

Effects of Vitamin C on Osteogenesis Imperfecta

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ABSTRACT. Biochemical and clinical effects of vitamin C on children with osteogenesis imperfecta have been documented. There is a significant drop in serum zinc correlated with ascorbate ingestion (25 to 50 mg/kg/day). This tendency contrasts to the trend in normal controls which is upward. Family members behave either like the affected child or normally whereas control families show only the expected increases.

Incidence of fracturing drops with chronic vitamin C ingestion. While encouraging this observation is not well controlled. Larger groups of patients must be observed before arriving at firm conclusions. *Pediatrics*, 53:56, 1974, OSTEOGENESIS IMPERFECTA, VITAMIN C, SERUM ZINC OR ZINC, TRACE METALS, FRACTURES.

Osteogenesis imperfecta is a metabolic bone disorder characterized by fractures following minor trauma, often associated with development of bony deformities. An associated finding, indicative of deficient collagen content, is blueness of the sclera. A number of investigators have found serum calcium, phosphorus, alkaline and acid phosphatase, potassium, hemoglobin, white blood cell count, and urinalysis to be normal.¹⁻⁴ Histological investigations have been interpreted as showing deficiency of mature collagen,^{3,5} diminished number of Haversian systems,⁴ irregular arrangement of the matrix and abnormal orientation of the organic fibers.^{3,4} It has been suggested that osteogenesis imperfecta is a qualitative defect, perhaps at the enzymatic level, which interferes with the mineralization process involved in bone formation.⁴

Zinc may play a role in bone metabolism through its activities as an enzymatic co-factor for enzymes such as the acid and alkaline phosphatases.⁶ The study reported here documents an abnormality in response of the serum zinc concentration of children with osteogenesis imperfecta to the oral administration of ascorbic acid (vitamin C). This agent has been reported to be a potentially useful treatment for this disorder.⁷ Further data support this impression.

MATERIALS AND METHODS

Sample Collection and Handling

Venous blood was drawn into polypropylene syringes through disposable needles. The blood was placed in nitric-acid-washed test tubes and allowed to clot for up to one hour at 25 C. The blood was then centrifuged at 2,500 rpm (1,200 G), for ten minutes, and the serum was transferred by Pasteur disposable pipettes to screw-capped vials. Samples were frozen until analysis. This process was shown to produce no measurable contamination by zinc.⁸

Zinc Analysis

Analyses of serum zinc were done according to our previously reported method using a Bausch and Lomb AC2-20 atomic absorption spectrophotometer in which the burner had been replaced with the Varian Techtron Model 61 Carbon Rod Atomizer.⁸ The mean normal serum zinc value obtained by this method is (mean \pm SD) $94.6 \pm 11.0 \mu\text{g}/100 \text{ ml}$ ($N = 25$); the average recovery of added zinc in serum is 97.5%; the coefficient of variation is 4.76% ($N = 76$); the qualitative concentration limit is $0.009 \mu\text{g}/100 \text{ ml}$; the sample requirement is $1.0 \mu\text{l}$ of serum per determination.⁹

CLINICAL INVESTIGATIONS

Thirteen patients from birth to 15 years of age, who had been diagnosed as having osteogenesis imperfecta and who were not receiving any specific medications, were chosen for this study. Clinical manifestations of osteogenesis imperfecta varied widely from severe deformities to only a few fractures and light blue sclera. Each patient was admitted to the Clinical Study Center for a three-day observation period. The parents and older patients were interviewed, with particular attention given to

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TABLE I
INCIDENCE OF FRACTURES AMONG PATIENTS WITH OSTEOGENESIS IMPERFECTA

