Effect of Glutamine Before Gets Cisplatin on Aif and Bcl-2 in the Evidence of Apoptosis Cell Tubulus Proximal in Rats Kidney of *Rattus Norvegicus* Strain Wistar

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Abstract

Background: Increased incidence of cancer in the world also increases the use of chemotherapy agents. Cisplatin is one of the most effective chemotherapy agents, often used for the treatment of various solid tumors. The therapeutic effect of cisplatin significantly increased with increasing dosage.

Aim: The aim of the study was to investigate the role of glutamine in the prevention of apoptosis in proximal renal tubular epithelial cells administered by cisplatin chemotherapy, via an intrinsic caspase-independent apoptotic (AIF).

Methods: The study was enrolled thirty male Wistar rats. The rats were divided into three groups of treatments, group A (negative control), group B has given intraperitoneal cisplatin dose of 20 mg/KgBB, group C administered the intravenous injection of glutamine 100 mg/KgBB for 7 days.

Results: Analyzed ANOVA, mean expression of BCL-2 group B = 56.80 ± 4.39 than group A (control) = 63.7 ± 5.53 decreased not significant (p = 0.06), and mean expression of BCL-2 group C = 113.30 ± 8.92 increase significant compared to group A = 63.7 ± 5.53 (p = 0.000001) and group B = 56.80 ± 4.39 (p = 0.000001). The average AIF expression group C = 236.20 ± 17.58 decreased significantly compared to group B = 309.50 ± 8.08 (p = 0.000001). The average number of group apoptosis C = 151.80 ± 21.87 decreased significantly compared to group B = 255.20 ± 27.82 (p = 0.000001).

Conclusion: Glutamine can inhibit the expression of AIF, increase BCL-2 expression, and decrease apoptosis of epithelial cells of the renal proximal tubules given cisplatin.

Keywords: *Glutamine, Cisplatin, Acute Kidney Injury*

Introduction

Cancer is the second leading cause of death that contributing 13% of deaths from 22% of deaths from non-communicable diseases in the world[1]. It is estimated that at least 22.2 million new cases will be diagnosed annually worldwide by 2030 with the estimated deaths from cancer worldwide will rise through 13.1 million deaths in 2030[2]. Ministry of Health Indonesia in 2007 reported that cancer is the leading cause of death number 7 in Indonesia with cancer prevalence 4.3 per 1000 population.

Cisplatin (cis-diammine-dichloroplatinum II) is one of the most effective chemotherapy agents, often used for

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the treatment of various solid human tumors including testicular and ovarian cancers[3]. This is evidenced by the use of cisplatin as a standard component in chemotherapy regimens in various types of cancer, such as head and neck cancer, ovarial cancer, testicular cancer, esophageal cancer, bladder cancer, cervical cancer, and non-small - small cell lung cancer[4].

The therapeutic effect of cisplatin significantly increases with increasing dosage[5]. However, increased doses of cisplatin in clinical use, often limited by adverse events, such as nephrotoxicity and neurotoxicity, are side effects that limit the dose of cisplatin[6]. The main side effect that limiting the dose of cisplatin is the effect of nephrotoxicity[7].

There are various percentages of nephrotoxicity effects of cisplatin, but the most serious and most frequent effect is acute kidney injury (AKI) occurring in 25-35% of patients[8]. Conceptually AKI is a rapid decline (in hours to weeks) of a generally reversible glomerular filtration rate (LFG), followed by renal failure to excrete residual nitrogen metabolism, with/without disturbance of fluid and electrolyte balance[9].

The diagnostic criteria for AKI according to acute kidney injury network (AKIN) are: abrupt decrease in renal function (within 48 hours) characterized by elevated serum creatinine levels>> 0.3 mg/dl (26.4 μmol/l) or serum creatinine more than 1.5 times (> 50%) when compared with previous levels or decreased urine output to less than 0.5 cc/h for more than 6 hours[10]. The nephrotoxic effects of cisplatin most commonly occur after 10 days of cisplatin administration[11]. Over the past few years, renal tubular epithelial cell apoptosis has been the focus of investigation on the mechanism of nephrotoxicity of cisplatin. There are several apoptotic pathways involved, including extrinsic pathways mediated by death receptor, mitochondrial-centered intrinsic pathways, and ER pathway reticulum (ER) stress[12].

