Anti-inflammatory and Wound Healing Activity of a Growth Substance in *Aloe Vera*

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Aloe vera improves wound healing and inhibits inflammation. Since mannose-6-phosphate is the major sugar in the *Aloe* gel, the authors examined the possibility of its being an active growth substance. Mice receiving 300 mg/kg of mannose-6-phosphate had improved wound healing over saline controls. This dose also had anti-inflammatory activity. The function of mannose-6-phosphate in *A. vera* is discussed.

The ability of an organism to activate the wound healing process effectively and promptly is essential for its survival. Wound healing, a necessary safeguard against long-term infection and subsequent death, has three major phases: inflammation, proliferation, and remodeling. After injury, fibroblasts migrate toward the wound site where they proliferate and produce collagen, elastin, and proteoglycans.[1] Proteoglycans form the ground substance in which collagen and other connective tissue fibers are embedded.[2,3] These substances remodel the connective tissue (Fig. 1). The movement of individual fibroblasts within the extracellular matrix produces the forces for tissue contraction and, therefore, wound healing.[1] An increase in fibroblast proliferation by platelet and mononuclear phagocyte products improves wound healing.[1]

Aloe vera is a complex plant containing many biologically active substances.[4] Evidence has shown that *Aloe* is effective in wound healing and inflammation reduction.[5-7] This is attributed to a growth factor-like substance in *Aloe* that activates the wound healing and inflammation reduction process.

The objective of this experiment is to determine whether mannose-6-phosphate is an active ingredient in *Aloe* for wound healing and anti-inflammation. It will be important to understand the role of mannose-6-phosphate (a major constituent of the *Aloe* leaf), and if linkage to a protein is necessary to initiate a growth response. Is wound healing a solitary event of mannose phosphate or a combined effort with other cellular substances in *Aloe*, such as glucose phosphate? Proposing a mechanism to explain how *Aloe* affects wound healing and inflammation will be instrumental in determining the most efficient way to use *Aloe* as a growth factor-like substance.

Cells in a wound communicate with each other through substances known as growth factors.[8] Growth factors are polypeptide hormones that are stored by most cells and are secreted into local tissues. Once the growth factor has been attracted to the wound area, it binds to a cell surface receptor, usually a fibroblast. This sequence initiates the biological response, wound healing. The recent experiments by Huang and Huang[9] recognized that the intracellular interaction of growth factors with their receptors may be important in generating the biological effects of these growth factors. Vladsky makes the link between the insulin-like growth fac-

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tor II receptor and fibroblast growth factor stimulation.[9] Evidence reveals that insulin-like growth factor II and mannose-6-phosphate bind to different binding sites of the same receptor on the fibroblast.[10–14] This relationship makes key the stimulation of the fibroblast surface receptor by the mannose-6-phosphate located in the mucopolysaccharide of the *Aloe* plant. This evidence offers a possible link between mannose-6-phosphate and growth factor stimulation of the fibroblast.

Materials and Methods

Wound Healing Assay. Adult male ICR mice (30 g; 15 animals/group) were anesthetized with ether and shaven on both sides of the back. A 6-mm wound was made on each side of the vertebral column. Anterior to posterior wound diameter measurements were made with a caliper on the first, fourth, and seventh days. Mice received daily subcutaneous injections of a mannose-6-phosphate solution at dosages of 30, 150, and 300 mg/kg, respectively. Control mice received daily injections of saline on a 10-mg/kg basis. Another group of animals was given a 150-mg/kg dose of glucose-6-phosphate to evaluate its effects on wound healing and inflammation. Glucose-6-phosphate served as a control for mannose-6-phosphate.

