

In vivo tissue uptake of intravenously injected water soluble all-*trans* β -carotene used as a food colorant

Tomoko T. YAMANUSHI *, Midori I. TORII, Najma JANJUA, and Hideaki KABUTO

Kagawa Prefectural College of Health Sciences

281-1 Mure-cho, Takamatsu City, Kagawa, 761-0123 Japan

* Corresponding author

Email addresses:

TTY: t.t.yamanushi@chs.pref.kagawa.jp

MIT: midoriitorii@yahoo.co.jp

NJ: janjua@chs.pref.kagawa.jp

HK: kabuto@chs.pref.kagawa.jp

Abstract

Water soluble β -carotene (WS-BC) is one of the carotenoid forms which have been developed as food colorant. WS-BC is known to contain 10 % of all-*trans* β -carotene (AT-BC). The aim of the present study was to investigate *in vivo* tissue uptake of AT-BC after the administration of WS-BC into rats. Seven-week-old male rats were administrated WS-BC (2 mg in saline) by intravenous injection into the tail vein. At 0, 6, 24, 72, 120 and 168 hours, rats were killed (n=7 for the each time) and plasma, liver, lungs, adrenal glands, kidneys and testes were obtained. The levels of AT-BC in these tissues were quantified with HPLC. After intravenous administration, AT-BC level in plasma first increased up to 6 h and returned to normal at 72 h. In the testis, the AT-BC level first increased up to 24 h, and then did not decrease but was retained up to 168 h. In the other tissues, the level first increased up to 6 h, then decreased over the period of 6 to 120 or 168 h, but did not return to a normal level. In the present study, after the administration of WS-BC intravenously, AT-BC was found to be accumulated in testes, but not in the other 5 tissues examined. This may suggest that AT-BC was excreted or metabolised in these tissues but not in testes. Although WS-BC is commonly used as a food colorant, its effects on body tissues are still not clarified. Results of the present study suggest that further investigations are required to elucidate effects of WS-BC on various body tissues.

Findings

Carotenoids are one of the main groups of coloring substances in nature [1-3]. The advantages to add carotenoids as food colorants are: high tinctorial potency, safety, stability, compatibility and availability. As color conveys a concept of freshness and wholesomeness by an ingrained color-taste expectancy relationship, the technical challenge of the food industry has been creating suitable application forms of carotenoids for food coloring needs [1-4]. Several application forms of the carotenoids have been developed for coloring both fat-based (margarine, cheese, butter, etc.) and water-based (juice, beverages, etc.) foods [4]. Since the carotenoids are water insoluble, three

approaches were used to overcome their solubility disadvantage: 1) reduction in crystal size; 2) preparation of emulsions in liquid and beadlet forms; and 3) development of colloidal preparations [4]. Since various application forms of β -carotene have been developed and are most widely used as food colorants, people intake β -carotene easily in their daily life. β -Carotene is one of the provitamin A carotenoids, which is cleaved to retinal, followed by its conversion to retinyl ester within the small intestine [5-8]. Vitamin A and its analogs (retinoids) are needed to maintain normal growth and development, immunity, reproduction and other essential physiologic processes [8-10]. Besides the provitamin A activity, β -carotene has other important biological functions such as quenching of singlet oxygen, interrupting peroxidation, reducing the free radicals, and so on [3, 6]. Many epidemiological studies have reported a negative relationship between β -carotene intake and chronic disease, over a long period [6, 11]. However, two large recent trials found that a pharmacological level of β -carotene increased lung cancer incidence and deaths in smokers and asbestos workers [11]. A larger trial with healthy American men, however, found no effect of β -carotene on cancer except an increased risk for thyroid and bladder cancer [11]. These contradictory reports suggest a possible dual response of β -carotene, whereby it promotes health when taken at dietary levels, but may have adverse effects when taken at higher doses [12]. The tissue distribution of β -carotene is still not clearly defined [12]. In the present study, we aimed to examine rat *in vivo* tissue uptake of all-*trans* β -carotene (AT-BC). The studies of β -carotene distribution are difficult because of several factors, such as the solubility conditions and *in vivo* digestion, influence its absorption. Therefore, the choice of solvent and the method of β -carotene administration have been controversial [13]. In the present study, we used water soluble β -carotene (WS-BC) which has been commonly used as one of the application forms of food colorants. The WS-BC is considered to overcome the β -carotene insolubility and its absorption difficulties.

