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Research Article

Efficacy of antioxidant vitamins (vitamin C, vitamin E, beta-carotene) and selenium supplement on D-galactosamine-induced lung injury

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Abstract

Effects of vitamin combination with selenium on acute lung injury in rats were examined in this study. Four experimental groups of rats were used as follows: group 1, animals administered intraperitoneally physiological saline solution; group 2, rats fed with vitamin C, vitamin E, beta-carotene and sodium selenate for three days; group 3, a single intraperitoneally injection of D-galactosamine (D-GaIN; 500 mg kg^{-1}) into rats; group 4, animals fed with the antioxidant vitamin combination with selenium for three days, and administered D-GaIN. Lung tissues were examined using light microscope, and the following biochemical parameters were measured glutathione (GSH), lipid peroxidation (LPO) levels and tissue factor (TF), lactate dehydrogenase (LDH), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), paraoxonase (PON), myeloperoxidase (MPO), xanthine oxidase (XO) and sodium potassium ATPase (Na^+/K^+-ATPase) activities in lung tissues. Extensive edema in peripheral areas, mononuclear cell infiltrations around venules and locally a honeycomb-like structure were observed in the lung of group 3 rats. GSH, GPx and PON activities were decreased, whereas LPO level, TF, LDH, CAT, SOD, MPO and XO activities were increased in rats treated with D-GalN. Administration of the antioxidant combination protected lung tissue against damage by enhancing biochemical chances and pulmonary edema in group 3 animals, while no significant effect on protection of pulmonary inflammation was observed. In conclusion, the antioxidant vitamin supplementation with selenium can be used in the prevention of acute lung injury.

Keywords: Antioxidant, ascorbic acid, β-carotene, D-galactosamine, selenium, vitamin E, lung injury, oxidative stress
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D-galaktozamin ile oluşan akciğer hasarında antioksidant vitaminlerin (vitamin C, vitamin E, beta-karoten) ve selenyumun etkisi

Özet

Bu çalışmada, akut akciğer hasarı üzerine selenyum ihtiva eden vitamin kombinasyonunun etkileri araştırılmıştır. Çalışmada sıçanlardan dört farklı deney grubu oluşturulmuştur; bu gruplar, grup 1, intraperitonal fizyolojik tuzlu su enjekte edilen sıçanlar; grup 2, üç gün süre ile vitamin C, vitamin E, beta-karoten ve sodyum selenat ile beslenen hayvanlar; grup 3, tek doz intraperitonal D-galaktozamin (D-GaIN; 500 mg kg^{-1}) enjekte edilen sıçanlar; grup 4, üç gün süre ile selenyum ve antioksidan kombinasyonu ile beslenen ve tek doz D-GaIN enjekte edilen sıçanlardan oluşturulmuştur. Akciğer dokuları ışık mikroskobu ile araştırılmış ve şu biyokimyasal parametreler ölçülmüştür; glutatyon (GSH), lipid peroksidasyonu (LPO) düzeyleri ve doku faktörü (TF), laktat dehidrojenaz (LDH), katalaz (CAT), superoksit dismutaz (SOD), glutatyon peroksidaz (GPx), paraoksonaz (PON), miyeloperoksidaz (MPO), ksantin oksidaz (XO) ve sodyum potasyum ATPaz (Na^+/K^+-ATPaz) aktiviteleri. Grup 3 sıçanların akciğer dokularında periferal alanlarda yaygın ödem, venüller etrafında mononuklear hücre infiltrasyonları ve bölgesel peteksi yapılar gözlenmiştir. D-GaIN injekte edilen sıçanlarda GSH, GPx ve PON aktiviteleri azalmasına rağmen, LPO düzeylerinin, TF, LDH, CAT, SOD, MPO ve XO aktivitelerinin arttığı saptanmıştır. Antioksidan kombinasyonunun verilmesinin, grup 3 hayvanlardaki biyokimyasal değişiklikleri ve pulmoner ödem bulgularını iyileştirerek, hasara karşı akciğer dokularını koruduğu, ancak pulmoner inflamasyona karşı önemli bir etkisinin olmadığı saptanmıştır. Sonuç olarak, selenyum ihtiva eden antioksidan vitamin uygulamasının akut akciğer hasarını önlemede kullanılabileceği kanısına varılmıştır.

