Association between Dose and Duration of Cisplatin Exposure with Sitotoksisity Effect on Nasopharyngeal Carcinoma Stem Cell

Widodo Ario Kentjono[1], Lakshmi Kusuma Wardhani[1], Achmad C Romdhoni[1]

[1]Department of Otorhinolaryngology, Faculty of Medicine, Universitas Airlangga, (60131), Indonesia

Abstract

Background: Nasopharyngeal carcinoma (NPC) is ranked 6th of malignant tumors in Indonesia, while cisplatin is an effective chemotherapy therapy but the side effects and resistance problems are two major constraints limiting its application.

Objective: To analyze the correlation of dose and duration of cisplatin exposure with cytotoxic effects on nasopharyngeal carcinoma stem cells

Methods: A true experimental laboratory *in vitro* with the factorial design was used in this study. The biopsy NPC tissue was cultured and processed to obtain NPC stem cells to be treated with cisplatin exposure at different doses (0.05, 0.1, 0.2, 0.4, 0.8, 1 and 2 µg) and different durations (24 and 48 hours). The number of dead cells after exposure will be calculated using a hemocytometer after 24, 48, and 72 hours of NPC stem cells free of cisplatin.

Results: Death stem cell density of NPC was mostly obtained at the exposure of 2 µg/ml cisplatin dose after 24 hours observation was 81.37%, while the smallest death cell density a dose of 0.05 µg/ml after a 72-hour observation was 21.3%. Statistical analysis with multiple regression correlation tests between the density of death cell and cisplatin dose was obtained the coefficient correlation 0,827 and value p = 0,000. The analysis of the correlation between cisplatin exposure duration and death cell was also significant with the correlation coefficient -0.357 and the value p = 0.001. The cutoff point that correlated the dose and death stem cell of NPC by 50% in both 24 and 48 hours of exposure was 1 µg (EC_{50}).

Conclusion: There was a correlation between the increased dose of cisplatin with the cytotoxicity effects on NPC stem cell.

Keywords: *Nasopharyngeal carcinoma (NPC), Dose, Duration Of Exposure, Cisplatin, Cytotoxicity*

Introduction

The incidence of Nasopharyngeal Carcinoma (NPC) in Indonesia is 6.2/100,000 inhabitants every year. NPC is ranked 6th of malignant tumors in humans after malignant tumors of the cervix, liver, breast, lung, and skin. According to research both national and international, it is reported that most of NPC patients (80%) come in advanced stages III and IV. During the last five decades, NPC handling is mainly in various forms of chemotherapy and radiation therapy. The damage effect caused by chemotherapy is called cytotoxicity, that most chemotherapy drugs work by interfering cell mitosis and primarily targeting cells with high cleavage rates [1].

Cisplatin is an effective chemotherapy, but side effects and resistance problems are two major constraints limiting its application. The biochemical mechanism of cisplatin cytotoxicity includes the correlation between

Corresponding Author:
Widodo Ario Kentjono
Department of Otorhinolaryngology, Faculty of Medicine, Universitas Airlangga, (60131), Indonesia
E-mail & phone : widododarioken@gmail.com, +62315020251

DNA and non-DNA targets which will induce death cell through apoptosis, necrosis or both. The cytotoxicity indicator used is EC50 (effective concentration 50) which is the dose/concentration of certain compounds that needed to produce a cytotoxic effect of 50% death cell in cell culture in vitro. This indicator is often used as a benchmark of eukaryotic cell cytotoxicity in culture [2].

Research that correlated the role of resistant cancer stem cells to cisplatin and the progression of many malignancies were included in ca mammae studies was reported that the presence of tumor cells were expressing the normal tumorigenic stem cell characteristics by 5.9% of the tumor cell population [3]. The population of these cells significantly increased to an average of 8.8% in primary transplants that only responded partially to cisplatin, while in secondary tumor transplants, the population increased to 22.8% [4].

In vitro research on the effect of cisplatin on DNA suggests that cisplatin toxicity is affected by dose and time/dose also time-dependent. The previous research mentioned the concentration of certain cisplatin was needed to kill 90% of cancer cells. While the other studies reported that 24-hour cisplatin exposure was significantly much more cytotoxic than the first hour duration of exposure [5]. Based on these descriptions, this study was conducted to reveal the cytotoxic effects of cisplatin chemotherapy drugs on NPC stem cells and the dose also duration that affect them. This approach useful for understanding how NPC stem cells process cisplatin exposure and determining the effect of the dose also the duration of cisplatin exposure resulting in cytotoxic effects on NPC stem cells [6].

