Cukurova Medical Journal
ÇUKUROVA ÜNİVERSİTESİ TIP FAKÜLTESİ

Cukurova Med J 2022;47(1):128-133
DOI: 10.17826/cumj.1030500

ARAŞTIRMA / RESEARCH

Effect of tacrolimus in the inner ear of rats

Ratlarda takrolimusun iç kulak üzerindeki etkisi

Fatmanur Uysal[1], Selim S. Erbek[2], Seyra Erbek[2], Belde Çulhaoğlu[3]

[1]Doğuş University, Department of Audilogy, İstanbul, Turkey
[2]Baskent University, School of Medicine, Department of Otolaryngology, Ankara, Turkey.
[3]Ondokuz Mayıs University, Faculty of Health Sciences, Audiologist, Samsun, Turkey.

Cukurova Medical Journal 2022;47(1):128-133

Abstract

Purpose: Tacrolimus, an immunosuppressive agent, is used especially after organ transplantation. It has been asserted that tacrolimus has protective effects on the auditory system in some studies while it has negative effects in other studies. The purpose of our study is to investigate the effect of tacrolimus on the inner ear of the rats.

Materials and Methods: 20 healthy Sprague Downey male rats weighing 250-350 grams were included in our study. The first group of rats were given 1mg/kg tacrolimus (n:7), the second group of rats were given 0.1 mg/kg tacrolimus (n:7), and the third group (n:6) was the non-administered control group. The first measurements of all rats were taken with distortion-product otoacoustic emission before starting the experiment. Then, tacrolimus drug was administered by gavage method to the 1st and 2nd groups along 30 days. The last measurement was repeated on the 30th day.

Results: According to the results of the first measurements, emission was obtained in all rats and the responses were found to have similar characteristics. Similarly, the difference between the signal noise rate values in the last measurements taken from the groups did not show any statistical significance.

Conclusions: Based on the distortion-product otoacoustic emission measurements, it can be said that Tacrolimus does not have ototoxic effects on the auditory system of rats considering the administered dosage and time.

Keywords:. Distortion-product otoacoustic emission, hearing loss, ototoxicity, tacrolimus

Öz

Amaç: İmmünosupresif bir ajan olan takrolimus, özellikle organ nakli sonrası kullanılmaktadır. Takrolimusun bazı çalışmalarda işitsel sistem üzerinde koruyucu, bazı çalışmalarda ise olumsuz etkileri olduğu ileri sürülmüştür. Çalışmamızın amacı ratlarda takrolimusun iç kulağa etkisini araştırmaktır.

Gereç ve Yöntem: Çalışmamıza 20 adet, ortalama 250-350gram ağırlığında, sağlıklı Spraquey Downey erkek ratlar dahil edildi. Birinci grup 1mg/kg takrolimus (n=7), ikinci grup 0.1mg/kg takrolimus (n=7) verilen ratlar ve üçüncü grup ise (n=6) ilaç verilmeyen kontrol grubudur. Deneye başlamadan önce DPOAE ile tüm ratların ilk ölçümleri yapıldı. Daha sonra 30 gün boyunca 1. ve 2. gruba belirlenen dozda takrolimus ilacı gavaj yöntemi günde bir kez uygulandı. Son ölçüm ise 30. günde tekrarlandı.

Bulgular: İlk ölçüm sonuçlarına göre tüm ratlarda emisyon elde edilmiş ve yanıtların benzer özelliklere sahip olduğu saptanmıştır. Aynı şekilde gruplara yapılan son ölçümlerdeki SNR değerleri arasındaki fark istatistiksel olarak anlamlılık göstermedi.

Sonuç: Yapılan tüm DPOAE ölçümlerinden yola çıkarak takrolimusun en azından verilen doz ve sürede ratlarda işitme sistemi üzerinde toksik bir etkisinin olmadığı söylenebilir. Bu çalışma farklı doz ve sürelerde verilen takrolimus ile başka çalışmalara öncü olabilir.

