Can aspirin protect or at least attenuate gentamicin ototoxicity in humans?

Fatholah Behnoud, MD, *Khashayar Davoudpur,* MD, *Mohammad T. Goodarzi,* PhD.

ABSTRACT

الأهداف: فحص وإثبات أن الأسبرين كعامل ضد الأكسدة يحمي أو على الأقل يخفف من تسمم الأذن للجنتامسين لدى البشر.

الطريقة: تم إجراء تجربة عشوائية استطلاعية ل 60 مريض أكملوا جميع المتطلبات. أجريت الدراسة عن طريق قسم الأنف والأذن والحنجرة وجراحة العظام – مستشفى بيست – جامعة حمدان للعلوم الطبية – إيران خلال الفترة من ديسمبر 2007 حتى نوفمبر 2008. تم تقسيم المرضى إلى مجموعتين، مجموعة التجربة ومجموعة التحكم التي كانت متشابه مع الأخذ بالاعتبار للجنس، والعمر، والوزن. تلقت مجموعة العلاج1.5g/day 500 mg/8h جرعة من الأسبرين، بينما تلقت مجموعة التحكم علاج إرضائي مشابه للمجموعة الأخرى.

النتائج: أظهرت مقارنة ثرومبوبلاستين البلازما PTAفي 1000و 2000 و 4000 و 8000HZ اختلافات بين متوسط PTAفي اليوم الأول، والثامن، والخامس عشر. كانت قيمة على التوالي p=0.03، و p=0.003 و p=0.001 و p=0.0010 كان مقدار عتبة PTAفي 250Hz مختلف بشكل مهم في اليوم الثامن والخامس عشر فقط p=0.004، كما كان تكرار 500Hz مهم، الاختلاف بين البداية مع اليوم الخامس عشر واليوم الثامن مع اليوم الخامس عشر مهم إحصائيا p=0.001.

خاتمة: هي هذه الدراسة الحالية وجدنا أن الأسبرين يحمي من أثر تسمم الأذن للجنتامسين في المرضى.

Objectives: To investigate and prove that Aspirin as an antioxidant protects, or at least attenuates Gentamicin ototoxicity in humans.

Methods: A prospective, randomized, double-blind trial was conducted in 60 patients that completed all requirements. This study was conducted in the Department of Otolaryngology and Orthopedics, Besat Hospital, Hamadan University of Medical Sciences, Hamadan, Iran, between December 2007 and November 2008. The patients were divided into 2 groups, the experimental and the control groups that were similar with respect to gender, age, and weight. The treatment group received 1.5 g/day (500 mg every 8 hours) Aspirin, and the control group received placebo similar to the other group.

Result: Comparison of the pure tone audiometry (PTA) at 1000 Hertz (Hz) (p=0.03), 2000 Hz (p=0.003), 4000 Hz (p=0.001), and 8000 Hz (p=0.0010) showed significant differences between mean of PTA at the beginning, 8th, and 15th day. The threshold of PTA at 250Hz was significantly different only at the 8th and 15th day (p=0.004), also at the frequency of 500Hz, the difference between the beginning within 15th day and 8th day with 15th day was significant (p=0.001).

Conclusion: In the present study, we had shown that Aspirin can protect the ototoxic effect of gentamicin in patients.

Saudi Med J 2009; Vol. 30 (9): 1165-1169

From the Department of Ear, Nose and Throat, Medical School, Hamadan University of Medical Sciences, Hamadan, Iran.

Received 22nd March 2009. Accepted 8th August 2009.

