Photodiagnosis and Photodynamic Therapy 18 (2017) 275–278

Contents lists available at ScienceDirect

Photodiagnosis and Photodynamic Therapy

journal homepage: www.elsevier.com/locate/pdpdt

Antimicrobial photodynamic therapy combined to periodontal treatment: Experimental model

Estéfani L. Belinello-Souza[a], Letícia H. Alvarenga[a,b,*], Cintia Lima-Leal[a,b], Patrícia Almeida[a], Carolina Guimarães Leite[b], Tairine R. Lima[b], Bianca Godoy-Miranda[a,b], Jhosepher Previati-Oliveira[b], Lucas de Pretto[c], Anderson Zanardi de Freitas[c], Adjaci U. Fernandes[d], Rodrigo Labat Marcos[a], Renato A. Prates[a,b,*]

[a] Dep. of Biophotonics, Universidade Nove de Julho (UNINOVE), São Paulo, Brazil
[b] School of Dentistry, Universidade Nove de Julho (UNINOVE), São Paulo, Brazil
[c] Center for Lasers and Applications – IPEN-CNEN/SP, São Paulo, Brazil
[d] Universidade Anhembi Morumbi, São Paulo, Brazil

ARTICLE INFO

Article history:
Received 1 December 2016
Received in revised form 3 March 2017
Accepted 13 March 2017
Available online 19 March 2017

Keywords:
Antimicrobial photodynamic therapy
Periodontitis
Photosensitizer
Porphyrin
LED

ABSTRACT

Background: Antimicrobial photodynamic therapy (aPDT) has been used as an adjuvant treatment for periodontitis. It combines a photosensitizer with a light source to induce reactive oxygen species and kill microbial cells. PpNetNI is a protoporphyrin derivative, and it has a chemical binding site at biofilm and great affinity to microbial cells. The aim of this study was to investigate the effects of aPDT as an adjuvant treatment for periodontitis.
Methods: Thirty healthy male rats Wistar (*Rattus norvegicus*) were used in this study (Approved by UNINOVE Ethical committee AN0029/2015). Periodontitis was induced by placing a cotton ligature around the first mandibular molar in a subgengival position. The contralateral mandibular first molar received neither a ligature nor any treatment, and was used as a control. After 7 days, the ligature was removed and all animals received scaling and root planing (SRP) and were divided according to the following treatments: SRP group (received SRP and irrigation with PpNetNI, 10 μM) and aPDT group (PpNetNI 10 μM followed by LED irradiation). aPDT was performed with a LED (630 nm) with an output power of 400 mW (fluence-rate 200 mW/cm^2; fluence 18 J/cm^2). Rats were euthanized at 24 h, 48 h and 7 days postoperatively. The area of bone loss in vestibular region of the first molar was evaluated by Optical Coherence Tomography (OCT, THORLABS LTD., Ely, UK). Data were analyzed statistically (ANOVA and Tukey tests, $p < 0.05$).
Results: The animals treated by aPDT showed bone gain of approximately 30% compared to the SRP group following 7 days from the treatment.
Conclusion: aPDT promoted bone recovery 7 days after periodontal intervention.

1. Introduction

Periodontitis is an inflammatory disease of supportive periodontal tissue characterized by gingival inflammation, loss of connective tissue attachment, and reabsorption of alveolar bone [1,2]. This disease is caused by microorganisms organized in biofilms that colonize tooth surface; the biofilm is a complex structure composed by microcolonies of bacteria imbibed in an extra-cellular matrix that promotes cell protection and adherence to the tooth [3,4]. The periodontal therapy is based on professional removal of the biofilm and calculus by scaling and root planing (SRP) in conjunction with personal plaque control [5]. However, the mechanical debridement may fail to remove some pathogenic organisms because of their location in subgengival tissue, like furcations or deep pockets and in some situations the use of systemic antibiotics may also be indicated [6,7].