Increased incidence of cancer in the world also increases the use of various chemotherapy agents, including cisplatin. The widespread use of cisplatin increases the side effects of cisplatin use, including the nephrotoxic effects of cisplatinie Acute Kidney Injury (AKI), caused by renal cell apoptosis. Glutamine is the most abundant amino acid found in the blood and in the storage of free amino acids from the body. One of the important characteristics of glutamine is that glutamine has a beneficial effect on critical illness, as it can increase the expression of heat shock protein (HSP)[13]. HSP is a group of essential proteins for cell endurance under stress conditions[14]. There is evidence that glutamine can increase HSP-70 and HSP-72 expression in pulmonary and epithelial cell macrophages[15]. Increased expression of HSP-72 has the ability of antiapoptosis by stimulating Bcl 2, Bcl-XL and inhibiting damage to the mitochondrial membrane[16]. Therefore, this study will examine the effect of glutamine injection as an antiapoptosis of proximal renal tubular cells treated with cisplatin chemotherapy.

Method

This research was a laboratory experimental research with "*Randomized Post-test only control group design*" design that used thirty male Wistar rats. Rats were divided into three treatment groups, group A (negative control), group B has given intraperitoneal cisplatin injection of a single dose of 20 mg/KgBB, group C given intravenous injection glutamine 100 mg/KgBB for 7 days and at day 7 were given intraperitoneal cisplatin dose of 20 mg/KgBB. After 72 hours of cisplatin injection, renal mouse tissue was processed immunohistochemically, to observe the number of proximal renal tubular cells expressing Bcl-2, AIF, and apoptotic cell counts.

The experimental animals used in this study were obtained from the Faculty of Veterinary Medicine of Airlangga University. Selection of sample for grouping and giving treatment using simple random sampling, with inclusion criteria is *Rattus novergicus* strain Wistar rats, healthy male rats, healthy-looking active, 2.5-3 months old, 150-200 gram weight.

The sample size in this study was determined based on Federer's formula, with dropout correction (0.1), resulting in a total sample of 30 mice with details of 10 rats for each treatment group. Animal treatment was performed for 7 days, then examination of kidney preparation on the 10th day was the number of cellsexpressing BCL-2, the number of cells expressing Apoptotic Inducing Factor AIF and the number of apoptotic cells in male rats proximal tubule rats Rattus novergicus wistar strain, with a 1000X light microscope, positive rectal proximal tubular cells were calculated (located in the renal cortical region, characterized by cylindrical tube lumen), positive cell counts in 20 viewing field (HPF) in each sample.

The duration of Injectable Glutamine Intra-Vein (IGIV) administration was 7 days, because based on previous studies glutamine will increase HSP after 7 days of IGIV. Observation time at day 10 or 72 hours after intraperitoneal cisplatin injection (on day 7) because apoptosis in proximal renal tubular cells was apparent after 72 hours of cisplatin administration. Renal kidney tissue is processed immunohistochemically, to observe the number of proximal renal tubular cells expressing Bcl-2, AIF, and apoptotic cell count. Data were analyzed with SPSS 17, ANOVA analysis followed by Post Hoc Tukey test, with p-value <0.05.

Results

Based on the results obtained the analysis of glutamine inhibit apoptosis proximal tubule renal cells. Increased HSP70 affects survival associated with the ability to inhibit apoptosis. Glutamine supplementation enhances HSP expression. It was concluded that in the 72-hour observation after cisplatin administration, glutamine could inhibit apoptosis of proximal renal tubule cells significantly, and was directly proportional to the glutamine effect that inhibited the expression of AIF.

The homogeneity test shown in Table. 1 was performed by using one-way ANOVA test, indicating that there was no significant difference between body weight before treatment and weight after treatment (sig >0.05). Here is table 1.

Table.1 Homogeneity Test Based on Weight Loss

GROUPS	F	p
Weight before treatment	1.486	0.222*
Weight after treatment	0.941	0.449*

From that table, its can suggests that animal weight data try to have a homogeneous variation. In this study, it appears that changes in epithelial cells of proximal renal tubules occur after 72 hours of observation.

Immunohistochemical renal tissue examination results showed that the control group's renal tissue showed an average amount of BCL-2 expression of 63.7 ± 5.538752. Giving cisplatin (Group B) in animals tries to decrease mean expression BCL-2 is not significant (p = 0.066028) after 72 hours to 56.80 ± 4.391912. Administration of cisplatin and glutamine (group C) in the experimental animals significantly increased the mean expression of BCL-2 to group A (p = 0.000001) and group B (p = 0.000001) after 72 hours to 113.30 ± 8.920015. Administration of cisplatin and glutamine (Group C), BCL-2 expression was increased when compared to the control group (Group A) and the cisplatin-treated group (Group B). Addition of cisplatin (Group B) did not significantly decrease BCL-2 expression compared to the control group (Group A). Histologic representation of BCL-2 expression with Immunohistochemical staining, using Olympus BX 50 magnification 1000x.

