Available online at www.sciencedirect.com

ScienceDirect

Journal of the Chinese Medical Association xx (2018) 1−7

www.jcma-online.com

Original Article

Warfarin accelerated vascular calcification and worsened cardiac dysfunction in remnant kidney mice

Ming-Tsun Tsai [a,b], Ying-Ying Chen [b,c], Wei-Jeng Chang [d], Szu-Yuan Li [b,c,*]

[a] *Division of Nephrology, Department of Medicine, Taipei Veterans General Hospital Taoyuan Branch, Taoyuan, Taiwan, ROC*
[b] *National Yang-Ming University, School of Medicine, Taipei, Taiwan, ROC*
[c] *Division of Nephrology, Department of Medicine, Taipei Veterans General Hospital, Taipei, Taiwan, ROC*
[d] *National Laboratory Animal Center, National Applied Research Laboratories, Taipei, Taiwan, ROC*

Received June 23, 2017; accepted August 7, 2017

Abstract

Background: Vascular calcification is highly prevalent in end-stage renal disease (ESRD) and is a significant risk factor for future cardiovascular events and death. Warfarin use results in dysfunction of matrix Gla protein, an inhibitor of vascular calcification. However, the effect of warfarin on vascular calcification in patients with ESRD is still not well characterized. Thus we investigated whether arterial calcification can be accelerated by warfarin treatment both *in vitro* and *in vivo* using a mouse remnant kidney model.
Methods: Human aortic smooth muscle cells (HASMC) were cultured in medium supplemented with warfarin and phosphate to investigate the potential role of this drug in osteoblast transdifferentiation. For *in vivo* study, adult male C57BL/6 mice underwent 5/6 nephrectomy were treated with active vitamin D3 plus warfarin to determine the extent of vascular calcification and parameters of cardiovascular function.
Results: We found that the expressions of Runx2 and osteocalcin in HASMC were markedly enhanced in the culture medium containing warfarin and high phosphate concentration. Warfarin induced calcification of cultured HASMC in the presence of high phosphate levels, and this effect is inhibited by vitamin K2. Severe aortic calcification and reduced left ventricular ejection fractions were also noted in 5/6 nephrectomy mice treated with warfarin and active vitamin D3.
Conclusion: Warfarin treatment contributes to the accelerated vascular calcification in animal models of advanced chronic kidney disease. Clinicians should therefore be aware of the profound risk of warfarin use on vascular calcification and cardiac dysfunction in patients with ESRD and atrial fibrillation.
Copyright © 2018, the Chinese Medical Association. Published by Elsevier Taiwan LLC. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Keywords: Left ventricular dysfunction; Uremia; Vascular calcification; Warfarin

1. Introduction

Atrial fibrillation (Af) is the most common cardiac dysrhythmia and is associated with a significant increased risk of stroke as well as subsequent cardiovascular (CV) morbidity and mortality.[1,2] The prevalence of Af increases with age, reaching approximately 8% in those aged ≥80 years compared with.4%−1.0% in the general population.[3] In patients with end-stage renal disease (ESRD), Af is common in the post-dialysis period, with a prevalence ranging from about 10% to 20% depending on both the definition and age of the population studied.[4] For the general population, anticoagulation therapy can substantially reduce the risk of stroke in patients with Af and a CHA2DS2-VASc score of more than two.[5,6] However, there are few prospective clinical trials about the protective effect of warfarin in patients with ESRD, and the

Conflicts of interest: The authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.

* Corresponding author. Dr. Szu-Yuan Li, Division of Nephrology, Department of Medicine, Taipei Veterans General Hospital, 201, Section 2, Shi-Pai Road, Taipei 112, Taiwan, ROC.
E-mail address: syli@vghtpe.gov.tw (S.-Y. Li).

https://doi.org/10.1016/j.jcma.2017.08.021
1726-4901/Copyright © 2018, the Chinese Medical Association. Published by Elsevier Taiwan LLC. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Please cite this article in press as: Tsai M-T, et al., Warfarin accelerated vascular calcification and worsened cardiac dysfunction in remnant kidney mice, Journal of the Chinese Medical Association (2018), https://doi.org/10.1016/j.jcma.2017.08.021

benefit of warfarin for stroke prevention in hemodialysis (HD) patients with Af is still debated in most retrospective studies.[7−9] Warfarin may cause excessive bleeding and calcification in vascular structures that can be life-threatening and even lead to death in patients with advanced chronic kidney disease (CKD).[7] Accordingly, the Kidney Disease Improving Global Outcomes (KDIGO) guideline suggests that routine anticoagulation in ESRD patients with Af for the primary prevention of stroke is not indicated because of these complications and lack of valid evidence for stroke prevention.[10]

In addition, vascular calcification is highly prevalent in ESRD and is a significant risk factor for future CV events and death. Matrix Gla protein (MGP) is a vitamin K−dependent protein highly expressed in bone and arteries, where it acts as a local regulator of vascular calcification.[11] Warfarin use results in undercarboxylation of MGP and impairs its biological effects.[12] This effect on MGP may explain the more rapid progression of coronary calcification in patients taking warfarin.[13] However, the effect of warfarin on vascular calcification and CV outcomes in patients with ESRD is still not well characterized. Thus, we investigated whether arterial calcification can be accelerated by warfarin treatment both *in vitro* and *in vivo* using a mouse remnant kidney model.

