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Dysfunction of aorta is prevented by whey protein concentrate-80 in venous thrombosis-induced rats

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ABSTRACT

A reciprocal association between venous thromboembolism (VTE) and arterial pathologies has been proposed recently. Whey protein concentrate-80 (WPC-80) is known to have beneficial effects on health, especially on the cardiovascular system. Therefore, the potential role of WPC-80 in arterial disease prophylaxis was studied in the model of venous thrombosis in male Wistar rats obtaining WPC-80 (0.3 or 0.5 g/kg body weight) or vehicle for 7, 14 or 21 days. Functional studies of aorta showed that venous thrombosis caused worse aortic relaxation response to acetylcholine, which was prevented by WPC-80 supplementation. WPC-80 also attenuated phenylephrine-induced contraction (in a dose of 0.3 g/kg for 7, 14 and 21 days; and in a dose of 0.5 g/kg for 21 days) in rats with thrombosis. The potential mechanisms may mainly involve nitric oxide as well as prostanoids and potassium channels.

1. Introduction

Cardiovascular diseases (CVD) are a serious health problem worldwide and the leading cause of death. Vessel dysfunction (including endothelial dysfunction) plays an important role in their pathogenesis. In recent years, the mutual association between venous thromboembolism (VTE) and arterial pathologies has gained increasing interest (Kleinegris, Ten Cate-Hoek, & Ten Cate, 2012; Kottke-Marchant, 2010; Prandoni et al., 2015; Sørensen, Horvath-Puho, Pedersen, Baron, & Prandoni, 2007). Numerous studies and analyses posed the hypothesis that the shared point between venous and arterial pathologies could be the same risk factors (age, obesity, smoking, and diabetes mellitus) (Ageno, Becattini, Brighton, Selby, & Kamphuisen, 2008; Riva, Donadini, & Ageno, 2015),

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Abbreviations: ACh, acetylcholine; CVD, cardiovascular diseases; INDO, indomethacin; KCa, Calcium (Ca^{2+})-activated potassium channels; L-NAME, L-N^G-Nitroarginine methyl ester hydrochloride; Phe, phenylephrine; TEA, tetraethylammonium; VTE, venous thromboembolism; vWF, von Willebrand factor; WPC-80, whey protein concentrate-80

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inflammation (Libby, Ridker, & Maseri, 2002; Riva et al., 2015) or endothelial disruptions (Becattini, Vedovati, Ageno, Dentali, & Agnelli, 2010; Le Brocq, Leslie, Milliken, & Megson, 2008; Mazzoccoli et al., 2012; Migliacci et al., 2007; Prandoni, 2007b; Sørensen et al., 2007). This is consistent with the recurring claim that spontaneous VTE may enhance endothelial activation and inflammatory response, thus augment the risk of atherosclerotic complications (Lerman & Zeiher, 2005, reviewed in Prandoni, 2007a; Riva et al., 2015), and is predictive for cardiovascular events (Becattini et al., 2010; Migliacci et al., 2007).

In contemporary medicine, there is still a need for new drugs and preparations (including functional food) that may play a role in CVD prophylaxis. Limiting cardiovascular risk factors, oxidative stress and inflammation, and improving endothelial function became new therapeutic goals (De Caterina, D'Ugo, & Libby, 2016; Tousoulis et al., 2006).

Whey Protein Concentrate-80 (WP-80, which means that it contains 80% proteins in its composition) is a rich source of peptides and amino-acids obtained from milk, which are used in the production of functional food (Król, Litwińczuk, Zarajczyk, & Litwińczuk, 2008; Szwajkowska, Wolanciuk, Barłowska, Król, & Litwińczuk, 2011), in infant formula (Heine, Klein, & Reeds, 1991), in the prevention and treatment of malnutrition, and furthermore by athletes and body builders to increase muscle mass. Whey proteins exert a wide range of health-beneficial properties affecting the cardiovascular system. In the literature, numerous studies on its positive properties: antihypertensive (Car, Koprowicz, Tokajuk, & Tokajuk, 2014; Erdmann, Cheung, & Schroder, 2008; Fekete, Givens, & Lovegrove, 2013; FitzGerald & Meisel, 1999; FitzGerald, Murray, & Walsh, 2004; Kawase, Hashimoto, Hosoda, Morita, & Hosono, 2000; Pal & Ellis, 2010) limiting vascular disorders (Pal & Ellis, 2010) and improving endothelial function (Ballard et al., 2009) can be found. However, the effects of WPC-80 on arterial tone and endothelial function have not yet been defined, particularly in the venous thrombosis model. Therefore, it is reasonable to determine whether WPC-80 affects vascular reactivity.

