

Lycopene restores trace element levels in ochratoxin A-treated rats

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This study was designed to investigate the *in vivo* effects of ochratoxin A (OTA) and/or lycopene on the levels of selenium, zinc, and copper in the liver, kidneys, and testes of male Sprague-Dawley rats. The rats were treated with OTA (0.5 mg kg⁻¹ day⁻¹) and/or lycopene (5 mg kg⁻¹ day⁻¹) by gavage for 7 or 14 days. Trace element levels were measured by atomic absorption spectrometry. OTA significantly lowered selenium (20 % in the liver, 17 % in the kidney, and 40 % in the testis), zinc (24 % in the liver, 23 % in the kidney, and 26 % in the testis), and copper levels (40 % in the liver and 10 % in the kidney). Lycopene alone did not affect the trace element levels in any of the organs. In combination with OTA, however, it significantly restored liver, kidney, and testis selenium and zinc levels compared to the group treated with OTA alone. Our results have confirmed that depletion of trace elements in different organs is one of the mechanisms of action of OTA. They also suggest that lycopene interferes with this depleting effect and restores trace element levels, the implications of which need to be further investigated.

KEY WORDS: *copper; kidney; liver; mycotoxins; selenium; testis; zinc*

Ochratoxins are a family of mycotoxins produced by several food-borne species of the genera *Aspergillus* and *Penicillium* as secondary metabolites. These toxins are ochratoxin A (OTA), B, and C, and OTA is the most abundant and the most toxic member of the ochratoxin family (1). It can contaminate a variety of food commodities like cereals, vegetables, dried fruits, spices, coffee, nuts, fermented beverages, and medicinal plants. Consumption of such food results in chronic exposure (1, 2). OTA is a potent kidney carcinogen in animals (3, 4) and is arguably involved in the Balkan endemic nephropathy and urinary tract tumours in humans (5, 6). However, a direct cause-effect relation has not yet been established, and the mechanism of OTA carcinogenicity remains to be clarified in humans, even though animal research has given sufficient evidence of its carcinogenic properties (7).

Researchers have proposed several underlying mechanisms of OTA toxicity, such as the inhibition of protein synthesis, membrane lipid peroxidation, and DNA damage (8-10). Our earlier studies (11-13) showed that OTA caused oxidative stress, lipid peroxidation, oxidative DNA damage, and apoptosis in rat kidney and liver, but we could not pinpoint the exact toxicity mechanism. There are only

a few reports investigating the effects of OTA on trace elements or *vice versa* (14-16).

Lycopene is the most prevalent antioxidant carotenoid in the Western diet. It is present in tomatoes, watermelon, pink grapefruit, and several other red fruits (16). The consumption of tomatoes and/or tomato products is associated with increased lycopene blood levels and reduced oxidative damage of lipids, proteins, and DNA (17). Recent studies showed that consumption of lycopene-containing food, especially tomato products and lycopene supplements, was associated with a decreased risk of chronic complex conditions, ranging from cancer to cardiovascular diseases (18-20). However, little is known about the effects of lycopene on trace element status.

In tissues such as liver, kidney, and testis, trace elements have substantial roles in preserving tissue and cellular integrity. The levels of trace elements have particular importance in maintaining tissue homeostasis, as they prevent the toxic action of different chemicals due to their presence in several intracellular antioxidant components. Changes in trace element levels can affect the cellular redox state, which may result in cell disruption and tissue damage (21). Furthermore, their levels affect the expression and synthesis of several antioxidant enzymes, superoxide dismutase (SOD), glutathione peroxidases (GPxs), and thioredoxin reductases (TrxRs) in particular. For example, selenium (Se) deficiency may lead to dysfunctional immune response, cardiovascular disease, neurodegeneration, and

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finally cancer (22-26). Zinc (Zn) is the cofactor of more than 300 enzymes. Copper (Cu) is common in organic complexes known as metalloproteins, which act as antioxidant enzymes. Together with iron (Fe), Cu is important for the activity of cytochrome c oxidase, an enzyme which is required for aerobic respiration whereas with Zn, it affects the activity of Cu,Zn-SOD, an enzyme that catalyses the breakdown of superoxides (27).

