Characterization of the Disposition of Lutein after i.v. Administration to Rats

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Lutein is a carotenoid that has antioxidant effects. Although lutein has received much attention recently due to its antioxidant activities, little information about the pharmacokinetic properties of lutein is available. The disposition of lutein after i.v. administration has not been investigated because lutein is now used as a supplement. The present study was undertaken to acquire additional data on the disposition of lutein after i.v. administration. After i.v. administration, lutein is preferentially distributed to the liver, spleen and lung. Intravenous administration of lutein may provide effective antioxidant activities in these tissues, not only the eye. The results of this study should provide valuable data for drug development.

Key words lutein; carotenoid; antioxidant

The hypothesized role of oxidation in development of disease has promoted interest in the role of antioxidants in treatment and prevention.¹⁾ One hypothesis for the etiology of age-related disease involves the breakdown of antioxidant systems within the body. The body has several defense mechanisms against oxidation. Dietary carotenoids have been implicated as possible protective agents against oxidation.^{2,3)} Man is not capable of synthesizing carotenoids *de novo* and, thus, their presence in human tissues is entirely of dietary origin. Lutein is a carotenoid that is present in deep-yellow vegetables such as spinach and kale.^{4,5)} In the human eye, lutein is located in the macula lutea, yellow spots, between incoming photons and photoreceptors.⁶⁾ Since yellow spots contribute to eyesight, a lack of the macular pigments causes age-related macular degeneration (ARMD).^{7,8)} It has been proposed that lutein may prevent light-initiated oxidative damage to the retina and retinal pigment epithelium and thus protect against ARMD.⁹⁾ Moreover, an increased intake of lutein appears to be associated with a lower risk of cataract development.¹⁰⁾

Although lutein has received much attention recently due to its antioxidant activities, there is little information about the pharmacokinetic properties of lutein because lutein is now used as a supplement. We have found that the bioavailability of lutein is less than 10% (data not shown). Thus, it is important to investigate its antioxidant effects through another route. Further characterization of its pharmacokinetic properties is needed to increase our understanding of clinical evaluation. In this study, we focused on the i.v. administration route. The present study was undertaken to acquire additional data on the disposition of lutein after i.v. administration. The results of this study suggest potentially favorable pharmacokinetic properties of lutein in humans.

MATERIALS AND METHODS

Chemicals Lutein (Mw. 568.9) was kindly supplied by Kemin Foods, L.C. (Tokyo, Japan) and Koyo Mercantile Co., Ltd. (Kyoto, Japan). Mixed emulsion was composed of 1.5%

Marigold extract, 22.0% Arabia gum, 12.5% propylene glycol, 9.0% sucrose fatty acid ester, 0.5% Dammar gum, 0.5% phosphate, 0.2% tocopherol, 0.2% L-ascorbic acid, 6.0% oil and 47.6% water (W/W%). This emulsion includes more than 0.23% (W/W%) of lutein. All other reagents were of the highest grade available and used without further purification.

Animals Male Wistar rats, aged 7 to 9 weeks (250–350 g in weight), were obtained from NRC Haruna (Gunma, Japan). The housing conditions were the same as those described previously.¹¹⁾ The experimental protocols were reviewed and approved by the Hokkaido University Animal Care Committee in accordance with the “Guide for the Care and Use of Laboratory Animals.”

Dosing and Plasma Collection Lutein was administered in emulsion as a 0.2% liquid solution (0.5 mg/kg body weight). Experimental rats were killed at a designated time after administration of lutein, and tissue samples were excised at that time. The liver, spleen, lungs, kidneys, intestine, brain and fat were removed rapidly, washed, weighed, and homogenized in distilled water using a glass Teflon homogenizer with 20 strokes. Protein content was measured by the method of Lowry *et al.* (1951).¹²⁾ Sequential blood samples were obtained from the femoral vein. Plasma was obtained by centrifugation (850×*g* for 15 min). These operations were done under dim yellow light to minimize isomerization and oxidation of lutein by light.

Analytical Procedures The concentration of lutein was determined by HPLC. One hundred microliters of a sample was diluted twice with distilled water, and 200 μ l of ethanol was added. After vortexing, the sample was extracted with five volumes of a mixture of *n*-hexane and chloroform (4 : 1, v/v). After shaking the mixture vigorously, the sample was centrifuged at 2000×*g* for 10 min. Eight hundred microliters of the organic layer was evaporated to dryness under a nitrogen gas stream. The residue was redissolved in 200 μ l of mobile-phase solution for HPLC injection. The concentration of lutein was determined using an HPLC system equipped with a JASCO 880-PU pump with a 870-UV UV-vis detector. The column was a GL Science Inertsil-CN (5 μ m in particle

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size, 4.6 mm in inside diameter $\times$ 250 mm). A mobile phase containing *n*-hexane/dichloromethane/methanol/diisopropylethylamine (750/250/4/1) was used. Column temperature and flow rate were 30 °C and 1.5 ml/min, respectively. The wavelength for detection was 444 nm. One hundred microliters of a sample was injected into the HPLC system. A standard solution of lutein was prepared with *n*-hexane/acetone/toluene/ethanol (10/7/7/6). Good linearity was obtained in the range of 2.5 ng/ml–100 ng/ml (r^2 value >0.999). Results are presented as lutein+zeaxanthin, due to their unsatisfactory resolution in plasma and tissue samples.