Anahtar Kelimeler: Antioksidan, askorbik asid, β-karoten, D-galaktozamin, selenyum, vitamin E, akciğer hasarı, oksidatif stres

Introduction

Acute lung injury can be induced by inhalation of some toxins, remote organ failure, and mechanic ventilatory, and local and systemic inflammations. It is characterized by epithelial and endothelial cell damage, inflammation and edema. Moreover, oxidative stress is the first sign of tissue damage in acute lung injury. Previously, various lung injury models in experimental animals were reported. For example, aspiration pneumonia induced acute lung injury (Puig et al. 2016). Lipopolysaccharide and carbon tetrachloride were used for acute lung injury model using rats (Kurt et al. 2016; Lin et al. 2016). Lung injury can also be modeled using hyperoxia and radiation treatment (Kayalar and Oztay 2014; Calik et al. 2016). Experimental animal injury models using chemical agents may lead to secondary organ dysfunctions. It was reported that ischemic-reperfusion model in the kidney causes acute lung injury (Karimi et al. 2016; Oztay et al. 2016). D-Galactosamine (D-GaIN) is a common agent used to sensitize mice and other animals to the lethal effects of tumor necrosis factor-alpha (TNF-α). Previously, Catal et al. (2010) reported D-GaIN-induced liver injury accompanied with the elevated oxidative stress and liver injury also causes kidney injury in rats. In this study, protective effects of antioxidant supplementation together with selenium against D-GalN-induced acute lung injury in rats were investigated. The present study reported lung damage caused by D-GaIN-treatment, and positive effects of an antioxidant combination containing selenium on an injury.

Materials and methods

Experiment

In this study, 2-2.5 months female Sprague-Dawley female rats were used. They were randomly divided into four groups as follows: Group I: rats injected physiological saline solution, intraperitoneally (IP). Group II: animals treated with the combination of vitamin C (100 mg $kg^{-1}.day^{-1}$), vitamin E (100 mg $kg^{-1}.day^{-1}$), beta-carotene (15 mg $kg^{-1}.day^{-1}$), and sodium selenate (0.2 mg $kg^{-1}.day^{-1}$) for three days via

gavage. Group III: rats injected D-GaIN (500 mg kg^{-1}; IP) as a single dose. Group IV: rats given the antioxidant combination for three days, then injected D-GaIN. Rats were sacrificed 6 hours after the injection in groups I and III, 7 hours after the last administration in groups II and IV.

Light microscopy

Samples from the lung were fixed in Bouin's solution for 24 h and embedded in paraffin. The sections 5 µm in thickness were stained with haematoxylin-eosin and examined under light microscope.

Biochemical analysis

Right lung samples were analyzed for biochemical studies. Tissue samples from right lung were washed with physiological saline and stored at –20 °C before the experiments. Right lung samples were homogenized in cold saline using a glass homogenizer in order to make 10 % (w/v) homogenate for spectrophotometric analyses. After centrifugation, the supernatant fraction was removed for biochemical determinations. Supernatants were used to determine reduced glutathione (GSH), lipid peroxidation (LPO), thromboplastic activity (TF) and total protein levels as well as for enzymatic analyses. GSH levels were determined by the method developed by Beutler (1975) by using Ellman's reagent. LPO levels in lung homogenates were estimated according to Ledwozyw et al. (1986). TF activities in the homogenates were performed according to Quick's one-stage method described by Ingram and Hill (1976). Lactate dehydrogenase (LDH) activity was assayed by the method proposed by Wroblewski (1957). Catalase (CAT) activity of the lung tissue was carried out by the method of Aebi (1984). Superoxide dismutase (SOD) activity was done according to Mylroie et al. (1986). Glutathione peroxidase (GPx) activity in the lung tissue samples was determined using the method described by Paglia and Valentine (1967) and modified by Wendel (1981). Determination of paraoxonase (PON) activity in the lung tissues was carried out by the method described by Furlong et al. (1988). Myeloperoxidase (MPO) activity of the lung tissue was assayed according to the method of Wei and Frenkel (1993). Xanthine oxidase (XO) activity was evaluated as uric acid production according to Corte and Stirpe (1968) with a few modifications. Sodium/potassium-ATPase (Na^+/K^+-ATPase) in the lung tissue homogenates was determined by the method developed by Ridderstap and Bonting (1969). The protein content in the supernatants was estimated by the method of Lowry et al. (1951) using bovine serum albumin as standard.

