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Effects of Vitamin E on the Changes of Mineral Contents in Chronic Cadmium-Poisoned Rats*

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This study was carried out to investigate the effects of vitamin E on the cadmium contents of bone and on the calcium and phosphorous contents of the blood, urine and feces. Male Sprague-Dawley rats weighing 100±10 g were randomly assigned to one normal group and three cadmium poisoned groups. The cadmium poisoned groups consisted of a vitamin E free diet (Cd-0E) group; a 40 mg vitamin E /kg diet (Cd-40E) group; and a 400 mg/kg diet (Cd-400E) group. Experimental animals were maintained on their respective diets for 20 weeks and were simultaneously administered 50 ppm Cd^{2+} dissolved in the drinking water. At the end of the trial, the average hematocrit value in the Cd-0E group was 28.13% lower than in the normal group. However, the average hematocrit value in the Cd-400E group was significantly higher than in the Cd-0E and Cd-40E groups. WBC levels in the cadmium-poisoned groups were lower than in the normal group, but Cd-400E group levels were significantly higher than in the Cd-0E and Cd-40E groups. The contents of calcium of tibia has no significant difference between normal group and cadmium exposed group at 10^{th} week After 20 weeks, the calcium contents of the tibia in the Cd-0E and Cd-40E groups were lower than in the normal group by 25.5% and 22.1%, respectively, although the calcium contents of the tibia in the Cd-400E group were higher than in the normal group. After 10 weeks, the calcium contents of the femur in the Cd-0E and Cd-40E groups were 19.25% and 15.45% lower than in the normal group, respectively, but the calcium contents of the femur in the Cd-400E group were at the same levels as in the normal group. The levels of calcium in the femur after 20 weeks were similar to the 10-week levels. Calcium levels of the urine in the Cd-0E and Cd-40E groups were 3.92 fold and 2.92 fold higher, respectively, than in the normal group, but levels in the Cd-400E group were significantly lower than in either the Cd-0E group or the Cd-40E group. Calcium levels of the feces in cadmium-poisoned groups were significantly higher than in the normal group, although levels in the Cd-400E group were significantly lower than in the Cd-0E and Cd-40E groups. Phosphorous levels of the blood in the Cd-0E group were 17% lower than in the normal group, although levels in the Cd-400E group were significantly higher than in the Cd-0E group. Phosphorous levels of the urine in the Cd-0E and Cd-40E groups were significantly higher than in the normal group, while Cd-400E group levels were found to be at the same level as in the normal group. Cadmium contents of the tibia in the Cd-40E and Cd-400E groups were 13% and 17% lower, respectively, than in the Cd-0E group. Regarding cadmium levels in the femur, only the Cd-400E group achieved lower levels (10% lower) than the Cd-0E group. In conclusion, vitamin E supplementation resulted in a suppression of the release of calcium from bone, and a reduction in the excretion of calcium via the urine and feces, thus having a normalizing effect on calcium metabolism in rats with chronic cadmium poisoning.

Key words : chronic cadmium poisoning, Vitamin E, mineral (calcium and phosphorous) contents

INTRODUCTION

Cadmium is responsible for inducing a great deal of acute and chronic disease, due to its long biological half-life (10-30 years) and its continuous accumulation and concentration in organisms higher in the food chain.[1] Cadmium can cause the malabsorption of essential minerals, such as zinc, copper, iron and calcium, and can especially induce bone disorders by interfering with calcium and vitamin D metabolism in the bone, kidney and intestines. Adams et. al.[2] reported that chronic cadmium exposure resulted in bone loss in humans. Slemenda et. al.[3] reported that cadmium reduced bone mineral density, leading to increased bone fractures, particularly in the case of smoking women.

In animal experiments, cadmium reduced calcium absorption by interfering with the action of 1,25 -dihydroxycholecalciferol or by inhibiting the synthesis

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of calcium binding protein, and resulted in a loss of bone minerals by increasing the excretion of calcium due to cardiac insufficiency.[4)-7)] Also, it was reported that cadmium induced osteoporosis and osteomalacia through enhanced bone resorption activities, caused by promoting the differentiation of osteoclasts and the formation of hydroxyapatite. Regunathan et. al.[8)] reported that cadmium reduced the bone mineral contents of mice, and increased the formation of, and the level of activity of, osteoclasts. These studies show that chronic cadmium poisoning leads to the disruption of calcium metabolism and to reduced levels of bone minerals.