This study in male Sprague-Dawley rats was designed to a) investigate the effects of OTA and/or lycopene on trace element (Se, Zn, and Cu) levels in the liver, kidney, and testis; b) evaluate OTA toxicity in relation to the changes in trace element levels; and c) determine protective effects of lycopene against OTA toxicity.

To the best of our knowledge, this is the first study in which such effects have been investigated *in vivo*.

MATERIALS AND METHODS

Chemicals and reagents

All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) or Merck Co (Darmstadt, Germany). Lycopene was a gift from Micro-Gen (Ankara, Turkey).

Animals and treatment

Male Sprague-Dawley rats (<200 g) were supplied from the Hacettepe University Experimental Animals Laboratory. The animals were housed in plastic cages with stainless-steel grid tops under standard laboratory conditions (temperature 23 °C; humidity 50 %, and the 12-hour light and dark cycle) and had free access to standard laboratory chow and drinking water. The study was approved by the Hacettepe University Ethics Committee.

The dose chosen for OTA treatment was 1/40 of the median lethal dose (LD₅₀) (24). The lycopene dosing was based on the literature. Limpens et al. (28) observed that daily supplementation with lycopene at 5 mg kg⁻¹ of body mass through oral gavage was well tolerated and did not change mean body weight in nude mice. In addition, Breinholt et al. (29) demonstrated that a 14-day lycopene treatment at 5 mg kg⁻¹ day⁻¹ for 14 days peaked the antioxidant enzyme activities in rats. Cavusoglu et al. (30) treated rats with 5 or 10 mg kg⁻¹ day⁻¹ lycopene in combination with mercury (Hg) and found that lycopene lowered Hg cytotoxicity at both doses.

The study consisted of six groups of six animals each. The control group (C7) received 1 mL of corn oil containing 10 % DMSO by intra-gastric lavage for 7 days. The lycopene control group I (L7) received daily intra-gastric lycopene doses of 5 mg kg⁻¹ bw for 7 days. The lycopene control group II (L14) received the same doses for 14 days. The OTA group (OTA) received daily intra-gastric OTA doses of 0.5 mg kg⁻¹ bw for 14 days. The OTA plus lycopene 7 group (OTA+L7) received OTA (in the same doses as

above) for 14 days and lycopene (in the same doses as above) for the last seven days of treatment. The OTA and lycopene 14 group (OTA+L14) received the combination in the above doses for 14 days.

Lycopene was dissolved in DMSO and later scaled to the required volume with corn oil. The rats were weighed at baseline (before receiving the first dose) and at the end of the (one or two-week) treatment, 24 h after which they were anaesthetised with thiopental and decapitated and their kidneys, testes, and liver removed.

Preparation of tissue homogenates and determination of trace element levels

For wet digestion, 100 mg of each tissue (liver, kidney, or testis) was taken into a tube containing 1 mL of 30 % HNO₃ and heated at 110 °C for at least one hour. This process is applied to digest the organic material and remove the inorganic part. The samples were then cooled to room temperature and kept at -80 °C until analysis.

Trace elements were determined with atomic absorption spectrometry (AAS). For Se and Cu we used Zeeman background correction AAS (SpectrAA 240Z, Agilent, Santa Clara, CA, USA) and for Zn fast sequential AAS (SpectrAA 240FS, Agilent). Both spectrometers were equipped with a graphite tube atomizer (GTA 120, Agilent).

