Effect of Oxidized Frying Oil and Vitamin C Levels on the Hepatic Xenobiotic-Metabolizing Enzyme System of Guinea Pigs

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Summary

The influence of oxidized frying oil (OFO) on the guinea pig hepatic microsomal xenobiotic-metabolizing enzyme system in the presence of different amounts dietary vitamin C was investigated. Weanling male guinea pigs were divided into four groups and were fed 15% oxidized frying oil diets supplemented with vitamin C at 300, 600, or 1,500 mg/kg (experimental diets) or a control diet that contained 15% fresh untreated soybean oil with 300 mg/kg of vitamin C, respectively. After 60 d, guinea pigs were euthanized and phase I and phase II xenobiotic-metabolizing enzymes in the liver were determined. Compared with the fresh oil diet fed the control group, the relative liver weight was higher in the OFO-fed groups. Hepatic microsomal protein and cytochrome P450 contents were significantly higher in OFO-fed guinea pigs than in the control group. Both values increased in response to increased intake of vitamin C. The activities of phase II relative components, including UDP-glucuronyl transferase, UDP-glucuronyl dehydrogenase and β-glucuronidase, of guinea pigs fed the OFO diets supplemented with 300 mg vitamin C/kg were significantly higher than those of guinea pigs fed the control diet. However, the phase II relative components decreased with increasing vitamin C content in the diet. The results demonstrate that both dietary OFO and vitamin C in guinea pigs induce hepatic xenobiotic metabolizing enzymes, but the level of induction is modulated by the dietary vitamin C level.

Key Words: cytochrome P450, guinea pigs, oxidized frying oil, vitamin C, xenobiotic-metabolizing enzymes

The nutritional and biological effects of thermally oxidized fat, which can be considered a kind of oxidative stress, have been studied and reviewed extensively (1–3). Appetite and growth depression, decreased fat absorption, and liver and kidney enlargement were the most common observations in long-term feeding studies. Liver enlargement is frequently accompanied by an increase in microsomal enzyme activities in animals dosed with xenobiotics. Most drugs and xenobiotics introduced in animals and humans undergo extensive transformation in the liver. The type, extent, and rate of these biotransformations determined the efficacy or toxicity of these agents. These biotransformations are conventionally considered to be carried out by phase I and phase II enzyme systems. Cytochrome P450 enzymes (CYP450), the major phase I components, have a double-edged role in animals. They are important in the bioactivation of chemical carcinogens, in the biotransformation of many endogenous (i.e., fat-soluble) vitamins, steroids, etc., and in the detoxification of numerous xenobiotics (4, 5). Phase II components, which include transferase, dehydrogenase, and glucuronidase, are concerned with conjugation and detoxification reactions. Both phase I and phase II enzymes have important health implications for animals and humans, and are reported to be influenced by various dietary factors (6, 7).

It is well established that vitamin C is an important antioxidant in mammalian systems. In addition to its antioxidant function, vitamin C has been shown to play an important role in drug oxidation via its influence on CYP450 (7–9). We have reported that guinea pigs fed with a diet containing 15% oxidized frying oil (OFO) had significantly lower vitamin C concentrations in the plasma and most tissues than guinea pigs fed a diet containing 15% fresh soybean oil (10). Furthermore, thermally oxidized fat is generally thought to contain potentially toxic lipid peroxidation products, which might have to be metabolized by the hepatic xenobiotic-metabolizing enzyme system in the rat model (11, 12). This study was designed to assess the influence of OFO on the hepatic microsomal xenobiotic-metabolizing enzyme activities in guinea pigs and to study the modulation of dietary vitamin C supplementation on hepatic xenobiotic-metabolizing enzymes in OFO-fed guinea pigs.

Materials and Methods

Diets and animal care. The OFO and diets used in this experiment were essentially from the same batch as those used in the experiment reported previously (10). The OFO diets contained 15 g of OFO per 100 g diet
supplemented with 300, 600 or 1,500 mg vitamin C/kg as D300, D600 and D1500 diets, respectively. The control diet contained 15 g of fresh soybean oil per 100 g diet supplemented with 300 mg vitamin C/kg.

Twenty-four weanling male guinea pigs (N: HART) were purchased from the Laboratory Animal Center, College of Medicine, National Taiwan University (Taipei). Animals were treated in accordance with the Guide for the Care and Use of Laboratory Animals (13) as reported previously (10).

Tissue sampling and preparation. After 60 d of feeding, guinea pigs were anesthetized with sodium pentobarbital. Blood was collected from the abdominal vena cava into a tube containing heparin and analyzed for hematological variables. Plasma was separated by centrifugation at 1,000 g for 10 min. Livers were excised, weighed, and a portion of the liver sample was immediately homogenized in ice-cold 0.01 mol/L potassium phosphate buffer (pH 7.4) containing 11.5 g/L KCl. Aliquots of the crude homogenate (25% w/v) were centrifuged at 12,000×g for 20 min to obtain the post-mitochondrial supernatant (PMS). Aliquots of PMS were centrifuged at 105,000×g for 60 min to obtain the microsomal pellets (MP). The MP were suspended in 0.05 M phosphate buffer (pH 7.7) containing 10 mmol/L EDTA and 30% glycerol. Both PMS and MP were stored at −80°C until analyzed. The remaining liver was frozen in liquid nitrogen and stored at −80°C until analyzed.