Ear Swelling Croton Oil Assay. Each mouse was given a 0.01-ml (25 µg/µl) dose of croton oil on the seventh day. The dose was applied topically to the right ear to induce inflammation. The left ear of each mouse was used as an internal control. After 6 hr, a sterile 6-mm biopsy punch was used to remove a specimen from each ear of every mouse. These specimens were then weighed with a Mettler®[1] balance. Standard errors for each mean value were calculated. The Student's *t*-test was used to determine significant differences between treatment and control groups. An analysis of variance was also calculated to determine the statistical significance of the study as a whole.

Results and Discussion

Wound Healing. On the seventh day, control animals receiving saline demonstrated an average decrease in wound diameter of 2.8 mm (42.1%). The group receiving 30 mg/kg of mannose-6-phosphate had an average wound decrease of 3.1 mm (43.8%), while animals injected with 150 mg/kg had a wound diameter decrease of 3.7 mm (47.3%). Neither response is considered significantly different from the control group ($p > 0.10$). Mice receiving a dose of 300 mg/kg of the mannose-6-phosphate had an average decrease of 4.3 mm (50.7%) This is considered significant ($p < 0.01$). Animals that received the 150-mg/kg dose of glucose-6-phosphate had an average decrease of 3.1 mm (40.3%), which is not significant ($p > 0.10$). These values are summarized in Table 1.

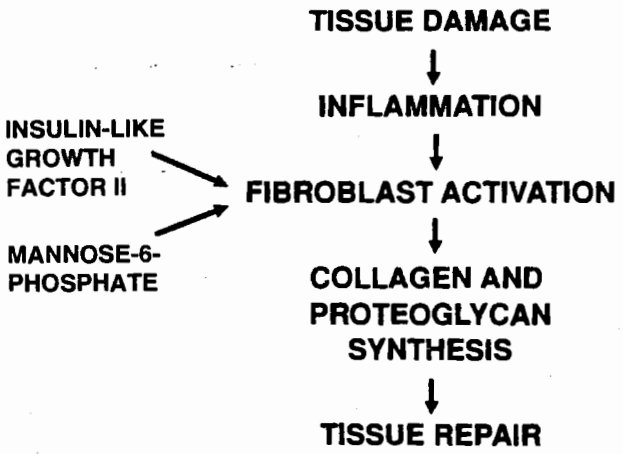

Figure 1. Wound healing process.

Inflammation. The average ear weight difference between the treated right ear and the control left ear of the saline control group was 7.3 mg. The glucose-6-phosphate group had an ear weight difference of 7.0 mg. The groups that were administered mannose-6-phosphate at doses of 30, 150, and 300 mg/kg were observed to have differences of 6.7, 5.6, and 5.5 mg, respectively. The group that received the dose of 300 mg/kg is the only group considered to be significantly different from the control group ($p = 0.05$).

Mannose-6-phosphate and glucose-6-phosphate are the main constituents of the polysaccharide chain in *Aloe*. These experiments have shown that mannose-6-phosphate demonstrates wound healing and anti-inflammatory activity in a dose response fashion (Figs. 2 and 3). Furthermore, the authors concluded that glucose-6-phosphate does not improve wound healing or reduce inflammation. Therefore, the evidence suggests that mannose-6-phosphate is a major structural constituent that stimulates wound healing and anti-inflammation. The data may implicate a structural blueprint of the mucopolysaccharide in *A. vera* (Fig. 4). This figure is a possible representation of a lock and key mechanism between the insulin-like growth factor II/mannose-6-phosphate receptor on the fibroblast and the active polysaccharide chain in *Aloe*.

Gowda et al[15] reported that there is approximately a 6:1 ratio of mannose to glucose in the *Aloe* polysac-

[1]Mettler Instrument Corp, Hightstown, NJ.

