide(s), should be investigated more extensively to elucidate some of the basic aspects of skin biology and repair, as well as in view of its potential implications in therapeutic wound healing.

5. Wound healing in catabolic states: the role of growth hormone

The balance between anabolic and catabolic states and hormones may affect wound healing, since the overall protein compartment status has a great influence on this process [101]. Protein synthesis restores and maintains lean body mass, composed of muscle, skin, and the immune system, all of them having a role during wound repair. When anabolic activity decreases, as occurs during stress, aging, or chronic disease, there is a derivation of proteins to the energy compartment and, therefore, affects wound healing as a result of protein depletion in the wound to restore lost lean mass. Impaired immunity and healing during catabolic states are directly proportional to the degree of lean mass loss [102, 103]. Protein depletion appears to delay wound healing by prolonging the inflammatory phase (inhibits fibroplasia, synthesis of collagen, and proteoglycans), affects the proliferation phase (neoangiogenesis) and inhibits wound remodeling [104]. It has been shown that protein depletion models produce a decrease
in tensile strength of wounds in animals, and rats fed with a protein-deficient diet showed a decrease in wound integrity and resistance as compared to control animals [105].

Burn injury induces acute and severe inflammation and a hypermetabolic state which are strongly correlated to the size of the burn [106]. The inflammatory process reaches a peak during the first week postburn and persists to a lesser extent throughout convalescence [107]. The hypermetabolic state begins 5 days after the burn, and may last up to 1 year after the injury, with energy requirements that reach 150–200% of the basal metabolic rate [108].

GH is one of the most important anabolic hormones and, like other anabolic hormones, has an anti-cortisol activity, lowering the catabolic response of this steroid, without altering its protective anti-inflammatory activity. Many studies have demonstrated the usefulness of anabolic hormones in existing wounds in catabolic states. However, it remains difficult to determine whether the benefit is due to the increase in the systemic anabolic state or to a direct effect on the anabolic state of the wound [109].

Starvation and intense exercise, both being catabolic states, are potent stimuli of GH, while acute or chronic injury or illness inhibits GH release, especially in the elderly [109]. GH leads to an increased influx of amino acids into the cell, decreasing the flow of these from the same. The increase in fatty metabolism that GH produces is also beneficial, since it preserves the amino acids for the synthesis of proteins, instead of being used as an energy resource.

Severe burns and injuries, people with HIV infection with wasting and elderly people, all of them catabolic states, are populations that could benefit from GH therapy. GH increases lean mass, muscle strength, and immune function in these states, but requires an intake of a high-protein, high-energy diet [109].

The skin is a target tissue for GH, and GHRs have been found on the surface of epidermal cells. Recent data indicate that IGF-1 and insulin also provide some of the anabolic effects of GH therapy in wounds [110, 111]. GH administered exogenously increases the thickness of the skin even in normal people [112]. It has been shown that GH can improve the re-epithelialization rate of sites where a skin graft has taken place in adults and children with severe burns or trauma [7, 10]. In addition, it has been seen, in experimental models, that GH also accelerates the healing by increasing wound collagen content, granulation tissue, and wound tensile strength, as well as the local production of IGF-1 by fibroblasts [109, 113].

A study conducted on burned children also supports the role of GH in catabolic states, since no differences were found in mortality, organ failure, or clinically significant morbidity between the groups, and the requirements for albumin supplementation were reduced by 65%, as well as episodes of hypocalcemia, an unexpected benefit of the hormone [114]. As it will be discussed at the end of the chapter, unlike it happens in children, it has been reported an increase of mortality in adult with burns when GH was used [115]. However, the authors of the study in pediatric population have been treating severely burned children with rhGH for more than 10 years, and they have reported that 0.2 mg/kg/day of rhGH in this catabolic state has some benefits, accelerating donor site wound healing by up to 30% and reducing a 25% the hospital stay and costs. They have also shown that GH increased protein synthesis by more than 25%. Another study has also found that GH causes significant serum elevations in
other different parameters as total catecholamines, insulin, glucagon, or free fatty acids. GH therapy even showed a rise in blood flow of the leg [114].