On the other hand, several studies that examined the relationship between cadmium detoxification and nutrients have shown that some dietary factors can alleviate the effects of cadmium poisoning or can reduce the level of cadmium accumulation in the body. Yin et. al.[9)] and several other researchers[10)-12)] reported that some nutrients, such as protein, fiber, calcium, iron, zinc, copper, selenium, vitamin C and vitamin E, reduce the effects of cadmium poisoning. Rena and Verma,[13)] and Kim et. al.[14)] reported that when antioxidative materials, such as glutathione (GSH), α-tocopherol, and selenium, were supplemented with cadmium, cadmium accumulation and absorption in the kidney and liver were reduced due to increased cadmium excretion and synergic effects of chelating elements. Tadon et. al.[15)] reported that, when vitamin E was supplemented, cadmium accumulation in the blood, liver and kidney were significantly reduced, thereby protecting the body from cadmium intoxication. Kim et al.[16)] reported that, under conditions of chronic cadmium poisoning, vitamin E supplementation reduced cadmium accumulation in the body by lowering cadmium absorption, promoting cadmium excretion, and assisting with mechanisms for cadmium detoxification.

Drawing on, and extending, previous research on the relationships between vitamin E and cadmium toxicity, this study was carried out to determine the effects of vitamin E on blood composition, and on calcium and phosphorous levels of bone, blood and urine, in chronic cadmium poisoned rats.

MATERIALS AND METHODS

1. Experimental animals and diets

Male Sprague-Dawley rats weighing 90-100g were purchased from KRITC (Korea Research Institute of Chemical Technology; Yusung, Korea). The animals were individually housed in stainless steel cages in a room with controlled temperatures (20-23℃) and lighting (alternating 12 hour periods of light and darkness). The animals were fed a pelleted, commercial, non-purified diet for 6 days after arrival, and were then randomly divided into one normal group and three cadmium groups. The cadmium groups included a vitamin E-free diet group (Cd-0E), a 40 mg vitamin E/kg diet group (Cd-40E), and a 400mg vitamin E/kg diet group (Cd-400E). The cadmium was supplied by adding $CdCl_2 \cdot 1/2H_2O$ to distilled water to generate a cadmium concentration of 50 ppm. The rats had unlimited access to water and diet over the 20 weeks experimental period. The experimental design was approved by the Catholic University of Daegu's committee for care and use of laboratory animals.

Table 1. Classification of experimental groups

Group	Vitamin E (mg/kg diet)	Cadmium* (50 ppm Cd drinking water)
Normal[1)]	40	-
Cd-0E[2)]	0	+
Cd-40E[3)]	40	+
Cd-400E[4)]	400	+

* : Experimental and normal groups fed with or without 50 ppm Cd ($CdCl_2$1/2 H_2O) in drinking water respectively,
[1)] Normal : Cd no treatment
[2)] Cd-0E : Cd treatment, vitamin E free diet
[3)] Cd-40E : Cd treatment, vitamin E supplementation (vitamin E 40 mg/kg diet)
[4)] Cd-400E : Cd treatment, vitamin E supplementation (vitamin E 400mg/kg diet)

2. Sample preparation

After the tibia, femur and feces were collected and weighed, they were dried using a drying oven at 110℃ and were then stored in a desiccator. After the urine volume were measured, the supernatant was obtained by centrifuging at 1500 x g for 10 minutes, and was then stored at -70℃ until analysis.

3. Measurement of blood composition

The hemoglobin contents of blood were measured using the cyanmethemoglobin method. The hematocrit value was measured as the packed red cell volume. The white blood corpuscles (WBC) and red blood corpuscles (RBC) contents of the blood were measured using the cell dyn 1300 (Abdott, U.S.A).

4. Measurement of calcium and phosphorous contents in bone, blood, urine and feces.

The calcium contents of bone and feces were analyzed by the dry ashing method,[17)] and the calcium contents of blood and urine were analyzed by the wet digestion method,[18)] using an atomic absorption spectrophotometer (AAs) at 422nm. The inorganic phosphorus contents of bone, blood and urine were measured using the Asan kit (Asan co., Korea).

5. Measurement of cadmium contents in bone

The cadmium contents of bone were analyzed by the dry ashing method[17] and were measured using an atomic absorption spectrophotometer (AAs).

6. Statistical analysis

All data were assessed by analysis of variance (ANOVA). If significance was found by ANOVA, comparisons among group means were made by Tukey's HSD test.

