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Small intestinal bacterial overgrowth and warfarin dose requirement variability

Vittorio Giuliano [a,*], Gabrio Bassotti [a], Evangelia Mourvaki [b], Danilo Castellani [a], Esmeralda Filippucci [b], Giuseppe Sabatino [a], Stefania Gizzi [a], Francesco Palmerini [b], Francesco Galli [c], Olivia Morelli [a], Monia Baldoni [a], Antonio Morelli [a], Alfonso Iorio [b]

[a] Gastrointestinal and Hepatology Section, Department of Clinical and Experimental Medicine, Italy
[b] Internal and Vascular Medicine Section, Department of Internal Medicine, Italy
[c] Department of Biochemistry Science and Molecular Biotechnologies, University of Perugia, Italy

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ABSTRACT

The dose of warfarin needed to obtain a therapeutic anticoagulation level varies widely among patients and can undergo abrupt changes for unknown reasons. Drug interactions and genetic factors may partially explain these differences. Intestinal flora produces vitamin K_2 (VK_2) and patients with small intestinal bacterial overgrowth (SIBO) rarely present reduced INR values due to insufficient dietary vitamin K. The present study was undertaken to investigate whether SIBO occurrence may affect warfarin dose requirements in anticoagulated patients. Based on their mean weekly dose of warfarin while on stable anticoagulation, 3 groups of 10 patients each were defined: *low dose* (LD, ≤17.5 mg/wk of warfarin); *high dose* (HD, from 35-70 mg/wk); and *very high dose* (VHD ≥70 mg/wk). Each patient underwent a lactulose breath test to diagnose SIBO. Plasma levels of warfarin and vitamin K-analogues were also assessed. Patients with an altered breath test were 50% in the VHD group, 10% in the HD group, and none in the LD group ($P = 0.01$). Predisposing factors to SIBO were more frequent in the VHD group, while warfarin interfering variables were not. VHD patients were younger and had a higher plasma vitamin K_1 (VK_1) concentration ($P > 0.05$). On the contrary, the plasma VK_2 levels tended to be lower. This pilot study suggests that SIBO may increase a patient's warfarin dose requirement by increasing dietary VK_1 absorption through the potentially damaged intestinal mucosa rather than increasing intestinal VK_2 biosynthesis. Larger studies are needed to confirm these preliminary data and to evaluate the effects of SIBO decontamination on warfarin dosage.

1. Introduction

Atrial fibrillation is considered the main risk factor for arterial thromboembolic disease and affects about 2.3 million adults in the United States; up to 79% of these patients are treated with vitamin K antagonists (VKAs) [1–3]. Meta-analyses of clinical trials indicate that in patients with atrial fibrillation, oral anticoagulation with dose-adjusted warfarin, the most commonly used coumarin derivative, reduces the risk of stroke by about 60% compared to placebo [4,5]. The same class of drugs is the mainstay of the long term treatment of patients with venous thromboembolic disease or heart prosthetic valves [6,7]. Due to wide inter- and intra-individual variation in dose requirements, VKAs require frequent dose tailoring. VKAs reduce the synthesis of functional vitamin K-dependent clotting factors (II, VII, IX, and X) and inhibitors (proteins C and S). Acquired conditions, such as concomitant administration of interacting drugs, other diseases and a variation in dietary intake of vitamin K can partly explain this variability [8]. Supplementation with Vitamin K of patients with unexplained variability in their response to warfarin significantly improved their response to treatment [9]. Additional factors, such as genetic polymorphisms for cytochrome CYP2C9 and VKORC1 (the target protein for coumarins), have also been shown to influence the VKA dose requirements [10]. Bleeding complications, with an incidence of total hemorrhagic events estimated at 7.6% per treatment year [11], can unexpectedly occur following abrupt variation of the anticoagulation level, even in patients with stable warfarin requirements and International Normalized Ratio (INR) values (the laboratory index used to monitor VKA therapy). All these issues make warfarin therapy cumbersome for the clinician [12].

