

The Effect of Glycyl-L-Histidyl-L-Lysine Copper Chelate on the Healing of Diabetic Ulcers: A Pilot Study

Patrick Massey, MD, PhD
Leonard M. Patt, PhD
Joan C. D'Aoust, MS

ABSTRACT: A preliminary, open-label, non-randomized, dose response study has been conducted to evaluate the wound healing response of diabetic ulcers to PC1020 [Glycyl-L-Histidyl-L-Lysine tripeptide copper chelate, Iamin®] treatment. The study consisted of a two week treatment phase followed by a two week observation period. Treatment consisted of an injection of a solution (0.03%, 0.3%, or 3.0%) of PC1020 or saline into the target ulcer on days 1, 2, 3, 5, 9, 11, and 15. The extent of healing was followed by wound tracings obtained prior to treatment, on days 4-5, and then at approximately weekly intervals. At the end of the treatment phase, the 0.3% PC1020 treatment group had a significantly increased average rate of wound closure relative to the saline injected group. PC1020 treatment also increased the average percent of the wound's surface area healed both at the end of the treatment phase and at the end of the observation phase.

WOUNDS 1992;4(1):21-28

From Rush Presbyterian-St. Luke's Medical Center,
Chicago, IL and ProCyte Corporation, Kirkland, WA.

Address correspondence to: Leonard M. Patt, PhD,
ProCyte Corporation, 12040 115th Avenue N.E. Suite
210, Kirkland, WA 98034

The use of agents which actively promote wound healing offers a potential therapeutic alternative to the physician presented with non-healing dermal lesions. PC1020 (Glycyl-L-Histidyl-L-Lysine tripeptide copper chelate, Iamin®) is a peptide factor which stimulates many biological activities which are associated with the wound healing process.¹⁻³ For example, PC1020 stimulates formation of new blood vessels,^{4,5} has been shown to be a chemoattractant

for macrophages and mast cells^{6,7} and stimulates the synthesis of collagen in cultured fibroblasts.⁸ Injection of PC1020 at the wound periphery of full-thickness excisions in rats accelerated closure.⁹

These biological properties have encouraged evaluation of the potential of PC1020 to accelerate wound healing in human subjects. In a preliminary study evaluating topical application of PC1020 in acute wounds following Mohs surgery, PC1020 increased the average rate of wound closure and decreased the average number of days to complete healing.¹⁰ These results were not statistically significant but there was a good correlation between the average rate of wound closure determined by the Gilman¹¹ method and the decrease in healing time. These preliminary results in acute wounds following Mohs' surgery have now been expanded to include the effects of PC1020 treatment in chronic wounds.

Methods

Patient population. The patient population consisted of male and female diabetic patients between the ages of 41 and 80 years of age presenting with unhealed ulcers of the lower extremities. All ulcers were full-thickness wounds with no evidence of bone involvement or infection. The majority of the treated wounds (76%, see Table 1) were located on the plantar surface. After entry into the study, no other form of wound treatment (cleansing agents, antiseptics, topical antibiotics, corticosteroids, contact casts) of the ulcer was allowed. Wound debridement consisted of taking the newly formed callus down to the viable tissue area using a #15 scalpel blade, usually without inducing a significant amount of bleeding. Debridement occurred approximately every four to five days. The ulcer bed itself was not enucleated. All the patients were ambulatory during the course of the study and were treated on an outpatient basis. Patients were instructed to keep the treated extremity elevated when sitting. No other form of activity curtailment was advised.

Exclusion criteria included no hypersensitivity to any of the components of the medication, no history of diseases associated with hyper-

cupremia (Wilson's disease), and no exposure to any systemic immunosuppressant or cytotoxic therapy within four weeks prior to the study or participation in an experimental clinical trial during the previous 30 days prior to enrollment. Exclusion criteria also included infection of the ulcer and bone involvement.

Informed consent forms were obtained from all subjects, IRB approval was obtained, and the study conformed to all relevant human subject guidelines.

Drug treatment. A novel application method, intralesional injection, was evaluated in this pilot clinical study. The method of application of factors to modify the healing response of chronic wounds is critical. The vehicle should not adversely affect the healing process. In this study, direct intralesional injection into tissue surrounding the ulcer bed was evaluated as a drug delivery method. This application method allows observation of the effects of PC1020 independent of any formulating agents other than physiologic saline. A limited treatment phase was permitted, two weeks of injections, followed by an additional two week observation period.

