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Research Article
Light Microscope Study of Intratympanic Methylprednisolone Effect on Cisplatin Induced Ototoxicity

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Abstract

Background and Objective: The use of intratympanic methylprednisolone has a significant effect on the improvement of the cochlear response. This research aimed to study the histologic imaging of the effect of intratympanic methylprednisolone on ototoxicity induced cisplatin. This research aimed to examine the histologic imaging of the effect of intratympanic methylprednisolone on ototoxicity induced cisplatin. **Materials and Methods:** This is an experimental study with pre-post test control group design. Data obtained from 11 New Zealand strain rabbits, were treated with 4.6 mg kg^{-1} cisplatin intraperitoneal followed by the treatment of 0.5 mg kg^{-1} intratympanic methylprednisolone. They underwent distortion product evoked otoacoustic emission testing at baseline, at day 3 and 9. At the day 3 and 9 of the experiment, the animals were sacrificed and their cochlear were retrieved and prepared for hematoxylin and eosin staining. The histopathologic changes that occurred were observed based on the scoring system. The difference of cochlear damage analyzed statistically with Independent sample by t-test. **Results:** Assessment of cochlear damage by the degree of stria vascular damage by using a scoring system, as indicated by the level of shrinkage of the intermediate cells. There is a difference of cochlear damage (stria vascularis) on day 9 of cisplatin administration between group 1 (cisplatin) and group 2 (cisplatin+methylprednisolone) ($p<0.026$). There is a difference in cochlear damage (stria vascularis) between day 3 and 9 in group 2 (cisplatin+methylprednisolone) but not statistically significant ($p>0.05$). **Conclusion:** Histologically, intratympanic methylprednisolone giving effect to the improvement of cochlear function.

Key words: Cisplatin, intratympanic, methylprednisolone, ototoxicity, stria vascularis

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cisplatin increases the risk of hearing loss during therapy. The use of anti-cancer drugs began in 1946 with the accidental discovery of nitrogen mustard that can be used to treat leukemia. Anti-cancer drugs are very toxic so its use must be prudent and on the right indications[1].

One of the many appropriate toxic effects on the ear, nose and throat is the ototoxicity that is marked by a sharp decrease in hearing in patients with malignant head-neck tumors post-chemotherapy[2].

The hallmark of ototoxicity associated with high frequency sensorineural hearing loss. Although it may also extend to an intermediate frequency, it may be temporary but irreversible[2,3].

Cisplatin used as a standard for the treatment of various malignancies including head and neck tumors. It is known to be the most anticancer drug that causes ototoxicity, 33% reported incidence in patients given single cisplatin dose 50 mg m^{-2} [3]. In several studies there were differences in the rate of ototoxic ranging from 11-33%[4,5].

Cisplatin damages the outer hair cells of the cochlear progressively from the base to the apex so that sensorineural hearing loss occurs starting at high frequencies. In the condition of all the outer hair cells are damaged, damage can affect the inner hair cells and supporting cells. The cisplatin ototoxicity is not limited to hair cells alone. It is also reported to occur atrophy of the stria vascularis, the collapse of the Reissner's membrane and the degeneration of the spiral ganglion. The mechanism of cisplatin ototoxicity occurs through the process of apoptosis which induced by the increase of free radicals in the inner ear which is induced by cisplatin[6-8].

The literature shows the benefits of corticosteroids to treat inner ear disorders. But systemic steroid use has poor complications[9,10]. Direct steroid injection into the middle ear space should be considered to reduce the effects of the corticosteroids. Some researchers used intratympanic methylprednisolone. Intratympanic administration has several advantages: The potential for steroid removal through a circular window membrane, resulting in higher perilymph levels and possibly a reduction in systemic steroid uptake and toxicity[9,10]. Wihartanti found that cisplatin administration showed a decreased cochlear response at various frequencies, but after intratympanic methylprednisolone administration, there was an improvement of cochlear response especially in the high frequency while at low frequency did not appear improvement[11].

This study aimed to find the histopathologic feature of the effect of intratympanic methylprednisolone on ototoxicity induced cisplatin.

MATERIALS AND METHODS

Study site: This study conducted in January-June, 2017 in Animal Laboratory of Hasanuddin University in Makassar.