Anahtar kelimeler: Ototoksisite, distorsiyon ürünü ototakustik emisyon, takrolimus, işitme kaybı

Yazışma Adresi/Address for Correspondence: Dr. Fatmanur Uysal, Doğuş University, Department of Audilogy, İstanbul, Turkey E-mail: ody.fnuruysal@gmail.com
Geliş tarihi/Received: 30.11.2021 Kabul tarihi/Accepted: 26.01.2022

INTRODUCTION

Ototoxicity is the condition of loss of balance, hearing loss, or existing of both conditions and deterioration in the inner ear structure as a result of vestibular and cochlear damage caused either by a chemical agent or drug. There are many agents that may cause ototoxicity[1,2]. The selective characteristics of the protocols created by immunosuppressive drugs used today are increasing gradually. Combined administration of drugs provides synergistic effect as well as preventing adverse side effects by reducing the dosage, therefore enabling the increase in quality of life[3].

Tacrolimus performs its immunosuppressive effect by inhibiting cytokine inhibition. When it enters from the outside of the cell, it binds to its intracellular receptor, FK-binding proteins (FKBP12). FKBP12 is a cytosolic protein commonly found in T lymphocytes. This protein prevents dephosphorylation by preventing the interaction of calcineurin, a calcium- and calmodulin-dependent phosphatase, with the transcription factor. Then, it makes NF-AT (nuclear factor of activated T cells) dependent gene transcription and suppresses the immune system. Tacrolimus also inhibits IL-2 transcription and other calcium-dependent events (nitric oxide synthetase activation, cell degranulation and apoptosis). It also blocks both degranulation and transcriptional activation of cytokines such as IL-3 and IL-5 in mast cells. As a result, the production of other cytokines such as IL-3, IL-4, IL-5, interferon (IF)-γ, tumor necrosis factor (TNF)-α and granulocyte macrophage colony stimulating factors (GM-CSF) is also reduced[4,5] (Figure 1).

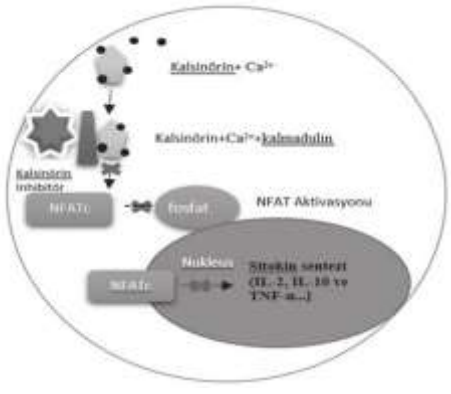

Figure 1. Mechanism of action of immunosuppressive drugs.

In the literature, it has been asserted that tacrolimus has protective effects on the auditory system in some studies while it has negative effects in other studies. Therefore, the purpose of our study is to investigate the effect of tacrolimus, one of the immunosuppressive agents, on auditory system by using the distortion product otoacoustic emission (DPOAE) test method[6-15]. The hypothesis of the study was to investigate the effects of tacrolimus, which is used as an immunosuppressive agent in treatment, on the auditory system. In the present study, it was observed that tacrolimus did not cause a significant change in the auditory system when the measured DPOAE results were considered. However, attention was drawn to the possible risk of hearing loss for patients who will need to use tacrolimus for a long time.

MATERIALS AND METHODS

This study was performed in Başkent University, animal experiments laboratory after obtaining the approval of Başkent University Animal Experiments Local Ethics Committee (DA/16/48). In the study, the rules regarding animal care and use set forth in the International Helsinki Declaration were followed. The anesthesia to be applied to the rats and the DPOAE measurements were performed by an experienced audiologist that had the "Experimental Animals Use Certificate". Before starting the study, power analysis was carried out using G*Power (3.1.9.3) statistical software.

Animals

A total of 20 healthy Sprague Downey male rats weighing 250-350 grams were included in our study. Rats were kept in cages in the same room, under equal conditions of 12 hours of light and 12 hours of darkness at a temperature of 20-22°C, where they could have food and water, with a background noise level below 50 dB SPL.

