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Effect of Phylloquinone on Dexamethasone-Induced Calcification of Heart Muscle and Media Elastocalcinosis in Aorta Artery in Rat Model

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Abstract: Background. Dexamethasone is a type of steroid medication which enhances the rate of pericyte differentiation and mineralization in vitro with concomitant suppression of calcification inhibitory molecule matrix Gla-protein (MGP). Vitamin K is an essential cofactor in the carboxylation of glutamate residues in a small group of proteins, including MGP. This study tries to assess the efficacy of vitamin K1 on dexamethasone-induced media elastocalcinosis in aorta artery and heart muscle in a rat model. Materials and Methods. 110 male rats with a normal weight range of 270 ± 20 were enrolled in this study. They received a calcification-inducing diet containing both vitamin K1 and dexamethasone during 6 or 12 weeks and were randomly assigned into two groups; a basic group (n=30), and an experimental group (n=80). The experimental group was divided into two groups receiving treatment during 6 and 12 weeks. Administration of dexamethasone was 0.5 mg/kg, intraperitoneal (IP). Vitamin K intakes were different including 5, 10, and 20 µg/kg, which were considered as low, moderate, and high intake, respectively. Results. Plasma concentrations of calcium were not affected by the different regimes and ranged between 2.27 and 2.31 millimolar (mM) (mean ± SD: 2.29 ± 0.02). According to the findings of pathologic biopsy of aorta artery and heart muscle, treatment of 0.5 mg/kg dexamethasone during 6 and 12 weeks did not induce media elastocalcinosis at all. Conclusion. Administration of 0.5 mg/kg dexamethasone during 6 and 12 weeks did not induce media elastocalcinosis at all. On the other hand, structure and histology of vessels did not change following intake of vitamin K1, therefore, different dosages of vitamin K could not affect the aorta artery status.

Keywords: Media Elastocalcinosis, Dexamethasone, Vitamin K, Aorta Artery, Heart Muscle

1. Introduction

Arterial calcification is an important independent risk factor for the development of atherosclerosis, Myocardial Infarction (MI), stroke, and renal disease.[1] Despite the recent thought that calcification is a passive process and end stage of cardiovascular disease, during the past 10 years, however, it has become clear that several osteoregulatory proteins, both stimulatory and inhibitory are involved in calcification of vascular tissue.[2] Atherosclerosis is characterized by remodeling and stiffening of large elastic arteries.[3]

The effect of glucocorticoids on vascular cell in vivo remains obscure. Pericytes are pluripotent cells that can differentiate into osteoblasts, and recent evidence suggests that they could participate in vascular calcification. Synthetic glucocorticoids dexamethasone would enhance the rate of

pericyte differentiation and mineralization in vitro with concomitant suppression of calcification inhibitory molecules "MGP".[1]

Vitamin K refers to a family of fat-soluble compounds with a common chemical structure.[4] Phylloquinone, or vitamin K1, is present in foods of plant origin, for example, green, leafy vegetables.[5] Bacterial and other forms of vitamin K, referred to as the menaquinones or vitamin K2, differ in structure from phylloquinone in their 3-substituted lipophilic side chain.[6] Vitamin K is an essential cofactor in carboxylation of glutamate residues in a small group of proteins, including MGP.[7] Observational studies are promising in terms of associations between vitamin K (either diet or biochemical measures) and bone health and other conditions of normal calcification in the elderly.[8] Main problem was evaluation of role of dexamethasone in induction of calcification of arteries and probable anti-calcification role of vitamin K, a hypothesis was designed about the effect of high intake of vitamin K on dexamethasone-induced media elastocalcinosis in rats.

2. Materials and Methods

2.1. Animals and Groups

After approving of study protocol and ethical aspect in ethical committee of animal studies in our university, 30 rats were selected as the basic group, of which 10 rats were killed at the beginning of the experiment for pathologic assessment. The remaining 20 rats received normal saline volume just equal to corticosteroids volume cases during 6 weeks and then 10 rats were killed for pathologic assessment. Treatment continued for the remaining 10 rats for further 6 weeks and then they were killed.