Statistical analysis

Biochemical test results were evaluated using one-way ANOVA and unpaired Student's t-test using the NCSS statistical computer package (NCSS 2001, Kaysville, UT, USA). Significant differences were considered when $p<0.05$. Data were expressed as the mean ± standard deviation (SD).

Results

Light microscopical results

Control rats exhibited healthy lung structure. In rats treated with D-GalN, lungs were characterized by extensive edema in peripheral areas, mononuclear cell infiltrations around venules and locally a honeycomb-like structure in the alveolar area. Pretreatments of the antioxidant vitamins combined with selenium preserved lung against injury by improving pulmonary edema in D-GalN-treated rats, whereas they had not an effect on prevention of pulmonary inflammation in these rats (Fig. 1).

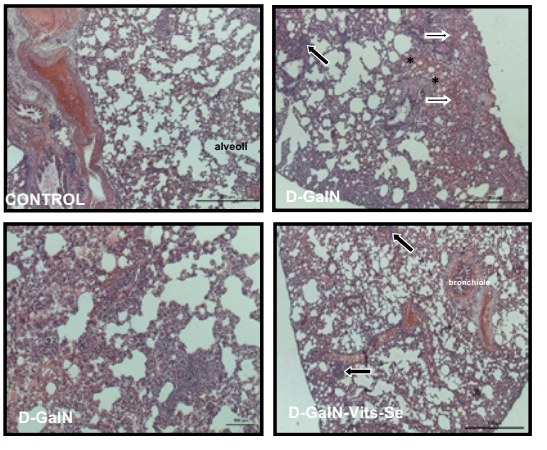

Figure 1. It shows general structure of lungs in experimental rats: Edema (Thin arrow), infiltration areas (Thick arrow) and honeycomb strucure in alveolar areas (*).

Biochemical results

Lung tissue GSH, LPO and TF levels are presented in Table 1. The GSH levels were significantly lower in the lung of treated control+antioxidant and D-GalN group than that of the control group ($p<0.05$). The TF levels were significantly decreased in D-GalN group as compared with control group ($p<0.0001$). In LPO levels, antioxidant treatment to control group and D-GalN administered group were significantly increased when compared to control group, respectively ($p<0.05$; $p< 0.0001$). Administration of antioxidant to D-GalN group reversed these effects ($p<0.05$; $p<0.0001$) (Table 1).

Table 1. Lung tissue glutathione (GSH), lipid peroxidation (LPO), and tissue factor (TF) levels of all groups.

Groups	GSH (nmol GSH/mg protein)*	LPO (nmol MDA/mg protein)*	TF (sec)*
Control	30.86 ± 1.82	3.68 ± 0.52	204.25 ± 17.63
Control + Antioxidant	24.65 ± 3.70[a]	4.43 ± 0.32[a]	194.12 ± 24.22
D-GalN	25.62 ± 1.87[a]	7.18 ± 1.39[c]	151.25 ± 13.66[c]
D-GalN + Antioxidant	38.24 ± 6.58[b]	3.22 ± 1.01[d]	227.69 ± 14.47[d]
P_{ANOVA}	0.002	0. 0001	0.0001

*Mean ± SD
[a]$p<0.05$ versus control group [c]$p<0.0001$ versus control group
[b]$p<0.05$ versus D-GalN group [d]$p<0.0001$ versus D-GalN group

Table 2. Lung tissue lactate dehydrogenase (LDH), catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GP_x) of all groups.

