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Whey protein concentrate limits venous thrombosis in rats.

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Abstract:

To study the influence of whey protein concentrate (WPC-80) on the development of thrombosis, rats were supplemented with two doses of WPC-80 (0.3 or 0.5 g/kg) for 7, 14 or 21 days. Then, one hour venous thrombosis model was performed in a half of the animals. Coagulation parameters, platelet count, and thrombus weight were assessed. Thrombus weight was decreased in rats obtaining WPC-80 and that was significant only for 14 and 21-day-supplementation. There were slight differences between groups in coagulation parameters and platelet count but without evident direction. Further research is needed to clarify the observed effects.

Keywords: whey, milk proteins, thrombosis, venous thrombosis, antithrombotic
Introduction

Whey is a by-product generated mainly in the production of cheese and casein. Powder forms of whey are used widely in the food industry as well as high-protein food for infants, for convalescents, by athletes and especially by bodybuilders to increase muscle mass during exercise (Huffman 1996; Naclerio et al. 2016; Sauser et al. 2018). The tested whey protein concentrate (WPC-80) contains almost 80% protein (78.2 %), fiber (0.5 %), carbohydrates (7.9 %), fat (6.72 %) (McDonough et al. 1974; Tokajuk et al. 2016) and also amino acids, growth factors and cytokines. In the last few years, there were many studies indicating the increasing role of nutrients in the prophylaxis and treatment of human diseases (Baer et al. 2011; de Soussa et al. 2012; Fekete et al. 2013; FitzGerald et al. 2004; Madureira et al. 2007; McDonough et al. 1974; Pal and Radavelli-Bagatini 2013; Płusa 2009; Togawa et al. 2002; Tokajuk et al. 2016).

So far, it is known that the beneficial health properties of whey proteins include antidiabetic effects, blood pressure lowering, improving cardiovascular system function, antibacterial, antiviral, and other effects (Bounous 2000; Car et al. 2014; Fekete et al. 2016; Foltz et al. 2007; Ikeda et al. 1998; Król et al. 2008; Pal and Radavelli-Bagatini 2013; Wu et al. 1996 and 1998). Interestingly, peptides from whey have angiotensin convertase enzyme (ACE) inhibitory potential (FitzGerald et al. 2004; Foltz et al. 2007; summed up by Car at al. 2014). Due to the fact that well-known ACE inhibitors have antithrombotic potential (Chabielska et al. 2005; Gromotowicz et al. 2011), studying the effects of whey proteins on thrombosis development seems to be rational. When venous thromboembolism is still a severe life-threatening illness, it is wise to search for natural sources of bioactive peptides for the prevention or treatment of venous thromboembolic complications, especially when commonly used drugs, anticoagulants, have well-known side effects (e.g. the risk of bleeding), contraindications and limitations of their administration. Therefore, it is reasonable to examine its effect on coagulation in young healthy rats and in rats with induced venous thrombosis.
Materials and Methods

Whey proteins

WPC-80 was obtained as a gift from Moniecka Spółdzielnia Mleczarska, Mońki, Poland; its content was examined and published by Tokajuk et al. 2016.

Animals

Male Wistar-Crl:WI (Han) rats of laboratory strain (6-7 weeks old, weighing 180-250g, from the Center of Experimental Medicine, Medical University of Bialystok) were housed in standard laboratory conditions with free access to rat chow (ssniff R/M-H, Ssniff, Soest, Germany) and tap water. The experimental protocol was in compliance with Polish law and international guidelines and was approved by the Animal Ethics Committee of the Medical University of Bialystok. The rats were divided into groups receiving WPC-80 in a dose of 0.3 or 0.5 g per kg\(^{-1}\) of body weight in a 0.9% NaCl solution (Garg et al. 2018; Hassan et al. 2012; Pérez-Cano et al. 2008; Tokajuk et al. 2016) or only vehicle for 7, 14 or 21 days by intragastric gavage. Before the sham operation or venous thrombosis induction, the rats were fasted for 24 h, however free access to water was maintained. Anaesthesia was induced by an intraperitoneal pentobarbital sodium injection (45 mg per kg\(^{-1}\) of b. w.). The venous thrombosis model was performed in half of the rats from each group according to the protocol featured by Reyers (Reyers et al. 1989) with modification by Chabielska (Chabielska et al. 2005) and Gromotowicz (Gromotowicz et al. 2011). The abdomen was opened, and 2 cm of inferior vena cava was unveiled and tightly ligated with a cotton thread at the level of the left renal vein. Subsequently, the abdomen was sutured and reopened after 1 hour for thrombus and blood sample extraction. In sham animals, all procedures were performed except for vena ligation (Tokajuk et al. 2016).

