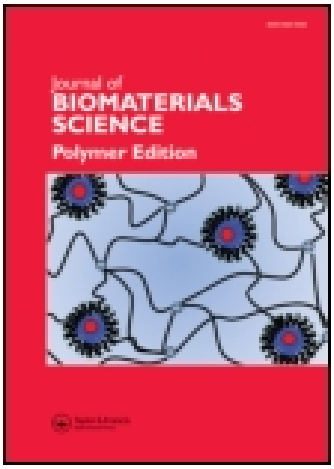

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The human tri-peptide GHK and tissue remodeling

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Review

The human tri-peptide GHK and tissue remodeling

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Abstract—Tissue remodeling follows the initial phase of wound healing and stops inflammatory and scar-forming processes, then restores the normal tissue morphology. The human peptide Gly-(L-His)-(L-Lys) or GHK, has a copper 2+ (Cu^{2+}) affinity similar to the copper transport site on albumin and forms GHK-Cu, a complex with Cu^{2+}. These two molecules activate a plethora of remodeling related processes: (1) chemoattraction of repair cells such as macrophages, mast cells, capillary cells; (2) anti-inflammatory actions (suppression of free radicals, thromboxane formation, release of oxidizing iron, transforming growth factor beta-1, tumor necrosis factor alpha and protein glycation while increasing superoxide dismutase, vessel vasodilation, blocking ultraviolet damage to skin keratinocytes and improving fibroblast recovery after X-ray treatments); (3) increases protein synthesis of collagen, elastin, metalloproteinases, anti-proteases, vascular endothelial growth factor, fibroblast growth factor 2, nerve growth factor, neutrotropins 3 and 4, and erythropoietin; (4) increases the proliferation of fibroblasts and keratinocytes; nerve outgrowth, angiogenesis, and hair follicle size. GHK-Cu stimulates wound healing in numerous models and in humans. Controlled studies on aged skin demonstrated that it tightens skin, improves elasticity and firmness, reduces fine lines, wrinkles, photodamage and hyperpigmentation. GHK-Cu also improves hair transplant success, protects hepatic tissue from tetrachloromethane poisoning, blocks stomach ulcer development, and heals intestinal ulcers and bone tissue. These results are beginning to define the complex biochemical processes that regulate tissue remodeling.

Key words: Gly-His-Lys; copper; wound healing; remodeling; regeneration; stem cells.

INTRODUCTION

Tissue remodeling

Tissue remodeling is a poorly understood process that restores normal tissue morphology after various types of injuries. This remodeling process stops the initial inflammatory events and scar-forming processes after injury, then slowly removes, over a period that can last from a few weeks to 20 years, the scar tissue and cellular

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debris arising from injuries. This process is most active in the young skin of children where most scars and blemishes are swiftly removed. However, the process slows as we age and it can take years for mature skin to remove scars.

When this remodeling stage of repair is incomplete, such as often occurs after radiation treatments or in elderly patients, the tissues can remain locked in a chronic and painful inflammatory stage that produces further scar formation for years after the original injury. Also, the failure of implanted medical devices such as heart valves and artificial hips is often due to the formation of scar-like fibrous tissue around the implant that disrupts the device's performance.

While the human tripeptide GHK (Glycyl-l-histidyl-l-lysine) was discovered in 1973 by Pickart and Thaler [1], and certain wound repair properties were observed in 1985 [2], it was only in 1999 that Maquart *et al.* postulated, on the basis of observed patterns of RNA and protein synthesis after administration of the copper (II) complex of GHK (or GHK-Cu) into implanted Shilling–Hunt wound chambers in rats, that GHK-Cu functioned as an activator of tissue remodeling [2].

The best direct evidence supporting this remodeling function role came from a series of facial studies in women that gave statistically significant evidence of skin remodeling. The blinded, placebo-controlled studies revealed that cosmetic GHK-Cu products, applied to uninjured skin, increase skin collagen, reduce skin irritation and redness, tighten loose skin, improve elasticity, thicken older skin, improve firmness, reduce fine lines and depth of wrinkles, smooth rough skin, improve overall appearance and reduce age spots, photodamage and hyperpigmentation. These studies are detailed below.

In the last few years, additional reports have given further support to this proposal and this paper is a review of the most important publications on the actions of GHK and GHK-Cu. The combined information arising from studies on GHK is beginning to provide the first unified understanding of the complex biochemical systems that mediate the remodeling of skin and other tissues.

Acute-phase fibrinogen, GHK and copper

GHK was discovered during studies on the causes of the increased "acute-phase" blood levels of fibrinogen that occur during normal human aging and also after tissue injuries. Fibrinogen is a blood protein that is polymerized into fibrin, a mesh that forms a hemostatic plug or clot. Pickart and Thaler found that a low-molecular-weight fraction in human plasma that suppressed the acute phase rise of fibrinogen in mice and that this activity was due to the presence of a complex of GHK and copper 2+ (Cu^{2+}) [3].

GHK is a normal constituent of human plasma, saliva and urine. The molecule has a very high affinity for Cu^{2+} and forms the chelate GHK-Cu. In plasma, the plasma level is about 200 ng/ml (10^{-7} M) at age 20 but declines to 80 ng/ml by age 60 [4–7]. GHK is generated during proteolytic degradation of proteins of the extracellular matrix after tissue injury [8] and probably during normal tissue turnover. Maquart *et al.* first pointed out that, while the sequence GHK is

a relatively rare sequence in most proteins, since its presence in a protein creates a copper-binding site, the sequence is relatively high in proteins of the extracellular matrix [9], especially SPARC (secreted protein acidic and rich in cysteine), a protein that regulates endothelial cell shape and function during repair processes [8].