Design and study variables: This study is an experimental study of the histopathological imaging of the effect of intratympanic methylprednisolone on ototoxicity induced cisplatin. The study variable consisted of independent variables that are cisplatin intraperitoneal and methylprednisolone intratympanic. The dependent variable is the histopathological imaging of cochlear response.

Animals: This study uses 11 rabbit strains of New Zealand, age 3-4 months and weight 1500-2000 g. The experimental animals are maintained in individual cages, cleaned daily. Temperature 28-32°C and there is adequate air circulation and light and adapted for 7 days with regular feeding and drinking.

Research ethics aspects: This study received permission from the Commission on Ethics of Biomedical Research on Human, Faculty of Medicine Hasanuddin University (Register No.: UH16121266).

Exclusion criteria: Rabbit has diarrhea, rabbit with narrow meatus, rabbits have otitis externa, otitis media and rabbit die at the time of the research.

Drugs: This study used cisplatin produced by PT. Kalbe located in Jakarta, Indonesia, with a 1 mg cisplatin composition, a 10 mg preparation applied intraperitoneally. Methylprednisolone injection production PT. Kalbe, packed with one vial box at 125 mg dry methylprednisolone and one ampoule at 4 mL solvent used intratympanic. The study also used Isoflurane USP 250 mL and xylazine 20 mg mL^{-1}.

Study groups: Rabbits, who entered the inclusion criteria were randomly assigned to 2 groups.

Group I: Rabbits in this cluster were given only intraperitoneal cisplatin on day 0 with a dose of 4.6 mg kg^{-1}. On day 3, DPOAE measurements and histology examination performed on three rabbits. The cisplatin administered on days 3, 6 and 9. On the day 9, DPOAE measurements and histopathologic examination performed for three rabbits

Group II: Rabbits in this group were given intraperitoneal cisplatin on day 0 with a dose of 4.6 mg kg^{-1}. On the day 3, DPOAE measurements and histology examination performed on two rabbits. In 3 other rabbits, followed by intratympanic injection of methylprednisolone at a dose of 0.5 mg kg^{-1} with a 27.0 G needle slowly in the inferior posterior quadrant on days 3, 6 and 9. On the day 9, DPOAE measurements and histology Imaging performed

The animals sacrificed and their cochlear were retrieved and prepared for hematoxylin and eosin staining at Pathology Anatomy Laboratory of Unhas hospital for examination.

Ototoxicity: Determination of ototoxicity using distortion product of auto acoustic emission (DPOAE) as a gold standard which resulted in the measurement of signal to noise (SN) and determination of cochlear damage degree using histopathology examination with scoring consisting of four levels of damage, i.e. (1) Cell depletion, (2) Mild shrinkage, (3) Moderate shrinkage and (4) Heavy shrinkage.

Statistical analysis: Data analyzed by using statistical package for social sciences (SPSS) software (version 23.0 for Windows; SPSS Inc., Chicago, IL). This study utilized independent sample t-test, with significant level of $p \leq 0.05$.

RESULTS

Functional hearing evaluation: The DPOAE measurements performed as clinical evidence to look at the occurrence of ototoxicity induced by cisplatin and to see the effect of intratympanic methylprednisolone. Data shows that cisplatin induced otoxicity based on decreasing of the signal to Noise (SN) Value. Signal to Noise (SN) value of all subjects were decreased after 3 days administration of Ciplastin, but subjects, who were also given methylprednisolone improved SN values at 2, 3 and 4 KHz on the 9th day of observation. (Table 1).

Morphological evaluation of ototoxicity: A total of 5 rabbits, i.e., 3 rabbits from group 1 and 2 rabbits of group 2 were sacrificed and performed histopathological examination and proved that all degree of stria vascular damage were exist (Fig. 1). Histologic changes that occurred were observed based on the scoring system that has been compiled. The degree of stria vascular damage where score 0 if no cell depletion, score one if mild shrinkage, score two if moderate reduction and score 3 for severe shrinkage.