Stock solutions for Se, Zn, and Cu were prepared by dissolving 1 g of high-purity metals in nitric acid and filling the volume up to 1000 mL with double-distilled water. When kept in polyethylene bottles at -20 °C, they can be used for at least 3 months. From these stock solutions, we prepared the basic set of 100 mL standard solutions containing 5 mL of hydrochloric acid to construct analytical curves. To prepare calibration curves for the standards we used the following concentrations: 0, 0.5, 1, 2, 5, 10, 25, and 50 µg mL⁻¹ for Se; 1, 5, 10, 50, 100, and 200 µg mL⁻¹ for Zn; and 0, 0.25, 0.5, 1, 2, 4, 8, and 10 µg mL⁻¹ for Cu. Optical densities were read at 196 nm, 213.9 nm, and 324.7 nm, respectively. To verify the assay accuracy, standard solutions were run on every 10th test. Trace element levels were calculated with the Spectr AA software (Agilent).

Recoveries from blank samples spiked with 1 µg mL⁻¹ Se, 25 µg mL⁻¹ Zn, or 5 µg mL⁻¹ Cu (30 samples for each element) were (mean±SD): 91±4.2 % for Se, 94±3.1 % for Zn, and 95±2.4 % for Cu. The variation coefficients for between-run precisions were 10.40±0.74 % for Se, 9.12±1.14 % for Cu, and 11.04±2.25 % for Zn. For within-day precisions these coefficients were 7.28±1.14 % for Se, 10.14±1.97 % for Zn, and 11.25±3.41 % for Cu.

The limit of detection (LOD) for Se, Zn, and Cu was 0.025 µg mL⁻¹, 0.027 µg mL⁻¹, and 0.025 µg mL⁻¹, respectively.

Statistical analysis

The results are expressed as means ± standard deviation (SD). The differences between the groups were analysed

with the Statistical Package for Social Sciences program (v. 17.0, SPSS, Chicago, IL, USA) using the Kruskal-Wallis one-way analysis of variance and then Mann-Whitney U test. The P values <0.05 are considered statistically significant.

RESULTS AND DISCUSSION

Liver trace element levels

In OTA-treated rats, all trace element levels decreased significantly (Se ~20 %, Zn ~24 %, and Cu ~40 %) compared to the control group (P<0.05) (Table 1). Similar to our findings, the *in vitro* study by Zheng et al. (15) in human hepatoma (HepG2) cells established that a 12-hour exposure to OTA lowered intracellular Zn levels to 80.67 % of the control cell levels. Adding Zn, however, counteracted OTA-induced DNA damage.

Lycopene treatment made no significant changes regardless of the treatment duration. Our results are in accordance with Watanabe et al. (31), who also showed that lycopene diet supplementation did not affect serum and liver Zn levels compared to control.

Given with OTA, however, lycopene in our study significantly restored Se and Cu levels compared to the OTA group, but the 7-day treatment failed to recover Cu and Zn to control levels. Zinc was fully recovered only with the 14-day lycopene treatment while Cu levels remained significantly lower than control (Table 1).

Judging by the findings reported by Banji et al. (32), lycopene shows better (synergistic) effects if combined with Zn supplements. We can assume that higher lycopene levels would have induced higher Zn transport to liver in the presence of OTA.

A similar explanation may also be valid for Cu; either higher levels of lycopene or longer supplementation periods with lycopene are needed to restore Cu levels to control ones. However, longer exposure to carotenoids at doses higher than expected in normal diet may not result in beneficial effects, which needs to be investigated further on a rat model.

Kidney trace element levels

Similar to the liver, kidney trace element levels dropped significantly in the OTA group compared to control (Se by ~17 %, Zn by ~23 %, and Cu by ~10 %), and lycopene given alone did not affect them. In combination with OTA it significantly restored Se and Cu levels, but not Zn compared to the OTA group, even though it did increase Zn levels by ~12 % (Table 2). Again, we can conclude that either higher levels of lycopene or longer supplementation is needed to restore Zn levels to normal in the kidney.

