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Bioefficacy of β-carotene is improved in rats after solubilized as equimolar dose of β-carotene and lutein in phospholipid-mixed micelles

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Abstract

β-Carotene (BC) is a potent dietary source of vitamin A for populations at risk of vitamin A deficiency, yet its bioavailability is influenced by several factors such as dietary fat, carotenoids type, and other components. We hypothesize that type of micellar phospholipids influence bioefficacy of carotenoids and activity of carotenoid metabolizing enzymes. This study determined the BC bioefficacy in rats (n = 5/time point) after an equimolar dose of BC and lutein (Lut) solubilized in micelles containing either phosphatidylcholine (PC) or lysophosphatidylcholine (LPC), or no phospholipid (NoPL). Results show that no BC and Lut was detected in the plasma of rats at 0 hour, but after gavage, the mean (SD) area under the curve (AUC; in picomoles per milliliter) of plasma BC for 6 hours in PC, LPC, and NoPL groups were 1145 (132), 965 (199), and 2136 (112), respectively. The AUC value of plasma Lut in LPC group (183 ± 23 pmol mL^{-1} h^{-1}) was higher than the other 2 groups. Similarly, liver BC and Lut levels in the LPC group were significantly higher than the other groups. The activity of BC 15,15′-monooxygenase in the intestinal mucosa of LPC and PC groups was higher than NoPL group. Plasma retinyl palmitate level in LPC (AUC, 647 ± 89 pmol mL^{-1} h^{-1}) group was 2-fold higher than that of PC and NoPL groups. Results indicate that phospholipids enhanced the BC and Lut absorption. β-Carotene uptake was not affected by Lut when given with micellar phospholipids, but reduced plasma Lut level was observed, which may be due to the conversion of absorbed Lut into its metabolites.

Keywords: β-Carotene; Bioavailability; Lutein; Mixed micelles; Rat; Vitamin A

Abbreviations: AUC, area under the curve; BC, β-carotene; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; HEPES, *N*-2-hydroxyethyl piperazine-*N*′-2-ethanesulfonic acid; HPLC, high-performance liquid chromatography; Lut, lutein; LPC, lysophosphatidylcholine; NoPL, no phospholipid; PC, phosphatidylcholine; RT, retinyl palmitate.

1. Introduction

Vitamin A deficiency remains a major public health problem in developing countries, including India [1]. An inadequate intake of animal and plant-based foods for various reasons, with low fat content was considered to be the major cause of the deficiency [2,3]. The provitamin A carotenoids are important sources of vitamin A for humans; among these, β-carotene (BC) has the highest vitamin A activity. For subjects dependent on BC to meet their vitamin A requirements, bioavailability of BC and its conversion to retinol should be maximized. There is, therefore, concern regarding BC bioavailability, especially the role of dietary factors that affect the absorption and cleavage of BC to vitamin A. There is continued interest in the nutrition of carotenoids, as supplements or from natural food sources, to prevent vitamin A deficiency and other chronic diseases [4,5].

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Factors that limit BC bioavailability include food matrices, amount and type of dietary fat that interfere with micelle formation, interaction with other nutrients, carotenoids such as xanthophylls, and malnutrition [6]. Studies with human subjects reported the plasma levels of absorbed carotenoids and interaction between carotenoids during absorption. Kostic et al [7] reported an interaction between lutein (Lut) and BC; in 5 subjects, Lut reduced the plasma BC response, whereas in 3 subjects, the response was increased. β-Carotene absorption was suppressed when fed in conjunction with other carotenoids [8]. These differences emphasize the need of bioavailability studies in other tissues using animal models (because human studies are limited to blood), to find out the possible role of dietary factors that interfere with absorption and metabolism of carotenoids. Studies with rats and mice reported the improved carotenoid uptake after supplementation with physiologic emulsions and micelles [9,10]. Although rats absorb little intact BC [11], they efficiently metabolize the carotenoids at intestinal level.