Address correspondence and reprint request to: Dr. Fatholah Behnoud, Ear, Nose, and Throat Department, Besat Hospital, Hamadan, Iran. Tel. +98 (91) 81115595. Fax. +98 (81) 12640064. E-mail: behnoud344@yahoo.com

Aminoglycosides are incorporated into the regimen against multi-drug resistant tuberculosis as per suggestion of the World Health Organizaton.[1] It is also used for treating gram-negative bacterial infections.[2] These drugs may be the most commonly used antibiotics worldwide, especially in developing countries.[3] Although these drugs are extremely efficacious, they can result in ototoxicity.[1,2] The incidence of cochleotoxicity has been reported up to 33% of patients, while the balance apparatus may be affected in approximately 15%.[1] The incidence of side effects may be higher in developing countries where the drugs are generally available over

the counter, and the drug serum levels in patients are not routinely obtained for an adjustment of dosing, to avoid levels that may be associated with a higher risk of ototoxicity.[1,3] The biochemical mechanisms leading to aminoglycosides ototoxicity are not fully understood,[4] but several evidences suggest that damage may result from the formation of reactive oxygen species that overwhelm the cellular antioxidant defense systems of the inner ear.[4,5] The resulting cellular redox-imbalance causes apoptotic or necrotic cell death.[6,7] In fact, the use of aminoglycosides lead to chromatin condensation and DNA fragmentation, 2 hallmarks of programmed cell death.[4] Caspases, a superfamily of cysteine aspartate-specific proteases, play a central role in apoptosis. Aminoglycosides can also induce the release of cytochrome-c with caspase 8 can play critical role in hair cell death.[7,8] According to this mechanism, several agents have been shown to reduce ototoxicity, mostly focusing on antioxidant therapy[3,9,10] for example, aspirin,[1,3,11] sodium thiosulfate[2] glutathione,[6,12] and melatonin.[13] Based on the report of Sha et al,[9] aspirin has the potential to attenuate gentamicin-induced hearing loss. The aim of this study is to evaluate the range of the effectiveness of Aspirin as an otoprotective agent against Gentamicin ototoxicity. Actually, it is a well known fact that Aspirin has several potential benefits in different fields of medicine. This drug is relatively safe, easily available and cheap.

Methods. A prospective, randomized, double-blind trial was conducted in the Department of Otolaryngology and Orthopedics, Besat Hospital, Hamadan University of Medical Sciences, Hamadan, Iran, between 2007 and 2008. Patients who were referred to these departments for treatment of various infections, and scheduled for gentamicin therapy were included in this study. Sixty patients completed all the requirements. The patients >18 years who were scheduled for treatment with gentamicin were recruited and patients were excluded if they had a preexisting hearing loss, or systemic disease and if they were pregnant. The patients were divided into 2 groups, the experimental and the control groups that were similar with respect to gender, age, and weight. The treatment group received 1.5 g/day (500 mg/8 hours) Aspirin, and the control group received placebo similar to the other group. The criteria for the selection of patients was at least being 18 years old. The duration of therapy was 7 days, and the dosage of gentamicin was 240 mg/day (80 mg every 8 hours). At the beginning of the study, the hearing threshold of all patients was determined by 2 tests: pure tone audiometry (PTA) and speech discrimination score, then they were retested 8 and 15 days later, but the result of the last test was used for conclusion. All patients were examined for any otologic defect including middle ear damage, and if there was a problem, that case would be excluded. Selection of all patients for the 2 groups was based on what part of the body had been infected, for example orthopedic, or head and neck infection. All patients in the 2 groups, in addition to gentamicin, received a second drug (Keflin [1 g/6 hours], a second generation of Cephalosporins, Daru Pakhsh Company, Iran). The study was approved by the Ethical Committee of Besat Hospital and Hamadan University of Medical Sciences. Initially, a questionnaire was completed by an authorized physician for each case and control, followed by a complete clinical exam, and then PTA was performed for all cases and controls.

Statistical analysis. Paired t-test and independent t-test were used to compare differences in means for continuous variables. A $p<0.05$ was used as significant level. All analyses were conducted using SPSS version 13.

