The Effectiveness of Oral Vitamin C on Post-extraction Wound Healing: A Pilot Study

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ABSTRACT

Background: Delayed wound healing is one of complications after tooth extraction. The role of vitamin C in the healing of wounds has been studied in many aspects, however there is no report about the effect of oral vitamin C supplement in different dosages on extraction wound healing in human.

Objectives: The purpose of this study was to evaluate the effect and proper dosage of oral vitamin C supplement on post-extraction wound healing.

Methods: This preliminary study was a split-mouth, double-blind, randomized controlled clinical trial of 18 patients who underwent symmetric bilateral non-infected premolar extractions. The patients were randomly divided into 3 intervention pairs (6 patients in each pair); Pair 1: placebo vs vitamin C 600 mg/d, Pair 2: placebo vs vitamin C 1,500 mg/d and Pair 3: vitamin C 600 mg/d vs vitamin C 1,500 mg/d. Each group was prescribed placebo and/or vitamin C three times a day for 10 days after each tooth extraction and the assessment of the wound was performed on day 0, 7 and 21 then the tooth on other side was extracted and studied with the same protocol. Socket's bucco-lingual and mesio-distal width, depth and 1% toluidine blue staining were recorded by two examiners. Pain score and high vitamin C-containing diets were also recorded during the first 3 days and after 7 days, respectively. Paired t-test was used in studying any difference in the reduction proportion of the size of the extraction socket between 2 extraction sites and Wilcoxon Signed-Rank test was used for comparing pain scores.

Results: The reduction proportion of socket depth between 7 and 21 days post-extraction in teeth receiving vitamin C 1,500 mg/d was higher than that in placebo (p=0.037). Pain score on day 1 after tooth extraction in teeth receiving vitamin C 1,500 mg/d was significantly lower than the placebo side (p=0.045).

Conclusion: Taking oral vitamin C 1,500 mg per day for 10 days tended to promote post-extraction wound healing between 7 days and 21 days after tooth extraction.

Keywords: Ascorbic acid, Extraction wound, Tooth extraction, Vitamin C, Wound healing

INTRODUCTION

Tooth extraction is a common dental procedure.[1] However, this simple procedure may cause post-operative complications including dry socket, post-operative bleeding, pain, infection and delayed wound healing, which has an impact on patients' quality of life and increases clinical workloads and cost.[2, 3]

Normal wound healing process starts with hemostasis and progresses through inflammation, proliferation and remodeling phases, all of which are overlapping.[3-5] Delayed wound healing is a failure to progress through the normal phases. Vitamin C deficiency is one of the significant factors[5, 6] since vitamin C plays an important role in all phases of wound healing. In the inflammatory phase, vitamin C is required for neutrophil apoptosis and clearance. In the proliferative phase, vitamin C is involved in the process of synthesis, maturation, secretion and degradation of collagen by acting as a cofactor for hydroxylation of proline and lysine during collagen synthesis and being associated with fibroblast proliferation which affects angiogenesis and capillary strength.[5-9]

The role of vitamin C in wound healing has been investigated many studies including both laboratory investigation and clinical trials.[1, 4, 10-14] However, there are few studies relating the effect of oral vitamin C supplement on the extraction wound healing in humans especially those comparing between various vitamin C dosages.[15, 16] Therefore, the objective of this study was to investigate the clinical effect and proper dosage of vitamin C oral supplement on extraction wound.

MATERIALS AND METHODS

This pilot study was a randomized, double-blinded clinical trial conducted at the Department of Oral and Maxillofacial Surgery, Chulalongkorn University between August 2018 and February 2019. The study was approved by the Human Research Ethic Committee of the Faculty of Dentistry, Chulalongkorn University. The inclusion criteria were healthy patients aged between 14-40 years who underwent symmetric bilateral non-infected premolar extraction. Smokers, alcoholics, pregnant or lactating women, psychiatric patients, patients who took corticosteroids or estrogen-containing contraceptive drugs were excluded. The study was designed as split-mouth in which 2 different interventions were given to each patient during a different period of time. There were 18 patients in this pilot study and they were randomly divided into 3 intervention pairs (6 patients for each pair) as follows; Pair 1: placebo vs vitamin C 600 mg/d, Pair 2: placebo vs vitamin C 1,500 mg/d and Pair 3: vitamin C 600 mg/d vs vitamin C 1,500 mg/d.

