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Comparative assessment of distribution of blackcurrant anthocyanins in rabbit and rat ocular tissues

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Abstract

Anthocyanins (ACs) are phenolic compounds that are distributed widely in fruits and vegetables. Although consumption of these compounds has been shown to improve visual function, the distribution of ACs in ocular tissue has not been examined in detail. The aim of this study was therefore to evaluate the ocular distribution of blackcurrant anthocyanins (BCAs) in rats and rabbits after oral, intravenous (i.v.) and intraperitoneal (i.p.) administration. Identification and quantification of ACs were carried out using high-performance liquid chromatography-electrospray ionization mass spectrometry (HPLC-ESI-MS) and high-performance liquid chromatography (HPLC) with UV-visible detection, respectively. BCAs were identified in the plasma and whole eye after oral and i.p. administration in rats. No other peaks were detected in either plasma or ocular tissues after administration when the absorbance of the eluate was monitored at 520 nm. This finding indicates that intact forms of ACs were present in rats after administration of BCA. In rats given i.p. administration, the concentration of total ACs in the whole eye and some ocular tissues was higher than that measured in plasma. These results suggested that ACs detected in the ocular tissues were not due to residual blood. Following i.v. administration in rabbits, four ACs were identified in the plasma and several ocular tissues including the aqueous humor, cornea, sclera, choroid, ciliary body, iris and retina. A small amount of ACs was also detected in the vitreous and lens. In conclusion, this study demonstrated that BCAs were absorbed and distributed in ocular tissues as intact forms. Our data show clearly that intact forms of BCAs pass thorough the blood–aqueous barrier and blood–retinal barrier in both rats and rabbits.

Keywords: blackcurrant; anthocyanin; ocular distribution; ciliary body; sclera; retina

1. Introduction

Anthocyanins (ACs) are a group of naturally occurring phenolic compounds responsible for the color of many flowers, fruits (particularly berries) and vegetables. Due to their widespread distribution and occurrence in fruits and vegetables, the daily intake of ACs in humans in the United States has been estimated to be 200 mg/day (Kühnau, 1976). Dietary ACs have attracted considerable interest due to their health-promoting benefits, such as reducing the risk of coronary heart disease and preventing several chronic diseases (Renaud and de Lorgeril, 1992).

Blackcurrant (*Ribes nigrum* L.) berries and juice are rich in ACs and are consumed in many countries of the world. The composition of blackcurrant anthocyanins (BCAs) is summarized in Fig. 1 and consists of four AC components. The typical AC profile in blackcurrant fruits is 47% of delphinidin-3-rutinoside (D3R), 13% of delphinidin-3-glucoside (D3G), 35% of cyanidin-3-rutinoside (C3R) and 5% of cyanidin-3-glucoside (C3G). These four ACs have been isolated and purified (Matsumoto et al., 2001a) and we have demonstrated in both humans and rats that they are absorbed through the gastrointestinal tract and are detectable in blood as unmetabolized forms (Matsumoto et al., 2001b). The bioavailability and metabolism

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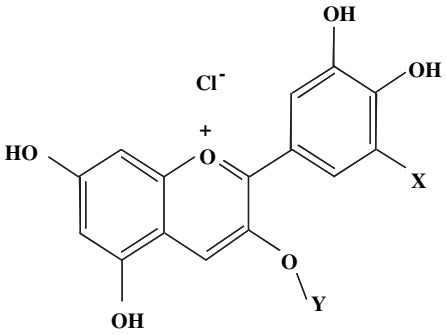

Fig. 1. Structure of the four anthocyanins in blackcurrants. Delphinidin-3-rutinoside (D3R; X = OH, Y = glucose-rhamnose), delphinidin-3-glucoside (D3G; X = OH, Y = glucose), cyanidin-3-rutinoside (C3R; X = H, Y = glucose-rhamnose) and cyanidin-3-glucoside (C3G; X = H, Y = glucose).

of ACs has been reported in several studies and although these mainly investigated cyanidin-glycosides, ACs were shown to be absorbed only as intact forms in human plasma. Neither cyanidin aglycone, conjugated form nor methylated anthocyanins were detected (Miyazawa et al., 1999; Felgines et al., 2003; Morazzoni et al., 1991; Mulleder et al., 2002). Recently, several metabolites were reported to form after oral ingestion of anthocyanins. These metabolites include a glucuronyl conjugate from C3G and both glucuronyl conjugate (Wu et al., 2002) and sulfate conjugate from pelargonidin (Felgines et al., 2003). However, the intake of D3G, D3R or C3R did not produce any glucuronides in rats (Ichiyanagi et al., 2004).