Results. Audiologic data in the 2 patients groups was comparable at the beginning of the study except in 2, 4, and 8 kiloHertz (kHz) frequencies that the threshold of PTA in the aspirin group was a little higher than the placebo group. This event was random, and no selection had been carried out. Interestingly, the mean of age in the Aspirin group was a little higher than the placebo group. At the 3 different periods of time (the beginning, 8th, and 15th day) in the placebo group, PTA test was measured on different frequencies (**Table 1**). Comparison of the PTA at 1000 Hertz (Hz), 2000 Hz, 4000 Hz, and 8000 Hz showed significant differences between mean of PTA at the beginning, 8th, and 15th day (for 1000 Hz [p=0.03], 2000 Hz [p=0.003], 4000 Hz [p=0.001] and 8000 Hz [p=0.001]). The threshold of PTA at 250 Hz, was significantly different only at 8th and 15th day (p=0.004), also at the frequency of 500 Hz, the difference between the beginning with 15th day and 8th with 15th day were significant (p=0.001). **Table 2** shows the results of PTA test in the aspirin group. According to the results, there were a significant difference in 4000 Hz and 8000 Hz at 3 settings of PTA testing (**Table 2**). The comparison between measured means of PTA threshold in the control group shows significant difference between them at the beginning, 8th, and 15th day, except for the first and 8th days at 2 frequencies of 250 and 500 Hz. Also, in the control group, 11 (36%) cases of patients showed changes equal to 15 dB, or more in their hearing threshold in 4000 Hz and 6 (20%) in 8000 Hz had this value, while in the aspirin group, there was only one case in each above mentioned frequency. Using Chi square test, these differences were statistically significant (**Table 3**). The results showed the mean of PTA threshold at the

2000, 4000 and 8000 Hz, had significant differences, and there were no differences in other frequencies. Also, comparison between the results of men and women tests showed no significant difference.

Discussion. The present study sought to determine the effectiveness of Aspirin as an otoprotectant against gentamicin ototoxicity in our patients. Significant otoprotection was demonstrated in patients receiving Aspirin by pretreatment and post-treatment PTA testing.[1,3,9] In the control group, at the 8000 Hz frequency, the mean hearing threshold was 14.2 dB on the first, and 27.5 dB on the 15th day. In fact, this result shows that the threshold was 2-fold. In contrast, in the Aspirin group at 8000 Hz frequency, the mean threshold was 22.8 dB on first day, and 28.8 dB on the 15th day, and this means that Aspirin has a protective role on hearing for gentamicin ototoxicity. Also, comparison between the results of men and women shows no significant differences. In the past studies, the audiometric data for determining changes in cochlear function and to show ototoxicity, several levels of thresholds were used. In this study, in accordance with previous clinical studies, a threshold shift of >15 dB at 6 and 8 KHz had been chosen as the criterion for hearing loss. Aminoglycosides in general, were found to displace calcium from its binding sites resulting in a limitation of calcium-dependent physiological mechanisms. In detail, aminoglycosides were found to be able to block the transduction channels at the tips of stereocilia and the N-type and P/Q type channels in neurons, as well as, acetylcholine-evoked K+ currents in outer hair cells.[14] Some of the antioxidants (mannitol, glutathione, and salicylate) have been shown to protect against aminoglycosides ototoxicity *in vivo*.[1,2,9,15] Interestingly, a self-protection phenomenon to high ototoxicic doses of gentamicin has been proposed by a research group on animals in Brazil.[16] They indicated low dose gentamicin

Table 1 - Pure tone audiometry (PTA) threshold dB in the placebo group in different frequencies.

Time	Frequency (Hz)					
	250	500	1000	2000	4000	8000
Pretreatment (0 day)	5.6 ± 2.8	6.2 ± 3.6	7.3 ± 4.5	9.7 ± 5.5	12.2 ± 7.4	14.2 ± 9.4
8th day of treatment	6.0 ± 3.3	6.5 ± 3.9	7.7 ± 4.8	11.5 ± 6.8	15.2 ± 8.7	18.7 ± 12.2
15th day of treatment	6.7 ± 3.5	7.8 ± 4.5	9.0 ± 5.3	14.8 ± 8.6	21.5 ± 12.5	27.5 ± 16.4
P value						
0 day - 8 days	NS	NS	0.03	0.003	0.001	0.001
0 day - 15 days	NS	0.01	0.03	0.003	0.001	0.001
8 day - 15 days	0.004	0.01	0.03	0.003	0.001	0.001

*Data are expressed as mean±SD, dB - decibel, Hz - Hertz

Table 2 - Pure tone audiometry (PTA) threshold dB Aspirin group in different frequencies.

