

The Effect of Vitamin E on Free Radical-Mediated Adriamycin Toxicity in Guinea Pig Kidney

VİTAMİN E'NİN KOBAY BÖBREĞİNDE SERBEST RADİKAL ARACILI ADRIAMİSİN TOKSİSİTESİ ÜZERİNE ETKİSİ

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Summary

Purpose: The anthracycline antitumor drug adriamycin causes severe nephrotoxicity in a variety of experimental animals and may be nephrotoxic to humans. This effect may be the consequence of oxidative stress. This study was performed to investigate the effect of adriamycin and the protective action of vit E against adriamycin induced toxicity in kidney.

Materials and Methods: A total of 27 guinea pigs were used (400-500 g) in this study. Animals were divided into three group. Group I served as the control group, group II Guinea pigs were injected with adriamycin (5 mg/kg) i.p. as a single dose. Group III animals were treated with 500 mg/kg vit E at 1st and 4th day after adriamycin injection. Malondialdehyde (MDA) level, Na-K ATP ase activity and phospholipid composition were determined in kidneys.

Results: MDA level and Na-K ATP ase activities were increased significantly after adriamycin treatment, phospholipid composition of kidneys were found to be changed: phosphatidyl glycerol (PG), phosphatidyl inositol (PI), phosphatidyl serine (PS) levels increased, but phosphatidyl choline (PC) and phosphatidyl ethanolamine (PE) decreased.

In our study it was observed that vit E has a protective effect against peroxidation, but it has no significant effect on phospholipid composition. On the other hand vit E reduced membrane Na-K ATP ase activity compared to control group.

Conclusion: It has been concluded that vit E is beneficial in ameliorating the biochemical changes due to adriamycin nephrotoxicity, but these results may be related to the dose schedule of vit E.

Key Words: Adriamycin nephrotoxicity, Phospholipid composition, Na-K ATP ase, Vit E

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Özet

Amaç: Antrasiklin grubundan antitumor ajanlardan olan adriamisin, çeşitli deney hayvanlarında böbrek toksisitesine neden olmakta ve insanlarda da nefrotoksik olabilmektedir. Bu etki oksidatif stresin bir sonucu olabilir. Bu çalışma böbrekler üzerine adriamisinin etkisi ve adriamisinin oluşturduğu toksisiteye karşı vitamin E nin koruyucu etkisini incelemek için yapıldı.

Materyal ve Metod: Bu çalışmada 400-500 gr. ağırlığında 27 kobay kullanıldı. Hayvanlar 3 gruba bölündü. Grup I kontrol grubu olarak kullanıldı. Grup 2 kobaylara adriamisin enjekte edildi. (5 mg/kg) i.p. tek doz. Grup 3'deki hayvanlara adriamisin verilmesinden sonraki 1. ve 4. günlerde 500 mg/kg vit E verildi. Böbreklerde malondialdhid (MDA) seviyesi, fosfolipid bileşimi ve Na-K ATP az aktivitesi tayin edildi.

Bulgular: Adriamisin verilmesinden sonra MDA seviyesi, Na-K ATP az aktivitesi önemli derecede arttı, fosfolipid bileşimi değişti: fosfatidil gliserol (PG), fosfatidil inositol (PI), fosfatidil serin (PS) arttı, fakat fosfatidil kolin (PC) ve fosfatidil etanolamin (PE) azaldı.

Çalışmamızda peroksidasyona karşı vit E'nin koruyucu etkisi gözlemlendi. Ancak fosfolipid bileşimi üzerindeki etki önemli değildi. Diğer taraftan vit E membran Na-K ATP az aktivitesini kontrol grubunun değerlerine düşürdü.

Sonuç: Vit E'nin adriamisinin neden olduğu böbrek toksisitesinde ortaya çıkan biyokimyasal değişimleri düzelttiği, ancak bunun doz semasıyla ilgili olabileceği sonucuna varıldı.

Anahtar Kelimeler: Adriamisinin böbrek toksisitesi, Fosfolipid bileşimi, Na-K ATP az, Vit E

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The anthracycline antitumor drug adriamycin causes severe nephrotoxicity in a variety of experimental animals and may be nephrotoxic to humans (1-3). The molecular mechanism by which anthracycline

cause renal damage is unknown. However, numerous studies support the suggestion that the host toxicities of anthracyclines may be a consequence of oxidative stress (4,5). It has been reported previously that adriamycin enhances the peroxidation of unsaturated membrane phospholipids in liver (5-7).

The fluidity of the plasma membrane, which depends on the lipid composition of the bilayer, has a profound effect on the activity of enzymes associated with that domain of the membrane. The activity of the Na-K ATP ase can be particularly affected (8-10).