Plasma preparation
Blood samples were obtained from the heart, added to 3.13% sodium citrate in a volume ratio of 9:1, and then centrifuged (20 minutes at 3500xg, 4°C). Plasma was deep-frozen at -80°C until the subsequent steps of the experiment.

**Thrombus weight and hemostatic parameters**

Number of platelets (PLT) was measured with hematological equipment ScilVet ABC Plus + (HORIBA ABX, France) using a volumetric impedance method. The thrombin time (TT), activated partial thromboplastin time (aPTT), prothrombin time (PT), international normalized ratio (INR) and fibrinogen levels were determined by routine laboratory test using the Coag Chrom 3003 coagulometer (Bio-ksel, Poland) and standard reagents (Bio-ksel, Poland). The thrombus weight was tested after 24 h of drying at room temperature.

**Statistical Analysis**

Data were showed as mean ± standard error of the mean (SEM). Lack of thrombus was presented as 0.0 mg. The statistical significance of the results was computed by the Mann-Whitney test. The level of significance was set at $P<0.05$ and $P<0.01$ as shown. Statistical analyses were carried out using GraphPad Prism 6.0 software (California, USA).

**Results**

The examined coagulation parameters in rats from the control groups were within normal limits. After WPC-80 supplementation, there was the tendency to prolonged aPTT, but in comparison the results did not prove significant. In animals that received WPC-80 0.3 g per kg for 21 days with and without induced thrombosis, PT and INR were somewhat decreased, remaining within the normal range, but the nature and significance of this observation is beyond the framework of the current study (Table 1). Moreover, the number of PLT was within the normal range in each group (Table 1). Interestingly, in all groups of animals that received WPC-80, the
thrombus formation was limited (Fig. 1). This observation was significant in rats receiving WPC-80 in both doses, for 14 and 21 days only; with stronger effect after 21-day-supplementation of WPC-80. Additionally, fibrinogen and thrombin time (TT) did not differ significantly between the groups.

Discussion

There are numerous studies showing that whey proteins lower blood pressure and improve endothelial function, suggesting their role in the prevention of cardiovascular system diseases (Ballard et al. 2009; Krissansen 2007; Seppo et al. 2003; Tokajuk et al. 2016). Our results show the promising potential of WPC-80 supplementation in limiting thrombosis development and that the effect was dependent on the duration of administration. The obtained results could result from the fact that whey proteins exert angiotensin convertase enzyme inhibitory effects. Going further along this trail, the renin-angiotensin-aldosterone system activation enhances the thrombotic potential via mechanisms dependent on platelets, oxidative stress, and endothelial function (Car et al. 2014; Chabielska et al. 2005; Gromotowicz et al. 2011). Our previous research showed prolongation of aPTT after WPC-80 supplementation in old hyperglycemic rats (Car et al. 2012). Nevertheless, there are reports presenting that WPC-80 in higher doses may cause liver and kidney overload (Żebrowska-Gamdzyk et al. 2018).

However, in our study INR and aPTT values seem to reflect normal levels of coagulation factors. Fibrinogen concentration and blood count parameters, including platelet number, were within the normal range. Additionally, WPC-80 supplementation improved endothelial function, which may help to limit thrombosis development (Tokajuk et al. 2016).