Procedure

Otoscopic examinations of all rats were performed under general anesthesia and the debris and the plugs in the outer ear canal were cleaned before the experiment. General anesthesia was provided by administering ketamine HCL (Ketalar Ampoule, Pfizer, Istanbul) 60 mg/kg intraperitoneal and xylazine HCl (Rompun Ampoule, Bayer, Istanbul) 6mg/kg intraperitoneal (ip).

DPOAE measurements were performed before starting the experiment for hearing evaluations of the rats. Since emission results can be different in each ear and are independent of each other, an otoacoustic emission test was applied to both ears of all subjects. As a result of DPOAE measurements, 20 rats with a Signal to Noise Ratio (SNR) above 3 dB were included in the study.

Statistical analysis

The statistical evaluation was performed by using the SPSS (Statistical Program forSocial Sciences) 20.0 statistical program. The continuous variable numerical data were expressed as mean ± standard deviation. The comparison of the means of the numerical data between the groups was made with the "Kruskal Wallis Test", and the intragroup comparisons were made with the "Mann Whitney Test". A P value less than 0.05 was considered statistically significant.

The significance level (p) was taken as 0.05 for comparison tests. Since there were no normal distributions in the variables (p>0.05), the analysis was continued with non-parametric test methods.

The Kruskal Wallis Test Analysis was made for the comparisons in the independent multiple groups. Since the p value would increase depending on the increased number of comparisons in the variables with difference, the Bonferroni Corrected p value was used and calculated with "(0.05/binary comparison)".

Since there were number of groups and number of comparisons in the study, the calculation was made as $\binom{Group\ number}{Comparison\ number}$= a, α_{BD}=0.05/a), the p values obtained with the Mann-Whitney Test after the Kruskal-Wallis Test were compared with this value, and the result was decided.

RESULTS

The 20 rats included in the study were divided into 3 groups, the first and second groups both consisted of 7 rats and the third group consisted of 6 rats. After the study, all rats were sacrificed by cervical dislocation method. The first group of rats were given 1mg/kg tacrolimus (n:7), the second group of rats were given 0.1 mg/kg tacrolimus (n:7), and the third group (n:6) was the non-administered control group. After anesthesia was administered, the body temperature of the rats was maintained under the heater during the waiting period for the test.

DPOAE measurements of all groups were performed simultaneously. After the head of the rat was placed in a horizontal position on the ground, the probe was placed into the outer ear canal of the ear to be measured. The measurement was initiated after having seen the probe indicator on the device and proper configuration stimulus waveform together with the device in an appropriate measuring position.

The ratio between the frequencies f2 and f1 (f2/f1) was kept at 1.22. The difference between the L1-L2 levels was set to 10 dB SPL (L1 = 65 dB SPL, L2 = 55 dB SPL). DPOAEs were measured at a frequency of 2f1-f2. As a result of DPOAE measurements, Signal to Noise Ratios (SNR) occurring at frequencies of 4004, 6064, 7998 and 9854 Hz were recorded.

In our study, DPOAE measurements were performed in all rats before tacrolimus drug was administered. According to the measurement results in all rats, emission values were obtained. According to Table 1, it is seen that the SNR ratios obtained as a result of DPOAE measured in each of the 3 groups increase with the increase in frequency. However, this increase was no statistically significant difference was observed between SNR values according to the frequencies observed in all 3 groups (p> 0.05). Table 1 shows the first DPOAE measurement results of the groups.

Table 1. Initial DPOAE measurements for all groups

Frequency	Group 1 (mean ± SD)	Group 2 (mean ± SD)	Group 3 (mean ± SD)	p value
4004 Hz	12.68 ± 9.64	11.7 ± 5.	16.87 ± 10.64	0.300
6064 Hz	21.67 ± 11.75	19.7 ± 5.93	24.61 ± 11.38	0.44
7998 Hz	30.12 ± 13.15	26.3 ± 55.1	29.99 ± 11.59	0.43
9854 Hz	33.02 ± 10.08	31.25 ± 6.05	34.56 ± 6.76	0.54

Sd; standart deviation, p; Kruskal Wallis test significance value; DPOAE; distortion-product otoacoustic emission, Hz; Hertz.

