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Short Communication

Pharmacokinetics of fucoxanthinol in human plasma after the oral administration of kombu extract

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Abstract

Dietary fucoxanthin has been reported to exert several physiological functions, and fucoxanthinol is considered to be the primary active metabolite of fucoxanthin. However, there is no information about the pharmacokinetics of fucoxanthinol in human subjects. In the present study, eighteen human volunteers were orally administered kombu extract containing 31 mg fucoxanthin, and their peripheral blood was collected 5 min before and 0·5, 1, 2, 4, 8 and 24 h after the treatment. Plasma fucoxanthinol concentrations were measured by HPLC, and the pharmacokinetics of fucoxanthinol were as follows: maximum concentration, 44·2 nmol/l; time at maximum concentration, 4 h; terminal half-time, 7·0 h; area under the curve (AUC) for 1–24 h, 578·7 nmol/l × h; $AUC_{(\infty)}$, 663·7 nmol/l × h. In addition to fucoxanthinol, we also attempted to detect amarouciaxanthin A, a hepatic metabolite of fucoxanthinol, using HPLC, but it was not present in the volunteers' plasma. On the other hand, a peak that was suspected to represent the *cis*-isomer of fucoxanthinol was found in the HPLC chromatogram. By comparing the present results with those of a previous study using mice, we found that the bioavailability and metabolism of fucoxanthinol differ between human subjects and mice.

Key words: Fucoxanthinol: Fucoxanthin: Human subjects: Plasma: Kombu (*Laminaria japonica*)

Fucoxanthin is one of the xanthophylls found in brown seaweed such as kombu (*Laminaria japonica*), hijiki (*Sargassum fusiforme*) and wakame (*Undaria pinnatifida*)[1]. It has been reported to have several physiological functions including anti-carcinogenic[2–7] and anti-obese activities[8,9] in several studies involving experimental animals and human subjects and murine cell lines. Recently, brown seaweed extract has been used as a source of fucoxanthin for commercial nutritional supplements worldwide. Most dietary fucoxanthin is absorbed as fucoxanthinol, a hydrolysed metabolite, in the small intestine[10]. Previous studies have suggested that fucoxanthinol is further converted to amarouciaxanthin A in the liver[11]. Thus, these metabolites are considered to be the active forms that exert physiological functions in the body. The bioavailability of these metabolites is required for the proper and safe usage of dietary fucoxanthin. Recent studies have described the pharmacokinetics of fucoxanthinol and amarouciaxanthin A, and demonstrated their accumulation after the oral administration of fucoxanthin to mice[12,13]. However, there are no reports about the pharmacokinetics of fucoxanthin and its metabolites in human subjects, although Asai *et al.*[14] reported that fucoxanthinol was detectable at a concentration of 0·8 nmol/l in human plasma after the daily intake of wakame containing 6·1 mg fucoxanthin for 1 week.

In the present study, kombu extract containing 31 mg fucoxanthin was orally administered to human volunteers, and the pharmacokinetics of fucoxanthin and its metabolites were investigated.

Abbreviations: AUC, area under the curve; BW, body weight.

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Experimental methods

Reagents

Fucoxanthin, fucoxanthinol and amarouciaxanthin A were prepared as described previously[12]. Astaxanthin was purchased from Extrasyntèse (Genay, France). Kombu extract was provided by Oryza Oil & Fat Chemical (Aichi, Japan). All other reagents were of the highest grade commercially available.

Human study

The study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Ethics Committee at Kobe University Graduate School of Medicine (permission no. 615). Written informed consent was obtained from all subjects. Verbal consent was witnessed and formally recorded. A total of eighteen volunteers (ten males and eight females), with a mean age of 33 (range 22–63) years, a mean body weight (BW) of 60 (range 45–71) kg and a mean BMI of 21·8 (range 19·0–24·6) kg/m^2, were enrolled in the single oral dose study. The participants were told to refrain from consuming brown algae such as kombu and wakame for 1 week and then fasted for 12 h. They were orally administered 10 ml of kombu extract dissolved in medium-chain TAG, which contained 31 mg fucoxanthin. Their peripheral blood was collected 5 min before the treatment and 0·5, 1, 2, 4, 8 and 24 h after the treatment. Plasma samples were prepared by centrifugation at 1000 ***g*** for 10 min at 4°C and stored at −80°C until the analysis.

HPLC analysis of fucoxanthin and its metabolites

Plasma (2·0 ml) was mixed with 20 μl of 20 μM-astaxanthin, as an internal standard, and added to 2·5 ml dichloromethane–methanol (1:2, v/v). This was then extracted three times with 5·0 ml dichloromethane. The dichloromethane layer was collected after centrifugation at 1500 ***g*** for 15 min, evaporated and then dissolved in dimethyl sulfoxide–methanol (1:1, v/v). Then, 50 μl of the samples were subjected to HPLC analysis to determine the levels of metabolites, as described previously[12].

