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Original Article

Evaluation of Nivolumab for Ototoxic Effects: An Animal Study in Rats

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OBJECTIVES: The aim of this study was to assess whether nivolumab is ototoxic in rats and whether this ototoxicity is dose-dependent.

MATERIALS and METHODS: Twelve rats were divided into two groups: Group 1 (control group, 6 rats, 12 ears) received intraperitoneal saline for 14 days. Group 2 (study group, 6 rats, 12 ears) and received two doses of 3 mg/kg intraperitoneal nivolumab within 14 days. Auditory brainstem responses (ABRs) were performed preoperatively and 4 and 8 weeks postoperatively. We compared between the groups, morphologic appearance of spiral ganglion cells and organ of Corti and density of spiral ganglion cells (measured with conventional light microscope connected to a personal computer).

RESULTS: In our control group, both spiral ganglion and organ of corti had a normal morphological appearance. In our study group, spiral ganglion cells had a normal morphological appearance. However, some sections showed possibly mild degenerative changes in the organ of corti. Of 12 samples in the study group, four had a significant loss of density of spiral ganglion cells compared to the control group. The baseline ABR thresholds did not significantly differ between the groups (p=0.713). There was no statistically significant difference between the groups regarding ABR thresholds at week 4 (p=0.347). However, a statistically significant difference was observed in the ABR thresholds at week 8 (p=0.045).

CONCLUSION: The results of our study showed that nivolumab treatment has ototoxic effects. Based on our results, we recommend monitoring the changes in the hearing ability of chemotherapy patients.

KEYWORDS: Ototoxicity, nivolumab, immunotherapy, animal study

INTRODUCTION

Antibodies targeting programmed cell death 1 (PD-1) immune control point pathways have begun to be used in the treatment of recurrent and metastatic tumors. Currently, nivolumab has been approved for the treatment of metastatic melanoma, small cell/non-small cell lung cancer, renal cell carcinoma, and head and neck squamous cell carcinoma after primary chemotherapy and will soon be used against other types of cancer [1]. Nivolumab, along with anti-tumor response, increases pre-existing adaptive immune response by directly blocking the PD-1 receptor, which is the control point of the effector phase of the immune responses. In contrast to conventional cancer therapies, immunotherapy with monoclonal antibodies that block the PD-1 pathway has the risk of causing the development of undesirable side effects associated with immunity [2, 3]. Treatment-related autoimmunity may occur in any system, such as neurological, respiratory, musculoskeletal, cardiac, and hematopoietic. The most commonly affected organs are the skin, intestines, liver, lungs, eyes, and endocrine glands [4, 5]. Currently, patients presenting with rare but life-threatening rare side effects, e.g., acute heart failure, rhabdomyolysis, and dyspnea due to myositis, have been reported under anti-PD-1 therapy [6-8]. In such cases, early diagnosis and proper medical treatment are crucial for reducing the morbidity rates.

This study was presented as a poster presentation at the 40th National Ear Nose Throat Head and Neck Surgery Congress, 7-11 November, Antalya, Turkey

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According to our literature review, the ototoxicity of nivolumab has not yet been investigated. The purpose of the present study was to determine whether nivolumab is ototoxic in rats and whether this ototoxicity is dose-dependent.

MATERIALS AND METHODS

The study was approved by the Ethics Committee for Animal Experiments (0046-05/31/2018) of Ankara Training and Research Hospital, Turkey. The study was conducted in the animal experiments laboratory of the university according to the principles of the Declaration of Helsinki. Twelve male rats aged 4-8 months were randomly divided into two groups. The rats were maintained in an environment with ad libitum access to food and water at 25°C in a 12-hour light/dark cycle. The noise level was <60 dB. All rats were subjected to an auditory brainstem response (ABR) test on day 0 after anesthetization with ketamine/xylazine. Intrauterine saline injections were applied to Group 1 (control group, six rats) for 14 days. Two doses of 3 mg/kg intraperitoneal nivolumab were injected in Group 2 (study group, six rats) within 14 days. On weeks 4 and 8, the audiological examination was repeated in rats under general anesthesia, and the rats were then sacrificed by decapitation. Removed temporal bones were preserved in 10% neutral buffered formalin for histopathological examination.

Auditory assessment

The examination of the outer ear canal and eardrum of the rats was made under a microscope (Möller-Wedel Optical®; Hamburg, Germany) on day 0 under general anesthesia and confirmed to be normal. The ABR test was performed using the Vivosonic Integrity System (Toronto, ON, Canada). ER-3A insert headphones were used during the tests. Electrode placements were on the apex of nasi (ground electrode), vertex (positive electrode), left mastoid (negative electrode), and right mastoid (another negative electrode) (Figure 1). The electrode-skin impedance was maintained at <5 kΩ.

