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Original Article

# Effect of Intraperitoneal Injection of Aspirin on Vascular Calcification in a Rat Model

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## ABSTRACT

**Background and Aim:** Aspirin (ASA) is crucial in safeguarding patients at high risk for cardiovascular events by reducing ischemic occurrences. Its protective effects are due to trans-acetylation, which involves a reaction between ASA and the amino groups found in lysine and N-terminal residues. To date, there have been no studies on aspirin injection in arterial calcification. The aim was to confirm by experiments that acetylated aspirin injection can significantly alleviate arterial calcification. **Methods:** The rat's model of diabetic arterial calcification induced by diabetes+WVK (Warfarin and Vitamin K) and the effect of aspirin injection was evaluated through the measurement of vascular calcification in aorta, smaller arteries. **Results:** Rats that received aspirin injections (60 and 90 mg/kg) for a week showed a significant reduction in calcification in both the aorta and peripheral arteries. **Conclusion:** These results suggested that aspirin injection may be a useful in treatment of vascular calcification.

**Keywords:** Aspirin injection, Diabetes, Experimental model, Vascular calcification, Warfarin.

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## INTRODUCTION

Vascular Calcification (VC) has long been an important concern in cardiovascular medicine. Vascular calcification is commonly linked to the underlying pathological processes in individuals with atherosclerosis, diabetes, and chronic kidney disease. Vascular calcification is considered an important risk factor for cardiovascular disease, with calcium deposits frequently found in advanced atherosclerotic plaque lesions.[1] Calcified blood vessels are stiffer than healthy vessels, making them less responsive to vasodilation. This increases the likelihood of thrombus formation and the rupture of atherosclerotic plaques. Vascular calcification typically occurs in the elastic lamellae and muscular arteries, including the aorta, carotid, and coronary arteries. The most accurate information available from recent studies is the fact that vascular calcification is a highly complex pathological process. Arterial calcification may occur in the intima and tunica media. Medial Elastocalcinosis (MEC), or medial arterial calcification, contrasts with intimal calcification seen in advanced atherosclerotic lesions. It involves accumulation of calcium minerals on a damaged elastic network in large arteries. The resulting microvascular and macrovascular complications stem from severe metabolic disruptions, leading to ongoing tissue damage. While vascular calcification was once considered a passive occurrence, growing evidence now suggests it is a regulated process, involving the activation of proteins that manage mineralization in the vascular wall.[4-6]

Osteopontin (OPN), a non-collagenous protein that binds calcium and hydroxyapatite in bones and teeth, has been found in both calcified atherosclerotic lesions and in Medial Elastocalcinosis (MEC).[7] So far, drugs such as losartan and atrobastatin have been used to treat arterial calcification. Aspirin (ASA) is widely recognized as the most commonly used non-steroidal anti-inflammatory drug, owing to its broad range of effects. Beyond its traditional use for pain, fever, and inflammation, ASA plays a significant role in clinical practice. It is extensively employed as a preventive treatment for atherothrombotic diseases due to its ability to inhibit the COX-1 enzyme in platelets. Additionally, ASA promotes the resolution of inflammation by enhancing COX-2 enzyme activity in the vasculature, thereby reducing the risk of cardiovascular ischemic events. ASA also contributes to lowering insulin resistance by decreasing levels of pro-inflammatory cytokines. Its therapeutic effects are further mediated through the transfer of its acetyl group to the amino groups of proteins at lysine and N-terminal residues. Furthermore, Endothelial Progenitor Cells (EPCs) are essential for neovasculogenesis and the preservation of vascular integrity.[8-10] Aspirin has been shown to enhance the pro-angiogenic potential of Endothelial Progenitor Cells (EPCs).[11] However, to date, no studies have explored the use of aspirin injections for treating Vascular Calcification (VC). Based on this, we hypothesize that aspirin injections could offer protective effects against arterial wall degeneration by reducing

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calcification, potentially through a combined mechanism of modulating inflammation and mobilizing EPCs.

## MATERIALS AND METHODS

### Materials

Aspirin injection was obtained from CHONGHUNG pharmaceutical company, DPR Korea and identified by comparison with the voucher specimen deposited at National drug certification institute of Pyongyang, DPR Korea.

### Animals

Male Wistar rats (260 g) were provided by Laboratory Animal Centre of Pyongyang University of Medical Sciences and adapted in a lab environment before experiments for a week. 50 rats are randomly chosen and during the experiment, food and water were available to rats at any time. The temperature was maintained at 20±2°C and the humidity was 55%. The study was approved by the Ethics Committee for Animal Experimentation, Faculty of Basic Medicine, Sinyuzu University of Medical Sciences.

