Copper-Peptides for Tissue Regeneration

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Dr Loren Pickart of Skin Biology, a pioneer in the field, describes the use of copper peptide complexes in tissue regeneration

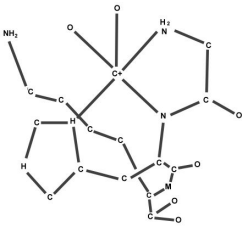
Certain types of copper peptide complexes possess both tissue protection and repair properties. Most information on these effects is based on a human copper peptide complex, glycyl-l-histidyl-l-lysine:copper (II) or GHK-Cu.

GHK-Cu has protective and regenerative actions on several organ systems including skin, hair follicles, bone, gastric mucosa and intestinal linings. These types of copper complexes are increasingly used in cosmetic skin and hair care products and after dermatological skin renewal procedures, such as chemical peels, laser resurfacing and dermabrasion, to improve post-treatment skin recovery.

Ageing reversal experiments

GHK-Cu emerged during my attempts to reverse certain changes that occur during human ageing. The goal was to suppress the synthesis of the blood fibrinogen, a protein that rises with age and rises even more after myocardial infraction. Its blood concentration is an excellent predictor of mortality. Elevated fibrinogen levels increase blood coagulation and decrease tissue perfusion, by increasing the thixotropic properties of blood in the microcirculation.
I found that the albumin fraction of human blood plasma has a suppressive action on fibrinogen synthesis and also improved the survival of the cultured liver cells that produce fibrinogen. Further isolations found these activities concentrated in a low molecular weight fraction that contained GHK-Cu.1 Subsequent work defined the three dimensional solution structure of GHK-Cu and the binding affinities between GHK and copper (II), as shown in Figure 1.2

Figure 1 - Solution structure of GHK-Cu



My colleagues at the University of Washington (Seattle) and I used the structure of GHK-Cu to create analogues that were very potent cell growth inhibitors, inhibiting fibroblast replication at concentrations equivalent to chemotherapeutic drugs, such as cisplatin and bleomycin. During surgical procedures to test these inhibitors on the suppression of tumour growth in mice, GHK-Cu was used as a control substance. It became apparent that GHK-Cu was rapidly healing the surgical incisions.3

Biogenesis, metabolism & structure of GHK-Cu

Later work has more clearly defined the role of GHK-Cu. The molecule is found in human plasma, saliva and urine. Its concentration in plasma is highly variable but approximately 200 ng/ml (10exp (-7) M) at age 20, which later declines to 80 ng/ml by age 60.
GHK is a very rare sequence in human proteins, mainly existing in inflammation-associated proteins and proteins of the extracellular matrix, such as collagen, thrombospondin, fibrin-chain, prokininogen, complement C1q, interleukin 4, skin collagenase, coagulation factor XI and SPARC. During episodes of tissue damage, GHK is generated by proteolysis after injury. Numerous cell culture studies have found that the biologically effective levels GHK-Cu are approximately 10 exp (-9) M. This contrasts with plasma levels of 10 exp (-7) M.4

In human plasma and wound areas, GHK is likely to exist as a mixture of GHK and GHK-Cu. GHK has a high binding affinity for copper (II) (pK=16.2) that is very close to albumin's affinity for copper (II) (pK=16.4). GHK effectively competes with albumin for copper. However, under physiological conditions only about 5% to 20% of GHK molecules would be expected to exist as GHK-Cu complexes with copper (II).5

Biological actions have been reported for both GHK and GHK-Cu, although GHK is likely to chelate available copper at very low concentrations while, conversely, GHK-Cu may lose copper to other binding agents. Thus, experiments using either molecule are actually studying a mixture of both molecules. GHK-Cu is a very fragile molecule that is sensitive to carboxypeptidase actions. In blood plasma, it is rapidly degraded.

Protecting, healing & remodeling

In 1985, I founded a company called ProCyte to develop GHK-Cu for clinical use. It was quickly established that GHK-Cu had repair actions on tissues such as the skin, the stomach, the intestine and the bone. GHK-Cu accelerated the healing of a variety of accidental and surgical wounds in rats, mice, pigs and horses. It also improved the establishment of skin grafts in mice and in pigs. GHK-Cu analogues with added hydrophobic residues strongly increased hair growth in mouse models.
It appears that, after tissue damage, GHK is generated by proteolysis of inflammatory and extracellular matrix proteins. A significant fraction of GHK converts to GHK-Cu by obtaining copper (II) from albumin. Wound healing of skin is basically a two-phase process. Initially, a variety of anti-microbial sterilising and tissue-destructive process are activated. Scar-producing growth factors such as Transforming Growth Factor (TGF) â-1 induce the production of copious quantities of collagen, and create a tough, protective layer over the injury. In the second phase, inflammation and scar formation is suppressed and remodeling processes remove scar tissue and rebuild normal skin.