The biochemical uniqueness of GHK resides both in its very small size, which would permit it to approach membrane receptors more easily than larger proteins, and its unique copper-binding characteristics that allows copper transfer into and from cells. GHK's affinity for Cu^{2+} (log stability constant = 16.4) is nearly equivalent to the copper transport site on the plasma protein albumin (log stability constant = 16.2) and much higher than similar peptides (Gly-His = 8.7, Gly-His-Gly = 8.5, Gly-Gly-His = 7.6). Lau and Sarkar, using dialysis experiments, found that GHK can exchange copper (II) with albumin. With equimolar albumin and GHK, 42% of Cu^{2+} binds to GHK. At physiologically relevant concentrations of albumin, GHK and histidine, about 6% of Cu^{2+} associates with low-molecular-weight components [10].

The albumin-copper log stability constant of 16.4 is a critical value associated with rapidly exchangeable Cu^{2+} that moves between the blood and the tissues. This fraction is about 5% of total plasma copper and has a turnover time of 2 h. Molecules with a higher or lower pK of binding cannot participate in this rapid exchange of copper. Most other plasma copper is associated with the anti-oxidant protein and acute phase reactant, ceruloplasmin, which has a turnover time of several days. [10] While both albumin and GHK can exchange Cu^{2+} with tissues, there are at least 1000 albumin molecules for each GHK molecule in human blood plasma. Albumin with copper(II) will not mimic GHK-Cu actions on cell functions such as collagen synthesis. Therefore, virtually all transport of exchangeable copper is mediated by albumin and the GHK-Cu actions must be mediated by specialized receptors.

The solution structure of GHK-Cu at pH 7.0 is given in Fig. 1. This structure is based on X-ray crystallography [11] but is consistent with additional studies that used methods such as optical studies, titration data [10], electron spin resonance and electron spin echo envelope spectroscopy [12], nuclear magnetic resonance and electron paramagnetic resonance [13] and proton magnetic resonance [14].

GHK vs *GHK-Cu*

Most reported GHK actions use the copper complex but both GHK and GHK-Cu molecules may each have distinct biological actions because of their interconversion between forms as a function of ionic copper availability. This is further complicated within mammals because of the competition among unsaturated binding sites for exchangeable ionic copper. Plasma albumin can bind 4 μg/ml of Cu^{2+} to its high-affinity site but only carries between 0.05 to 0.18 μg/ml under physiological conditions [10, 15].

Some researchers have tested both GHK or GHK-Cu, others have tested only one of the forms; therefore, I cite the actual version(s) use in various studies.

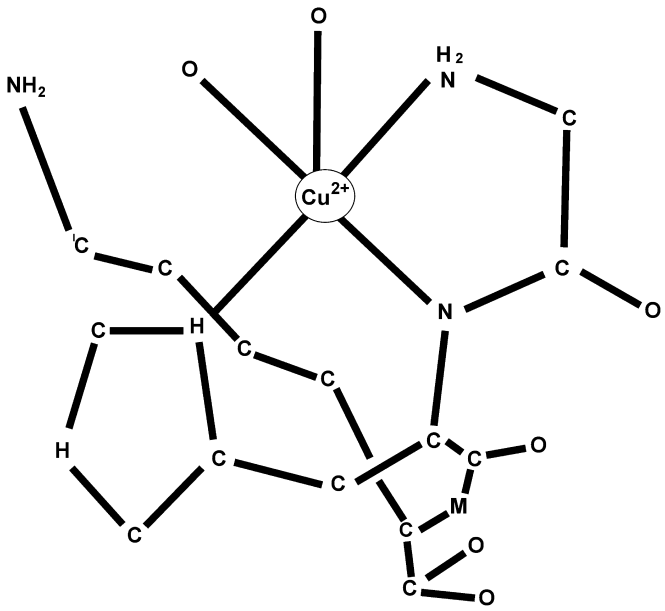


Figure 1. X-ray structure of GHK-Cu.

However, the actual balance of these two molecules depends on the conditions in the physiological milieu of the test system.

BIOCHEMICAL AND CELLULAR ACTIONS OF GHK/GHK-CU

The best understanding of the function of GHK/GHK-Cu is by an analysis of its basic biochemical and cellular actions.

Anti-inflammatory actions

The GHK-Cu system possesses a wide array of actions that suppress the "acute-phase" inflammatory and scar-forming events after wounding. The acute phase is a localized and systemic response to tissue injury that serves to stop bleeding, destroy invading bacteria, and quickly cover the wound surface with a protective layer. If this phase is not suppressed and remodeling is not complete, as often occurs after radiation burns, then scars and inflammation may persist for months or years.

In addition to the suppressive actions on the acute phase increase in fibrinogen synthesis cited above, the molecule also suppresses cellular synthesis or activity of key acute phase cytokines such as tumor necrosis factor-alpha in wounds [16] and interleukin-1 [17], both of which induce further tissue damage after injuries. GHK-Cu also suppresses the production of the scar forming protein, transforming growth factor-beta-1 by cultured normal and keloid-producing human fibroblasts [18, 19].

Since TGF-beta-1 also stimulates the production of other acute-phase reactants, this provides a secondary inhibition of the acute phase response [20].

