Note

Improved Bioavailability of β -Carotene by Amorphous Solid Dispersion Technology in RatsShuichi OTANI^{1,2}, Shohei MIKI³, Yuta NAKAMURA³, Kenji ISHIMOTO^{1,4}, Yukio AGO^{1,3,4} and Shinsaku NAKAGAWA^{1,3,4,*}¹Laboratory of Innovative Food Science, Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamadaoka, Suita, Osaka 565-0871, Japan²R&D Group, Mitsui Norin Co. Ltd., 223-1 Miyabara, Fujieda, Shizuoka 426-0133, Japan³Laboratory of Biopharmaceutics, Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamadaoka, Suita, Osaka 565-0871, Japan⁴Global Center for Medical Engineering and Informatic, Osaka University, 2-2 Yamadaoka Suita, Osaka 565-0871, Japan

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Summary β -Carotene (BC) is a natural lipophilic carotenoid mainly present in vegetables and fruits. Although it has various beneficial pharmacological activities, its bioavailability is low owing to its low water solubility. Recently, we reported that BC solid dispersion prepared using hot-melt technology with polyvinylpyrrolidone and sucrose fatty acid esters was in an amorphous state and showed the highest solubility. We hypothesized that the absorption of BC solid dispersion would be better because of its increased water solubility. To verify this, we conducted a pharmacokinetic analysis of BC for application in functional foods. Crystalline or amorphous BC was orally administered to rats. Blood was collected at various time points, and the BC concentration in the plasma was measured by HPLC. Oral administration of amorphous BC showed increased absorption in rats compared with that of BC crystals. Using blood samples from rats that were intravenously injected with the plasma of rats that had been orally administered BC, pharmacokinetic parameters could be calculated without using organic solvents or surfactants. It was possible to calculate various pharmacokinetic parameters under physiological conditions according to amorphous BC characteristics. Thus, we were able to determine the bioavailability of BC after oral administration. This simple technology to improve BC solubility without the use of organic solvents can be applied not only in the pharmaceutical industry but also in the food industry, and it therefore has high utility value.

Key Words absorption, β -carotene crystal, bioavailability, physiological condition, polyvinylpyrrolidone

β -Carotene (BC) is a natural lipophilic carotenoid mainly contained in vegetables and fruits. It is known to act as provitamin A because it is metabolized into vitamin A in vivo. Therefore, BC intake is expected to exhibit the pharmacological actions of vitamin A, including maintenance of healthy skin and mucous membrane and improvement of visual acuity (1).

In addition, BC is known to have a strong antioxidant activity, and it can remove excess active oxygen produced in vivo. It has been reported to be effective in preventing degenerative diseases such as cardiovascular diseases, diabetes, and some types of cancer (2). Therefore, BC is used in various fields such as pharmaceuticals, food supplements, and cosmetics. On the contrary, BC has a very low solubility in water and thus poor absorption. Baskaran et al. (3, 4) have reported that BC composite mixed micelles increased the solubility of BC in water, thereby improving its absorption in mice and rats.

Recently, we reported that BC solid dispersions pre-

pared using hot-melt technology with polyvinylpyrrolidone (PVP) and sucrose fatty acid esters was in an amorphous state; it showed the highest solubility ever reported (5). As this preparation method can realize mass production without using an organic solvent, its application in the food industry is expected. However, we did not examine whether the absorption of amorphous BC actually improved.

In this study, we evaluated the absorbability of amorphous BC with improved water solubility in rats. In addition, BC was administered intravenously (i.v.) without using an organic solvent or surfactant, and then pharmacokinetic analysis was performed under conditions similar to physiological conditions to determine the distribution volume (V_d), which has not been reported. We also determined the bioavailability of BC.

Materials and Methods

Chemicals. β -Carotene and chloral hydrate were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). High performance liquid chromatography (HPLC)-grade acetonitrile (MeCN), methanol (MeOH), tetrahydrofuran

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(THF), analytical-grade *n*-hexane, dibutylhydroxytoluene (BHT), acetic acid, ammonium acetate, sodium acetate, and *N*-ethyl-diisopropylamine were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). *trans*- β -Apo-8'-carotenal, the internal standard (I.S.), was purchased from Sigma-Aldrich Japan Inc. (Tokyo, Japan). Polyvinylpyrrolidone (Kolidon 25) was procured from BASF Japan Ltd. (Tokyo, Japan). Sucrose fatty acid ester (S-1670) was provided by Mitsubishi Chemical Foods Co., Ltd. (Tokyo, Japan).

