Effect of cholestin on toxicity of vitamin A in rats

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ABSTRACT

A study was undertaken to investigate the effect of cholestin on the toxicity of vitamin A in male wistar rats. The rats were divided into six groups and fed different diets with or without supplement of 1% cholestin and 25,000–50,000 (IU) vitamin A for 2 months. Hence, the symptoms of vitamin A toxicity in rats included loss of body weight, hepatotoxicity and nephrotoxicity. However, these toxic effects of vitamin A were significantly reduced when the rats fed a diet supplemented with cholestin. Furthermore, the level of vitamin A in the serum of rats treated with cholestin and vitamin A was higher than that of the rats treated with vitamin A alone. It indicated that cholestin might play a role in reducing the toxic effect of vitamin A in rats.

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1. Introduction

Vitamin A is an essential nutrient for humans because it cannot be synthesized de novo within the body. The term vitamin A used generically for all β-ionone derivatives (other than carotenoids) that have the biological activity of all-trans retinol. Forms of vitamin A include retinol, retinal (also called retinaldehyde), and various retinyl esters (Sauberlich et al., 1974). Retinoic acid can perform some but not all of the biological functions of vitamin A. In the past, most cases of systemic and hepatic toxicity due to vitamin A resulted from excessive ingestion of animal liver with its enormous quantities of vitamin A. This has been recognised for more than 50 years (Rodahl & Moore, 1943). The main clinical features of vitamin A poisoning are fever, anorexia, nausea, vomiting, headache, drowsiness, skin changes, papilledema, skeletal pain, hair loss, pseudotumor cerebri, liver disease, and psychiatric complaints have been described (Shekelle, Lepper, & Liu, 1981). Hepatic injury associated with the clinical use of vitamin A has been reported in the United States and Western Europe (Elias & Williams, 1981; Josephs, 1944; Stimson, 1961), but cases resulting from excessive intake of yellow–green vegetables, such as carrots and pumpkin are rare (A de Francisco, Zaman, Chowdhury, Kransinski et al., 1989; Leo & Lieber, 1988). Red yeast rice is commonly used in China for centuries to enhance the colour and flavour of food, as well as a traditional medicine for digestive and vascular functions (Ma et al., 2000). In the late 1990s, dietary supplement companies decided to commercialise red yeast extracts because of their ability to reduce cholesterol as efficiently as statin drugs. It was placed on market in 2001 (Journoud & Jones, 2004; Qin et al., 1999; Rippe et al., 1999). However, in the spring of 2001, the US Food and Drug Administration (FDA) banned the sale of dietary supplement containing red yeast extracts or xuezhikang (SoRelle, 2000), which was found to contain lovastatin. Because lovastatin belongs to the drug category, the FDA made an administrative decision to remove this dietary supplement (often sold as cholestin in earlier times) from the health food store. Cholestin is the fermented product of rice on which red yeast (Monascus purpureus) has been grown; and it is a dietary staple in many Asian countries with typical consumption ranging from 0.5 to 2 oz/person/day (Stuart, 1979). This product has been used as a food preservative for maintaining taste and colour in fish and meat, and/or as functional medicine (Mei, 1990).

The medicinal properties of red yeast extract were described by pharmacologists of the Ming Dynasty (1368–1644) as cited by Ma et al. (2000). Increased levels of cholesterol and triglycerides are known to be risk factors for developing coronary artery diseases. Lipid-lowering agents that inhibit 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase are now prominent among the drugs of choice for treating hypercholesterolaemia. It is another effective way to control the cholesterol level with diet and food supplements (Brosche, Kral, Summa, & Platt, 1996). Cholestin also

contains 2–6% fatty acids including palmitic acid, linoleic acid, oleic acid, and stearic acid (Zhang & Duan, 1998), some of which have been shown to have the ability to reduce the blood–lipid level in animal models and humans (Su, Liu, & Cheng, 2007). Indeed, diets enriched with cholestin were effective in reducing cholesterol in high cholesterol rabbits and rats (Wang, Lin-Shiau, Chen, & Lin, 2000). Recently, cholestin played an important role in reducing the toxic effect of lipid peroxidation in rats (Wang et al., 2000). Cholestin may play an important role in reducing the toxic effect of oxidised cholesterol and oxidised fish oil in rats (Yeh, Lee, Hsieh, & Hwang, 2010). As the above description, hypervitaminosis A is a modern toxic vitamin. Hence, it prompted us to investigate the effect of cholestin on the toxicity of vitamin A.