In summary, the use of GH together with adequate nutrition and protein intake, at the appropriate doses, clearly improves anabolic activity and, as a consequence, positively impacts wound healing, even in patients with spinal cord injuries, as Figure 8 shows. Although many data suggest that the effect of GH on wound healing can be direct, it is still unknown whether some other hormones could contribute to this positive effect.

5.1. Ghrelin, GH, and wound healing

Ghrelin (GH-releasing peptide or GHRP) is a small peptide found in the gastrointestinal tract in 1999 [116]. Although it is mainly secreted by the stomach, it is known that Ghrelin is also produced in other territories, such as the intestine or placenta, for example.

In addition to its known actions on the regulation of appetite and energy expenditure, it has also been discovered that this hormone plays a role in the control of inflammation and metabolism, as do leptin and adiponectin. In fact, all three hormones are interrelated in chronic disease states [117–119]. Interestingly, in a study that addressed the relationship between these hormones in burns, the authors came to the surprising conclusion that they acted in two different ways: one in normal physiological conditions or chronic disease states, and another after severe acute stresses such as burn injury [120]. This can be an adaptive mechanism that depends on the physiological situation or the type of the pathological condition.

Recently, it was demonstrated that Ghrelin improves hemodynamic and metabolic alterations and attenuates cancer, heart affectations, and cachexia induced by burns, and also again protects the damage induced by burns and facilitates the healing of wounds [121].

In relation to the hemodynamic role of this hormone, receptors for Ghrelin have been found in the aorta, the left cardiac ventricle, and the left cardiac atrium in rats. In healthy humans, the intravenous infusion of Ghrelin decreases blood pressure, increases the cardiac index, and produces a greater volume of the pulse [122].

Ghrelin also has an anti-inflammatory effect, by inhibiting the secretion of IL-6 and TNFα from monocytes and T5 cells [119, 123]. The protective role of Ghrelin appears to depend on the integrity of GH/IGF-1 axis, since in studies of inflammation with pancreatitis, protection against inflammation did not occur in hypophysectomized rats unless they received IGF-1 in parallel with Ghrelin. In these studies, when normal GH secretion was reached, the

Figure 8. Evolution of a pressure ulcer in the foot of a quadriplegic patient (complete spinal cord injury, C5-C6) treated with GH applied topically (0.4 mg/day, 5 days/week), before the treatment (10/10/2012) and throughout it until the healing of the wound (02/27/2013).
inflammation was reduced in severity, with a more rapid regeneration of the pancreas, resulting in a reduction in the serum concentrations of interleukin 1-β pro-inflammatory (IL-1β) as well as the amylase and lipase activities. In addition, there was an increase in pancreatic blood flow, and DNA synthesis increased in this organ. This demonstrates that the possible role of Ghrelin during catabolic states needs an adequate functioning of the GH/IGF-1 axis [124]. This last statement has also been supported by models of colitis in which treatment with Ghrelin clearly improved the area of damage in the colonic mucosa in intact pituitary rats, but increased it in hypophysectomized animals. In addition, it was shown that rats with a normal production of GH-IGF-1 had improved blood flow in the colonic mucosa and increased mucosal cell proliferation while treated with Ghrelin, as well as reduced levels of IL1-1β and myeloperoxidase; just the opposite of what was found in hypophysectomized rats [125].

The therapeutic effect of Ghrelin on wound healing has also been evaluated using a rat model in which the administration of radiation was combined with the induction of a wound. The altered healing of a wound caused by radiation often occurs in clinical practice and the exact mechanisms by which this occurs are not yet clear. In this wound model, the administration of Ghrelin promoted the healing of skin wounds, and also reduced the average time of wound closure [126]. Ghrelin inhibited the induction of serum pro-inflammatory mediators, especially TNFx, and promoted wound healing in a dose-dependent manner [127]. After the isolation and analysis of the granulation tissues, a greater synthesis of DNA, hexosamine, nitrate, and nitrite, a high content of collagen and an enhanced neovascularization was observed after treatment with Ghrelin. The hormone also increased the expression of VEGF and TGFβ, responsible for wound healing as described. Again, when a GH 1a secretagogue receptor blocker (GHS-R1a) was administered, all of these therapeutic effects of Ghrelin were affected [126]. These results identify Ghrelin as a peptide that could be used for the affected wound healing induced by radiation, although it is necessary that there is a normal secretion of GH so that its effects occur. These effects of Ghrelin are shown in Figure 9.