2. Methods

2.1. Ethics statement

The experimental animal procedures were conducted in accordance with the *European Commission Directive 86/609/EEC for animal experiments* and were approved by the Institutional Animal Care and Use Committee of the study hospital.

2.2. Cell culture and induction of calcification

Human aortic vascular smooth muscle cells (HASMC) were obtained from Cambrex Bioscience (Wokingham, United Kingdom) and cultured in medium-199 (Sigma−Aldrich, St. Louis, MO) containing 15% fetal bovine serum (FBS), 2 mmol/L L-glutamine, 100 U/mL penicillin G, and 100 μg/mL streptomycin. Calcification of HASMC cultures was induced by the method of Wada et al.[14] Briefly, HASMC were cultured in normal growth medium for 4 days and then switched to medium containing vehicle control, high phosphate levels (final concentration 5 mM), warfarin (10 μM; Sigma−Aldrich, St. Louis, MO), or warfarin plus high phosphate levels for 10 days. The cell culture medium was replaced with fresh medium every other day. The antagonistic effect of vitamin K1 (phytomenadione, 5 μM; Sigma−Aldrich, St. Louis, MO) and vitamin K2 (menaquinone, 25 μM; Sigma−Aldrich, St. Louis, MO) on mineral deposition in HASMC induced by warfarin was also examined. The extent of transdifferentiation from HASMC into osteoblasts was evaluated using α-smooth muscle actin, osteocalcin, and Runx2 levels that were examined by immunoblotting as described below. Cell cultures were stained for mineral deposition using the von Kossa method as previously described.[14]

2.3. Western blot to detect osteocalcin and Runx2

Expression levels of both osteocalcin and Runx2 were evaluated using cell lysate that was electrophoresed in 12.5% SDS-PAGE. Western blotting was performed with a rabbit polyclonal anti-human α-smooth muscle actin antibody at a 1:400 dilution (Abcam, Cambridge, UK), rabbit polyclonal anti-human osteocalcin antibody at a 1:2000 dilution (Abcam, Cambridge, UK) or with rabbit anti-human Runx2 antibody (Invitrogen, Carlsbad, CA) at a 1:500 dilution followed by horseradish peroxidase−labeled anti-rabbit IgG antibody. Antigen-antibody complexes were visualized with the horseradish peroxidase chemiluminescence system (GE Healthcare, Buckinghamshire, UK).

2.4. Animals

Twelve-week-old male C57BL/6 mice were purchased from the National Laboratory Animal Center (Taipei, Taiwan) and allowed free access to standard rodent chow and water. Animals were housed in a temperature-controlled and light-controlled environment (23 °C, 50 ± 5% humidity and a 12-h light/dark cycle).

2.5. 5/6 Nephrectomy and study groups

The remnant kidney model was induced in animals following the two-step surgical procedure under Avertin anesthesia. Briefly, the left kidney was exposed, and the upper and lower poles were electrocauterized. Two weeks later, a right total nephrectomy was performed.

The C57BL/6 mouse strain is known to be resistant to the development of vascular calcification, despite uremic status induced by both the 5/6 nephrectomy and feeding with high dietary phosphate.[15] In experimental animal models, excessive dietary intake of active vitamin D3 may induce vascular calcification via complex, systemic feedback regulatory mechanisms that control calcium-phosphate metabolism.[16,17] Therefore, we evaluated the effects of warfarin on both arterial calcification and cardiovascular function in mice that underwent a 5/6 nephrectomy and were fed a diet containing high-dose active vitamin D3.

At 3 days post-5/6 nephrectomy, mice were divided into five groups of 10−12 mice per group: the sham-operated control group fed vehicle, the 5/6 nephrectomy group fed vehicle, the 5/6 nephrectomy group fed warfarin (3 mg/kg/day), the 5/6 nephrectomy group fed active vitamin D3 (1α,25-dihydroxyvitamin D3,.1 μg/kg/day; Sigma−Aldrich, St. Louis, MO), and the 5/6 nephrectomy group fed warfarin plus active D3. All mice were orally administered vitamin K1 (Sigma−Aldrich, St. Louis, MO) to prevent internal bleeding and were sacrificed 16 weeks after the 5/6 nephrectomy.

Please cite this article in press as: Tsai M-T, et al., Warfarin accelerated vascular calcification and worsened cardiac dysfunction in remnant kidney mice, Journal of the Chinese Medical Association (2018), https://doi.org/10.1016/j.jcma.2017.08.021

Download English Version:

https://daneshyari.com/en/article/8758940

Download Persian Version:

https://daneshyari.com/article/8758940

Daneshyari.com