Pharmacokinetic analysis

The pharmacokinetic parameters of fucoxanthinol were calculated from the changes in its concentration over time using a non-compartmental pharmacokinetic analysis program[15].

Results and discussion

To investigate the pharmacokinetics of dietary fucoxanthin and its metabolites in human plasma, eighteen healthy volunteers were orally administered 10 ml of kombu extract containing 31 mg fucoxanthin, and their peripheral blood was collected 5 min before and 0·5, 1, 2, 4, 8 and 24 h after the treatment. Fucoxanthinol was detectable in the plasma of all participants, but fucoxanthin was not (Fig. 1(A)). The plasma concentration of fucoxanthinol increased until 4 h after the treatment and then gradually decreased to 7·6 (SD 0·8) nmol/l at 24 h after the treatment (Fig. 1(B)). The pharmacokinetic parameters described below were calculated from these data. The maximum concentration (C_{max}), time at maximum concentration (T_{max}), terminal half-life ($t_{1/2}$), area under the curve (AUC) for 1–24 h and AUC$_{(\infty)}$ of fucoxanthinol in plasma were 44·2 nmol/l, 4 h, 7·0 h, 578·7 nmol/l × h and 663·7 nmol/l × h, respectively. Novotny *et al.*[16] reported that the AUC of [^{13}C]β-carotene and [^{13}C]lutein in human

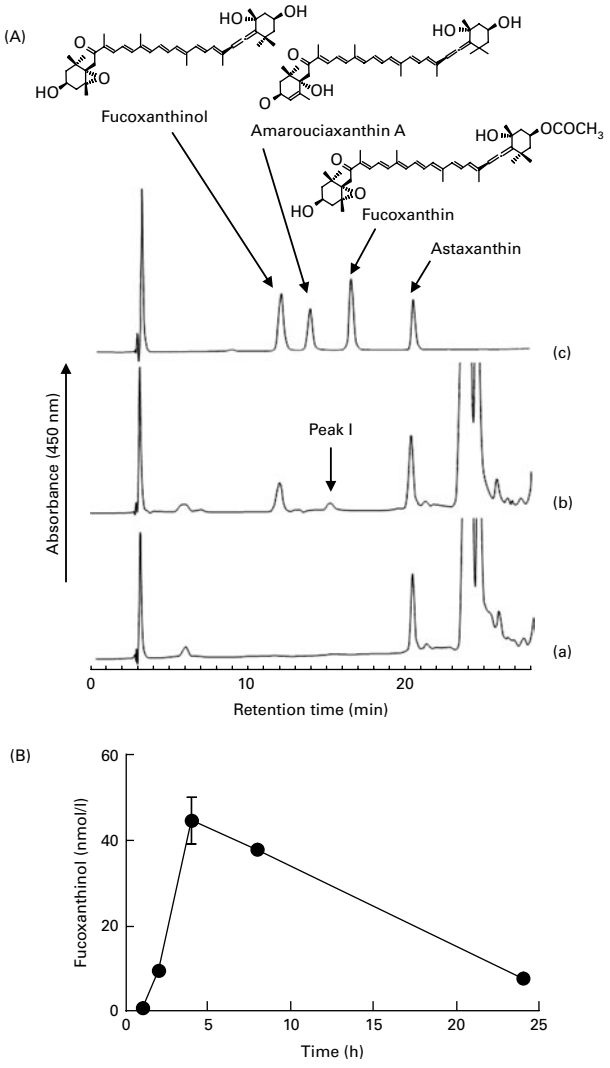

Fig. 1. (A) Representative HPLC chromatograms of human plasma. HPLC analysis was performed as described in the Experimental methods section. Chromatograms of the plasma extract obtained at (a) 5 min before and (b) 4 h after the administration of a single dose of 31 mg fucoxanthin-containing kombu extract. (c) Chromatogram of fucoxanthin (retention time, 11·9 min), fucoxanthinol (13·8 min) and amarouciaxanthin A (16·4 min) standards and astaxanthin (20·4 min) as an internal standard. (B) Time-course profile of fucoxanthinol in human plasma after the administration of a single dose of fucoxanthin. Eighteen volunteers were administered kombu extract containing 31 mg fucoxanthin. The amount of fucoxanthinol in their plasma was determined by HPLC as described in the Experimental methods section. Values are means, with standard deviations represented by vertical bars, *n* 18.

subjects fed isotopically labelled kale were 13·6 and 42·8 μM, respectively, when the ingested doses of the labelled carotenoids were 34 and 33 μM, respectively. Mercke Odeberg *et al.*[17] demonstrated that the $AUC_{(\infty)}$ of astaxanthin was 2·26 μmol/l × h after the administration of a single oral dose of 40 mg (67 μmol) astaxanthin to healthy male volunteers, and changes in the formulation enhanced the AUC (range 3·71–8·31 μmol/l × h). Thus, the bioavailability of fucoxanthinol seems to be lower than that of other dietary carotenoids such as β-carotene, lutein and astaxanthin.

