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Effects of Saskatoon berry powder on monocyte adhesion to vascular wall of leptin receptor-deficient diabetic mice

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Abstract

Hypothesis:
Atherosclerotic cardiovascular complications are the leading cause of death in diabetic patients. Monocyte adhesion is an early event for atherogenesis. Previous studies demonstrated that dark-skin berries had cardiovascular protective effects. We hypothesize that Saskatoon berry (SB) powder may reduce monocyte adhesion in leptin receptor-deficient (db/db) diabetic mice.
Methods: Wild-type and db/db mice were fed with chow or supplemented with SB powder. Anthocyanins in SB powder were identified using mass spectrometry. Mouse monocytes were incubated with mouse aorta. Monocyte adhesion was counted under microscopy. Inflammatory or metabolic markers in blood or tissue were analyzed using immunological or biochemical methods.
Results: SB powder significantly reduced monocyte adhesion to aorta from diabetic db/db mice compared to regular chow. The increased monocyte adhesion to aorta was normalized in db/db mice treated with ≥5% of SB powder for 4 weeks. Increased contents of Nicotinamide adenine dinucleotide phosphate oxidase (NADPH) oxidase-4, heat shock factor-1, monocyte chemotactic protein (MCP)-1, intracellular adhesion molecule (ICAM)-1, P-selectin, tumor necrosis factor-α, plasminogen activator inhibitor (PAI)-1 and urokinase plasminogen activator in aorta or heart apex, elevated plasma PAI-1 and MCP-1 were detected in db/db mice on chow compared to wild-type mice on the same diet; 5% SB powder inhibited the increases of inflammatory, fibrinolytic or stress regulators in aorta or heart apex of db/db mice. Monocyte adhesion positively correlated with blood glucose, cholesterol, body weight, heart MCP-1, PAI-1 or ICAM-1.
Conclusion: The findings suggest that SB powder attenuated monocyte adhesion to aorta of db/db mice, which was potentially mediated through inhibiting the inflammatory, stress and/or fibrinolyic regulators.

Keywords: Saskatoon berry; db/db mice; Monocyte adhesion; Inflammatory mediators; PAI-1

1. Introduction

The prevalence of diabetes mellitus (DM) rapidly increased in most countries during last 30 years, and the trend is expected to continue. Nine out of ten diabetic patients are type 2 DM (T2DM) [1]. Atherosclerotic cardiovascular complications are the predominant cause of death in diabetic patients. Vascular inflammation plays a critical role in the etiology of atherosclerosis [2]. Monocyte adhesion on endothelium has been considered as an early event in vascular inflammation [3]. Previous studies demonstrated that monocyte adhesion may be regulated by cytokines, adhesion molecules, metabolic factors and oxidative stress [4,5]. Elevations of inflammatory mediators, including interleukins, tumor necrosis factor (TNF)-α, monocyte chemotactic protein (MCP)-1 and intracellular adhesion molecule (ICAM)-1, were detected in diabetic patients [6,7]. Hyperglycemia and hyperketonemia are associated with increased monocyte adhesion to endothelial cells (EC) [8,9]. Previous studies in our laboratory demonstrated that glycated low-density lipoprotein (LDL) may increase the production of reactive oxygen species, activate NADPH oxidase (NOX) and impair mitochondrial respiration in cultured EC or fibroblasts [10–12]. The effect of diabetes on monocyte adhesion and relevant regulators in animal models remains to be investigated.

Previous studies demonstrated that products of a number of dark-skin fruits had cardiovascular protective effects in animal models or in small trials of human study [13,14]. Anthocyanins are common flavonoids in the dark-skin phyto-products. A recent study found that purple sweet potato extracts and cyanidin inhibited TNF-α-induced monocyte adhesion and the production multiple cytokines or adhesion molecules in cultured EC [15]. Previous studies by our

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group demonstrated that cyanidin-3-glucoside or delphindin-3-glucoside inhibited glycated or oxidized LDL-induced oxidative stress and cell injury in vascular EC [16,17]. Saskatoon berries (SB) naturally grow or are planted in Canada prairie areas and Northern states of USA. Previous studies by Hosseinian and Beta demonstrated that SB powder had relatively higher contents of anthocyanins compared to blue berries, strawberries, raspberries, chokecherry or seabuckthorn [18]. The effect of SB on monocyte adhesion or vascular inflammation has not been documented.