Preparation of BC amorphous solid dispersions. Ten grams of a physical mixture (PM) was prepared with BC/PVP/S-1670 at a ratio of 1 : 7 : 2 (w/w/w). This PM was hot melt extruded at 180°C using a twin-screw extruder (Technovel Co., Ltd., Osaka Japan) to obtain BC amorphous solid dispersion (BCASD).

Animal. All animal studies were approved by the Animal Care and Use Committee of the Graduate School of Pharmaceutical Science, Osaka University (Approval number: 29-3-4). All experimental procedures were conducted in accordance with the guidelines of the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996).

Male Sprague-Dawley (SD) rats (9-wk-old) were purchased from Charles River Laboratories Japan, Inc. (Yokohama, Japan) and allowed to acclimatize for least 1 wk before the start of the experiment. Three rats were housed per cage and maintained in air-conditioned quarters with room temperature of $20 \pm 2^\circ\text{C}$, relative humidity of $50 \pm 10\%$, and alternating 12 h light : dark cycle. The rats were fed a Certified Diet MF (Oriental Yeast Co., Ltd., Kyoto, Japan) and water ad libitum. The rats were fasted for 15 h before the experiment.

Intragastric (i.g.) administration. The rats were administered BC crystals as the PM (control) or amorphous BC as BCASD. The dose of BC crystal was 100 mg/kg and that of amorphous BC was 50 or 100 mg/kg B.W. Each administration liquid was suspended in ion-exchange water.

Blood (500 μL) was collected from the jugular vein with the rats under isoflurane anesthesia using a heparin-treated syringe at 1, 2, 3, 4, 6, and 8 h after administration into a vacuum blood collection tube (plain). Plasma was isolated by centrifuging the blood samples at $2,000 \times g$ for 15 min at 4°C and stored at -80°C for extraction.

Extraction of BC from plasma (i.g.). To 200 μL of plasma, 200 μL of 1 $\mu\text{g}/\text{mL}$ I.S. in MeCN/MeOH/THF (2 : 2 : 1, v/v) containing 0.1% BHT was added. The sample was diluted to 1 mL with 1 M sodium acetate buffer (pH 5.0). After mixing the sample using a vortex, 2 mL of *n*-hexane containing 1% BHT (w/v) was added to the samples. After extraction, the samples were centrifuged at $2,000 \times g$ for 15 min at 4°C and frozen at -80°C for 20 min. The upper organic layer was decanted into a glass tube. This operation was performed 3 times. The extracts were concentrated under a steam of nitrogen gas at room temperature (6). The extract was dissolved in 50 μL of MeCN/MeOH/THF (27.5 : 17.5 : 55, v/v/v) containing 0.5% BHT. The samples were transferred

into insert vials and placed in an HPLC auto-sampler. The injection volume was 10 μL .

HPLC conditions: Quantification of plasma BC after i.g. administration. The concentration of BC in the plasma after oral administration was measured by HPLC. The HPLC conditions were the same as those reported previously (5, 7).

Intravenous (i.v.) administration study. Male SD rats (8-wk-old) were purchased from Charles River Laboratories Japan, Inc. and allowed to acclimatize for least 1 or 4 wk before the start of the experiment. The rats were reared under the same conditions as mentioned for the oral administration test. The rats were i.g. administered amorphous BC three times every 20 min by oral gavage at a dose of 100 mg/kg B.W. ion exchange water suspension (10 mL/kg). Blood was collected from the heart, with the rats under chloral hydrate anesthesia at 2 h and 40 min after the first administration. The blood sample was centrifuged at $2,000 \times g$ for 15 min at 4°C to obtain plasma.

The plasma was i.v. administered (1,747.2 ng/kg, 4.1 mL/kg) to 9-wk-old SD rats ($n=3$) under chloral hydrate anesthesia. Blood samples (800 μL) were collected from the jugular vein with the rats under anesthesia using a heparin-treated syringe at 3, 6, 12, and 24 min after the administration of plasma samples. The blood was treated in the same manner as described previously and stored at -80°C until extraction. β -Carotene from the plasma was extracted in the same manner as described in the i.g. administration experiment.