The obtained results are preliminary in their nature. In the above research, fibrinolysis was not examined due to the short duration of thrombosis in the model, which may be a limitation of the study. Moreover, assessment of platelet function may bring new light to explaining the WPC-80 action. Controversial data that components of WPC-80 (lactalbumin) promote blood
coagulation can be found in the literature (Chan et al. 1993). Therefore, the exact effect of WPC-80 on the coagulation system is still elusive and requires further thorough research including mechanisms of action.

Determining the potential clinical application of WPC-80 requires the selection of the optimal dose and duration of supplementation.

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The authors declare no conflict of interests.

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Bounous, G. 2000. Whey Protein Concentrate (WPC) and Glutathione Modulation in Cancer Treatment. Anticancer Res. 20(6C): 4785–4792.


Table 1. Coagulology parameters in healthy rats and in rats with induced thrombosis.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>Control</th>
<th>WPC-80 0.3 g·kg⁻¹</th>
<th>WPC-80 0.5 g·kg⁻¹</th>
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<tr>
<td></td>
<td>Durati...</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Model</td>
<td>7</td>
<td>14</td>
<td>21</td>
</tr>
<tr>
<td>aPTT [s]</td>
<td>w/t</td>
<td>25.7±3.6</td>
<td>22.38±1.63</td>
<td>25.7±3.6</td>
</tr>
<tr>
<td></td>
<td>t</td>
<td>30.01±1.72</td>
<td>28.73±2.04</td>
<td>26.97±2.14</td>
</tr>
<tr>
<td>PT [s]</td>
<td>w/t</td>
<td>23.94±3.46</td>
<td>20.84±1.94</td>
<td>23.94±3.46</td>
</tr>
<tr>
<td></td>
<td>t</td>
<td>20.05±1.65</td>
<td>19.16±1.57</td>
<td>18.76±0.84</td>
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<tr>
<td>INR</td>
<td>w/t</td>
<td>1.3±0.12</td>
<td>1.32±0.12</td>
<td>1.3±0.12</td>
</tr>
<tr>
<td></td>
<td>t</td>
<td>1.43±0.17</td>
<td>1.22±0.11</td>
<td>1.19±0.05</td>
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<tr>
<td>Fibrinogen [g/L]</td>
<td>w/t</td>
<td>2.36±0.15</td>
<td>2.44±0.26</td>
<td>2.36±0.15</td>
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<tr>
<td></td>
<td>t</td>
<td>2.19±0.2</td>
<td>2.04±0.26</td>
<td>2.26±0.14</td>
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<td>TT [s]</td>
<td>w/t</td>
<td>19.65±1.1</td>
<td>18.25±1.94</td>
<td>19.65±1.1</td>
</tr>
<tr>
<td></td>
<td>t</td>
<td>21.65±1.1</td>
<td>18.25±1.94</td>
<td>19.65±1.1</td>
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<tr>
<td></td>
<td>t</td>
<td>10.64 ± 3.42 (n=5)</td>
<td>14.76 ± 4.68 (n=7)</td>
<td>11.71 ± 3.0 (n=8)</td>
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<tr>
<td>w/t</td>
<td></td>
<td>806.7 ± 24.7 (n=7)</td>
<td>814.0 ± 23.1 (n=7)</td>
<td>806.7 ± 24.7 (n=5)</td>
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<tr>
<td>PLT [x10^3/mm^3]</td>
<td></td>
<td>753.2 ± 47.11 (n=5)</td>
<td>677.3 ± 34.5 (n=7)</td>
<td>744.4 ± 56.16 (n=8)</td>
</tr>
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</table>

* p<0.05 vs respective control group (e.g. WPC-80 0.3 g·kg⁻¹ for 7 days vs Control for 7 days)

** P<0.01 vs respective control group

Data are expressed as mean ± SEM, n – number of rats

aPTT, activated partial thromboplastin time; PT, prothrombin time; INR, international normalized ratio; t-with induced thrombosis; w/t, without induced thrombosis; TT, thrombin time
Caption:

Fig. 1 The weight of dry thrombus in particular groups of rats (n=5-10). *p<0.05 vs respective control group, **p<0.01 vs respective control group (e.g. WPC-80 0.3 g·kg⁻¹ for 7 days vs Control for 7 days)