Patient	Sex	Age (yr) Started Study	Total No. Fractures	Ascorbic Acid (mg/day)	No. Months on Study	No. Fractures Equal Time Prior	No. Fractures on Study
M.P.	M	6	57	1,000	40	32	10
A.M.	M	8	60	1,250	40	14	5
M.A.	M	15	7	1,500	36	2	1
B.H.	F	10	6	1,000	31	4	1
C.P.	F	10	50	1,000	42	14	2
S.E.	F	11	30	1,000/3,000	25	10	3
E.S.	F	14	75	1,000	21	1	0
N.W.	M	6	17	1,000	10	3	1
T.H.	F	Birth	22	250	17	20 (at birth)	2
J.K.	F	Birth	5	250	18	3 (at birth)	2
S.B.	F	Birth	2	100/400	43	2 (at birth)	0
D.H.	M	5	8	1,000	40	6 (1 yr prior)	2
A.B.	F	1	4	600	31	2 (in 3 mo)	2

the following areas of the history: familial incidence of osteogenesis imperfecta; number of fractures and the ages at which they occurred; the apparent cause of each fracture; the number of accidents and falls not resulting in fracture; obvious deformities and their influence on physical performance; appetite and diet; general well-being, including emotional, mental, and sexual development; heat intolerance; measures of physical size (weight, height, etc.); and blueness of the sclerae. Fasting blood samples were obtained as previously described and 24-hour urine samples were collected. In addition to routine analysis, the following laboratory tests were performed: in *serum*—ascorbic acid, carbon dioxide, chloride, sodium, potassium, urea nitrogen, creatinine, calcium, alkaline and acid phosphatase, inorganic phosphorus, glutamic oxaloacetic transaminase (SGOT), lactic dehydrogenase (LDH), total cholesterol, hemoglobin, hematocrit, and leukocyte counts; in *urine*—specific gravity, pH, qualitative estimates of protein, glucose, and occult blood, and microscopic examination.

The patients then began receiving a daily dosage of between 1 and 2 gm of ascorbic acid (standard 250-mg tablets taken in four divided doses). The infants received between 250 and 600 mg of natural liquid vitamin C (Rose Hips and Acerola extract) which was given in milk. They were discharged to their regular physician's care with instructions to continue the ascorbic acid supplement.

During the study period children were readmitted for repetition of laboratory tests, serum zinc analysis, and the standard interview. Special attention was given to noting any changes in physical well-being, appetite, diet, bowel habits, physical measures of growth, frequency of fractures, and particularly those accidents not resulting in any known fracture.

RESULTS

At no time during this study were the results for the routine laboratory tests outside the accepted normal ranges. Although the serum ascorbic acid levels consistently increased (means, 1.0 mg/100 ml prior to and 1.9 mg/100 ml during treatment), all remained within the accepted normal range.¹⁰

Most patients reported increased physical activities along with increased stamina and a feeling of more energy (see Comments on Patients) while on the ascorbic acid therapy.

Fracture Rate

To facilitate the discussion concerning the fracture rate both on and off ascorbic acid therapy, the study patients have been divided into two groups: group 1 (the first eight patients listed in Table I) consists of the patients for whom a control period, equal in length to the time on the study, and during which they received standard medical care from their physicians, exists. Group 2 (the last five patients listed in Table I) includes the patients for whom no such control period exists. Three of the five (T.H., J.K., S.B.) were placed on the study at birth; the other two (D.H., A.B.) have now been on the study for a much longer period of time than elapsed from the first fracture until placed on the study.

All eight patients in group 1 showed a decrease in the number of fractures while on ascorbic acid therapy as compared to the control period immediately prior to initiation of the ascorbic acid supplementation. Six of the patients showed a significant decrease in fractures, although all six demonstrated increased physical activities during this same treatment period. The other two (M.A., E.S.) were placed on the study after puberty, and had already experienced the decrease in fracture rate that is

TABLE II
CHANGE IN SERUM ZINC WHILE ON ASCORBIC ACID

Patient	Age (yr) Started Study	Ascorbic Acid (mg/24 hr)	Initial Serum Zinc ($\mu\text{g}/100\text{ ml}$)	Time Between Samples (mo)	Serum Zinc ($\mu\text{g}/100\text{ ml}$)	Zinc
M.P.	6	1,000	100.0	3	76.1	-23.9
A.M.	8	1,250	139.5	16	89.0	-50.5
M.A.	15	1,500	76.0	20	80.0	4.0
B.H.	10	1,000	132.0	12	100.0	-32.0
C.P.	10	1,000	109.0	3	91.0	-18.0
E.S.	14	1,000	91.0	3	70.0	-21.0
N.W.	6	1,000	99.0	4	62.0	-37.0
T.H.	Birth	250	97.0	6	87.2	-9.8
J.K.	Birth	250	90.0	8	72.2	-17.8
D.H.	5	1,000	89.0	18	60.0	-29.0
A.B.	1	600	139.0	5	109.0	-30.0

often associated with puberty in patients with osteogenesis imperfecta.