HPLC conditions: Quantification of plasma BC after i.v. administration. The concentration of BC in the plasma after i.v. administration was measured by HPLC. The HPLC conditions, except the mobile phase, were the same as those reported previously (5). The mobile phase and gradient conditions were optimized with reference to Sugawara et al. (8). The solvent system consisted of a gradient system with solvent A (MeCN/THF/1% ammonium acetate solution = 50 : 30 (v/v/v)), solvent B (MeCN/THF/1% ammonium acetate solution = 50 : 44 : 6 (v/v/v)). Gradient elution was performed at 1.0 mL/min with the following concentration: a 2-min hold at 100% A, 4.7-min linear gradient in solvent B from 0% to 85%, 8-min hold at 85% B, and 5.3-min linear gradient in solvent A from 15% to 100%. The HPLC system was calibrated with the standard solutions of 31.25–4,000 ng/mL BC dissolved in MeCN/MeOH/THF (4 : 4 : 2, v/v).

Pharmacokinetic analysis. Pharmacokinetic analysis after i.g. and i.v. administrations of BC was carried out using free software "Momentl. xls," provided by Kyoto University (9).

Results and Discussion

The rats were orally administered 100 mg/kg BC crystals and 50 and 100 mg/kg amorphous BC, and then the plasma level of BC was quantified at 1, 2, 3, 4, 6 and 8 h after administration. The results are shown in Fig. 1. β -Carotene was not detected in the plasma after administration of BC crystal, whereas it was detected

in the plasma after administration of amorphous BC at both 50 and 100 mg/kg concentration. The maximum plasma concentration ($C_{\max}$) after administration of 50 mg/kg amorphous BC was 211.0 ± 96.0 ng/mL, time of maximum concentration ($T_{\max}$) was 2.2 ± 0.4 h, and $AUC_{0-\infty h}$ was 850.7 ± 620.9 ng/mL·h. The $C_{\max}$ after administration of 100 mg/kg amorphous BC was 284.9 ± 69.7 ng/mL, $T_{\max}$ was 2.8 ± 0.4 h, and $AUC_{0-\infty h}$ was $1,579.8 \pm 477.7$ ng/mL·h. The results indicate that the absorption of BC ($AUC_{0-\infty h}$) was improved by water solubilization in a dose-dependent manner.

The BC solubilization techniques have already been reported as a method to improve BC absorption, which has been validated in mice and rats. These include BC-mixed micelles for oral administration (3, 4), BC-loaded organogel-based nanoemulsion for improved bioaccessibility (10), and BC-encapsulated solid lipid nanoparticles (11). These previous studies have reported an increase in the $C_{\max}$ and $AUC_{0-\infty h}$ due to the solubilization of BC compared with those of oral administration of crystal BC. A common feature of BC solubilization techniques reported is BC dissolved in an organic solvent such as ethanol, hexane, and MeOH, and then formulated through desolvation. On the contrary, our method is simple, as it does not use any organic solvent;

Fig. 1. Plasma level of β -carotene (BC) in male SD rats was determined by HPLC after a single dose of 100 mg/kg (BC crystal) or 50 or 100 mg/kg (amorphous BC). Each point represents the mean $\pm$ SD, $n=5$.

it only employs hot-melt extrusion using an extruder after mixing the raw materials. This production method can be applied not only in the pharmaceutical industry, but also in the food industry, as no organic solvents are used. Furthermore, it is an eco-friendly manufacturing method, which does not require special equipment.

In the present study, the $T_{\max}$ varied depending on the dose of amorphous BC. Alama et al. reported the effects of co-administration of alendronate and various sucrose fatty acid esters at different concentrations on BC absorption. It has been reported that the AUC, $C_{\max}$, and $T_{\max}$ change when the co-dose of sucrose fatty acid ester (S-1670) is increased (12). This has been attributed to the effect of sucrose fatty acid esters contained in BCASD. That is, as the dose of BCASD increases, not only the dose of BC but also that of sucrose fatty acid ester increases. Therefore, we considered that the $T_{\max}$ changes when the dose of amorphous BC changes.