Method

This study is experimental laboratory in vitro with the factorial design. Biopsy specimens from a patient suspected of NPC were taken in sufficient quantities, and some were sent to the Anatomical Pathology Installation Dr. Soetomo Genera hospital in formalin solution. Nasopharyngeal carcinoma stem cell culture divided into two plates [7].

After the culture procedure, stem cells nasopharynx carcinoma confirmed with CD44 + staining that divided into two plates of each containing 24 well. Each well is filled with 100,000 stem cells of nasopharyngeal carcinoma. One plate was incubated for 24 hours and another plate for 48 hours, after 24 hours exposure, NPC stem cells in one plate were cleared of cisplatin and observed at 24, 48, and 72 hours before calculation. The same procedure for 48 hours of cisplatin exposure was performed in a similar method [8]. The density of the dying cell from the original mixture will be calculated according to the percentage of total number of dead NPC stem cells divided by total number of NPC stem cells (dead and alive cells) [5].

Results

The data shows the number of cells experiencing mortality at 24 hours cisplatin exposure and observation periods at 24, 48 and 72 hours. The number of cells that died after cisplatin administration increased along with increasing the doses (compared with controls). The pattern increase occurs with a little fluctuation were the decrease in the dose of 0.4 μg at 24 and 48 hours of observation then increase again. At 72 hours observation the decrease occurred at a dose of 0.8 μg and then the number of dead cells increased (Table 1).

Death stem cell density of NPC mostly in the 24 hours cisplatin exposure was obtained at 2 μg/ml with the post-observation time after exposure was 81.37%, while the smallest death cell density at 0.05 μg/ml dose calculated after 72 hours observation was 21.3%. The proportion of dead cells was relatively high after post-24 hour observation, while post-observation 48 and 72 hours of the relatively close coincident pattern but not as high as post-24 hour observation.

Table 2 shows a similar pattern to 24-hour cisplatin exposure that is an increase in dose-dependent death cell, there was a slight decrease in the dose of 0.1 μg, then increased again. On a 48-hour observation, death cell was high at a dose of 0.05 μg, dropping at 0.1 μg, then just increasing again at 0.4 μg. On a 72-hour observation, death cell dropped at a dose of 0.1 μg then increased at a dose of 0.2 μg. Density stem cells of NPC mostly at 48 hours cisplatin exposure was obtained at 2 μg/ml cisplatin dose exposure with a post-observation time at 72 hours after exposure was 51.32%,.

Table 2 shows that death cell density after 48 hours of cisplatin exposure tends to increase according to the observation period. The density of death cell at 48 hours of observation was relatively higher than 24 hours, while after 72 hours observation showed the highest density. From statistical analysis with multiple regression correlation tests between the density of death cell

(proportion of dead cells) and cisplatin dose was obtained the correlation coefficient 0,827 and p = 0,000. Table 1 and 2 show tendency pattern of increased death cell with higher cisplatin doses. Both of these showed that there was a significant correlation between the increased dose of cisplatin and cytotoxicity in NPC stem cells (p <0.05). Data correlation of cisplatin exposure duration with death cell was obtained coefficient correlation -0,357 and p = 0,001 indicating that there was the correlation between duration of cisplatin exposure with cytotoxicity profile on NPC stem cells which also significant (p <0,05).

Table 1. Number and density of dead cells in post-exposure solution of 24-hour cisplatin

Concetration (mg/ml)	**Observation 24-hour**			**Observation 48-hour**			**Observation 72-hour**		
	Live	**Dead**	**Death cell density (%)**	**Live**	**Dead**	**Death cell density (%)**	**Live**	**Dead**	**Death cell density (%)**
0,05	43500	23000	34,58	81000	27000	25,00	90500	24500	21,30
0,1	55000	30500	35,67	67000	34500	33,99	89000	31500	26,14
0,2	58500	42500	42,07	95000	45000	32,14	96500	40500	29,56
0,4	54500	38500	41,39	68500	41000	37,44	89000	44000	33,08
0,8	42000	40000	48,78	82500	49000	37,26	59500	41000	40,79
1	16000	30000	65,21	64500	47000	42,15	57500	47500	45,23
2	9500	41500	81,37	53500	60500	53,07	32500	39500	54,86
Control	70000	20000	22,22	117000	15000	11,36	98500	13500	12.05

Table 2. Number and density of dead cells in the original solution of post-exposure to cisplatin 48 hours