Time	Frequency (Hz)					
	250	500	1000	2000	4000	8000
Pretreatment (0 day)	6.2 ± 4.5	6.8 ± 4.8	9.3 ± 6.3	12.3 ± 7.8	16.8 ± 13.9	22.8±18.1
8th day of treatment	6.0 ± 3.2	7.7 ± 5.2	10.2 ± 7.5	13.5 ± 7.8	19.3 ± 14.2	24.5± 19.0
15th day of treatment	6.9 ± 3.5	7.7 ± 5.0	11.2 ± 8.3	15.0 ± 11.2	21.8 ± 16.1	28.8± 19.5
P value						
0 day - 8 days	NS	NS	NS	0.017	0.001	0.023
0 day - 15 days	NS	NS	NS	NS	0.004	0.001
8 day - 15 days	NS	NS	NS	NS	0.033	0.002

*Data are expressed as mean±SD, dB - decibel, Hz - hertz

Table 3 - Comparison of number of patients showing changes in Pure tone audiometry (PTA) in 2 different frequencies.

Group	PTA changes at 4000 Hz		PTA changes at 8000 Hz	
	≥ 15 dB	< 15 dB	≥ 15 dB	<15 dB
Control	11*	19	6†	24
Aspirin	1	29	1	29

Hz - Hertz, dB - decibels, *Chi square = 10.4, degrees of freedom (df)=1, p=0.001, †Chi square= 4.04, df=1, p=0.04

(10 mg/kg/day for 30 days) followed by a higher dose (160 mg/kg/day for 10 days) showed normal hearing and normal outer hair cells.

The use of Aspirin should be appealing to developing countries, where the low cost of Aspirin would make such a treatment affordable. Furthermore, a combination therapy with Aspirin may also provide further benefits for combating bacterial diseases. Salicylate can reduce or eliminate bacterial biofilm production, and potentiate antimicrobial therapy.[1] Bacterial biofilms are 3-dimensional aggregates of bacteria that have been shown recently to play a major role in many chronic infections.[17] Chen et al[1] on their research on the efficacy of Aspirin for attenuation of gentamicin ototoxicity found a significant hearing loss of 15 dB or more, at 6 and 8 kHz in 13% of patients who received placebo. In contrast, only 3% of the patients that received Aspirin showed evidence of hearing loss. In the present study, we showed that Aspirin can protect the ototoxic effect of gentamicin in our patients. Although at present, very little is known about this potentials.[1] As already mentioned, patients in our study had hearing loss at the high frequencies, the same in Chen et al's[1] study. Although Aspirin significantly has positive effect on gentamicin ototoxicity, it does not provide complete protection. In general, none of our patients in 2 groups complained of vertigo during the period of hospitalization. Also, all patients were satisfied with their treatment and nobody pointed out any hearing loss, probably due to the fact that their hearing loss occurred at levels above speech frequencies. Because aminoglycosides remain in the cochlea for a long period after therapy ends, patients should be advised to avoid noisy environments for 6 months after therapy completion, because they remain more susceptible to noise-induced cochlear damage. In the past studies, the doses of Aspirin were high,[1,5] whereas presently we use a lower dose of Aspirin (1.5 g/day) and future works are required to clarify whether a dosage lower than that might provide protection against gentamicin-induced hearing loss.