RESULTS

1. Changes in blood composition

1-1 Hemoglobin contents and hematocrit value

The measurements of hemoglobin contents and hematocrit values are shown in Figure 1. The hemoglobin contents in the cadmium-poisoned groups were significantly lower than in the normal group. Hemoglobin contents were not affected by vitamin E supplementation. The average hematocrit value in the Cd-0E group was 28.13% lower than in the normal group. The hematocrit value in the Cd-400E group, which had the highest level of vitamin E supplementation, was significantly higher than in the Cd-0E group and the Cd-40E group ($p<0.05$).

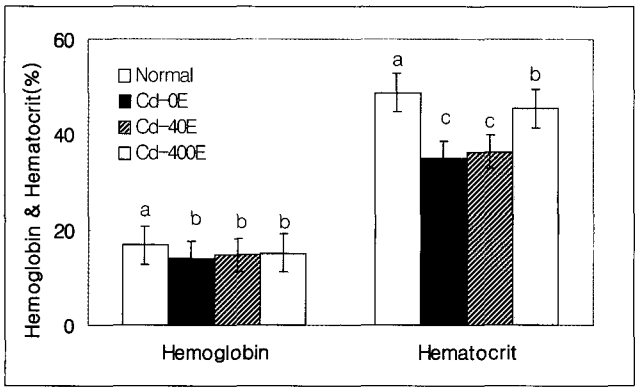

Fig 1. Effects of vitamin E on hemoglobin concentration and hematocrit values in chronic cadmium poisoned rats(values taken at 20 weeks).

Values are mean ±SE (n=10).
Values with different superscript letters (a,b,c) are significantly different at $p<0.05$ by Tukey's test.
The experimental conditions were the same as those described in Table 1.

1-2 WBC and RBC contents.

In order to observe changes in blood composition, the WBC and RBC contents were assessed (Figure 2). The WBC levels in cadmium-poisoned groups were lower than in the normal group ($p<0.05$), but WBC levels were significantly higher in the Cd-400E group than in the Cd-0E and Cd-40E groups ($p<0.05$). RBC levels were found to be not significantly different between the normal group and the cadmium-poisoned groups.

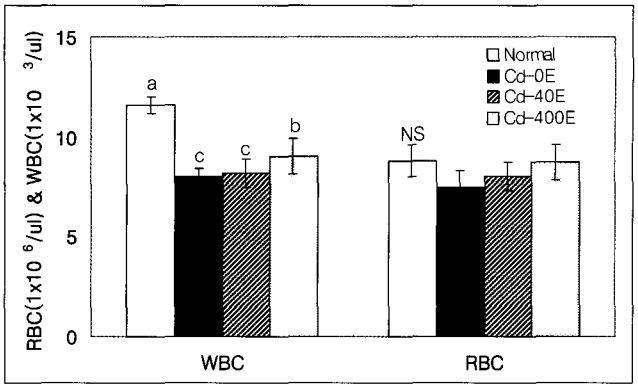

Fig 2. Effects of vitamin E on RBC and WBC values in chronic cadmium poisoned rats(values taken at 20 weeks).

Values are mean±SE (n=10).
Values with different superscript letters (a,b,c) are significantly different at $p<0.05$ by Tukey's test.
The experimental conditions were the same as those described in Table 1.

2. Mineral contents

2-1. Calcium contents of the tibia and femur

Results for the calcium contents of the tibia and femur are shown in Table 2. The calcium contents of the tibia showed no significant difference between the normal group and the cadmium poisoned groups at the 10th week of the experiment. The tibia calcium levels of the Cd-0E and Cd-40E groups were 25.5% and 22.1% lower, respectively, than the normal group. At the 20th week, the tibia calcium levels of the Cd-400E group were higher than in the Cd-0E and Cd-40E groups. At the 10th week, the calcium contents of the femur in the Cd-0E and Cd-40E groups were 19.25% and 15.45% lower, respectively, than the normal group, while levels in the Cd-400E group were the same as the normal group. The levels of calcium in the femur at the 20th week showed a similar trend as at the 10th week.

Table 2. Effects of vitamin E on calcium levels of the tibia and femur in chronic cadmium poisoned rats.

Group	Tibia		Femur	
	10wks	20wks	10wks	20wks
	(mg/g wet wt)			
Normal	115.61±16.50^{NS}	115.50±5.45^{a}	103.50±10.75^{a}	123.60±2.15^{a}
Cd-0E	95.32±8.51	87.51±9.01^{b}	83.87±7.56^{b}	90.34±9.78^{b}
Cd-40E	98.51±7.32	91.53±6.75^{b}	87.50±3.45^{b}	93.50±3.45^{b}
Cd-400E	109.80±10.22	108.34±6.45^{ab}	95.67±5.67^{a}	115.21±4.56^{a}

All values are mean ±SE(n=10).
Values with different superscript letters (a,b) in the same column are significantly different at $p<0.05$ by Tukey's test.
The experimental conditions were the same as those described in Table 1.

