ORIGINAL ARTICLE

Effect of Cornelian cherries on brain nitric oxide synthase activity in obese Zucker rats

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Abstract

Due to their significant antioxidant capabilities, Cornelian cherries have a positive effect on cardiovascular and metabolic disorders, atherosclerosis and, most recently, neurodegenerative diseases. We aimed to study the effects of three varieties of Cornelian cherries on nitric oxide synthase (NOS) activity in brain cortex and cerebellum of obese rats. Male obese Zucker rats were divided into the control group and groups treated with Cornelian cherry varieties like Wild Type, Koralovij Marka, and Radost (5 g/kg/day, n = 6 in each group) for 6 weeks. Blood pressure and body weight were determined. NOS activity was measured by the formation of [3H]-L-citrulline from [3H]-L-arginine. None of the varieties was able to lower blood pressure. Koralovij Marka decreased body weight of obese Zucker rats. Only Wild Type increased NOS activity in both brain cortex and cerebellum. In conclusion, varieties of Cornelian cherries had different effect on brain NOS activity, which may be relevant in the selection of natural antioxidant substances against different neurodegenerative diseases.

Introduction

Brain tissue is characterized by high energy consumption and high level of polyunsaturated fatty acids, catecholamines, iron, and other factors that may generate an increased amount of reactive oxygen species (ROS). Thus, due to its high oxidative metabolic activity, the brain may be particularly vulnerable to oxidative stress. Indeed, ROS increase susceptibility to neuronal damage and functional decline in different neurodegenerative diseases like Alzheimer's disease (AD), Parkinson's disease (PD), cerebrovascular disorders, and others (Rao and Balachandran, 2002, Niedzielska *et al.* 2016). However, brain tissue has relatively low levels of antioxidants and low repair capacity (Niedzielska *et al.* 2016, Lee *et al.* 2020). Only a small part of the whole brain, which contains noradrenergic, dopaminergic, and serotonergic neurons, is characterized by higher antioxidant enzyme activity (Sun and Sun 2001).

The antioxidant system is indeed important for the rescue of neuronal cells from oxidative stress and for maintaining the physiological redox balance in the brain tissue by promoting antioxidant defenses to neutralize ROS. Currently, there is a growing interest in the development of new exogenous anti-

oxidant supplements, mainly on a natural basis, which may protect the central nervous system (CNS) from different disorders and provide supportive therapy (Pechanova 2022, Dayar and Pechanova, 2021). Many studies have shown that the antioxidant properties of natural products are mainly due to the polyphenolic substances they contain (Cebova *et al.* 2017, Pechanova *et al.* 2020a). Polyphenols belong to a group of powerful antioxidants that supplement and enhance the function of endogenous antioxidants and enzymes involved in defensive action against the increased oxidative load (Tsao 2010). Many polyphenols have been demonstrated to modify expression of various genes or antioxidant enzyme activities (Dayar *et al.* 2021, Tsao 2010). Moreover, different polyphenols have been shown to increase the activity/expression of endothelial (eNOS) or neuronal nitric oxide synthase (nNOS) with subsequent enhanced nitric oxide generation (Galleano *et al.* 2010, Dayar *et al.* 2021).

Recently, several studies have pointed to the beneficial effects of polyphenolic substances contained in Cornelian cherry (*Cornus mas* L.). Cornelian cherry has a high biological value, mainly associated with their antioxidant and anti-inflammatory activities which are attributed just to a rich polyphenolic composition (Kazimierski *et al.* 2019, Lietava *et al.* 2019) Cornelian cherry includes mainly anthocyanins, flavonoids, iridoids, phenolic acids, and tannins. Except for polyphenols, Cornelian cherries is famous for being a rich source of ascorbic acid, and essential minerals like potassium and magnesium (Gąstoł *et al.* 2013, Lietava *et al.* 2019).

Our recent studies have documented increased NOS activity in the aorta of obese Zucker rats after the treatment with Wild Type or Koralovij Marka variety of Cornelian cherry (Dayar *et al.* 2020). Similarly, treatment of rabbits on high-cholesterol diet with loganic acid or anthocyanins extracted from Cornelian cherry led to increased mRNA expression of eNOS in thoracic aortas. (Sozański *et al.* 2019). Moreover, Francik *et al.* (2014) demonstrated that addition of freeze-dried fruit of Cornelian cherry to fructose or high-fat diet increased activity of both catalase and paraoxonase in the brain of male Wistar rats.

Thus, the aim of our study was to investigate the effects of three varieties of Cornelian cherry, namely Wild Type (WT), Koralovij Marka (KM) and Radost on nitric oxide synthase (NOS) activity in the brain cortex and cerebellum of obese Zucker rats.

Materials and methods

Chemicals and Cornelian Cherry Preparation

Most of the chemicals were obtained from Sigma-Aldrich (Saint-Louis, MO, USA); if not, the company is indicated.