Recently, we reported a series of studies that showed oral intake of BCAs prevented myopic refractory shift caused by work on visual display terminals (VDT) (Nakaishi et al., 2000). In addition, we demonstrated that D3R, the main component of BCAs, has a relaxing effect on bovine ciliary smooth muscles (Matsumoto et al., 2005). Ciliary muscle relaxation has been studied extensively in the search for drug therapies to treat both myopia and glaucoma (Beauregard et al., 2001). We have also reported that C3G and C3R stimulate the regeneration of rhodopsin in frog rod outer segment (ROS) membranes (Matsumoto et al., 2003). Although ACs are widely available in the United States and Japan as nutritional supplements for improving visual function, their bioavailability in ocular tissue has not been studied. In order to clarify the physiological ability of BCAs to improve visual function, we investigated the ocular absorption, distribution and elimination of BCAs in rats after oral and intraperitoneal (i.p.) administration and in rabbits after i.v. administration.

2. Materials and methods

2.1. Chemicals and solutions

The BCA powder was prepared from commercial blackcurrant juice by the methods described in our previous report (Matsumoto et al., 2001a). The total AC content in the BCA was 21.6%, consisting of D3R (10.2%), C3R (7.5%), D3G (2.9%) and C3G (1.0%) (Fig. 1).

2.2. Oral administration in rats

Thirty-five male Wistar rats aged 8 weeks with a mean body weight of 218.38 ± 2.82 g were obtained from Clea Japan Co., Ltd. (Tokyo). The rats were housed individually in stainless-steel wire-mesh cages at 23 ± 2 °C with a 12-h light–dark cycle. The animals were given free access to tap water and a commercial diet (MF, Oriental Yeast. Co. Ltd, Tokyo, Japan). All rats were handled in accordance with the NIH Guide for the Care and Use of Laboratory Animals. After a 1 week feeding period, food was withheld for 12 h. The rats were then assigned randomly to seven groups. BCA powder dissolved in physiological saline (23.15 mg/ml) was administered orally to the rats in the designated group by direct stomach intubation at a dosage of 463 mg BCA powder per kg body weight (100 mg ACs per kg body weight). Five rats were killed at each of the following time points after administration of BCA (0 min, 30 min, 1 h, 2 h, 4 h, 8 h and 24 h) by withdrawing blood from the inferior vena cava under diethyl ether anesthesia using a heparinized needle and syringe. The whole eyes were then enucleated, with both eyes from each animal being pooled. In order to minimize AC contamination from blood, the blood samples were withdrawn from a vessel on the sclera. The plasma and eyes were used to determine the AC levels at various times after administration of BCAs.

2.3. Intraperitoneal administration in rats

Thirty-five male Wistar rats aged 9 weeks, with a mean body weight of 255.24 ± 4.35 g (Clea Japan) were used for these experiments. The rats were housed using the same domestication procedures as in the oral administration study. After the feeding period, food was withheld for 12 h. BCA powder dissolved in physiological saline (500 mg/10 ml) was then administered intraperitoneally at a dosage of 500 mg BCA powder per kg body weight (108 mg AC per kg body weight). Five rats were killed at each of the following time points after administration of BCAs (0 min, 30 min, 1 h, 2 h, 6 h and 24 h). Blood and whole eye samples were collected for measurement of ACs using the same procedures as in the oral administration study.

To examine the distribution of ACs in ocular tissue, the remaining five rats were killed at 60 min post-administration by withdrawing blood under diethyl ether anesthesia from the inferior vena cava using a heparinized needle and syringe. The aqueous humor was aspirated into a sterile syringe after enucleation of the eye globe and then acidified with a 1/40 volume of 6 N HCl. The aqueous humor samples from both eyes of the animals were pooled from each time point and kept at 5 °C for measurement of ACs. The eyes were then dissected carefully into six parts (cornea, sclera with choroid, ciliary body with iris, retina, vitreous and lens). The plasma and corresponding tissues of both eyes from each animal were pooled, with the