The present study examined the effect of SB powder on the adhesion of monocytes to aorta and relevant inflammatory, stress and fibrinolytic regulators in leptin receptor-knockout (db/db) mice, a common rodent model of T2DM

2. Materials and methods

2.1. SB powder

Smoky SB was purchased from Prairie Lane Saskatoons (Portage, Manitoba). The berries were cleaned and frozen at −70°C and then lyophilized using a freeze-drier without an addition of supplement. Dried whole berries were powered and stored in vacuum-sealed bags at −20°C. All processes were conducted in sterile stainless steel containers.

2.2. Animals

Male db/db mice and C57BL/J counterparts (6 weeks of age) were obtained from the Jackson Laboratory (Bar Harbour, ME, USA). Mice were housed in an air-conditioned room and received regular chow for 1 week for stabilization. Animals were randomized into indicated groups (n = 5–8/group). Mice in control group received regular mouse chow, and animals in the SB treatment groups received chow supplemented with 0.2–20% (w/w) SB for up to 5 weeks. Body weights were measured at weekly basis. Heart, aortae, and blood were harvested from db/db and wild-type mice after euthanasia. The protocol of animal experiments was approved by the Animal Protocol Management and Review Committee in the University of Manitoba.

2.3. Monocyte adhesion to intima of aorta

Aortae were freshly harvested at the level of aortic arch to the level of abdominal aorta beyond renal arteries and submersed in ice-cold Hank's balance salt solution (HBSS). Perivascular adipose tissue was removed from freshly isolated aortae. The aortae were longitudinally opened in fresh ice-cold HBSS and fixed with 27-gauge needles on the bottom of 35-mm culture dish within 1 h after harvesting. Mouse WEHI-274.1 monocytes were labelled with 3 μg/mL of TRITC (Molecular Probes, Burlington, ON) for 15 min. Free dye was removed from cells through overlaid on fetal calf serum and a centrifugation at 400×*g* as previously described [19]. The abundance of labeled monocytes represents >95% of total cells assessed using flow cytometer. Fluorescently labelled or unlabeled monocytes (1×10^5) were added to each dish containing aorta. Mouse monocytes were incubated with aortic strips at room temperature for 30 min on a rotator mixer. Un-adhered monocytes were removed using 2 washes with HBSS. Adhered monocytes were fixed using 2% glutaraldehyde in HBSS. Five fields (2 mm in diameter/field) were observed for each aortic strip for monocyte counting under fluorescent microscopy using 10× magnification. One field was at center and 4 fields at each corner of aortic strip. The combined examining areas represent approximately 1/4 of total area (typically 10 mm×6 mm) of each aortic strip (Fig.1B). The averages of adhered monocytes to the fields in each aorta were used for data analysis.

2.4. Western blotting

Targeted proteins in protein extracts of heart apex and ascending aorta were analysed using Western blotting analysis as previously described [20]. Monoclonal or polyclonal antibodies against mouse PAI-1, urokinase plasminogen activator (uPA), heat shock factor-1 (HSF1), ICAM-1, P-selectin, TNF-α, MCP-1 or β-actin were obtained from Santa Cruz (Santa Cruz, CA, USA), Cedarlane (Burlington, ON) or Abcam (Cambridge, MA, USA). Corresponding secondary antibody conjugated with horse radish peroxidase (Santa Cruz) and enhanced chemiluminescence reagents (GE Healthcare, Buckinghamshire, UK) were applied to visualize targeted antigens on nitrocellulose membranes. The abundances of the antigens was assessed using Chemi-Doc system and Quantity One software (BioRad, Hercules, CA, USA), and normalized with the abundance of β-actin in corresponding samples.

