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WILEY
The Effectiveness of Platelet-Rich Plasma in an Anosmia-Induced Mice Model

Ahmet Gorkem Yasak, MD; O zgur Yigit, MD; Ela Araz Server, MD; Sevgi Durna Dastan, PhD; Mehmet Gul, PhD

**Objective:** In this study, we aimed to functionally and morphologically demonstrate the effectiveness of platelet-rich plasma (PRP) on anosmia in a mouse model of anosmia.

**Study Design:** Animal study.

**Methods:** A total of 16 male mice were included. When selecting the mice, the food-finding test (FFT) was used to make sure that the animals could smell, and anosmia was induced by administration of intraperitoneal 3-methylindole. The mice were randomly divided into two groups of eight (groups A and B). After 1 week, topical PRP was administered to the mice in group A and topical saline was administered to the mice in group B. The FFT was again administered at 7, 14, and 21 days. The mice were sacrificed on day 21, the olfactory neuroepithelium was histopathologically examined, and the epithelial damage scores and epithelial thickness were measured.

**Results:** After topical administration of PRP and saline, the difference in the average FFT values of the groups was statistically significant at 7, 14, and 21 days ($P < 0.005$). During the histopathological examination, the epithelial damage score was statistically significantly lower in the PRP group ($P = 0.001$) than in the saline group, and epithelial thickness was statistically significantly greater in the PRP group compared to the saline group ($P = 0.003$).

**Conclusion:** We showed that PRP administration has a curative effect on olfactory functions in an anosmia-induced mouse model. However, there is a need for further research before PRP can be considered for use in patients with anosmia in clinical practice.

**Key Words:** Smell, anosmia, hyposmia, 3-methylindole, platelet-rich plasma.

**Level of Evidence:** NA.

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INTRODUCTION

In addition to affecting quality of life, loss of sense of smell significantly impacts the inability to recognize harmful smells, such as those of rotten foods, gas, and smoke. The prevalence of smell disorders has been reported at between 1% to 25% in previous studies, whereas hyposmia, defined as a reduced ability to smell, is found in 16% of the general population. The prevalence of anosmia, or the loss of sense of smell, is 5% and that has been shown to vary. Because the pathophysiological mechanism of anosmia and hyposmia cannot be clearly determined, there are difficulties in treating these disorders. Degeneration in the neurons of the olfactory fossa due to any cause may result in anosmia or hyposmia. However, it has been shown that growth factors induce neuronal regeneration in animal models, and the use of stem cell applications has been reported in various studies conducted in such models.

Platelet-rich-plasma (PRP) is a platelet concentration that is obtained by centrifuging the blood sample of a patient or experimental animal. It contains more growth factors than the blood, and promotes and accelerates healing through these and neurotrophic factors. In addition, PRP is easily accessible, can be prepared quickly, and is biocompatible and reversible. We aimed to investigate the usefulness of PRP in the treatment of anosmia via the growth and neurotrophic factors involved in accelerating and enhancing healing.

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**MATERIALS AND METHODS**

Our study was approved by the Istanbul University Animal Experiments Ethics Board. A total of 16 male mice (aged 9–10 weeks and weighing 18–20 grams) were included. When selecting the mice, the food-finding test (FFT) was used to make sure that the animals could smell. The mice were randomly divided into two groups of eight, and each mouse was given a number. Anosmia was induced via intraperitoneal injection of 300 $\mu$g/g 3-methylindole (3-MI) in 1 mL saline. A week later, the FFT was used to determine whether the animals had developed anosmia. For topical applications and PRP preparation, the mice were given general anesthesia. The mice in group...
A were administered topical PRP, whereas the mice in group B were administered topical saline. The FFT test was then used on days 7, 14, and 21 after administration. The mice were sacrificed on day 21, the olfactory neuroepithelium was histopathologically examined, and epithelial thickness was measured in each mouse. Both groups were compared in terms of FFT results, degeneration in epithelial neuroepithelium, and epithelial thickness.