Patients' vital signs were recorded before every extraction. Premolar teeth on both sides were randomly selected and the extraction was done by the same operator with standardized technique, using elevators and extraction forceps under 2% mepivacaine local anesthetic with 1:100,000 epinephrine. Bleeding was controlled using gauze pressure. Acetaminophen 500 mg was prescribed as needed.

The vitamin C was prescribed 3 times a day as shown in Table 1. Vitamin C used in the study was manufactured by Patar Lab Co. (Pathumthani, Thailand), whereas placebo containing calcium carbonate, sucrose and coated with carnauba wax was manufactured under the license of the Faculty of Pharmaceutical Sciences, Chulalongkorn University.

Table 1 The interventions assigned in three intervention pairs.

	Extraction site	Placebo (tab)	Vitamin C 100 mg (tab)	Vitamin C dosage per day (mg)
Pair 1	1st site	5	0	0
	2nd site	3	2	600
Pair 2	1st site	5	0	0
	2nd site	0	5	1,500
Pair 3	1st site	3	2	600
	2nd site	0	5	1,500

Moreover, all patients received a form to record their diets and a checklist of top 5 high vitamin C-containing vegetables and fruit during the wound healing period for 7 days. Pain score was also recorded using a 10-point Visual Analog Scale (0 = no pain, 10 = extreme pain) during the first three days post-extraction at 8 am, 12 pm and 6 pm. Daily pain scores were calculated from the average of the three time points.

The measurement of a wound site was performed 3 times for each tooth including immediately after tooth extraction, on day 7 and day 21 post-extraction by two examiners who were not the operator. Inter-examiner reliability was calibrated between them. The measurement procedures were described as follows:

- The sizes of the extraction socket (in mm) in both bucco-lingual (BL) width and mesio-distal (MD) width were measured by a caliper using the adjacent teeth as reference points.
- The depth of the extraction socket was measured at buccal plate by a periodontal probe.

On day 21 post-extraction, the wound was stained with 1% toluidine blue solution, rinsed and recorded for the site with less staining. If the extraction site became normal gingival tissue, there would be no stain.[17]

When the measurement of the first socket was finished, the tooth on the other side was extracted with the same protocol. Patients were prescribed placebo or vitamin C after the extraction and blinded of the prescription.

To analyze the data, the statistical program SPSS version 22 was used. Patients' demographic data were presented. Chi-square was used for comparing the difference in gender and the location of the extraction site among 3 intervention pairs. Kruskal Wallis H test was used for comparing the age of the patients among the 3 groups.

The proportion of socket size reduction was calculated for BL, MD widths and socket depth as (dimension at time $_{before}$) - (dimension at time $_{after}$)/ (dimension at time $_{before}$).

The normality of the data was tested by Kolmogorov-Smirnov test. Because of the normal distribution, the proportions of the wound size reduction were compared between 2 extraction sites using Paired t-test. Wilcoxon Signed-Rank test was used for comparing pain scores due to the non-normal distribution. Toluidine blue staining of the 2 extraction sites was compared by two observers after 21 days and the less staining site of each pair was recorded and defined as 'better healing'. Patients' vitamin C-containing diet after each extractions was also compared and presented as the percentage difference.

RESULTS

Nine males and 9 females participated. The mean age of the patients was 19.56 years. The extraction site was mostly in the maxilla. The demographic data and *p*-value were shown in Table 2. The age of the patients between the three intervention groups was significantly different but gender and the location of extraction sites were not.

Table 2 Age, gender and location of extraction sites of the patients (*$p < 0.05$)

	All	Pair 1	Pair 2	Pair 3	*p*-value
Age (years) mean±SD	19.6±3.7	21.3±2.8	21.0±2.7	16.3±3.7	0.023*
Gender (n,%)					
Male	9 (50%)	5 (83.3%)	2 (33.3%)	2 (33.3%)	0.135
Female	9 (50%)	1 (16.7%)	4 (66.7%)	4 (66.7%)	
Extraction site (n,%)					
Maxilla	12 (66.7%)	5 (83.3%)	4 (66.7%)	3 (50%)	0.472
Mandible	6 (33.3%)	1 (16.7%)	2 (33.3%)	3 (50%)	

Socket size reduction

The proportion of socket size reduction (BL, MD, depth) between any time points was not significantly different in Pair 1 and Pair 3 (Table 3 and Table 5). However, the proportion of socket size reduction in Pair 2 was significantly different between 7 days and 21 days post-extraction (Table 4) (p=0.037).