Dry β -carotene beadlets were kindly donated by Hoffmann La Roche Japan, Co., Ltd. (Tokyo, Japan) (Trade name: Dry β -Carotene 10 % Cold Water-Soluble). The beadlets contained 10 % of

AT-BC. In addition to AT-BC, the beadlets consisted of vehicle (starch, gelatin, sucrose and plant oil) and antioxidant (vitamin E and vitamin C). In this paper, the dry β -carotene beadlets refer to as water soluble β -carotene (WS-BC). The reagents used for the WS-BC extraction were purchased from Wako Pure Chemical Industries Inc. (Osaka, Japan). The solvents for high performance liquid chromatography (HPLC) were purchased from Nacalai Tesque Inc. (Kyoto, Japan). Sprague-Dawley male rats (7 weeks old, body weight 210 - 230 g) were purchased from Clea Japan, Inc. (Tokyo, Japan) and housed under constant temperature on a 12-hour light/dark cycle. Before the intravenous injection of WS-BC, rats were fed standard laboratory diet (CE-2, Clea Japan. Inc., Tokyo, Japan) and given access to tap water, *ad libitum* for 5 days. The intravenous injection of WS-BC was performed as described previously (13). In the present study, WS-BC was dissolved in saline at a concentration of 2 mg/ml. One ml of this solution was injected in the tail vein of the rat. The actual amount of AT-BC administered into the rat was 200 μ g. At 0, 6, 24, 72, 120 and 168 hours after the intravenous administration of WS-BC, blood was drawn from the abdominal vein and centrifuged to obtain plasma. Rats were killed, and liver, lungs, adrenal glands, kidneys and testes were removed and frozen in liquid nitrogen. For each time point, 7 rats were used. All organs and plasma were kept at -80 °C until further analysis. All experimental protocols were conducted in accordance with Japanese Act on Welfare and Management of Animals (Act No. 105 of October 1, 1973). The quantification of AT-BC in the tissues was performed using HPLC as described before [13]. Data were presented as means \pm SD. Statistical analyses were carried out using Origin (Microcal Software Inc., USA) or Excel (Microsoft, USA) software. Differences were considered significant at the level of $p < 0.05$.

The mean (\pm SD) AT-BC levels in the tissues were examined at various times after the intravenous administration (Fig 1). The AT-BC levels in all tissues increased up to 6 h (Fig.1). It is considered that the AT-BC circulated in the blood stream and was distributed to these tissues within 6 h. In plasma, the AT-BC level decreased rapidly after 6h and the level at 72 h was not significantly different from that at 0 h (Fig 1a). It is indicating that the AT-BC level returned to

normal at 72 h. It suggests that all the administrated AT-BC in plasma was distributed to other body tissues or excreted from blood at 72h. The AT-BC level in the liver decreased over a period of 6 h to 120 h (Fig 1b). In the lung, adrenal gland and kidney, the AT-BC levels decreased gradually over the period of 6 h to 120 or 168 h (Fig 1c, d and e). It is suggested that the AT-BC was excreted from these tissues or was metabolised to the possible metabolites of AT-BC, retinoids and carotenoid isomers [5-8, 12]. In these tissues, the AT-BC levels at 120 and/or 168 h were significantly different from those at 0 h (Fig 1c, d and e). It is indicating that the possible amount of AT-BC was still retained at 120 or 168 h in these tissues. In the testis, AT-BC level first increased up to 24 h, and then did not decrease but was retained up to 168 h (Fig 1f).

In the present study, rat *in vivo* uptake of AT-BC was examined after the intravenous administration of WS-BC (Fig.1). The *in vivo* emulsifying conditions have been shown to affect β -carotene absorption [12, 14]. In our pilot experiment fed the rats with refined diet containing the determined amount of WS-BC, the level of AT-BC in each tissue was largely different by the individuals. This was suggested that *in vivo* tissue uptake of AT-BC was affected by the individual's absorption conditions of WS-BC. Therefore, oral administration of WS-BC is considered to be difficult to examine *in vivo* tissue uptake. Furthermore, in our previous work involving intravenous administration of the emulsified AT-BC crystals in solvent, high levels of AT-BC accumulated in the lung [13]. This was suggested to be due to the trapping of the solvent used for dissolving AT-BC [13]. To overcome these problems of absorption and insolubility difficulties of AT-BC, in the present study, WS-BC was dissolved in saline and administered intravenously. However, like our previous study [13], in some tissues including lung, accumulation of a large amount of AT-BC was not found in the present study. This would suggest that the WS-BC was not trapped by the solvent in the present study whereas it was in the previous study [13].

