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Inhibitory Effect of Bovine Milk on the Progression of Atherosclerosis in Rats

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우유가 흰쥐의 동맥경화증 유발억제에 미치는 영향

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ABSTRACT—This study was performed to investigate the inhibitory effect of bovine milk on the atherosclerotic rats. Eighty male rats of 5-weeks of age were divided into 4 groups, control, active treatment control fed the atherogenic feed, and skim milk and whole milk groups fed powdered skim or whole milk mixed with the atherogenic feed and observed for 13 weeks. Growth, clinical and pathological changes of the rats were examined. Rats of the 4 groups did not show significant difference of feed intake and weight gain. The level of serum cholesterol, high density lipoprotein cholesterol (HDL-cholesterol) fraction, and inorganics between skim milk and whole milk groups were not significantly different though significant difference was shown between active treatment control and milk groups. Milder calcification and necrosis in aorta, heart and kidney and fat degeneration in liver were seen in the milk groups than were in active treatment control. Marked difference, however, was not found between the skim milk and whole milk groups. Both powdered skim and whole milks could have a helpful effect of vitamin D_2-and-cholesterol-induced atherosclerosis in rats.

Keywords □ cholesterol, vitamin D_2, atherosclerosis, rats, inhibitory effect, skim milk, whole milk

It has been of great importance to find ways to lower blood cholesterol levels in man. A report from the Lipid Research Clinics Program[8] included results from a 7-year study indicating that reduction of total plasma cholesterol can lower the incidence of coronary heart disease in the population suffering from primary hypercholesterolemia. Much effort also has been made to reduce the blood cholesterol levels of hypercholesterolemic patients by drugs or diets to prevent the development of atherosclerosis.

Many investigators have attempted to explain the hypocholesterolemic effect of fermented and unfermented milks in man and animals since the existence of a hypocholesterolemic milk factor was first proposed by Mann and Spoerry[10] in 1974. Subsequent studies have confirmed that supplementing yogurt produced a decline in blood cholesterol levels.[1,3,9,11] The cholesterolemic response by consuming milk, however, has been more variable,[5,14] producing a decline,[3-6,15] no change[3,12] or an elevation[15] in blood cholesterol levels. Keim *et al.*[5] pointed that the variability in cholesterol response of man and animals consuming milk could be due to several differences in experimental design of condition. Recently, Naito[13] performed randomized cont-

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rolled studies and concluded that neither regular whole milk or skim milk decrease serum cholesterol level in human, suggesting that cholesterol lowering factors might not exist in milk. More studies need to be conducted along this line.

This study was, therefore, performed to examine whether milk affects the development of atherosclerosis as well as hypercholesterolemia. Thakur and Jha[17] and Kiyosawa *et al.*[7] suggested the possibility that yogurt and milk prevented the development of atherosclerosis in rabbits by the degree of sudanophilia. However little or no information has been reported on the histopathological changes in animal. This study reports the effect on serum and histopathological findings in rats fed skim or whole milks mixed with cholesterol and vitamin D_2.

Materials and Methods

A total of 80 male Sprague-Dawley rats of 5-weeks of age were used in the experiment. The animals were assigned to 4 groups according to diet; the first group served as control group fed the basic ration of a commercial rodent chow which contains approximately 22.1% protein, 3.5% fat, 5.0% fiber, 8.0% ash, 0.6% calcium, 0.4% phosphorous and 60.4% nitrogen-free-extract (by difference) by the manufacturer's analysis. The second, active treatment group, was fed the atherogenic feed, basic ration containing 1.0% of cholesterol and 50,000 IU/100 g of vitamin D_2. The concentrations of cholesterol and vitamin D_2 in feed for the induction of atherosclerosis was followed by Lee *et al.*[9] The other two groups, skim milk group and whole milk goup received the basic ration containing 1.0% of cholesterol and 50,000 IU/100 g of vitamin D_2 and powdered skim milk or whole milk of recommended daily allowance. All feed was made into pellet form.

All animals were housed in cages for rats and allowed to have feed and water at libitum throughout the study. Room conditions were maintained at 22± 2℃ with a light-dark cycle. Body weight and feed intake were measured and recorded.