Table 1 The effects of OTA and/or lycopene on liver trace element levels in Sprague-Dawley rats

GROUP (N=6 each)	Liver trace element levels		
	Se (µg g ⁻¹ tissue)	Zn (µg g ⁻¹ tissue)	Cu (µg g ⁻¹ tissue)
C7	1.01±0.18 [#]	30.12±2.95 [#]	5.64±0.37 [#]
L7	1.00±0.10 [#]	30.48±3.65 [#]	5.40±0.27 [#]
L14	1.01±0.10 [#]	30.43±1.06 [#]	5.31±0.31 [#]
OTA	0.80±0.14 [*]	22.87±3.32 [*]	3.33±0.66 [*]
OTA+L7	0.94±0.16 [#]	22.31±2.05 [*]	4.31±0.46 ^{**}
OTA+L14	0.97±0.15 [#]	25.80±3.47 ^{**}	4.29±0.85 ^{**}

All results are given as mean ± SD. C7: control group; L7: lycopene group on the last 7-day treatment; L14: lycopene group on the 14-day treatment; OTA: OTA group; * significantly different from control (P<0.05); [#] significantly different from OTA group (P<0.05)

Table 2 The effects of OTA and/or lycopene on kidney trace element levels in Sprague-Dawley rats

GROUP (N=6 each)	Kidney trace element levels		
	Se ($\mu\text{g g}^{-1}$ tissue)	Zn ($\mu\text{g g}^{-1}$ tissue)	Cu ($\mu\text{g g}^{-1}$ tissue)
C7	1.38 \pm 0.19 [#]	23.85 \pm 2.51 [#]	6.11 \pm 0.32 [#]
L7	1.24 \pm 0.19	22.78 \pm 0.54 [#]	5.81 \pm 0.13 [#]
L14	1.44 \pm 0.34 [#]	22.44 \pm 1.91 [#]	5.97 \pm 0.55 [#]
OTA	1.14 \pm 0.25 [*]	18.15 \pm 1.02 [*]	5.53 \pm 0.34 [*]
OTA+L7	1.20 \pm 0.27 [#]	20.34 \pm 3.97	5.69 \pm 0.45
OTA+L14	1.21 \pm 0.06 [#]	20.49 \pm 2.48	5.87 \pm 0.20

All results are given as mean \pm SD. C7: control group; L7: lycopene group on the last 7-day treatment; L14: lycopene group on the 14-day treatment; OTA: OTA group; [#]significantly different from control ($P < 0.05$); ^{*}significantly different from OTA group ($P < 0.05$)

Testicular trace element levels

As in the other organs, OTA treatment significantly lowered Se (~47 %) and Zn (~26 %) but did not affect the Cu levels (Table 3). Malekinejad et al. (34) suggested that OTA could cause reproductive abnormalities, and our findings suggest that decreasing Zn levels may be involved, as Zn has a critical role in sperm production (35).

Lycopene alone, again, did not affect the trace element levels, but in combination with OTA it significantly restored Se (~47 % in the OTA+L7 group and ~79 % in the OTA+L14 group) and Cu levels (~21 % in the OTA+L7 group and ~48 % in the OTA+L14 group) compared to the OTA group. It was not, however, effective in counteracting OTA-induced drop in testicular Zn levels (Table 3).

The protective roles of selenium, copper, and zinc against OTA toxicity have been well established in research conducted so far, and lycopene seems to prevent their depletion.

Atroshi et al. (36) have established that OTA reduces selenoenzyme activity and that Se supplementation (in combination with vitamin E) compensates for this effect. Lycopene in our study seems to have achieved the same, but not through compensation but rather by preventing Se depletion. It is quite likely that it improves selenoenzyme expression as described by Gan et al. (37) in porcine kidney 15 (PK15) cells.

As for Cu and Zn, the effects of lycopene against OTA toxicity are not that clear-cut, especially in the testes. Cu levels in rats receiving OTA+lycopene rose above those observed in the control and lycopene-alone groups (Table 3). This may be the consequence of lycopene changing the oxidative/antioxidative equilibrium. Future studies might look into how it affects this equilibrium.

