Cosmetic

Oral Nutritional Supplementation Accelerates Skin Wound Healing: A Randomized, Placebo-Controlled, Double-Arm, Crossover Study

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Nutritional therapy is critical for wound healing in people with severe malnutrition or specific metabolic deficiencies. Medical claims from manufacturers of many oral supplements are marketed to surgical patients for decreasing edema, bruising, and discomfort. The effect of supplementing nutrients on soft-tissue wound healing in otherwise normal, healthy adults is an area of clinical importance, but little information is available. Proteolytic enzymes have been reported to moderate the inflammatory cycle and may up-regulate the healing process. The goal of this study was to perform a clinical trial in normal, healthy adults that examined the effects of an oral nutritional supplement (InflammEnz, Enzymes, Inc., Parkville, Mo.) on soft-tissue healing times. Twenty-six normal, healthy volunteers were recruited into a randomized, crossover, placebo-controlled, clinical trial consisting of two phases, each lasting 21 days. In phase I, subjects were subjected to a 3-mm forearm skin biopsy and randomly received a placebo or oral supplement (four capsules per day for 7 days). After a 2-week washout period, a second biopsy was performed to start phase II, with each subject receiving the respective placebo or supplement capsules. Digital photographs were taken during wound healing in both phases and analyzed for wound areas (in square millimeters) and perimeters (in millimeters). Twenty-two subjects completed the clinical trial. On the basis of wound surface areas, 17 subjects had improved wound healing and five subjects did not respond or responded only slightly to the supplement treatment. The mean ± SD healing time of the subjects responding to supplement-treated wounds was 15 ± 2.2 days, compared with 18 ± 2.5 days for the placebo group. The 17 percent acceleration of wound-healing time was significant (p < 0.005). In subjects responding to oral supplements, less redness in the wounds was observed that may have been associated with less inflammation. The authors’ results demonstrate that InflammEnz oral supplementation accelerated soft-tissue wound healing in 77 percent of normal, healthy subjects studied. The authors’ study validates observations made that this supplement modulates the wound-healing process and suggests that many patients with minor soft-tissue wounds may benefit from treatment. (Plast. Reconstr. Surg. 114: 237, 2004.)

The healing of soft-tissue wounds constitutes a complex interaction of cascading cells and respective secreted products with extracellular matrix.1–3 Maximal effectiveness of these cellular processes in discrete time sequences requires nutrients for optimal performance. To the best of our knowledge, no clinical human trials have established specific tissue or oral intake levels of nutrients required for optimal soft-tissue wound healing. Nutritional therapy for people with severe malnutrition or specific metabolic deficiencies is well known to accelerate healing.4 The effect of supplementing nutrients on soft-tissue wound healing in otherwise normal, healthy adults is an area of clinical importance, but little information is available.

Nutritional therapy in the regulation of wound healing has been predicated on two concepts.5 The first concept is that malnutrition is associated with an increased risk of wound-related complications. The second concept is that dietary intervention, in the form of complete nutrition support or as single-nutrient supplementation, may improve or accelerate the wound-healing response. Although frequently stated and extensively discussed, these two concepts are not always supported by objective data. However, an increasing number of reports are examining the
clinical effectiveness of herbal or natural biological agents.6–8

High concentrations of proteolytic enzymes have been demonstrated to moderate the inflammatory cycle and up-regulate the healing processes encountered postoperatively.9,10 Elevated concentrations of proteolytic enzymes are extracted from plants. The therapeutic uses of proteolytic enzymes in preclinical and clinical trials in rheumatic disorders and oncology have been reviewed.10,11 In clinical trials in rheumatic disorders, enzyme preparations containing bromelain, papain, trypsin, and chymotrypsin were used. After ingestion and clearance from the gastrointestinal tract into soft tissue, the exact mechanisms of action in specific wound-healing steps are not known.

InflammEnz (Enzymes, Inc., Parkville, Mo.) is an oral enzyme product (U.S. patent 6,413,512) derived from plant or microbial origin that contains proteases, bromelain, vitamin C (from calcium ascorbate), calcium (ascorbate and citrate), rutin, and grape seed extract (pycnogenol). Enzymes, Inc., has recorded increased wound healing in surgical wounds from individual physicians. The purpose of this clinical trial was to directly determine whether InflammEnz influenced soft-tissue wound healing in a normal, healthy population.