2. Materials and methods

2.1. Animals

Male weanling wistar rats were purchased from the National Laboratory Animal Center. Our Institutional Animal Care and Use Committee approved the protocols for the animal study, and the animals were cared for in accordance with the institutional ethical guideline. They were kept in an air-conditioned room (23 ± 1 °C, 50–60% humidity) lit for 12 h per day (07:00–19:00 h). After acclimatizing for 2 weeks with a commercial non-purified diet (Rodent Laboratory Chow 5001, Pruida Co., USA), 36 rats were divided into six groups. Six rats in each group were assigned to receive an 8-week course of one of six formulated diets (Table 1). The diets were formulated as described previously by the American Institute of Nutrition (1977) (AIN) because this formula is still commonly used in spite of the new one recommended by AIN in 1993. The vitamin A used was from Sigma (St. Louis, MO, USA). Water and food were always available. After feeding, all rats were weighed. The blood of the rats was taken at a 2 weeks interval from the tail vein. Then, the plasma samples were collected by centrifugation (2000g, 15 min) from blood and examined for the level of thiobarbituric acid-reactive substances (TBARS), and the activities of aspartate transaminase (AST) and alanine transaminase (ALT) in the plasma were also assayed by a Vitalab Selectra (E. Merck, Germany) using an enzymatic kit. At the last 2 days of 8-week course of diets, the serum of rats was collected and assayed for vitamin A, and then the rats were weighed and anesthetized with diethyl ether. The liver and kidney of rats were quickly excised without perfusion and weighed. Both ratios of liver and kidney weight to body weight were obtained. Then, the liver and kidney samples were stored at −40 °C for vitamin A, glutathione (GSH), and TBARS determinations. The plasma was analysed for TBARS, AST, ALT, blood urea nitrogen (BUN), and creatinine using enzymatic kit (E. Merck, Germany).

2.2. Preparation of cholestin

Cholestin (Red yeast rice) is described as the fermented product of rice on which red yeast (M. purpureus) has been grown. M. purpureus strain BCRC 31498 was purchased from the Bioresources Collection and Research Center (Food Industry Research and Development Institute, Taiwan, ROC). The fungus was maintained on malt extract broth (MEB) agar, containing 4 g/l yeast extract, 20 g/l malt extract, 20 g/l glucose, and 20 g/l agar (pH 7.0). Freshly inoculated cultures were incubated at 28 °C for 5 days, after which stock cultures were kept at 4 °C and transferred to fresh medium monthly.

M. purpureus strain BCRC 31498 was grown in liquid medium by inoculating one loop of stock culture into a 500 ml Erlenmeyer flask containing 50 ml of malt extract broth (MEB) agar growth medium containing malt extract 20 g/l, glucose 20 g/l, peptone 1 g/l, pH 4.7, and incubating the culture at 30 °C on a rotary shaker at 220 rpm. Lovastatin esterase activity was induced by the addition of lovastatin ammonium salt (LAS) to each flask to a final concentration of 0.5 mg/ml. The culture was then allowed to incubate for another day before it was harvested.

Dried red yeast rice was extracted with boiling water at 100 °C for 4 h. The extract was then filtered through a Büchner funnel and freeze-dried red yeast rice was stored at −20 °C until use.

2.3. TBARS production

Lipid peroxidation activities in blood and liver were assayed by measurements of malondialdehyde (MDA), an end-product of peroxidized fatty acids, and thiobarbituric acid (TBA) reaction product. Twenty microlitre of plasma and 20% liver homogenate were separately mixed with 1.0 ml of 0.4% TBA in 0.2% HCl and 0.15 ml of metaphosphoric acid was added to the liver preparations. Supernatants were collected after centrifugation and analysed using HPLC supplied with fluorescence at an excitation wavelength of 515 nm and an emission wavelength of 550 nm (Tatum, Changchit, & Chow, 1990).