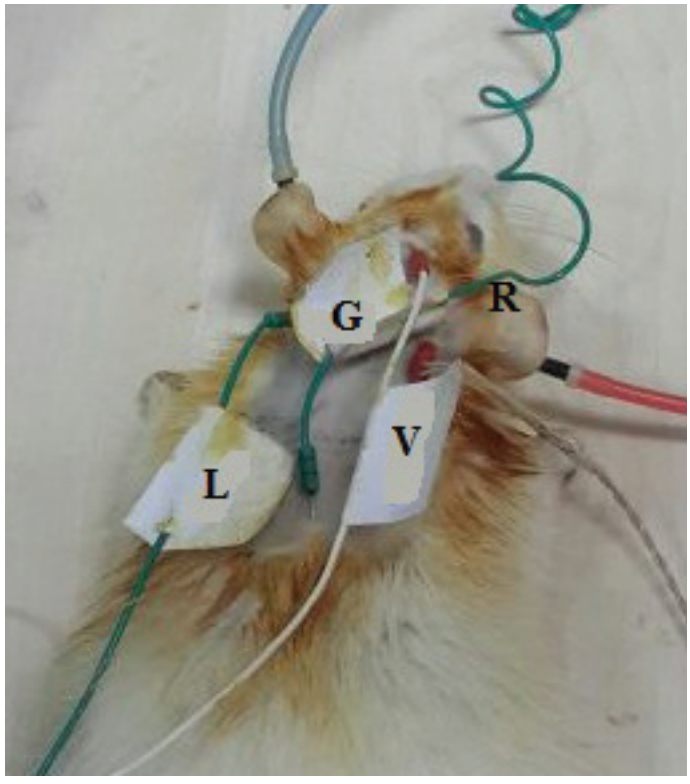

Figure 1. Position of rat during ABR test and placement of recording electrodes.
L: left; R: right; V: vertex; G: ground

The ABR tests were performed on the rats. Basal hearing thresholds were measured to exclude rats with hearing loss prior to the procedure. Thresholds were determined for each frequency by reducing the sound intensity in 10 dB increments and then in 5 dB steps near the threshold. The threshold was defined as the lowest intensity capable of producing a reproducible ABR waveform with a good morphology of wave III response. At least two readings of the same stimulus strength were obtained for each ear. The ABR test was repeated on weeks 4 and 8, and comparisons of the findings were made with the baseline ABR recordings.

Histopathological assessment

Inner ear samples were prepared for histological study by light microscopy. The temporal bones were kept in formalin fixation solution. The bones were placed into formic acid for decalcification at 25°C room temperature for 5 days. The bones were dehydrated in an increasing ethanol series of 70%, 80%, 90%, and 100% and then subjected to a diaphanization process in xylene after which the samples were then embedded in paraffin blocks. Serial sections of 5-micrometer thickness were obtained from paraffin blocks using a Leica RM2255 rotary microtome. Then, deparaffinization, moistening, and staining with hematoxylin and eosin (H&E) were performed.

Morphometric evaluations of spiral ganglion cells (SGCs) were made for each cochlear rotation in the sections stained with H&E. Cochlear samples were observed and photographed using an Olympus CX40 light microscope (Olympus, Tokyo, Japan), and digital images were recorded. For each cochlear turn, all neurons meeting the size and shape criteria for type 1 SGCs were counted in each profile of the Rosenthal channel. SGC density was determined as the number of cell nuclei per 10,000 μm^2 Rosenthal channel. The density of the SGC was calculated as previously described in three-midmodiolar sections 30 µm apart from each cochlea, with the average defined as the data for animals [9].

Statistical Analysis

The results are presented as mean±standard deviation and minimum and maximum values. The Shapiro–Wilk test was used to assess the normality of distribution of the groups. The Kruskal–Wallis H test was used to compare the groups for quantitative data. Data were analyzed using the Statistical Packages for the Social Sciences (SPSS) version 22.0 software for Windows (IBM Corp.; Armonk, NY, USA). A $p<0.005$ was considered statistically significant.