It will be interesting to investigate whether Aspirin can protect the auditory system from other types of damage, such as exposure to different drugs, noise, or aging. In humans, a dosage of 1.95 g/day of Aspirin for 7 days is sufficient to induce tinnitus,[18] and dosage for our patients was lower than the amount mentioned above (1.5 g/day for 7 days). The prevalence of Aspirin intolerance is estimated to be between 0.6% and 2.5% in the general population.[18] In contrast, the benefits of Aspirin in several kind of therapy has been documented, for example, as antifungal,[19] protects striatal dopaminergic neurons from neurotoxin-induced degeneration,[20] helping the treatment of major depression,[21] protective role on head and neck cancer.[22] It should be mentioned that Reye's syndrome can be related to use of Aspirin in the children group.[23] In addition, a group of researchers in India have suggested that Aspirin can lead to the accumulation of gentamicin inside the body, and could result in toxicity.[24]

One of the limitations on this study was the inability to determine Aspirin and Gentamicin serum level. If this procedure had been carried out the results would have been more accurate.

In conclusion, in the present study, we showed that Aspirin can protect the ototoxic effect of gentamicin in our patients, and future works are needed to clarify whether the dose lower than what used in this study might provide protection against gentamicin-induced hearing loss.

Acknowledgment. *The authors would like to thank Mrs. Faranak Imami, Audiologist, for helping the patients in the audiometry test.*

References

1. Chen Y, Huang WG, Zha DJ, Qiu JH, Wang JL, Sha SH, et al. Aspirin attenuates gentamicin ototoxicity: from the laboratory to the clinic. ***Hear Res*** 2007; 226: 178-182.
2. Hochman J, Blakley BW, Wellman M, Blakley L. Prevention of aminoglycoside-induced sensorineural hearing loss. ***J Otolaryngol*** 2006; 35: 153-156.
3. Sha SH, Qiu JH, Schacht J. Aspirin to prevent gentamicin-induced hearing loss. ***N Engl J Med*** 2006; 354: 1856-1857.
4. Corbacella E, Lanzoni I, Ding D, Previati M, Salvi R. Minocycline attenuates gentamicin induced hair cell loss in neonatal cochlear cultures. ***Hear Res*** 2004; 197: 11-18.
5. Sha SH, Taylor R, Forge A, Schacht J. Differential vulnerability of basal and apical hair cells is based on intrinsic susceptibility to free radicals. ***Hear Res*** 2001; 155: 1-8.
6. Ruiz JW, Guzman J, Polak M, Eshraghi AA, Balkany TJ, Van De Water TR. Glutathione ester protects against hydroxy nonenal-induced loss of auditory hair cells. ***Otolaryngol Head Neck Surg*** 2006; 135: 792-797.
7. Jiang H, Sha SH, Forge A, Schacht J. Caspase-independent pathways of hair cell death induced by kanamycin in vivo. ***Cell Death Differ*** 2006; 13: 20-30.
8. Bae WY, Kim LS, Hur DY, Jeong SW, Kim JR. Secondary apoptosis of spiral ganglion cells induced by aminoglycoside: Fas-Fas ligand signaling pathway. ***Laryngoscope*** 2008; 118: 1659-1668.
9. Sha SH, Schacht J. Antioxidants attenuate gentamicin-induced free radical formation in vitro and ototoxicity in vivo: D-methionine is a potential protectant. ***Hear Res*** 2000; 142: 34-40.
10. Hockenbery DM, Oltvai ZN, Yin XM, Milliman CL, Korsmeyer SJ. Bcl-2 functions in an antioxidant pathway to prevent apoptosis. ***Cell*** 1993; 75: 241-251.
11. Sha SH, Schacht J. Salicylate attenuates gentamicin-induced ototoxicity. ***Lab Invest*** 1999; 79: 807-813.
12. Meister A. Glutathione deficiency produced by inhibition of its synthesis, and its reversal; applications in research and therapy. ***Pharmacol Ther*** 1991; 51: 155-94.