Eighty rats were enrolled in the experimental group. To induce vascular calcification, 40 rats received dexamethasone (0.50 mg/kg) subcutaneously every 12 hours for 6 weeks (the first dexamethasone dose at 8 AM and the second one at 8 PM with no accompanying vitamin K). After 6 weeks of treatment 10 rats were killed and pathologically assessed and the remaining 30 were subdivided into 3 groups of 10 rats for another 6 weeks. Dexamethasone was discontinued in the new 3 groups. One group received 5µg/kg of vitK1, the second group 10 µg/kg of vitK1, and the third group 20 µg/kg of vitK1 every 24 hours (at 8 AM during the treatment period), after 6 weeks of treatment they were killed and assessed. The other 40 rats received corticosteroids (0.50 mg/kg) subcutaneously every 12 hours for 12 weeks (the first dexamethasone dose at 8 AM and the second one at 8 PM with no accompanying vitamin K). After 12 weeks of treatment 10 rats were killed and pathologically assessed and the remaining 30 rats were subdivided into 3 groups of 10 for another 6 weeks. Dexamethasone was discontinued in these 3 groups. The first group received 5 µg/kg of vitK1, the second group 10 µg/kg of vitK1, and the third group 20 µg/kg of vitK1 every 24 hours (at 8 AM during the treatment period). After 6 weeks of treatment they were killed and assessed.

2.2. Place & Food

The rats were housed in normal cages with free access to water and the indicated foods. Male rats were given free access to rodent diet 5001 (Prina Mills), which is comprised of 0.67% phosphorus and 0.95% calcium by weight. This diet contains 500 µg phylloquinone/kg with no added menadione and all injections are administered subcutaneously in the back of animals. To reduce trauma injection sites, 25-gauge needles were used for all injections and subcutaneous injection sites were rotated between the 4 quadrants of the back.

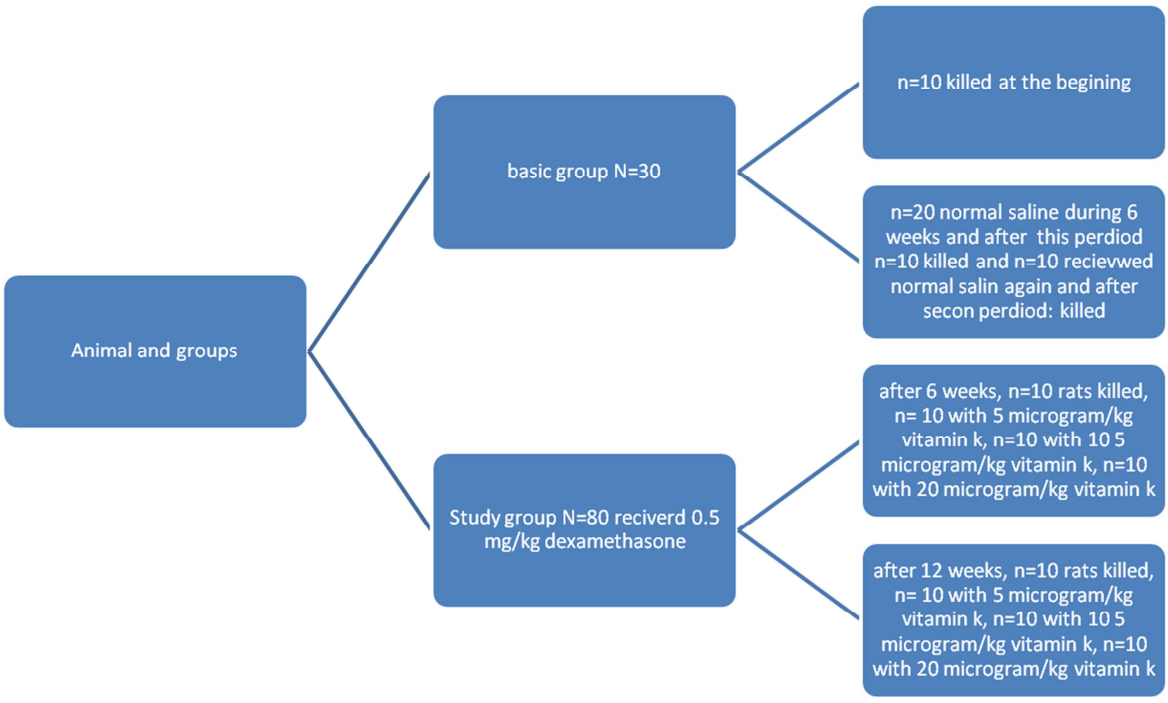

Chart 1. *Included animals in this experimental study.*

2.3. Biopsy

The rats were anesthetized with sodium pentobarbital. The segment of thoracic aorta between the aortic arch and renal branch was removed at necropsy and fixed in 1% buffered formalin while longitudinal sections were stained with von Kossastain to detect areas of mineralization. Abdominal aorta artery (between the renal branch and the iliac bifurcation) was dried, weighed and extracted with a 10-fold excess (wt/vol) of 10% formic acid for 16 hours at 4 C° for assessment of the calcium (Ca) content. Ca concentrations were measured using atomic absorption spectrometry.