This was a dose ranging study comparing three concentrations of PC1020, 0.03%, 0.3%, and 3.0%, and saline. Subjects were treated on an outpatient basis and were seen on the treatment or evaluation days. Prior to the injections, the ulcer bed was wiped with an alcohol pad. No further cleansing procedures were done before injections. Each ulcer was injected on days 1, 2, 3, 5, 9, 11, and 15 at four equally spaced locations around its periphery (at approximately 3, 6, 9, and 12 o'clock). Each injection consisted of 0.1 ml injected from a 1 cc syringe equipped with a 30 gauge needle. The injections were administered approximately 1mm inside the ulcer perimeter, with the needle angled towards the center of the ulcer. The needle was inserted until wound bed resistance was felt and then continued for approximately an additional millimeter in depth. After delivery of the 0.1 ml of the injection solution, the needle was left in place for approximately 10 seconds to facilitate dispersion of the drug solution into the wound bed. This procedure was repeated for each of the remaining locations around the wound. The wound was then covered with a fine

Table 1

		Demographic Summary			
		Saline	Glycyl-L-Histidyl-L-Lysine Copper Chelate		
			0.03%	0.3%	3.0%
Number		5	4	5	6
Age (Years)	Mean	58	67	66	63
	SEM	7	5	1	3
	Range	(41 - 77)	(56 - 80)	(65 - 69)	(53 - 76)
Sex (N)	Male	1	4	5	4
	Female	4	0	0	2
Ulcer Duration (Months)	Mean	13	9	20	14
	SEM	5	5	6	4
	Range	(7 - 24)	(3 - 20)	(5 - 31)	(3 - 33)
Baseline Lesion Size (Sq cm)	Mean	2.1	2.1	2.8	2.4
	SEM	0.8	1.1	0.9	0.7
	Range	(0.7-4.0)	(0.6 - 5.5)	(0.8 - 5.8)	(0.7 - 4.0)
Lesion Location	Plantar	4	3	2	5
	Non-plantar	1	1	3	1

Table 2

Effect of Glycyl-L-Histidyl-L-Lysine Copper Chelate on The Closure of Diabetic Foot Ulcers

		Average Closure in mm/day			
		Treatment group — PC1020 Concentration			
		Saline	0.03%	0.3%	3.0%
End of Treatment Phase		0.034	-0.018	0.055	0.101
		0.066	0.007	0.119	0.021
		0.000	0.094	0.093	0.031
		-0.013	-0.001	0.076	0.102
		-0.065		0.064	0.097
					-0.015
Median		0.000	0.003	0.076	0.064
Mean		0.004	0.021	0.081	0.056
SEM		0.022	0.025	0.011	0.021
Sig.			ns	P < .05	ns

Statistical comparison to saline group by Student's *t*-test and Mann-Whitney *U*-Test, ns = not significant

mesh gauze and a non-occlusive bandage and secured with tape.

Groups of consecutive patients were entered into the study groups in the following sequence, 0.03% PC1020, 0.3% PC1020, 3.0% PC1020, and finally the saline group. The attending physician was not blinded as to the treatment group of the subjects.

Wound Healing Evaluation. Wound healing was evaluated on days 1, 5, 15, 22, and 29 during the course of the clinical study. The wound area of each lesion on each evaluation day was obtained by tracing the edge of the wound on a piece of clear polyethylene film. Area and perimeter measurements of the unhealed portion of the wound were obtained from the tracing by planimetry with a BioQuant™ image analysis system. These parameters were measured by a person independent of the clinical investigator.

The progress of wound healing was followed by calculating the rate of closure as described by Gilman¹¹ and by determining the percent of the wound surface area healed relative to the day zero area. Briefly, the method measures the daily linear advance (in mm) of the wound edge normalized for differences in wound area. The method provides a better comparison of healing when wounds of different areas are to be compared.¹ Comparison of the percent healed versus time may not always provide a valid comparative method when wounds of different sizes are compared.

The percent of the wound healed relative to the area of the day 0 of the study was also calculated. The rate of closure was calculated at the end of the treatment phase of the study [days 14 and 15] and the percent healed was calculated at both the end of the treatment phase and at the end of the complete study [days 26 through 29].

Statistical analysis. Group mean differences were compared statistically employing the Mann-Whitney or Student's t-test for unpaired samples. All comparisons were made comparing an individual test group with the saline control group.

The closure parameter is calculated as follows.