2.5. Identification of anthocyanins in SB powder

Anthocyanins in SB powder were extracted and analyzed using Waters high performance liquid chromatography-mass spectrometry (HPLC-MS) as previously described [18]. Purified anthocyanins were loaded on a solid-phase extraction C18 cartridge of a Water HPLC system. Anthocyanins were eluted with acidified methanol and the solution was dried at ambient temperature under nitrogen gas. The residue was re-dissolved in HPLC mobile-phase solution for the determination of anthocyanins using Waters Quatro Micro API mass spectrometer (Waters Corp, Milford, MA, USA) equipped with an ESI Multi-Mode Ionization probe as described [18]. Standards of anthocyanins were obtained by Polyphenols Laboratories (Sandnes, Norway).

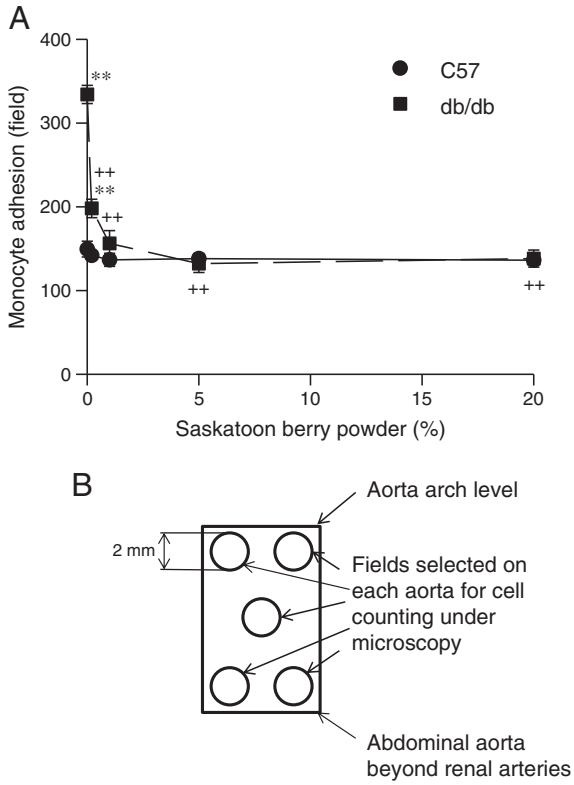

Fig. 1. Dose responses of Saskatoon berry (SB) powder on monocyte adhesion in leptin receptor-knockout (db/db). (A) C57 control mice and db/db mice (male, 7 weeks of age) were treated with 0, 0.2, 1.0, 5.0 and 20.0% of SB powder (w/w) for 5 weeks. Freshly isolate aorta were incubated with 1×10^5 of TRITC labelled WEHI.274.1 mouse monocytes for 30 min at room temperature at a rotating plate. Unattached monocytes were washed away from aorta by 2 washes. Monocytes remaining or the surface of aorta were fixed and counted under microscope with 10× magnification. Values were expressed in mean±SD (n=5 mice/group, averages of monocytes/field from 5 fields/aorta). (B) A scheme for the size and location of selected fields on aortic strip selected counting monocyte adhesion on aorta under microscopy. $^{**}P < .01$ versus wild-type mice on regular chow; $++P<.01$ versus db/db mice without SB powder treatment.

2.6. Analyses of variables in blood

The levels of glucose in mouse blood were measured using AlphaTRAK glucose monitoring system from Abbott. Serum cholesterol was measured using Sekisui Diagnostics SL reagents. Plasma PAI-1 and MCP-1 were measured using ELISA kits (Cedarlane for mouse PAI-1, Thermo Scientific for mouse MCP-1).

2.7. Statistical analysis

Continuous data were presented as means of values±S.D. Data from multiple groups were analyzed using the one-way variance assay followed with post-hoc tests using Microsoft Excel software. Data from two groups using Student's *t* test. Correlations between 2 groups on continuous data were analyzed using liner regression analysis using Sigma Window software. Differences at $P<.05$ were considered as significant.

3. Results

3.1. Anthocyanin composition of SB powder

SB powder contained 11.5% moisture. The total anthocyanins in freeze-dried powder of Smoky SB were 5011.0 mg/kg (dry weight