It also remains to elucidate why lycopene was not efficient in counteracting Zn-lowering OTA effects in all organs. Perhaps because OTA had more severe effects on Zn than on Se and Cu levels. What we can conclude from these findings is that Zn-deficient individuals may suffer detrimental reprotoxic effects, both due to OTA toxicity and Zn deficiency. However, it remains for the future studies to prove it.

CONCLUSION

Being the first *in vivo* study of OTA effects on trace elements and of the lycopene action against OTA toxicity, our study is limited in many ways. It investigates treatment with one OTA dose, one antioxidant (lycopene), and only three trace elements. Even so, it has confirmed that at least one of the mechanisms underlying OTA toxicity is its lowering of trace element levels in different organs. It has also shown that lycopene improves trace element distribution in various tissues after OTA exposure. People who take sufficient amounts of vitamin A analogues are therefore better protected from the toxic effects of OTA.

Table 3 The effects of OTA and/or lycopene on testis trace element levels in Sprague-Dawley rats

GROUP (N=6 each)	Testicular trace element levels			
	Se (µg g ⁻¹ tissue)	Zn (µg g ⁻¹ tissue)	Cu (µg g ⁻¹ tissue)	
C7	0.57±0.05 [#]	28.18±2.67 [#]	1.37±0.15	
L7	0.58±0.06 [#]	28.17±0.76 [#]	1.42±0.26	
L14	0.55±0.048 [#]	28.75±1.34 [#]	1.47±0.46	
OTA	0.34±0.033 [*]	20.81±1.65 [*]	1.24±0.22	
OTA+L7	0.50±0.05 [#]	21.51±5.01 [*]	1.51±0.54	
OTA+L14	0.61±0.11 [#]	21.43±2.56 [*]	1.84±0.54 ^{**#}	

All results are given as mean ± SD. C7: control group; L7: lycopene group on the last 7-day treatment; L14: lycopene group on the 14-day treatment; OTA: OTA group; [#]significantly different from control (P<0.05); ^{*}significantly different from OTA group (P<0.05)

This protection should be enhanced with a well-balanced diet that includes vitamins, trace elements, and minerals.

Conflicts of interest

The authors declare no conflicts of interest.

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Likopen obnavlja razine elemenata u tragovima u štakora izloženih okratoksinu A

Svrha ovoga *in vivo* istraživanja bila je utvrditi djelovanje okratoksina A (OTA) i/ili likopena na razine selenija, cinka i bakra u jetri, bubrezima i testisima mužjaka štakora Sprague-Dawley. Štakori su primali OTA ($0,5 \text{ mg kg}^{-1} \text{ dan}^{-1}$) i/ili likopen ($0,5 \text{ mg kg}^{-1} \text{ dan}^{-1}$) gavažom u trajanju od 7 odnosno 14 dana. Elementi u tragovima izmjereni su atomskom apsorpcijskom spektrometrijom. OTA je značajno snizila razine selenija (20 % u jetri, 17 % u bubregu te 40 % u testisu), cinka (24 % u jetri, 23 % u bubregu te 26 % u testisu) i bakra (40 % u jetri te 10 % u bubregu). Kad je davan sam, likopen ni u jednom organu nije utjecao na razine elemenata u tragovima. Međutim, u kombinaciji s OTA-om značajno je obnovio razine selenija i cinka u jetri, bubregu i testisu u usporedbi sa skupinom izloženom samo okratoksinu A. Naši rezultati potvrđuju da je jedan od mehanizama djelovanja okratoksina A nestanak elemenata u tragovima u pojedinim organima. Također upućuju na to da likopen sprečava takvo djelovanje i obnavlja razine elemenata u tragovima. Buduća bi istraživanja trebala rasvijetliti implikacije ovih saznanja.

KLJUČNE RIJEČI: *bakar; bubreg; cink; jetra; mikotoksini; selenij; testis*