GHK-Cu acts indirectly as an extremely potent chemoattractant at 10 exp (-12) M for cells that stimulate repair, such as macrophages and mast cells, which release protein growth factor proteins that stimulate tissue repair. Tissue areas deficient in copper (II) will not support the ingrowth of new blood vessels or angiogenesis. In rabbit models, GHK-Cu induces angiogenesis by promoting the synthesis of a family of six proteins from 35K to 66K, and by acting as a chemoattractant for capillary cells at 10exp (-12) M.6

In cell culture systems, and in vivo in rats and mice, GHK-Cu, at approximately 10 exp (-9) M, acts directly on fibroblasts by increasing the production of m-RNA for collagen, elastin, proteoglycans, glycosaminoglycans and decorin. In addition, GHK-Cu simultaneously stimulates the m-RNA production of, and synthesis of, certain metalloproteases and anti-proteases that clear damaged protein and remove scars. Thus, GHK-Cu links the processes of removal of damaged scar tissue and deposition of new tissue.7

Francois Maquart and collegues at Reims have argued that GHK-Cu acts on the second phase of healing as an inducer of tissue remodeling processes. Further support for this concept is that the molecular weight of collagen

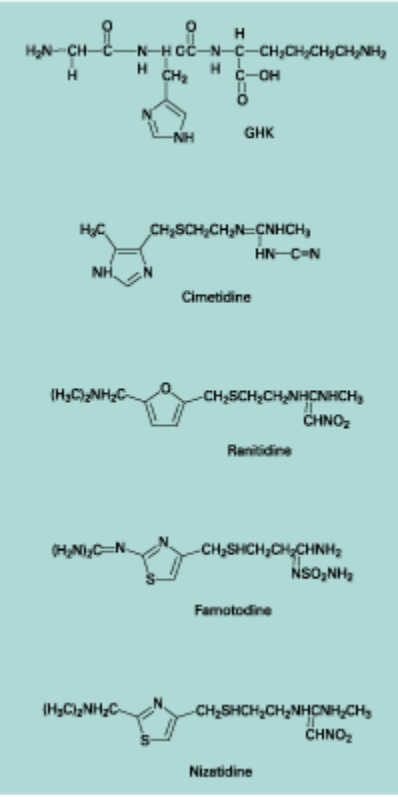
fragments induced by GHK-Cu are much smaller than those produced in the early phase of wound repair. This suggests that, with the copper complex, collagen synthesis and degradation are simultaneously occurring. Also, in cell culture, GHK-Cu reduces the secretion of TGFâ-1 by normal fibroblasts and keloid-producing fibroblasts. This, combined with GHK-Cu's healing activities, suggests that scar-free healing needs both an activation of metalloproteinases and a reduction in TGFâ-1 production.8

In culture systems, the complex promotes the differentiation, viability and axon outgrowth in cultured chick and rat neurons. In isolated rat hepatocytes, GHK stimulates the activity of phosphorylase A, an activity that converts glycogen into bioavailable glucose for energy production.9

GHK-Cu as a tissue protective molecule

Another important function of GHK-Cu may be as a circulating human non-steroidal anti-inflammatory. Virtually all NSAIDs avidly bind copper (II). There are striking similarities between GHK and anti-ulcer drugs such as cimetidine, ranitidine, famotidine and nizatidine (see Figure 2).

Figure 2 - Similarity between GHK-Cu and anti-ulcer drugs



The correspondences include (1) an N-terminal side change, (2) a central imidazole ring, and (3) a C-terminal lysine-like basic group. 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. One small study of 16 patients with distal inflammatory bowel disease, who were treated with rectally administered solutions of GHK-Cu, found that after the 12 weeks of treatment, there was a 60% reduction in severity as measured by endoscopy, histopathology, and symptoms.