The antioxidant properties of β -carotene are strictly dependent on oxygen partial pressure (OPP) [15, 16]. *In vitro* experiments at different OPP have demonstrated ambiguous behavior of

β -carotene [15]. At OPP less than the oxygen pressure in normal air, β -carotene behaved as an antioxidant whereas at higher values, it was found to lose its antioxidant activity and actually showed a pro-oxidant effect [15]. A number of reports have now confirmed this phenomenon in purified systems [17], microsomes [18], cell lines [19] and bacteria [20]. In addition, after β -carotene administration to rats, cytochrome P450 isoforms were induced and reactive oxygen species (ROS) were increased in kidney, lung, intestine and liver which was the most affected tissue [16]. From these findings, it was suggested that β -carotene may have two contradicting behaviors, antioxidant and pro-oxidant [16]. Oxidative stress plays a major contributory role in pathogenesis of many generative and chronic diseases [16]. Many epidemiological studies have been reported which show a negative relationship between dietary β -carotene intake and chronic disease [6, 11]. On the other hand, recent intervention trials suggest that β -carotene supplementation may promote health when taken at dietary levels but have adverse effects when taken in higher amounts [11, 12]. The conflicting behaviour of β -carotene may explain why the contradictory reports were obtained in the reported studies.

In the present study, the observation of accumulation of AT-BC over a period of 168 h after the intravenous administration (Fig. 1f) suggests that under certain conditions, testis may have the ability to store AT-BC for several days. In an immunohistochemical study [7], human β -carotene 15,15'-mono-oxygenase (BCO1), which is involved in the symmetrical cleavage of β -carotene into two retinal molecules, was detected in steroidogenic cells in testis, ovary, and adrenal gland [21]. In mouse testis, the level of mRNA of carotene cleavage enzyme (CCE), which cleaves provitamin A carotenoid to retinol, was highest in all 4 tissues examined including the small intestine [8]. From these observations, it was suggested that in testis, β -carotene could act as a local source of retionids, which have been shown to be important during proliferation, differentiation, and maturation of germinal cells [7, 8, 21]. In male rats treated with fenvalerate [22] or cadmium [23], administration of β -carotene was reported to ameliorate the induced toxicity in the testes. Moreover, β -carotene administration increased semen quality [22]. Overall, these

findings suggested that β -carotene may be essential for the function of testes. As β -carotene is commonly used commercially to colour food, people intake it easily from food in their daily life. Since β -carotene has ambiguous behaviour to become antioxidant or pro-oxidant depending on its partial oxygen pressure, results of the present study suggest further investigations are required to elucidate its effect on body tissues under various physiological conditions.

List of abbreviations

WS-BC: water soluble β -carotene; AT-BC: all-*trans* β -carotene; HPLC: high performance liquid chromatography; OPP: oxygen partial pressure

Competing interests

The author(s) declare that they have no competing interests.

Authors' contributions

TTY was the main author of the manuscript and contributed in the design of the study, preparation of protocols, assistance of all the experiments, interpretation of data and preparation of manuscript. MIT participated in all the experiments, the statistical analyses, interpretation of data and preparation of manuscript. NJ took part in the editing of manuscript and the proof reading of English. HK contributed in interpretation of data and preparation of manuscript.

References

1. Gordon HT, Bauernfeind JC: Carotenoids as food colorants. *Crit Rev Food Sci Nutr* 1982, 18:59-97
2. Delgado-Vargas F, Paredes-Lopez O: Carotenoids. In: *Natural Colorants for Food and Nutritional Uses*. Written by Delgado-Vargas F, Paredes-Lopez O. Boca Raton, FL, CRC