2-2. Calcium contents in blood, urine and feces

The results for calcium contents of the blood, urine and feces are shown in Table 3. The calcium contents of the blood in the cadmium-poisoned groups were lower

than in the normal group, but there were no significant differences between the cadmium-poisoned groups. The calcium contents of the urine in the Cd-0E and Cd-40E groups were 3.92 fold and 2.92 fold higher, respectively, than in the normal group, while the calcium levels of the Cd-400E group were significantly lower than in the Cd-0E and Cd-40E groups ($p<0.05$). The calcium contents of feces in the cadmium-poisoned groups were significantly higher than in the normal group ($p<0.05$). The calcium contents of feces in the Cd-400E group were lower than in the Cd-0E and Cd-40E groups.

Table 3. Effects of vitamin E on calcium levels of blood, urine and feces in chronic cadmium poisoned rats(values taken at 20 weeks).

Group	Blood	Urine	Feces
	(mg/dL)	(mg/day)	(mg/day)
Normal	8.75 ± 0.50^{NS}	0.25 ± 0.05^{c}	113.45 ± 8.75^{c}
Cd-0E	7.49 ± 0.56	0.98 ± 0.08^{a}	170.56 ± 15.89^{a}
Cd-40E	7.65 ± 0.78	0.63 ± 0.05^{a}	167.84 ± 20.13^{a}
Cd-400E	7.95 ± 0.85	0.54 ± 0.06^{b}	150.45 ± 13.69^{b}

All values are mean ±SE(n=10).
Values with different superscript letters (a,b,c) in the same column are significantly different at $p<0.05$ by Tukey's test.
The experimental conditions were the same as those described in Table 1.

2-3. Phosphorous contents in blood and urine

The results for the phosphorous contents of blood and urine are shown in Figure 3. The phosphorous levels of blood in the Cd-0E group were 17% lower than in the normal group, while levels in the Cd-400E group were significantly higher than in the Cd-0E group. The phosphorous levels in the urine of the Cd-0E and Cd-40E groups were higher than in the normal group ($p<0.05$), while the Cd-400E group levels were the same as in the normal group.

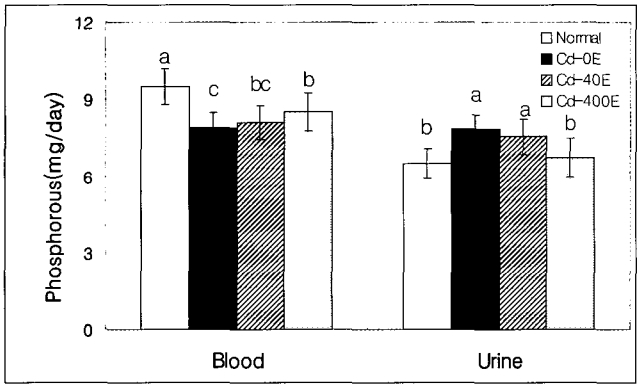

Fig 3. Effects of vitamin E on phosphorous levels of blood and urine in chronic cadmium poisoned rats(values taken at 20 weeks).

All values are mean ± SE (n=10).
Values with different superscript letters (a,b,c) in the same column are significantly different at $p<0.05$ by Tukey's test.
The experimental conditions were the same as those described in Table 1.

3. Cadmium contents of the tibia and femur

The measurements of the cadmium contents of the tibia and femur are shown Table 4. The cadmium contents of the tibia in the Cd-40E and Cd-400E groups were 13% and 17% lower, respectively, than in the Cd-0E group. For the femur cadmium contents, only the Cd-400E group was lower (by 10%) than the Cd-0E group.

Table 4. Effects of vitamin E on cadmium concentrations in the tibia and femur in chronic cadmium poisoned rats(values taken at 20 weeks).

Group	Tibia	Femur
	(ug/g wet wt)	
Normal	0.25 ± 0.01^{c}	0.14 ± 0.01^{c}
Cd-0E	3.63 ± 0.29^{a}	4.03 ± 0.11^{a}
Cd-40E	3.15 ± 0.43^{b}	3.91 ± 0.15^{ab}
Cd-400E	3.02 ± 0.08^{b}	3.62 ± 0.13^{b}

All values are mean±SE (n = 10).
Values with different superscript letters (a,b,c) in the same column are significantly different at $p<0.05$ by Tukey's test.
The experimental conditions were the same as those described in Table 1.