The presence of iron complexes in damaged tissues proves detrimental to wound healing since they increase local inflammation and microbial infection by supplying iron. GHK-Cu stops the release of oxidizing iron from ferritin and inhibits lipid peroxidation by physically blocking the channels in ferritin involved in the release of Fe(II) [21]. The administration of GHK to mice increased their survival after infection with pathogenic bacteria [22].

In wound healing models, biotinylated GHK acts to increase the production of anti-inflammatory proteins such as copper, zinc-superoxide dismutase that detoxifies oxygen radicals [23, 24], while GHK-Cu increases decorin [25], an anti-inflammatory proteoglycan that protects diabetic rats from kidney damaging fibrosis induced by TGF-beta-1 [26, 27].

GHK blocks lethal ultraviolet radiation damage to cultured skin keratinocytes by binding and inactivating reactive carbonyl species such as 4-hydroxynoneal, acrolein, malondialdehye and glyoxal. This protection was found at a relatively high level of GHK, 20 mg/ml or 0.2%, but this concentration can be easily added to protective sunscreens [28]. Current sunscreens are built around molecules, such as oxybenzone, avobenzene, menthyl anthranilate and octyl methoxycinnamate, that strongly absorb ultraviolet radiation; they later shed the energy by releasing it as free radicals. Such free radical absorbing chemicals prevent sunburn but many of these chemicals have strong estrogenic activities and there is concern over their accumulation in human tissues [29].

GHK also inhibits the damaging glycation of copper, zinc-superoxide dismutase caused by fructose. GHK is more active than carnosine in this respect [28]. GHK-Cu accelerates the recovery of irradiated fibroblasts to the point where treated irradiated fibroblasts approximate the population-doubling time of normal controls [19].

Chemoattraction of healing cells

GHK-Cu at 10^{-12} to 10^{-10} M is a powerful attractant for capillary cells that build new blood vessels [30, 31] and on macrophages and mast cells that remove damaged cellular debris and secrete a cornucopia of 20 or more proteins (fibroblast growth factor, epidermal growth factor, etc.) important for tissue healing. Mast cells stimulate wound contraction [32, 33].

Concomitant removal and synthesis of extracellular matrix macromolecules

GHK-Cu simultaneously activates the production of metalloproteinases and anti-proteases that remove damaged proteins from the extracellular matrix macromolecules while activating the synthesis of new proteins for rebuilding the extracelluar matrix. Ehrlich first noted a simultaneous increase in both synthesis of new type 1 collagen and the breakdown of established collagen in wounds treated with GHK-

Cu [34]. Borel and colleagues, using implanted wound chambers, observed that GHK-Cu simultaneously induced both the production of messenger RNAs (mRNA) for new skin ingredients needed to rebuild the skin's extracellular matrix after injury such as collagen, proteoglycans, glycosaminoglycans, chondroitin sulfate and dermatan sulfate; in addition, they observed increased production of the mRNA metalloproteinases (such as MMP-2 and MMP-9) that dissolve damaged proteins plus the tissue inhibitors of metalloproteinases such as TIMP-1 and TIMP-2. In cultured fibroblasts, GHK-Cu stimulates mRNA and protein synthesis with peak stimulation around 10^{-9} M [9, 25, 35, 36]. They ultimately concluded, based on GHK-Cu's biochemical actions, that the molecule functions to activate the skin's remodeling processes [2].

Angiogenesis, anti-coagulation and vascular dilation

GHK-Cu helps re-establish blood flow into damaged tissues through a mixture of three actions: angiogenesis, anti-coagulation and vasodilation. GHK-Cu increases the expression of basic fibroblast growth factor and vascular endothelial growth factor from the wound repair fibroblasts, both of which aid blood vessel formation [19] and is a chemoattractant for capillary endothelial cells at 10^{-10} to 10^{-12} M [30, 31].

GHK-Cu induces angiogenesis (new blood-vessel formation) in rabbit eye models [37]. In rats, it also increases the level of erythropoietin, the protein that induces red blood cell formation [38]. During safety studies, I found that GHK-Cu inhibits platelet aggregation and the formation of vasoconstrictive thromboxane, an action that may reduce localized blood coagulation after tissue damage. GHK at 10^{-8} M binds to the blood pressure regulating protein, angiotensin II AT 1 receptor and competes for binding with the anti-hypertensive drug Losartan which suggests that GHK could exert actions similar to vasodilatory drugs [39]. High levels of GHK-Cu cause extreme vasodilation but this may not be physiological. During safety studies, I found that the intravenous perfusion of 1 g GHK-Cu into goats produces a dramatic fall in blood pressure. In perfused isolated rabbit hearts, GHK-Cu at 20 mg/ml increases blood flow through heart tissue by its vasodilatory action [40]. This may be due to an activation of tissue Cu, Zn-superoxide dismutase which supplies copper ions. Normally only about 50% of Cu, Zn-superoxide dismutase is loaded with the copper required for activity. The persistence of the vasodilatory molecule, nitric oxide, is lengthened by protective actions of superoxide dismutase [41].

Nerve outgrowth

When wound healing proves inadequate, the partially repaired area often lacks sensory abilities. GHK, at 10–200 ng/ml, enhanced nerve axonal outgrowth of cultured cells from chick embryo cerebral hemispheres [42] and from central nervous system tissue from embryonal rats (hippocampus) [43]. When severed nerves are placed in a collagen tube impregnated with biotinylated GHK, there is an increased production of nerve growth factor and the neurotrophins NT-3 and NT-4 [44].