For a wound where

Time	Area	Perimeter
t_1	a_1	p_1
t_2	a_2	p_2

Closure- \bar{d}

$$\bar{d} = \frac{\Delta a}{p}$$

where

\bar{d} = average progress toward closure, a representation of the length of advance of the wound margin over time T

$$\text{Time } T = t_2 - t_1$$

ΔA = difference in area before and after time T

$$A = a_2 - a_1$$

\bar{p} = average wound perimeter before and after time T

$$\bar{p} = [p_1 + p_2] / 2$$

The rate of closure is calculated by dividing the closure determined on an individual evaluation day by the number of days in the evaluation period.

$$\text{closure rate} = \bar{d} / T$$

The average closure rate is obtained by averaging the closure rates obtained at each evaluation period during the treatment phase of the study.

CLOSURE RATE OF DIABETIC ULCERS AT END OF TREATMENT PHASE

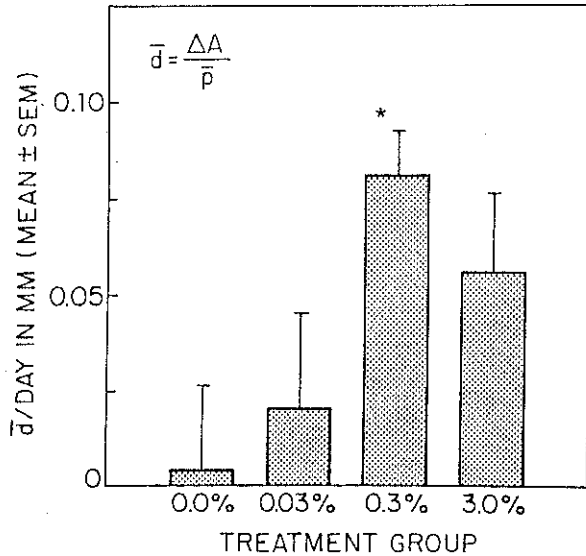


Figure 1. The effect of PC1020 treatment on the mean closure rate of diabetic ulcers at the end of the treatment phase (day 15). The closure rate was calculated as described in the Methods section. The * denotes significantly ($P < .05$) different from the saline (0.0%) group.

PERCENT HEALED AT END OF TREATMENT PHASE

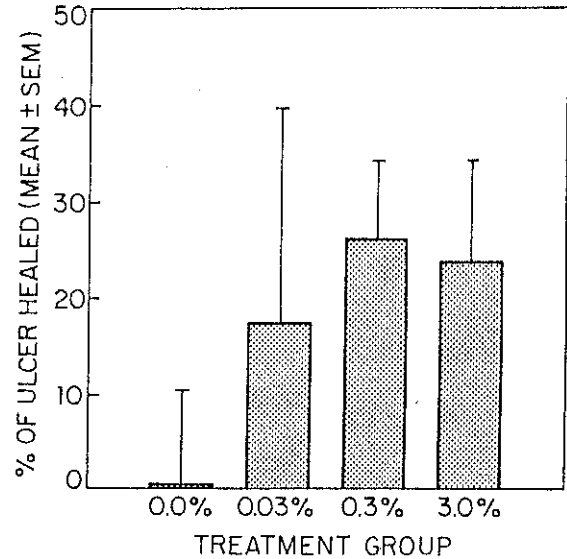


Figure 2. The effect of PC1020 treatment on the mean percent healing of diabetic ulcers at the end of the treatment phase (day 15).

Drug supplies. PC1020 is produced by ProCyte Corporation, Kirkland, Washington. The complex consists of a two to one molar complex of the peptide Glycyl-L-Histidyl-L-Lysine with copper (II) at neutral pH. Sterile, pyrogen-free, isotonic, solutions were prepared at concentrations of 0.03%, 0.3%, and 3.0% by weight of PC1020. Single unit dose vials containing 2.0 ml were stored at 4 degrees Fahrenheit throughout the study. Vehicle controls consisted of Sodium Chloride, 0.9%, for injection (Abbott Laboratories).

Results. Seventeen subjects were entered into this dose ranging study to evaluate the efficacy of intralesional injection of PC1020 on wound healing in diabetic ulcers. A total of 20 ulcers were treated during the course of the study. Two subjects had ulcers which were treated at two different doses of PC1020 and one subject had two ulcers at different locations which were treated separately at different doses and different times.

The subject age, ulcer duration, initial ulcer size and location are shown in the demographics of the patient population. (See Table 1.)

