Ascorbic Acid Treatment on Early Collagen Production and Wound Healing in the Guinea Pig

by
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CLAIMS HAVE BEEN made that ascorbic acid taken in large amounts can accelerate wound healing. Such amounts, usually far above recommended daily allowances, have been suggested by some as a rational approach for patients recovering from periodontal or other surgical procedures, a precaution against possible inadequate tissue levels of ascorbic acid that may be required for optimal wound healing collagenation.[1-12] On the other hand, others argue that the ascorbic acid status of the individual patient has little to do with wound healing collagenation.[13-18]

Much of the research describing suboptimal healing has been done on animals receiving "maintenance" levels of ascorbic acid. Therefore, such recommended daily requirements may provide most of the physiologic needs of a healthy organism but may not take into sufficient account the higher than normal requirements that may be needed during special situations, such as in wound healing. It has been observed by some that raising the level of ascorbic acid above the recommended daily allowance did not increase the tensile strength of the wound, or the collagenation within the healing wound.[5,16,19,20] Others found that the higher amounts of ascorbic acid increased wound healing collagenation.[1,8,9] The present study is an attempt to pursue this question further by determining if therapeutic levels of ascorbic acid can have any value in increasing wound healing collagenation. The experimental animal selected was the guinea pig.

PROCEDURES

English, short-haired, male guinea pigs of similar age and weight were selected for this research. The animals received water and a diet containing all the essential nutrients except ascorbic acid.[21] The nine animals were divided into three groups and fed twice daily: Group A received the ascorbic acid deficient diet; Group B received 5 mg, and Group C received 200 mg of ascorbic acid supplemention per day. All groups were maintained on their diets through the 21 day experimental period.

Foreign body sponges were implanted in all the animals 7 days after they were placed on the above regimens. This 7 day period was to assure that there was depletion of tissue and blood stores of ascorbic acid in the group receiving the ascorbic acid-free diet (group A) and compare these animals with those receiving either the adequate daily allowance of ascorbic acid (group B), or a megadose supplement (group C).

Polyvinyl alcohol sponges[22] when implanted in living tissue are rapidly permeated by connective tissue. They were first used in experimental surgical procedures in 1949; this technique is now widely used for studying wound healing collagenation.[23,24] The sponge implants were placed in the subcutaneous connective tissue on the middorsal aspect in the guinea pig; four 7 mm × 3 mm discs were placed in each experimental animal. After a 7 day healing period half the sponges were removed; one sponge from each animal was placed in a test tube and immediately frozen for hydroxyproline analysis. The other sponge, was placed in a specimen bottle containing formalin for histologic analysis. After a 14 day healing period, the remaining sponges were removed and also studied.

Tissue specimens were prepared and treated as follows: Hematoxylin and Eosin (Harris), Goldman-Bloom stain, Periodic Acid Schiff, Ammoniacal Silver Impregnation. Duplicate specimens were analyzed for hydroxyproline according to the spectrophotometric method of Stegemann.[25]

DISCUSSION OF RESULTS

From a review of the literature it was difficult to determine precisely the daily requirement of ascorbic acid for the guinea pig, but it was estimated that a 5 mg daily dose of ascorbic acid is more than adequate.[26] An attempt was then made to determine if an ascorbic acid supplement above and beyond the recommended daily allowance would increase the amount of early collagen formation in experimental wounds produced in guinea pigs. Determinations of collagen production were made histologically and by hydroxyproline analysis of collagen ingrowth into implanted foreign body sponges. Analyses of collagen production were made at 7 and 14 day healing periods. From this, the following findings were made:

1. There was no normal collagen production in animals receiving the ascorbic acid free regimen at the 7 day, or 14 day healing period. A macroscopic examination of sponges from the scorbutic group showed no fibrous encapsulation and there appeared to be no collagen ingrowth. The sponges were removed easily with no connective tissue adhesions at either of the healing periods and all sponge removals were accompanied by excessive hemorrhaging. A microscopic examination

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showed the presence of inflammatory cells throughout the 14 day implantation period. This inflammation increased from the 7 day examination period to the 14 day period; no normal collagen formation was present at either period. However, at the 7 day implantation period there was the presence of an extracellular amorphous mass instead of normal collagen. This extracellular mass seemed to increase in size by the 14 day period. However, at no time did it organize into normal collagen.

2. There was greater collagen production in animals receiving ascorbic acid supplements than in animals receiving no ascorbic acid.