Administration of antibiotic treatments has been decreasing over the years because its side effects, such as stomach pain and increase of bacterial resistance to antibiotic. In some cases, patient leaves the antibiotic treatment before its end, and it gives

* Corresponding authors at: Biophotonics Program, Nove de Julho University (UNINOVE), Rua Vergueiro 235/249, Liberdade, São Paulo ZIP: 01504–001, Brazil.
E-mail addresses: leticiahalvarenga@uni9.pro.br (L.H. Alvarenga), pratesra@uni9.pro.br (R.A. Prates).

http://dx.doi.org/10.1016/j.pdpdt.2017.03.008
1572-1000/

a chance to select resistant bacteria [6,8]. Antimicrobial photodynamic therapy (aPDT) has been proposed as adjunctive to periodontal treatment [9–11]. aPDT combines a non-toxic photosensitizer (PS) and low intensity visible light, which in the presence of oxygen produce cytotoxic reactive oxygen species (ROS), such as singlet oxygen [12,13], that are toxic to bacterial cells.

The effect of aPDT has been evaluated by several rat ligature models, with induction of experimental periodontitis by ligature placement [14–16]. The ligature leads to biofilm accumulation, resulting in loss of attachment and bone predictably in 7 days [17].

Considering that the results of clinical trials evaluating the benefits of aPDT are still inconclusive [18–20], the purpose of this study was to evaluate the efficacy of aPDT as an adjuvant to periodontal treatment in rats using proto-porphyrin PpNetNI as PS [21]. Porphyrins-PDT have demonstrated good results in the inactivation of bacteria [22–24]. PpNetNI was used for the first time in this animal model associated with irradiation with LED. Optical coherence tomography (OCT) assessment was used to evaluate the effects provided by aPDT in periodontal tissue.

2. Materials and methods

The experiments were approved by the Ethics Committee for Animal Research of UNINOVE/SP (n. AN0029/2015). All rats were housed under the same conditions and maintained on food and water *ad libitum*.

2.1. Study design

Thirty healthy male Wistar rats (*Rattus norvegicus*) with a body mass of approximately 250 g were used for this study. Periodontitis was induced by placing a cotton ligature around the right inferior first molar in a subgingival position. After 7 days, the ligatures were removed from the animals and they were divided into two groups. SRP group (n = 15) received SRP and irrigation with 10 μM PpNetNI, without irradiation and aPDT group (n = 15) received 10 μM PpNetNI followed by LED irradiation for 90 s. Rats were euthanized at 24 h, 48 h and 7 days postoperatively (n = 5 animals per group) by the administration of a lethal dose of anesthetics (150 mg/kg thiopental).

The experimental schedule is illustrated in Fig. 1.

2.2. Ligature induction of experimental periodontitis

The rats were anesthetized with ketamine (80 mg/kg) and xylazine (10 mg/kg) by intraperitoneal injection. A cotton ligature was placed around the right first molar of the jaw in a submarginal position to induce periodontal inflammation and the contralateral was used as control, without periodontitis induction (Fig. 2).

2.3. Photodynamic therapy

The PS (10 μM PpNetNI) was applied around the right inferior first molar and a pre irradiation time of 5 min was set. The tested size received LED irradiation during 90 s through the buccal side with the parameters described above in Table 1.

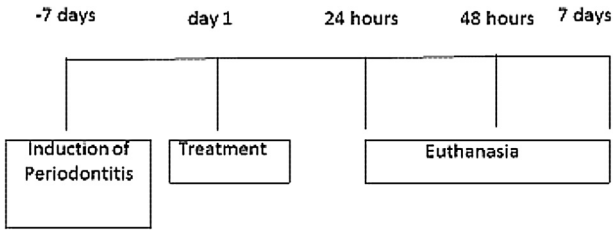

Fig. 1. Induction of periodontitis on day −7. All treatments were performed at day 1. The animals were euthanized following 24 h, 48 h, and 7 days from the treatment.