RESULTS

Histopathological findings after 8 weeks

Examination of the H&E-stained sections revealed normal morphological appearance of the spiral ganglion and organ of Corti in the control group (Figure 2). The nivolumab group had normal morphological appearance of the spiral ganglion. However, some sections showed

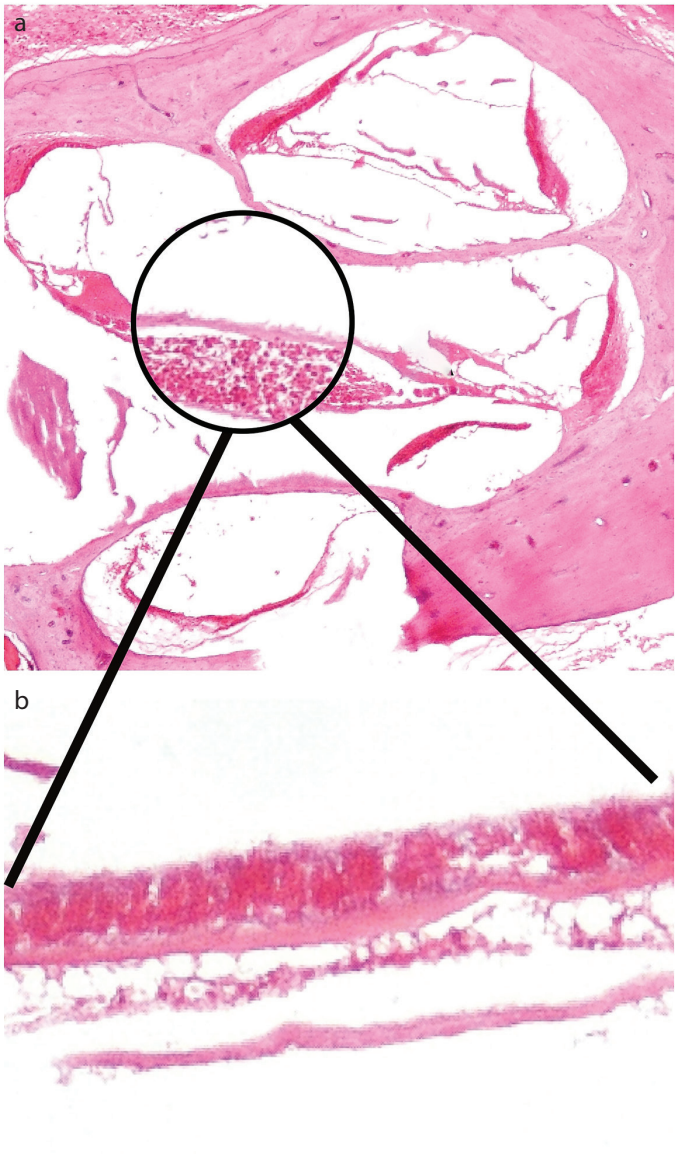

Figure 2. a, b. Light photomicrograph of cochlea in control group. Normal morphological appearance of organ of corti and spiral ganglion at low (a) and high (b) magnification.

possibly mild degenerative changes in the organ of Corti. Of the 12 samples in the nivolumab group (6 rats, 12 ears), 4 had a significant loss of SGCs (Figure 3).

ABR recording changes after 8 weeks

The mean ABR thresholds for each of the two groups on day 0, week 4, and week 8 are presented in Table 1. The baseline ABR thresholds did not significantly differ between the groups (p=0.713). There was no statistically significant difference between the groups regarding ABR thresholds on week 4 (p=0.347). However, a statistically significant difference was observed in the ABR thresholds on week 8 (p=0.045).

DISCUSSION

Nivolumab, a human immunoglobulin G4 anti-PD-1 monoclonal antibody, binds to the PD-1 receptor, preventing the ligand PD-L1 from binding, and results in disruption of T cell regulation and activation.

