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Citation	Biological & Pharmaceutical Bulletin, 29(8), 1764-1766 https://doi.org/10.1248/bpb.29.1764
Issue Date	2006-08
Doc URL	https://hdl.handle.net/2115/14885
Type	journal article
File Information	B&PB29-8.pdf

Protective Effect of Lutein on Ischemia-Reperfusion Injury in Rat Small Intestine

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Lutein is a carotenoid and it has antioxidant effects. Lutein may have a protective effect on ischemia reperfusion (I/R) injury induced by free radical species. However, little is known about the protective effect of lutein on I/R injury *in vivo*. The present study was undertaken to clarify the protective effects of lutein on I/R injuries in the rat small intestine. Administration of lutein before intestinal I/R attenuated the damage to villi and deciduation of enterocytes and suppressed the increase in lipid peroxide.

Key words   lutein; ischemia-reperfusion; carotenoid; antioxidant

Lutein is a carotenoid that is present in deep-yellow vegetables such as spinach and kale. It has a structure similar to that of beta-carotene, which is the forerunner material of vitamin A.[1] Vitamin C and vitamin E have received much attention recently due to their antioxidant activities.[2—4] It has been reported that lutein modulates cellular oxidative status. Since lutein has maximum absorption at 445 nm, it can act as a blue-light filter (400—460 nm).[5—7] It is well known that carotenoids, such as lutein and zeaxanthin, are macular pigments that are located in the macula lutea, yellow spots, between incoming photons and photoreceptors. Since yellow spots contribute to eyesight, the lack of the macular pigments bring age-related macular degeneration (ARMD).[8—10] Lutein has been shown to play an important role in the prevention of ARMD, cataract and other blinding disorders. It has been reported that lutein improves visual function in ARMD patients, according to the results of the randomized, double-masked Lutein Antioxidant Supplementation Trial (LAST).[11] Recently, it has been revealed that reactive oxygen is responsible for ARMD. The protective effects of the carotenoids on ARMD have been extensively studied.[12—14] Besides ARMD, it has been shown that oxygen free radicals contribute to the development of ischemia-reperfusion (I/R) injuries, cataract, glaucoma and cancer. In this study, we focused on the protective effect of lutein on intestinal I/R injury.

Since a large amount of oxygen flows in tissue at reperfusion, reactive oxygen is thought to play a major role in I/R failure. Reactive oxygen participated in the I/R failure have received attention.[15—19] It has been shown that oxidation can be prevented by pretreatment with an antioxidant such as allopurinol or N^G-nitro-L-arginine methyl ester.[20—22] Disruption of the intestinal mucosal barrier predisposes to extraluminal egress of potentially harmful bacteria and/or their toxins. Attention has been focused on the role of the gastrointestinal tract as a source of pathogens that may cross a disrupted mucosal barrier initiating a septic process and perpetuating multiorgan failure.[23,24]

The present study was undertaken to clarify the protective effect of lutein on I/R injury of rat small intestine.

MATERIALS AND METHODS

Chemicals   Lutein was kindly supplied by Kemin Foods, L.C. (Tokyo, Japan) and by Koyo Mercantile Co., Ltd. (Kyoto, Japan). 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.[25] 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".

Intestinal I/R Model   Surgical procedures were carried out as described in a previous report with some modification.[26] Rats were not fed for 16 h prior to the experiments but were allowed free access to water. The animals were anesthetized with sodium pentobarbital (30 mg/kg body weight, i.p. injection). Through a midline laparotomy, each rat was subjected to 30 min of ischemia by occluding the superior mesenteric artery (SMA), and reperfusion was produced by removing the clamp. The abdomen was then covered with a sterile plastic wrap. Experimental rats were killed at 1 h after reperfusion, at this time tissue samples were excised. Lutein was administered in emulsion as a 0.2% liquid solution (0.5 mg/kg body weight).