Injection of the PC1020 did not result in any observable side effects. The intralesional injections were well tolerated in this patient population. All patients had a significant peripheral neuropathy and most did not feel the injections. Of those who could feel the injection, the discomfort was mild and transient (lasting a few seconds). In addition, the intralesional injections did not result in an increase in local or systemic infections. None of the patients treated developed infections in the ulcer beds or surrounding tissues.

The initiation and subsequent progress of healing was assessed by two methods, determination of the wound closure rate and by the percentage of the ulcer healed at the end of the treatment phase of the study.

At the end of the two week treatment phase of the study the closure rate of the two high dose PC1020 groups (0.3% and 3.0%) was greater than

Table 3

Effect of Glycyl-L-Histidyl-L-Lysine Copper Chelate on the Relative Change in Wound Area

	Percent Healed			
	Treatment group — PC1020 Concentration			
	Saline	0.03%	0.3%	3.0%
End of Treatment Phase	20.1%	-2.6%	5.9%	48.1%
	27.5%	-11.2%	55.2%	10.7%
	-13.9%	83.6%	16.0%	14.6%
	-6.3%	0.0%	27.8%	57.9%
	-25.1%		25.8%	24.2%
				-12.8%
Median	-6.3%	-1.3%	25.8%	19.4%
Mean	0.5%	17.5%	26.1%	23.8%
SEM	10.1%	22.2%	8.2%	10.6%
Sig.		ns	ns	ns
End of Study	-11.3%	-10.1%	19.9%	65.3%
	-19.8%	19.0%	86.4%	-67.4%
	-16.8%	93.6%	2.1%	0.1%
	-47.2%	-14.8%	26.6%	44.4%
	*		*	*
				-64.5%
Median	-18.3%	4.5%	23.3%	0.1%
Mean	-23.8%	21.9%	33.8%	-4.4%
SEM	8.0%	25.0%	18.3%	27.2%
Sig.		ns	P < 0.5	ns

* = Subject was not followed after the treatment phase of the study. Statistical comparison to saline group by Student's t-test and Mann-Whitney U-test, ns = not significant

that observed in both the saline group and the lowest PC1020 group (0.03%). (See Figure 1.) The mean closure rate observed in the 0.3% PC1020 group differed significantly ($P < .05$) from that of the saline group. (See Table 2.)

The percent of the ulcer healed was also greater in the higher dose PC1020 groups. The percentage of each ulcer healed at the end of the treatment phase was approximately 20% in both the 0.3% and 3.0% PC1020 groups. (See Figure 2.) The means of these treatment groups were not

statistically different from that of the saline control group. (See Table 3.) Table 3 shows that 10 of 11 ulcers injected with the high dose PC1020 solutions responded with a positive healing response (defined as a decrease in ulcer area relative to the day 0 value) within the treatment phase of the study. Only 2 of 5 saline injected ulcers responded with a positive healing response and 1 of 4 of the 0.03% PC1020 group, a combined 3 out of 9 response rate. There was no change in the distribution of the data if only plantar ulcers were

7

included in the comparisons.

Although none of the wounds healed fully in the two weeks of observation following treatment, those individuals who received effective doses of PC1020 fared better when compared with the saline group. None of the saline injected group showed a positive response relative to the initiation of the study (Table 3, bottom panel). The saline group had a median increase in ulcer size relative to day 0 of approximately 18% compared to a median decrease in ulcer size of 23% in the 0.3% PC1020 group. The mean percentage of the wound healed in the 0.3% PC1020 group at the end of this preliminary study was significantly increased ($P < .05$) over that of the saline group.

Discussion. This preliminary clinical study evaluated the potential of PC1020 to initiate a healing response in diabetic ulcers. The intraleisional injection mode of application permitted evaluation of the effects of the PC1020 free of any formulation components other than normal saline. The study was designed to assess the effects of short term treatment of PC1020 on the wound healing process. Non-healing ulcers in diabetics present a serious challenge to the physician and these ulcers, if unsuccessfully treated, represent a significant risk of amputation of the involved limb.¹² Numerous treatment regimes are currently used including wound debridement of necrotic tissue, antibiotic application, and various forms of bandaging, both dry and occlusive. Vascular surgical intervention is also indicated in some cases to correct perfusion deficits. Preventive measures such as corrective or weight distributing footwear are also prescribed.