3. There was no histologic difference in the amount of collagen formed between the group receiving the recommended daily allowance and the group receiving the megadose supplement of ascorbic acid.

The histologic examination of the implanted sponges is of questionable value in determining the amount of collagen present. It had been established previously that histologic examination gave no indication of the tensile strength of a wound and that an increase in tensile strength parallels an increase in collagenation.[27] One can deduce that histologic analysis does not give an indication of the true amount of collagen present in healing wounds and the amount of collagen is at best only a gross index. Histologic analysis did reveal, however, that there was no normal collagen formed in the scorbutic guinea pigs. Therefore, hydroxyproline analysis was carried as a better index of collagen formation and a significantly greater amount was found to be present in those animals receiving either of the ascorbic acid supplements.

4. Animals receiving no ascorbic acid showed some hydroxyproline production at 7 and 14 day healing periods. At 7 days, there was a minimal level of hydroxyproline (0.542 μg); at 14 days the hydroxyproline concentration was higher but it was still low (0.620 μg).

5. Animals receiving 5 mg of ascorbic acid daily show significantly greater amounts of hydroxyproline than animals receiving no ascorbic acid, at both the 7 and 14 day healing periods ($P < 0.05$). There was good hydroxyproline formation during the 7 day healing period (2.863 μg), and at 14 days the amount of hydroxyproline increased sharply to 37.13 μg.

6. Animals receiving a megadose supplement of ascorbic acid show significantly greater amounts of hydroxyproline than animals receiving the lower amounts of ascorbic acid at the 7 day healing period ($P < 0.05$) but this difference is not significantly different at 14

FIGURE 1. *Ammoniacal silver impregnation of a 14 day sponge in an animal receiving no ascorbic acid (Group A) (Medium power, original magnification, × 150). Sponge is the light gray amorphous material. Collagen fibers are the darker gray material located within the sponge. There is an increase in the number of nuclei and also an increase in the amount of extracellular material. However this material remains amorphous and does not appear to be formed into normal collagen. There appears to be inflammation present.*

FIGURE 2. *Ammoniacal silver impregnation of a 14 day sponge in an animal receiving a recommended daily allowance of ascorbic acid (Group B) (Medium power, original magnification, × 150). Sponge is the light gray amorphous material containing no nuclei. Collagen fibers are the darker gray material located within the sponge. There appears to be a well organized, dense collagen ingrowth into the sponge. There appears to be no inflammation present.*

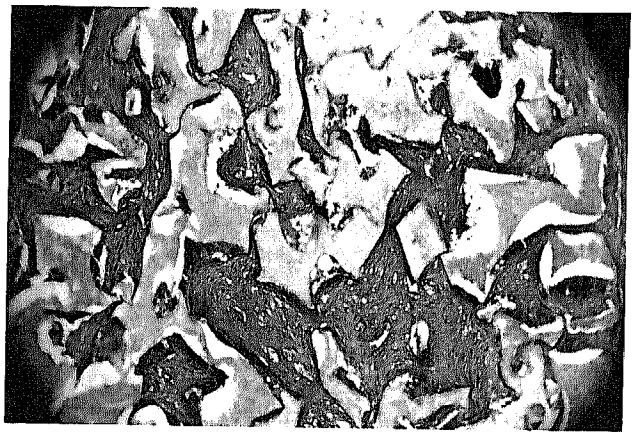


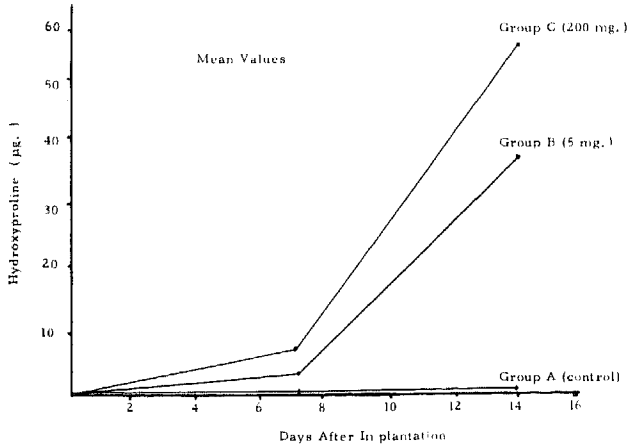
FIGURE 3. *Ammoniacal silver impregnation of a 14 day sponge in an animal receiving a megadose supplement of ascorbic acid (Group C) (Medium power, original magnification, × 150). Sponge is the light gray amorphous material containing no nuclei. Collagen fibers are the darker gray material located within the sponge. There appears to be a well organized, dense collagen ingrowth into the sponge. There appears to be no inflammation present.*