Fruits of Koralovij Marka and Radost were provided by the National Botanical Garden in Kiev, Ukraine while Wild Type originating in the White Carpathians, Slovakia. All fruits of Cornelian cherry were stored and dried under the same conditions. The fresh fruit was mixed with the standard feed and the addition of water so that the mixture was mouldable into the desired cuboid form 9 cm^3. The blocks were dried for 6 h at 50 °C to 90% dry weight on a tray dryer and used for feeding the rats.

Animals and Treatment

All procedures and experimental protocols were approved by an Ethical committee of the Institute of Normal and Pathological Physiology Slovak Academy of Sciences (Ro-3601/17-221/3) according to the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purpose, Directive 2010/63/EU of the European Parliament.

Obese Zucker rats were obtained from Charles River, USA and housed in groups of 2 animals, under a 12 h light- 12 h dark cycle, at a constant humidity (45–65%) and temperature (20–22 °C). Twelve-week-old male Zucker rats were divided into the control group and groups treated with Wild Type, Koralovij Marka, or Radost. Each group consisted of 6 animals. Control group was fed with a standard diet ad libitum, Cornelian cherry groups were fed with special diet which contained dry fruit (5 g/kg/day) and mixed with standard diet (30 g/day). The treatment lasted for 6 weeks. Daily water consumption was estimated individually for every animal and adjusted, if necessary. Body weight and blood pressure were monitored weekly. Blood pressure was measured noninvasively, using tail-cuff plethysmography weekly. At the end of the treatment, the animals were sacrificed, and samples of the brain cortex and cerebellum were used to determine NOS activity.

Total NOS Activity Determination

Total NOS activity was determined in crude homogenates of the brain cortex and cerebellum by measuring the formation of [3H]-L-citrulline from [3H]-L-arginine as previously described by Jendekova *et al.* (2006) with minor modifications (Paulis *et al.* 2009). Briefly, 50 µl of crude homogenate of the brain part (7.5 mg of wet tissue) was incubated in the presence of 50 mmol/l Tris/HCl, pH 7.4, containing 1 µmol/l [3H]-L-arginine (specific activity 5 GBq/mmol, approx. 100000 d.p.m.), 0.5 mg/ml calmodulin, 0.5 mmol/l β-NADPH, 250 µmol/l tetrahydrobiopterin, 4 µmol/l FAD, 4 µmol/l flavin mononucleotide and 1 mmol/l Ca 2+, in a total volume of 100 µl. After a 30-min incubation at 37 °C, the reaction was stopped (by adding 0.02 M Hepes containing 2 mM EDTA, 2 mM EGTA and 1 mM [3H]-L-citrulline), the samples were centrifuged, and supernatants were applied to 1-ml Dowex 50WX-8 columns (Na + form). [3H]-L-citrulline was eluted with 2 ml of water and radioactivity was

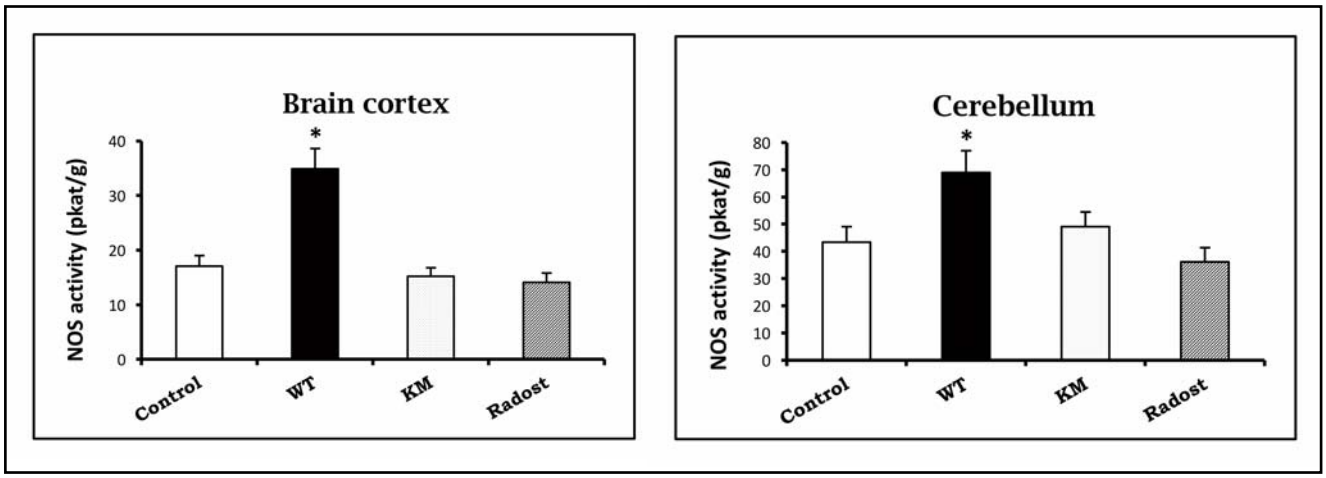

Fig. 1. Effect of Wilde Type (WT), Koralovij Marka (KM), and Radost on total nitric oxide synthase (NOS) activity in the brain cortex and cerebellum of obese Zucker rats. Data are means ± SEM from 6 animals in each group. * $p < 0.01$ compared to the control group.

determined by liquid scintillation counting. Total NO synthase activity was expressed as pkat/g of proteins.