Table 3 Comparison of socket size reduction between 2 extraction sites in Pair 1 (*$p < 0.05$)

Pair 1 (Placebo vs Vitamin C 600 mg/d)				
	Mean proportion of socket size reduction site 1	**Mean proportion of socket size reduction site 2**	**Mean difference (site 2 - site 1)**	***p*-value**
Between immediate and 7 days				
Bucco-lingual	0.406	0.403	-0.003±0.13	0.960
Mesio-distal	0.392	0.526	0.134±0.23	0.209
Depth	0.548	0.568	0.020±0.15	0.765
Between immediate and 21 days				
Bucco-lingual	0.496	0.563	0.067±0.97	0.152
Mesio-distal	0.439	0.555	0.116±0.23	0.272
Depth	0.803	0.858	0.055±0.11	0.276
Between 7 days and 21 days				
Bucco-lingual	0.145	0.251	0.106±0.16	0.162
Mesio-distal	0.079	0.057	-0.022±0.07	0.484
Depth	0.546	0.643	0.097±0.23	0.364

Table 4 Comparison of socket size reduction between 2 extraction sites in Pair 2 (*$p < 0.05$)

Pair 2 (Placebo vs Vitamin C 1,500 mg/d)				
	Mean proportion of socket size reduction site 1	**Mean proportion of socket size reduction site 2**	**Mean difference (site 2 - site 1)**	***p*-value**
Between immediate and 7 days				
Bucco-lingual	0.443	0.455	0.012±0.21	0.894
Mesio-distal	0.366	0.176	-0.190±0.32	0.231
Depth	0.506	0.494	-0.012±0.35	0.935
Between immediate and 21 days				
Bucco-lingual	0.518	0.581	0.063±0.17	0.405
Mesio-distal	0.460	0.345	-0.115±0.29	0.373
Depth	0.783	0.853	0.070±0.14	0.280
Between 7 days and 21 days				
Bucco-lingual	0.113	0.220	0.107±0.19	0.231
Mesio-distal	0.125	0.218	0.093±0.12	0.129
Depth	0.510	0.731	0.221±0.19	0.037*

Table 5 Comparison of socket size reduction between 2 extraction sites in Pair 3 (*$p < 0.05$)

Pair 3 (Vitamin C 600 mg/d vs Vitamin C 1,500 mg/d)				
	Mean proportion of socket size reduction site 1	**Mean proportion of socket size reduction site 2**	**Mean difference (site 2 - site 1)**	***p*-value**
Between immediate and 7 days				
Bucco-lingual	0.495	0.489	-0.006±0.15	0.930
Mesio-distal	0.455	0.296	-0.159±0.19	0.103
Depth	0.655	0.697	0.042±0.13	0.449
Between immediate and 21 days				
Bucco-lingual	0.560	0.574	0.014±0.09	0.722
Mesio-distal	0.496	0.404	-0.092±0.22	0.352
Depth	0.867	0.854	-0.013±0.06	0.614
Between 7 days and 21 days				
Bucco-lingual	0.144	0.159	0.015±0.19	0.850
Mesio-distal	0.074	0.168	0.094±0.16	0.215
Depth	0.606	0.506	-0.100±0.15	0.168

Post-operative pain

Patients receiving vitamin C 600 mg/d and 1,500 mg/d, tended to have lower post-operative VAS score during the first 3 days than those receiving placebo (Fig. 1 and Fig. 2), but the difference was statistically significant only between placebo vs vitamin C 1,500 mg/d on day 1 (p=0.045). There was no significant difference in pain scores between patients who received vitamin C 600 mg/d compared to vitamin C 1,500 mg/d during the first 3 days (Fig. 3).