DPOAE measurements were repeated after 30 days. And it is seen in Table 2 that the SNR ratio increases as the frequency increases, similar to the initial measurement. In addition, it was observed that the SNR ratios of the control group were high in the first and last measurement. However, this situation was not statistically significant (p> 0.05). When all the groups were compared with each other in terms of DPOAE measurements at the end of the study, the difference between the SNR values did not show statistical significance (p> 0.05). The final DPOAE measurement results of the groups can be found in Table 2.

Table 2. Final DPOAE measurements for all groups

Frequency	Group 1 (mean ± SD)	Group 2 (mean ± SD)	Group 3 (mean ± SD)	p value
4004 Hz	6.96 ± 7.49	7.62 ± 6.87	7.76 ± 7.34	0.88
6064 Hz	12.09 ± 11.39	13.52 ± 9.19	12.67 ± 7.29	0.83
7998 Hz	15.38 ± 12.20	18.77 ± 14.64	20.8 ± 10.52	0.43
9854 Hz	21.35 ± 13.66	23.38 ± 11.22	24.48 ± 13.64	0.77

Sd; standart deviation, p; Kruskal Wallis test significance value; DPOAE; distortion-product otoacoustic emission, Hz; Hertz.

DISCUSSION

Today, it is known that diuretics, antibiotics, anti-inflammatory agents, antineoplastic agents, and certain other drugs can cause ototoxicity[1,2]. In our study, we compared SNR values between the groups by administering different doses of tacrolimus to rats. SNR values measured at the initial phase of and 30 days after the administration did not differ significantly between these groups. This result suggested that tacrolimus did not produce a toxic effect in the administered dose and time in our study. On the other hand, SNR values decreased in all groups compared to the first measurement. This may be due to stress and anesthesia.

Tacrolimus (FK506) and cyclosporine are potent immunosuppressive agents which are called calcineurin inhibitors. Especially after organ transplantation, they are widely used at therapeutic levels in patients to prevent rejection. However, in the literature, cases of neurotoxicity associated with calcineurin inhibitors, depending on the dose, are also seen[11].

In patients taking tacrolimus after liver transplantation, side effects such as hearing loss, tinnitus, and otalgia have been reported[12]. Norman et al. reported the development of bilateral hearing loss and tinnitus ten weeks after transplantation in a patient who used tacrolimus two times a day. In the treatment of the patient, with a new arrangement, they discontinued tacrolimus, and initiated sirolimus instead. It was observed that there were also decreases in complaints after 14 days. Tacrolimus was, therefore, thought to be able to cause severe hearing loss even at therapeutic levels, although its exact mechanism of action is unknown[13].

Lakshmi et al. reported that a patient who used tacrolimus following organ transplantation had tinnitus with a sudden hearing loss 17 days later. They also reported hearing loss in 2 pediatric cases in approximately 4 years after renal transplantation. In this way, they concluded that tacrolimus might have toxic effects with its cumulative effect in the longer term, similar to our study[16].

In another group of patients evaluated by pure sound audiometry after transplantation, tacrolimus has been shown to affect particularly high-frequency tones. Besides, Gülleroğlu et al.[8] stated that two patients using 0.1 mg/kg of tacrolimus per day had a sudden hearing loss after pediatric renal transplantation. Therefore, for patients who will use a long-term immunosuppressive agent, they recommended periodic hearing examinations[14]. For patients who are planned to take tacrolimus medication, pure tone audiometry testing should be planned at regular intervals together with immitansmetric examination before and after the medication.

