ABSTRACT: The mice were fed daily with lycopene nanoliposomes or lycopene-rich oil by gavage. Liver and serum levels of lycopene and activities of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and the content of malonaldehyde (MDA) in liver were determined. Liver lycopene content as well as activities of SOD, POD, and CAT were higher in the mice treated with lycopene nanoliposomes compared to that of lycopene-rich oil treated mice. In contrast, MDA content was significantly decreased by lycopene nanoliposomes treatment. These results indicate that nanoliposomes can robustly increase the antioxidant capability of lycopene in vivo. In summary, our results suggest that nanoliposome delivery systems may be useful as a means to improve the bioavailability of lycopene in liver.

KEY WORDS: Absorption, Antioxidant, Bioavailability, Lycopene, Nanoliposomes.

INTRODUCTION

Lycopene is a kind of carotenoid that cannot be synthesized by body. It is a natural food pigment that has been suggested by some, but not at all, to play a preventive role in a variety of conditions, such as prevention of cancer and cardiovascular diseases, enhancement of immunity, as well as anti-aging properties (Agarwal and Rao, 2000; Levy et al., 1995; Livny et al., 2002; Breinholt et al., 2000; Stahi et al., 2006). While lycopene has been called the gold hidden in tomatoes, because of its insolubility in water and low oral bioavailability, its application is greatly limited. Consumption of lycopene with dietary fat can improve the absorption of lycopene; however this practice may lead to dietary imbalance with excessive fat intake (Fan and Huang, 2007).

In recent years, nanotechnology has been steadily incorporated in agriculture and food science known as “Agrifood Nanotechnology” (Kuzma and VerHage, 2006). Much of this research and the development of nano foods and nutrients has been carried out in the United States, Japan and other developed countries. In particular, nanocarriers for embedding nutrients in the production of functional food ingredients have become a hot spot of nanotechnology research (Tarver, 2006; Chen and Shahidi, 2006). Nanocarrier research involves the development of micro-capsule technologies including nanoliposomes, solid lipid nanoparticles, polymer nanocapsules, nanosphere, nano-dispersion, etc.

Liposomes are self-assembled colloidal particles that can be found naturally or can be artificially prepared. Historically, liposomes have been used as artificial membranes through which to study the structure and function of biofilms. In the 1970s, after Gregoriadis proposed using liposomes as carriers for β-galactosidase to treat glycogen storage disease, investigators began to use liposomes as carriers to control drug release with water-soluble drugs encapsulated in the aqueous phase and fat-soluble drugs contained within lipid bilayers. For several decades, liposomes were mostly used in the fields of medicine and cosmetics; however, in the 1990s liposomes began to be used in the food industry, especially in dairy products. In their application to the dairy industry liposomes have been used to embed enzymes (rennet) to ripen cheese (Ehab et al., 2003; Walde and Ichikawa, 2001), Nisin Z to prevent microbiological contamination (Laridi et al., 2003), and vitamin D (Bavlille et al., 2000) and FeSO4 (Xia and Xu, 2005) to improve the nutritional quality of dairy products. Standard liposomes (1-100 μm) are limited in their application because they do not penetrate cells due to their relatively large size. Nanoliposomes, on the other hand, have the capacity to penetrate cells because of their small size and they can also target specific tissues with surface markers (Weiss et al., 2006).

In order to improve the bioavailability of lycopene, we propose to deliver lycopene with nano-materials. Nano-carriers (such as nanoliposomes) as delivery systems can improve water-solubility and bioavailability of many lipophilic nutrients. Furthermore liposomes and biofilms have good compatibility...
because of their similar compositions and membrane bilayer structures. When consumed orally, nanoliposomes can also increase absorption of compounds into the intestinal epithelium and can extend the absorption time. Lycopene embedded within nanoliposomes may be both more stable and more bioavailable than lycopene alone.