Stria vascularis damage: Scoring of stria vascularis damage based on histologic changes (day 9) is difference between group 1 and group 2. Mean scoring of group 1 is 3,00, means most of subject given cisplatin has severe shrinkage damage while mean scoring of group 2 was 1,00, means most of subject given Cisplatin, followed by intratympanic injection of methylprednisolone has mild shrinkage damage. Analysis showed that there is a significance difference stria vascular damage between group 1 and group 2 ($p = 0.026$, $p \leq 0.05$). (Table 2).

Table 1: Signal to noise (SN) value before and after treatment

		SN value											
		Before cisplatin administration (day 0)				After cisplatin administration (day 3)				After cisplatin administration (day 9)			
		Frequency (KHz)				Frequency (KHz)				Frequency (KHz)			
Rabbits	Weight (kg)	2	3	4	5	2	3	4	5	2	3	4	5
Group 1 (n = 6)													
1*	2.0	13	32	26	31	13	16	13	12	-	-	-	-
2*	2.0	17	36	28	24	27	10	31	28	-	-	-	-
3*	2.0	13	17	21	27	12	27	17	25	-	-	-	-
4	1.9	8	6	17	13	14	-1	32	31	3	2	0	0
5	2.0	10	23	30	0	16	32	27	36	3	5	8	6
6	1.9	2	17	15	8	-2	11	12	22	-16	10	8	27
Group 2 (n = 5)													
1*	2.0	16	7	8	18	-2	7	4	-2	-	-	-	-
2*	1.9	-2	7	7	5	-8	8	0	3	-	-	-	-
3	2.0	21	15	31	34	-5	0	0	13	-12	3	2	0
4	2.0	19	25	25	19	-5	4	-5	2	1	-4	8	-4
5	2.0	8	5	0	6	6	-5	5	3	7	15	14	29

*Sacrifice on day 3 for histopathological examination, Group 1 was given ciplastin, Group 2 was given ciplastin+methylprednisolone

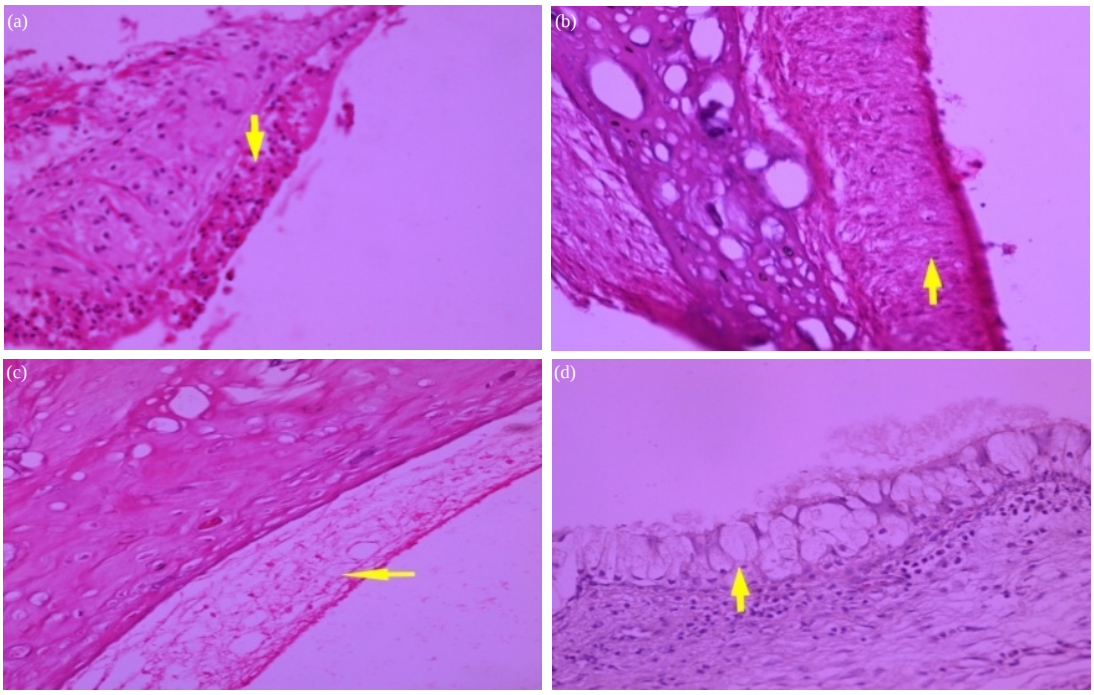

Fig. 1(a-d): Histopathological examination of rabbit for stria vascularis damage, (a) No cell depletion, (b) Mild shrinkage, (c) Moderate shrinkage and (d) Heavy shrinkage