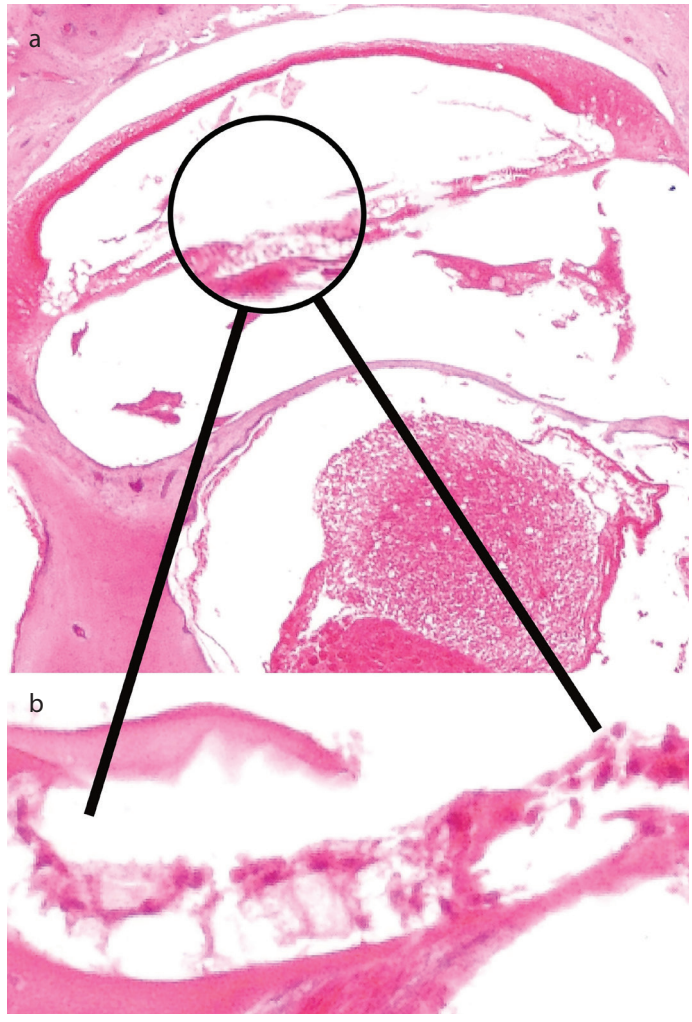

Figure 3. a, b. Light photomicrograph of cochlea in study group. Mild degenerative changes in the organ of corti and loss of SGCs can be seen at at low (a) and high (b) magnification.

Table 1. Changes in ABR thresholds on day 0, week 4, and week 8

ABR thresholds		Experimental group (n=12)	Control group (n=12)	p
0 day	Min–max (median)	10-15 (15)	10-20 (12.5)	0.713
	Mean±SD	13.33±2.21	13.12±3.39	
4 weeks	Min–max (median)	12.5-17.5 (15)	10-15 (13.75)	0.347
	Mean±SD	14.37±1.88	13.33±1.94	
8 weeks	Min–max (median)	12.5-17.5 (15)	10-15 (45)	0.045
	Mean±SD	15.00±1.06	13.12±2.16	

ABR: auditory brainstem response; SD: standard deviation; min: minimum; max: maximum. p<0.05 was considered to be significant

Blockage of this interaction results in the loss of inhibitory signals in T cells and recognition of the tumor by cytotoxic T cells, thus restoring T cell function [10]. The single agent anti-PD-1 treatment with nivolumab is generally well tolerated and exhibits an appropriate safety profile [11-13]. Generally, all control point inhibitors can potentially induce autoimmune events in the human body. Since anti-PD-1 treatment is administered continually, autoimmune events can occur after the beginning of treatment or possibly following discontinuation of therapy.

The mean onset time of the development of autoimmune reactions depends on the affected organ but usually takes 5-15 weeks after the initiation of treatment. Autoimmune events associated with late-onset immunity are rare and observed during ongoing anti-PD-1 treatment [11]. Zimmer et al. [5] reported that 27.8% of the patients (n=138) receiving nivolumab and pembrolizumab for skin cancer have documented rare or unexpected side effects of these drugs. In 116 patients, the side effects affected the skin, gastrointestinal system, liver, endocrine, and kidney. Rare side effects included pancreatic insufficiency due to diabetes mellitus, lichen planus, and pancreatitis. They also reported that 77 patients had neurological, respiratory, musculoskeletal, cardiac, ocular, and hematopoietic side effects, which were successfully treated with local or systemic steroids [5].

Ototoxicity is a side effect caused by the use of chemotherapy drugs [14, 15]. In the literature, there is only one case of autoimmune inner ear disease (AIED) observed in the environment of ongoing anti-PD-1 treatment. In that previous study, the authors reported an AIED in a patient with advanced sinonasal melanoma treated with pembrolizumab [16]. The patient's sensorineural hearing loss was successfully treated with intratympanic dexamethasone injections. The authors concluded that the possible mechanism of hearing loss was secondary to a cross-reactive autoimmune response of T cells to melanocytes in the inner ear [16]. The patients' response to steroid treatment also supports an autoimmune process in the development of hearing loss. Interestingly, to the best of our knowledge, there is no reported case of hearing loss associated with the use of nivolumab. Therefore, the effect of the drug dose was determined to be similar to other toxicity studies [5]. In the present study, the animals in the nivolumab group received 3 mg/kg drug twice in 2 weeks. The important findings of histopathological examination in the inner ear samples were mild degenerative changes of the cortical organ and loss of SGCs in four animals in the nivolumab group. However, both the Corti organs and the SGCs had normal morphological appearance. The ABR thresholds on week 8 were significantly higher in the nivolumab group than in the control group.

It is also important to enumerate some of the limitations of the present study. First, the number of animals was limited. Second, light microscopy is known to have limitations even when the best material is used. Thus, further investigations using transmission and scanning electron microscopy methods are needed.