The results of immunohistochemical renal tissue examination of the average AIF group A (control) expression amounted to 48.4 ± 3.134042. Giving cisplatin (Group B) in animals tries to significantly increase the average of AIF expression (p = 0.000001) after 72 hours to 309.50 ± 8.086340. However, administration of cisplatin and glutamine in group C in animals tended to decrease mean AIF expression significantly against group B (p = 0.000001) after 72 hours to 236.20 ± 17.586611. It can be concluded that in the administration of cisplatin and glutamine (Group C), the expression of AIF decreased significantly compared with the group given cisplatin (Group B). A histological representation of AIF expression with immunohistochemical staining using Olympus BX 50 1000x magnification microscope.

The results of the renal tissue examination with apoptosis detection kit In Situ Cell Death Detection Kit, POD showed that the group A (control) kidney tissue showed an average number of cell apoptosis of 47.40 ± 18.572381. Giving cisplatin (Group B) in animals tries to significantly increase cell apoptosis averages (p = 0.000001) after 72 hours to 255.20 ± 27.828043. However, the administration of cisplatin and glutamine in group C in animals tended to decrease the mean cell apoptosis significantly against group B (p = 0.000001) after 72 hours to 151.80 ± 21.877436. It can be concluded that in the administration of cisplatin and glutamine (Group C), rectal proximal renal tube apoptosis decreased significantly compared to the cisplatin group (Group B). Histologic features of rectal proximal tubular apoptosis with detection kit In Situ Cell Death Detection Kit, POD, using Olympus BX 50 1000x magnification.

The results of data analysis obtained showed that cisplatin and glutamine can increase BCL-2 expression, so BCL-2/Bax ratio can be increased which will inhibit

the leakage of mitochondrial membrane, and inhibit the release of apoptogenic factors (cytochrome c, apoptosis inducing factor (AIF), Second Mitochondria-Derived Activator of Caspase (SMAC), and others)[12]. Similar results were also obtained from previous studies which concluded that HSP-72 expression enhancement has anti-apoptotic ability by stimulating Bcl 2, Bcl-XL and inhibiting damage to mitochondrial membranes[16].

In other side cisplatin (cis-diammine-dichloroplatinum II) is one of the most effective chemotherapy agents, often used as chemotherapeutic reagents of various solid human tumors including testicular and ovarian cancers[3]. This is evidenced by the use of cisplatin as a standard component in chemotherapy regimens in various types of cancer[4].

The therapeutic effect of cisplatin significantly increases with increasing dosage[5]. However, increased doses of cisplatin in clinical use are often limited by adverse events, such as nephrotoxicity and neurotoxicity, which are side effects that limit the dose of the cisplatin[6]. Giving glutamine to malignancy is still controversial. There are groups who argue that glutamine is the main food needed for tumor cell metabolism, so the administration of glutamine can enlarge the tumor and resistant to chemotherapy[17]. Yet another group believes that glutamine intake is necessary as a nutritional intake of cancer and has antioxidant effects by increasing Glutathione (GSH) that can inhibit tumor growth otherwise it is known to reduce the side effects of chemotherapy agents Doxorubicin[18]. Previous studies proving cisplatin may increase AIF expression in the renal proximal tubular cytosol, which then AIF out of the mitochondria, will accumulate in the cell nucleus that will induce apoptosis via the caspase-independent pathway[19].

Hsp 72 renal epithelial cells may decrease mitochondrial membrane injury by Hsp 72 interaction with BCL-2. Furthermore, the potential of L-glutamine to HSP 72 is associated with increased intestinal epithelial resistance to apoptotic injury, and the reduction of HSP 72 may be associated with increased caspase activity in glutamine deficiency. Thus, it is hoped that the administration of glutamine would be useful in increasing heat shock protein (HSP) 72 and BCL2 which ultimately decrease apoptosis of renal proximal tubule cells due to cisplatin.

Glutamine administration inhibits the expression of AIF so it can inhibit the occurrence of apoptosis. This result is not in accordance with previous research, the resistance of AIF expression is not due to increased expression of BCL-2, this indicates if the effect of cisplatin on decreasing expression of BCL-2 is very low. Therefore, observation for 72 hours after cisplatin administration, glutamine administration can inhibit AIF expression significantly. Administration of cisplatin may increase apoptosis of proximal renal tubular cells that are mechanically nephrotoxic of cisplatin(15). Apoptosis due to cisplatin administration occurs through increased AIF expression. AIF that comes out of the mitochondria, will accumulate in the cell nucleus that will induce apoptosis through the caspase independent pathway.