5.2. Cellular senescence and wound healing: benefit of GH therapy

Cellular senescence is the consequence of DNA damage secondary to oxidative stress associated with aging or chronic morbid conditions such as diabetes. This seems to be an antitumor mechanism [128]. The number of senescent cells is low in young individuals, while it increases with age in all tissues, including the skin [129, 130].

At skin level, senescence has been reported in keratinocytes, melanocytes, endothelial cells, epithelial cells, T-lymphocytes, and even in stem cells [131–133].

This concept has emerged as a possible cause of general tissue dysfunction [134, 135], since, although senescent cells are unable to divide, they remain metabolically active. This high metabolic activity is associated with the release of a multitude of cytokines, chemokines, and pro-inflammatory growth factors, which leads to its denomination as the secretion phenotype associated with senescence (SASP) [136]. These factors would include interleukin (IL) 6 and IL-8, chemokines such as monocyte chemoattractant protein (MCPs), macrophage inflammatory proteins, and growth factors as VEGF, granulocyte/macrophage colony-stimulating factor (GMCSF), TGFβ, and proteinases such as matrix metalloproteinases [128, 137]. All these
Figure 9. Ghrelin effects on a wound. While many positive effects appear at very different levels during wound healing, there is a need for a normal pituitary secretion of GH, so that these Ghrelin effects can occur. Therefore, it is not clear whether these effects depend on Ghrelin or on GH, although the possibility exists that GH could induce Ghrelin expression in the wound.

Factors can act in an autocrine and paracrine way, also having effects on the surrounding cells and their environment. Therefore, the senescent cell itself could initiate a feedback mechanism by spreading this phenomenon to nearby cells [138].

Characteristically, the inflammation resulting from cellular senescence is sterile or is not associated with pathogens [137]. It has been suggested that chronic low-level inflammation that is often observed during aging in tissues without obvious infection is due to senescent cells and SASP [139]. In addition, a low number of senescent cells can have systemic effects, and it is already evident that the senescence process can be transmitted to normal cells by SASP in a paracrine or autocrine manner [128].

The basis of this senescence is mitochondrial dysfunction, which in turn causes oxidative stress, which has been implicated as a cause of aging [140].

Understanding this process would help develop different strategies that could mitigate chronic inflammation and, therefore, cellular senescence. These dysfunctional and destructive signs are also found in the wounds of diabetic or elderly patients, altering the normal healing process.

At this point, it is important to note that GH is a mitochondrial protector [141–143], therefore, playing a positive role in this process. GH restores the redox imbalance, improving the mitochondrial respiratory chain and the production of energy.

In situations of GH deficiency (GHD) there is an accelerated aging process. In mice with GHD, GH replacement therapy increases stress resistance by altering the functional capacity of the glutathione S-transferase system (GST) through the regulation of specific members of the GST family [144]. The hormone also affects the regulation of thioredoxins (TRX) and glutaredoxins (GRX), which are factors that regulate the post-translational modification of proteins and the redox balance, also influencing resistance to stress [144]. Patients with GHD show a decrease in their life expectancy with a twice higher risk of death from cardiovascular disease. In this regard, after 24 weeks of GH replacement therapy in the GREAT
study, the hormone significantly lowered plasma diacron-reactive oxygen metabolites and improved endothelial function, as measured by reactive hyperemia index [145]. This indicates that GH can exert a protective role in redox balance in GHD, in which predominates a pro-oxidant environment, corrected by short-term GH administration [146]. Klotho, a GH-releasing factor that currently is gaining in interest, also lowers the oxidative stress, decreasing apoptosis and senescence of the vascular system in an atherogenic risk rat model [147]. The hormone also affects the regulation of TRX and GRX, which are factors that regulate the post-translational modification of proteins and the redox balance, also influencing resistance to stress [41]. As a consequence of the antioxidant action of GH, the hormone produces a benefit in the inflammatory state associated with senescence [22]. It has been reported that this protection against oxidative stress is mediated by GH induction of the RAS/ERK pathways [148].