After 13 weeks, rats were fasted 12~14 hours and sacrificed by exsanguination through cardiac puncture for the collection of blood. Serum samples were obtained by centrifugation of the blood. Immediately after exsauguination, selected organs were collected and fixed with 10% neutral buffered formalin solution for histopathological examination. Paraffin sections were made and stained with hematoxylin and eosin (HE) and examined by microscope.

Serum samples were tested by a biochemical analyzer (Ciba Corning Diagnostics, Express 550, U.S.A.). Total cholesterol by enzyme method, high-density lipoprotein cholesterol (HDL-cholesterol) by Finley method, triglyceride by enzyme method, calcium by o-cresolphthalein complexone (OCPC) method, inorganic phosphorous by modified Daly and Ertingshausen's method were determined. Data were analyzed by Duncan's Multiple Range Tests following Analysis of Variance procedures.

Results

No animals had any clinical sign throughout the experimental period, showing almost an equal level of final body weight. The growth responses, weight gain and feed efficiency ratio were not significantly different among groups (Table 1). However the relative liver weight of the control group was significantly lower than that of the other groups (Table 2).

Milk groups did not show lower cholesterol level than did the control group although no significant difference between the two milk groups was observed. However the rats in both skim milk and whole milk groups had significantly lower cholesterol level and higher HDL-cholesterol fraction than did the rats in the active treatment group ($p<0.05$) (Table 3). Calcium and inorganic phosphorous concentrations in serum in active treatment group and milk groups were significantly higher than in control group ($p<0.05$) (Table 4).

The histopathological findings of aorta and organs, kidney, liver, stomach and adrenal, were ob-

Table 1. Growth responses of rats fed milk with cholesterol and vitamin D_2 for 13 weeks

Group classification	Dose levels in feed			Body weight (g)		Feed efficiency ratio
	Cholesterol (g/100 g)	Vitamin D_2 (IU/100 g)	Milk[1)]	Initial	Final	
Control	0	0	0	163.50± 23.18	452.00± 48.79	0.13± 0.01
Active treatment control	1.0	50,000	0	166.25± 20.83	462.50± 47.00	0.13± 0.01
Skim milk group	1.0	50,000	RDA[2)]	154.75± 16.18	469.75± 36.04	0.13± 0.02
Whole milk group	1.0	50,000	RDA	184.25± 11.73	441.50± 24.87	0.14± 0.01

[1)] Powdered milk, [2)] Recommended daily allowance.

Table 2. Relative organ weight of rats fed milk with cholesterol and vitamin D_2 for 13 weeks (g/100 g body weight)

Group classification	Organ				
	Liver	Heart	Kidney	Spleen	Stomach
Control	3.09± 0.21^b	0.35± 0.10	0.38± 0.08	0.25± 0.08	0.46± 0.08
Active treatment control	3.71± 0.49^a	0.31± 0.09	0.37± 0.08	0.23± 0.08	0.44± 0.07
Skim milk group	3.68± 0.60^a	0.29± 0.07	0.35± 0.04	0.20± 0.06	0.39± 0.07
Whole milk group	3.83± 0.57^a	0.30± 0.07	0.38± 0.07	0.23± 0.06	0.42± 0.09

All values represent means± S.D.
Significance between means in the same organ is indicated with different superscript ($p<0.05$).

Table 3. Content of lipid in serum of rats fed milk with cholesterol and vitamin D_2 for 13 weeks

Group classification	Total-cholesterol (mg/dl)	HDL-cholesterol (mg/dl)	HDL-cholesterol / Total-cholesterol (%)	Triglyceride (mg/dl)
Control	45.4± 3.7^c	34.0± 2.6^b	75.1± 5.1^a	35.4± 6.5^c
Active treatment control	80.3± 11.8^a	36.3± 7.7^b	45.6± 9.9^c	56.0± 11.4^{a,b}
Skim milk group	74.2± 9.0^b	46.9± 8.5^a	63.7± 10.5^{a,b}	60.8± 10.0^a
Whole milk group	73.0± 9.3^b	38.9± 5.7^b	53.6± 7.4^b	47.4± 8.6^b

All values represent means± S.D.
Significance between means in the same item is indicated with different superscript ($p<0.05$).