Groups	LDH (U/g protein)*	CAT (U/g protein)*	SOD (U/g protein)*	GP_x (U/g protein)*
Control	17.82 ± 6.88	1.54 ± 0.42	4.77 ± 1.49	35.67 ± 4.27
Control + Antioxidant	11.95 ± 2.64	3.75 ± 0.72[c]	5.64 ± 2.48	39.30 ± 3.32
D-GalN	31.48 ± 2.30[a]	4.68 ± 0.88[c]	9.12 ± 2.10[a]	26.59 ± 4.07[a]
D-GalN + Antioxidant	12.39 ± 4.56[b]	1.42 ± 0.64[d]	4.07 ± 1.43[e]	48.63 ± 6.65[e]
P_{ANOVA}	0.0001	0.0001	0.002	0.001

*Mean ± SD
[a]$p<0.05$ versus control group [d]$p<0.001$ versus D-GalN group
[b]$p<0.0001$ versus D-GalN group [e]$p<0.05$ versus D-GalN group
[c]$p<0.001$ versus control group

Lung tissue LDH, CAT, SOD and GPx, activities of all groups are shown in Table 2. According to the table 2, LDH, CAT, and SOD activities were remarkably increased in D-GalN group as compared with the control group, respectively ($p<0.05$; $p<0.001$; $p<0.05$). However, treatment with an antioxidant to D-GalN group gave rise to in a remarkable decrease in the activities of these enzymes according to D-GalN group, respectively ($p<0.0001$; $p<0.001$; $p<0.05$). The GPx activity was notably lower in the lung tissue of treated D-GalN when compared to the control group ($p<0.05$). However, administration of antioxidant caused a significant increase in lung GPx activity in D-GalN group ($p<0.05$) (Table 2).

PON, MPO, XO, and Na^+/K^+-ATPase activities of tissue homogenates are presented in Table 3. There were a significant decrease in PON ($p<0.05$) and an insignificant decrease in Na^+/K^+-ATPase activity in D-GalN treated group when compared with control group.

Administration with an antioxidant to D-GalN group led to both notable increase in activities of PON ($p<0.0001$) and Na^+/K^+-ATPase ($p<0.05$). The MPO activities of tissue homogenates were remarkably higher in the lung of treated control+antioxidant and D-GalN group than that of the control group, respectively ($p<0.001$; $p<0.0001$). Treatment with antioxidant to D-GalN group resulted in a significant decrease in the activity of MPO ($p<0.0001$). A significant increase in the activity of XO was observed in D-GalN given the group as compared to control group ($p<0.001$). Supplementation of antioxidant to D-GalN group gave rise to in an unremarkable decline in the lung XO activity.

Table 3. Lung tissue paraoxonase (PON), myeloperoxidase (MPO), xanthine oxidase (XO) and sodium potassium ATPase (Na^+/K^+-ATPase) activities of all groups

Groups	PON (U/mg protein)*	MPO (U/g tissue)*	XO (U/mg protein)*	Na^+/K^+-ATPase (µmol P_i/mg protein/h)*
Control	13.70 ± 4.31	1.34 ± 0.62	8.89 ± 1.30	1.46 ± 0.17
Control + Antioxidant	17.21 ± 1.99	2.52 ± 0.38[c]	12.11 ± 2.99	2.58 ± 0.99
D-GalN	6.91 ± 1.22[a]	5.34 ± 0.75[d]	16.60 ± 2.04[c]	1.06 ± 0.39
D-GalN + Antioxidant	17.32 ± 1.40[b]	1.83 ± 1.14[b]	14.64 ± 2.20	3.20 ± 0.81[e]
P_{ANOVA}	0.0001	0.0001	0.001	0. 026

*Mean ± SD
[a]$p<0.05$ versus control group [d]$p<0.0001$ versus control group
[b]$p<0.0001$ versus D-GalN group [e]$p<0.05$ versus D-GalN group
[c]$p<0.001$ versus control group