2.4. Levels of GSH measurement

Glutathione levels were measured using the glutathione assay kit (Calbiochem, San Diego, CA). An equal volume of ice cold 10% metaphosphoric acid was added to the liver preparations. Supernatants were collected after centrifugation and analysed for total GSH as per manufacturer’s instructions. Total GSH in the samples was normalised with protein.

Table 1

<table>
<thead>
<tr>
<th>Ingredient (%)</th>
<th>Control</th>
<th>Cholestin</th>
<th>Vitamin A 25,000</th>
<th>Cholestin + vitamin A 25,000</th>
<th>Vitamin A 50,000</th>
<th>Cholestin + vitamin A 50,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Casein</td>
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<td>35</td>
<td>35</td>
<td>35</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
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<td>30</td>
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<tr>
<td>Cellulose</td>
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<td>5</td>
<td>5</td>
<td>5</td>
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<tr>
<td>Corn oil</td>
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<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Methionine</td>
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<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Choline</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
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<td>0.2</td>
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<td>AIN mineral mix</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>AIN vitamin mix</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Cholestin</td>
<td>0</td>
<td>0</td>
<td>25,000</td>
<td>25,000</td>
<td>50,000</td>
<td>50,000</td>
</tr>
</tbody>
</table>

*Cholesterol: 1% cholestin in diet; vitamin A 25,000: 25,000 (IU) vitamin A in diet; cholestin + vitamin A 25,000: 1% cholestin and 25,000 (IU) vitamin A in diet; vitamin A 50,000: 50,000 (IU) vitamin A in diet; cholestin + vitamin A 50,000: 1% cholestin and 50,000 (IU) vitamin A in diet.

2.5. Vitamin A analyses in liver, kidney, and serum

Vitamin A in liver and kidney homogenates (20% w/v in water) were extracted using diisopropyl ether, essentially according to Nilsson, Hanberg, Trossvik and Håkansson (1996) and separated on a Nucleosil C-18 5 μm high performance liquid chromatography (HPLC) column using an ethanol:water gradient elution. Retinol, retinal acetate, and retinyl palmitate were detected with a fluorescence detector with an excitation wavelength of 325 nm and emission wavelength of 475 nm (Model 821-FP, Jasco). Internal (retinyl acetate) and external (retinol and retinyl palmitate) standards were used for quantification. Serum analyses of retinol and retinyl esters were being performed by simple extraction, with different concentrations of retinyl palmitate, was used to quantify all retinyl esters. The intra-assay variation was 5.1%.

2.6. Histopathological examination

A.0 portion of the median lobe of liver was dissected and fixed in 10% buffered formalin for at least 24 h, dehydrated with a sequence of ethanol solutions and processed for embedding in paraffin. Sections of 5–6 mm in thickness were cut, deparaffinized, rehydrated, stained with haematoxylin and eosin (H & E) and were subjected to photomicroscopic observation. The histological scoring of hepatic damage and fibrosis were expressed using the following score system: 0, no histopathologic change; 1≤, mild histopathologic change; 2≤, moderate histopathologic change; 3≤, severe histopathologic change (Kondou et al., 2003).

2.7. Statistical analysis

Statistical analysis for differences among rats in the experimental groups was performed by the two-way analysis of variance procedure and Duncan’s new multiple range tests (Puri & Mullen, 1980). All statistical analyses were performed using the statistical software SPSS 11.0 (SPSS Ltd., Surrey, UK). A P value <0.05 was considered statistically significant.