METHODS

Subjects

Twenty-six normal, healthy volunteers were recruited on campus and consented to the Institutional Review Board on Human Subjects–approved protocol at the University of Texas Southwestern Medical Center. All subjects were counseled to maintain a usual lifestyle during the study time period. All clinical wound procedures were performed in the Department of Plastic Surgery’s Aston Clinic. Persons who did not or were unable to stop taking oral supplements or were allergic to any ingredient in the oral supplement were excluded.

Trial Design

A randomized, crossover, placebo-controlled, clinical trial in two phases was performed in which subjects were randomly assigned to the placebo group or the oral supplement group for phase I as they were entered into the study. The study consisted of two phases, each lasting 21 days, separated by 2 weeks. Enzymes, Inc., provided identical-appearing capsules that contained either the placebo or InflammEnz. Each subject had two bottles, one for each phase. All subjects were assigned a number, and a code was determined by Enzymes, Inc., to identify which subject would receive the placebo or InflammEnz capsules in each phase of the study. The identification code was revealed at the end of data closure.

In phase I, subjects were subjected to local lidocaine injection and waited for 15 minutes before the excision of a 3-mm forearm skin biopsy under sterile conditions. A standardized template was used to generate similar biopsy sites for all wounds. Immediately after the biopsy, all subjects were assigned by randomization to receive either a placebo or InflammEnz capsules. Each subject took four capsules per day on an empty stomach (1 hour before eating or 2 hours after eating) for 7 days.

Standardized photographs with measuring scales were taken at each visit (Nikon 900, Nikon Corp., Tokyo, Japan) and digital images were transferred to a computer that had the Web-based National Institutes of Health Image program (Scion Corp., Frederick, Md.). Planimetric measurements were performed on days 1, 3, 5, 8, 10, 12, 15, 17, 18, 19, 20, and 21; some exceptions were made because of scheduling difficulties. After a 2-week washout period, a second biopsy was performed to initiate phase II, and each subject received either the placebo or InflammEnz capsules (whichever one was not used in phase I). Each subject received four capsules per day on an empty stomach (1 hour before eating or 2 hours after eating) for 7 days. Photographs with planimetric measurements were obtained on days 1, 3, 5, 8, 10, 12, 15, 17, 18, 19, 20, and 21. Data collection and analysis were based on these photographs, which were digitized for wound area and perimeter measurements.

Statistical Analysis

Data from all photographs were adjusted to millimeters and expressed as mean ± SD. A paired t test was used to compare differences between the two arms of the study in all subjects. Significant statistical difference was defined as p < 0.05.

RESULTS

Twenty-six subjects started the clinical trial. Four subjects withdrew from the study because
of concerns about possible hypertrophic scar formation. No punch biopsy sites became infected or were treated with conventional therapy. Each wound on each photograph was analyzed for wound closure, perimeter, and total wound area. Three individuals confirmed calculated wound measurements.

Individual subjects’ wound-healing times were determined from photographic data that were blinded to observers (Table I and Figs. 1 through 3). Using wound closure as an endpoint, 17 subjects, or 77 percent of the study subjects, had a significantly improved wound-healing time of 17 percent (p < 0.005) in the InflammEnz-treated wounds compared with the placebo-controlled wounds. The normal wound-healing time of 18 ± 2.5 days for these subjects was improved to 15 ± 2.2 days (Fig. 4). The greatest mean differences between placebo and treated arms were observed between 5 and 7 days. In five subjects, little or no difference was observed in the wound-healing times of the placebo- versus InflammEnz-treated time periods (17.5 ± 3.0 days versus 18.5 ± 2.4 days; p < 0.62).

The response to wound healing was evenly distributed among the subjects, as the majority of subjects had accelerated wound-healing times of 2 to 3 days. Two subjects had accelerated wound-healing times of 5 to 6 days and five subjects experienced little or no change.

Observations of serial photographic data over time in subjects that responded to treatment versus control demonstrated reduced redness at the wound site or the loss of the scab at an earlier time (Figs. 1 through 3).

**DISCUSSION**

Our data demonstrate that ingesting an oral supplement during the early phase of wound healing results in accelerated soft-tissue wound healing compared with placebo in normal, healthy individuals. In the majority of subjects, wound-healing time was decreased by 2 or 3 days when the supplement was ingested. This supplement may modulate the inflammatory process, as decreased redness in the wounds was observed for subjects receiving the supplement. It is unknown which of the ingredients is most effective or whether an additive or synergistic effect is responsible for the clinical results seen. The ingredients consist of a special formulation of natural supplements commonly considered to be safe with generally unfounded claims of health benefits.