Discussion

Several protective compounds against lung injury models have been reported using experimental animal models. For example, infliximab was reported as a protective compound against carbon tetrachloride-induced lung damage (Kurt et al. 2016). Dexamethasone, nitric oxide synthase inhibitors prevent lung damage (Kozan et al. 2016). Epigallocatechin gallate protects lung damage against fluoride-induced oxidative stress (Shanmugam et al. 2016). Imatinib reduced lung injury in ischemia/reperfusion injury in rats (Tanaka et al. 2016). Protective effects of emodin on lung damage were also reported (Xu et al. 2016). Sivelestat shows beneficial effects on sepsis-related lung damage (Li et al. 2016). Dexmedetomidine protects lung ischemia-reperfusion damage in rats (Zhang et al. 2016). Administration of dexamethasone treatment before lung injury induced by ventilation shows beneficial effects in rats (Reis et al. 2016). Ghorbel et al. (2016) suggested that extra virgin olive oil may be a novel strategy to protect lung tissue injury. Magnolol was reported as a protective agent against lung injury inhibiting nitric oxide and TNF-α (Tsai et al. 2016). Hyperoxia-induced lung injury can be prevented by etanercept and retinoic acid treatments in rats and mice (Kayalar and Oztay 2014; Kaya et al. 2016). It was reported that isoflurane post-conditioning attenuates lipopolysaccharide-induced lung injury induced by reactive oxygen species (ROS) (Yin et al. 2016).

Some protective agents against the liver may also show beneficial effects on the lung. For example, silymarin which is used to protect the liver can also inhibit activation of enzymes such as caspases in the lung in rats (Jin et al. 2016). Previously, a vitamin E-derivative, ETS-GS, was reported as a ROS scavenger improving the lung in crush injury in rats (Nakagawa et al. 2016). Catal and Bolkent (2008) and Catal et al. (2010) reported protective effects of vitamin E, beta-carotene and selenium on liver and kidney injury through inactivation of caspase-3 and ROS scavenging. Various biochemical parameters such as elevated LPO levels, increased activities of MPO, LDH, CAT, SOD and GPx,

and reduced GSH levels were reported in lung and kidney injury models (Catal et al. 2010; Arda-Pirincci et al. 2012; Oztay et al. 2016). Also, it is well known the excessive oxidative stress-induced tissue damage in the acute lung injury. Pulmonary endothelial and epithelial cells and activated alveolar macrophages produce ROS in response to inflammatory. The generated ROS cause pulmonary endothelial/epithelial damage, endothelial/epithelial barrier disruption and pulmonary edema (Arda-Pirincci et al. 2012). In the present study, antioxidant enzymes as well as compounds, such as XO and MPO were shown as an important indicator of D-GaIN-mediated oxidative stress accompanied with inflammation and the damage of alveolar structure in lung tissues. Additionally, it is known that decreased TF activity in tissue samples contributes to high thromboplastin level and cellular damage. In the present study, D-GaIN-mediated biochemical alterations mentioned above resulted in structural damage, inflammation, and pulmonary edema. Because oxidative stress is effective on acute lung injury, antioxidant therapy is useful in the regression of damage. For example, the therapeutic administration of *N-acetylcysteine* to rats after induction of acute lung injury partially attenuated oxidative stress and defects in lung structure (Choi et al. 2012). On the other hand, patients with acute respiratory stress syndrome have a significant decrease at concentrations of GSH, ascorbic acid, α-tocopherol, β-carotene and selenium (Richard et al. 1990; Bowler et al. 2003). In the present study, the antioxidant vitamin supplementation containing vitamin C, vitamin E, beta-carotene and selenium regressed pulmonary edema and structural damage, by inducing of antioxidant enzymes, PON, TF and Na^+/K^+-ATPase activities in D-GalN-treated rats, whereas it had not an effect on prevention of pulmonary inflammation in these rats. In conclusion, the antioxidant vitamins combined with selenium can be used to protect lung tissues against acute lung injury. However, this therapy needs of using of additive anti-inflammatory reagents.

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