Table 4. Content of inorganics in serum of rats fed milk with cholesterol and vitamin D_2 for 13 weeks

Group classification	Calcium (mg/dl)	Inorganic phosphorous (mg/dl)
Control	9.6± 1.0^b	6.3± 1.1^b
Active treatment control	11.3± 1.1^a	7.1± 1.0^a
Skim milk group	11.3± 1.4^a	7.7± 1.0^a
Whole milk group	11.8± 1.3^a	7.2± 0.8^a

All values represent means± S.D.
Significance between means in the same item is indicated with different superscript ($p<0.05$).

served. Aorta showed irregular appearance of the intima with swelling, necrosis and calcification of the endothelial cell in active treatment control (Photo 1), but no significant histopathological view was observed in control and milk fed groups. Coronary artery of the heart showed necrosis and calcification in active treatment control (Photo 2), but no significant histopathological view was observed in control and milk fed groups. Kidney showed moderate tubular calcification in the medulla and papilla in active treatment control (Photo 3), showed

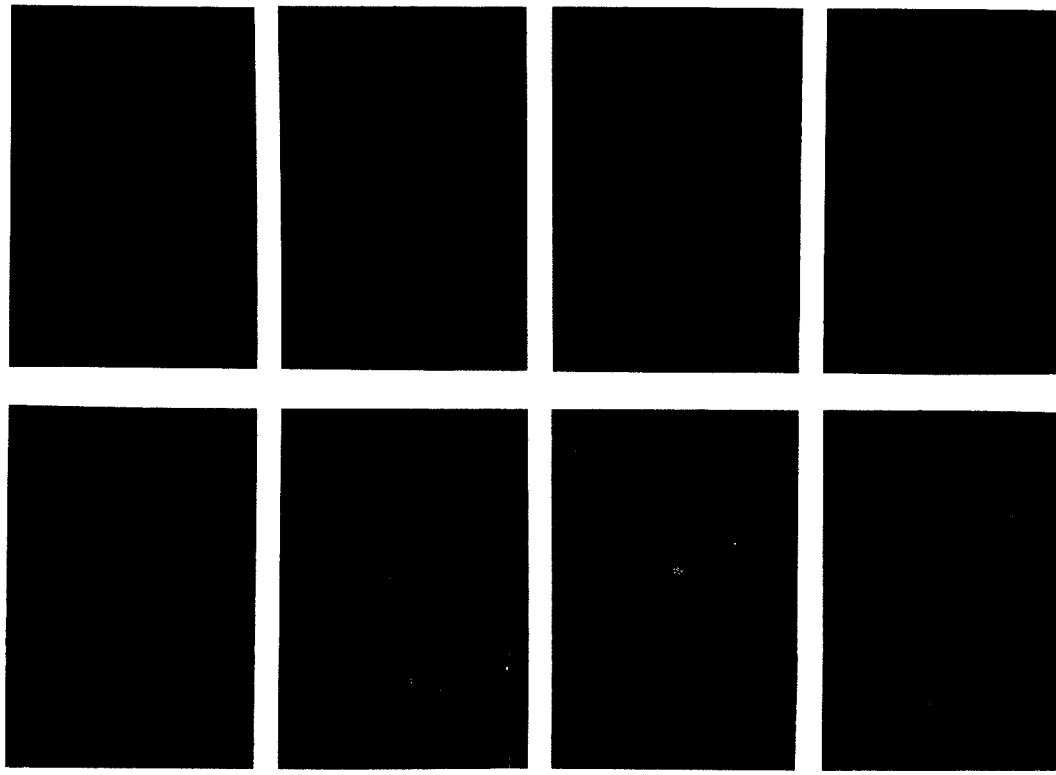

Photo 1. Swelling of endothelium in aorta of rat fed 1.0% cholesterol and 50,000 IU/100 g feed of vitamin D_2 for 13 weeks (×200).
L: Lumen

Photo 2. Calcification and necrosis of coronary artery in heart of rat fed 1.0% cholesterol and 50,000 IU/100 g feed of vitamin D_2 for 13 weeks (×200).

Photo 3. Moderate tubular calcification in kidney of rat fed 1.0% cholesterol and 50,000 IU/100 g feed of vitamin D_2 for 13 weeks (×100).

