Drug-induced Reduction of Gamma Carboxylation in Osteocalcin: What is the Fallback?

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Abstract

Osteoporosis is a medical condition in which bone becomes fragile and weak. In this condition, the quality and density of the bone are reduced. Vitamin K is vital for bone mineralization as it plays a vital role in the gamma-carboxylation of osteocalcin. Therefore, if there is a deficiency of vitamin K, it can lead to osteoporosis due to undercarboxylated osteocalcin.

Warfarin is the most widely used anticoagulant in the elderly. In this article, we reviewed how Warfarin, an inhibitor of vitamin K, affects bone remodeling and leads to osteoporosis.

Categories: Internal Medicine, Preventive Medicine, Orthopedics
Keywords: osteoporosis, fracture, warfarin, anticoagulants

Introduction And Background

Warfarin is a traditional oral anticoagulant used in elderly patients for stroke prevention with non-valvular atrial fibrillation (NVAF). It is used in many other diseases such as myocardial infarction, deep vein thrombosis (DVT), and pulmonary embolism (Table 1). It has many side effects, among which hemorrhage is the most common; other side effects are purple toe syndrome, warfarin necrosis, osteoporosis, and calcification. Warfarin is a vitamin K antagonist that interferes with the gamma-carboxylation of glutamic acid residues. Therefore, it inhibits the activation of bone matrix proteins [1]. Warfarin effect should be monitored every one to four weeks by assessing the prothrombin time/International Normalized Ratio (INR).
1. Long-term prophylaxis of thrombosis

- Prevention of thrombosis during atrial fibrillation
- Prevention of thromboembolism (in patients with prosthetic heart valves)
- Prevention of venous thrombosis and associated pulmonary embolism
- Treatment of Myocardial infarction and stroke

2. Not useful in emergencies

### TABLE 1: Uses of warfarin

Several analyses have reported an amplified risk of osteoporotic fractures with the use of Warfarin [1-4]. This concern was confirmed by a population-based study of 14,564 Medicare patients in the United States in 2006, which highlighted a higher risk of osteoporotic fracture in patients diagnosed with Atrial Fibrillation (AF) (odds ratio: 1.25) and long-term (one year) warfarin users as compared with Warfarin non-users [3].

Two hundred fifty thousand people in the United States are hospitalized with hip fractures per year [5]. All patients admitted with these fractures, between 7.8% and 10.3% are receiving chronic warfarin therapy [6-9]. In the coagulation cascade, vitamin K acts as a necessary cofactor in the gamma-carboxylation of glutamate to γ-carboxyglutamate (Gla) residues in many coagulation proteins (proteins C and S) and clotting factors (factors II, VII, IX, and X). By inhibiting vitamin K, coagulation is also inhibited. Warfarin antagonizes vitamin K-dependent processes, including the gamma-carboxylation of osteocalcin and bone matrix proteins such as Gla proteins that are required in bone mineralization (Table 2). Previous studies have shown that there is an association between the augmented level of undercarboxylated osteocalcin in warfarin users and amplified fracture risk, caused due to reduced bone mineral density. Osteocalcin in the carboxylate form binds to calcium and then to hydroxyl-apatite crystals in the matrix of the bone [10-12].

| NAME                          | TISSUE              | FUNCTION                        |
|-------------------------------|---------------------|---------------------------------|
| Osteocalcin (OC)              | Bone                | Contribute to bone health       |
| Matrix Gla Protein (MGP)      | Aorta, heart valve  | Inhibit calcification in arteries|
| Coagulation factors/Anti-coagulation factors | Liver             | Contribute to normal coagulation |
| GAS6                          | Aorta, brain        | Modulate cell growth            |