The aims of the study were to investigate the effects of venous thrombosis on aortic function and the morphology of aortic rings isolated from healthy rats and rats with induced 1-hour venous thrombosis, and the potential influence of prior WPC-80 supplementation on the observed relation.

2. Material and methods

2.1. *WPC-80 composition*

The WPC-80 used in the study was a gift from Moniecka Spółdzielnia Mleczarska in Mońki, Poland. The WPC-80 was examined in the accredited laboratory SJ Hamilton Poland LTD (Gdynia, Poland) which holds certificates of good manufacturing practice. Particular protein content tests were performed by Rtech laboratory from Land O'Lakes Laboratories (St. Paul, MN, USA).

2.2. *Experimental model*

Animal experiments were approved by Animal Ethics Committee in Bialystok (Poland) and conducted strictly in accordance with the institutional and international regulations (Giles, 1987). Male Wistar-Crl:WI (Han) rats (6–7 weeks old, weighting 180–250 g) were housed in a conventional state 12 h light/12 h dark cycle and at a constant temperature 22 ± 2 °C, and provided with open access to standard rat chow and tap water. The rats were divided randomly into groups and obtained by intragastric gavage WPC-80 solution in a dose of 0.3 or 0.5 g/kg body weight (Hassan, Abdel-Aziem, & Abdel-Wahhab, 2012), or vehicle (0.9% NaCl solution) established according to the body mass for 7, 14 or 21 days. Then rats were fasted for 24 h prior to the sham operation or induction of venous thrombosis but free access to water was allowed. The animals were anaesthetized by an intraperitoneal injection of pentobarbitone sodium (45 mg/kg). In the half of rats from each group venous thrombosis was performed according to the protocol described by Reyers, de Gaethano, and Donati (1989) with modification by Chabielska, Mogielnicki, Kramkowski, and Buczko (2005) and Gromotowicz et al. (2011). After opening the abdomen by an incision along the linea alba, 2 cm length of vena cava inferior was exposed, carefully separated from the surrounding tissues and tightly ligated with a cotton thread below left renal vein. Afterwards, the abdomen was closed with sutures and reopened after 1 hour to collect tissue samples. In sham operated rats all procedures were performed despite vena ligation.

2.3. *Vessel preparation*

The aorta was removed carefully to prevent endothelium injury and placed into cold Krebs–Henseleit solution of the following composition (in mM) NaCl 118; KCl 4.8; $CaCl_2$ 2.5; $MgSO_4$ 1.2; $NaHCO_3$ 24; KH_2PO_4 1.2; glucose 11; EDTA 0.03 and pH 7.4.

The thoracic aorta rings (3–5 mm long) were carefully cleaned of adherent tissue and suspended on stainless-steel wires in 10 ml organ baths containing Krebs–Henseleit solution.

Muscle tension was recorded by a force displacement transducer (PIM 100RE, BIO-SYS-TECH, Białystok, Poland) and displayed on a computer.

All vessels were kept at 37 °C Krebs–Henseleit solution and were gassed with 95% O_2 and 5% CO_2 and were allowed to equilibrate for 60 min (resting tension ~14.7 mN). Afterwards, 120 mM KCl solution was given to achieve the maximal contractile response of isolated aorta rings. Contractile responses were expressed as a percentage of the response to 120 mM KCl (Kozłowska, Szymska, Schlicker, & Malinowska, 2003).

2.4. *Concentration–response curves*

Vessels were washed and the integrity of endothelium was assessed by pre-constricting rings with the α_1-adrenoceptor agonist, (–)-phenylephrine (0.03 μM) followed by relaxation induced by ACh (1 μM). The tissues were considered as endothelium-intact when the relaxation to ACh reached at least 80–90%. After washouts, main experiments were performed (in each individual preparation only one experimental curve was determined) (Kozłowska et al., 2003).

To test the vascular contractile function, endothelium-intact aorta was exposed to cumulative concentration of phenylephrine, 0.0001–30 μM (Kozłowska et al., 2003).

To examine the vasorelaxant response of ACh (0.001–30 μM) arteries were pre-constricted sub-maximally with