### Model of Vascular Calcification

The rat vascular calcification model was established as previously described.[12,13] Briefly, 40 rats were used for treatment groups of a vehicle control, VC model and aspirin injection (30, 60 and 90 mg/kg). 30 rats were given vitamin K1 (15 mg/kg, intramuscularly) plus warfarin (150 mg/kg, intramuscularly) at 08:00 on day 1, and warfarin was re-administered at 20:00 on the same day for 7 weeks. After 2 weeks, 30 rats were added the treatment with aspirin injection (30, 60 and 90 mg/kg/day, intraperitoneal) for a week as the aspirin group and the remaining rats served as the VC group.

### Quantification of Vascular Calcification

To assess and quantify calcification in the aortic wall, thoracic aorta sections were stained using Von Kossa's method. After isolation, the thoracic aorta was fixed in neutral buffered formalin for 90 min, then cut into 15-20 segments, each 2 to 3 mM in length, and embedded in paraffin blocks. Thin sections (4 uM) were stained with Von Kossa's technique and counterstained with hematoxylin and eosin to identify calcification. The percentage of calcified area was determined using Axiovision image analysis software (Release 4.5; Carl Zeiss, Oberkochen, Germany), which analyzed the total tissue area and Von Kossa-positive area through two-color separation thresholds. The calcified area percentage was calculated as the ratio of Von Kossa-positive area to the total tissue area.

Further quantification of calcification in the aorta and smaller arteries was performed by measuring calcium content. The proximal abdominal aorta and left carotid and femoral arteries were isolated and weighed using a precision balance. The samples were then digested in 65% nitric acid ($HNO_3$) at 60°C for 6 hr. The calcium content in the digests was measured by flame atomic absorption spectrometry, with results expressed as milligrams of calcium per gram of wet tissue.

### Statistical Analysis of Data

Quantitative data are reported as mean±standard error of the mean. Statistical differences in basal characteristics between the groups were calculated by one-way analysis of variance and t-test for continuous variables. $p<0.01$ was considered statistically significant. All statistical analyses were performed using the SPSS 16.0 software.

## RESULTS

The effects of aspirin injection on vascular calcification, the calcium content was evaluated in the aorta and smaller arteries such as femoral and carotid arteries of rats. The results were presented as Table 1.

In the model group, the calcium content was highest across all arterial segments: aorta (4.1±0.6 mg/g), femoral artery (2.1±0.2 mg/g), and carotid artery (1.6±0.3 mg/g). Treatment with aspirin resulted in a dose-dependent reduction in calcium content in all arteries.

At a dose of 30 mg/kg (Aspirin-1), calcium levels decreased moderately in the aorta (3.7±0.4 mg/g), femoral artery (1.8±0.2 mg/g), and carotid artery (1.2±0.1 mg/g), although the reduction was not statistically significant compared to the model group.

Higher doses of aspirin demonstrated significant reductions in arterial calcium content. At 60 mg/kg (Aspirin-2), calcium content in the aorta, femoral, and carotid arteries was significantly lower (2.9±0.23 mg/g, 0.9±0.1 mg/g, and 0.4±0.03 mg/g, respectively; $p<0.01$). The most pronounced effect was observed at 90 mg/kg (Aspirin-3), where calcium levels in the aorta, femoral, and

**Table 1: Effects on treatment of aspirin injection on calcium content in arteries (mg/g wet tissue).**

| Group | Dose | Aorta | Femoral | Carotid |
|---|---|---|---|---|
| Model | - | 4.1±0.6 | 2.1±0.2 | 1.6±0.3 |
| Aspirin-1 | 30mg/kg | 3.7±0.4 | 1.8±0.2 | 1.2±0.1 |
| Aspirin-2 | 60mg/kg | 2.9±0.23** | 0.9±0.1** | 0.4±0.03** |
| Aspirin-3 | 90mg/kg | 2.1±0.2** | 0.7±0.1** | 0.3±0.04** |

Each value represents the mean±SEM of 10 rats per group. **$p<0.01$ as compared with model group.

