THE EFFECT OF VITAMIN E (α-TOCOPHEROL) TO TNF-α SERUM LEVELS IN WISTAR WHITE STRAIN RATS EXPOSED TO CISPLATIN

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ABSTRACT

Objective: *To analyze the protective effect of vitamin E on TNF-α levels in white Wistar strains exposed to Cisplatin.* ***Material & Methods:*** *The design of this study was an experimental laboratory with post-test only control group design, with the evaluation of TNF-α levels carried out after the animals were treated. The grouping of experimental animals was carried out by randomization. This study using male Wistar white rats as samples. The control group in this study included a negative control group (CN), which was given an injection of 1 cc intravenous normal saline 0.9% on the 7th day as a placebo, then on the 10th day the blood sample was taken. The positive control group (CP), which was given cisplatin treatment at a dose of 5 mg/kg intraperitoneally, once on the 7th day. Treatment group (P1) was treated using cisplatin 5 mg/kg intra-peritoneally and Vitamine E 100 mg/KgBW, and Treatment group (P2) was treated using cisplatin 5 mg/kg intra-peritoneally and Vitamine E 200 mg/KgBW. Blood samples were taken on the 10th day, intra-cardiac and TNF-α levels were analyzed using ELISA.* ***Results:*** *There were significant differences in the mean TNF-α levels in the negative control group for all treatment groups with a p-value <0.05. There was also a significant difference in TNF-α levels in the positive control group for treatment group 1 and treatment 2 with p<0.05. On the other hand, further analysis showed that there was no significant difference between treatment group 1 and treatment group 2 (p>0.05).* ***Conclusion:*** *TNF-α levels in mice given cisplatin was much higher compared with the control group. Vitamin E 100 and 200 mg/kgBW cause a decrease in TNF-α protein levels in mice injected with cisplatin when compared with controls. There is no difference in TNF-α levels in mice receiving vitamin E at doses of 100 and 200 mg/kgBW.*

Keywords: *Cisplatin, TNF-α, vitamin E.*

ABSTRAK

Tujuan: *Menganalisis efek protektif vitamin E terhadap kadar TNF-α pada tikus putih strain Wistar yang terpapar Cisplatin.* ***Bahan & Cara:*** *Rancangan penelitian ini adalah eksperimental laboratorium dengan post test only control group design, dengan evaluasi kadar TNF-α yang dilakukan setelah hewan coba diberikan perlakuan. Pengelompokan hewan coba dilakukan dengan cara randomisasi. Penelitian ini menggunakan tikus strain Wistar sebagai sampel. Kelompok kontrol dalam penelitian ini meliputi kelompok kontrol negatif (CN), yang diberikan perlakuan injeksi normal saline 0.9% 1 cc 1x intra peritoneal pada hari ke-7 sebagai placebo, kemudian pada hari ke 10 kelompok ini akan diambil sampel darah. Kelompok kontrol positif (CP), yang diberikan perlakuan cisplatin dengan dosis 5 mg/kgBB intra peritoneal 1x pada hari ke-7. Kelompok perlakuan dibagi manjadi dua, yaitu: P1 yang mendapatkan paparan cisplatin 5 mg/KgBB intraperitoneal dan Vitamin E 100 mg/KgBB, dan P2 yang mendapatkan paparan cisplatin 5mg/KgBB dan Vitamin E 200 mg/KgBB. Pengambilan sampel darah dilakukan pada hari ke-10. Pengambilan sampel darah melalui intra kardiak. Selanjutnya dilakukan analisis kadar TNF-α dari darah yang telah diambil dengan menggunakan ELISA.* ***Hasil:*** *Terdapat perbedaan yang signifikan rerata kadar TNF-α pada kelompok kontrol negatif terhadap semua kelompok perlakuan dengan nilai p<0.05. Selain itu juga terdapat perbedaan kadar TNF-α secara signifikan pada kelompok kontrol positif terhadap kelompok perlakuan 1 dan perlakuan 2 dengan p <0.05. Disisi lain, analisa lanjutan menunjukan bahwa tidak ada perbedaan yang signifikan terhadap kelompok perlakuan 1 dengan kelompok perlakuan 2 (p>0.05).* ***Simpulan:*** *Terdapat peningkatan kadar TNF-α pada tikus yang diberikan cisplatin dibandingkan dengan control. Vitamin E 100 dan 200 mg/KgBB mengakibatkan terjadinya penurunan kadar protein TNF-α pada tikus yang diinjeksikan cisplatin bila dibadingkan dengan kontrol. Tidak ada perbedaan kadar TNF-α pada tikus yang mendapat vitamin E dengan dosis 100 dan 200 mg/KgBB.*