In evaluating the patients in group 2, because of the lack of a comparable control period, it becomes more important to examine each individual's history. D.H. suffered six fractures (five leg, one arm) within one year prior to initiation of ascorbic acid treatment; in 3½ years since that time, he has only had two further fractures—a broken clavicle suffered when he fell off a bed, and a broken finger. His activities are completely normal, and he is an extremely active young boy. He has blue sclera which have appeared to lighten while on the ascorbic acid.

A.B. was placed on the study after suffering two fractures within three months. Her father, his sister, and his mother also have osteogenesis imperfecta. In the 31 months on the study, A.B. has received only two fractures, neither of which were detected at the time of occurrence, but following healing were noticed. She is a very active little girl with completely normal activities, and has suffered a number of falls and accidents not resulting in fractures.

S.B. is the oldest child on the study who was started on ascorbic acid at birth. She was born with two fractured femurs and classical radiological findings of osteogenesis imperfecta. In the 3½ years she has been on the study, she has not suffered any further fractures. She is extremely active with her activities limited only by her size. She has blue sclera, which her parents feel have lightened recently, and hyperelastic joints. Although still quite tiny, she experiences a growth spurt following each increase in ascorbic acid dosage.

J.K. was born with two fractured femurs and one femur fracture that had healed. She was placed on ascorbic acid therapy at birth; she has had two fractures of the left femur which healed uneventfully. She is now 18 months old; she uses her arms a great deal, and rolls around on the floor. She does not sit up alone or crawl yet.

T.H. was born with over 20 fractures, apparent pseudoarthroses of the radii, and such a lack of skull formation that almost the entire brain could be palpated through the skin. She is now 17 months old, and has had only two further fractures, both of the humerus. She has good skull formation, uses her arms extensively, and is very alert. She creeps, but does not yet crawl. Deformity is still considerable, although the apparent pseudoarthroses have healed.

Serum Zinc

Eleven of the 13 study patients had blood drawn for a serum zinc determination prior to initiation of the ascorbic acid therapy (Table II). These patients were readmitted for follow-up studies after a variable length of time, at which point a second blood sample for serum zinc determination was drawn. Ten of the 11 showed a decrease in their serum zinc levels while on ascorbic acid. This decrease (using the Student *t* test for paired observations, $N = 11$) is significant at the $p = 0.001$ level. The decrease occurs during the first several months of therapy, as subsequent serum zinc determinations on these patients show only slight fluctuations in their serum zinc levels.

Eight normal controls (four adults and four children) were placed on 1 gm of ascorbic acid to be taken in four divided doses daily for three months. Blood was drawn for serum zinc determination before and after the three-month period. Six of the controls showed a significant increase in their serum zinc levels at the end of three months; two showed no significant change. Taking the group as a whole, the increase in the serum zinc level (using the Student *t* test for paired observations, $N = 8$) is significant at the $p = 0.005$ level.

Eight siblings of four of the study patients were also placed on 1 gm of ascorbic acid daily for three months. Of these eight, five showed an increase in

their serum zinc levels; three (all siblings of N.W.) showed a significant decrease in their serum zinc levels.

Follow-up blood samples were taken three months after the ascorbic acid was discontinued from three control children, one control adult, and the five siblings whose serum zinc rose; all had returned to approximately their original value. Three study patients (M.A., B.H., C.P.) were taken off ascorbic acid for three months, and blood was drawn for serum zinc determinations at the end of the three months. They showed no significant difference in serum zinc levels.

DISCUSSION AND CONCLUSIONS

The drug study as presented suffers from two important deficiencies. First, the population of patients is small and variable as to extent of disease. Therefore, a matched control series for a double-blind control study is not available. Second, immobilization time for fractures in those patients treated by the authors was probably less than had been used previously. We believe that the abbreviated immobilization, i.e., two to four weeks, allows for stabilization of the bone while minimizing osteoporosis and subsequent fractures. Normal control patients were matched to test subjects as to age. Accurate size matching was not possible.