Table 2: Analysis of stria vascularis damage on day 9 between group 1 (cisplatin) and 2 (cisplatin+methylprednisolone)

Variable	Groups	n	Mean	Std. deviation	p-value
Stria vascularis damage	1	3	3.00	±0.000	0.026*
	2	2	1.00	±1.000	

*Independent sample t-test

Table 3: Analysis stria vascularis damage between day 3 and 9 in group 2 (cisplatin+methylprednisolone)

Variables	Days of observation	n	Mean	Std. deviation	p-value
Stria vascularis damage	3	3	2.50	±0.707	0.170*
	9	3	1.00	±1.000	

*Independent sample t-test

All subjects given ciplastin followed by intratympanic injection of methylprednisolone were sacrifice on day 3 and 9, data showed that stria vascularis damage on day 3 is heavier (mean = 2.50) than day 9 (mean = 1). There is a difference of cochlear damage (stria vascular) between day 3 and 9 in group 2 (cisplatin+methylprednisolone) but not statistically significant (Table 3).

DISCUSSION

This study showed that there was a significant effect on the improvement of the cochlear response. Furthermore, there was an increase based on the morphological changes that occurred.

Histopathologic examination using a light microscope with cross sections of modiolus can be performed to identify any damage to the stria vascular, spiral ganglion and Reissner's membrane structures[9,10].

Lynch *et al.*[12] use Haematoxylin and Eosin staining to evaluate cell morphological changes that occur confined to stria vascularis damage. In further studies, this staining technique provides a real and definite picture of the structure, with a clear view of the nucleus and cytoplasm and efficiently provides a clear vision of the cellular damage. Other studies also used toluidine blue[13,14]. Staining or 1% methylene blue+1 percent Azur II in 1% of sodium tetraborate[12,15-22].

Cisplatin is pharmacokinetically hydrolyzed and monohydrate complex (MHC) is formed which is a toxic biotransformation product and occurs after 15 min or 1 h after injection bolus but this is found to be an individual variation in the occurrence of the ototoxicity effect[23].

The effects of cisplatin can occur 2 days after the start of therapy may also appear after 7 days of cessation of treatment, there is some literature to conduct audiometric evaluation 1, 2, 5 and 14 days after treatment[24].

In most studies of the effects of cisplatin on cochlear reported damage to the structure of the outer hair cell and striavascularis[9,11,16,18,19,20]. Where the harm is intended for depreciation in intermediate cells[9,11,16,18,19,20] blending of marginal cells[9,16,20] protrusion from peripheral cells into

endolymphatic space[13,18], edema and atrophy[25] with the use of cisplatin dose of 2 mg kg^{-1} for 8 consecutive days as proposed by De Groot *et al.*[17], O'Leary *et al.*[22] and Van Rujven *et al.*[26,27] where they also observed any changes of stria vascularis.

Campbell *et al.*[15] limited their research to only finding the damage that occurs to the stria vascular regardless of the damage taking place in the outer hair cell. Lynch *et al.*[12] reported damage to the outer hair cell in rats similar to that of marmots.

In animal studies, intratympanic steroid administration led to a very high increase in steroid concentrations in perilymph compared with oral and intravenous administration[28].

Research by Chandrasekhar *et al.*[24] also showed an increase in steroid levels in perilymphs administered intratympanic compared with systemic administration. Pinar *et al.*[10] revealed that intratympanic administration of methylprednisolone was highly significant in providing high protection against cisplatin induced ototoxicity rather than intravenous administration.

Wihartanti[11] find that cisplatin administration showed a decreased cochlear response at various frequencies but after intratympanic methylprednisolone administration, there was an improvement of cochlear response especially in the high frequency while at low frequency did not appear improvement.

This study implies that all patients receiving cisplatin therapy should be examined for the risk of ototoxicity as early prevention. The use of methylprednisolone may be considered by the patient's condition to be given along with cisplatin.