A correlation between warfarin requirements and intestinal flora vitamin K production is inferred by different evidence from literature: the intestinal flora produces vitamin K_2 (menaquinones, a series of

Abbreviations: SIBO, small intestinal bacterial overgrowth; VK_2, vitamin K_2 or menaquinone; LD, low dose; HD, high dose; VHD, very high dose; VK_1, vitamin K_1 or phyloquinone; VKAs, vitamin K antagonists; INR, International Normalized Ratio; LBT, lactulose breath test; PPI, proton pump inhibitors; MKs, menaquinone-n; OR, odds ratio; CI, confidence interval; BMI, body mass index; SEM, standard error of the mean.

* Corresponding author. Gastrointestinal and Hepatology Section, Department of Clinical and Experimental Medicine, Ospedale S. Maria della Misericordia, S.Andrea delle Fratte, 06132 Perugia Italy.
E-mail address: vittorio.giuliano@unipg.it (V. Giuliano).

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vitamers with multi-isoprene units; [13,14]), INR is often increased in subjects receiving broad spectrum antimicrobials [15], there is an association between the use of antibiotics and the reduction in hepatic menaquinone concentration [16], and hemorrhagic symptoms in experimental germ-free rats (*gnotobiotic animals*) can be reverted by bacterial intestinal contamination from wild type rats [17]. Thus, conditions leading to pathologic modifications of intestinal flora in the small bowel could influence warfarin requirements in VKA-treated patients. Among these conditions, small intestine bacterial overgrowth (SIBO), a condition characterized by an abnormal increase in the number of colonic type bacterial flora in the small bowel [18], may represent a suitable experimental model in human beings for testing this hypothesis. SIBO can be easily and effectively assessed without invasive methods using the lactulose breath test (LBT; [19]).

This study was planned as a pilot investigation to assess whether patients requiring different doses of VKAs to obtain a therapeutic INR value may have a different prevalence of SIBO, hypothesizing that SIBO is a potential responsible factor for the increase in VKA dose requirements. Plasma concentrations of warfarin and vitamin K analogues (K_1 and K_2) were evaluated and correlations with SIBO-related clinical symptoms were studied.

2. Materials and methods

2.1. Study population

Patients were on chronic treatment with VKAs for atrial fibrillation, venous thromboembolic disease or prosthetic aortic valve and should have been assigned an INR range of 2 to 3. Three groups of patients were recruited based on the mean weekly dose of warfarin prescribed, as follows: *low dose* (LD, lower 25% of the mean weekly dose distribution), *high dose* (HD, from 75%-90%), and *very high dose* (VHD, above 90%). These three cohorts included patients with a stable mean weekly dose of warfarin during the last 6 months. Exclusion criteria were use of laxatives, antibiotics, or probiotics during the previous 2 months, or a previously performed LBT. The weight and height of each patient were recorded, and the subjects were asked to complete a questionnaire about the use of proton pump inhibitors (PPI) and other drugs, symptoms consistent with SIBO (bloating, flatulence, chronic diarrhoea, and abdominal pain), diseases known to be associated with SIBO (diabetes, scleroderma, chronic pancreatitis, Parkinson disease, hypothyroidism, small bowel diverticula, and Crohn disease), and surgical procedures predisposing to SIBO (gastric resection, vagotomy, right hemicolectomy, and total colectomy).

2.2. Lactulose breath test (LBT)

To minimize the basal excretion of hydrogen (H_2) and methane (CH_4), the patients were invited to eat a standardized diet the day before testing, and to refrain from smoking for at least 2 hours before testing. After 12 hours of fasting, patients performed a chlorexidine gargle and collected two basal end-alveolar expiratory air samples. After ingestion of 10 g of lactulose, breath samples were taken every 20 minutes for 4 hours using a two-bag system, as previously described [20].

Samples were evaluated for H_2 and CH_4 within 24 hours since collection, using a Microlyzer™ DP Quintron Gas Chromatograph (Quintron, Milwakee, WI, USA). Results were expressed as parts per million (ppm). An abnormal LBT was defined as a rise of > 20 ppm in H_2 or CH_4 within 90 minutes since lactulose ingestion or at least two consecutive peaks ≥ 12 ppm above the basal values.