press 2003, 113-166

3. Olson JA, Krinsky NI: Introduction: the colorful, fascinating world of the carotenoids: important physiologic modulators. *FASEB J* 1995. 9: 1547-1550
4. Sambale C: Processing characteristics and stability of chemically synthesized carotenoids in food systems. *Arch Latinoam Nutr* 1999. 49: 48S-51S
5. Blaner WS, Olson JA: Retinol and retinoic acid metabolism. In: *The retinoids: Biology, Chemistry, and Medicine*. Edited by Sporn MB, Roberts AB, Goodman DS. New York, NY, Raven Press 1994, 229-256
6. Krinsky NI: Effects of carotenoids in cellular and animal systems. *Am J Clin Nutr* 1991, 53: 238S-246S
7. Lindqvist A, Andersson S: Cell type-specific expression of beta-carotene 15,15'-monooxygenase in human tissues. *J Histochem Cytochem* 2004, 52:491-499
8. Paik J, Vogel S, Quadro L, Piantedosi R, Gottesman M, Lai K, Hamberger L, Vieira Mde M, Blaner WS: Vitamin A: overlapping delivery pathways to tissues from the circulation. *J Nutr* 2004, 134: 276S-280S
9. Hofmann C, Eichele G: Retinoids in development. In: *The Retinoids, Biology, Chemistry, and Medicine*. Edited by Sporn MB, Roberts AB, Goodman DS. New York, NY, Raven Press 1994, 387-442
10. Gudas LJ, Sporn MB, Roberts AB: Cellular biology and biochemistry of the retinoids. In: *The Retinoids, Biology, Chemistry, and Medicine*. Edited by Sporn MB, Roberts AB, Goodman DS. New York, NY, Raven Press, 1994, 443-520
11. National Institute of Health: NIH State-of-the Science Conference Statement on Multivitamin/Mineral Supplements and Chronic Disease Prevention. *Ann Intern Med* 2006, 145: 364-371
12. Rao AV, Rao LG: Carotenoids and human health. *Pharmacol Res* 2007, 55: 207-216
13. Yamanushi T, Igarashi O: The mobilization and tissue distribution of beta-carotene in the rat by the venous injection method. *J Nutr Sci Vitaminol* 1995, 41: 169-177

14. Goodman DS, Blomstrand R, Werner B, Huang HS, Shiratori T: The intestinal absorption and metabolism of vitamin A and beta-carotene in man. *J Clin Invest* 1966, 45: 1615-1623
15. Burton GW, Ingold KU: Beta-Carotene: an unusual type of lipid antioxidant. *Science* 1984, 224: 569-573
16. Paolini M, Antelli A, Pozzetti L, Spetlova D, Perocco P, Valgimigli L, Pedulli GF, Cantelli-Forti G: Induction of cytochrome P450 enzymes and over-generation of oxygen radicals in beta-carotene supplemented rats. *Carcinogenesis* 2001, 22: 1483-1495
17. Zhang P, Omaye ST: Beta-carotene and protein oxidation: effects of ascorbic acid and alpha-tocopherol. *Toxicology* 2000, 146: 37-47
18. Salgo MG, Cueto R, Winston GW, Pryor WA: Beta carotene and its oxidation products have different effects on microsome mediated binding of benzo[a]pyrene to DNA. *Free Radic Biol Med* 1999, 26: 162-173
19. Palozza P, Luberto C, Calviello G, Ricci P, Bartoli GM: Antioxidant and prooxidant role of beta-carotene in murine normal and tumor thymocytes: effects of oxygen partial pressure. *Free Radic Biol Med* 1997, 22: 1065-1073
20. Bianchi L, Melli R, Pizzala R, Stivala LA, Rehak L, Quarta S, Vannini V: Effects of beta-carotene and alpha-tocopherol on photogenotoxicity induced by 8-methoxypsoralen: the role of oxygen. *Mutat Res* 1996, 369: 183-194
21. Lindqvist A, Andersson S: Biochemical properties of purified recombinant human beta-carotene 15,15'-monooxygenase. *J Biol Chem* 2002, 277: 23942-23948
22. El-Demerdash FM, Yousef MI, Kedwany FS, Baghdadi HH: Role of alpha-tocopherol and beta-carotene in ameliorating the fenvalerate-induced changes in oxidative stress, hemato-biochemical parameters, and semen quality of male rats. *J Environ Sci Health part B* 2004, 39: 443-459
23. El-Demerdash FM, Yousef MI, Kedwany FS, Baghdadi HH: Cadmium-induced changes in lipid peroxidation, blood hematology, biochemical parameters and semen quality of

male rats: protective role of vitamin E and beta-carotene. *Food Chem Toxicol* 2004,
42:1563-1571

Figure legend

Figure 1 - Time dependent changes in AT-BC levels in 6 tissues after WS-BC intravenous administration in rat

Values plotted are mean \pm SD. (n=7 for each time point)

For each tissue, individual AT-BC values at each time point were adjusted by subtracting the respective mean values at 0 hour, and mean \pm SD were then calculated.