In a case report, tacrolimus that was initially prescribed at a dose of 8 mg/day to a 48-year-old patient during the first two years after transplantation continued by decreasing the dose to 2.5 mg/day. During the treatment, as a result of pure sound audiometers performed in the first examination, in the 14th, and 26th months, improvements in hearing thresholds were observed. Therefore, the authors stated that tacrolimus could be involved in treatment;

however, they also reported that further studies are needed for the correct dose and duration of treatment[7].

In experimental studies on acoustic trauma, dexamethasone, melatonin, and tacrolimus were used. Each drug was applied at a dose of 1mg/kg on the first day and during 14 days. The groups were compared with each other by performing DPOAE, ABR, and cytococleogram. As a result, after acoustic trauma, it was observed that tacrolimus has protected cochlea against trauma since the beginning of the 1st week, and eventually provided full recovery of hearing loss. This is achieved by inhibiting the expression of c-fos and TNF-alpha mRNA, and it reduced the loss of outer hair cells[6]. In an acoustic trauma model, tacrolimus, cyclosporin A and rapamycin were compared. In that study, tacrolimus was administered to rats at a dose of 1 mg/kg, 5 mg/kg, or in a combination with other drugs. Ultimately, tacrolimus has been found to have a protective effect[15].

Tacrolimus is used approximately 1 mg per day in patients after transplantation. However, in the future, dose adjustment is made according to the level in the blood. Uemaetomari et al.[15] applied 5mg/kg tacrolimus to the animals after acoustic trauma. As a result of the study, they observed that although tacrolimus was administered at a high dose, it had protective effects. In our study, we also found that there was no ototoxic effect when we applied 1 mg/kg and 0.1 mg/kg tacrolimus.

According to above mentioned articles, there is an inconsistency to explain the effects of tacrolimus on the auditory system. Additionally, many experimental studies in the literature investigated the effect of tacrolimus after acoustic trauma. Our aim was to determine whether the tacrolimus caused deterioration in functions of hair cells or not by producing a cumulative ototoxic effect in healthy adult rats. The results of the present study did not reveal a decrease in DPOAEs after administration of tacrolimus to rats.

In our study, different doses of tacrolimus were administered to the rats and SNR values in DPOAE measurements were compared. No significant difference was observed in the measurements made between the groups at the beginning and after 1 month, suggesting that tacrolimus did not have a toxic effect on the inner ear in the dose and time administered in our study. On the other hand, SNR values decreased in all groups compared to the first measurement. This may be due to the stress and anesthesia.

This study also has its limitations. The number of rats included in the study was relatively low. The one-month period may have been low for the toxic effect to occur. In addition, higher doses may produce toxicity. There is no ABR test in the test battery, therefore, no information on neurotoxicity could be obtained. In subsequent studies, higher doses of tacrolimus can be given for longer periods of time and ABR can be added to the test battery. Studies investigating the effects of tacrolimus on the auditory system should be continued using different study methods.

Our results suggested that tacrolimus did not have a toxic effect in the inner ear of the rats in the administered dosage and time. The present study is thought to be a guide for future studies.

Yazar Katkıları: Çalışma konsepti/Tasarımı: FU; Veri toplama: BÇ, FU; Veri analizi ve yorumlama: FU, SSE; Yazı taslağı: FU; İçeriğin eleştirel incelenmesi: SE, SSE; Son onay ve sorumluluk: FU, SSE, SE, BÇ; Teknik ve malzeme desteği: FU, SE; Süpervizyon: SSE, SE; Fon sağlama (mevcut ise): yok.
Etik Onay: Bu çalışma için Başkent Üniversitesi Hayvan Deneyleri Yerel Etik Kurulundan 05.12.2016 tarih ve 16/48 sayılı kararı ile etik onay alınmıştır.
Hakem Değerlendirmesi: Dış bağımsız.
Çıkar Çatışması: Yazarların beyan edecek çıkar çatışması yoktur.
Finansal Destek: Bu çalışma Başkent Üniversitesi Araştırma Fonu tarafından desteklenmiştir.