**Food-Finding Test**

Prior to selecting the mice, a group of mice was fed with the same type, color, and flavor of cheese for a week, allowing them to become accustomed to the smell and taste of the cheese. The test is conducted in a labyrinth, which is designed in a T-shape. The labyrinth was covered with sawdust, which was replaced during each test. The cheese was randomly placed at the end of one of the T-arms and hidden under the sawdust. After the food had been hidden, the mice were individually placed at the starting point of the labyrinth, following a 24-hour fasting period. When each mouse was released, the timer was started and then stopped when the mouse found the cheese. All tests were recorded with a video camera. The FFT was first applied during animal selection. Following the first test, conducted to establish that the mice that found cheese in 3 minutes were included in the study. The second test, to show that anosmia had developed, was conducted on day 7 after 3-MI injection. The mice did not find the cheese within at least 3 minutes were considered anosmic. Platelet-rich plasma and saline were administered to the mice that had developed anosmia. The next test was repeated on days 7, 14, and 21 after the PRP or saline lavages. Five different FFT results were enumerated as follows: 1) during the selection of the mice; 2) on day 7 after 3-MI injection; 3) on day 7 after PRP and saline injection; 4) on day 14 after PRP and saline injection; 5) on day 21 after PRP and saline injection.

**Preparation of PRP and Application to the Mucosa**

The mice received intraperitoneal ketamine hydrochloride 100 mg/kg and 7 mg/kg of xylazine anesthesia. A total of 0.25 mL retroorbital sinus blood was taken from each mouse and centrifuged for 10 minutes at 2,000 revolutions per minute (rpm) in a sodium citrate tube. After centrifuging, the plasma over the dense region, which had precipitated at the bottom of the tube and was composed of blood cells, was taken with the aid of a micropipette and centrifuged a second time for 5 minutes at 4,000 rpm in a tube with CaCl₂. After centrifugation, the PRP was made into an active gel. Approximately 125 μL of PRP, observed as a thin layer in the middle of the tube, was taken with the aid of a micropipette. A total of 125 μL of PRP was administered to the mice in group A, and 125 μL of saline was administered to the mice in group B.

The anesthetized mice were placed head-up in a conical handle that exposes the nose. A 4-mm 25-gauge was advanced 3 mm inward through the injector nostril. The lavages were made by forcefully expelling the contents of a syringe containing PRP and saline. The first lavage was carried out in the right naris and then in the left naris. The mice were held for approximately 30 seconds upside down. Lavages were carried out once.

**Histopathological Examination**

For histopathological examination, the mice were sacrificed at week 4 after injection of 3-MI, using high doses of anesthetic (at least 200 mg/kg of ketamine and over 10 mg/kg of xylazine). After decapitation, the brains and palates were removed, and the remaining tissues were stored in neutral buffered 10% formaldehyde solution for 48 hours at room temperature. After determination of the nasal mucosa, the tissue samples were washed under running tap water and then dehydrated by passing them through increasing amounts of ethanol (50%, 70%, 80%, 96%, and 99.9%). The tissue samples were then passed through a series of xylene and subjected to molten paraffin infiltration (62°C). Following the infiltration process, the samples were buried in paraffin blocks, and 6-μm thick sections were taken from these blocks and placed on slides using a microtome. Hematoxylin-eosin was used to stain the sections taken, and these sections were then examined using a Nikon Optiphot-2 light microscope, Nikon DS-Fi2 camera, and Nikon DS L3 image analysis system (Nikon Corporation, Tokyo, Japan).

Olfactory mucosal injury parameters in the sections (inflammatory cell infiltration, vacuolization in epithelial cells, and epithelial cilia damage) were scored semiquantitatively (none = 0, mild = 1, moderate = 2, and severe = 3; total maximum score = 9). Olfactory epithelium thickness was calculated as the average of the mucosal thickness measured at 3 points (two peripheral, one central) of the olfactory mucosa in each section.

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**TABLE I. Food-Finding Test Values (seconds).**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Subject</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>11</td>
<td>87</td>
</tr>
<tr>
<td>2</td>
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<td>50</td>
</tr>
<tr>
<td>8</td>
<td>32</td>
<td>63</td>
</tr>
</tbody>
</table>

1: during the selection of the mice; 2: day 7 after 3-MI injection; 3: day 7 after PRP and saline injection; 4: day 14 after PRP and saline injection; 5: day 21 after PRP and saline injection.

3-MI = 3-methylindole; PRP = platelet-rich plasma.
Statistical Analysis

Statistical Package for the Social Sciences for Windows, version 15.0 (SPSS Inc., Chicago, IL) was used for statistical analysis. Descriptive statistics are given as a number and percentage for categorical variables and as a mean for numerical variables, standard deviation, and median. Because the parametric test condition was not fulfilled, comparisons between two independent groups of numerical variables were made using the Mann Whitney U test, and in the multiple dependent groups using the Friedman analysis. The rates of categorical variables in independent groups were tested by chi-square analysis. Monte Carlo simulation was applied when conditions were not met. The statistical significance level of alpha was accepted as \( P < 0.05 \).