2.4. Statistical Analysis

Values were expressed as mean ± SD (standard deviation). The difference between 2 groups was determined by Wilcoxson ranked non-paired test. Differences for multiple comparisons were determined by ANOVA with Bonferroni correction. Differences were considered to be significant at *p*-vlue less than 0.05.

3. Results

110 male rats with a normal weight range of 270 ± 20 were enrolled in this study. The plasma concentration of calcium was not affected by the different regimens and ranged between 2.27 and 2.31 mM (mean ± SD: 2.29 ± 0.02). Treatment of 0.5 mg/kg dexamethasone during 6 weeks did not induce media elastocalcinosis at all in this category. In addition, structure and histology of vessels did not change following intake of vitamin K1, therefore, different dosages of vitamin K could not affect heart muscle and aorta artery status (Figure 1).

In rats who received dexamethasone for 12 weeks, there was no evidence of calcification in the heart muscle or elastocalcinosis in the aorta (Figure 2). Calcification was not observed in rats who received dexamethasone followed by vitamin K treatment (Figure 3).

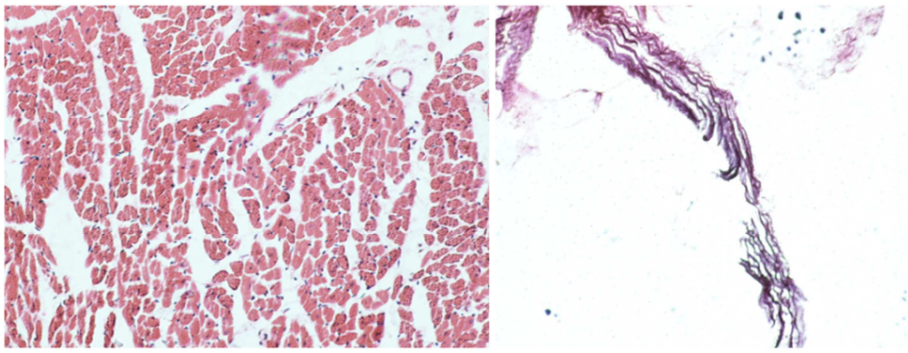

Figure 1. *Right: heart muscle without calcification during 6 weeks of treatment [phenomenal section, ×20], Left: Aorta artery without calcification during the 6 weeks [longitudinal section, ×20].*

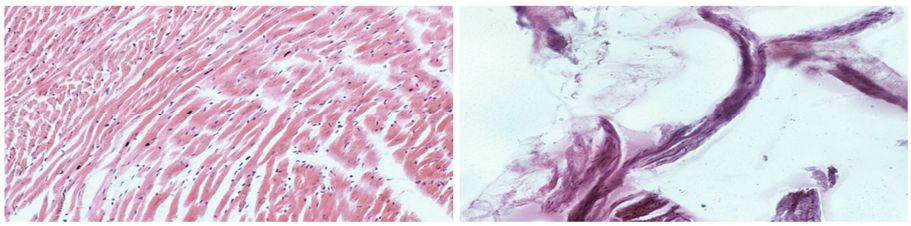

Figure 2. *Right: Heart muscle without calcification during 12 weeks of treatment [×20], Left: Aorta artery without media elastocalcinosis during 12 weeks of treatment [×20].*

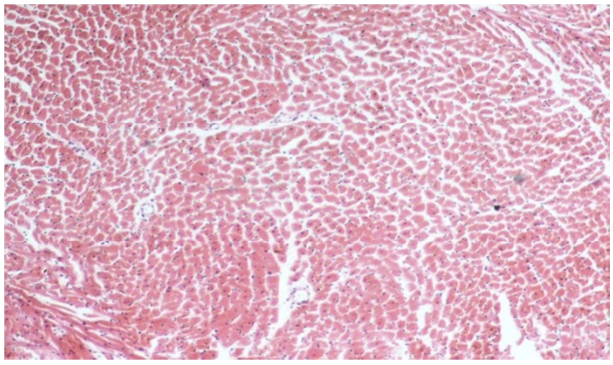

Figure 3. *Heart muscle without calcification during 12 weeks of treatment [×20].*