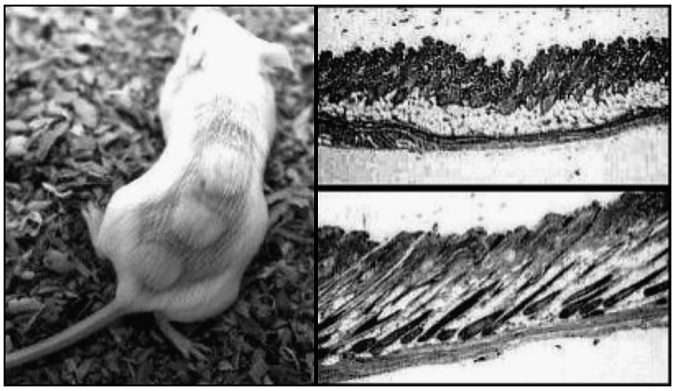


Figure 2. (Left) Mouse was shaved then intradermally injected once with GHK-Cu. (Right) Top, control hair follicles; bottom, greatly enlarged hair follicles.

Stem cell proliferation and differentiation

During wound-healing studies, we noted the GHK-Cu induced greatly enlarged hair follicles at the periphery of the wound (see Fig. 2, changes in mouse follicles after intradermal injection of GHK-Cu). Burn surgeons have long observed that the influx of hair follicles into a burned area predicts a good healing response. It is now established that dermal hair follicles provide a major source of stem cells used for dermal healing [45].

Stem cell proliferation requires extremely low copper concentrations created by the use of copper chelating agents. However, when stem cells are exposed to higher copper levels, they progress into differentiated cells [46, 47]. The lead author of the two last cited references, a member of a stem cell biotech firm (Gamida Cell, Jerusalem, Israel), has claimed in a patent that GHK increases proliferation of stem cells, while GHK-Cu increases their progression into differentiated cells [48].

Further supporting the above are the findings that low tissue copper, in itself, may increase stem cell proliferation and availability in animals. For instance, copper deprivation contributes to the neogenesis of alpha and beta cells in the pancreatic ducts of diabetic rats [49]. Also, feeding diabetes-prone BioBreeding (BBdp) rats a hydrolyzed-casein-based diet, a diet that binds nutritional copper and lowers tissue copper, promotes islet cell neogenesis and results in 2–3-fold fewer diabetes cases compared with feeding cereal-based diets [50].

TISSUE REPAIR STUDIES

A key problem in developing tissue repair strategies is that clinical wounds present a variety of different conditions and situations that inhibit the healing. These include conditions such a extreme age, diabetes, immune depression, difficult-to-treat internal tissues, fresh surgical wounds, radiation ulcers, gastrointestinal ulcers, venous stasis ulcers, infected wounds, attachment of joint implants to bony tissue,

tissue poisoning, and so on. Correspondingly, GHK-Cu has been tested on a variety of healing situations and has been found to have repair actions on the various types of wounds, hair follicles, the stomach lining, the intestinal lining, bone tissue and the liver.

Dermal wound healing

GHK-Cu accelerates healing of uncomplicated surgical wounds models in mice, rats, rabbits, pigs, dogs and horses. GHK-Cu gives a dose-dependent increase in healing up to about 1% ionic copper in the application vehicle. Higher levels of GHK-Cu produce skin irritation.

Initial experiments on dermal healing studied full thickness surgical wounds in mice and rats. When GHK-Cu was injected at the periphery of the wound, the rate of wound closure accelerated. In mice, it proved possible to reattach a full thickness skin graft, with a 40% success rate, and have it reestablished in the wound area. Surgical experts considered this impossible at that time [51].

Ischemic dermal wounds in mice and rats

Ischemic wounds, such as venous stasis skin ulcers, that are common in the elderly, have a inadequate blood supply that fails to supply adequate nutrients and oxygen. These are difficult to heal and often become infected. In rats, a 2% GHK-Cu cream applied to a lesion within an ischemic bipedicle skin flap, produced a significantly faster decrease in the injured area as compared to the control group. By day 13, initial wound area had decreased by 64.5% in the GHK-Cu group, 45.6% in the vehicle group, and 28.2% in the untreated control group. GHK-Cu treated wounds contained significantly lower concentrations of the tumor necrosis factor-alpha (which causes inflammation, swelling, redness and pain) and matrix metalloproteinases 2 and 9 (which dissolve collagen) than control wounds. Thus, while GHK-Cu simulates the production of both proteases (MMPs) and anti-proteases, the balance is toward more anti-protease activity [16].

GHK-Cu vs *zinc oxide in rabbit full-thickness wounds*

Zinc oxide creams are widely used to treat wounds in hospitals. A comparison study of the healing of full thickness surgical wounds in rabbits found that a GHK-Cu cream gave faster coverage of the wound bed with granulation tissue, more wound contraction and a faster reduction of the unhealed area than a zinc oxide cream or the untreated control [52].

Full-thickness pad wounds in dogs

The foot pads of dogs are considered difficult to heal. When tested for improving healing of full thickness pad wounds in dogs, GHK-Cu was injected in a saline solution. GHK-Cu improved the healing rate by day 6 and increased the content of

type-1 collagen in comparison to control wounds. The GHK-Cu benefit was reduced if used under a damp bandage [53].

Localized pig punch biopsy wounds

In pigs, punch biopsies were used to create puncture wounds. The defects were filled with graded amounts of GHK-Cu in a cream or control substances. In a dose dependent manner, GHK-Cu markedly stimulated wound healing and collagen synthesis. The effect is highly localized to the immediate skin area that is treated [54]. Mild thermal burns to the backs of pigs provide an excellent method for determining the healing activity of various formulations of GHK-Cu; however, there have been no descriptive publications on this method.