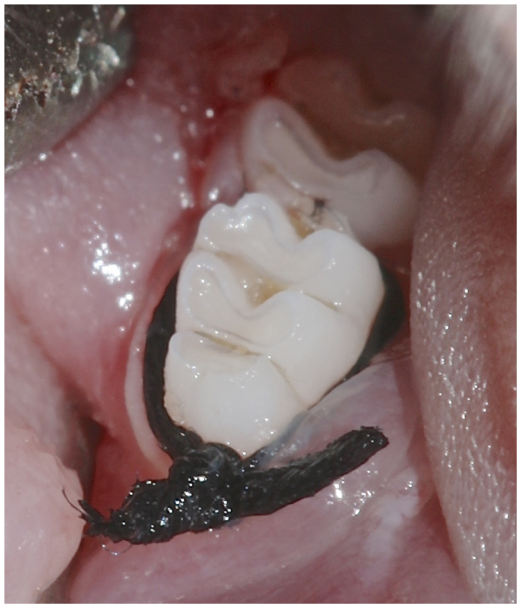

Fig. 2. Cotton ligature around the right inferior first molar in a subgingival position.

Table 1
Irradiation parameters used in the experiment. The buccal area of the right first molar was irradiated once.

Irradiation parameters	
Wavelength	630 nm
Radiant output power	400 mW
Irradiance	200 mW/cm^2
Radiant exposure	18 J/cm^2

2.4. Optical coherence tomography

The maxilla of the rats was removed and the samples were immediately fixed in 4% paraformaldehyde in small glasses and identified according the respective groups.

The area of bone loss in buccal region of the first molar was evaluated by Optical Coherence Tomography (OCT, THORLABS LTD., Ely, UK). The analysis was made by measuring the distance of the coronal margin of the bone crest to the cementoenamel junction (CEJ). Each sample was evaluated and a percentage value of the amount of bone loss was calculated by using the contralateral tooth as parameter.

To assess the effect of the treatments, One-Way ANOVA test was used followed by TUKEY test. The results were considered statistically significant when ($p < 0.05$). Data is presented as the mean and standard deviation of each group.

3. Results

Optical Coherence Tomography was able to detect bone loss in the samples and it was a nondestructive method for this experimental model (Fig. 3).

After 7 days from the treatment, the animals treated by PDT showed bone gain of approximately 30% compared to the SRP group (Fig. 4).

Furthermore, there was an increase in bone loss in the SRP group, while the aPDT group presented regression in the size of bone loss after 7 days (Fig. 5).

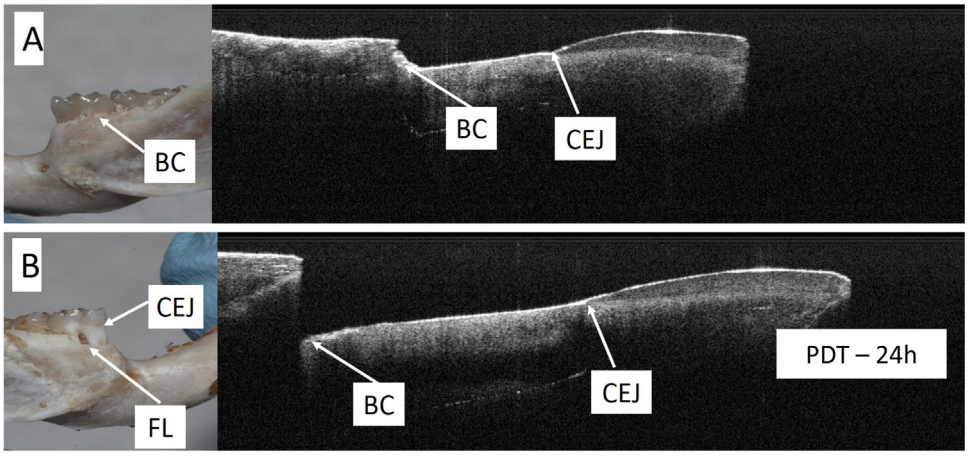

Fig. 3. Samples photographs (left) and OCT images (right) from an animal 24 h after treatment from aPDT group. We can observe the distance between the cementoenamel junction (CEJ) and the bone crest (BC). Note the Furcation Lesion (FL) and the magnitude of bone loss. A – control side; B – test side.