The main antioxidant enzymes in liver are glutathione peroxidase, CAT, SOD and POD. We chose to assess the activity of CAT, SOD and POD in this study. SOD is the only enzyme in vivo that can remove free radicals directly, and the higher the activity of SOD, the greater the ability to remove free radicals. When too many free radicals attack unsaturated fatty acids in cell membranes, lipid peroxidation generates MDA. The content of MDA in the body represents the degree of lipid peroxidation (Macmillan Crow and Cruthirds, 2001).

In this paper, lycopene nanoliposomes were prepared by film-ultrasound. Mice were treated daily with lycopene nanoliposomes or lycopene-rich oil. The content of lycopene in blood and liver were compared and antioxidant effects were assessed. The mechanisms through which nanoliposomes may improve the bioavailability and antioxidant efficacy of lycopene are also discussed.

MATERIALS AND METHODS

Materials

Lycopene (90%) was purchased from North China Pharmaceutical (Shijiazhuang, China). Cholesterol was obtained from Huixing Biochemical Reagents (Gangdong, China). Lecithin was provided by Poly Biological Technology Co. (Shanghai, China). All other reagents were of analytical grade.

Preparation of lycopene nanoliposomes and lycopene-rich oil

To prepare the lycopene nanoliposomes 0.01 g lycopene, 0.15 g cholesterol and 1.0 g lecithin were dissolved in 20 ml chloroform. The solution was rotated at 34 °C in the dark for 10 min. After the solution formed a uniform film the residual solvent was removed, the chloroform was recycled, and 30 ml of pH 7.4 PBS buffer, 0.05 ml of Tween-80 and glass beads were added and the sample was rotated to wash the membrane. After about three hours the membrane was completely eluted into the buffer. After that the mixture was ultrasonicated in an ice bath for 15 min, where it formed a transparent colloidal solution of lycopene nanoliposomes with a pink opalescence. The solution was filtered through a 0.8 µm microporous membrane to remove unwanted compounds and metal particles. This process yielded lycopene nanoliposomes containing 180 µg lycopene /ml.

To prepare the lycopene-rich oil 0.0027 g lycopene was dissolved in 15 ml soybean oil and stirred with a magnetic stirrer until completely dissolved. The lycopene-rich oil contained 180 µg lycopene /ml.

Morphology of lycopene nanoliposomes

The freeze-dried powder of lycopene nanoliposomes was dispersed in the top of the conductive adhesive. The morphology of lycopene nanoliposomes was observed with a scanning electron microscope (SEM, JSM-6490LV, Japan).

Detection of lycopene nanoliposomes

The membranes of lycopene nanoliposomes were lysed to release lycopene, and the color change was compared. The UV absorption was measured at 518 nm (Hou et al., 2007) using a UV–Vis spectrophotometer (Rayleigh Analytical Instrument Corporation, Beijing, China).

Animal experiment

Male mice with weights of about 20 ± 2 g were fed with a standard mouse chow for one week to adapt to the laboratory conditions and then they were divided into three groups of ten each. The three groups were as follows:

1) Control group: standard diet, gavaged daily with 4 ml saline for four weeks.
2) Lycopene-rich oil group: standard diet, gavaged daily with 4 mg/kg dosed by body weight for four weeks.
3) Lycopene nanoliposomes group: standard diet, gavaged daily with 4 mg/kg dosed by body weight for four weeks.

After completion of the treatment period the mice were fasted overnight, blood was collected by retro-orbital puncture, and the mice were euthanized by cervical dislocation. Livers were collected for biochemical analysis.

Pretreatment of the liver and serum

The blood was centrifuged at 3000 rpm for 10 min and the serum was stored at -40°C until analysis. The liver homogenate (0.5g liver tissue added to 4.5ml chilled pH 7.4 PBS buffer) was centrifuged at 15,000 rpm for 15 min at 4°C, and the supernatant was used for enzymatic analyses. The liver homogenate and serum were pretreated as described previously (Tian et al., 2007). One hundred µl of sample was used for HPLC analysis where the peak area (Y) was recorded and the concentration (X) was calculated from the standard curve equation.