The mean percentage for all study subjects was approximately 14 percent in our study. In wound-healing studies, this would appear to be a significant increase in wound closure in otherwise normal, healthy individuals. In patients who are malnourished and present with multiple clinical problems, the effect of a single factor may not be fully measurable or appreciated. In terms of statistical significance, the phase II randomized, double-blind, placebo-controlled study of becaplermin gel in the treatment of pressure ulcers with 124 subjects had far less statistical power than in this study.12 Our results of wound closure from a 3-mm skin punch biopsy are similar to others.13

The use of proteolytic enzymes has been examined in various clinical orders.11 The reason for the lack of research is that there are many conditions and illnesses, as well as nutritional states, that affect the wound-healing process. Any condition that affects blood circulation or microcirculation, such as trauma, shock, cardiovascular disease, and diabetes, will alter the wound-healing process.1-3 If nutrient and oxygen delivery to a wound is impaired, healing will not occur or will be delayed. Patients enrolled in a wound-healing study would need to be free of these conditions or all have the conditions to the same degree. Patients with diabetes are at high risk for

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**TABLE I**

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*Wound-healing closure times (in days) for placebo or treated arms for each subject. Final wound closure was defined as no observation of a micro-exudate at the wound site for 24 hours. Subjects 4, 6, 9, and 15 did not complete the entire study.*
primary and secondary malnutrition. Although profiles exist defining the extent of the deficiency, the process of wound healing and the interactions of the macronutrients and micronutrients necessary to accomplish it must first be understood.14–17

Wound healing is a dynamic biological process of repairing insults to the integumentary system. It is commonly divided into three phases: inflammatory, proliferative, and maturation. Each phase has unique cellular and substance constituents without which it cannot progress normally. A large variety of intrinsic factors may influence any part of wound healing, including local factors such as bacteria, oxygen tension, and bleeding, and systemic factors such as the mental and physical health of the patient. There are also extrinsic factors that can be influenced by the caretakers of the wound to enhance wound healing. One area of intervention that requires careful clinical study is the effect of nutritional status on wound healing.
Standardizing wound healing in human wounds is difficult, and often animal models are investigated.\textsuperscript{18-20} To acquire a patient population with wounds that are uniform in location, size, and depth and that are free of other complications is extremely difficult. Therefore, an experimental wound in a clinical trial must be standardized in the parameters listed above, and all wounds must receive similar postoperative care and management. The small biopsy wounds were generated and allowed to heal without the aid of sutures or extended wound dressings. Larger biopsies would necessitate sutures, thus interfering with the photographic recording of the wound-healing process.

Fig. 3. Comparison of two time points in skin wounds, day 15 (left) and day 17 (right). No difference was observed on day 15 in placebo-exposed wounds (control) (above, left) versus supplement-exposed wounds (treated) (below, left). On day 17, the placebo-exposed skin wound (control) (above, right) had a scab that was no longer present in the supplement-exposed wound (treated) (below, right).
The time period of intervention was the first 7 days postoperatively. From our results, the greatest difference between treated and control subjects occurred at days 5 to 8 (Fig. 4). This time period would correspond to the inflammatory period that typically is initiated on approximately days 3 to 4, depending on the marker used for this wound-healing mechanistic response. It would seem logical that the combined decreased wound surface area data and erythema of the wounds in the treated group versus control group data would point to decreased inflammation associated with the treatment group. Even under these controlled conditions, the distribution of results points to the inherent heterogeneity of responses often observed in wound-healing studies.

These data demonstrate that there was a wide range of subjects who responded and a small minority of subjects who did not respond. The limitation of this study was that an intensive pretreatment dietary assessment was not performed. In addition, no blood chemistries were performed to determine whether subjects had any abnormal analyte levels, including hyperlipidemia or diabetes. The design of this study did not allow us to further examine the possible reasons for nonresponsiveness. Likewise, we cannot explain how several subjects were highly responsive to this complex of nutrients and proteases.