Conclusion

Based on the results of the study it can be concluded that glutamine can increase BCL-2 expression in epithelial cells of the renal proximal tubules given cisplatin.

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Ethical Clearance: This study was approved by Ethical Commission of Health Research Faculty of Medicine University of Airlangga.

References

1. Shibuya K, Mathers CD, Boschi-Pinto C, Lopez AD, Murray CJL. Global and regional estimates of cancer mortality and incidence by site: II. Results for the global burden of disease 2000. BMC Cancer. 2002;2(1):37.
2. Bray F, Jemal A, Grey N, Ferlay J, Forman D. Global cancer transitions according to the Human Development Index (2008–2030): a population-based study. Lancet Oncol. 2012;13(8):790–801.
3. Ognjanović BI, Djordjević NZ, Matić MM, Obradović JM, Mladenović JM, Štajn AŠ, et al. Lipid peroxidative damage on cisplatin exposure and alterations in antioxidant defense system in rat kidneys: a possible protective effect of selenium. Int J Mol Sci. 2012;13(2):1790–803.
4. Noda K, Nishiwaki Y, Kawahara M, Negoro S, Sugiura T, Yokoyama A, et al. Irinotecan plus cisplatin compared with etoposide plus cisplatin

for extensive small-cell lung cancer. N Engl J Med. 2002;346(2):85–91.

5. O'Dwyer PJ, Stevenson JP, Johnson SW. Clinical status of cisplatin, carboplatin, and other platinum-based antitumor drugs. Cisplatin Chem Biochem a Lead Anticancer drug. 1999;31–69.
6. Hanigan MH, Devarajan P. Cisplatin nephrotoxicity: molecular mechanisms. Cancer Ther. 2003;1:47.
7. Sastry J, Kellie SJ. Severe neurotoxicity, ototoxicity and nephrotoxicity following high-dose cisplatin and amifostine. Pediatr Hematol Oncol. 2005;22(5):441–5.
8. Lee KW, Jeong JY, Lim BJ, Chang Y-K, Lee S-J, Na K-R, et al. Sildenafil attenuates renal injury in an experimental model of rat cisplatin-induced nephrotoxicity. Toxicology. 2009;257(3):137–43.
9. Broden CC. Acute renal failure and mechanical ventilation: reality or myth? Crit Care Nurse. 2009;29(2):62–75.
10. Bagshaw SM, George C, Bellomo R. A comparison of the RIFLE and AKIN criteria for acute kidney injury in critically ill patients. Nephrol Dial Transplant. 2008;23(5):1569–74.
11. Arany I, Safirstein RL. Cisplatin nephrotoxicity. In: Seminars in nephrology. Elsevier; 2003. p. 460–4.
12. Danial NN, Korsmeyer SJ. Cell death: critical control points. Cell. 2004;116(2):205–19.
13. Morrison AL, Dinges M, Singleton KD, Odoms K, Wong HR, Wischmeyer PE. Glutamine's protection against cellular injury is dependent on heat shock factor-1. Am J Physiol Physiol. 2006;290(6):C1625–32.
14. Fleshner M, Campisi J, Amiri L, Diamond DM. Cat exposure induces both intra-and extracellular Hsp72: the role of adrenal hormones. Psychoneuroendocrinology. 2004;29(9):1142–52.
15. Eliasen MM, Brabec M, Gerner C, Pollheimer J, Auer H, Zellner M, et al. Reduced stress tolerance of glutamine-deprived human monocytic cells is associated with selective down-regulation of Hsp70 by decreased mRNA stability. J Mol Med. 2006;84(2):147–58.
16. Knowlton AA, Salfity M. Nuclear localization and the heat shock proteins. J Biosci. 1996;21(2):123–32.
17. Erickson JW, Cerione RA. Glutaminase: a hot spot for regulation of cancer cell metabolism? Oncotarget. 2010;1(8):734.
18. Liu Y, Lao J, Gao K, Gu Y, Xin Z. Outcome of nerve transfers for traumatic complete brachial plexus avulsion: results of 28 patients by DASH and NRS questionnaires. J Hand Surg (European Vol. 2012;37(5):413–21.
19. Pabla N, Dong Z. Cisplatin nephrotoxicity: mechanisms and renoprotective strategies. Kidney Int. 2008;73(9):994–1007.