3. Results

The effects of cholestrol and vitamin A on the growth of rats are shown in Fig. 1. After 2-week feeding, the weight of the rat was significantly decreased (P < 0.05) when the concentration of vitamin A in the diet was more than 25,000 (IU), but significantly increased when the concentration of vitamin A in the diet was more than 25,000 (IU), but significantly increased when the diet was supplemented with cholestelin (P < 0.05). It also indicated that cholestrol could improve the growth of rats, which were fed diets with supplement of 25,000–50,000 (IU) vitamin A. The effects of cholestrol and vitamin A in the plasma were shown in Fig. 1. After 8-week feeding, the ratios of liver and kidney weight to body weight in rats are shown in Fig. 1. After 8-week feeding, the ratios of liver and kidney weight to body weight in rats were significantly different from those for the rats fed a diet supplemented with vitamin A. This means that cholestrol might not significantly reduce the toxicity of vitamin A in the rats based on the ratios of liver and kidney weight to body weight. The effects of cholestrol and vitamin A on the activities of AST and ALT in the plasma are shown in Fig. 1. It was found that the activities of AST and ALT in the plasma of the rats fed diets supplemented with vitamin A gradually increased with the feeding time course. The activities of AST and ALT in the plasma of the rats increased with increasing the level of vitamin A. The activities of AST and ALT in those rats fed a diet supplemented with cholestelin significantly reduced the toxicity of vitamin A (P < 0.05), indicating that cholestelin might have a protective effect on vitamin A toxicity in rats. The effects of cholestrol and vitamin A on the TBARS production in the plasma in rats are shown in Fig. 1. After 6-week feeding, the level of TBARS in the plasma of the rats fed a diet supplemented with vitamin A was higher than that of the control group (P < 0.05). After 8-week feeding, the level of TBARS in the plasma of the rats fed a diet supplemented with vitamin A was also higher than that of control group (P < 0.05). The level of TBARS in the plasma and liver of the rats fed a diet supplemented with cholestelin and vitamin A was less than that of the rats fed an unsupplemented diet (P < 0.05). The level of GSH in the liver of the rats decreased with increasing the concentration of vitamin A in the diet. The level of GSH in the liver of rats fed a diet supplemented with cholestelin and vitamin A was higher than that of the rats fed a diet only supplemented with vitamin A (P < 0.05) (Fig. 2). The effects of cholestrol and vitamin A on the level of BUN and creatinine in the plasma were shown in Fig. 2. After 8-week feeding, the level of BUN and creatinine in the plasma was higher in the groups fed a diet with supplemented with vitamin A than in the control group. The level of BUN and creatinine in the plasma of the rats increased with increasing the dose of vitamin A in the diet. When the diet was supplemented with cholestelin, the level of BUN and creatinine was significantly reduced (P < 0.05). The effects of cholestrol and vitamin A on the level of vitamin A in the liver, kidney and serum of rats are shown in Fig. 3. After 8-week feeding, the level of vitamin A in the liver, kidney, and serum was obviously higher in the control group. The level of vitamin A in the liver, kidney, and serum increased with increasing the dose of vitamin A in the diet. When the diet was supplemented with cholestelin, the level of vitamin A in the liver was significantly reduced, and the level of vitamin A in the serum was slightly higher (P < 0.05).

The histological observations supported the results obtained from serum enzymes assays. Liver sections from control mice showed normal lobular architecture and hepatic cells with a well-preserved cytoplasm and well-defined nucleus and nucleoli (Fig. 4). The results of the hepatic histopathological examination are shown in Table 2. When compared with the normal liver tissues of vehicle controls, the liver tissue in the rats treated with acetaminophen revealed extensive liver injuries, characterised by moderate to severe hepatocellular hydropic degeneration and necrosis around the central vein, lipidosis, hepatic fibrosis, and cholangiocyte hyperplasia. However, the histopathological hepatic lesions induced by administration of vitamin A were only remarkably ameliorated in the central lobular necrosis, hepatic lipidosis, and hepatic fibrosis by pre-treatment with cholestolin in rats.

4. Discussion

The symptoms of vitamin A toxicity in rats included reduced body weight, ratios of liver and kidney weight to body weight, and level of GSH in the liver, and increasing activities of AST and ALT in the plasma, levels of TBARS, BUN, and creatinine in the plasma.
and/or liver, and concentrations of vitamin A in the liver and the kidney of rats. In the clinical plasma examination, the activities of AST and ALT in the plasma represent biomarkers for liver functions (Ronald & Koretz, 1992). The activities of AST and ALT in the plasma of the rats were significantly elevated by vitamin A, indicating vitamin A-related injury to the liver. This result is also reported by other papers (Leo et al., 1989). Since cholestin significantly reduced the AST and ALT activities in the plasma of the rats, the hepatic injury by vitamin A could be ameliorated by cholestin. This result was similar to that of body weight in rats. However, the levels of TBARS and GSH in the liver are additional indicators of liver injury. TBARS is an end product of lipid peroxidation. The level of TBARS of the plasma and liver increased with increasing the dose of vitamin A and the level of TBARS in the plasma also increased with exposure time. The data does not prove that the mechanism of vitamin A injury is by lipid peroxidation, but it is likely that it plays an important role.