Kata Kunci: *Cisplatin, TNF-α, vitamin E.*

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INTRODUCTION

Cancer is the second highest cause of death globally, with an estimated 9.6 million cancer deaths in 2018. Bladder cancer is the 7th most common malignancy in men and 17th in women. The worldwide incidence is 9 per 100.000 for men and 2 per 100.000 for women.[1] The incidence of bladder cancer in Indonesia is not known certainly. According to GLOBOCAN data in 2008, the incidence in Indonesia reached 5.8 per 100.000 population. One of the therapies in cancer is chemotherapy. Cisplatin is one of the most widely used chemotherapy including malignancies in urology: bladder cancer, testicular cancer, penile cancer, and several other cancers. Cisplatin is the first-line chemotherapy in solid tumour. One mechanism by which cisplatin is effective against solid tumour is through the effects of proapoptotic.[2] An important side effect of cisplatin is acute kidney failure; it is estimated that one third of patients are proven to have kidney dysfunction due to cisplatin therapy.[3] Although there are several strategies to prevent nephrotoxicity from cisplatin, no specific treatment recommendations are available to date.

The pathophysiology of acute kidney failure induced by cisplatin is associated with damage to the renal proximal tubules and fundamental mechanisms such as oxidative stress, inflammation, and vascular damage. Recent studies have focused on tubular cell apoptosis and many apoptotic pathways, including tumour necrosis factor receptors (TNFs) or extrinsic pathways, mitochondrial intestinal pathways (Bax pathways) that are controlled by Bcl-2 exits, and endoplasmic stress reticulum pathways, have been described as correlated with renal tubular cell death.[3]

Vitamin E (α-Tocopherol) is a fat-soluble antioxidant, which has the protective effect of biological cell membranes from lipid peroxidation so that it may have an anti-inflammatory effect. Vitamin E supplements are useful in reducing and slowing kidney damage that occurs due to increased oxidative stress, also vitamin E prevents the decrease in levels of other antioxidants in the kidneys such as GSH, CAT, and SOD caused by cisplatin.[4] The protective effect against ROS, other antioxidants and anti-inflammatory effects of vitamin E might reduce TNF-α levels in the kidneys caused by cisplatin administration. Therefore, vitamin E is assumed to be a nephroprotector agent in subjects receiving cisplatin treatment.

OBJECTIVE

To analyze the protective effect of vitamin E on TNF-α levels in white Wistar strains exposed to Cisplatin.

MATERIAL & METHODS

The design of this study was an experimental laboratory with post-test only control group design, with the evaluation of TNF-α levels carried out after the animals were treated. The grouping of experimental animals was carried out by randomization, with repetition of 7 experimental animals in each group and there was a control group as a comparison (positive control and negative control).

Samples are grouped into 4 groups randomly by using random numbers to increase internal validity because this research is a case study (causality). Samples of male white rats (Rattus norvegicus) strain of Wistar, which were newly obtained from Lembaga Penelitian Terpadu (LPT) of Gadjah Mada University, Jogjakarta, began with an adaptation process in the cage/research environment for 2 weeks with a cycle of 12 hours of light, 12 hours of dark. The control group in this study included a negative control group (CN), which was given an injection of 1 cc intravenous normal saline 0.9% on the 7th day as a placebo, then on the 10th day the blood sample was taken. The positive control group (CP), which was given cisplatin treatment (Cisplatin, Kalbe Farma, Indonesia) at a dose of 5 mg/kg intraperitoneally, once on the 7th day. Treatment group (P1) was treated using cisplatin 5 mg/kg intra-peritoneally and Vitamine E 100 mg/KgBW, and Treatment group (P2) was treated using cisplatin 5 mg/kg intra-peritoneally and Vitamine E 200 mg/KgBW.

Blood samples were taken on the 10th day, intra-cardiac. Furthermore, TNF-α levels were analyzed using ELISA. The TNF-α value data will be tested for Kolmogorov-Smirnov normality to find out whether the data is normal or not and will also be tested for variance to find out whether the data variant is same or not. If the data distribution is normal and the variant is homogeneous, then the One-Way Anova hypothesis test is used, if the variant is not homogeneous, One-Way Anova Brown-Forsythe is used.

The hypothesis is determined based on the significance value obtained. If the significance value

<0.05, then the next step is to do a multiple comparison test or Post Hoc Test by LSD, which is to find out in more detail the pairs of treatment groups that are significantly different and those that are not significantly different. If the data variant is not homogeneous then the next step is to do a multiple comparison or post hoc test using Games-Howel to find out the comparison of differences between treatment groups. If there are differences, then proceed with the next statistical test to find out different data pairs (to see differences from each group). This test uses Mann Whitney as a further Kruskal Wallis test.