Pharmacokinetic Calculations The elimination rate constant (k_e) was estimated from the plasma concentration–time curves by least squares regression analysis. Area under plasma concentration–time curve (AUC) was calculated by the trapezoidal rule. The volume of distribution (V_d) was obtained using following equation:

$$V_d = \text{Dose} / AUC \cdot k_e$$

Systemic clearance (CL) was calculated using following equation:

$$CL = \text{Dose} / AUC$$

RESULTS AND DISCUSSION

In the first part of this study, we investigated the plasma concentration of lutein after i.v. administration (Fig. 1). Plasma concentration of lutein had decreased at 1 h after i.v. administration. Pharmacokinetic parameters are summarized in Table 1. We then investigated the tissue distribution of lutein. It became clear that large amounts of lutein were distributed to the liver, spleen and lung (Fig. 2). Next, we investigated the time course of the tissue concentration of lutein after i.v. administration. The concentration of lutein in the small intestine reached a maximal level at 6 h after i.v. injection

Fig. 1. Time Profile of the Blood Concentration of Lutein+Zeaxanthin after i.v. Administration (0.5 mg/kg) in Rats

Each point represents the mean with S.D. of three determinations.

Table 1. Kinetic Parameters for Lutein+Zeaxanthin after i.v. Administration (0.5 mg/kg) in Rats

k_e (min^{-1})	0.120
AUC ($\mu\text{g} \cdot \text{min}/\text{ml}$)	4.82
V_d (ml/kg)	864
CL (ml/min/kg)	104

(Table 2). We have recently reported that treatment with lutein by i.v. injection 6 h before ischemia prevented tissue peroxidation and loss of villi following ischemia-reperfusion of the small intestine.¹³ These findings suggest that tissue concentration of lutein is responsible for its antioxidant activities. Although the concentration of lutein in the intestine is smaller than the concentrations in the liver, spleen and lung, lutein has a protective effect on ischemia-reperfusion injury in the small intestine. Taking these findings into consideration, it is possible that the administration of lutein provides effective antioxidant activities in the liver, spleen and lung. It has been reported that oxidative stress induces injury in these tissues.^{14,15} Further studies are needed to assess the antioxidant activity of lutein in these tissues.

Since the spleen acts as a reservoir of erythrocytes,^{16,17} it is possible that lutein is preferentially distributed to erythrocytes. We investigated the blood disposition of lutein after i.v. administration. After i.v. injection, lutein is preferentially distributed to erythrocytes rather than plasma (Fig. 1). Although lutein has high lipid solubility, there was little distribution of lutein to the brain and fat (data not shown). Brain capillary endothelial cells mediate the exchange of solute between blood and brain interstitial fluid. P-Glycoprotein (P-gp/MDR1/ABCB1) works as an efflux pump for various lipophilic compounds in the luminal membrane of brain capillary endothelial cells.¹⁸ The structure of lutein is similar to that of vitamin A. Considering the fact that vitamin A is a substrate for P-gp,¹⁹ it is possible that a low distribution of lutein to the brain is responsible for the P-gp-mediated efflux.

In this study, we investigated the disposition of lutein after i.v. administration because of its low oral bioavailability. To

Fig. 2. Tissue Distribution of Lutein+Zeaxanthin at 1 h after i.v. Administration (0.5 mg/kg) in Rats

Each point represents the mean with S.D. of three determinations.

Table 2. Time Courses of Tissue Distribution of Lutein+Zeaxanthin after i.v. Administration (0.5 mg/kg) in Rats

	Accumulation (ng/mg protein)		
	1 h	4 h	6 h
Liver	12.6 $\pm$ 3.55	36.2 $\pm$ 4.30	11.3 $\pm$ 2.63
Spleen	21.4 $\pm$ 13.0	12.8 $\pm$ 4.92	12.3 $\pm$ 4.43
Lung	9.96 $\pm$ 5.68	1.59 $\pm$ 0.52	1.09 $\pm$ 0.47
Kidney	0.26 $\pm$ 0.13	0.12 $\pm$ 0.07	0.06 $\pm$ 0.03
Intestine	0.25 $\pm$ 0.04	0.12 $\pm$ 0.03	0.69 $\pm$ 0.35

Each value represents the mean with S.D. of three determinations.

evaluate the potential for further development of lutein as a clinical compound, it is important to investigate pharmacokinetics and disposition of lutein through other routes such as percutaneous and pulmonary routes. Since lutein has high lipid solubility, a percutaneous route may be a desirable route.

In summary, lutein is preferentially distributed to the liver, spleen and lung. Intravenous administration of lutein may provide effective antioxidant activities in these tissues, not only the eye. The results of this study should provide valuable data for clinical drug development.

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