Differences in the Absorption Efficiency Between α- and β-cryptoxanthin from Carrot Leaves and Papaya Fruit

Abstract

A corn-oil based diet comprising β-cryptoxanthin from papaya puree and α-cryptoxanthin from green carrot leaves (17.3 and 9.2 nmol per day, respectively) was fed to five female Wistar rats for eight consecutive days. The identity of the xanthophylls in the supplement was ascertained by LC-(APCI)MS analyses, xanthophylls present in liver and plasma samples were determined by HPLC/DAD. The β-cryptoxanthin concentrations of rat livers of the treatment group were statistically distinguishable ($P<0.01$) from those present in livers of the control group, fed a basic diet. α-Cryptoxanthin, the second xanthophyll present in the supplement, was not found in rat livers of the treatment group. Plasma samples were free of xanthophylls. This is the first study proving that β-cryptoxanthin has a higher absorption efficiency than α-cryptoxanthin in rats, at least from a minimally processed oil-based xanthophyll supplement.

INTRODUCTION

Xanthophylls, such as lutein and zeaxanthin, have gained increasing interest because of positive correlations between their consumption and prevention of eye diseases as age-related macular degeneration (AMD) and cataract formation. Unlike α- and β-carotene, lutein and zeaxanthin are not considered to be provitamin A, but are supposed to act by absorbing blue light which damages the retina and by enhancing the antioxidative status of the environmental tissues. Several factors influence the bioavailability of xanthophylls, including the chemical nature (presence of $E/Z$-isomers or esters) and dietary factors as fat and fiber content and the level of food processing. Since xanthophylls in human blood are typically found in their free form, it was suggested that saponification of native xanthophyll esters could improve their bioavailability in dietary supplements. Only a few human intervention studies have discussed possible differences in the absorption efficiency of lutein and zeaxanthin, and as far as we know, no study directly compared the lutein and zeaxanthin plasma levels reached after consumption of a formulated product. To date, long-term intervention studies about zeaxanthin bioavailability as sole component are rare. Human intervention studies dealing with lutein are much more frequent. With respect to β-cryptoxanthin only a few human intervention studies have been conducted. As far as we know there is no study directly comparing the absorption efficiency of the monohydroxylated counterparts of lutein and zeaxanthin, α- and β-cryptoxanthin. The main goal of this study was to obtain basic data about the absorption efficiency of monohydroxylated xanthophylls.

MATERIALS AND METHODS

Fresh green carrot leaves, corn oil and fresh chicken liver were obtained from a local market. Papaya puree was kindly provided by SGF International (Schutzgemeinschaft der Fruchtsaftindustrie e.V, Nieder-Olm, Germany). β-Cryptoxanthin was isolated from papaya puree (Carica papaya) (Breithaupt et al., 2007). The β-cryptoxanthin containing fraction was obtained by elution with light
petroleum:acetone 95:5 (v/v). α-Cryptoxanthin was isolated from fresh green carrot leaves (Daucus carota). To prepare the xanthophyll supplement, aliquots of both solutions were combined to give a concentration ratio of β- : α-cryptoxanthin = 2:1. The solvent was evaporated (35°C, 50 mbar), the dry residue was suspended in ethanol (1 ml), and dissolved in corn oil (22 ml). A blank supplement was obtained by mixing ethanol (1 ml) with corn oil (22 ml).

Ten female Wistar rats (24 days of age) were obtained from a breeding colony. All animals were housed singly in hanging numbered cages, and were maintained on a 12 h light/dark cycle at a constant temperature of 20-24°C and were provided access to a NIH-31 basic diet and water ad libitum. All animals received the basic diet for the whole study time (23 days). After a clearance phase of 15 days, animals were randomly allocated to a control (C) and a treatment (T)-group of five animals each. The C-group was fed additionally the blank supplement while the T-group received additionally the xanthophyll supplement (0.5 ml/day) for 8 days each. Animals were sacrificed and necropsied. Terminal heart blood was obtained using a venous blood collection tube containing an anti-coagulant solution (tri sodium citrate (13.2 g/L), citric acid (4.8 g/L), glucose (14.7 g/L); 1 ml). Immediately after collection, plasma was separated by centrifugation (5.000 rpm, 10 min, 10°C) and stored in plastic caps at -20°C until analysis. Livers were removed, washed with sodium chloride solution (0.9 %, w/v), dried with a tissue, weighed, and stored in plastic bags at -20°C until analysis.