Concentration (mg/ml)	**Observation 24-hour**			**Observation 48-hour**			**Observation 72-hour**		
	Live	**Dead**	**Death cell densty (%)**	**Live**	**Dead**	**Death cell density (%)**	**Live**	**Dead**	**Death cell density (%)**
0,05	92000	8500	8,45	70000	17500	20	66000	21500	24,57
0,1	85000	14500	14,5	63500	13500	17,53	59000	15000	20,27
0,2	73000	14000	16,09	52000	9500	15,44	47000	16500	25,98
0,4	91500	13000	12,44	46500	12500	21,18	41000	17000	29,31
0,8	44500	12500	21,92	53500	16500	23,57	33500	17500	34,31
1	35000	16500	32,03	46000	24000	34,28	32500	21500	39,81
2	34500	18500	34,90	27500	24500	47,11	27500	29000	51,32
Control	117500	3500	2,89	90000	2000	2,17	80000	5000	5,88

Discussion

In a population of tissues or cells, apoptosis and necrosis are two extremes of death cell. Low cisplatin concentrations that correlated with apoptotic death cell and high doses cause the death cell due to necrosis. Apoptosis is a response to cellular stress at the intensity of exposure below the necrotic threshold. High doses of cisplatin resulted in the damage of a number molecules that involved in the supply of cell energy adenosine triphosphate (ATP) [9].

The proteins directly involved or indirectly in the apoptotic process leading to the death of necrotic cells, as evidenced by the appearance of necrotic cell features at exposure to high doses of cisplatin cisplatin resistant keratinocyte tissue. Exposure to high doses of cisplatin causes the reduction of ATP cell levels resulting in severe ATP depletion. Then, it will cause a rapid metabolic collapse resulting in necrotic death cell. The fewer ATP depletions correlated with lower doses of cisplatin cause apoptosis by the release of mitochondrial cytochrome [10].

Some studies suggest two lag phase in cisplatin cell growth inhibition in accordance with the results of this study that within the first 6 hours, no cisplatin inhibition effect was detected. It was estimated that in that period cisplatin accumulates and reaches the DNA genome to then express its pharmacological activity. After that period there was a rapid decrease in cell viability up to 20 post-exposure hours [11]. The second lag phase of static cell/plateau growth occurs at 20-24 hours, which estimated to occur due to inactivation of cisplatin by thiol compound, only by then, there will be a significant decrease in cell viability. At the length of exposure duration up to 48 hours, there was an extensive membrane blockade of platelet function, the proportion of dead cells did not parallel with the drug content of assumption that saturation at the receptor has been achieved [12].

In the 48-hour duration of cisplatin exposure, cell proliferation has lasted for 2-4 generations of cells (assuming doubling the time of NPC cell line time in varies from 10.5 to 28.5 hours). The highest level of cisplatin uptake (passive diffusion) at the early time, that the more death cell at the beginning of the duration exposure with the proliferation of cells have lasted 2 to 4 cells generations within 48 hours, the number of dead cells becomes less than the new living cells resulting from the proliferation that occurs after the cell undergoes recovery [13].

The EC_{50} indicator was the concentration or dose that required by a drug to achieve the desired effect of 50% in vitro. EC_{50} for cytotoxicity means at concentrations of 50% cells showing the effect of death cell [14]. Measurement of drug concentrations or doses usually follows a rapidly increasing pattern of sigmoid curves in relatively small dose changes. The effective dose point mathematically determined by drawing the corresponding line that was more easily determined by a graph than a complex statistical equation [15].

A number of studies used both EC_{50} and IC_{50} indicators with similar results. Another study reported that the cisplatin dose of 0.5 µg in the cell line of NPC CNE1 cellular was damage but the cells still respond actively that characterized by the inhibition of cell growth in the early period of observation but then recovered [16]. Kadashiet conducted a study of cisplatin cytotoxicity on several cell culture types using IC_{50} indicators obtained different values. Different doses that effect cytotoxic effects dissimilar between cell types because the mechanisms of apoptosis induced by cisplatin were unlike, and highly specific in each cell . This difference might also be due to the doubling time difference between cell types, especially in the growth-regulated neoplastic cells [17].

Conclusion

There was the correlation between the dose increased of cisplatin and cytotoxicity in NPC stem cells. Moreover, there was a correlation between the duration of cisplatin exposure and cytotoxicity in NPC stem cells. The effective dose of cisplatin resulting in a cytotoxic effect on NPC stem cells was by 1 µg at 24-hours exposure duration.

Ethical Clearance: This research involves participants in the process using a questionnaire that was accordant with the ethical research principle based on the regulation of research ethic regulation. The present study was carried out in accordance with the research principles. This study implemented the basic principle ethics of respect, beneficence, non-maleficence, and justice.

Conflict of Interest: The authors have not found any conflict of interest related to this research so far.

Source of Funding

All of the cost and fees related with this research are paid by the authors only with no sponsorship nor external funds.