Recently, we have reported the pharmacokinetics of fucoxanthinol in mouse plasma after the administration of a single oral dose of 160 nmol (0·105 mg) fucoxanthin, i.e. 3·5 mg fucoxanthin/kg BW[12]. The T_{max} of fucoxanthinol was 4 h, and the plasma concentration of fucoxanthinol decreased gradually until 24 h. The time-course profile of fucoxanthinol in human plasma obtained in the present study was similar to that detected in mice. On the other hand, our previous study[12] also indicated that the C_{max}, $t_{1/2}$ and $AUC_{(\infty)}$ of fucoxanthinol were 132 nmol/l, 4·5 h and 1430 nmol/l × h, respectively, and that the plasma concentration of fucoxanthinol at 24 h after its administration was 8·2 (SD 4·5) nmol/l. The mean dose in the present study was 0·52 mg fucoxanthin/kg BW (range 0·44–0·69 mg/kg BW), which was one-seventh (15 %) of that administered to the mice in our previous study[12]. However, the C_{max} and $AUC_{(\infty)}$ of fucoxanthinol in the human study were estimated to be 33 and 46 % of the values found in mice, respectively. Furthermore, the $t_{1/2}$ of fucoxanthinol in human subjects was 7·0 h, and its concentration at 24 h after its administration was 7·6 (SD 3·2) nmol/l (Fig. 1(B)). These results suggest that the bioavailability of fucoxanthinol is higher in human subjects than in mice. Mordenti[18] reported that smaller, short-lived animals generally clear drugs from their bodies more rapidly than larger, long-lived animals, and that the pharmacokinetic profiles of different species were strikingly different, with elimination being most rapid for mice and least rapid for human subjects among the species compared. This seems to be the reason why the bioavailability of fucoxanthinol in human subjects is higher than that in mice.

In the present study, amarouciaxanthin A was not detected in the blood of any of the participants (Fig. 1(A)). This result agrees with the findings of a previous human study[14]. On the other hand, amarouciaxanthin A was detected as a hepatic metabolite of fucoxanthinol in mice *in vivo*[11]. Also, our previous mouse study[12] established the pharmacokinetics of amarouciaxanthin A, i.e. C_{max}, 230 nmol/l; $t_{1/2}$, 6·7 h; $AUC_{(\infty)}$, 2040 nmol/l × h. Kistler *et al.*[19] reported that the metabolism of astaxanthin, a xanthophyll, differed between human subjects and rats because culturing human hepatocytes with astaxanthin and the single oral administration of astaxanthin to rats resulted in the induction of different P450 and the production of different metabolites. Thus, fucoxanthinol might also induce different P450 and be converted to unknown metabolites. On the other hand, Asai *et al.*[14] also detected the *cis*-isomer of fucoxanthinol in human plasma after the daily intake of wakame containing 6·1 mg fucoxanthin for 1 week. In the present study, an unknown metabolite (peak I in Fig. 1(A)) was detected at 15·1 min. This peak might have been due to the *cis*-isomer of fucoxanthinol. These results indicate that xanthophylls are metabolised differently in human subjects and rodents. Further studies are needed to examine this assumption in relation to the metabolism of fucoxanthin and fucoxanthinol.

An additional experiment, in which healthy volunteers (five males and five females) with a mean age of 26 (range 22–34) years and a mean BMI of 21·5 (range 19·1–23·9) kg/m^2 were administered five soft-gel capsules containing a total of 0·31 mg fucoxanthin daily for 28 d, showed that this dose did not cause the accumulation of fucoxanthin metabolites in the body (data not shown). This amount is almost equal to the amount of brown algae that is consumed each day in the Japanese diet according to a previous report[20]. Moreover, no harmful effects were observed in general blood tests or biochemical blood tests (data not shown), suggesting that the daily intake of fucoxanthin from sea algae is safe. Although the outer colour and internal tissues of mice became orange when they were fed a 0·1 % fucoxanthin-containing diet for 1 month (approximately 100 mg/kg BW per d), they did not suffer any toxic symptoms[13]. It seems that the toxicity of dietary fucoxanthin is low, even if its metabolites accumulate at low levels. These results suggest that the dose (31 mg; mean intake 0·52 mg/kg BW) used in the present study is safe and is sufficient to induce health benefits.

The present study is the first report on the pharmacokinetics of fucoxanthinol, which is the primary metabolite of fucoxanthin and is considered to be the active form in the body, and also discussed the accumulation of fucoxanthin metabolites in human subjects. Recently, Abidov *et al.*[21] demonstrated that the administration of 2·4 mg fucoxanthin for 16 weeks to obese premenopausal women with non-alcoholic fatty liver disease improved their liver function test results; however, they did not provide any data about the accumulation of fucoxanthin metabolites. Further studies on metabolite accumulation after the administration of fucoxanthin at functional concentrations are needed to clarify the safety of administering fucoxanthin to humans.

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