In spite of the volume of research carried out, the biochemical mechanism by which adriamycin causes nephrotoxicity has not been defined clearly. It is interesting to discover which of the phospholipids affect the Na-K ATP ase activity in adriamycin toxicity in kidney.

In this study, we aimed to investigate the biochemical changes occur in the kidney of guinea pigs which were treated with adriamycin only or vitamin E+adriamycin. Phospholipid composition, malondialdehyde (MDA) level (lipid peroxidation product) and Na-K ATP ase activity were measured to verify the biochemical alterations in kidney and to determine to what extent vit E could reverse biochemical changes that may be caused by adriamycin.

Material and Method

Adriamycin was obtained from Carlo Erba, vitamin E was purchased from Roche and other chemicals were obtained from Sigma. A total of 27 guinea pigs (male and female) were used (400-500 gr) in the study. Animals were divided into three groups. They were fed a standard diet and allowed free access to water.

Group I served as control. Group 2 guinea pigs were injected with adriamycin 5 mg /kg intraperitoneally as a single dose (11) and four days later they were sacrificed. Group 3 animals were treated with 500 mg/kg vit E intraperitoneally at 1st and 4th day after administration of adriamycin. The dose of vit E was based on acceptable human doses (11). After the sacrifice of animals, kidneys were immediately removed, washed with ice -cold saline and kept at -70°C until use.

Evaluation of lipid peroxidation: Kidney MDA levels were estimated by the method of Uchiyama and Mihara (12).

The Isolation of cortex membrane was based on the method described by Kinsella and Holohan (13).

The Na-K ATP ase activity of the cortex membrane was assayed by the measurement of the produced inorganic phosphate (14).

Phospholipid Measurement: Phospholipids were isolated by thin-layer chromatography (TLC).

The complete evaluation of individual phospholipids was achieved by three step procedure: 1-Extraction using organic solvents, 2-fractionation by TLC, 3-quantitation of each fraction.

Extraction of phospholipids: 1 volume of 200 mg kidney cortex was homogenized with 4 ml 2:1 chloroform - methanol mixture (v/v) to a final dilution 20 fold the volume of the tissue sample, and is mixed thoroughly with 0.2 its volume of water. The mixture is allowed to separate into two phases by centrifugation (3 minutes at 3000 rpm). After upper phase is removed the lower phase of 2 ml is mixed with water and centrifugation. Then, the upper phase is removed again. Finally, the lower phase is diluted to any desired final volume by the addition of 2:1 chloroform - methanol mixture before application.

Fractionation by TLC: samples (30 ml) of extracts and phospholipid standards were applied to silica gel plates. The plates were placed in a tank which contained chloroform- methanol-water (70: 30: 5 v/v). This solvent was allowed to rise within 0.5 cm of the top of the adsorbent.

Quantitation of each fractions : Phospholipid fraction were visualized on the plate using iodine vapors. After the iodine was evaporated from the plate, each outlined spots were scraped off and transferred into glass test tubes. Acid digestion was performed using sulphuric / perchloric acid 1:1 v/v at 100°C 1 h and phosphorus level was measured in each sample (17).

Assuming that 1 mmol phosphorus is equivalent to 1mmol phospholipid, the results were converted into 1mmol phospholipids / gr tissue (15).

Other Procedure: Protein concentration in cortex membranes was estimated by the Lowry method (16). Inorganic phosphate was measured by the method of Ames (17). Statistical analysis was done by using Mann Whitney U test.

Results

In this study, MDA level increased significantly after adriamycin treatment and the phospholipid composition of kidney changed. PG, PI, PS levels increased significantly but PC and PE decreased insignificantly. On the other hand, Na-K ATP ase activity of kidney tissue was found to be increased after adriamycin treatment (Table 1).

In our study it was observed that vitamin E had a protective effect against MDA, but it had no significant effect on phospholipid composition.

As seen in our results, vitamin E reduced Na-K ATP ase activity to control levels (Table 1).

Discussion

Adriamycin nephrosis in rats is a widely accepted model of experimental nephrosis which mimics several aspects of human idiopathic nephrosis and glomerular sclerosis (1). Adriamycin induces the formation of free radicals by direct oxidation to quinone or stimulates superoxide anion synthesis by an enzymic route or both (3,5,6).

Adriamycin modulates several membrane activities such as glycoprotein synthesis and phospholipid composition. Adriamycin is also actively cytotoxic without entering the cells. This fact has led to consideration that the membrane is the target of adriamycin cytotoxicity via a mechanism that is

so far unknown (1). In our study we observed that adriamycin treatment raised MDA levels and changed the phospholipid composition of cortex membrane in guinea pigs.