Photo 4. Severe fat change in liver of rat fed 1.0% cholesterol and 50,000 IU/100 g feed of vitamin D_2 for 13 weeks (×200).
PV: Portal vein

Photo 5. Mild tubular calcification in kidney of rat fed powdered skim milk with 1.0% cholesterol and 50,000 IU/100 g feed of vitamin D_2 for 13 weeks (×100).
Pa: Papilla

Photo 6. Mild tubular calcification in kidney of rat fed powdered whole milk with 1.0% cholesterol and 50,000 IU/100 g feed of vitamin D_2 for 13 weeks (×200).

Photo 7. Moderate fat change in liver of rat fed powdered skim milk with 1.0% cholesterol and 50,000 IU/100 g feed of vitamin D_2 for 13 weeks (×200).
CV: Central vein, PV: Portal vein

Photo 8. Moderate fat change in liver of rat fed powdered whole milk with 1.0% cholesterol and 50,000 IU/100 g feed of vitamin D_2 for 13 weeks (×200).
PV: Portal vein

mild tubular calcification in the medulla in skim milk group and showed mild tubular calcification in the medulla and papilla in whole milk group (Photo 5~6). Liver showed severe fat change with swelling and microvesicular fat droplet in active treatment control (Photo 4), showed moderate fat change with swelling and fat droplet around portal triad in skim milk group (Photo 7), and showed moderate fat change with swelling and fat droplet around portal triad and midzonal region in whole milk group (Photo 8). Stomach and adrenal showed no significant histopathological view in all groups.

Discussion

We did not observe any significant differences among groups in body weight in spite of the excess intake of calories corresponding to whole milk. It is very difficult for us to explain this result. Naito[13] also observed this phenomena.

The findings of our study that both skim milk and whole milk-fed rats had lower serum cholesterol levels than did the atherogenic diet-fed rats is in agreement with data from other studies.[7,17] However they compared the influence of fermented milk to that of skim milk or whole milk and the comparision of the effect of skim milk and whole milk on the atherogenic rats had not been previously tested. We could not find that the cholesterol level in milk groups was lower than that in control group which was observed by Thakur and Jha.[17] We could explain it by the difference of cholesterol intake of experimental animals. Cholesterol intake of rats in our study (1.0 g/100 g feed) was higher level than that of rabbits in their study (0.1 g/kg body weight).

Many studies of vitamin D status have included measurement of calcium and phosphorous concentration in serum and/or urine as a biochemical parameters.[2] The elevation of these two inorganics in serum in our study provides the evidence for hypervitaminosis D.

The histopathological findings of organs of rats in 13 weeks showed no or milder changes in aorta, heart, kidney, and liver in milk groups while severe changes in active treatment group. We could not find marked difference between the skim milk and whole milk groups. Whole milk has been considered to be related to a coronary health hazard because of its fat content.[16] However our data of growth, clinical and pathological findings showed benefits of both skim milk and whole milks in rats fed vitamin D_2 and cholesterol. This study, therefore suggests that supplementing milks could have a helpful effect on the development of atherosclerosis.

국문요약

본 연구는 우유가 흰쥐의 동맥경화증 유발억제에 미치는 효과를 관찰하기 위하여 수행되었다. 생후 5주령의 Sprague-Dawley계 흰쥐 수컷 80마리를 4개군(대조군, 처치집단 대조군, 탈지유군, 전지유군)으로 나누어 13주간 비교 관찰한 결과 사료섭취량 및 체중은 유의한 차이를 나타내지 않았다. 혈청 중 총콜레스테롤, HDL-콜레스테롤 분획은 모두 탈지유 및 전지유군간에 유의한 차이를 보이지 않았으나 처치집단대조군과 우유군간에는 유의한 차이를 보였다($p<0.05$). 각 장기에 대한 병리조직학적 관찰 결과 대동맥, 심장, 신장 및 간에서 조직의 변화가 관찰되었으며 석회침착, 괴사 등의 정도 및 지방변성 소견이 처치집단 대조군에 비하여 우유군에서 양호하였고, 탈지유군과 전지유군간의 현저한 차이는 없는 것으로 판단되었다. 전지유와 탈지유는 모두 흰쥐에 있어 콜레스테롤과 비타민 D_2로써 실험적으로 유발된 동맥경화증에 억제적 역할을 할 수 있으며 그 효과는 비슷한 것으로 평가된다.

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