Table 1. Effect of Mannose-6-Phosphate on Wound Healing and Topical Croton Oil Induced Inflammation Over 7 Days

	Doses (mg/kg x 7 days)	Wound Healing		Edema	
		mm	% Decrease	mg	% Decrease
Saline	10	2.8 ± 0.2	42.1 ± 2.0	7.3 ± 0.8	
Glucose-6-phosphate	150	3.1 ± 0.2	40.3 ± 1.7	7.0 ± 0.8	4.1 ± 0.5
Mannose-6-phosphate	30	3.1 ± 0.2	43.8 ± 1.9	6.7 ± 1.0	8.2 ± 1.2
	150	3.7 ± 0.3	47.3 ± 2.4	5.6 ± 0.7	23.3 ± 2.9
	300	4.3 ± 0.2	50.7 ± 1.6[a]	5.5 ± 0.7	24.7 ± 3.1[b]

[a]$p < 0.01$.
[b]$p = 0.05$.

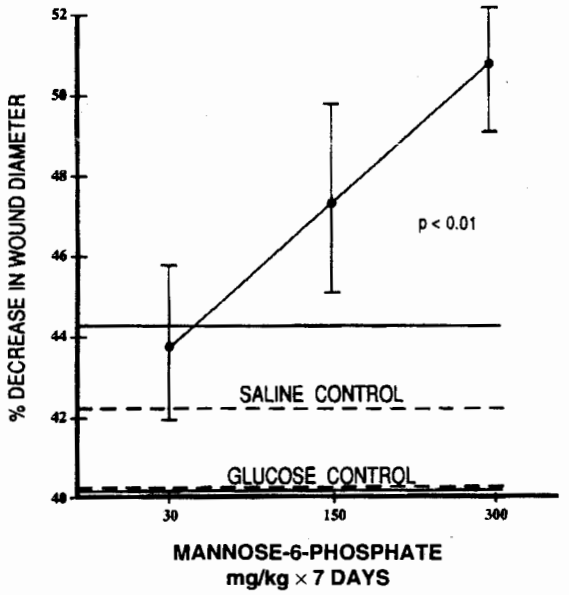

Figure 2. Effect of mannose-6-phosphate on wound healing.

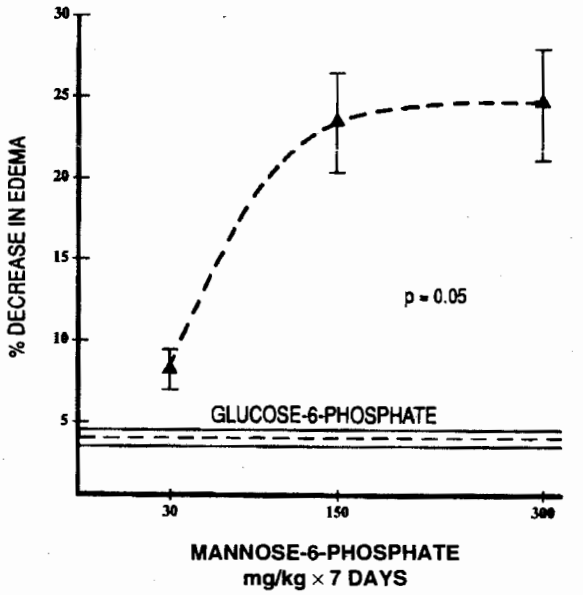

Figure 3. Effect of mannose-6-phosphate on topical croton oil-induced inflammation.

charide chain. The authors believe that the protein is noncovalently attached to the polysaccharide chain by a glucose binding site. The polysaccharide can dissociate from the protein in the same manner as it dissociates from the insulin-like growth factor II/mannose-6-phosphate receptor.

Current theory suggests that mannose-6-phosphate needs to be protein linked to yield a wound healing or an anti-inflammatory response.[16] The authors' data demonstrate that free mannose-6-phosphate effectively reduces inflammation and heals wounds. The laboratory has previously shown that *A. vera* extract improves wound healing and reduces inflammation.[4-6] A comparison of data would suggest that mannose-6-phosphate linked to a protein, thus forming a mucopolysaccharide, may yield even greater wound healing and inflammation reduction.