2.3. Determination of warfarin plasma levels

Fasting blood samples were obtained by venous puncture using a 19-gauge butterfly needle and collected into tubes containing EDTA. After centrifugation, plasma samples were stored at -20 °C until testing. Warfarin was purified from acidified plasma samples, and added to oxybenzone (internal standard) by liquid-liquid extraction with diethyl ether as described by Ring and Bostick [21]. After centrifugation, the organic layer was evaporated to dryness under a stream of nitrogen at 40 °C and the residue was reconstituted in 200 µL of phosphate buffer (50 mM, pH = 7.4) methanol (50/50 [v/v]). Fifty µL were injected into the HPLC system (PU-1580; Jasco-Europe, Lecco, Italy). equipped with a UV-detector (UV 2075 Plus; Jasco-Europe) set at 308 nm. Separation was carried out on a reversed-phase C_{18} column (Waters Spherisorb ODS-2, 5 µm particle size, 4.6 × 150 mm internal diameter; Waters Corporation, Milford, MA, USA) coupled with a guard column packed with the same material (5 µm, 4.6 × 10 mm). The mobile phase consisted of a potassium phosphate buffer (25 mM, pH = 3 adjusted with 1 M HCl) and methanol in the proportion of 2/3 (v/v), and was pumped at a flow rate of 1 ml/min. All solvents were of HPLC grade and purchased from Carlo Erba Reagents (Milan, Italy). Quantification was achieved by direct comparison of peak area ratios (warfarin to oxybenzone) generated from the calibration standard to those generated by the sample. All reagents were purchased from Sigma-Aldrich (Milan, Italy).

2.4. Assessment of vitamin K analogues in plasma

Plasma levels of vitamin K_1 and K_2 (sum of menaquinone-n [MKs]) were measured using a modification of the procedure described by Davidson and Sadowski [22]. Briefly, 0.25 mL of plasma were mixed with a proper amount of internal standard ($K_{1(25)}$) and two volumes of ethanol to remove proteins. Extraction was carried out using n-hexane (1.5 mL) and deionized water (0.5 mL) to increase phase separation. The upper hexane layer was quantitatively transferred to a disposable glass tube following a 3 min thorough mixing and centrifugation (2500 rpm for 5 min). The extracted K-vitamers were further purified by a solid phase extraction. For this purpose, Bond Elut silica cartridges (500 mg; Varian, Palo Alto, CA, USA) were placed in a solid phase extraction manifold and washed with n-hexane (6 ml) prior to sampling. The extracts were then loaded and drawn through the cartridges under vacuum. After washing the solid phase extraction cartridges with n-hexane (6 ml) the K-vitamin fraction was eluted with 6 ml of diethyl ether/n-hexane (3.5/96.5 [v/v]), and then evaporated to dryness under a stream of nitrogen. The residue was reconstituted in a 100 µL mobile phase. Purified K_1 and MKs were separated and quantified by means of HPLC with an electrochemical detector (ESA Coulechem 5100 with a glassy carbon working electrode operated in the oxidation mode +0.6 V versus Ag/AgCl; ESA Biosciences, Chelmsford, MA, USA) after a post-column reduction with metallic zinc. The reactor (2.1 × 50 mm column bed packed with zinc metal dust, particle size <45 µm; Merck & Co. Inc, Whitehouse Station, NJ, USA) was connected in series between the analytical column (Beckman Ultrasphere ODS, 5 µm particle size, 4.6 × 250 mm; Beckman Coulter, Fullerton, CA, USA) and the detector. To remove oxygen from mobile phase, an oxygen-scrubber column (4 × 125 mm) packed with 10% platinum-on alumina was placed in front of the injector (Rheodyne 7725 with a loop of 100 µL; IDEX Health & Science, Wertheim-Mondfeld, Germany). Optimal separation was achieved by using an ethanol-methanol (1:1 [v/v]) mobile phase containing sodium perchlorate (0.025 M) with a flow rate of 0.6 ml/min. All solvents were of HPLC grade and purchased from Carlo Erba Reagents. Quantification was achieved by the internal standard method. MKs, K_1, and $K_{1(25)}$ were either purchased from Sigma Chemical Co. (Milan, Italy) or received as gifts from Hoffman-La Roche and Co. (Basel, Switzerland) and Eisai Co. (Tokyo, Japan).

2.5. Data analysis

The distribution of the mean weekly dose of warfarin was analysed through plotting and visual inspection. The Kolmogorov-