Fig. 1. Effect of vitamin A and cholestin on the ratios of liver and kidney weight to body weight, body weight, activity of aspartate transaminase (AST), transaminase (ALT) and the level of thiobarbituric acid relative substances (TBARS) in the plasma of rats. a–e: Values with different letters are significantly different at \( P < 0.05. \)

Another paper has also indicated that vitamin A might increase the level of TBARS in the tissues of experimental animals (Minuk, Kelly, & Hwang, 1988). The level of TBARS in the plasma and liver of rats was significantly reduced when the rats were fed a diet supplemented with cholestin. The level of GSH in the liver of rats was reduced by contaminated vitamin A, which was similar to that of another report (Geoffrey, 1994), while it was raised significantly when the rats were fed a diet supplemented with cholestin. This suggests that cholestin may play an important role in the metabolism of GSH and in preventing lipid peroxidation, but the related mechanism should be studied further. On the other hand, the levels of BUN and creatinine in the plasma of the rats are tested as indicators for kidney functions (Hendriks, Bosma, & Brouwer, 1993). Judging from both indicators and the ratio of kidney weight to body weight, vitamin A significantly induced the dysfunction of kidney. Although the supplementation with cholestin in diet did not...

Fig. 2. Effect of vitamin A and cholestin on the levels of TBARS and GSH in the liver and BUN and creatinine in the plasma of rats after 8-week feeding. a–e: Values with different letters are significantly different at $P < 0.05$. 

Ameliorate the ratio of kidney weight to body weight, the levels of BUN and creatinine in the plasma of the rats were significantly reduced when the rats were fed a diet supplemented with cholestin. Furthermore, the level of vitamin A in the liver and kidney was significantly increased with increasing the exposure to vitamin A in the diet. The accumulated amount of vitamin A was higher in the kidney than in the liver, which was the same as in a previous report (Vecchini et al., 1994). Accumulation of vitamin A is the net consequence of uptake, biotransformation and elimination processes within an individual. Once vitamin A is absorbed, cholestin exerts synergistic actions in scavenging and vitamin A may be transformed into vitamin-thionerin. Although the half-life of vitamin-thionerin in the liver and kidney is not known exactly (Sakamoto et al., 2001) there is a progressive accumulation in these tissues. The accumulated amount of vitamin A in the tissue was effectively reduced by cholestin. Cholestin is a special amino acid, which possesses an amino group and a sulphonate group. These functional groups might bind with vitamin A, and then stimulated the excretion of such compounds. In this study, it was also found that the amount of vitamin A in the serum of the rats fed a diet supplemented with cholestin was slightly increased. There is no evidence that cholestin directly reduces the production of free radicals but it may well operate by binding vitamin A which is then not absorbed or is more rapidly excreted. In other words it may act by reducing the overall bioavailability of vitamin A or the intracellular availability of absorbed vitamin A. Hence, dietary cholestin may play a role in reducing the toxic effect of vitamin A in the liver and kidney of rats.

Fig. 3. Effect of vitamin A and cholestin on the level of vitamin A in the liver, kidney and serum of rats after 8-week feeding. a–d: Values with different letters are significantly different at $P < 0.05$. 

Y.-H. Yeh et al. / Food Chemistry xxx (2011) xxx–xxx

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References


Table 2

Effects of cholestin on hepatic histopathology of liver damage in mice treated with vitamin A.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Design of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Hepatocellular hydropic degeneration</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Central lobular necrosis</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Hepatic lipidosis</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Hepatic fibrosis</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Cholangiocyte hyperplasia</td>
<td>0.0 ± 0.0</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD (n = 6) in each group.

0, no histopathologic change; 1±, mild histopathologic change; 2±, moderate histopathologic change; 3±, severe histopathologic change.

*P < 0.05 indicates statistically significantly different from control.

Fig. 4. Microscopic cross section of liver lobules in rat after 8-weeks fed diet with (A) control, (B) cholestin, (C) vitamin A 25,000, (D) cholestin + vitamin A 25,000 (E) vitamin A 50,000, and (F) cholestin + vitamin A 50,000 (×400 H & E). Bar represents 0.01 mm.
