Calcium and phosphate concentrations, and alkaline and acid phosphatase activities in serum of the rat fed with low calcium and beryllium diets

AKIRA MATSUMOTO, YOH HISADA, YOSHITAKA YOSHIMURA

Abstract: Calcium and phosphate contents, and alkaline and acid phosphatase (ALPase and ACPase) activities in serum of Wistar Strain rat fed with low Ca and Be diets were examined.

Fourty 4-week-old male rats of the Wistar Strain with initial body weight of 50-60 gr were used. Those animals were divided into 4 groups and were respectively fed with a normal well balanced diet (control group), a low Ca diet (low Ca group), a Be diet (Be group) and a Be-Ca diet (Be-Ca group) for 4 weeks.

At the end of the experiment period, serum were obtained from the carotid artery of all rats. Using this serum, we measured Ca and P with atomic absorption spectrophotomotry, ALPase and ACPase activity with Kind and King's method, and protein with Lowry's method.

The conditions of growth and development were worse in the order of the Be-Ca, the Low Ca and the Be groups than those in the control group.

Serum calcium decreased in the low Ca and Be groups, but in the Be-Ca group, it was similar to that in the control group.

Serum P decreased in low Ca group and furthermore decreased in the Be group. It also decreased in the Be-Ca group, but its value was similar to that in the low Ca group.

ALPase activity increased remarkably in the low Ca group. This acceleration was inhibited in the Be and the Be-Ca groups.

From these facts, it was suggested that low Ca diets accelerated ALPase activity and beryllium inhibited phosphate metabolism.

Key words: Serum Ca and P, Alkaline phosphatase activity, low Ca and Be diet

Introduction

It is known that hypocalcemia, acceleration of serum ALP and osteoporosis have occurred in rats fed with a low Ca diet1). We have also reported that increase of femur-head size, acceleration of ALPase activity and chondroitin sulfate synthesis have occurred in the femur cultured in a low Ca environment2).

The relationship between low Ca and beryllium remain unexamined.

It has been reported that when beryllium was intravenously injected to rats and rabbits, DNA synthesis3) and enzyme activity4,5) were inhibited.

In addition, it has also been reported that beryllium6,7,8,9) rickets has occurred in the rats administered beryllium, and the cause of this pathogenesis must be due to the inhibition of intestinal absorption of phosphate.

On the other hand, it has been reported that DNA synthesis was inhibited in the cultured liver cells administered beryllium10,11), and ALPase activity was accelerated in cul-
tered bone cells administered beryllium\textsuperscript{12}.

Dental clinically, it is known that beryllium included in metal plate and clasp becomes hapten, binds to protein, turn to antigen and is associated with sensitization\textsuperscript{13}.

However, it seemed that very little is known about the relationship between low Ca environment or beryllium and the movements of serum calcium, phosphate, ALPase and ACPase activities in the rats fed with low Ca and beryllium diets.

In the present study, we undertook to examine the effect of low Ca environment and beryllium on the parameters described above.

Materials and methods

Animals

Forty 4-week-old male rats of the Wistar Strain, weighing 50 to 60 gr. divided into four equal groups of rats and were fed with a normal well balanced diet (control group), a low calcium diet (low Ca group), a low calcium diet+beryllium diet (Be group) and a normal well ballanced diet+beryllium diet (Be•Ca group) respectively for 4 weeks (Table 1). During the experimental period, all rats were given demineralized water \textit{ad libitum}. The composition of diets was shown in Table 1. The body weight were measured every day.

Blood of all rats was obtained from the carotid artery under ether anesthesia at the end of the experimental period, and then, after the centrifugation at 750×g for 10 min, serum was obtained. Using this serum, we measured calcium content with atomic absorption spectrophometry, phosphate content with Eastoe's method\textsuperscript{15}, ALPase and ACPase activities with Kind and King's method\textsuperscript{16}, protein with Lowry's method\textsuperscript{17}.

Results

Growth chart

As shown in Fig. 1, the growth rate of body weights in the low Ca group was worse than that in the control group. In the case of the Be group, it was much worse than that of the low Ca group. With the addition of beryllium to normal well balanced diet, it was better than those of the low Ca and the Be groups.

Serum calcium concentration decreased significantly in the low Ca and the Be groups, compared with that of the control group (p<0.01) (Fig. 2) and was similar to that of the control group in the Be•Ca group (Fig. 2).

Serum phosphate concentration decreased significantly in the low Ca and the Be group, compared with that in the control group (p<0.01) (Fig. 3). The degree of decrease was higher in the Be group than in low the Ca group (Fig. 3). In the Be•Ca group, its level increased more than that in the Be

<table>
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<th>Table 1 Composition of diets</th>
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<tr>
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<td>Fe\textsubscript{2}(SO\textsubscript{4})\textsubscript{2}(NH\textsubscript{4})\textsubscript{2} SO\textsubscript{4} • 24H\textsubscript{2}O</td>
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<td>MgSO\textsubscript{4} H\textsubscript{2}O</td>
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* Salley and Bryson (1957)\textsuperscript{14}
Serum Ca₃P and ALPase activity in rat fed low Ca. and Be. diets

Fig. 1 Growth chart in four groups of rats.

Note that the growth rate is worse in the order of low Ca, Be·Ca and Be groups, compared with that in control group at the end of experimental period. Each point shows mean value (n=10).