Extraction and quantification of xanthophylls and retinol from liver were done as described in Breithaupt et al. (2007).

RESULTS AND DISCUSSION

For unequivocal identification of the xanthophylls, LC-MS analyses were performed using APCI interface operated in the positive mode, generating protonated xanthophyll ions. For identification, two characteristic mass traces were used (Fig. 1): α-cryptoxanthin (3) was identified due to an intensive fragment ion at m/z 553.5, generated by loss of water from the quasimolecular ion ([M+H - H₂O]⁺), while β-cryptoxanthin (4) was identified on the basis of its quasimolecular ion at m/z 553.5 ([M+H]⁺).

The average amount of blood which was obtained from rat hearts was 0.93 ml. In samples obtained by extraction without previous addition of the internal standard, no carotenoids were found, thus, plasma samples were not further investigated. The average weight of rat livers was 7.8 g. Using the 325 nm trace in HPLC analyses, liver extracts showed one peak corresponding to free retinol as well as several peaks being most probably related to different retinol esters. There was an increase in the concentration of β-cryptoxanthin in the T-group (Fig. 2). At the retention time expected for α-cryptoxanthin no peak appeared in neither chromatogram of livers belonging to the T-group. One compound eluting in front of α-cryptoxanthin (3) was already present in liver extracts of the C-group. Although the UV/Vis spectrum pointed to a lutein-derived metabolite (λmax 421(sh)/445/474 nm), identification was not doubtless. Further differences in the peak pattern of liver samples of groups C and T were not found. Trace S (Fig. 2) shows a chromatogram obtained by spiking extract T with two drops of the diluted xanthophyll supplement (300 µl/2 ml TBME/methanol; 1:1), resulting in the appearance of an α-cryptoxanthin peak (3) and in enhancement of the β-cryptoxanthin peak area (4). Additionally, several peaks corresponding to Z-isomers of β-cryptoxanthin, already present in the xanthophyll supplement (Fig. 1), appeared. Since those peaks were not present in extracts of the T-group, it was concluded that no in vivo isomerization occurred.

Quantitative analyses of β-cryptoxanthin and retinol in livers of both groups (results not shown) showed that in the C-group, the average concentration was 33 pmol/g whereas in the T-group the average concentration was 58 pmol/g; both data sets were statistically distinguishable. The retinol concentration found in both groups (C: 548 nmol/g, T: 547 nmol/g) was statistically not distinguishable.
Quantitative results of this study showed indeed an enhancement of the β-cryptoxanthin concentration in livers of the T-group compared to those of the C-group, being near to the expected level. This suggested that β-cryptoxanthin was efficiently absorbed even from a minimally processed formulation and that the main part was stored in the liver. This observation is in accordance with the fact that no xanthophylls were found in the plasma. Since the level of retinol in the basic diet was rather high, it was not possible to detect minute changes in the liver retinol concentration caused by enzymatic cleavage of β-cryptoxanthin to form vitamin A. Since the xanthophyll supplement comprised additionally α-cryptoxanthin a consequence should be the appearance of an α-cryptoxanthin peak in liver extracts. However, α-cryptoxanthin was not present in any liver sample. Consequently, the xanthophyll may be not absorbed or may be readily metabolized to vitamin A or apocarotenals. A preference in absorption of α- compared to β-cryptoxanthin can clearly be ruled out. Further clinical trials, particularly with human participants, have to prove whether the absorption efficiency of xanthophylls decreases within the order “lutein – zeaxanthin – β-cryptoxanthin – α-cryptoxanthin” using similar formulations. Since a high absorption rate is a prerequisite for development of xanthophyll health benefits, the results will have a direct impact on selection of xanthophylls used in human dietary supplements.

Literature Cited

Figures

Fig. 1. LC-(APCI)MS analysis (extended sections) of the xanthophyll supplement. The lower trace corresponds to UV/Vis detection (450 nm, DAD), the other traces show selected molecular masses, suitable for detection of α-cryptoxanthin (3; m/z 535.5, [M+H-H2O]⁺) and β-cryptoxanthin (4) (m/z 553.5, [M+H]⁺, molecular ion).
Fig. 2. HPLC analyses (extended sections; 450 nm, DAD) of liver extracts of one rat of the control group (C) and of the treatment group (T). Trace S shows a chromatogram obtained by spiking extract T with an aliquot of the diluted xanthophyll supplement. Peak assignment is as follows: 3: α-cryptoxanthin, 4: β-cryptoxanthin, 5: β-carotene (internal standard).