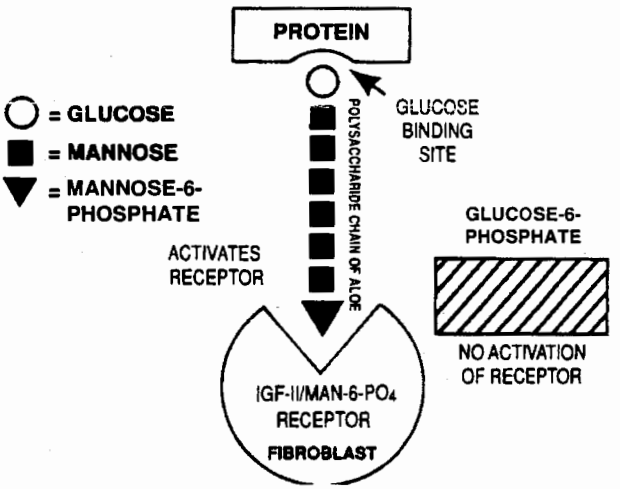

Figure 4. Mannose-6-phosphate activates the insulin-like growth factor II/mannose-6-phosphate receptor.

Possible Mechanism of Action of Mannose-6-Phosphate in *Aloe*. It is well established that insulin-like growth factor II and mannose-6-phosphate bind to the same receptor on the fibroblast.[10–14] These two ligands bind at separate binding sites within the insulin-like growth factor II/mannose-6-phosphate receptor.[9] However, the exact effect of these ligands binding to their individual binding sites is still unclear. There are several possible mechanisms of action of these ligands. One possible theory is that the binding of either ligand is capable of activating fibroblast proliferation. This would indicate that free mannose-6-phosphate is a growth substance capable of yielding the same response as insulin-like growth factor II. In *Aloe*, mannose-6-phosphate is part of a polysaccharide chain that is attached to a protein. This may be important in understanding how *Aloe* produces its wound healing and anti-inflammatory activity.

Another possible theory of fibroblast activation by binding of the ligands is that they work through a combined effort. Nolan et al[9] report that the binding of a ligand at one binding site is capable of influencing ligand binding at the other binding site of the same receptor. It is therefore possible that the binding of mannose-6-phosphate to its binding site preferentially increases the affinity of insulin-like growth factor II to its binding site.[17, 18] This would increase the rate of endocytosis of insulin-like growth factor II. In this manner, insulin-like growth factor II is delivered at a higher rate to the cells and thereby increases fibroblast activity and the wound healing response.

Further experiments are required to clarify the exact mechanism of the insulin-like growth factor II/mannose-6-phosphate receptor in yielding fibroblast activation. One possible experiment would use an antibody against the insulin-like growth factor II binding site. In the past, research using a receptor antibody has been unable to identify the exact mechanism of ligand binding.

Nissley et al[19] used an antibody that blocked the entire insulin-like growth factor II/mannose-6-phosphate receptor. This not only blocked the binding of insulin-like growth factor II to the receptor, but it also blocked mannose-6-phosphate binding as well. They reported no decrease in autocrine growth. They concluded that the insulin-like growth factor II/mannose-6-phosphate receptor is not involved in autocrine growth. This experiment failed to clarify the insulin-like growth factor II/mannose-6-phosphate receptor mechanism for two reasons. First, the entire receptor was blocked and not the individual insulin-like growth factor II binding site. Therefore, mannose-6-phosphate was also blocked from binding to the receptor. Second, insulin-like growth factor II is apparently capable of binding to cell surface binding proteins other than its receptor. Nolan et al[9] reported this phenomenon. Nolan et al found that cells lacking the insulin-like growth factor II/mannose-6-phosphate receptor still bound sufficient levels of insulin-like growth factor II to yield a growth response. This may be the reason that Nissley et al found no reduction in autocrine growth.