RESULTS

Food-Finding Test

The FFT values of the mice are shown in Table I. There was no statistically significant difference between the groups in the normal FFT mean values (\( P = 0.397 \)), and there was no statistically significant difference between the groups in the FFT mean values at week 1 after 3-MI injection (1.000). Following PRP and saline lavage, the difference in FFT mean value between the groups was statistically significant at 7, 14, and 21 days. A statistically significant decrease in the FFT mean value was found in group A (\( P < 0.001 \)); the anosmia of all mice had gone by day 7. There was no statistically significant decrease in FFT mean values in group B (\( P = 0.779 \)). The statistical results are summarized in Table II.

Histopathological Findings

Group A: In the sections examined, the olfactory mucosa epithelium generally resembled a normal histological structure. However, minimal subepithelial and intraepithelial inflammatory cell infiltration was observed in some areas. The olfactory glands were generally normal in structure, but dilatation in intercellular spaces between several gland epithelial cells and a heterochromatic increase in glandular epithelial cell nuclei were detected. In addition, thinning in local areas of the olfactory mucosa epithelium was noted, as well as irregularity in cilia and cilia formation (Fig. 1A, A). Epithelial thickness was 79.91 \( \pm \) 4.39 \( \mu \)m on average, and the thickness was statistically significant when compared to group B (\( P = 0.003 \)). The epithelial thickness results are summarized in Table III.

Group B: In the examined sections, cilia degeneration in the large area of the olfactory mucosal epithelium, locally diffuse cilia loss, inflammatory cell infiltration, and numerous cells with dense eosinophilic cytoplasm and heterochromatic pyknotic nuclei were noted. In addition, large areas of necrosis were found in the olfactory gland epithelium (Fig. 2A, B). Epithelial thickness was 69.49 \( \pm \) 5.35 \( \mu \)m on average, and was statistically significantly thinner when compared with group A. The histopathological findings are summarized in Table IV (\( P = 0.003 \)).

DISCUSSION

We aimed to investigate whether PRP would be effective in the treatment of anosmia. Platelet-rich plasma lavage was applied to the olfactory epithelium of anosmia-induced mice via 3-MI injection. When compared to the

![Fig. 1. (A) PRP group: cilia damage (arrow) in olfactory mucosa epithelium, olfactory glands (star). H&E, scale = 100 \( \mu \)m. (B) PRP group: olfactory mucosa epilepsy (arrow), dilatation in intercellular spaces between olfactory gland cells (star). H&E, scale = 10 \( \mu \)m. H&E = hematoxylin and eosin; PRP = platelet-rich plasma.](image-url)

Laryngoscope 128: May 2018 Yasak et al.: PRP Treatment in Anosmic Mice
saline group, significant improvement was detected in both the FFT and the histopathological examination of the anosmic mice after PRP lavage.

The pathologies of smell disorders may result from pathologies that occur anywhere, from the nasal cavity to the central nervous system of the olfactory system. The most common etiologic factor is sinonasal pathologies, whereas postinfectious and posttraumatic disorders often occur in nonsinonasal pathologies.14 When the underlying disease in sinonasal pathologies is treated by medical or surgical treatment, the smell disorder may be improved, but treatment is far more difficult in nonsinonasal pathologies because etiopathogenesis remains to be fully identified. Hyposmia resulting from neuroepithelial degeneration in an injury to the olfactory epithelium due to nonsinonasal pathologies, or anosmia in more serious cases, may occur.4 Anosmia and hyposmia can be temporary or permanent according to the degree of degeneration. There is no clear medical option to treat these conditions, although it has been claimed that a variety of drugs, such as systemic steroids, topical vitamin A, and topical sodium citrate, are effective.1,15,16

The olfactory epithelium is called the neuroepithelium due to both its epithelial structure and the neurons it contains. Anosmia is perhaps the only pathology that can be treated with correct medical or surgical treatment in neurodegenerative diseases. There are four different types of cells in the olfactory neuroepithelium: mature, immature, basal cells, and supporting cells. Basal cells are located in the bottom epithelial layer and are the only neuronal cells capable of regeneration, unlike in other sensory organs.6,17 Therefore, treatment is possible by using the regenerative potential of the basal membrane. If local growth factors or stem cells are applied to this region, they may trigger this potential. Lee et al. reported a significant improvement in anosmia after olfactory neuroepithelial stem cell transplantation in mice.5 Nota et al. found significant improvement in anosmia in their study group (anosmia-induced mice) after infiltration of basic fibroblast growth factor (bFGF).6