However, the exact role of GH in the redox equilibrium has not been fully understood, since in some cases of oxidative stress, overproduction, or administration of GH in excess may enhance oxidation [149]. Thus, both the overproduction of GH and its deficiency are closely related to increased oxidative stress.

5.3. Contrary studies not supporting a GH role in wound healing

As described in the introduction, GH needs specific stimuli to exert its effects. In fact, there is a study carried out to determine the effect of rhGH on the rate of wound healing in normal individuals. In each subject was performed a split-thickness wound in one buttock and a full-thickness wound in the other. The full-thickness wound healed significantly more slowly in the group treated with rhGH compared to the control group treated with placebo, while no statistically significant difference was observed in the healing of the split-thickness wounds. This study concluded that rhGH may delay healing in normal patients with full-thickness wounds, although it could not be ruled out if the healing delay associated to rhGH group was due to the quality of the scab, thereby, appearing only as an alteration of the wound healing process [18].

In another trial, the serum levels of some hormones, GH, insulin, and cortisol were analyzed in normal and diabetic rats during wound healing. It was shown that the rate of wound healing in normal rats is faster than that of diabetics. The serum insulin concentrations were lower in the diabetic rats compared to the normal and control groups and showed a correlation with the wound healing process in diabetic rats. Serum cortisol concentrations decreased in the normal and diabetic groups during wound healing, but did not show a significant correlation with this process. Serum GH levels did not change significantly in any of the groups, nor did they show a significant correlation with the wound healing process [150]. As described above, a possible explanation for these findings is that the main effect of GH in this case could occur as a consequence of the local production of the hormone, something that was overlooked because it was not measured. A small wound on the back of the animal is not a stimulus strong enough to increase systemic GH, which seems to be related, as demonstrated, to more intense catabolic states.

A recent report from two prospective, randomized, double-blind, placebo-controlled Phase III trials conducted in Europe, which studied the effects of rhGH in critically ill burned adult patients, in
an intensive care unit, revealed a significant increase in mortality among catabolic patients treated with rhGH (42 vs. 18%) [115]. GH, in fact, can increase cell adhesion molecules (CAM), since the serum of healthy patients treated with GH significantly increased the expression of VCAM-1 in cultured umbilical vein endothelial cells [151], and this could be the mechanism involved, but it must be taken into account that in these studies high doses of GH were used (10–20 times greater than the usual treatment dose), which would facilitate the appearance of side effects produced by the hormone. In contrast to these data, when the same study was carried out in burned children, no differences were found in mortality, but other beneficial effects were found.

6. Conclusion

Despite all data here presented, it is necessary to remember that the patient with a problem in the wound healing needs to be addressed in a holistic way. That is, “we do not treat a hole in the patient, but the whole patient”. Normal wounds in healthy people are not a problem. However, a delaying wound always appears in a patient with a morbid condition, normally in an elderly patient or that with a catabolic state or a chronic disease as diabetes mellitus. Therefore, using only a topical wound treatment seems to be an unrealistic approach to healing. However, a total approach will be more beneficial to not only accelerate the healing process but also decrease the possibility of a new wound.

The knowledge of the molecular aspects related to wound repair and tissue regeneration, as well as the whole circumstances affecting also the patients is crucial to success dealing with this topic.

We cannot overlook the high amount of data regarding the role of GH and its secretagogues, not only in the healing process, but also improving the pro-oxidant state of the patients. GH therapy is a cheap and well known drug, and may increase many growth factors when is locally used in wounds. Maybe the combination of appropriate doses of systemic GH and topical application in the wound would be a good option. The combination of GH or its secretagogues and IGF-1 in a topical way, could be also a beneficial approach for wounds repair.

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Conflict of interest

The authors declare that no conflict of interest exists.
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