days. The megadose group showed good hydroxyproline formation at the 7 day healing period (6.913 μg); at 14 days, the hydroxyproline concentration reached 54.67 μg for this group. Table 1 compares the mean values of the amount of hydroxyproline formation and this representation suggests there is greater collagen production when a megadose supplement is administered. Wound healing collagenation is even above the amount formed in animals receiving the recommended daily allowance of ascorbic acid (Graph 1). A statistical analysis of the data is presented in table 1.

TABLE 1. *Hydroxyproline Content (μg.) of Implant Sponges*

Animal Number	7 days	14 days
Group A—No ascorbic acid (control)		
A-1	0.475	0.710
A-2	0.700	0.610
A-3	0.450	0.540
mean	0.542	0.620
±SD	± 0.112	± 0.070
Group B—5 mg of ascorbic acid per day		
B-1	3.425	52.50
B-2	1.165	27.50
B-3	3.550	31.40
mean	2.863	37.13
±SD	± 0.884	± 10.98
Group C—200 mg of ascorbic acid per day		
C-1	6.965	88.50
C-2	9.935	50.20
C-3	3.840	25.30
mean	6.913	54.67
±SD	± 3.561	± 25.99

Group A is different from Group B at 7 days ($P < 0.05$).
Group A is different from Group B at 14 days ($P < 0.05$).
Group A is different from Group C at 7 days ($P < 0.05$).
Group A is different from Group C at 14 days ($P < 0.05$).
Group B is different from Group C at 7 days ($P < 0.05$).
Group B is *not significantly* different from Group C at 14 days (Mann-Whitney μ statistical test).



GRAPH 1. *Hydroxyproline content (μg) in implanted sponges.*

In conclusion, megadose supplements of ascorbic acid appear to have at least a transient effect on wound healing collagenation and the results support megadose ascorbic acid therapy during acute phases of healing.

SUMMARY

The purpose was to determine if megadoses of ascorbic acid have any value in wound healing. Guinea pigs were divided into three experimental groups: one group was fed a placebo, a second was fed the "recommended daily allowance" of ascorbic acid (5 mg per day), and the third received a megadose supplement (200 mg per day). The animals were maintained on these regimens for a total of 21 days during which time foreign body sponges were placed within the subcutaneous connective tissue to act as matrices upon which there would be collagen ingrowth. Sponges were removed at various time intervals and collagenation was examined qualitatively by a histologic technique and quantitatively by hydroxyproline analysis.

After a 7 day maintenance period, four sponges were implanted subcutaneously in the connective tissue on the dorsal aspect of each experimental animal. Seven days after implantation two of these sponges were removed from each animal: one for histologic analysis, the other for hydroxyproline analysis. Fourteen days after the original implantations the remaining two sponges were removed from the experimental animals, again for histologic and hydroxyproline analyses.

Histologic analyses revealed differences between the scorbutic group and the groups receiving ascorbic acid treatment. At the end of the 7 day and 14 day periods, the scorbutic animals showed no normal collagen formation. However, the groups receiving 5 mg, or 200 mg doses, appeared to form significant amounts of collagen. Histologically, no difference could be determined between the groups receiving ascorbic acid, and collagenation in both of these groups appeared to advance at the same level.

Hydroxyproline analysis of the sponges within the scorbutic group showed there was a small amount of hydroxyproline formed at 7 and 14 days. Both groups receiving ascorbic acid (5 or 200 mg per day) showed a significantly greater production of hydroxyproline at both the 7 day and the 14 day healing periods ($P < 0.05$), and the group receiving the megadose supplement showed the highest production of hydroxyproline at the 7 day healing period ($P < 0.05$), but at the 14 day healing period there was no statistical difference.

Results indicate megadose ascorbic acid therapy may be of value during the acute phase of healing but its value is less apparent in long term healing.

Note: The conclusions drawn in this particular experiment do not precisely reflect the wound healing phenomenon at the dentogingival junction in the wound healing during periodontal repair; the interface is between connective tissue and cementum subsequently cov-

ered by epithelium rather than two connective tissue interfaces covered with epithelium which results in first intention wound healing. During periodontal wound healing, the fact that there is epithelial downgrowth along the tooth may, in fact, alter even the early connective tissue healing.

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