Diabetic rat dermal wounds treated with biotinylated GHK

Diabetic skin ulcers have become a major health problem with the increase in the elderly population. In the United States alone, currently 650 000 diabetic patients suffer limb amputations yearly due to unhealed wounds that become infected.

A problem with injecting GHK-Cu into wounded tissue is that it is rapidly cleared (within 30 s) from the injection area. To increase its persistence in tissue, biotin was attached to GHK (biotin-glycyl-histidyl-lysine) then incorporated into collagen pads used in rat models. The biotinylated GHK rapidly accumulated copper ion and raised tissue copper 9-fold. This approach was compared to wounds treated with collagen pads only or without collagen (control). The biotinylated GHK increased wound contraction, increased cell proliferation, and increased expression of the antioxidant Cu, Zn superoxide dismutase [23]. This approach was then tested in (streptozotocin-induced) diabetic rats and those treated with biotinylated GHK demonstrated faster healing, an increased rate of wound contraction, higher levels of glutathione and ascorbic acid levels in the skin, increased growth and activation of fibroblasts, and higher levels of mast cells [55].

Systemic wound healing in impaired healing situations

Enhancement of systemic tissue repair would be especially helpful after internal surgeries and after procedures such as implanted artificial joints in elderly patients. GHK-Cu and certain analogs induce a systemic healing response in rats, mice and pigs. The best results are obtained if a series of intramuscular injections are given to rodents (0.1 mg GHK-Cu in 0.1 ml water to 25 g mice; 1 mg in 0.1 ml water to 200 g rats, 30 mg in 1 ml water to 14 kg pigs) over the course of the healing. For example, mice were injected on days 1, 2, 3, 6, 7, 8, 9, 10 and 13, while pigs were injected on days 0, 1, 2, 5, 6, 7, 8, 9, 12 and 13. These treatments markedly increased healing parameters, such as collagen production, angiogenesis and wound closure, in both wound chambers and full-thickness wounds.

To mimic healing patterns common in the elderly or immune depressed patients, rats were implanted with wound chambers, then one group was injected with 1 mg

cortisone acetate for three days to induce an impaired healing response. This cortisone treatment reduces collagen production and angiogenesis approximately 70%. Saline-treated rats served as a control. One group of cortisone-treated rats was treated with 1 mg GHK-Cu in 0.1 ml saline on days 5, 7, 8, 12, 13, 15 and 19. The GHK-Cu treatment raised the production of collagen and angiogenesis above the cortisone-only treated group and increased the healing response to that observed in normal animals [56].

Human wound-healing trials

Early open clinical trials in France on human diabetic wounds and venous stasis ulcers gave evidence that GHK-Cu accelerated healing of skin ulcers [57]. Unfortunately, this type of apparently successful healing cream, containing very low levels of preservatives, was never tested in larger FDA clinical trials. A later controlled clinical trail on venous stasis ulcers failed to reach clinical significance [58, 59]. Another subsequent open study used a 2% GHK gel on 120 diabetic patients, after their skin ulcers were surgically excised of dead, devitalized, or contaminated tissue. The percentage of closure of plantar ulcers (98.5% median area percentage closure compared with 60.8% for vehicle; $P < 0.05$) increased the rate of wound closure three-fold greater than with standard care and vehicle. The incidence of ulcer infections significantly lessened (7% incidence compared with 34% for vehicle, $P < 0.05$) [60]. However, a later blinded study of 530 patients, comparing the GHK-Cu gel with the best standard care failed to demonstrate efficacy. In short, the GHK-Cu gel appeared to improve wound recovery in patients receiving standard care; but the FDA requires that the drug demonstrate effectiveness in comparison with the highest level of standard diabetic skin care (e.g., aggressive standardized debridement of wounds, daily application of a metered dose of insulin, pressure-relieving footwear, patient education and activity modifications). This level of care is rarely found in nursing homes.

Cosmetic remodeling of human skin

Cosmetics containing GHK-Cu are widely used for skin renewal. A clinical study compared the effect on the skin's production of collagen after applying creams containing GHK-Cu, vitamin C, or retinoic acid. Twenty volunteers applied the various creams to their thighs daily for one month. New collagen production was determined by studying skin biopsy samples using immunohistological techniques. After one month, GHK-Cu increased collagen in 70% of those treated with GHK-Cu against 50% treated with the vitamin C cream, and 40% treated with retinoic acid [61].

A GHK-Cu facial cream, after 12 weeks of application to the facial skin of 71 women with mild to advanced signs of photoaging, reduced visible signs of aging, improved skin laxity, clarity, and appearance, reduced fine lines and wrinkles, and increased skin density and thickness [62]. A GHK-Cu eye cream, tested on the

eye area skin of 41 women for 12 weeks with mild to advanced photodamage, was compared to a placebo control and an eye cream containing vitamin K, and performed better than both controls in terms of reducing lines and wrinkles, improving overall appearance, and increasing skin density and thickness [63]. In another 12-week facial study of 67 50–59-year-old women with mild to advanced photodamage, a GHK-Cu cream was applied twice daily and improved skin laxity, clarity and appearance, reduced fine lines, coarse wrinkles and mottled hyperpigmentation, and increased skin density and thickness. Five of the women in this study also applied the GHK-Cu cream to one forearm (the other being the control) followed by histological analysis of biopsies. The GHK-Cu cream strongly stimulated dermal keratinocyte proliferation in forearm skin of the women [64].