After episodes of tissue damage, ferric ion is released from ferritin and catalyses damaging tissue oxidations. GHK-Cu counters this action by blocking ferritin channels, and the release of oxidising iron ions. GHK blocks the oxidation of low density lipoproteins by loosely bound copper. Interleukin-1-â is also released after tissue injury producing cellular damage. At 10 exp (-10) M, GHK-Cu was found to prevent damage to pancreatic cells by interleukin-1. GHK-Cu inhibits platelet aggregation and thromboxane production; this action may reduce localised blood coagulation after tissue damage.10

Wound healing

After my initial finding that GHK-Cu stimulated wound healing11, numerous other laboratories extended these observations. A few examples of healing actions include the healing of pad wounds in dogs, the increased wound closure and the contraction and production of granulation tissue. Healing with GHK-Cu was best with light bandaging. Wet bandages nullified the effect.12
In immune-suppressed rats, healing is impaired and collagen synthesis is 23% of that in normal rats. GHK-Cu more than tripled collagen synthesis in these rats, raising it to 77% of normal and restored normal wound healing and wound contraction in the immune-suppressed animals. In studies of the healing of punch biopsies wounds in pigs, the effect of intradermal injections of GHK-Cu produced highly localised patterns of healing.

Thus, in animal or human studies, it is possible to test several potential formulations on a given test subject.13

A GHK-Cu cream used after Moh's surgery increased wound healing and skin re-epithelialisation. An open study of wounds in 60 patients in 1987 with diabetic and venous stasis ulcers gave evidence of rapid healing. This study used high concentrations of GHK-Cu and very low concentrations of anti-microbial agents. This apparently successful formulation was never used in later clinical studies, which failed to achieve therapeutic goals in FDA trials for clinical uses.14

Ageing reversal of skin

The greatest interest in copper peptides is in the area of reversing the effects of human ageing and ultraviolet damage on human skin. During ageing, skin becomes thinner and tends to accumulate various skin lesions and imperfections. The dermis and epidermis thin and the subcutaneous fat cells diminish in number.
A number of studies at ProCyte in 1988, which were repeated by several other laboratories between 1998 and 2002, have found that the application of GHK-Cu creams to the human skin was more effective in promoting collagen development than retinoic acid or vitamin C. It also increased the thickness of the epidermis and dermis, increased skin elasticity, reduced wrinkles and resulted in a removal of skin imperfections such as blotchiness and sun damage marks. In skin healing models using mice, a very significant increase in subcutaneous fat cells was noted.15

Hair retention & growth stimulation

In 1985, a series of GHK-Cu analogues with added hydrophobic residues (fatty acids or hydrophobic amino acids) was found to stimulate hair growth strongly around healing wounds in mice. It was possible to obtain striking increases in hair follicle size and the rate of localised hair growth in mice.
Later, Hideo Uno and colleagues at the University of Wisconsin reported that these copper complexes produced a stimulation of the follicular cell proliferation, resulting in an enlargement of the anagen follicles, and converted vellus hair into terminal hair. GHK-Cu analogues also minimised hair loss after experimental chemotherapy in rats and accelerated new hair growth. These actions on hair growth may be secondary to improvements in skin vitality that increase nutrient flow to hair follicles.16

In humans, the results are less striking but do exist. An unpublished study by ProCyte reported that one GHK-Cu analogue increased terminal hair growth in adult men approximately 30% more than was reported for the control substance (2% minoxidil). GHK-Cu has been shown to increase hair outgrowth from hair transplants in men.17

Bone healing

GHK-Cu increases collagen synthesis by bone chondrocytes (chick and guinea pig) and increases the growth of human marrow stromal cells and promotes the attachment of human osteoblastic cells. Milan and collegues in Prague developed a GHK-Cu gel that promotes the filling of bone defects in femurs and bone attachment to cementless endoprostheses. The GHK-Cu gel, when used with cementless endoprostheses, produced vivid osteogenic activity at the interface of bone and metal stem. Such gels may aid in the establishment and retention of artificial joints.18

Developing breakdown-resistant complexes

The first generation products designed around GHK-Cu performed well in many controlled tests, however, the products failed in FDA clinical trials on the healing of very difficult-to-heal human wounds (as have many other approaches).

In 1975, during attempts to isolate GHK from human blood, we found that the molecule was especially vulnerable to carboxypeptidases and was rapidly degraded by blood enzymes. Intradermal injections of GHK are cleared from the skin in approximately 30 seconds. If added to blood, GHK is rapidly degraded into constituent amino acids by blood enzymes.

This fragility and rapid breakdown of GHK and other simple copper peptide complexes is the major problem in developing products for clinical and cosmetic use. In the human body, the GHK-Cu complex can be generated constantly. However, when used as a single dose therapy, its fragility leads to rapid breakdown, clearance from the dermis and a loss of effectiveness.