Author Contributions: Concept/Design : FU; Data acquisition: BÇ, FU; Data analysis and interpretation: FU, SSE; Drafting manuscript: FU; Critical revision of manuscript: SE, SSE; Final approval and accountability: FU, SSE, SE, BÇ; Technical or material support: FU, SE; Supervision: SSE, SE; Securing funding (if available): n/a.
Ethical Approval: For this study, ethical approval was obtained from Başkent University Animal Experiments Local Ethics Committee with the decision dated 05.12.2016 and numbered 16/48.
Peer-review: Externally peer-reviewed.
Conflict of Interest: The authors have no conflict of interest to declare.
Financial Disclosure: This study was supported by the Baskent University Research Fund.

REFERENCES

1. Bisht M, Bist S. Ototoxicity: the hidden menace. Indian J Otolaryngol Head Neck Surg. 2011;63:255-9.
2. Hatzopoulos S, Di Stefano M, Albertin A, Martini A. Evaluation of cisplatin ototoxicity in a rat animal model. Ann N Y Acad Sci. 1999;884:211-5.
3. Akbaba D. Evaluation of immunosuppressive blood drug level monitoring and the relationship between the test results obtained and biochemical parameters (Master thesis). İstanbul, Sağlık Bilimleri University. 2009.
4. Mok CC, Tong KH, To CH, Siu YP, Au TC. Tacrolimus for induction therapy of diffuse

proliferative lupus nephritis: an open-labeled pilot study. Kidney Int. 2005;68:813-7.
5. Yarosh DB, Pena AV, Nay SL, Canning MT, Brown DA. Calcineurin inhibitors decrease DNA repair and apoptosis in human keratinocytes following ultraviolet B irradiation. J Invest Dermatol. 2005;125:1020-5.
6. Bas E, Martinez-Soriano F, Láinez JM, Marco J. An experimental comparative study of dexamethasone, melatonin and tacrolimus in noise-induced hearing loss. Acta Otolaryngol. 2009;129:385-9.
7. Delmas J, Varoquaux A, Troude L, Salburgo F, Lavieille J-P, Montava M. Incidental effect of long-term tacrolimus treatment on sporadic vestibular schwannoma volume shrinkage and clinical improvement. Otol Neurotol. 2020;41:89-3.
8. Gulleroglu K, Baskin E, Aydin E, Ozluoglu L, Moray G, Haberal M. Hearing status in pediatric renal transplant recipients. Exp Clin Transplant. 2015;13:324-8.
9. Minami SB, Yamashita D, Schacht J, Miller JM. Calcineurin activation contributes to noise-induced hearing loss. J Neurosci Res. 2004;78:383-2.
10. Norman K, Bonatti H, Dickson RC, Aranda-Michel J. Sudden hearing loss associated with tacrolimus in a liver transplant recipient. Transplant International. 2006;19:601-3.
11. Rifai K, Klempnauer J, Manns M, Strassburg CP. Sudden hearing loss associated with high levels of calcineurin inhibitors after liver transplantation. Transplantationsmedizin. 2006;18:33-5.
12. Rifai K, Kirchner GI, Bahr MJ, Cantz T, Rosenau J, Nashan B et al. A new side effect of immunosuppression: high incidence of hearing impairment after liver transplantation. Liver Transpl. 2006;12:411-5.
13. Rifai K, Bahr M, Cantz T, Klempnauer J, Manns M, Strassburg C et al. Severe hearing loss after liver transplantation. Transplantation Proc. 2005;37,1918-9.
14. Fortes ME, Marroni CA, Coser PL, de los Santos CA. Audiometric changes in patients undergoing liver transplantation using distinct immunosuppressive protocols. Liver Transpl. 2008;14:509-1.
15. Uemaetomari I, Tabuchi K, Hoshino T, Hara A. Protective effect of calcineurin inhibitors on acoustic injury of the cochlea. Hearing Res. 2005;209:86-0.
16. Lakshmi B, Vidya B, Reddy M, Kumar A, Ram R, Kumar V. Sensorineural deafness following tacrolimus use. Exp Clin Transplant. 2020;18:110-1.