Laser-treated human skin

GHK-Cu at 2% in a cream did not reduce post-treatment erythema. However, patient satisfaction was significantly higher for those who used GHK-Cu after laser skin resurfacing [65].

Hair growth

We observed exceptionally large hair follicles develop at the periphery of wounds treated with GHK-Cu from the very first experiments. GHK analogs with added hydrophobic amino acid residues were more effective than GHK-Cu in rat models [66–69]. The details of hair stimulation were studied by (1) phototrichogram, (2) folliculogram (micro-morphometric analysis) and (3) the rate of DNA synthesis in the follicular cells. The effects demonstrated a stimulation of the follicular cell proliferation, resulting in an enlargement of the anagen follicles from vellus to terminal type (therapy to increase hair growth) or a maintenance of the piebald terminal follicles (prevention of hair loss) [70–72].

When rats, pretreated with GHK-Cu analogs, were exposed to cancer chemotherapeutic drugs, hair loss diminished. If rats were initially treated with chemotherapeutic drugs, the GHK-Cu analogs hastened hair re-growth [73].

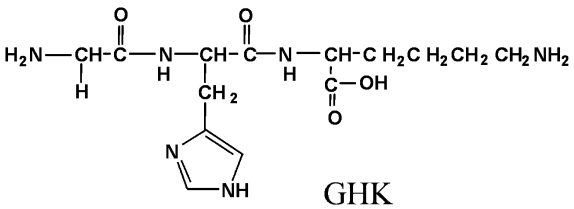
A commercial GHK-CU analog product (Tricomin®), developed to increase hair growth, claims to have boosted hair growth 33% more than that of 2% minoxidil, a proven agent [74].

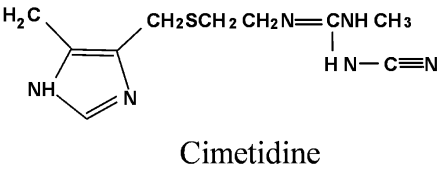
Hair transplants

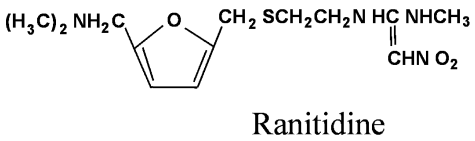
Analogs of GHK-Cu with added hydrophobic amino acid residues are commercially used for hair transplantation in a product called Graftcyte®. In two clinical studies, these analogs reduced skin crusting after transplantation 10 to 14 days to five days and the shedding of hair transplants from thirty percent with saline to ten percent. Re-growth of new hair accelerated by 50%. Patient satisfaction after transplantation rose from 80 to 95% [75, 76].

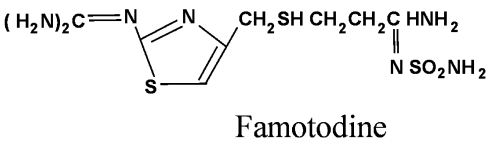
Gastrointestinal repair

GHK has striking similarities to several gastrointestinal anti-ulcer medications such as cimetidine, ranitidine, famotidine and nizatidine (see Fig. 3). The correspondences include (1) an N-terminal side change, (2) a central imidazole ring and (3) a C-terminal lysine-like basic group. Virtually all non-steroidal anti-inflammatory drugs avidly bind copper (II) [77] (see Fig. 3, comparison of GHK and anti-ulcer medications). Saliva has a relatively high level of copper ion (0.22 mg/l) [15] and a variable concentration of GHK (about 20 ng/ml). With this abundance of free copper and little other copper-binding proteins, all of the GHK should exist as GHK-Cu.









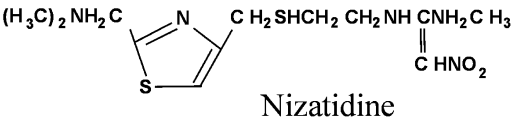


Figure 3. Comparison of GHK and anti-ulcer medications.

When tested in rat ulcer models, GHK-Cu reduces gastric acidity, increases mucous production and inhibits the development of gastric ulcers. Likewise, in intestinal ulcer models, GHK-Cu inhibits ulcer development [78–80]. GHK-Cu and other copper complexes produced a 75% reduction of gastric mucosa homogenates of lipid peroxidation in the range 10–100 mM, suggesting that copper-peptide complexes are able to neutralize oxygen-derived free radicals [81].

One small study of 16 patients with distal inflammatory bowel disease, who were treated with rectally administered solutions of GHK-Cu, found that after 12 weeks' treatment there was a 60% reduction in severity as measured by endoscopy, histopathology and symptoms [82].

Implant attachment to bony tissue

GHK-Cu accelerates the healing of bones by increasing the formation of healing granulation tissue in damaged bones. GHK-Cu has also been shown to increase chondrocyte proliferation and chondrocyte synthesis of collagen types I, II and III. The complex stimulated all collagens but most strongly stimulated collagen type II. In culture, it also increases the growth of human marrow stromal cells and promotes the attachment of human osteoblastic cells. A GHK-Cu gel was shown to promote the filling of bone defects in femurs and bone attachment to cementless endoprostheses. The GHK-Cu gel, tested in rats and guinea pigs for the implantation of cementless endoprotheses, produced vivid osteogenic activity at the interface of bone and metal stem. Such gels may aid in the establishment and retention of artificial joints [83–86].