Therapeutics which actively promote or accelerate a healing response in chronic wounds would be a valuable additional treatment modality. PC1020 possesses many biological properties which suggest that the compound could enhance a wound healing response. Topical application of PC1020 in a cream base has been previously evaluated in acute wounds following Mohs surgery.¹⁰ A trend toward accelerated wound closure rate and shortening of the length of time for wound healing was observed. In the current study, injection of increasing concentrations of PC1020

increased the mean wound closure rate during the two week treatment phase of the study. This increase in closure rate was accompanied by an increased percentage of the wound area which healed in the highest PC1020 dosage groups. The mean percent healed was approximately 20% in the two highest PC1020 groups compared to no change in the saline injected group.

This preliminary study with a limited treatment duration was not designed to effect complete healing of chronic foot ulcers. The increased healing seen in the optimal PC1020 dosage group was, however, extended into the observation phase of the study. At the end of the study, two weeks after cessation of treatment, the mean percent healed in the 0.3% PC1020 group was maintained at approximately 20% compared to a mean increase in wound area in the saline group of approximately 18%. These data indicate that PC1020 may initiate a healing response in a group of chronic diabetic ulcers. The effects of long term application of PC1020 on the wound healing response in diabetic ulcers will need to be confirmed and statistically validated by randomized, double-blind clinical studies to fully determine the potential of this compound in non-healing wounds.

The mechanism by which PC1020 initiates a healing response in a chronic wound is unknown at this time. PC1020 enhances many biologic processes implicated in wound healing. Increases in collagen synthesis,⁸ chemoattraction for macrophages and mast cells,^{6,7} and angiogenic activity,^{4,5} have been described for PC1020. A similar enhancement of these processes have been noted for many polypeptide growth factors, many of which have also been shown to enhance wound healing in both animal models and in clinical trials.¹³⁻¹⁶ The activity of PC1020 in wound healing may be associated with its effects on collagen synthesis and new blood vessel growth and through attraction of macrophages to the wound site.

References

1. Pickart L, Lovejoy S: Biological activity of human plasma copper binding growth factor Glycyl-L-Histidyl-L-Lysine. *Method Enzymol* 1987;147:314-328.

2. Pickart L: The biological effects and mechanism of action of the plasma tripeptide Glycyl-L-Histidyl-L-Lysine. *Lymphokines* 1983;8:425-446.
3. Pickart L: The use of Glycyl-L-Histidyl-L-Lysine in culture systems. *In Vitro* 1981;17:459-466.
4. Raju KS, Alessandri G, Ziche M, et al: Ceruloplasm, copper ions, and angiogenesis. *J Natl Can Inst* 1982;69:1183-1188.
5. Raju, KS, Alessandri G, et al: Characterization of a chemoattractant for endothelium induced by angiogenesis effectors. *Cancer Res* 1984;44:1579-1584.
6. Zetter BR, Rasmussen N, Brown L: An *in vitro* assay for chemoattraction activity. *Lab Invest* 1985;53:362-368.
7. Poole TJ, Zetter BR: Stimulation of rat peritoneal mast cell migration by tumor derived peptides. *Cancer Res* 1983;43:5857-5861.
8. Maquart F-X, Pickart L, Laurent M, et al: Stimulation of collagen synthesis in fibroblast cultures by tripeptide-copper complex Glycyl-L-Histidyl-L-Lysine-Cu²⁺. *FEBS Letters* 1988;238:343-346.
9. Downy D, Larrabee WF, Voci V, et al: Acceleration of wound healing using Glycyl-L-Histidyl-L-Lysyl-cu(II). *Surg Forum* 1985;23:573-575.
10. Fish FS, Katz I, Hien NT, et al: Evaluation of Glycyl-L-Histidyl-Lysine:copper complex in acute wound healing. *WOUNDS* 1991;3:171-177.
11. Gilman TH. Parameter for measurement of wound closure. *WOUNDS* 1990;2:95-101.
12. Most RS, Sinnock P: The epidemiology of lower extremity amputations in diabetic individuals. *Diabetes Care* 1983;6:87-91.
13. Ksander GA. Exogenous growth factors in dermal wound healing. *Annual Reports in Medicinal Chemistry* 1989;24:223-232.
14. Brown GL, Nanney LB, Griffen J, et al: Enhancement of wound healing by topical treatment with epidermal growth factor. *New Eng J Med* 1989;321:76-79.
15. Rothe M, Falanga: Growth factors. Their biology and promise in dermatologic diseases and tissue repair. *Arch Dermatol* 1989;125:1390-1398.
16. Poucher RL, Leahy JD, Howells G: Active healing of diabetic wounds utilizing growth factor therapy. *WOUNDS*; 1991;3:65-69.