All 13 patients on this study have experienced a decreased number of fractures and increased physical activities. They also showed a significant decrease in their serum zinc levels. Some of the patients initially had an abnormally high serum zinc level, but this does not seem to be correlated directly with the degree of severity of the disease or with the amount of improvement while on ascorbic acid or even with the amount of decrease in their serum zinc levels. Although most of the study patients had initially normal serum zinc levels, they did have higher levels than their parents or siblings, with the exception of M.A. (whose family was not available for blood samples) and N.W. (whose siblings were discussed above).

Ascorbic acid was chosen as a possible therapeutic agent for patients with osteogenesis imperfecta because of its known activities as a co-factor for protocollagen proline hydroxylase and protocollagen lysine hydroxylase.¹¹⁻¹⁷ It is also known that the cross links in collagen are derived from lysyl side chains. It has been reported that the collagen of osteogenesis imperfecta patients shows a deficiency of mature collagen, irregular arrangement of the matrix, and abnormal orientation of the organic fibers. It was hypothesized that perhaps this deficiency of mature collagen and abnormal orientation of the organic fibers was in part due to decrease

hydroxylation of proline and lysine prior to extrusion of the fibers. By greatly increasing the availability of one of the required co-factors, it was hoped that hydroxylation would be increased, and thus increase the amount of mature collagen and normal cross linkage and orientation of the fibers. Whether or not this has indeed happened has not yet been shown. The patients receiving large doses of ascorbic acid have shown a decreased tendency to fracture and several have seemed to show a whitening of the sclera.

The increase in the serum zinc levels of the normal controls while on ascorbic acid was expected, as it has been reported that ascorbate enhances the intestinal transport of zinc.¹⁸ The decrease in the serum zinc levels of the osteogenesis imperfecta patients while on ascorbic acid is evidently an abnormal response associated with the disease. Why this is found is unclear, as is what, if any, role this may play in the physical improvement demonstrated by the patients while on ascorbic acid. It is interesting to note, however, in light of reports of elevated pyrophosphate levels in osteogenesis imperfecta¹⁹ that inorganic pyrophosphatase, the enzyme that catalyzes the hydrolysis of inorganic pyrophosphate to orthophosphate, while activated by magnesium, is inhibited by zinc.²⁰ It is possible that the ascorbic acid therapy, by decreasing the zinc level, allows for increased activity of this enzyme and thus decreased levels of pyrophosphate, much as treatment with magnesium oxide would do through overcoming the zinc inhibition.

The varied response of serum zinc levels found in the siblings is of interest in that osteogenesis imperfecta is a genetic disease of an uncertain type. It is generally agreed that the common type of osteogenesis imperfecta is attributable to an autosomal dominant gene with variable expressivity.²¹ It is possible that the three siblings of N.W. actually carry the gene, but with subclinical expression, and may therefore transmit it to their offspring. Since there is no well-accepted diagnostic test for osteogenesis imperfecta, this hypothesis cannot be directly tested, but the possibilities it suggests lead to further investigations along this line, including doing similar studies on the parents of the patients.

SUMMARY

The daily oral administration of ascorbic acid to patients with osteogenesis imperfecta leads to a decreased tendency to fracture. At the same time it allows for greater physical activity. Associated with the ascorbic acid therapy is an abnormal decrease in the serum zinc levels of the patients. The mechanism of action of the ascorbic acid and the role the decrease in zinc may play are both unclear