CONCLUSION

Recently, nivolumab is frequently used during chemotherapy in clinical practice. Our results, especially the histopathological findings in the inner ear of rats, support that nivolumab treatment has ototoxic effects. Based on our results, we recommend monitoring the changes in the hearing ability of chemotherapy patients.

Ethics Committee Approval: Ethics committee approval was taken from the Ethics Committee for Animal Experiments (0046-05/31/2018) of Ankara Training and Research Hospital, Turkey.

Informed Consent: N/A.

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Conflict of Interest: The authors have no conflict of interest to declare.

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REFERENCES

1. Lim JS, Sundar R, Chénard-Poirier M, Lopez J, Yap TA. Emerging biomarkers for PD-1 pathway cancer therapy. Biomark Med 2017; 11: 53-67. [Crossref]
2. Ribas A. Tumor immunotherapy directed at PD-1. N Engl J Med 2012; 366: 2517-9. [Crossref]
3. O'day SJ, Maio M, Chiarion-Sileni V, Gajewski TF, Pehamberger H, Bondarenko IN, et al. Efficacy and safety of ipilimumab monotherapy in patients with pretreated advanced melanoma: a multicenter single-arm phase II study. Ann Oncol 2010; 21: 1712-7. [Crossref]
4. Naidoo J, Page DB, Li BT, Connell LC, Schindler K, Lacouture ME, et al. Toxicities of the anti-PD-1 and anti-PD-L1 immune checkpoint antibodies. Ann Oncol 2015; 26: 2375-91. [Crossref]
5. Zimmer L, Goldinger SM, Hofmann L, Loquai C, Ugurel S, Thomas I, et al. Neurological, respiratory, musculoskeletal, cardiac and ocular side-effects of anti-PD-1 therapy. Eur J Cancer 2016; 60: 210-25. [Crossref]
6. Läubli H, Balmelli C, Bossard M, Pfister O, Glatz K, Zippelius A. Acute heart failure due to autoimmune myocarditis under pembrolizumab treatment for metastatic melanoma. J Immunother Cancer 2015; 3: 11. [Crossref]
7. Min L, Hodi FS. Anti-PD1 following ipilimumab for mucosal melanoma: durable tumor response associated with severe hypothyroidism and rhabdomyolysis. Cancer Immunol Res 2014; 2: 15-8. [Crossref]
8. Yoshioka M, Kambe N, Yamamoto Y, Suehiro K, Matsue H. Case of respiratory discomfort due to myositis after administration of nivolumab. J Dermatol 2015; 42: 1008-9. [Crossref]
9. Nakaizumi T, Kawamoto K, Minoda R, Raphael Y. Adenovirus-mediated expression of brain-derived neurotrophic factor protects spiral ganglion neurons from ototoxic damage. Audiol Neurootol 2004; 9: 135-43. [Crossref]
10. Wang C, Thudium KB, Han M, Wang XT, Huang H, Feingersh D, et al. In vitro characterization of the anti-PD-1 antibody nivolumab, BMS-936558, and in vivo toxicology in non-human primates. Cancer Immunol Res 2014; 2: 846-56. [Crossref]
11. Weber JS, Hodi FS, Wolchok JD, Topalian SL, Schadendorf D, Larkin J, et al. Safety profile of nivolumab monotherapy: a pooled analysis of patients with advanced melanoma. J Clin Oncol 2017; 35: 785-92 [Crossref]
12. Schachter J, Ribas A, Long GV, Arance A, Grob JJ, Mortier L, et al. Pembrolizumab versus ipilimumab for advanced melanoma: final overall survival results of a multicentre, randomised, open-label phase 3 study (KEYNOTE-006). Lancet 2017; 390: 1853-62. [Crossref]
13. Ribas A, Hamid O, Daud A, Hodi FS, Wolchok JD, Kefford R, et al. Association of pembrolizumab with tumor response and survival among patients with advanced melanoma. Jama 2016; 315: 1600-9. [Crossref]
14. Lin HW, Roberts DS, Kay J, Stankovic KM. Sensorineural hearing loss following imatinib (Gleevec) administration. Otolaryngol Head Neck Surg 2012; 146: 335-7. [Crossref]
15. Attili VSS, Bapsy PP, Anupama G, Lokanatha D. Irreversible sensorineural hearing loss due to Imatinib. Leuk Res 2008; 32: 991-2. [Crossref]
16. Zibelman M, Pollak N, Olszanski AJ. inner ear disease in a melanoma patient treated with pembrolizumab. J Immunother Cancer 2016; 4: 8. [Crossref]