Chromatographic conditions

Analysis was performed using a HPLC system (Waters Corporation, USA) comprised of a 515 Pump, 2487 UV detector. A C18 column, 4.6x150mm, 5µm particle size was used. The mobile phase was methanol, the ratio of acetonitrile to dichloromethane was 70:70:20. The detection wavelength was 472 nm. The injection volume was 20 µl. The instrument room was maintained at 22 °C.

The standard curve of serum and liver

Twenty mg lycopene and 20 mg 2, 6-di-tert-butyl-p-cresol (BHT) was dissolved in LC dichloromethane to prepare 0.8 mg/ml lycopene solution. Then the solution was progressively diluted to 12.8µg/ml, 6.4 µg/ml, 3.2 µg/ml, 1.6 µg/ml, and 0.8 µg/ml for the standard solutions.
One-tenth ml of 0.8 μg/ml, 1.6 μg/ml, 3.2 μg/ml, 6.4 μg/ml, and 12.8 μg/ml standard solutions were each added to 0.1 ml serum, then the mixture was processed as described above in the section on pretreatment of the liver and serum. From the HPLC chromatogram, a standard curve was drawn with the peak areas used for the ordinate and the concentrations of the lycopene standard solutions used for the abscissa. The standard curve for liver lycopene content was drawn with the same method.

**Precision test**
Serum and liver homogenate samples containing 0.8 μg/ml, 6.4 μg/ml, and 12.8 μg/ml lycopene were prepared in triplicate for each group and the differences were measured.

**Oxidation experiment**
Protein level, MDA content and POD, CAT, and SOD activity were determined in the liver extracts with commercially-available kits (Nanjing Jiancheng Bioengineering Institute, China).

**Statistical analysis**
Experiments were performed in triplicate with the results expressed as means ± SD. Statistix 8.1 (Analytical Software, St.Paul, MN, USA) was used for statistical analysis. Significant differences (P < 0.01, P < 0.05) between sample averages were identified using t-test.

**RESULTS AND DISCUSSION**

The electron microscope morphology of lycopene nanoliposomes
The SEM photographs at different magnifications (2×10^4, 4×10^4, 8×10^4) (Figure 1) showed that liposomes were round or oval microspheres with an average size of 40 nm or less.

**FIGURE 1.** SEM photographs to observe the morphology of lycopene nanoliposomes at different magnifications: (a) ×2×10^4; (b) ×4×10^4; (c) ×8×10^4.

Detection of lycopene nanoliposomes
It can be seen from Figure 2 that the color of a solution containing lycopene nanoliposomes was completely different before and after rupture of the liposome membranes. Specifically the color changed from red to orange (the color of lycopene dissolved in chloroform). Figure 3 shows that the UV absorption spectrum of the solution after membrane rupture is consistent with the spectrum of lycopene alone. This indicates that indeed lycopene was embedded in the liposomes.
FIGURE 3. UV spectrogram of ruptured lycopene nanoliposomes. The membranes of lycopene nanoliposomes were lysed to release lycopene and the UV absorption was measured at 518 nm. The UV absorption spectrum after rupture of the lycopene nanoliposomes was consistent with the spectrum of lycopene alone.

FIGURE 4. HPLC chromatogram of lycopene. A standard solution of lycopene was analyzed by HPLC.

FIGURE 5. (a) HPLC chromatogram of serum in the control group; (b) HPLC chromatogram of serum in the experimental group.
HPLC Chromatogram
The retention time of lycopene was 6.805 (Figure 4), which is consistent with an earlier report (Xu et al., 2006).

The control serum extract had no absorption peak at 6.805, so there was no interference in the determination of lycopene content in serum (Figure 5). Likewise the control liver extract had no absorption peak at 6.805 (Figure 6), so there was no interference in the determination of lycopene content in liver.

Precision test
Under the chromatographic conditions of the current study the intra-day and inter-day precision of lycopene in the serum and liver (RSD d’ 4%) meet the requirements necessary for accurate results (Table 1).