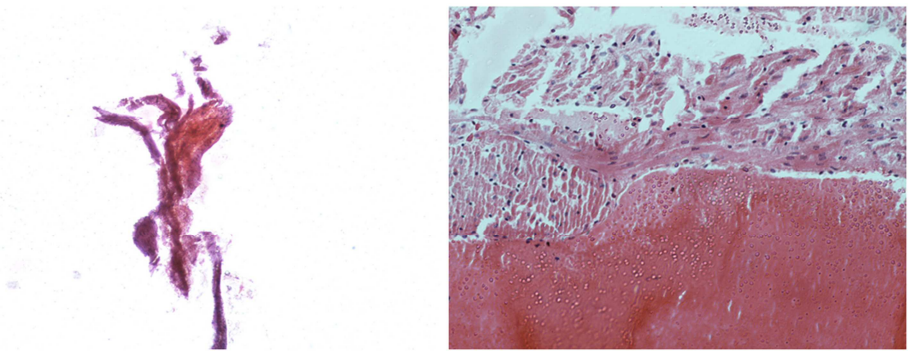

Figure 4. *Thrombosis of aorta artery and heart muscle during treatment [×20].*

In the 6-week follow-up, 5 rats died of which three were for high dose vitamin K group and two for moderate dose group. However, in the 12-week follow-up, 4 rats died, including one in moderate dose and three in high dose group. A tertiary rate was seen more in high dose of vitamin K group. For more evaluations of thrombosis, rats that died were assessed and pathology findings found thrombosis in heart muscle and aorta tissue as the cause of death (Figure 4).

4. Discussion

Arterial calcification is an important independent risk factor for the development of atherosclerosis, MI, stroke, and renal disease. Synthetic glucocorticoids dexamethasone would enhance the rate of pericyte differentiation and mineralization in vitro with a concomitant suppression of calcification inhibitory molecules "MGP".[1] This study tried to assess the efficacy of vitamin K1 on dexamethasone-induced media elastocalcinosis in aorta artery in rat models. Willenberg T et al. suggested that long-term treatment with corticosteroid was associated with calcifying peripheral atherosclerosis inducing arterial incompressibility and distally accentuated.[9] A study carried out by Nashel DJ indicated that prolonged corticosteroid therapy accelerated the development of atherosclerosis. On the other hand, prolonged corticosteroids therapy, particularly in younger persons, would be avoided whenever possible.[10] Miyahara T et al., suggested that corticosteroids and especially dexamethasone had a role in osteogenic cells calcification and also bone mineralization.[11] These clinical studies were in contrast with our study. Many studies have reported glucocorticoids to induce osteoporosis, it appears that in vitro, glucocorticoids promote calcification and media elastocalcinosis. We used dexamethasone for induction of media elastocalcinosis in rats, according to previous findings, however, our results were not in line with previous studies. A study conducted by Kirton JP revealed that the administration of dexamethasone at a physiological concentration of 10 nmol/L induced the differentiation of human bone marrow stromal cells in vitro. They claimed that their study was the first report showing that dexamethasone could enhance the osteogenic differentiation of pericytes and downregulates genes associated with inhibition of mineralization.[12] This finding was in agreement with our study. In the present study, also 0.5 mg/kg dexamethasone could not induce media elastocalcinosis in rat models.

Vitamin K is one of the most important cofactors in the production of proteins that inhibit calcification of vascular. According to previous studies, a low vitamin K intake has been associated with aortic and coronary calcifications. In our study, vitamin K1 could not affect histopathology of aorta artery. A study carried out by Maas AH et al., suggested that mean vitamin K2 levels were lower in the participants with breast artery classification (BAC) compared to participants without BAC, therefore, calcifications in breast arteries were not associated with a lower intake of vitamin K.[13] A study conducted by Rees K et al., indicated that there was no evidence for an effect of vitamin K1 on calcification. This finding was consistent with our study.[14]

Dexamethasone downregulates genes associated with inhibition of mineralization, depending on half-life of genes products in serum, this downregulation signs may observed in different intervals perhaps after several months (there is a reservoir of protein (genes product) in serum content depending on that of half-life. Finally, it was concluded that administration of 0.5 mg/kg dexamethasone during 6 and 12 weeks did not induce media elastocalcinosis at all. Furthermore, structure and histology of vessels did not change following intake of vitamin K1, therefore, different dosages of vitamin K could not affect aorta artery status. We suggest to carrying out researches about other corticosteroids with different dosages in other models of animals such as rabbits.

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Conflict of Interest

None Declared

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