APPENDIX
COMMENTS ON PATIENTS

- M.P. In braces; walks with walker; crawls on floor by self. Was in braces during control period also. Increased activity.
- A.M. Activities have increased; now very active, limited by arm deformities. Plays goalie on school soccer team, does stunts in trampoline.
- M.A. Past puberty when started on study; no significant change in zinc level, fracture rate. Blue sclera have lightened. Extremely active; large, normal-looking boy.
- B.H. Extremely active. Completely normal activities. Only fracture while on study occurred while doing tumbling stunts in school gym class.
- C.P. Nonambulatory. Since treatment is able to transfer self from chair to bed, sofa; can now scoot on floor.
- S.E. Nonambulatory. Since on study can transfer to wheelchair, wheel self around, crawl on floor.
- E.S. Started on study past puberty. Following surgery while on study, can walk in braces with crutches. Had been nonambulatory.
- N.W. Extremely active 7 year old boy. Only on study fracture was bone in hand fractured in fist fight with older brother.
- T.H. Is progressing well; has good skull formation now. Active; not yet walking. Both study fractures were of humerus. Considerable residual deformities.
- J.K. Both study fractures occurred during first week of treatment. Active, alert, progressing well. Not yet walking.
- S.B. Extremely active, completely normal activities. Blue sclera, hyperplastic joints, tiny.
- D.H. Had had six major fractures within one year prior to initiation of study; has had only two minor fractures in over three years on study. Extremely active; many falls with no fractures.
- A.B. Suffered two fractures in three months prior to treatment; in 31 months on study, has had two fractures, neither of which was noticed until seen on routine X-rays following healing. Extremely active; normal activities for age; has blue sclera; familial history of osteogenesis imperfecta.

at this time. Further studies to elucidate these roles are needed.

SPECULATIONS

The opposite effects of vitamin C on serum zinc in normal and osteogenesis imperfecta subjects could be on the basis of the serum protein of unusual affinity for zinc being elaborated in osteogenesis imperfecta. Thus circulating zinc would not be available to osteoblasts. Vitamin C serves either as a chelator of zinc or as an inactivator of the abnormal protein, liberating zinc for action.

If the abnormal serum protein were a defective or precursor enzyme for collagen synthesis only one enzyme defect would have to be invoked. Since the synthetic deficit would be incomplete, vitamin C, acting on products of the reaction, could stimulate synthesis without completely correcting the defect.

This latter case seems to hold, as seen by one patient, essentially freed of fractures, who remains severely retarded in growth.

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A CASE OF A BOY WHO IN 1813 SWALLOWED A PIECE OF COPPER AS REPORTED BY JAMES JACKSON, M.D.

James Jackson (1777-1867) has been described as the most conspicuous character in the medical annals of Massachusetts. He was the first physician to the Massachusetts General Hospital when it was opened in 1821. In 1812, he succeeded Benjamin Waterhouse as the Hersey Professor of the Theory and Practice of Medicine at the Harvard Medical School. The following case history will interest readers of PEDIATRICS both for its clarity of presentation and for Dr. Jackson's clinical reasoning.

In October, 1813, G. B. a boy in his fourth year, swallowed a half cent. There ensued almost immediately a nausea and a great flow of saliva from the mouth. Within twenty-four hours the patient began to vomit. He continued to vomit at intervals for eight days; and at sometimes it was almost incessant for hours, and in the highest degree distressing. He threw off his victuals and some bile; but much of the time, he was troubled with vain retchings. About the fifth day, he had three very offensive and copious stools, which were uncommonly green. He derived some relief from these evacuations. He often complained of pain in the abdomen, which seemed to be in the umbilical region.

The first solid food which he retained was in the night of the eighth day. He then awoke and called for brown bread; and he continued to demand this, with great vehemence, until he had eaten three large slices. He retained them all, and from that time was convalescent. For several weeks he experienced nausea whenever he attempted to swallow animal food, although he chewed it abundantly. His health has, since that time, been perfectly restored.

At first he did not take any medicine, but when the symptoms became distressing, I was induced to attempt giving relief. Two remedies evidently benefited him; the one consisted in hot fomentations to the epigastrium; the other was magnesia administered internally.

It seemed evident in this case, that the copper was undergoing solution in the stomach. This does not always take place when pieces of copper are swallowed. That it was taking place in this instance seemed evident by the nausea and vomiting; and afterwards by the relief obtained while the piece of coin was not discharged in its solid state.

I had two views in directing the use of magnesia. One was to lessen the acid in the stomach, so as to make the solution go on more slowly, and thus prevent the stomach from being charged at any time with so large a dose of the offensive salt which resulted from the solution as it would otherwise have. The other was to promote the peristaltic motion, and thus carry off the cause of offence (sic) as it was formed. The magnesia was, therefore, given as freely as possible. The benefit seemed to me unequivocal.¹

Noted by T. E. C., Jr., M.D.

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