An important experiment may be to produce an antibody specific for the insulin-like growth factor II binding site within the insulin-like growth factor II/mannose-6-phosphate receptor. This antibody should also be specific for the cell surface binding proteins. If this antibody is successful in blocking all insulin-like growth factor II binding sites, the exact role of mannose-6-phosphate will become clear. If a growth response still occurs with mannose-6-phosphate treatment, then mannose-6-phosphate alone is capable of stimulating the fibroblasts and is a growth substance. If no growth response is observed, then mannose-6-phosphate functions only to increase insulin-like growth factor II binding and it does not directly stimulate fibroblast activation.

In conclusion, the authors are convinced that mannose-6-phosphate in *Aloe* directly or indirectly stimulates fibroblast activation. Therefore, it is clear that mannose-6-phosphate is an important factor in the wound healing process and plays a significant role in the biological activity of *A. vera*.

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Lisfranc's Fracture-Dislocation

An Unusual Case Presentation

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A fracture of the tarsometatarsal joint was named after Lisfranc, who described amputation through that joint.[1] Dislocations and fractures of the Lisfranc joint are said to be rare, occurring at a rate of one person per 55,000 per year.[1] While the literature is replete with discussions concerning injuries to this joint, few cases of a spontaneous dislocation have been reported.

Mechanism and Classification

Two types of forces are said to cause injury to the Lisfranc's joint: direct and indirect. Direct injury occurs when some type of external object causes a crushing force concentrated at Lisfranc's joint.[1-3] Examples of this include a car or forklift running over a foot. Indirect forces are forces that cause twisting of the forefoot or axial loading of the plantarflexed foot and are much more common.[4] Examples of this mechanism include stepping off a curb or stepping into a hole. There are many classifications of these injuries.[1, 5, 6] The classification of Hardcastle et al[1] has gained widespread popularity because of its ease and its usefulness in prognosis. Wilson's[5] classification probably represents the most thorough description of the mechanism of injury and resulting fracture pattern. His studies on 11 cadaver specimens yielded insight into the stages of the Lisfranc's fracture-dislocation. This classification, however, gives little information to the practitioner on the injury's prognosis or treatment. Spontaneous dislocation has been mentioned in the literature and is thought to be possible in those patients with some type of neuropathic disease.[2, 7-9] Hennessy[8] reported the incidence of Lisfranc's lesions in diabetics as 0.1% to 0.22%. His report cited spontaneous dislocation of the Lisfranc's joint caused by repetitive subclinical microtrauma.[8] No reports of spontaneous dislocation were found in nonneuropathic subjects. Spontaneous ruptures of ligaments and tendons, however, have been reported in association with rheumatoid arthritis, systemic lupus erythematosis, gout, primary and secondary parathyroidism, chronic renal failure on dialysis, long-term steroid use, and local steroid injections.[10] Any of these conditions could weaken the ligamentous structures surrounding Lisfranc's joint, through the same mechanisms, to the point of failure.

Diagnosis

Diagnosis of a Lisfranc's fracture-dislocation is crucial since redislocation of an unfixated injury is common and has a poor prognosis and high incidence of painful arthrosis.[1, 3, 5] Clinically, the foot with a Lisfranc's fracture-dislocation presents as edematous, ecchymotic, and tender over the entire forefoot. In the neuropathic patient, severe pain and swelling may not be present; however, complete absence of pain is rare, and the affected segment is usually warm and pink. In the neuropathic patient, radiographic examination remains the mainstay of diagnosis. This becomes especially important in the spontaneous dislocation since many of these patients are neuropathic and do not present with acute trauma. In a study by Giesecke et al[7] of seven cases of spontaneous dislocations, five cases were incidental findings after plain films were taken of neuropathic, diabetic, ulcerated feet. Three plain film views of the foot are essential. Contralateral foot films may aid in the diagnosis as well, since anatomical variances are common in this area of the foot. Likewise, subtle fracture-dislocations can be difficult to diagnose because of the extensive overlapping of bone seen at the tarsometatarsal joint. Familiarity with normal anatomical alignment, therefore, is essential.

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