Block poisoning of liver tissue

Tetrachloromethane (carbontetrachloride) is a toxic organic solvent that causes severe damage to liver tissue. In rats, ten daily intraperitoneal injections of GHK in doses of 1.5 to 450 mg/kg stimulated mitotic activity of hepatocytes and dose-dependently suppressed immune reactivity (number of antibody-producing cells and delayed-type hypersensitivity reaction) [87]. To test GHK's action on tetrachoromethane poisoning, rats were pre-treated with an intraperitoneal administration of GHK at 2.5 mg/kg for 5 days before the administration of a lethal dosage of tetrachloromethane poisoning and 5 days after the poisoning. The GHK protected rats from tetrachloromethane-induced liver damage and the hepatic tissue maintained normal functional activity and immunological responsiveness [88].

PHYSIOLOGICAL FUNCTION OF GHK

Diverse reports from over 40 laboratories (referenced in this article) demonstrate an array of GHK and GHK-Cu actions that is biochemically consistent with a role related to tissue repair after various types of damage. Figure 4 is a possible version of a skin renewal cycle based on the actions GHK and GHK-Cu.

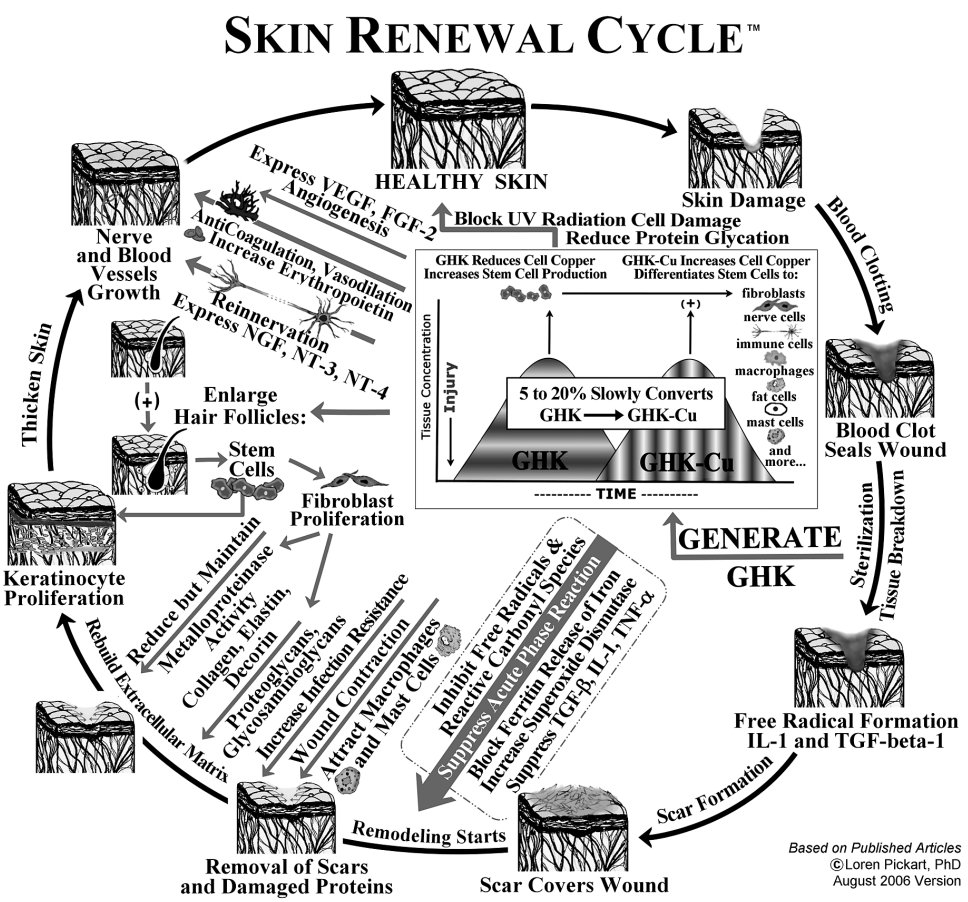


Figure 4. Skin renewal cycle.

These results raise two questions: First, why does this molecule have so many repair actions? It may be that GHK originated from an ancient marine signaling system dependent on levels of copper ion, that was swept into mammals on the tides of evolution, and predates more modern growth factors and cytokines. Free ionic copper proves highly toxic to primitive marine invertebrates such as mollusks, crustaceans, worms, corals, sponges, barnacles and jellyfish. GHK, a potent chemoattractor at 10^{-9} M of barnacle larvae, is structurally similar to natural, but still undefined, larval attractor. Barnacles date back at least 400 million years. Copper plates and copper-containing paints were used in the past to protect vessels from barnacle larvae that settled on the hulls. GHK at the same concentration also induces the metamorphosis of horseshoe crab larvae into the adult form. Horse crabs are one of the oldest classes of marine arthropods and have exhibited little physical change in the last 350–400 million years. GHK may chelate toxic ionic copper into a benign chelate [89]. Even on human skin, ionic copper is very irritating, whereas copper-peptide chelates give anti-inflammatory and healing actions.

The second question is: What is the physiological role of GHK? The molecule has attributes that may maintain tissues in a healthy state by suppressing inflammation and stimulating repair processes. Inflammatory processes, especially types of chronic inflammation, are predictors or contributors to chronic diseases of aging such as cardiovascular disease, kidney failure, osteoarthritis, osteoporosis, Alzheimer's Disease, insulin resistance and diabetes, muscle wasting and frailty. Cytokines and other mediators of inflammation are also predictors of mortality associated with age-related chronic diseases [90–92].