This study was significant if p-value <0.05. All data processing techniques were analyzed using Statistical Product and Service Solution 25 for Windows (SPSS 25) software.

RESULTS

In this study randomization of subjects was carried out to reduce research bias by using simple randomized sampling methods. Kolmogorov-Smirnov homogeneity test was conducted to assess the success of randomization. The results of normality weight data test subjects showed normal body weight of rats (p>0.05). Further analysis using the One-Way Anova parametric test was carried out to assess differences in the mean body weight of rats between groups (Table 1). The results found no mean difference between treatment groups (p>0.05). So it can be concluded that the randomization of subjects in this study has been successfully carried out. The sample characteristics were shown in table 1.

Table 1. Sample body weight characteristics.

Group	(Mean ± SD)	Normality	P value
CN	202.4 ± 2.9	0.160	0.506
CP	201.5 ± 4.1	0.173	
P1	199.8 ± 2.4	0.210	
P2	201.1 ± 2.9	0.224	

In this study, a statistical test was performed using the mean absorbance value in the form of optical density (OD) obtained from the ELISA method with serum samples of research mice. The normality test was carried out using the Kolmogorov-Smirnov test, the results of the data distribution were normal (p<0.05), therefore One-Way Anova was performed. One-Way Anova test showed that there were significant mean differences between groups with p<0.05. Mean data between groups was further analyzed to determine whether there were differences in variability using the Leven's Test, the result was a difference in variance between groups with p<0.05. Furthermore, the Post Hoc Games-Howell test was carried out to compare the mean differences between study groups (Table 3).

Table 2. Comparison of Tumour Necrosis Factor-α (TNF-α) levels in study subjects.

Group	(Mean ± SD).	Lower Bound - Upper Bound	Normality	P Value
CN	11.39 ± 0.65	10.70 – 12.08	0.200*	0.0001*
CP	31.10 ± 0.82	30.22 – 31.97	0.200*	
P1	20.56 ± 0.09	20.46 – 20.65	0.200*	
P2	20.45 ± 0.27	20.17 – 20.74	0.200*	

Table 3. Comparison of Tumour Necrosis Factor-α (TNF-α) levels between treatment groups.

Comparison of TNF-α levels between groups	Mean Difference	Confidence interval 95%		P value
		Lower bound	Upper bound	
CN Vs CP	19.705*	9.49	37.17	0.000
CN Vs P1	9.165*	18.37	21.03	0.000
CN Vs P2	9.061*	8.09	10.03	0.000
CP Vs P1	10.540*	9.29	11.78	0.000
CP Vs P2	10.643*	9.41	11.87	0.000
P1 Vs P2	0.103	-13.84	13.84	0.813

From the results of the Post Hoc Games-Howell statistical analysis, it was found that there were differences in the mean TNF-α levels in the negative control group for all treatment groups with a p-value <0.05. There was also a significant difference in TNF-α levels in the positive control group for treatment group 1 and treatment 2 with p<0.05. On the other hand, further analysis showed that there was no significant difference between treatment group 1 and treatment group 2 (p>0.05) (Table 3).

DISCUSSION

Cisplatin (dichlorodiamino platinum) is an inorganic platinum-based chemotherapy agent that is widely used in the treatment of various malignant solid tumours. Cisplatin has been used in the treatment of testicular, ovarian, bladder, head and neck cancers, esophagus, lungs, breast, cervix, stomach, prostate cancer, Hodgkin's and non-Hodgkin's lymphoma, neuroblastoma, sarcoma, multiple myeloma, melanoma, and mesothelioma. The main limitation of cisplatin chemotherapy agent is the nephrotoxicity side effects about 25%-35% of patients experience a significant decline in kidney function after a single dose of cisplatin.[5-6]

Previous studies have suggested that pro-inflammatory cytokines such as TNF-α are involved in cisplatin-induced nephrotoxicity.[7] TNF-α is also known as one of the main sources of nephrotoxicity in patients treated by cisplatin. TNF-α induction occurs when kidney tissue experiences a pathological condition which in turn leads to acute kidney failure. Various TNF-α inhibitors in mice induced with nephrotoxicity provide better progression in the occurrence of acute renal failure.[8]

