Chemico-Biological Interactions xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Chemico-Biological Interactions

journal homepage: www.elsevier.com/locate/chembioint

Protective effect of *Aronia melanocarpa* polyphenols against cadmium-induced disorders in bone metabolism: A study in a rat model of lifetime human exposure to this heavy metal

Malgorzata M. Brzóska [a,*], Joanna Rogalska [a], Malgorzata Galazyn-Sidorczuk [a], Maria Jurczuk [a], Alicja Roszczenko [a], Michal Tomczyk [b]

[a] *Department of Toxicology, Medical University of Bialystok, Adama Mickiewicza 2C street, 15-222 Bialystok, Poland*
[b] *Department of Pharmacognosy, Medical University of Bialystok, Adama Mickiewicza 2A street, 15-230 Bialystok, Poland*

A R T I C L E   I N F O

Article history:
Received 8 August 2014
Received in revised form 28 December 2014
Accepted 26 January 2015
Available online xxxx

Keywords:
Cadmium
Polyphenols
Aronia melanocarpa
Bone metabolism
Protection
Rat

A B S T R A C T

It was investigated, in a female rat model of low and moderate lifetime human exposure to cadmium (Cd), whether polyphenols from *Aronia melanocarpa* berries (chokeberry; AMP) may offer protection from this heavy metal-induced disorders in bone metabolism. For this purpose, numerous indices of bone formation (osteocalcin, alkaline phosphatase, osteoprotegerin) and resorption (carboxy-terminal cross-linking telopeptides of type I collagen, soluble receptor activator of nuclear factor-κB ligand) in the serum and/or distal femur epiphysis (trabecular bone region), as well as bone mineral status (volumetric bone mineral density of the femur and content of mineral components, including calcium, in the bone tissue at the distal femur epiphysis) were evaluated in female Wistar rats that received a 0.1% aqueous extract of AMP, as the only drinking fluid (prepared from lyophilized extract by Adamed Consumer Healthcare), and/or Cd in diet (1 and 5 mg/kg) for 3, 10, 17, and 24 months. Examination of the phytochemical profile of the aronia extract revealed high content of polyphenols (612.40 ± 3.33 mg/g), including anthocyanins, proanthocyanidins, phenolic acids, and flavonoids. Among detected compounds anthocyanins were identified as dominating. The exposure to Cd, dose- and duration-dependently, enhanced resorption and inhibited formation of the bone tissue resulting in its decreased mineralization. The administration of AMP under the exposure to 1 and 5 mg Cd/kg diet provided important protection from this heavy metal-induced disturbances in the bone turnover and changes in the bone mineral status, and the beneficial impact of polyphenols resulted from their independent action and interaction with Cd. These findings suggest that consumption of *Aronia melanocarpa* polyphenols may play a role in prevention against female skeleton damage due to chronic exposure to Cd and that chokeberry represents the good natural plant candidate for further investigations of its prophylactic use under environmental exposure to this heavy metal.

1. Introduction

Recently, due to the common exposure of the general population to numerous xenobiotics, including toxic heavy metals, more and more attention has been focused on the possibility of using of natural plant ingredients in protection against the unfavorable health effects of their action [1–3]. A promising group of such compounds seem to be polyphenols being the most abundant bioactive compounds in plants. Some experimental data indicates that polyphenolic compounds may play a role in protection from the toxic action of heavy metals, including cadmium (Cd), but the data are sparse [2,4–6].

Abbreviations: AAS, atomic absorption spectrometry; ALP, alkaline phosphatase; AMP, *Aronia melanocarpa* polyphenols; AW, bone ash weight (mineral components weight); b-ALP, alkaline phosphatase in the bone tissue; BMD, bone mineral density; Ca, calcium; Ca/AW, the ratio of calcium content in bone and the bone ash weight; Ca/DW, the ratio of calcium content in bone and the bone dry weight; CA, chlorogenic acid; Cd, cadmium; CTX, carboxy-terminal cross-linking telopeptides of type I collagen; Cy-3-ara, cyanidin 3-O-α-arabinoside; Cy-3-gal, cyanidin 3-O-β-galactoside; Cy-3-glu, cyanidin 3-O-β-glucoside; DW, bone dry weight; HNO_3, nitric acid; OC, osteocalcin; OPG, osteoprotegerin; RANK, receptor activator of nuclear factor-κB; RANKL, receptor activator of nuclear factor-κB ligand; s-ALP, alkaline phosphatase in the serum; sRANKL, soluble receptor activator of nuclear factor-κB ligand; sRANKL/OPG, the ratio of sRANKL and OPG; % mineral comp., percentage content of mineral components in the bone tissue; UPLC, Ultra Performance Liquid Chromatography.