A variety of chemical modifications to GHK have produced bioactive copper complexes with enhanced breakdown resistance. The problem with this classical organic chemistry approach is that each new chemical becomes, in FDA regulatory terms, a new chemical entity. This increases the possibility of undesirable side effects and much slower regulatory approvals.

In 1994, I set up Skin Biology to develop more stable copper peptides with tissue regenerative actions. To increase resistance to proteolysis, I used a fraction of peptide fragments that remained after partial proteolysis of soy proteins. Such soy peptides have a very low antigencity and long history of safe use in cosmetic products and in solutions used clinically for intravenous alimentation. When copper (II) is chelated to this peptide fraction, this creates skin regenerating copper peptides that can be used with skin exfoliating hydroxy acids for more rapid skin renewal and for scar reduction. These peptides have enhanced potency, breakdown resistance, a longer duration of action and very high adherence to skin.

In veterinary studies, creams made from these new copper complexes produced rapid and scar-free healing in dogs after spaying operations and in young horses after leg-straightening operations. This allowed the dogs to be returned to their owners in four days instead of the usual five, while the foals were returned in five days instead of seven. In humans, four small, placebo-controlled studies found faster skin healing after skin injuries induced by tape stripping, acetone burns (removal of skin lipids), 24-hour detergent irritation and nickel allergy inflammation.19

Importance of careful formulation

With copper-peptide products, great care must be taken to produce a product that has minimal interactions with the ionic copper in the product. Magnetic proton resonance measurements found that copper (II) exchanged slowly between GHK molecules, despite the very high binding affinity of GHK for copper (II). Often the chemical ingredients of creams, lotions, and solutions interact with the ionic copper and neutralise the positive copper-peptide actions and, in some cases, generate copper-complexes that inhibit cell replication. Any product should be tested carefully for its effect on skin repair. Some companies have recently sold cosmetic skin products using EDTA-copper, but this complex inhibits fibroblast function and skin repair.

Future uses

GHK-Cu remains the best molecule for internal medical treatments. The newer breakdown resistant, highly adhesive copper peptides under development at Skin Biology should prove better for cosmetic and superficial uses such as post-procedure dermatological healing, and development of scarless surgical procedures.
It is possible that GHK-Cu could be used clinically to protect and speed the repair of damaged organs. H. Paul Ehrlich found that intra-muscular injection of GHK-Cu into the thigh muscle of rabbits raised circulating wound macrophages in the blood and accelerated the healing of distant wounds in the rabbit ear. Patients might be pretreated with GHK-Cu before surgery to enhance post-surgical repair. Based on rabbit models, a dosage of 30 mg of GHK-Cu should suffice. The molecule is also very beneficial on kidney organ culture. Thus, GHK-Cu might

be infused into patients with kidney failure to exert its tissue protective and repair actions.

The newer, second generation copper peptides produced at Skin Biology appear to be very useful for post-procedure recovery after skin peels, dermabrasion and laser resurfacing. The combination of hydroxy acids and these peptides slowly, over a period of several months, reduces old scars and skin lesions. This method is economical and avoids the complications that often occur after chemical peels or laser treatments. In experimental studies, the use of such types of copper peptides after surgical procedures often results in scarless or nearly scarless healing.20

References

1. Pickart, Ph.D. thesis, Univ. of California, San Francisco, 1973; Pickart, Thaler, Nature New Biol. 243, 1973, 85; Pickart, Thaler, J. Cell Physiol. 102, 1980, 129, Pickart, In Vitro, 17 (6), 1981
2. Pickart, Freedman, Loker, Peisach, Perkins, Stenkamp, Weinstein, Nature 288, 1980, 715; Kwa, Bor-Sheng, Rose, Weinstein, Pickart, Peptides, 8, 1983, 805; Freedman, Pickart, Weinstein, Mims, Peisach, Biochemistry, 21, 1982, 4540

3. Johnson, Pickart, Rose, Inorg. Chem. Acta, 67, 1982, 159, Pickart, Goodwin, Burgua, Murphy, Johnson, Biochem. Pharmacol., 32, 1983, 3868; Pickart, Lovejoy, Methods Enzymol. 147, 1987, 314.

4. Maquart, Gillery, Monboisse, Pickart, Laurent, Borel, Ann. N.Y. Acad. Sci., 580, 1990, 573; Sage, Vernon, J. Hypertens. Suppl. 12, 1994, S145.