For pharmacokinetic parameter calculation, the kinetics after i.v. administration was confirmed, and the results are shown in Table 1. Plasma (with BC concentration of 417.7 ng/mL) after oral administration of BC amorphous was used as the administration solution without using an organic solvent or surfactant. With this method, the actual pharmacokinetic parameters of absorbed BC under physiological conditions could be obtained. The results revealed that the V_d was as low as 0.030 ± 0.001 , and we considered that BC, which was absorbed, was present in the blood with almost no migration to the tissues. Moreover, the bioavailability of BC was determined for the first time, and the value was approximately 0.03%. Furthermore, the elimination half-life ($t_{1/2}$) was 1.35 ± 0.84 h. On the contrary, this value was lower than the $t_{1/2}$ deduced from Fig. 1 after oral administration. This suggests that BC was absorbed slowly until 8 h after oral administration.

Several reports on pharmacokinetic analysis and organ distribution after i.v. administration of BC and other carotenoids have been reported (13–15). When carotenoid, a compound with poor water solubility compound, is i.v. administered, the dosing solution is first dissolved in an organic solvent or surfactant, and then

Table 1. Pharmacokinetic parameters of BC in SD rats after its i.v. or i.g. administration.

Parameter	Intragastric		Intravenous
	50 mg/kg	100 mg/kg	
V_d (L/kg)	NA	NA	0.030 ± 0.001
$AUC_{0-\infty h}$ (ng/mL·h)	850.7 ± 620.9	$1,579.8 \pm 477.7$	105.1 ± 64.6
K_a (1/h)	0.20 ± 2.81	0.30 ± 0.05	NA
K_e (1/h)	0.31	0.25 ± 0.04	0.51 ± 0.23
$t_{1/2}$ (h)	2.22	2.80 ± 0.4	1.35 ± 0.84
$T_{\max}$ (h)	2.2 ± 0.4	2.8 ± 0.4	NA
$C_{\max}$ (ng/mL)	211.0 ± 96.0	284.9 ± 69.7	NA
F (%)	0.028 ± 0.020	0.026 ± 0.008	NA

The values are means $\pm$ SD, $n=5$ (i.g.), $n=3$ (i.v.).

The rats were administered a single dose of either 50 or 100 mg/kg amorphous BC i.g. or 1,747.2 ng/kg i.v. Plasma level of BC was determined by HPLC analysis and pharmacokinetic parameters were determined using Momentl. xls.

V_d , volume of distribution; AUC, area under the curve; K_a , absorption rate constant; K_e , elimination rate constant; $t_{1/2}$, elimination half-life time; $T_{\max}$, time of maximum concentration; $C_{\max}$, maximum plasma concentration; F , absolute bioavailability; NA, not applicable.

diluted with saline or PBS. Therefore, BC is expected to be different from that present under physiological condition, and therefore, it is difficult to confirm that BC in organic solvents reflects the dynamics of BC absorbed from the digestive tract. Therefore, we collected plasma with the highest BC concentration after oral administration and used this plasma sample for the i.v. administration study. PVP was hardly absorbed in the oral gavage test in rats (16). In contrast, there is no evaluation report available on the absorption of sucrose fatty acid esters from the digestive tract. Moreover, carotenoids absorbed from the gastrointestinal tract have been reported to be transported into the blood after being taken up by very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) (17). This method strongly reflects the state of BC in the blood under physiological conditions.

In this study, the bioavailability of BA could be accurately calculated, and the value was as low as 0.03%. To the best of our knowledge, this is the first report of the bioavailability of BC. BC, which is provitamin A, is consumed in vivo as vitamin A, but many functions as BC have been reported. It is important to formulate BC with increased bioavailability. We were successful in preparing an amorphous formulation of BC with increased bioavailability.

However, it can be seen that most of the administered BC is not absorbed from the intestines, and further research on the formulation technology for increasing its bioavailability, safety, and functional properties will be important. We also found that there are many carotenes other than BC that are not absorbed. This puts further emphasis on developing formulations of BC with increased bioavailability. In this study, detailed BC pharmacokinetics was clarified using amorphous BC. In the food industry, safety has always been an issue; hence, this study was conducted using food grade products that are guaranteed to be safe for consumption—PVP and sucrose fatty acid esters were used as base materials. In this study, the dose was considered appropriate. We also found that raising the bioavailability through better formulation technology is an issue in terms of resource efficiency. In the future, we will evaluate the safety and functionality of BC with improved absorption and develop functional foods.

Disclosure of state of COI

SO is an employee of Mitsui Norin Co., Ltd. All the other authors declared no competing interests.

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