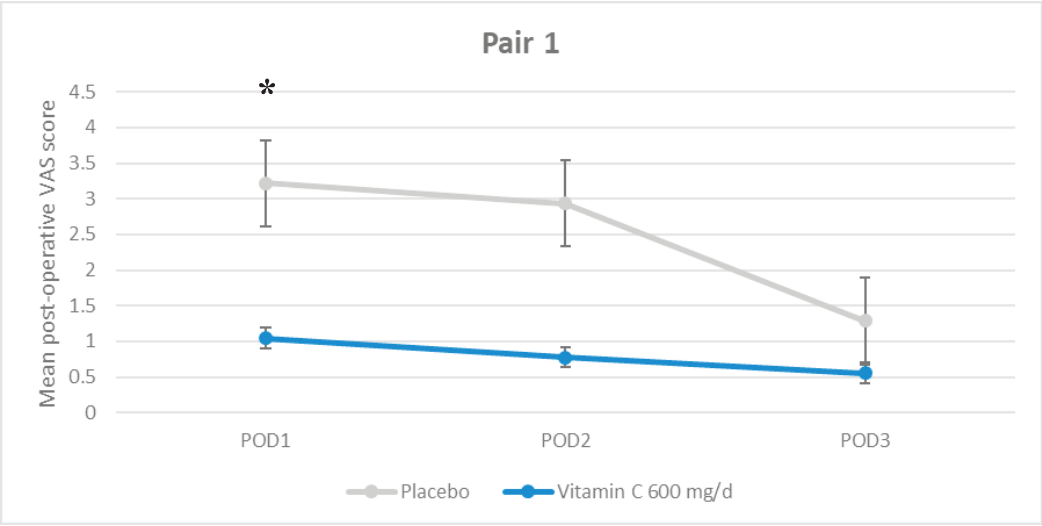

Fig. 1 Mean post-operative VAS score in Pair 1 (POD = post-operative day)

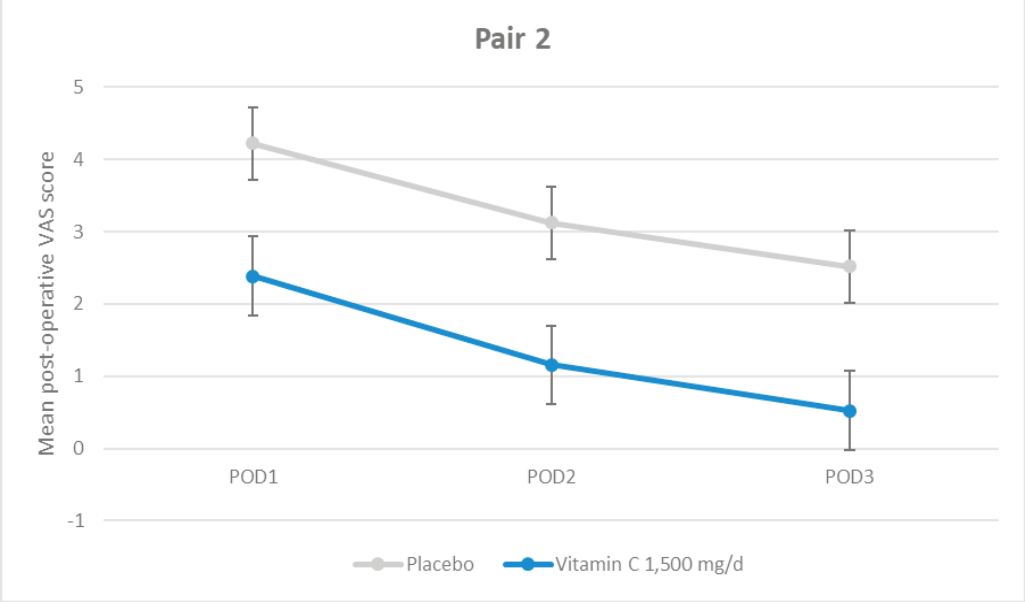

Fig. 2 Mean post-operative VAS score in Pair 2 (POD = post-operative day) (*p-value < 0.05)