POTENTIAL FUTURE USES OF GHK/GHK-CU

Human wound healing

Despite the successes in the use of various growth factors and cytokines, including GHK-Cu, in laboratory wound-healing models, none of these factors have proven effective for the clinical healing of indolent human wounds and skin ulcers. The failures in these studies seem to arise from two causes. The major problem in using growth factors appears to be the previous unknown influence of bacteria biofilms that colonize skin ulcers and are resistant to antibiotics and anti-microbial agents. These biofilms secrete powerful proteases that can degrade peptide and proteinaceous growth factors in minutes. GHK is quickly broken down by the wound proteases found in diabetic and venous stasis skin ulcers. The second problem is that most wound-healing models use young animals while the major clinical problems exist in older humans with slower healing responses.

Learning from these mistakes, we might try an alternative two-step therapy to clinically repair skin as in the case of diabetic ulcers. During this therapy, we would first increase scar formation and stem cell production. We could accomplish this by using scar-forming proteins such as TGF-beta-1 or smaller peptides that have TGF-beta-1 actions (used in cosmetics to plump and harden skin) followed by the use of copper-binding peptides (without copper) to lower tissue copper in the wounded tissue and facilitate stem cell production. During step one, which takes about three days, we would have created a protective wound-covering scar and hopefully stimulated stem cell production needed for new repair cells. For step two, we would apply copper-binding peptides containing about 0.4% ionic copper or ten times the copper level used in previous clinical studies.

To solve the problem of protease action of the fragile GHK molecule, that is easily broken down by bacteria proteases from wound fluid, we could use mixtures of small peptides of 200–700 Da from soy protein. It should remembered that the proteolysis following wound damage creates a blizzard of small peptides, including GHK, in the damaged area, so wounded tissue can metabolize such peptides. While GHK-Cu has a very strong affinity to bind copper(II) ions and can obtain copper ion from tissue fluids, we tested other small peptides that might stimulate wound healing if pre-loaded with copper ions. Most peptides other than GHK could

not obtain Cu^{2+} from albumin, since their binding constants for Cu^{2+} are only about 10^{+6} to 10^{+9} in contrast to albumin's $10^{+16.2}$. Peptide fragments from soy protein proteolytic digests possessed significant resistance to further proteolysis when chelated to Cu^{2+}. Such peptides have a long history of safe use in cosmetic products. In human and veterinary studies, creams made from these new copper complexes showed superior healing actions in all of my standard animal tests. They accelerated wound closure, produced greatly enlarged hair follicles, possessed anti-inflammatory actions as good as cortisone, increased the synthesis of collagen and elastin, and produced rapid and scar-free healing in dogs after spaying operations and in young horses after leg straightening operations [93, 94].

Safety studies in humans found that creams made from the new copper complexes produced significantly faster skin healing and reduced redness and inflammation after mild skin injuries brought on by tape stripping [95], acetone burns (removal of skin lipids) [96], 24-h detergent irritation [97] and nickel allergy inflammation [98].

Treatment of the inflammatory diseases of aging

The anti-inflammatory actions of GHK/GHK-Cu may open a door to the treatment of various chronic inflammatory conditions that are postulated to be a causative factor in the development of many diseases of aging as mentioned above. For example, low levels of GHK-Cu (that allow an increased expression of TNF-alpha) could increase the incidence of psoriasis, psoriatic arthritis, ulcerative colitis, rheumatoid arthritis, ankylosing spondylitis and Crohn's disease. Very expensive pharmacological TNF-alpha inhibitors, administered by injection or infusion, are used to treat such conditions but produce many side-effects, such as skin redness, itching, pain, swelling, bleeding and bruising. Unlike the pharmacological drugs, GHK-Cu is remarkably non-toxic; the acute lethal dosage in mice and rabbits at 300 mg/kg body weight, far exceeds presumed potential therapeutic dosages for humans.

Among GHK-Cu anti-inflammatory actions, this molecule also suppresses TGF-beta-1 and increases decorin synthesis. When levels of GHK-Cu drop too low, this may encourage an increase of scar-forming TGF-beta-1 and diminished levels of the anti-inflammatory protein decorin, which blocks TGF-beta-1 actions. The increased incidence of kidney fibrosis that comes with age is considered to be a result of excessive production of TGF-beta-1. Experimental therapies that lower TGF-beta-1 by genetically increasing endogenous levels of decorin or by direct administration of decorin have blocked kidney damage in rat models.

The above consideration may give us a window into a reason that organ function declines with age. Low tissue copper is associated with many degenerative diseases and deleterious conditions of human aging. There was much previous confusion on the role of copper in various disease states since plasma copper was elevated in conditions such as rheumatoid arthritis or cardiovascular disease but tissue levels of copper were low in humans and animal models of the conditions. However, supplementation with copper has been reported to increase dehydroepiandros-

terone levels [99], raise brain enkephalins [100], reduce carcinogenesis and cancer growth [101–103], reduce the development of cardiovascular disease [104], reduce the markers of osteoporosis [105], reduce rheumatoid arthritis [101], improve immune function [106], reduce protein glycation [107] and deleterious peroxidation of fats and red blood cells [108], reduce brain developmental defects in offspring [109] and increase anti-oxidant defenses by activation of superoxide dismutase [110]. Sorenson emphasized that the role of copper as a possible approach to treatment of chronic diseases [103]. GHK-Cu may point a way to develop copper based treatments for certain inflammatory diseases of aging.

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