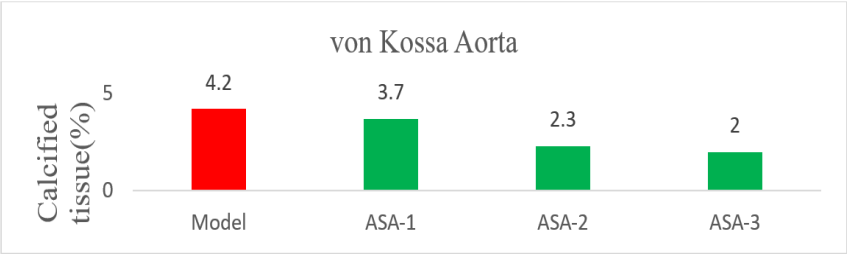


**Figure 1:** Effect of aspirin injection on calcified tissue. ASA-1: 30mg/kg, ASA-2: 60mg/kg, ASA-3: 90mg/kg. Each value represents the mean±SEM of 10 rats per group. $^{\triangle\triangle}$ $p$<0.01 as compared with Model group.

carotid arteries were reduced to 2.1±0.2 mg/g, 0.7±0.1 mg/g, and 0.3±0.04 mg/g, respectively ($p$<0.01).

The effect of aspirin injection on vascular calcification was evaluated by measuring the percentage of calcified tissue in the aorta, as assessed using Von Kossa staining (Figure 1). In the model group, the percentage of calcified tissue was highest at 4.2%. Treatment with aspirin significantly reduced the calcified tissue in a dose-dependent manner. At a dose of 30 mg/kg (ASA-1), the percentage of calcified tissue decreased to 3.7%, showing a moderate reduction compared to the model group. Higher doses of aspirin demonstrated more pronounced effects, with calcified tissue percentages of 2.3% at 60 mg/kg (ASA-2) and 2.0% at 90 mg/kg (ASA-3). The reductions at doses of 60 mg/kg and 90 mg/kg were statistically significant ($p$<0.01) compared to the model group.

## DISCUSSION

The present study examined the effects of aspirin injection on reducing the calcification in VC rat model. This work is significant because vascular calcification is a critical pathological feature associated with various cardiovascular diseases, and effective therapeutic interventions remain limited. We found that aspirin injection dose-dependently reduced calcium contents in the aorta and smaller arteries.

As shown in Table 1, rats in the VC model exhibited increased calcium content in the aorta and smaller arteries such as the carotid and femoral arteries. These elevated calcium levels represent a hallmark of vascular calcification, mimicking human pathological conditions. However, the administration of aspirin injection at doses of 60 mg/kg and 90 mg/kg significantly ($p$<0.01) decreased this parameter, indicating reduced vascular calcification in these arteries.

Further supporting these findings, histological analysis of the aorta demonstrated a marked reduction in the calcified area in rats treated with 60 mg/kg and 90 mg/kg aspirin injections, as evidenced by Von Kossa-stained sections. As shown in Figure 1, at doses of 60mg/kg and 90 mg/kg of aspirin injection calcified areas were significantly decreased compared to VC model group. This underscores the efficacy of aspirin in mitigating vascular calcification at both macro- and micro-structural levels.

Endothelial Progenitor Cells (EPCs) or Angioblast Cell (AGCs) play a critical role in neovasculogenesis and maintenance of vascular integrity. On the other hand, aspirin has been reported to increase the proangiogenic potential of EPCs.[11] This proangiogenic effect could contribute to improved vascular repair and resilience against calcification by promoting endothelial regeneration. Since inflammation triggered by abnormal hemodynamic stresses and endothelial damage is a key factor in the development of vascular calcification, we proposed that aspirin could reduce monocyte/macrophage infiltration into injured tissue by improving endothelial function and interfering with the interaction between endothelial cells and monocytes/macrophages.

The experiments presented in this study represent the experimental evidence that aspirin injection decreases vascular calcification. These findings also highlight the potential mechanistic role of aspirin in enhancing endothelial function and reducing inflammation, which are key contributors to vascular health.

In addition, the present study suggests that the model has important similarities with the human pathological condition. The ability of aspirin to attenuate calcification in this model suggests its potential translational relevance in managing vascular calcification in clinical settings. Further investigations are warranted to explore the long-term efficacy and underlying mechanisms in human populations.

## CONCLUSION

Aspirin injection significantly reduced vascular calcification in a dose-dependent manner, as evidenced by decreased calcium content and calcified areas in arteries. The findings suggest aspirin's potential to enhance endothelial function and reduce inflammation, key factors in mitigating calcification. The rat model used shares similarities with human pathology, supporting the translational relevance of these results. Aspirin shows promise as a therapeutic agent for managing vascular calcification, warranting further clinical investigation.

## ACKNOWLEDGEMENT

Nil.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## ABBREVIATIONS

**ASA:** Aspirin; **VC:** Vascular Calcification; **EPCs**: Endothelial Progenitor Cells; **MEC:** Medial Elastocalcinosis; **OPN:** Osteopontin; **COX-1:** Cyclooxygenase-1; **COX-2:** Cyclooxygenase-2; **WVK:** Warfarin and Vitamin K.

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