Existing clinical observations on InflammEnz indicate that it significantly reduces the healing time for the majority of individuals with small soft-tissue injuries. A recent study demonstrates that in terms of pain relief, enzyme products match or outperform the effects of nonsteroidal anti-inflammatory drug products without the side effects. All ingredients in the supplement are on the Food and Drug Administration’s list of products generally recognized as safe. Bromelain is one of a group of proteolytic enzymes. It is widely believed that most orally ingested enzymes are destroyed by the digestive juices before being absorbed. However, there is evidence that significant amounts of bromelain can be absorbed intact.1 Bromelain is an anti-inflammatory agent and, for this reason, is helpful in healing minor injuries, particularly sprains and strains; muscle injuries; and the pain, swelling, and tenderness that accompany sports injuries.3–5 Also, as a result of its anti-inflammatory effect, bromelain has been found to dramatically reduce postoperative swelling in controlled human research.9 Double-blind research has found bromelain effective in reducing swelling, bruising,10 and pain for women having minor surgery in conjunction with giving birth (epi-
siotomy).11 Bromelain is a natural blood thinner because it prevents excessive blood platelet stickiness.16

Preliminary human studies suggest that vitamin C supplementation in nondeficient people can speed healing of various types of wounds and trauma, including surgery, minor injuries, herniated intervertebral disks, and skin ulcers.10,11 The best-characterized function is the synthesis of collagen connective tissue protein at the level of hydroxylation of prolyl and lysyl residues of procollagen. This vitamin is an antioxidant that has been proven to be important in wound healing. Patients with peptic ulcers will heal faster on vitamin C than those without extra vitamin C. It improves the strength of the walls of capillaries and may help decrease the easy bruising seen with some patients.

Rutin is a bioflavinoid reported to be vital in its ability to increase the strength of the capillaries and to regulate their permeability. It assists vitamin C in maintaining collagen in healthy condition; it is essential for the proper absorption and use of vitamin C; it prevents vitamin C from being destroyed by oxidation; it is beneficial in hypertension; and it builds a protective barrier against infections.

Grape seed extract is very similar to pine bark extract, as it contains a unique type of bioflavonoids called proanthocyanidins, which are synergistic with vitamin C. Some evidence indicates that grape seed extract helps vitamin C enter cells, thus strengthening the cell membranes and protecting the cells from oxidative damage.

The success of this specific product of nutrient supplements in normal, healthy individuals was unexpected. None of the subjects appeared to be nutritionally deficient. In subsequent interviews with some subjects, no one consumed a strict vegetarian diet, as subjects consumed what would be considered an “American diet.” Therefore, the effects of this complex of nutrients would appear to act specifically on unique mechanisms that are generally not operating at maximum effectiveness during wound healing. An alternative explanation would be that this product provides unique catalysts or enzyme activities that act synergistically with natural body defenses to accelerate wound healing.

The clinical trial was designed to examine each person as his or her own control and was placebo-controlled in small, soft-tissue wounds. In clinical studies with pressure ulcers, nutritional supplementation with vitamin C, vitamin A, vitamin E, zinc, protein, and individual amino acids did not result in accelerated wound healing.21

One limitation of this study was that compliance with the ingestion of placebos or supplement was not determined by a biomarker. All subjects were asked whether they were taking the capsules, and the verbal responses were that they were. In addition, the dose of the supplement was not adjusted to weight or body mass index. The biopsy wounds that were generated were small compared with surgical or trauma wounds that are treated on a daily basis. These results may be directly extended to smaller wounds typically observed with blepharoplasty and other skin transfer procedures. The extrapolation to large wounds is a logical next step in translating this pilot study to clinically important wounds. We were not able to test to determine whether wound healing with the oral supplement was related to the subjects’ age, sex, or race. Future studies will consider these issues.

CONCLUSIONS

Extending into plastic surgery procedures, future studies will be undertaken to examine the effect of nutritional supplements in various medical indications. It is logical to assume that the wound-healing mechanisms in soft tissue are uniform in the body. From a basic science standpoint, future investigation will characterize the active ingredients for determining the maximum doses and time periods for maximal wound healing. Subjects undergoing plastic surgery procedures are commonly counseled to avoid various natural products, as they may interfere with perioperative procedures and postoperative wound healing. These data would suggest that physicians may now be able to provide a proactive approach in the care of their patients to ensure a greater degree of success in postoperative wound healing.

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ACKNOWLEDGMENT

This study was supported by an unrestricted educational grant from Enzymes, Inc., Parkville, Mo.
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