5. Lau, Sarkar, Biochem J. 199, 1981, 649

6. Poole, Zetter, Cancer. Res. 43, 1983, 5857; Zetter, Rasmussen, Brown, Lab. Invest., 53, 1985, 362, Raju, Alessandri, Gullino, Cancer Res. 44, 1984, 1579

7. Maquart, Pickart, Laurent, Gillery, Monboisse, Borel, FEBS Lett. 238, 1988, 343; Oddos, Jumeau-Lafond, Ries, Amer. Acad. Derm. Abstract P72, Feb. 2002; Wegrowski, Maquart, Borel, Life Sci. 51, 1992, 1049; Simeon, Wegrowski, Bontemps, Maquart, J. Invest. Dermatol., 115, 2000, 962; Maquart, Bellon, Chaqour, Wegrowski, Monboisse, Chastang, Birembaut, Gillery, J. Clin. Invest., 92, 1993, 2368; Simeon, Monier, Emonard, Gillery; Birembaut, Hornebeck, Maquart, J. Invest. Dermatol., 112, 1999, 957; Simeon, Emonard, Hornebeck, Maquart, Life Sci., 67, 2000, 2257

8. Maquart, Simeon, Pasco, Monboisse, J. Soc. Biol., 193, 1999, 423; McCormack, Nowak, Koch, Arch. Facial Plast. Surg., 3, 2001, 28

9. Sensenbrenner, Jaros, Moonen, Mandel, Neurobiology, 5, 1975, 207; Lindner, Grosse, Halle, Henklein, Z. Mikrosk. Anat. Forsch., 93, 1979, 820; Garcia-Sainz, Olivares-Reyes, Peptides, 16, 1995, 1203

10. Levine, Patt, Koren, Joslin, 25th Ann. Meeting of Digest. Dis. W., May 1995; Miller, DeSilva, Pickart, Aust, Adv. Exp. Med. Biol., 264, 1990, 79; Vinci, Caltabiano, Santoro, Rabuazzo, Buscema, Rizzarelli, Vigneri, Purrello, Diabetologia, 38, 1995, 39-45, Thomas, Biochem. Biophys. Acta, 1128, 1992, 50

11. Downey, Larrabee, Voci, Pickart, Surg. Forum, 1985, 573; Pickart in Biology of Copper Complexes, Plenum Press, 1987, 273

12. Swaim, Vaughn, Kincaid, Morrison, Murray, Woodhead, Hoffman, Wright, Kammerman, Am. J. Vet. Res. 57, 1996, 394

13. Ehrlich, Symposium on Collagen and Skin Repair, Reims, Sept. 12, 1991; Counts, Hill, Turner-Beatty, Grotewiel, Fosha-Thomas, Pickart, Fed. Am. Soc. Exp. Biol., 6, 1992, A1636

14. Aupaix, Maquart, Salagnac, Pickart, Gillery, Borel, Kalis, J. Invest. Derm., 94, 1990, 390; Fish, Katz, Hien, Briden, Johnson, Patt, Wounds, 3, 1991, 171.

15. Abulghani, Shirin, Morales-Tapia, Sherr, Solodkina, Robertson, Gottlieb, J. Invest. Derm., 110, 1998, 686; Amer. Acad. Derm. Meeting, February 2002, (Appa, Barkovic, Finkey, Stephens, Abstract P66; Leyden, Grove, Barkovic, Appa, Abstract P67; Leyden, Stephens, Finkey, Barkovic, Abstract P68; Leyden, Stephens, Finkey, Barkovic, Abstract P69).

16. Uno, Kurata, J. Invest. Dermatol., 101, 1993, 143S, Awa, Nogimori, J. Dermatol. Sci., 10, 1995, 99.

17. ProCyte Corporation, 1997; Perez-Meza, Int. J. Cosm. Surg., 6, 1998, 80.

18. Pesakova, Novotna, Adam, Biomaterials, 16, 1995, 911; Pohunkova, Stehlik, Vachal, Cech, Adam, Biomaterials, 17, 1996, 1567; Godet, Marie, Cell. Mol. Biol. (Noisy-le-Grand), 41, 1995, 1081.

19. Zhai, Chang, Singh, Maibach, Contact Dermatitis, 40, 1999, 205; Zhai, Poblete, Maibach, Int. J. Dermat., 37, 1998, 386; Zhai, Leow, Maibach, Skin Res. Tech., 4, 1998, 24; Zhai, Leow, Maibach, Clin. Exp. Derm., 23, 1998, 11.

20. More extended references to copper peptide actions on tissue regeneration are posted at www.skinbiology.com/copperpeptideregeneration.html

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