* Corresponding author. Tel.: +48 85 7485604; fax: +48 85 7485834.
E-mail address: malgorzata.brzoska@umb.edu.pl (M.M. Brzóska).

http://dx.doi.org/10.1016/j.cbi.2015.01.031
0009-2797/© 2015 Published by Elsevier Ireland Ltd.

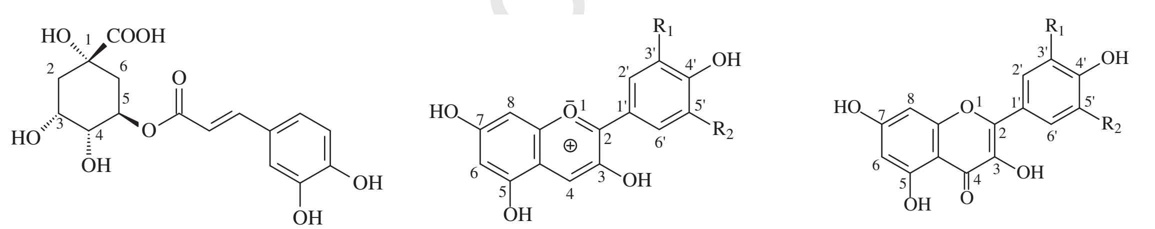
Cd belongs to the group of xenobiotics to which humans are exposed throughout their lifetime [7,8]. Numerous data show that under chronic exposure to this heavy metal, bone (besides the kidneys) is a target organ for its toxicity [9–16]. A growing body of epidemiological studies provides evidence that even relatively low chronic exposure to Cd, nowadays taking place in industrialized countries, may contribute to the development of osteoporosis and bone fractures [14–16]. This, together with the forecast that the general population's exposure to this metal will increase [7,8], makes the search for effective ways of protection from the unfavorable effects of its action, including skeleton damage, necessary.

Polyphenols occur naturally in plant-derived food, and are present in commonly consumed fruits, vegetables, grains, herbs, and drinks such as tea (especially green and white tea), fruit and/or vegetable juices, and wine [17]. These compounds possess numerous biological activities, including mainly antioxidative, anti-carcinogenic, anti-inflammatory, antiviral, and anti-hypertensive action [17,18]. Polyphenolic compounds are also known to have osteo-protective action in human and experimental animals; however, most of the data refer to green tea and soybeans polyphenols [19–21].

Some literature data [4–6] and our recent findings [22,23] suggest that polyphenols possess the potential to protect against Cd accumulation in the body and its toxicity. Due to the presence of hydroxyl groups (—OH groups), these compounds are capable of chelating ions of metals, including Cd^{2+} ions (Fig. 1) [24–26]. Choi et al. [4] reported that catechin (present in great amounts in green tea) was capable of providing protection from bone damage due to relatively high Cd exposure, while Paik et al. [6] revealed the beneficial impact of genistein (isoflavone occurring in soybeans) on the skeleton of Cd-exposed ovariectomized rats. Recently, using a rat model of low and moderate lifetime human exposure to this metal (1 and 5 mg Cd/kg diet, respectively, for up to 24 months), we revealed that polyphenols from the berries of *Aronia melanocarpa* (chokeberry, [Michx.] Elliott, Rosaceae; AMP) may decrease gastrointestinal absorption and body burden of this xenobiotic, including its accumulation in the liver, kidney and bone tissue (Table 1) ([22,23], submitted for publication).