PA, PI, and PS levels increased, but PC and PE decreased insignificantly. Our results were consistent with a recent published study by Fajardo et al (18). It was reported that adriamycin raised MDA level 2 fold in mouse kidney 4 days after adriamycin treatment.

Okosora et al. (19) have reported that a single i.v injection of adriamycin results in glomerular morphological changes that are similar to minimal - change disease in human and suggested that toxic oxygen metabolites were responsible for adriamycin toxicity in kidney (2). Mimnaugh (5) suggested adriamycin mediated oxyradical production markedly increase the peroxidation of membrane polyunsaturated phospholipids and this lipid peroxidation level in vivo may be involved in the nephrotoxicity of adriamycin.

Wolf et al. (20) reported that adriamycin binds to various cellular membranes incorporation into the lipid matrix of the membrane seems to govern passive drug transport as well as drug action. Their data clearly showed that anionic phospholipids are important determinants of adriamycin binding in membranes but zwitterion phospholipids have almost negligible active. We also observed that PG, PE, PS (anionic phospholipids) are raised following the administration of adriamycin.

The other aim of this study was to investigate the alteration of outermost cortex membrane Na-K ATP ase activity and the relationship between enzyme and phospholipid composition after adri-

Table 1. Na-K ATP ase, MDA, phosphatidylethanolamin (PE), phosphatidylglycerol (PG) phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidylserin (PS) Values

	Control (n=9)	Adriamycin (n=9)	Adriamycin + vit E (n=9)	P*
Na-K ATP ase (u mol Pi / h / mg pr)	0.09 ± 0.01	0.26 ± 0.03	0.09 ± 0.02	PO.05
MDA (nmol / g tissue)	43.89 ± 6.41	58.00 ± 5.29	30.22 ± 3.92	PO.05
PE (p mol Pi / g tissue)	19.12 ± 3.41	14.39 ± 3.58	15.86 ± 2.70	P> 0.05
P (p mol Pi / g tissue)	10.22 ± 2.21	13.64 ± 2.13	14.88 ± 1.13	P<0.05
PC (p mol Pi / g tissue)	18.37 ± 4.93	14.54 ± 1.88	16.56 ± 3.49	P>0.05
PI (u. mol Pi / g tissue)	5.73 ± 1.88	12.99 ± 2.33	12.51 ± 1.74	P<0.05
PS (p mol Pi / g tissue)	5.69 ± 1.74	13.31 ± 1.47	13.38 ± 1.23	P<0.05

* Kruskal - Wallis Variance Analysis

adriamycin treatment. The results showed that, there is an increased Na-K ATP ase activity in the adriamycin treated group.

Our results were in accordance with the results reported by Si-Guang et al. (21) They observed that the activity of Na-K ATPase increases in nephrotoxic rats treated by a single dose of adriamycin and they suggested that adriamycin may act directly on the tubular cells leading to an increase in the Na-K ATP ase activity through an unknown mechanism.

We showed that there is a close relation between phospholipid composition and Na-K ATP ase activity following adriamycin treatment. Na-K ATP ase activity and PG, PS, PI increased. As is well known, membrane lipid composition has a profound effect on the Na-K ATP ase activity (8-10).

In the previous studies it has been suggested that PS, PG, PI but not PE and PC associated with the Na-K ATP ase and PI served as the lipid activator of this enzyme in membrane (22,23).

Several agents have been investigated in an attempt to reduce adriamycin toxicity. One of the most important agents is vitamin E (24,25). It was postulated that vit E is a free radical scavenger and it can minimise cellular peroxidation after an injection of i.v. dose (5 mg/kg) of adriamycin (11).

Mimnaugh et al. (3) observed that adriamycin - enhanced microsomal and mitochondrial membrane lipid peroxidation could be potentially inhibited by vit E.

In the present study, we observed the protective effect of vit E (2x500 mg/kg) against lipid peroxidation induced by adriamycin treatment. But it has no significant protective effect on phospholipid composition.

We considered our results may be explained by the dose schedule of vit E. It has been previously reported that, trials of vit E in preventing adriamycin toxicity in several species of animals have produced conflicting results, probably because of the timing of vit E administration (25-27).

On the other hand our study indicated that vit E may have a direct effect on Na-K ATP ase. As seen in our results, vit E + adriamycin injection, re-

duced enzyme activity to control levels. As proposed previously it could be explained by its peroxide terminating property, not by affecting phospholipid composition (28).

Vit E has been reported to quench the production of free radicals and alleviate lipid peroxidation in different tissues (29). J

In another study, it has been suggested that higher Na-K ATP ase, but lower vit E content and lipid peroxidation are expressions of parallel events (30).

We concluded that vit E is beneficial in ameliorating the biochemical changes, due to adriamycin nephrotoxicity, at the dose used in this study.

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