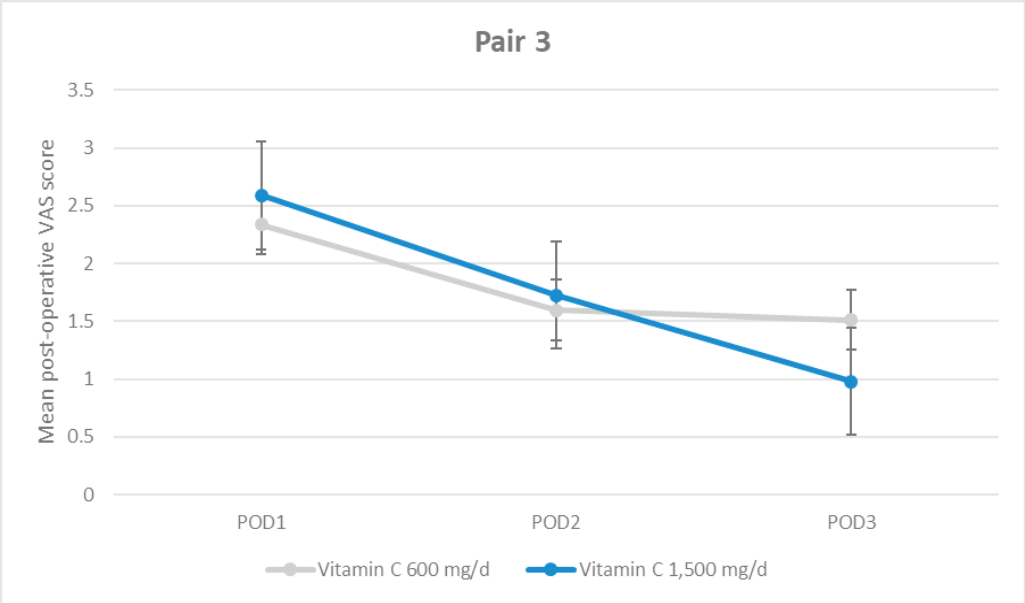

Fig. 3 Mean post-operative VAS score in Pair 3 (POD = post-operative day)

Toluidine blue staining

In Pair 1, the number of less stained sites in placebo was equal to vitamin C 600 mg/d, whereas in Pair 2, placebo had less stained sites than vitamin C 1,500 mg/d. In Pair 3, vitamin C 600 mg/d had less stained sites than vitamin C 1,500 mg/d.

Patients' diet during 7 days post-extraction

In Pair 1, half of the patients had the same diet after 2 extractions. There were 33.3% of the patients who had higher vitamin C-containing diet after the first than the second extraction, whereas 16.7% had higher vitamin C-containing diet after the second extraction. In Pair 2, most of the patients had higher vitamin C-containing diet after the first extraction and in Pair 3, there was no difference in diet between two extractions. The data were shown in Fig. 4.

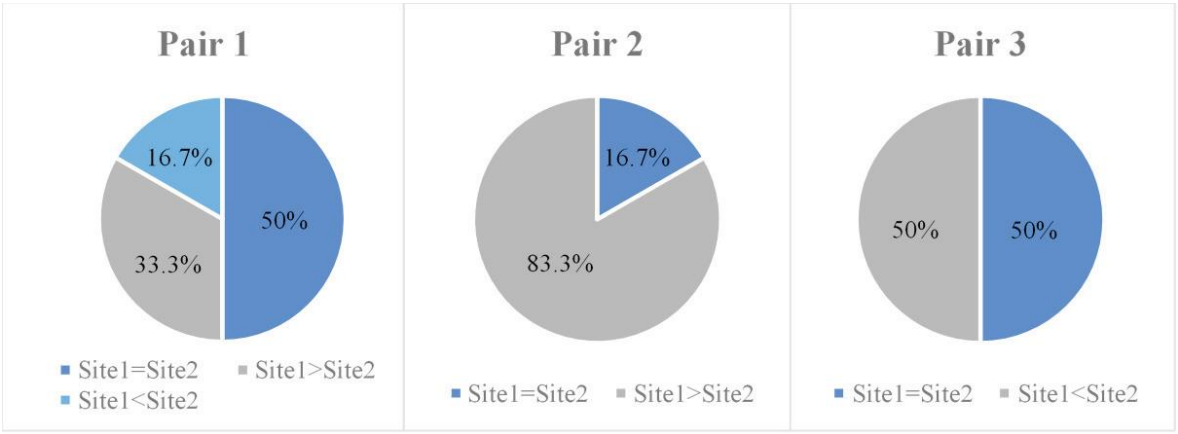

Fig. 4 The differences of patients' vitamin C-containing diet after each tooth extraction in 3 intervention pairs.