The use of cisplatin (5 mg/kgBW) in this study has shown a significant increase in TNF-α levels compared to controls. The results of this study are in accordance with the results of previous studies which showed that the cisplatin chemotherapy agent has the effect of nephrotoxicity through an increase in pro-inflammatory cytokines TNF-α.[7,9] Another study that used the same research design as this study was a study conducted by Liu et al., In 2006 which showed that intra-peritoneal cisplatin administration in mice would have an effect on nephrotoxicity and eventually there would be acute renal failure in mice.[10] The dose used in this study is similar to the dose in previous studies that used doses are between 2-40 mg/kgBW with the end result of nephrotoxicity in rat kidneys.[3,7]

Nephrotoxicity due to cisplatin can be caused by mitochondrial dysfunction and increased production of reactive oxygen species (ROS) through a respiratory chain that is disrupted by the cytochrome P450 (CYP) system.[11] An increase in various oxygen radical species such as hydrogen peroxide H2O2 and superoxide anion O2 is known to cause an increase in mRNA from TNF-α.[12] The TNF-α response invitro causes it to bind to two main TNF-α receptors, namely: TNFR1 and TNFR2. Both of these receptors will act as regulators of the process of apoptosis and proinflammation in kidney cells. TNFR1 will act as a ligand in the activation of various proapoptotic proteins and culminate in the expression of caspase effector 3 and 7 proteins.[13] On the other hand, TNFR2 will act as a ligand for further expression of various cytokines and chemokines that support the inflammatory process. Thus, an increase in ROS expression after exposure to cisplatin can increase TNF-α expression which ultimately can work simultaneously in resulting in increased tubular cell death, kidney tissue damage and increased inflammation.[10]

Various studies have been conducted to look for antioxidant agents that can prevent cisplatin nephrotoxicity without reducing its effectiveness. Vitamin E (α-tocopherol) is a natural antioxidant that can protect the integrity of cell membranes throughout the body from oxidation reactions caused by ROS.[14] Several studies have shown the protective effect produced by vitamin E and its derivatives on nephrotoxicity and ototoxicity due to cisplatin. Giving vitamin E as a single agent or in combination with other antioxidant agents can cause changes in biomarkers of oxidative stress such as decreased levels of malondialdehyde, decrease in serum urea and serum creatinine and also increase the antioxidant activity of the kidney antioxidant enzymes renal catalyst glutathione S-transferase and superoxide dismutation.[15]

In the results of this study, the exposure of low doses of vitamin E (100 mg/kgBW) gives a statistically significant difference in TNF-α levels compared to groups exposed to cisplatin alone. This is in line with previous studies that Vitamin E can reduce nephrotoxicity due to the cisplatin chemotherapy agent without interfering in its effectiveness.[16] There is research that says otherwise, that vitamin E cannot be a nephroprotector against cisplatin toxicity. But in that study a mouse sample was given estrogen supplement, which can increase cisplatin toxicity.[4]

Previous research concluded that Vitamin E can reduce the production of NO radicals and superoxide (endothelial cells and neutrophils) by inhibiting the production of protein kinase C. Besides having an antioxidant effect, vitamin E also has an anti-inflammatory effect. Several studies have shown that the administration of vitamin E can reduce the production of TNF-α in nephrotoxicity due to dichromate and acetic acid agents.[17-18] This can support the results of this study where the provision of vitamin E can reduce TNF-α production in mice given cisplatin significantly. However, in this study the administration of high-dose Vitamin E (200 mg/kgBW) did not provide a significant difference in the reduction of TNF-α in mice given cisplatin when compared with the administration of low-dose vitamin E (100 mg/kgBW).

The increased dose of vitamin E in this study did not increase the effect on TNF-α. This can occur because vitamin E in the body can act as a dual agent. Vitamin E at the right dose can ward off free radicals by binding to free electrons, but in high doses vitamin E can act as a prooxidant molecule. This is caused by every time an anti-oxidative mechanism occurs, an α-Tocopherol radical (α-Toc*) will also be formed. Physiologically the increase in the radical effect of vitamin E can be avoided by the conversion of α-Toc* which involves vitamin C (L-Ascorbic Acid, Asa). Reduction of α-Toc* requires the enzymes Glutathione (GSH) in the cytosol and Ubiquinol (UQH2) in cell membranes which in turn convert these excess molecules into stable tocopherol molecules.[19] This condition allows the absence of an effect of increasing the dose of vitamin E which leads to a decrease in TNF-α levels in the treatment.

CONCLUSION

TNF-α levels in mice given cisplatin was much higher compared with the control group. Vitamin E 100 and 200 mg/kgBW cause a decrease in TNF-α protein levels in mice injected with cisplatin when compared with controls. There is no difference in TNF-α levels in mice receiving vitamin E at doses of 100 and 200 mg/kgBW.