### TABLE 2: Vitamin K-dependent proteins

GAS6 - Growth Arrest Specific 6

If Vitamin K is low, it will lead to a low level of undercarboxylated osteocalcin, which increases
the fracture risk in the elderly (Tables 3-4) [13-14]. Further, the serum concentration of undercarboxylated osteocalcin has been revealed to increase with warfarin [15-16]. Despite the fact that warfarin increases fracture risk, it was an unavoidable treatment choice for years because no other comparable alternatives were available. For the prevention of atrial fibrillation and treatment of medical conditions such as acute coronary syndromes, venous thromboembolism. Several alternate options for warfarin are being investigated including oral direct thrombin inhibitors (Table 5). A new oral direct thrombin inhibitor Dabigatran has been approved for stroke prevention in patients diagnosed with atrial fibrillation. Dabigatran has a better basal characteristic profile as compared to warfarin (Table 5). Unlike warfarin, Dabigatran is being administered by a fixed-dose without any need for dosing adjustments and routine coagulation monitoring [17]. In this article, we tried to find an alternative coagulation therapy (as opposed to traditional warfarin therapy), as a means to avoid the risk of osteoporosis associated with warfarin.

| Alfa alfa       | Cabbage       | Cauliflower  | Cheese       | Dairy products | Egg yolk       | Green leafy   | Liver         | Meat         | Tomato       | Spinach       | Vegetables  |
|-----------------|--------------|--------------|--------------|----------------|----------------|--------------|---------------|--------------|--------------|--------------|-------------|
| Promotes bone calcification | Prevents blood vessel calcification | Assists in blood clotting |

TABLE 3: Sources of vitamin K

TABLE 4: Function of vitamin K
Apixaban | Rivaroxaban | Edoxaban | Dabigatran
--- | --- | --- | ---
**Mode of Excretion** | 75% feces 25% renal | 66% renal 34% feces | 50% renal 50% feces | 80% renal 20% fecal
**Max Concentration** | 3 hours | 2-4 hours | 1-2 hours | 1 hour
**Dosing** | Twice a day | Daily | Daily | Twice a day
**Half-life** | approximately 12 hours | Healthy Patients: 5-9 hours, Elderly Patients: 9-13 hours | 10-14 hours | 12-17 hours
**Cascade Target** | Factor Xa | Factor Xa | Factor Xa | Factor Xlla

### TABLE 5: Pharmacokinetics and pharmacodynamics of newer anticoagulants

#### Review

**Mechanism of osteoporosis by warfarin**

Osteoporosis is an illness in which bone loss is more than bone formation leading to the weak and brittle bones in the older age group. The bone becomes so soft that even minor stress can lead to fractures. The most common bones involved are vertebrae, spine, hip, and forearm. The symptom does not appear until the bone is fractured, and the most common symptom is back pain, short height, and stooped posture. In 1970, the sequence of amino acid in the bone G1a protein was determined, which is known as osteocalcin. It was confirmed that it plays a major role in bone mineralization [18]. The unique structure of G1a protein allows the binding of calcium ions and calcium mineral surface at position 17, 21, and 24 with three gamma-carboxyglutamic acid residues [19]. Vitamin K is a cofactor in the gamma-carboxylation of osteocalcin that helps in bone mineralization. If vitamin K is low, it can increase the risk of osteoporosis. High levels of γ-carboxylation make strong bones, and low-levels lead to more bone loss. It has been reported that undercarboxylated osteocalcin (uOC) increases after menopause and much higher after the age of 70, which causes a decrease in BMD and an increase in bone loss, resulting in hip fracture. Warfarin inhibits the vitamin- K dependent γ-carboxylation of osteocalcin, leading to osteoporosis, through two ways, directly and indirectly: (1) Directly by inhibiting gamma-carboxylation of osteocalcin and (2) indirectly because patients on Warfarin are recommended to take diet low in vitamin-k (Figure 1).
Warfarin increases skeletal fragility by decreasing bone mineral density and inducing the risk of vertebral and rib fractures through vitamin K deficiency. There is some controversy that there is warfarin-induced impairment of bone quality rather than quantity and also depends on the period of treatment, as one of the studies showed that there was no amplified osteoporotic fracture risk in patients who were using Warfarin for less than one year [19-21].