Platelet-rich plasma treatment is a process in which a small amount of blood from a test subject is separated into its components by a special centrifugation process, and the resulting PRP is administered to the same subject via injection. It has been used in numerous fields for almost 30 years, and its popularity is increasing. It has the advantages of being biocompatible and reversible9,10 and contains many growth factors such as platelet-derived growth factor, transforming growth factor-β, epidermal growth factor, vascular endothelial growth factor, and insulin-like growth factor; as well as numerous neurotrophic factors such as neurotrophin-3 (NT-3), angiopoietin-1, and glial cell line-derived neurotrophic factor (GDNF).8,9,18,19 In many studies, it has been shown that the growth factors mentioned had a neuroregenerative feature as well as a therapeutic effect.19–23 Cho et al. showed that PRP had an accelerating effect on neurotrophic factors (nerve growth factor, bFGF, angiopoietin-1, brain-derived neurotrophic factor, GDNF, and NT-3) and recovery of the facial nerve within a regeneration period following facial nerve injury.18 Platelet-rich plasma can be used in the treatment of anosmia via the above-mentioned methods and by using the regeneration property of the basal membrane of the olfactory neuroepithelium. The practicability of PRP, which is also preferred in many neurodegenerative diseases, has been tested in some animal studies, particularly focusing on otorhinolaryngology, facial nerve damage, vocal cord damage, and post-eardrop damage.10,18

Table III.

<table>
<thead>
<tr>
<th>Group</th>
<th>Average ± SD</th>
<th>Median</th>
<th>Average ± SD</th>
<th>Median</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Olfactory epithelial thickness (µm)</td>
<td>79.91 ± 4.39</td>
<td>80.45</td>
<td>69.49 ± 5.35</td>
<td>69.2</td>
<td>0.003</td>
</tr>
</tbody>
</table>

SD = standard deviation.

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Fig. 2. (A) Saline group: diffuse cilia loss (arrow) in the olfactory mucous epithelium, necrosis in the epithelial layer, and intraepithelial inflammatory cell infiltration in the basal region (star). H&E, scale = 100 µm. (B) Saline group: diffuse cilia degeneration in the olfactory mucosal epithelium (arrow), necrosis in the olfactory gland epithelium (star). H&E, scale = 10 µm. H&E = hematoxylin and eosin.

Laryngoscope 128: May 2018
Yasak et al.: PRP Treatment in Anosmic Mice
E160
Because anosmia treatment is not yet fully understood, studies are being conducted on animal models of induced anosmia. 3-MI, frequently preferred to induce anosmia when administered intraperitoneally, causes selective dose-dependent degeneration in the olfactory mucosa after getting into the systemic circulation. It shows its olfactotoxic effect with a 300 or 400 µg/g dose by causing severe hyposmia or anosmia.11 Food-finding test is a valuable method in the formation of anosmia and follow-up of treatment.11,24 We used 3-MI to create anosmia and evaluated the results with FFT. After PRP and saline lavages, there was a statistically significant improvement in the time of finding food in the PRP group in all three FFTs on days 7, 14, and 21. Only three of the eight mice that received saline were successful, but at a longer duration than the average of PRP group. In the remaining five mice, anosmia was still present on day 21. We attributed the improvement in the three mice to the temporary effect of 3-MI, which begins with decomposition in the basal membrane after 48 hours. This effect may start to decrease after 1 week, and this regeneration process lasts for several weeks.13,25 In our study, two mice showing FFT in the saline group succeeded in finding food on day 7, and the other mouse succeeded on day 14. Degeneration scores in the olfactory epithelium of the mice that were successful in finding food were lower than those that failed. We observed that the degeneration in the failed mice continued histopathologically and the epithelium thinned. All mice in the PRP group were successful in the FFT on day 7. On days 14 and 21, the time to find food was not statistically significant but was better than on day 7. In the histopathological examination, the epithelial damage score was significantly lower, and the epithelial thickness was significantly higher in the PRP group compared to the saline group.

We showed that PRP had a positive effect on olfactory functions in terms of FFT and histopathology. However, there were some limitations to our study. The use of more objective methods, such as the examination of olfactory marker protein-positive cells, Western blot analysis, and electro-olfactogram, may provide more significant improvement in olfactory neuroepithelial function. Platelet-rich plasma should be studied in a greater number of subjects, using more objective data, prior to any clinical use.

CONCLUSION
The results of our study showed that PRP positively affected olfactory neuroepithelial regeneration and improved olfactory functions in an anosmia-induced mouse model. However, there is a need for more objective studies before PRP can be considered for use in clinical practice.

BIBLIOGRAPHY