Nowadays, consumption of aronia berries is widely recommended as one of the most abundant sources of polyphenols [27–29]. The polyphenolic profile of *Aronia melanocarpa* berries is well-known and widely reported [27–30]. Anthocyanins (mainly cyanidin 3-O-α-arabinoside – Cy-3-ara, cyanidin 3-O-β-galactoside – Cy-3-gal, cyanidin 3-O-β-glucoside – Cy-3-glu, and cyanidin-3-xyloside, and relatively low amounts of pelargonidin-3-galactoside and pelargonidin-3-arabinoside), proanthocyanidins (oligomeric and polymeric catechins), flavonols (quercetin, kaempferol and several quercetin mono- and di-glycosides: quercetin-3-galactoside, quercetin-3-glucoside, quercetin-3-rutinoside, quercetin-3-vicianoside, and quercetin-3-robinobioside), and hydroxycinnamic acids (chlorogenic acid – CA and neochlorogenic acid) are present in the extract.

To our knowledge, the impact of AMP on bone metabolism has not been investigated so far; however, some subclasses of polyphenolic compounds present in chokeberries (anthocyanins, phenolic acids, flavonols) have been reported to have a favorable impact on bone metabolism [21,31,32]. Taking the above into account, including our own finding of decreased Cd concentration in the bone tissue due to AMP administration under moderate exposure to this metal (Table 1), we have hypothesized that consumption of these compounds under exposure to Cd may, at least partly, provide protection from its damaging impact on the skeleton. The present paper is the first report from our comprehensive study on this subject, and it was aimed at investigating whether AMP consumption under low and moderate chronic exposure to Cd may protect from disorders in bone metabolism. For this purpose, the influence of AMP on the bone turnover and bone mineral status



***trans*-Chlorogenic acid** **R_1 = OH, R_2 = H: Cyanidin** **R_1 = OH, R_2 = H: Quercetin**

Fig. 1. Chemical structures of the most common polyphenols present in the berries of *Aronia melanocarpa*.

Table 1
Effect of polyphenolic compounds from the berries of *Aronia melanocarpa* (AMP) on cadmium (Cd) concentration in the bone tissue at the distal femur epiphysis of rats exposed to this metal.

Experiment duration (months)	Exposure to 1 mg Cd/kg diet			Exposure to 5 mg Cd/kg diet		
	Cd concentration in rats exposed to this metal alone (µg/g)	Cd + AMP administration		Cd concentration in rats exposed to this metal alone (µg/g)	Cd + AMP administration	
		Effect of Cd + AMP	Effect of AMP		Effect of Cd + AMP	Effect of AMP
3	0.043 ± 0.004[1]	↑ 1.7-fold	↔	0.062 ± 0.007	↑ 2.1-fold	↔
10	0.039 ± 0.003	↑ 2.0-fold	↔	0.078 ± 0.005	↑ 2.9-fold	↘ 12%
17	0.038 ± 0.003	↑ 2.3-fold	↔	0.117 ± 0.005	↑ 6.0-fold	↘ 12%
24	0.049 ± 0.004	↑ 2.3-fold	↔	0.127 ± 0.010	↑ 4.5-fold	↘ 25%

In this table only Cd concentration in the bone of the rats treated with this metal alone (mean ± SE for 8 rats, except for 7 animals in the animals exposed to 1 and 5 mg Cd/kg diet alone for 24 months) and statistically significant ($p < 0.05$) changes: a factor of increase (↑) compared to the control group or percentage decrease (↘) compared to the respective group that received Cd alone are indicated. ↔ and ↔ without statistically significant ($p > 0.05$) change compared to the control (0.028 ± 0.002, 0.023 ± 0.001, 0.018 ± 0.002, and 0.021 ± 0.003 µg/g after 3, 10, 17, and 24 months, respectively) and respective group treated with Cd alone, respectively.

Please cite this article in press as: M.M. Brzóska et al., Protective effect of *Aronia melanocarpa* polyphenols against cadmium-induced disorders in bone metabolism: A study in a rat model of lifetime human exposure to this heavy metal, Chemico-Biological Interactions (2015), http://dx.doi.org/10.1016/j.cbi.2015.01.031