Statistical Analysis

The results are expressed as mean ± S.E.M. One-way analysis of variance and Bonferroni test were used for statistical analysis. Values were considered to differ significantly if the probability value was less than 0.05.

Results

Body Weight and Blood Pressure

Body weight of control obese Zucker rats was 698.5 ± 20.4 g. Neither WT nor Radost were able to significantly reduce body weight (664 ± 10.4 and 657 ± 22.8, respectively). Only KM decreased body weight of obese Zucker rats significantly (611.5 ± 15.0).

Blood pressure of control obese Zucker rats was 147 ± 2.5 mmHg at the end of experiment. None of the varieties was able to lower blood pressure significantly (WT: 137 ± 3.9 mmHg, KM: 143 ± 5.4 mmHg, Radost: 131 ± 8.9 mmHg).

Total NOS Activity

Total NOS activity of control obese Zucker rats in the brain cortex was 17.1 ± 1.9 pkat/g protein and in the cerebellum 43.4 ± 5.6 pkat/g protein. Neither KM nor Radost were able to significantly increase total NOS activity in the brain cortex or cerebellum. Only WT increased total NOS activity significantly in both brain cortex and cerebellum of obese Zucker rats (Fig. 1).

Discussion

There is still increasing evidence that NO is an important mediator and alterations in the NO signaling pathway may be involved in a wide range of neurological diseases (Džoljić *et al.* 2015). Within the nervous system NO plays an important role in neurotransmission, endothelium-dependent vasodilatation, and in host-defense mechanisms (Džoljić *et al.* 2015, Pechanova *et al.* 2020a). In addition to their antioxidant activity, several polyphenols found in different natural substances have the ability to increase endothelial or neuronal NOS activity and to increase NO production in CNS during different pathophysiological conditions (Vauzour 2012, Serreli and Deiana, 2023).

In our experimental study we have demonstrated that Cornelian cherry may belong among such natural substances. Three Cornelian cherry varieties like Wild Type, Koralovij Marka, and Radost have been studied. However, only Wild Type increased NOS activity in both brain cortex and cerebellum of obese Zucker rats. Fruits of Koralovij Marka and Radost were provided by the National Botanical Garden in Kiev, Ukraine while Wild Type originating in the White Carpathians, Slovakia. The study that monitored the levels of total polyphenols and anthocyanins in individual varieties showed that Wilde Type contained the most polyphenols (408.4 ± 49.0 mg/100 g) and anthocyanins (50.3 ± 6.0 mg/100 g) compared to Koralovij Marka (151.2 ± 18.1 and 41.9 ± 5.0 mg/100 g, respectively) and Radost (154.9 ± 18.5 and 18.6 ± 3.2 mg/100 g, respectively) (Panghyová *et al.* 2018). A sufficient amount of polyphenolic substances, especially anthocyanins, could be responsible for increasing the total activity of NOS. Wilde Type, but also Koralovij Marka increased NOS activity in the aorta of obese Zucker rats as well (Dayar *et al.* 2020). Different mechanisms, however, have been implicated in this increase. Koralovij Marka increased eNOS expression and did not affect ROS production, while Wilde Type increased superoxide dismutase and decreased NADPH oxidase without affecting eNOS expressions (Dayar *et al.* 2020). Thus, we hypothesised that decreased oxidative stress after WT treatment may stabilised eNOS and/or nNOS dimers and may be responsible for increased NOS activity in the brain cortex and cerebellum. Of course, further studies, espe-

cially monitoring the activity/expression of antioxidant enzymes, eNOS and nNOS, are necessary to prove this hypothesis.

Several studies also pointed to the fact, that increased blood pressure in genetic hypertension is usually caused by high activity of sympathetic nervous system which is enhanced by central angiotensin II but lowered by central nitric oxide (Hojná *et al.* 2007). In our experimental study none of the varieties was able to lower blood pressure significantly, not even Wilde Type, which significantly increased NOS activity in both studied brain regions. This may be related to the fact that obese Zucker rats are only mildly hypertensive and their sympathetic nervous system may not be overactivated. Indeed, results of Levin *et al.* (1983) suggest an organ-specific decrease of norepinephrine synthesis in the obese Zucker rat, possibly due to decreased dopamine beta-hydroxylase activity. Wilde Type was not able to reduce body weight either. Only Koralovij Marka decreased body weight of obese Zucker rats significantly. It may be related to a different content of polyphenolic substances than Wilde Type or Radost. Different varieties can also contain different proportions of sugar, ascorbic acid and other substances that can affect body weight.

In conclusion, we have demonstrated that varieties of Cornelian cherries that have been studied had different effect on brain NOS activity. This fact may be relevant in the selection of natural antioxidant substances against different neurodegenerative diseases.

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