Tissue Sampling   The 15-cm-long portion of the intestine was excised to measure the protein and lipid peroxide contents and the amounts of lutein that accumulated in the intestine. The intestine was cleansed in ice-cold saline and then homogenized in 2.0 ml distilled water using a glass Teflon homogenizer with 20 strokes. Protein content was measured by the method of Lowry *et al.*[27]

TBA Analysis   The amount of lipid peroxide in the intestine was determined as that of malondialdehyde (MDA) by the method of Ohkawa *et al.* with some modification.[28] Thiobarbituric acid (TBA) solution consisted of 2.6 mM TBA, 918 mM trichloroacetic acid, 0.3 mM HCl, and 1.8 mM 2,6-di-*tert*-butyl-4-metylphenol (BHT) in 22% ethanol. The reaction mixture contained 0.2 ml of tissue homogenate, 0.2 ml of 8.1% sodium dodecyl sulfate (SDS), 1.5 ml of 20%

acetic acid solution (pH 3.5), and 1.5 ml of 0.8% aqueous solution of TBA. The mixture was heated at 95 °C for 60 min. After cooling with tap water, 1.0 ml of distilled water and 5.0 ml of n-butanol were added, and the mixture was shaken vigorously. After centrifugation at $3000 \times g$ for 10 min, the absorbance of the organic layer (upper layer) was measured at 535 nm with 1,1,3,3-tetraethoxypropane (TEP) as a standard.

Measurements of Intestinal Concentration of Lutein
The protocol used for analyzing lutein was kindly donated by Daiichi Pure Chemicals Co., Ltd. (Tokyo, Japan). The concentration of lutein in the intestine was determined by HPLC. One hundred microliters of sample was diluted twice with distilled water, and 200 μl 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 $2000 \times g$ for 10 min. Eight hundred microliters of the organic layer was evaporated to dryness under a nitrogen gas stream. The residue was redissolved in 0.2 ml 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 μm in particle size, 4.6 mm in inside diameter$\times$250 mm). A mobile phase containing n-hexane/dichoromethane/methanol/diisopropylethlamine (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 sample was injected into the HPLC system. We used lutein in corn oil as a 20% liquid solution for a standard solution. A standard solution of lutein was prepared with n-hexane/ethanol/acetone/toluene (7/6/5/5). Good linearity was obtained in the range of 2.5—100 ng/ml (r^2 value >0.999).

Histological Study Samples of jejunum or ileum were taken after reperfusion and immediately fixed in 10% buffered formalin. The fixed tissue was embedded in paraffin and thin-sectioned (5.0 μm). Slides were stained using hematoxylin and eosin to evaluate the intestinal morphology under a light microscope for classification.

Data Analysis Statistical significance was evaluated using one-way analysis of variance (one-way ANOVA) or unpaired Student's t-test. A value of $p<0.05$ was considered significant.

RESULTS AND DISCUSSION

Intestinal ischemia-reperfusion (I/R) is a grave condition caused by acute mesenteric ischemia, small bowel transplantation, abdominal aortic aneurysm, traumatic or septic shock or severe burns. Ischemia itself produces the intestinal damage, but the major damage is caused by reperfusion.[15] I/R occurs in other tissues, including the stomach, pancreas, and cardiac and skeletal muscle.[15,29,30] Production of free radical species in tissue is known to cause many pathological disorders.[16—19] Therefore, free radical scavengers play an important role in the prevention of various human diseases. It has been reported that the transport of lutein in Caco-2 cells is facilitated and involves the scavenger receptor SR-BI.[31] In this study, we investigated the antioxidative activity of lutein using a rat I/R injury model.
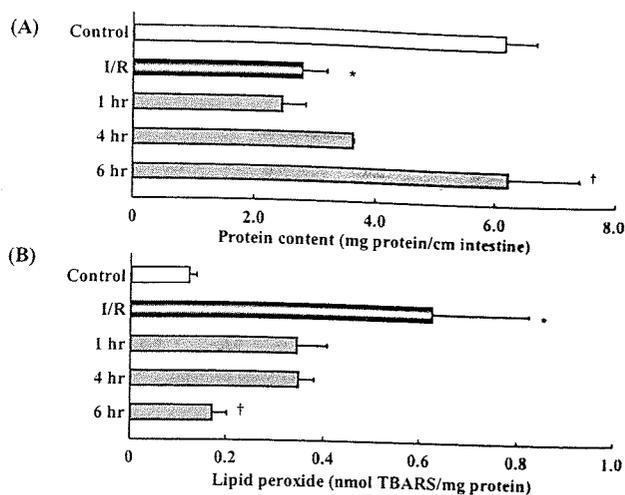

Fig. 1. Effect of Lutein on the Protein Content (A) and the Amount of MDA (B) in the Small Intestine after i.v. Injection (0.5 mg/kg Body Weight)

Each column represents the mean with S.E.M. of 3—6 measurements. * $p<0.05$, significantly different from the control. †$p<0.05$, significantly different from that in the absence of lutein.