REFERENCES

1. Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, et al. GLOBOCAN 2012: Estimated Cancer Incidence, Mortality and Prevalence Worldwide in 2012 v1.0. International Agency for Research on Cancer, editor. World Health Organization; 2013.
2. Fuertes M, Castilla J, Alonso C, Pérez J. Cisplatin Biochemical Mechanism of Action: From Cytotoxicity to Induction of Cell Death Through Interconnections Between Apoptotic and Necrotic Pathways. Curr Med Chem. 2012; 10(3): 257-66.
3. Miller RP, Tadagavadi RK, Ramesh G, Reeves WB. Mechanisms of cisplatin nephrotoxicity. Toxins (Basel). 2010; 2(11): 2490-518.
4. Nematbakhsh M, Nasri H. The effects of vitamin E and selenium on cisplatin-induced nephrotoxicity in cancer patients treated with cisplatin-based chemotherapy: A randomized, placebo-controlled study. J Res Med Sci. 2013; 18(7): 626-7.
5. Lebwohl D, Canetta R. Clinical development of platinum complexes in cancer therapy: An historical perspective and an update. Eur J Cancer. 1998; 34(10): 1522-34.
6. Kunze D, Wuttig D, Füssel S, Meye A, Wirth MP. Sirna-Mediated Inhibition of Antiapoptotic Genes in Human Bladder Cancer Cells. Eur Urol Suppl. 2006; 5(14): 800.
7. Zhang B, Ramesh G, Norbury CC, Reeves WB. Cisplatin-induced nephrotoxicity is mediated by tumor necrosis factor-? produced by renal parenchymal cells. Kidney Int. 2007; 72(1): 37-44.
8. Pabla N, Dong Z. Cisplatin nephrotoxicity: Mechanisms and renoprotective strategies. Kidney Int. 2008 May; 73(9): 994-1007.
9. Kumar P, Barua CC, Sulakhiya K, Sharma RK. Curcumin ameliorates cisplatin-induced nephrotoxicity and potentiates its anticancer activity in SD rats: Potential role of curcumin in breast cancer chemotherapy. Front Pharmacol. 2017; 8(APR): 1-12.
10. Liu M, Chien CC, Burne-Taney M, Molls RR, Racusen LC, Colvin RB, et al. A pathophysiologic role for T lymphocytes in murine acute cisplatin nephrotoxicity. J Am Soc Nephrol. 2006; 17(3): 765-74.
11. Volarevic V, Djokovic B, Jankovic MG, Harrell CR, Fellabaum C, Djonov V, et al. Molecular mechanisms of cisplatin-induced nephrotoxicity: A balance on the knife edge between renoprotection and tumor toxicity. J Biomed Sci. 2019; 26(1): 1-14.
12. Kosmidou I, Vassilakopoulos T, Xagorari A, Zakynthinos S, Papapetropoulos A, Roussos C. Production of interleukin-6 by skeletal myotubes: Role of reactive oxygen species. Am J Respir Cell Mol Biol. 2002; 26(5): 587-93.
13. Ramesh G, Reeves WB. TNF-α mediates chemokine and cytokine expression and renal injury in cisplatin nephrotoxicity. J Clin Invest. 2002; 110(6): 835-42.
14. Combs GF, McClung JP. The Vitamins: Fundamental Aspects in Nutrition and Health. 5th ed. Academic Press, Inc., San Diego; 2017. 628 p.

15. Pace A, Giannarelli D, Galiè E, Savarese A, Carpano S, Della Giulia M, et al. Vitamin e neuroprotection for cisplatin neuropathy: A randomized, placebo-controlled trial. Neurology. 2010; 74(9): 762-6.
16. Kalkanis JG, Whitworth C, Rybak LP. Vitamin E Reduces Cisplatin Ototoxicity. Laryngoscope. 2004; 114(3): 538-42.
17. Mehany HA, Abo-youssef AM, Ahmed LA, Arafa E-SA, Abd El-Latif HA. Protective effect of vitamin E and atorvastatin against potassium dichromate-induced nephrotoxicity in rats. Beni-Suef Univ J Basic Appl Sci. 2013; 2(2): 96-102.
18. Roberts NJ, Zhou S, Diaz LA, Holdhoff M. Systemic use of tumor necrosis factor alpha as an anticancer agent. Oncotarget. 2011; 2(10): 739-51.
19. Rizvi S, Raza ST, Ahmed F, Ahmad A, Abbas S, Mahdi F. The role of Vitamin E in human health and some diseases. Sultan Qaboos Univ Med J. 2014; 14(2): 157-65.