The limitation of this study is still using a light microscope and not yet using an electron microscope and also dose response methylprednisolone also not studied.

CONCLUSION AND FUTURE RECOMMENDATION

The studies concluded that the intratympanic methylprednisolone treatment reduced cisplatin induced ototoxicity, intratympanic methylprednisolone is giving effect to the improvement of cochlear function based on the morphological changes. Histologically, intratympanic methylprednisolone giving effect to the improvement of cochlear function.

This study recommends for further study with the addition of different doses and frequencies for faster and better cochlear enhancement.

SIGNIFICANCE STATEMENTS

This study provides an overview of cochlear morphology, the treatment response of intratympanic methylprednisolone that can be beneficial for treatment in cisplatin induced ototoxicity. This study help the physician to considered for the clinical application of intratympanic methylprednisolone in patients undergoing cisplatin induced ototoxicity to prevent ototoxicity effects to became worst.

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REFERENCES

1. Sukardja, I.D.G., 2000. Dasar-Dasar Kemoterapi Kanker. Airlangga University Press, Jakarta, pp: 239-255.
2. Riggs, L.C., 1998. Ototoxicity. In: Head and Neck Surgery-Otolaryngology: Self-Assessment, Bailey, B.J. (Ed.). 2nd Edn., Lippincott-Raven, Philadelphia, ISBN: 9780397518050, pp: 2165-2168.
3. Rademaker-Lakhai, J.M., M. Crul, L. Zuur, P. Baas and J.H. Beijnen *et al.*, 2006. Relationship between cisplatin administration and the development of ototoxicity. J. Clin. Oncol., 24: 918-924.
4. Wright, A., 1997. Ototoxicity. In: Scott-Brown's Otolaryngology, Scott-Brown, W.G. (Ed.). 6th Edn., Butterworth Heinemann, Great Britain, ISBN: 9780750623681, pp: 3-20.
5. Duta, A., M.D. Venkatesh and R.C. Kashyap, 2005. Study of the effects of chemotherapy on auditory function. Indian J. Otolaryngol. Head Neck Surg., 57: 226-228.
6. Rybak, L.P., A.E. Talaska and J. Schacht, 2008. Drug-Induced Hearing Loss. In: Auditory Trauma, Protection and Repair, Schacht, J. (Ed.). Springer, Philadelphia, ISBN: 978-0-387-72560-4, pp: 219-256.
7. Ramirez Camacho, R., J.R. Garcia Berrocal, J. Bujan, A. Martin Marero and A. Trinidad, 2004. Supporting cells as a target of cisplatin-induced inner ear damage: Therapeutic implications. Laryngoscope, 114: 533-537.
8. Daldal, A., O. Odabasi and B. Serbetcioglu, 2007. The protective effect of intratympanic dexamethasone on cisplatin-induced ototoxicity in guinea pigs. Otolaryngol.-Head Neck Surg., 137: 747-752.