**Warfarin versus dabigatran**

An oral anticoagulant ethoxylate dabigatran is a pro-drug that is converted by serum esterase to more potent form, Dabigatran directly inhibits thrombin by preventing the conversion of fibrinogen into fibrin (Figure 2). Dabigatran has a half-life of 12 to 17 hours and bioavailability of 6.5%. Dabigatran does not need routine monitoring as compared to warfarin [22]. In a pilot trial that involved patients with atrial fibrillation, Dabigatran has been given for the prevention of venous thromboembolism, doses of 220 mg once daily, and 150 mg twice daily, were promising [23-24]. Another large randomized trial also compared the same treatments of dabigatran with warfarin in patients who were diagnosed with atrial fibrillation, and the result was very assuring. Lower rates of hemorrhage and comparable rates of systemic embolism and stroke were associated with the 110-mg dose of dabigatran when compared to Warfarin; A similar rate of major hemorrhage and lower rates of systemic embolism and stroke was associated with a 150-mg dose of dabigatran (Table 6).
FIGURE 2: Comparison of the mechanism of action between warfarin and Dabigatran

| Property                        | Warfarin                                                                 | Dabigatran                                              |
|---------------------------------|--------------------------------------------------------------------------|----------------------------------------------------------|
| Mechanism of action             | Reduced synthesis of prothrombin and other clotting factors              | Direct inhibition of thrombin                            |
| Onset of action Elimination half-life | 36-72 hours 20-60 hours 48-96 hours                                      | 0.5-2 hours 12-14 hours 24 hours                        |
| Duration of action              |                                                                          |                                                         |
| Indication for AF               | Valvular or non-valvular atrial fibrillation                             | Non-valvular atrial fibrillation                         |
| Dosing                          | Individualized to each patient and target INR                           | Fixed-dose, dependent on creatinine clearance and age    |
| Administration                  | Oral Once daily                                                          | Oral Twice Daily (for AF)                                |

TABLE 6: Comparison of basal characteristics between warfarin and dabigatran
AF, atrial fibrillation

As compared to warfarin, dabigatran is not vitamin K-dependent anticoagulant, which is approved to be used in patients with NVAF. In recent animal studies, it was reported that the application of dabigatran lowers bone turn over, increases bone volume leading to a low risk of osteoporotic fracture as compared to Warfarin. There was another in vivo study done recently, which indicated less trabecular separation, higher bone volume compared with warfarin use in
rats, but no similar study was conducted in humans. In this study, they compared the effect of warfarin and dabigatran on bone structure in rats without any renal impairment. Histomorphometry study was done, and the results indicated that there was increased Warfarin mediated osteoclastic activity leading to a significant decrease in bone volume and an increase in trabecular separation which determines alteration in bone quality and high risk of fractures. As compared to warfarin, dabigatran-treated group was safe as it lacks the increased osteoclastic activity and any structural impairment, being more reliable in this context. However, after chronic use of warfarin, which is associated with the amplified osteoclastic activity, a significant reduction in bone volume and increases in trabecular separation might establish a higher propensity to fractures and alterations in bone quality [25]. Therefore, the amplified risk of warfarin-induced fracture in patients is because of quality, not the quantity of bone, also based on the period of the use. Biologically, dabigatran is vitamin- k independent anticoagulant, other than that patients are not asked to reduce vitamin K in the diet; this could be another reason that Dabigatran is not causing osteoporosis. These studies have claimed that dabigatran is an excellent alternative to warfarin if we want to avoid osteoporotic fractures.

Conclusions

The patients needing anticoagulants such as warfarin are often at risk for osteoporosis. This happens because the patients on Warfarin are suggested to be on a vitamin K-free diet. However, vitamin K is necessary for bone mineralization. This review suggests that the anticoagulant Dabigatran is relatively safe in comparison to warfarin - as it is vitamin K independent - and does not require any dietary restrictions of vitamin K. However, the data currently present on this subject is not sufficient. From this article, the questions that arise are “if warfarin use induces osteoporosis, why don't all patients on warfarin suffer from osteoporosis?”, "what are the factors that protect warfarin users from developing osteoporosis?" and "what are the factors that limit the transition of warfarin to dabigatran in routine medical practice?". Therefore, we recommend more studies in the near future on this subject to find more treatment options for the patients already who have osteoporosis needing anti-coagulants.

Additional Information

Disclosures

Conflicts of interest: In compliance with the ICMJE uniform disclosure form, all authors declare the following: Payment/services info: All authors have declared that no financial support was received from any organization for the submitted work. Financial relationships: All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. Other relationships: All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

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