TABLE 1. Precision test. In order to verify accuracy of the method, the intra-day and inter-day precision of lycopene in the serum and liver was measured. Data points expressed as means ± SD (n = 3).

<table>
<thead>
<tr>
<th>Tissues</th>
<th>C (μg/ml)</th>
<th>Intra-day X±SD(μg/ml) (%)</th>
<th>RSD</th>
<th>Inter-day X±SD(μg/ml) (%)</th>
<th>RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>0.8</td>
<td>0.78±0.11</td>
<td>1.12</td>
<td>0.81±0.45</td>
<td>2.06</td>
</tr>
<tr>
<td></td>
<td>6.4</td>
<td>6.37±0.26</td>
<td>2.49</td>
<td>6.38±0.67</td>
<td>4.54</td>
</tr>
<tr>
<td></td>
<td>12.8</td>
<td>12.74±0.67</td>
<td>3.66</td>
<td>12.83±0.23</td>
<td>1.35</td>
</tr>
<tr>
<td>Liver</td>
<td>0.8</td>
<td>0.77±0.24</td>
<td>2.8</td>
<td>0.78±0.02</td>
<td>2.55</td>
</tr>
<tr>
<td></td>
<td>6.4</td>
<td>6.39±0.71</td>
<td>1.72</td>
<td>6.35±0.32</td>
<td>2.61</td>
</tr>
<tr>
<td></td>
<td>12.8</td>
<td>12.78±0.10</td>
<td>2.52</td>
<td>12.77±0.88</td>
<td>2.18</td>
</tr>
</tbody>
</table>

Lycopene concentrations in serum and liver
The content of lycopene in liver was significantly higher (p < 0.01) in the mice treated with lycopene nanoliposomes than in the mice treated with lycopene-rich oil group (Table 2). Serum lycopene was not significantly changed (Table 2).

These results indicate that nanoliposomes can significantly increase the content of lycopene in liver compared with traditional oil-based delivery methods. Nanoliposomes can easily penetrate the small intestine because of their large surface area, small particle size and surface hydrophilicity. The lycopene nanoliposomes also seemed to promote the accumulation of lycopene in the liver. Therefore nanoliposomes as a means of drug delivery could enhance the efficacy of drugs, and achieve the purpose of targeted drug delivery.

Antioxidant effect
Table 3 shows the activities of POD, CAT, and SOD were significantly higher in the livers of mice treated with the lycopene-rich oil while the content of MDA was lower compared to the control group (p < 0.01). These results
are consistent with a previous report (Fan and Huang, 2007). The activities of POD, CAT, and SOD in the mice treated with lycopene nanoliposomes were significantly higher (p < 0.01) and the content of MDA was lower (p < 0.05) than in the mice treated with lycopene-rich oil.

Our analysis of antioxidant capacity in the mice indicate that the activities of POD, CAT, and SOD in both lycopene-treated groups were significantly higher than in the control group and the content of MDA was lower. Our study further shows that the activity of liver antioxidant enzymes was most strongly increased by treatment with lycopene embedded in nanoliposomes and that lycopene nanoliposomes were targeted to the liver. This suggests that nanoliposome carriers could improve the biological effects and bioavailability of oral lycopene in mammals.

### CONCLUSION

In this paper, lycopene nanoliposomes were prepared by nanocarrier technology and were fed to mice to assess the effects on antioxidant status. Our aim was to increase the applications for which nanotechnology could be applied to food nutrients and to show that lycopene is not only a nutrient, but can also be used as a drug. By employing nanotechnology we showed that encapsulation of lycopene in nanoliposomes improves the water solubility of lycopene and increases its oral bioavailability. The experiment also showed that lycopene nanoliposomes exhibit passive targeting to the liver because the antioxidant effect in the livers from mice treated with lycopene nanoliposomes was more robust compared with livers from mice treated with lycopene-rich oil. Taken together our results indicate that lycopene nanoliposomes could improve the biological effects and bioavailability of oral lycopene and provide a basis for further study of food nanoliposomes.

### REFERENCES