Serum ALPase activity increased significantly in the low Ca group, compared with that in the control group (p<0.01) (Fig. 4). It was very remarkable. In the Be group, its activity decreased significantly, compared with that in the low Ca group (p<0.01) and came near to control value (Fig. 4). In the Be·Ca group, its activity was lower than that in the control and the Be groups (Fig. 4).

Serum ACPase activities were shown in Fig. 5.

It decreased in the low Ca group. In the Be group, its activity showed a slightly lower value, compared with that in the low Ca group. Serum ACPase activity decreased in the Be·Ca group than that in the control group, and was similar to that in the low Ca group. The movement of serum ACPase activities was smaller than that of ALPase activities.

Serum protein concentration of the 4 groups showed slight and not significant differences (Fig. 6).

Discussion

Calcium, phosphate, ALPase and ACPase activities were examined in the serum of the rats fed with low Ca and Be diets in the present study. In the rats fed with low Ca diet, the condition of growth and development was worse than that in control group.

There are many reports which show that the growth rate is worse in the rats fed with low Ca diet than in those fed with normal diet\textsuperscript{18,19,20}. However, the difference was larger in the present study than that in many other reports. This discrepancy may be due to the differences of animal condition at the present experiment period and diets. Be seemed to inhibit growth and development\textsuperscript{9}. This inhibition was remarkable in the rats fed with the Be added low Ca diet, and this inhibition was lessened when calcium was
added to that diet. There seemed to be competitive action calcium and beryllium. These phenomena were similar to that of the animals with "Srrickets". As both Be and Sr are alkaline earth metals, the decrease of growth rate may be due to toxic action of the metals for the body.

The calcium level decreased remarkably in low Ca group as many reports have stated. In Be group as well, its level was almost the same as that in low Ca group. This fact may mean that Be hardly affect calcium metabolism. In the Be-Ca group, the calcium level was similar to that in control group.

The evidences which showed that the calcium level in the Be group (low Ca diet+ Be) or the Be-Ca group (normal diet+ Be) was similar to those in the low Ca and con-
Serum Ca, P and ALPase activity in rat fed low Ca. and Be. diets

Note that acid phosphatase activity shows the inclination to decrease in low Ca, Be and Be-Ca groups, compared with control group, but the deviation of change was not so great as that in alkaline phosphatase activity.

Means ± S. D are shown (n=10)

ACPase activity decreased in the low Ca, Be and Be-Ca groups, compared with that in the control group, but the degree of change was not as great as that in ALPase activity. Further examination is necessary to clarify the movements of ACPase activity.

The 4 groups showed hardly any difference in protein concentration in the present study.

Dental clinically, as beryllium is included in the materials of metal plate and crown, it is conjectured that metal allergy may occur after the long term-application in oral cavity. However, further investigation will be required to clarify between beryllium and dental.
clinical symptom.

**Conclusion**

Calcium, phosphate contents, and ALPase and ACPase activities in serum of rats fed with a normal well balanced, low Ca, Be and Be-Ca diets were examined, and the results obtained are as follows.

1. Growth and development were worse in the order of the Be-Ca, the Low Ca and the Be groups, compared with those in the control group.
2. Serum calcium decreased in the low Ca and Be groups, but not in the Be-Ca group.
3. Serum phosphate decreased in the low Ca group and further decreased in the Be group. In the Be-Ca group, it decreased as well, compared with that of the control group. The value was higher than that in the Be group.
4. ALPase activity increased in the serum of the rats fed with low Ca diet.
5. This acceleration was inhibited in the serum of rats fed with the Be added normal well balanced diet and the Be added low Ca diet.
6. It was suggested that the low Ca diet accelerated ALPase activity and beryllium was inhibitory for phosphate metabolism.

**References**

13) 白砂美輝雄，他：歯科医学大辞典，p 478，医歯薬出版 東京 1989
低カルシウム食並びにペリリウム食で飼育されたラット血清中のカルシウム、磷酸、アルカリ性並びに酸性ホスファターゼ活性について

松本 章

生後4週齢の雄性ウイスター系ラットを正常食（対照群）、低Ca食（低Ca群）、Be食（Be群）、Be・Ca食（Be・Ca群）で行ったイオン水を飲ませて4週間飼育した。飼育後、エチルナトリウム溶液で頭動脈より採血し、750×gで遠心し、血清を得た。この血清中のカルシウム並びに酸濃度、単位蛋白質量当たりのアルカリ性並びに酸性ホスファターゼ活性を調べた。

成長発育状況は、対照群で比べBe・Ca群、低Ca群、低Ca群、Be群の順で劣悪化した。

血清カルシウム濃度は対照群に比べて低Ca群とBe群で有意に減少していた（p<0.01）が、Be・Ca群では殆ど変化しなかった。

血清酸濃度は対照群に比べ低Ca群で有意に減少（p<0.01）し、Be群では更に減少した。Be・Ca群でも低下したがBe群程ではなかった。

血清アルカリ性ホスファターゼ活性は対照群に比べ、低Ca群で有意に上昇した（p<0.01）。Be・Ca群では低Ca群と比べ減少（p<0.01）、対照群と同様の値を示した。Be・Ca群ではBe群と比べ更に減少した（p<0.01）。

血清酸性ホスファターゼ活性は対照群に比べ低Ca群、Be群、Be・Ca群で低下する傾向を示した。

これら的事実から、低Ca食でラットを飼育すると主にCa量は低下することと著明にアルカリ性ホスファターゼ活性が上昇することが認められた。Be含有食で飼育されたラットではCa代謝への影響は少なく、P代謝を強く促進することが示唆された。