In the first part of this study, we investigated the time course of the effect of lutein on the protein content and the amount of MDA in the small intestine after i.v. administration. Treatment with lutein by i.v. injection 6 h before ischemia prevented the decrease in protein in the small intestine (Fig. 1A). Moreover, the increase in lipid peroxide after I/R was significantly inhibited by the treatment with lutein (Fig. 1B). These findings suggest that lutein that accumulated in the small intestine reduced the tissue peroxidation and loss of villi following I/R of the small intestine.

Oral delivery is generally the most desirable means of drug administration, mainly because of patient acceptance, convenience in administration, and cost-effective manufacturing. Lutein is now used as a supplement. Intake of antioxidants from nutrients as well as nonnutrients is related to health outcomes. Plasma concentration of lutein in rats reached a maximal level at 3 h after oral administration (unpublished data). Lutein was therefore administered orally at three hours before ischemia. The gastrointestinal tract works as the first barrier to the absorption of nutrients. The absorption of drugs from the gastrointestinal tract is one of the important determinants for oral bioavailability. In the second part of this study, we investigated the protective effect of oral administration of lutein on I/R injury in the rat small intestine. Treatment with lutein by oral administration 3 h before ischemia prevented the decrease in protein in the small intestine (Fig. 2A). The increase in lipid peroxide after I/R was significantly inhibited by the treatment with lutein (Fig. 2B).

Multiple erosions were observed microscopically after I/R injury. Damage to the crypt and shortening of the villi are typical histological findings after I/R injury. Although pretreatment with lutein prevented damage to the crypt, the loss of the villi was not attenuated by pretreatment with lutein (Fig. 3). The recovery of intestinal mucosa starts on the crypt layers. Thus, crypt layers play an important role in tissue regeneration. Intestinal mucosa is sensitive to I/R. In the case of intestinal I/R, the crypt has high reproduction ability in order to keep intestinal mucosa normal. It is possible that administration of lutein strengthens the tissue reproduction

(A)

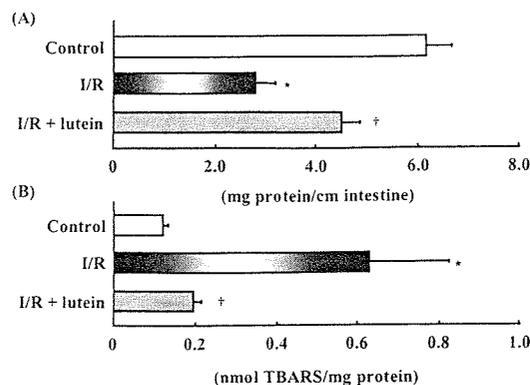(mg protein/cm intestine)

(B)

(nmol TBARS/mg protein)

Fig. 2. Effect of Lutein on the Protein Content (A) and the Amount of MDA (B) in the Small Intestine after Oral Administration (0.5 mg/kg Body Weight)

Each column represents the mean with S.E.M. of 3—7 measurements. $*p<0.05$, significantly different from the control. $\dagger p<0.05$, significantly different from that in the absence of lutein.
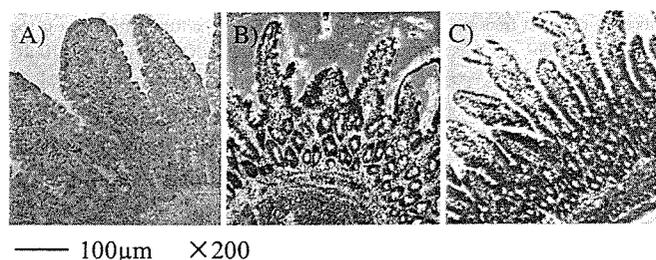

100μm ×200

Fig. 3. Microscopic Appearance after Hematoxylin and Eosin Staining of Intestinal Tissue after I/R (×200)

(A) Untreated control intestine. (B) I/R-treated intestine. (C) I/R-treated intestine pretreated with lutein by oral administration.

ability.

In this study, pretreatment with lutein could not prevent the loss of villi but could prevent I/R injury of crypt layers. Since intestinal mucosa has an extremely high reproduction ability, pretreatment with lutein advances the regeneration of intestine, and decreases the tissue injury from I/R.

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