DISCUSSION

This study was carried out to investigate the effects of vitamin E on the cadmium contents of bone and on calcium and phosphorous levels in blood, urine and feces.

The hemoglobin contents in the cadmium-poisoned group were significantly lower than in the normal group. Hemoglobin contents were not affected by vitamin E supplementation. The hematocrit value for the Cd-0E group was 28.13% lower than for the normal group. The hematocrit value for the Cd-400E group was significantly higher than for the Cd-0E and Cd-40E groups. The WBC levels in the Cd-0E and Cd-40E groups were significantly lower than in the normal group, although Cd-400E group levels were significantly higher than Cd-0E and Cd-40E group levels. RBC levels were not significantly different between the normal group and the cadmium-poisoned groups.

These results agree with those of Decker et al.[19] who reported that, following three months of cadmium exposure, hemoglobin levels were reduced. Choi and Rhee[20] reported that hemoglobin contents and hematocrit values were reduced with chronic cadmium exposure. In several research studies,[21),22)] the levels of plasma transferrin and total iron binding capacity (TIBC) were reduced as a result of cadmium exposure, while hypochromic microcytic anemia was induced due to a reduction in size of red blood cells. Especially, Fox[23] emphasized that iron deficiency, induced by cadmium poiso-

ning, was mitigated by a high protein diet containing sufficient vitamin C and iron supplementation. In this research, hemoglobin levels and hematocrit values in cadmium-poisoned groups were reduced due to reduced food intake and malnutrition, and it can be assumed that sufficient vitamin E supplementation improved iron metabolism.

The calcium content of the tibia was not significantly different between the normal group and the cadmium-exposed groups, at the 10th week. However, at 20th week the Cd-0E and Cd-40E groups were 25.5% and 22.1% lower, respectively, than the normal group; the Cd-400E group was found to have higher levels of calcium in the tibia than the Cd-0E and Cd-40E groups, at the 20th week. The calcium contents of the femur in the Cd-0E and Cd-40E groups were 19.25% and 15.45% lower, respectively, than the normal group at 10th week. The Cd-400E group was found to have the same femur calcium level as the normal group at the 20th week. The levels of calcium in the tibia and femur of the animals at the 20th week showed similar trends to those observed at the 10th week. Calcium poisoning promoted calcium excretion, and bone resorption and cadmium accumulation increased at the expense of calcium. Vitamin E supplementation allowed tibia and femur calcium levels in cadmium-poisoned rats to increase to normal levels, because vitamin E inhibited bone resorption and cadmium accumulation.

In this study, the calcium contents of the tibia in the Cd-40E and Cd-400E groups were 13% and 17% lower, respectively, than in the Cd-0E group. In the case of the calcium contents of the femur, the Cd-400E group showed a slightly lower level (10% lower) than the Cd-0E group.

In this study, the calcium contents of blood in the cadmium-poisoned groups were low, but there were no significant differences between the experimental groups. Therefore it can be assumed that serum calcium was maintained at its normal level because of homeostasis.[24]

On the other hand, regarding the bone renewal rate, if the serum calcium level is high, bone resorption decreases. If plasma phosphorus levels are increased, then bone resorption is reduced and bone formation is promoted.[25]

The phosphorous contents of blood in the Cd-0E group were 17% lower than in the normal group, while the Cd-400E group achieved the levels of the normal group. The phosphorous contents of urine were higher in the Cd-0E and Cd-40E groups, compared to the normal group, while the Cd-400E group achieved the same level as the normal group. Therefore it can be assumed that vitamin E supplementation promotes bone formation by increasing the phosphorus contents of the plasma; this result agrees with the study of Choi and Rhee.[20]

The calcium contents of the urine of the Cd-0E and Cd-40E groups were 3.92 fold and 2.92 fold higher, respectively, than in the normal group, although the Cd-400E group was found to have lower urine calcium levels than the Cd-0E and Cd-40E groups. The calcium contents of feces in the Cd-0E and Cd-40E groups were higher than in the normal group, and the Cd-400E group was found to have lower levels than the Cd-0E and Cd-40E groups.

In this study, in order to maintain serum calcium levels under conditions of cadmium poisoning, calcium was released from bone, bone resorption increased, and the mineral contents of bone were reduced. Calcium excretion through the urine and feces was increased. Vitamin E supplementation reduced the levels of urinary and fecal calcium excretion under conditions of cadmium poisoning, and it can therefore be assumed that vitamin E is effective in normalizing calcium metabolism.

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