* significantly different compared with AT-BC level at 0 h

significantly different compared with AT-BC level at the preceding time point

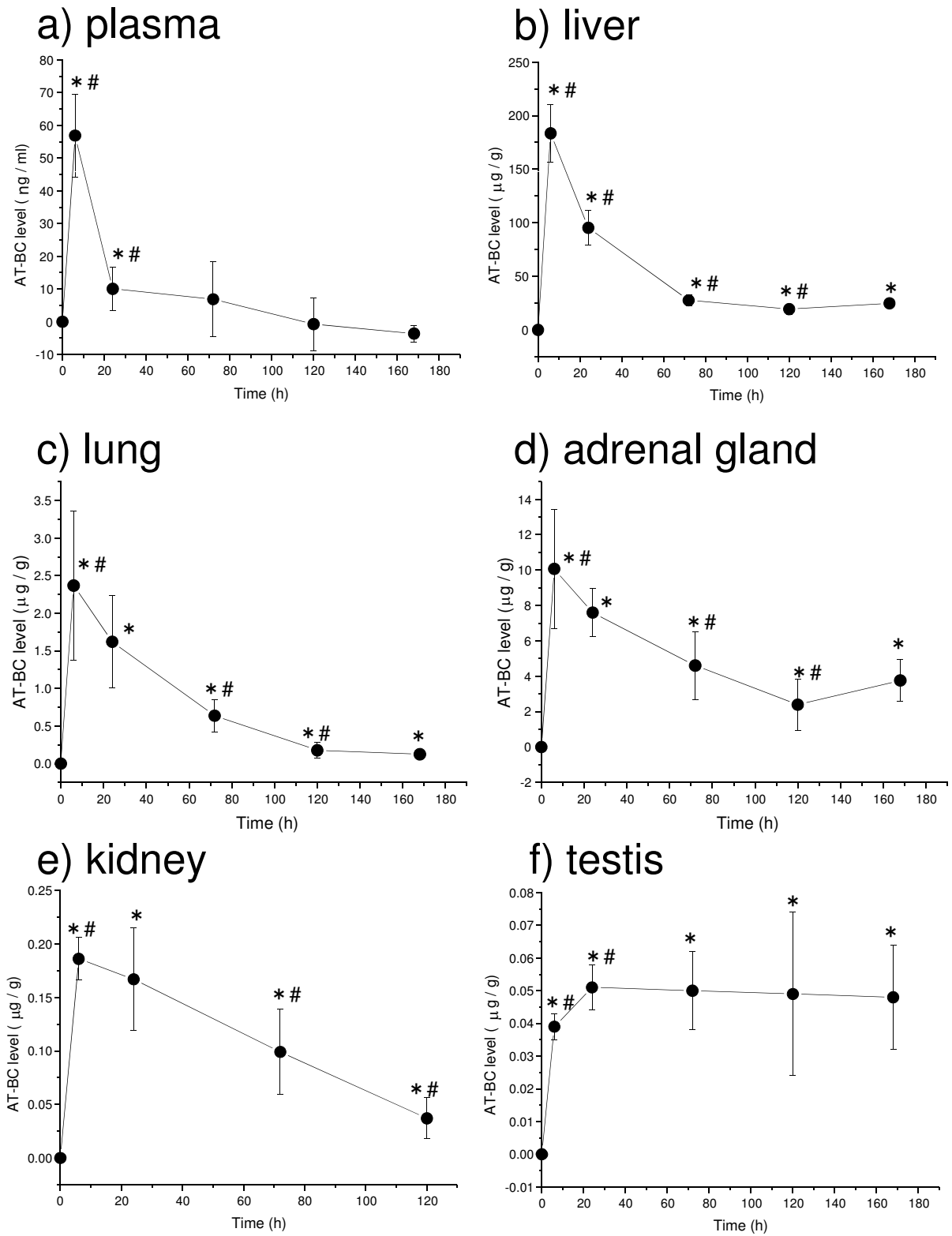


Figure 1