13. Ye LF, Tao ZZ, Hua QQ, Xiao BK, Zhou XH, Li J, et al. Protective effect of melatonin against gentamicin ototoxicity. ***J Laryngol Otol*** 2009; 123: 598-602.
14. Heinrich UR, Helling K, Sifferath M, Brieger J, Li H, Schmidtmann I, et al. Gentamicin increases nitric oxide production and induces hearing loss in guinea pigs. ***Laryngoscope*** 2008; 118: 1438-1442.
15. Lautermann J, Crann SA, McLaren J, Schacht J. Glutathione-dependent antioxidant systems in the mammalian inner ear: effects of aging, ototoxic drugs and noise. ***Hear Res*** 1997; 114: 75-82.
16. Maudonnet EN, de Oliveira JA, Rossato M, Hyppolito MA. Gentamicin attenuates gentamicin-induced ototoxicity-self-production. ***Drug Chem Toxicol*** 2008; 31: 11-25.
17. Psaltis AJ, Ha KR, Beule AG, Tan LW, Wormald PJ. Confocal scanning laser microscopy evidence of biofilms in patients with chronic rhinosinusitis. ***Laryngoscope*** 2007; 117: 1302-1306.
18. Muller M, Klinke R, Arnold W, Oestreicher E. Auditory nerve fibre responses to salicylate revisited. ***Hear Res*** 2003; 183: 37-43.
19. Leeuw NJ, Swart CW, Ncango DM, Pohl CH, Sebolai OM, Strauss CJ, et al. Acetylsalicylic acid as antifungal in Eremothecium and other yeasts. ***Antonie Van Leeuwenhoek*** 2007; 91: 393-405.
20. Di Matteo V, Pierucci M, Di Giovanni G, Di Santo A, Poggi A, Benigno A, et al. Aspirin protects striatal dopaminergic neurons from neurotoxin-induced degeneration: an in vivo microdialysis study. ***Brain Res 2***006; 1095: 167-177.
21. Mendlewicz J, Kriwin P, Oswald P, Souery D, Alboni S, Brunello N. Shortened onset of action of antidepressant in major depression using acetylsalicylic acid augmentation: a pilot open-label study. ***Int Clin Psychopharmacol*** 2006; 21: 227-231.
22. Jayaprakash V, Rigual NR, Moysich KB, Loree TR, Nasca MA, Menezes RJ, et al. Chemoprevention of head and neck cancer with aspirin: a case-control study. ***Arch Otolaryngol Head Neck Surg*** 2006; 132: 1231-1236.
23. Glasgow JF. Reye's syndrome: the case for a causal link with aspirin. ***Drug Saf*** 2006; 29: 1111-1121.
24. Shivprakash, Gandhi TP, Patel RB, Sheikh MA, Jhala A, Santani DD. Pharmacokinetics of gentamicin in rabbits pretreated with nonsteroidal anti-inflammatory drugs: an iteraction study. ***J Pharm Sci*** 1994; 83: 542-544.

Related topics

Almasswary AA. Very late stent thrombosis in a bare-metal stent, 9 years after implantation. ***Saudi Med J*** 2009 Jun;30(6):847-50.

Zubaid M, Rashed WA, Al-Khaja N, Almahmeed W, Al-Lawati J, Sulaiman K, Al-Motarreb A, Amin H, Al-Suwaidi J, Al-Habib K. Clinical presentation and outcomes of acute coronary syndromes in the gulf registry of acute coronary events (Gulf RACE). ***Saudi Med J*** 2008 Feb;29(2):251-5.

Fabijanic D, Banic M, Kardum D, Sutlic Z, Radic M, Rudez I, Biocina B. Gastroduodenal lesions in coronary artery disease patients. Frequency, endoscopic characteristics and risk factors. ***Saudi Med J*** 2007 Jul;28(7):1137-9.

Sait KH, Alkhattabi MA, Alkushi AO, Alqahtani MH. Ovarian mucinous cystadenoma in a female with Turner syndrome. ***Saudi Med J*** 2004; 25: 1270-1273.