References

1. Hussein YM, Gharib AF, Etewa RL, Amal S, Abdel-Ghany ME, Elsawy WH. The melanoma-associated antigen-A3,-A4 genes: relation to the risk and clinicopathological parameters in breast cancer patients. Mol Cell Biochem. 2011;351(1–2):261–8.
2. Skoog T, van't Hooft FM, Kallin B, Jovinge S, Boquist S, Nilsson J, et al. A common functional polymorphism (C→ A substitution at position−863) in the promoter region of the tumour necrosis factor-α (TNF-α) gene associated with reduced circulating levels of TNF-α. Hum Mol Genet. 1999;8(8):1443–9.
3. Kumar JN, Wu Y-L, Loh XJ, Ho NY, Aik SX, Pang VY. The effective treatment of multi-drug resistant tumors with self-assembling alginate copolymers. Polym Chem. 2019;10(2):278–86.
4. Soetrisno S, Mulyani S, Nurinasari H, Budi E. The Influence of Psychocurative on Cortisol Level, Anxiety, and Quality of Life in Advanced Stage Cervical Cancer Patients. Folia Medica Indones. 2019;55(3):202–5.
5. Rahmawati D, Indrawati R, Roestamadji RI, Setiawatie EM, Yuliati A, Bramantoro T. Osteogenic ability of combined hematopoetic stem cell, hydroxyapatite graft and platelet rich fibrin on rats (Rattus novergicus). J Krishna Inst Med Sci. 2017;6(4).
6. Sari GM. THE EFFECT OF LONG TERM ADMINISTRATION OF GLUCOCORTICOID TO BONE LINING CELLS APOPTOSIS. Folia Medica Indones. 2017;52(4):251–7.
7. Ahsan A, Nursalam N, Efendi F. Model Development of Nursing Service Loyalty. Indian J Public Heal Res Dev. 2019;10(1).
8. Sari NIP, Mertaniasih NM, Maruyama F. Application of serial tests for Mycobacterium tuberculosis detection to active lung tuberculosis cases in Indonesia. BMC Res Notes. 2019;12(1):313.
9. Hendrijantini N, Kusumaningsih T, Rostiny R, Mulawardhana P, Danudiningrat CP, Rantam FA. A potential therapy of human umbilical cord mesenchymal stem cells for bone regeneration on osteoporotic mandibular bone. Eur J Dent. 2018;12(3):358.
10. Sole SS, Srinivasan BP, Akarte AS. Anti-inflammatory action of tamarind seeds reduces hyperglycemic excursion by repressing pancreatic β-cell damage and normalizing SREBP-1c concentration. Pharm Biol. 2013;51(3):350–60.
11. Maceli M. Tech-Savvy Librarian Versus (Library) Technologist: Understanding the Future Role of Librarians in Technology Practice. In: Re-envisioning the MLS: Perspectives on the Future of Library and Information Science Education. Emerald Publishing Limited; 2018. p. 153–78.
12. Chernyaeva E, Rotkevich M, Krasheninnikova K, Yurchenko A, Vyazovaya A, Mokrousov I, et al. Whole-genome analysis of mycobacterium tuberculosis from patients with tuberculous spondylitis, Russia. Emerg Infect Dis. 2018;24(3):579.
13. Sienel W, Varwerk C, Linder A, Kaiser D, Teschner M, Delire M, et al. Melanoma associated antigen (MAGE)-A3 expression in Stages I and II non-small cell lung cancer: results of a multi-center study. Eur J cardio-thoracic Surg. 2004;25(1):131–4.
14. Jikrona R, Suharjono S, Ahmad A. Thiamine supplement therapy improves ejection fraction value in stage ii heart failure patients. Folia Medica Indones. 2017;53(2):139–43.
15. Feng Y, Zhang S, Zhang Z, Guo J, Tan Z, Zhu Y, et al. Understanding Genotypes and Phenotypes of the Mutations in Voltage-Gated Sodium Channel α Subunits in Epilepsy. CNS Neurol Disord Targets (Formerly Curr Drug Targets-CNS Neurol Disord. 2019;
16. Zuhaira S, Nizam NM, Ridzuan PM. The Efficacy of Psidium guajava Linn Leaf Extractsfrom Selangor Region Against Gram-Positive and Gram-Negative Bacteria. Folia Medica Indones. 2018;54(4):294–300.
17. Rachmawati SA, Hikmawati D, Budiatin AS, Putra AP. Physicochemical and Cytotoxicity Characterization of Injectable Bone Substitute Based on Hydroxyapatite-Chitosan-Streptomycin for Spinal Tuberculosis Cases. In: Materials Science Forum. Trans Tech Publ; 2019. p. 133–8.