DISCUSSION

The result of this study tended to correlate with the earlier study that oral vitamin C was effective in promoting wound healing after tooth extraction.[15, 16] This study was designed to be split-mouth so that the results could be compared in each patient to eliminate the confounding factors. Socket size measurement was performed to provide reliable objective data. Age of patients which was significantly different among 3 groups might be due to the small sample size.

There is no specific guideline of vitamin C for wound healing.[18] Because the evidence of toxicity is low, vitamin C is often supplemented in high doses.[6] In the hypermetabolic state, the dosage recommended was between 500 mg/d and 2,000 mg/d, which was more than 10 times the daily intake recommended by Food Standards Agency (FSA).[19] For promoting the healing process, the recommended dose of vitamin C supplement was 500 to 1,000 mg/d.[6] In order to maximize uptake and plasma concentrations of vitamin C, it was recommended to prescribe vitamin C in divided doses over the day.[20] Until recently, vitamin C regimen has been controversial. Previous studies investigated the effect of vitamin C 1,500 mg/d and 2,000 mg/d,[15, 16] whereas in our study, we compared two regimens of vitamin C, i.e. 600 mg/d vs 1,500 mg/d. Higher dose of vitamin C (1,500 mg/d) showed no difference in socket healing compared to lower dose of vitamin C (600 mg/d). Both vitamin C 600 mg/d and 1,500 mg/d possibly enhanced wound healing but the difference was not obvious.

In patients who received vitamin C 1,500 mg/d, the reduction of depth of the socket was significantly higher than that of the placebo site between 7 and 21 days. Vitamin C possibly promotes granulation tissue healing in the proliferation phase resulting in the reduction of socket depth. This was supported by the histological study in animals which reported that granulation tissue fibroblast maturation in vitamin C group was significantly higher than the control group on post-operative day 14. Moreover, collagen deposition and neovascularization were also higher in vitamin C group at that time.[12] The study in Gulo$^{-/-}$ mice, a humanized model for wound healing, revealed that there was a higher expression of healing mediators, induction of self-renewal genes and fibroblast proliferation in sufficient vitamin C group and deficient with vitamin C supplemented group and concluded that vitamin C promoted tissue repair and shortened healing duration.[4] In dental socket healing, the replacement of the granulation tissue by the connective tissue which contained spindle-shaped fibroblasts, collagen and ground substance occurred in 14- to 16-day

post-extraction.[21] Another study showed that vitamin C administration could reduce the incidence of dry socket, a painful complication caused by improper formation of the granulation tissue bed.[15]

The result of the toluidine blue staining (in Pair 2) showed that the placebo side had better healing than vitamin C 1,500 mg/d, inconsistent with the result of extraction wound size reduction. Although the size proportion became smaller, the connective tissue seemed to have incomplete epithelialization. More sample size will be needed for confirmation of this finding.

According to pain, the results showed that patients who received vitamin C tended to have lower pain scores comparing to the placebo. Although the analgesic mechanism is unclear, there were clinical studies showing that vitamin C administration decreased pain in patients with chronic regional pain syndrome and suggested that vitamin C was an effective adjunctive therapy for reducing acute and chronic pain in patients.[22] The previous animal study showed that vitamin C promoted healing by accelerating termination of the inflammatory phase of wound healing.[4] As pain is one of the inflammation symptoms, the shorter inflammation may cause less pain and results in rapid healing. Moreover, there was evidence suggesting that vitamin C might act as an analgesic in various parts of the body.[15]

Limitation of this study was the patient's compliance. The intervention was prescribed 3 times (5 tablets each) a day for 10 days after tooth extraction. Patients were asked to return the vitamin C package for counting how many tablets they had and we found that some patients could not follow the entire prescription. In addition, since vitamin C can be obtained from food such as fruits and vegetables, split-mouth design was used to reduce this error. However, there were some patients who had different diet after 2 extractions.

CONCLUSION

Taking oral vitamin C 1,500 mg/d for 10 days tended to promote post-extraction wound healing between 7 and 21 days after tooth extraction in terms of the depth of the extraction wound and post-operative pain.

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