Xenobiotic-metabolizing enzyme system analyses. Plasma and liver vitamin C concentrations were analyzed using the 2,4-dinitrophenylhydrazine method (14), as reported previously (10). The PMS was assayed for UDP-glucuronol transferase (UDPGT), UDP-glucuronol dehydrogenase (UDPGDHase), and β-glucuronidase activities. UDPGT activity was assayed according to the method of Thurman et al. (15) using p-nitrophenol as the substrate. The change in absorbance at 340 nm of NADH was measured in the UDPGDHase activity assay (16). The β-glucuronidase activity was determined according to the spectrophotometric method of Marsh (17). The microsomal protein was quantified using the Lowry method. Total cytochrome P450 (CYP450) content was determined by the dithionite CO binding difference spectrum according to the procedure described by Omura and Sato (18).

Statistical analyses. Significant differences between groups were analyzed using ANOVA and Duncan’s multiple range test using the General Linear Model of the SAS package. Linear regression analysis was employed to assess the dietary vitamin C effect on hepatic microsomal protein and CYP450 content, cytosolic UDPGT, UDPGDHase, and β-glucuronidase among the three OOF-fed groups. Differences of p<0.05 were considered significant.

Results and Discussion

Guinea pigs fed the OOF diet had significantly lower body weight gain over the 60-d feeding (10). Variations in vitamin C intake were reflected in the plasma and liver vitamin C concentrations. The relative liver weight was slightly higher in OOF-fed guinea pigs than in the control group. Guinea pigs fed the D300 diet had lower plasma and hepatic vitamin C concentrations than did those in the control group (Table 1). Guinea pigs fed the D300 diet had lower plasma and hepatic vitamin C concentrations than did those in the control group (Table 1). Guinea pigs fed the OOF diet supplemented with higher vitamin C levels (600 mg/kg, 1,500 mg/kg) had higher vitamin C concentrations in the plasma and liver than did the control and D300 groups. The hepatic microsomal protein and CYP450 content were significantly higher in the OOF-fed groups than the control group. Hepatic microsomal protein and CYP450 content increased significantly with rising vitamin C supplementation. Guinea pigs fed OOF diets had elevated phase II enzyme—UDPGT and
Table 2. Correlation between dietary vitamin C and hepatic protein, cytochrome P450 content, activities of phase II relative components.

<table>
<thead>
<tr>
<th></th>
<th>R</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>0.761</td>
<td>0.0002</td>
</tr>
<tr>
<td>Cytochrome P450</td>
<td>0.606</td>
<td>0.0077</td>
</tr>
<tr>
<td>UDPGT</td>
<td>-0.732</td>
<td>0.0006</td>
</tr>
<tr>
<td>UDPGDHase</td>
<td>-0.710</td>
<td>0.0010</td>
</tr>
<tr>
<td>β-glucuronidase</td>
<td>-0.719</td>
<td>0.0008</td>
</tr>
</tbody>
</table>

* Analyzed using linear regression analysis of data from the three OFO-fed groups (D300, D600, and D1500).

a UDPGT: UDP-glucuronyl transferase, UDPGDHase: UDP-glucuronyl dehydrogenase.

with an increase in dietary vitamin C level and hepatic vitamin C concentration was found among the three OFO-fed groups. So, it appears that the level of hepatic CYP450 was not only affected by OFO feeding but also by dietary vitamin C content. The increase in total CYP450 content would point towards increased detoxification efficiency with higher dietary vitamin C. Thus, vitamin C promotes degradation of xenobiotics and reduction of oxidative damage from xenobiotics in these animals.

Concerning the other relative enzymes, UDPGT is the major conjugation enzyme in the phase II system. UDPGDHase and β-glucuronidase are the major enzymes for the synthesis and degradation of glucuronic acid, respectively. Glucuronic acid is the dominant substrate of the phase II enzyme system. The amount of glucuronic acid affects the ability to metabolize xenobiotics in animals. The UDPGT, UDPGDHase and β-glucuronidase activities were markedly elevated in guinea pigs fed with OFO. However, a negative correlation was observed between dietary vitamin C levels and the activities of these three enzymes. Based on these results, it seems that the potentially toxic products of OFO are degraded and metabolized primarily through the phase I enzyme system. Thus, fewer OFO products were metabolized and degraded through the phase II enzyme system of OFO-fed guinea pigs, especially when supplemented with vitamin C. If the ability to metabolize xenobiotics in OFO-fed animals increased through the phase I enzyme system with increased dietary vitamin C levels, the oxidative damage from OFO would decrease. The activities of phase II relative enzymes were also decreased correspondingly in vitamin C-supplemented OFO-fed guinea pigs. It is suggested that the possibility of protection afforded by vitamin C could be mediated by modifying the activities of these xenobiotic-metabolizing enzymes in the liver.

In conclusion, the results obtained in this study clearly indicate that both OFO and dietary vitamin C levels have a marked effect on the induction of xenobiotic-metabolizing enzymes in guinea pigs. Dietary vitamin C is an important and required factor for xenobiotics metabolism in animals and humans who can not synthesize vitamin C in the body. However, the major CYP450 isofrom profiles that participate in the OFO metabolism remain to be investigated.

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