Earlier, we reported enhanced intestinal uptake of BC and Lut in rats when administered either of the carotenoids with micellar phospholipids [12,13]. However, the fate of micellar phospholipids carrying hydrophobic carotenoids after their intestinal uptake is unknown. We hypothesize that micellar phospholipids undergo intestinal processing particularly micellarization of carotenoids and thereby facilitate the bioavailability of carotenoids. It is imperative to elucidate their intestinal handling and plasma appearance after micellar dose and to assess their role on carotenoid cleavage enzymes. To improve our understanding of micellar phospholipids on BC and Lut bioavailability, we determined the bioefficacy of BC in rats after an equimolar dose of BC and Lut solubilized in mixed micelles. The activity of BC cleavage enzyme in the intestinal mucosa and the level of retinol formed from the newly absorbed BC were measured. We also investigated the usefulness of the plasma lipid profile as a measure of carotenoid absorption from mixed micelles. This study provides new insight into the possible role of micellar phospholipids on carotenoid uptake and metabolism.

2. Methods and materials

2.1. Chemicals

All-*trans*-BC (type II, ≥95%), all-*trans*-retinal (≥98%), retinol (≥95%), Lut (98%), retinyl palmitate (RP; >99%), monooleoyl glycerol, D-α-tocopherol, sodium taurocholate, phosphatidylcholine (PC), lysophosphatidylcholine (LPC), triolein, dipalmitoyl PC, and cholesterol were purchased from Sigma Chemical Co (St. Louis, Mo). Dithiothreitol (DTT), *N*-2-hydroxyethyl piperazine-*N*′-2-ethanesulfonic acid (HEPES), ethylenediaminetetraacetic acid (EDTA), *N*-tris(hydroxymethyl)-methylglycine (Tricine buffer), Tween-40 and high-performance liquid chromatography (HPLC)-grade acetonitrile, hexane, methanol, and dichloromethane were purchased from Sisco Research Laboratories (Mumbai, India). Sephadex G-25 was obtained from Pharmacia Biotech (Uppsala, Sweden). Standard fatty acids were obtained from NU-Check Prep. Inc, (Elysian, Minn). Other reagent grade chemicals and solvents were purchased from E-Merck Co, Ltd (Mumbai, India).

2.2. Preparation of mixed micelles

Freshly prepared mixed micelles in phosphate-buffered saline (pH 7.0) were composed of 2.5 mmol/L monooleoyl glycerol, 7.5 mmol/L oleic acid, 12 mmol/L sodium taurocholate, and 200 μmol/L BC and 200 μmol/L Lut. Three types of mixed micelles were prepared using (1) 3 mmol/L PC, or (2) LPC, or (3) no phospholipid (NoPL) [10]. The micellar components were dissolved in hexane or methanol/dichloromethane (2:1, vol/vol) separately, and the appropriate concentrations (as above) were added and dispersed well by vortexing. The solvent was evaporated to dryness under nitrogen gas, and the mixture was resuspended in phosphate-buffered saline with vigorous mixing and sonication to obtain an optically clear solution. Micellar solutions were prepared on the same day of feeding to animals, and all the steps were performed under dim yellow light and cold condition to minimize isomerization of carotenoids and to maintain the stability of gavage solution. An aliquot (100 μL) of the micellar solution was extracted and analyzed by HPLC, and carotenoids were found to be stable under the experimental conditions. The equimolar (0.2 mmol/rat) concentration of BC and Lut fed was 0.671 mg/kg body weight, compared with the human subjects [14].

2.3. Rats and the experimental design

The Animal Ethics Committee of Central Food Technological Research Institute (Mysore, India) approved the study, and the use and care of rats followed all necessary protocols to ensure the humane treatment of the animals. Male rats (OUTB-Wistar, IND-cft (2c)), with mean (SD) weight of 42 (2) g, were housed in steel cages at room temperature (28 ± 2°C) with a 12-hour dark/light cycle. Rats were fed ad libitum with fresh-pelleted diet (Amrut feeds, Sangli, India) and had free access to tap water. After 7 days of acclimatization, rats were deprived of diet for 12 hours before administering micellar BC and Lut.

Groups of rats (n = 25/group) were administered a single dose of BC and Lut solubilized in mixed micelles (PC, LPC, and NoPL groups). Each group was further divided into 5 subgroups (n = 5/subgroup) to measure the time-course plasma response of BC and Lut for 6 hours. The BC and Lut in micelles (0.2 mL/rat) were administered by intubations to the stomach. Rats in the 0 hour and in each treatment group (n = 5/time point) were killed at 0 (baseline data), 1, 2, 3, and 6 hours after gavage. The rats were exsanguinated under ether anesthesia, blood was collected from heart into heparinized tubes, and plasma was separated immediately by centrifuging at 1000 × *g* for 15 minutes at 4°C. Liver and upper part of small intestine were removed and washed with