9. Doyle, K.J., C. Bauch, R. Battista, C. Beatty and G.B. Hughes *et al*., 2004. Intratympanic steroid treatment: A review. Otol. Neurotol., 25: 1034-1039.
10. Pinar, E., C. Calli, B. Serbetcioglu, S. Oncel, H.A. Bagriyanik and O. Yilmaz, 2010. Protective effect of methylprednisolone against cisplatin-induced ototoxicity: Comparison of route of administration. Int. Adv. Otol., 6: 53-59.
11. Wihartanti, 2015. [The effect of intrtimpanic metilprednisolone on the improvement of koklea response post cisplatin treatment *in vivo*]. M.Sc. Thesis, Perpustakaan Pusat Universitas, Makassar, Indonesia.
12. Lynch, E.D., R. Gu, C. Pierce and J. Kil, 2005. Reduction of acute cisplatin ototoxicity and nephrotoxicity in rats by oral administration of allopurinol and ebselen. Hearing Res., 201: 81-89.
13. Cardinaal, R.M., J.C.M. de Groot, E.H. Huizing, J.E. Veldman and G.F. Smoorenburg, 2000. Dose-dependent effect of 8-day cisplatin administration upon the morphology of the albino guinea pig cochlea. Hearing Res., 144: 135-146.
14. Wang, J., R.V.L. Faulconbridge, A. Fetoni, M.J. Guitton, R. Pujol and J.L. Puel, 2003. Local application of sodium thiosulfate prevents cisplatin-induced hearing loss in the guinea pig. Neuropharmacology, 45: 380-393.
15. Campbell, K.C.M., R.P. Meech, L.P. Rybak and L.F. Hughes, 1999. D-Methionine protects against cisplatin damage to the stria vascularis. Hearing Res., 138: 13-28.
16. Sergi, B., A. Ferraresi, D. Troiani, G. Paludetti and A.R. Fetoni, 2003. Cisplatin ototoxicity in the guinea pig: Vestibular and cochlear damage. Hearing Res., 182: 56-64.
17. De Groot, J.C.M., F.P.T. Hamers, W.H. Gispen and G.F. Smoorenburg, 1997. Co-administration of the neurotrophic $ACTH_{(4-9)}$ analogue, ORG 2766, may reduce the cochleotoxic effects of cisplatin. Hearing Res., 106: 9-19.
18. Heijmen, P.S., S.F.L. Klis, J.C.M.J. de Groot and G.F. Smoorenburg, 1999. Cisplatin ototoxicity and the possibly protective effect of α-melanocyte stimulating hormone. Hearing Res., 128: 27-39.
19. Cardinaal, R.M., J.C.M. de Groot, E.H. Huizing, J.E. Veldman and G.F. Smoorenburg, 2000. Cisplatin-induced ototoxicity: Morphological evidence of spontaneous outer hair cell recovery in albino guinea pigs? Hearing Res., 144: 147-156.
20. Cardinaal, R.M., J.C.M. de Groot, E.H. Huizing, J.E. Veldman and G.F. Smoorenburg, 2000. Histological effects of co-administration of an $ACTH_{(4-9)}$ analogue, ORG 2766, on cisplatin ototoxicity in the albino guinea pig. Hearing Res., 144: 157-167.
21. Smoorenburg, G.F., J.C.M.J. de Groot, F. Hamers and S.F. Klis, 1999. Protection and spontaneous recovery from cisplatin-induced hearing loss. Ann. N. Y. Acad. Sci., 884: 192-210.
22. O'Leary, S.J., S.F.L. Klis, J.C.M.J. de Groot, F.P.T. Hamers and G.F. Smoorenburg, 2001. Perilymphatic application of cisplatin over several days in albino guinea pigs: Dose-dependency of electrophysiological and morphological effects. Hearing Res., 154: 135-145.
23. Ekborn, A., 2003. Cisplatin-induced ototoxicity: Pharmacokinetics, prediction and prevention. Ph.D. Thesis, Departement of Otorhinolaryngology and Head and Neck Surgery, Karolinska Institute, Stockholm, Sweden.
24. Chandrasekhar, S.S., R.Y. Rubinstein, J.A. Kwartler, M. Gatz, P.E. Connelly, E. Huang and S. Baredes, 2000. Dexamethasone pharmacokinetics in the inner ear: comparison of route of administration and use of facilitating agents. Otolaryngol.-Head Neck Surg., 122: 521-528.
25. Sluyter, S., S.F.L. Klis, J.C.M.J. de Groot and G.F. Smoorenburg, 2003. Alterations in the stria vascularis in relation to cisplatin ototoxicity and recovery. Hearing Res., 185: 49-56.
26. Van Ruijven, M.W.M., J.C.M.J. de Groot and G.F. Smoorenburg, 2004. Time sequence of degeneration pattern in the guinea pig cochlea during cisplatin administration.: A quantitative histological study. Hearing Res., 197: 44-54.
27. Van Ruijven, M.W.M., J.C.M.J. de Groot, S.F.L. Klis and G.F. Smoorenburg, 2005. The cochlear targets of cisplatin: An electrophysiological and morphological time-sequence study. Hearing Res., 205: 241-248.
28. Parnes, L.S., A.H. Sun and D.J. Freeman, 1999. Corticosteroid pharmacokinetics in the inner ear fluids: an animal study followed by clinical application. Laryngoscope, 109: 1-17.