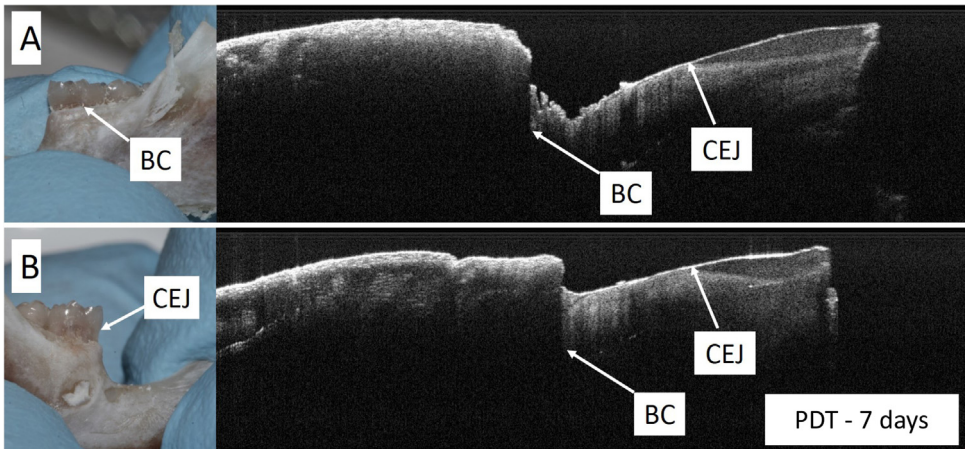

Fig. 4. Samples photographs (left) and OCT images (right) from an animal 7 days after treatment from aPDT group. We can observe the distance between the cementoenamel junction (CEJ) and the bone crest (BC). A – control side; B – test side.

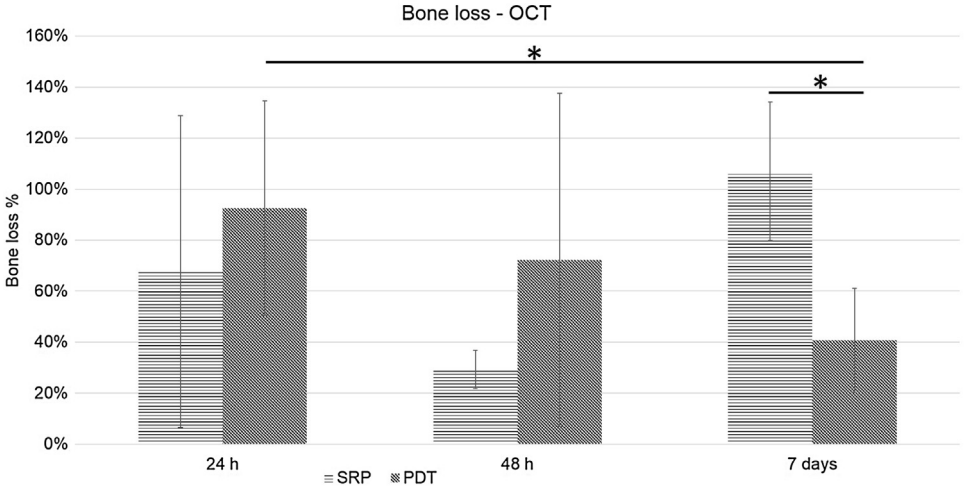

Fig. 5. Quantification data of bone loss comparing aPDT and SRP group. The animals were euthanized following 24 h, 48 h, and 7 days from the treatment. In the Y axis we can observe the relation between the control and the test side. Data are means and standard deviation. Statistical significant difference is represented by the symbol (*).

4. Discussion

In the present study, we reproduced a previously reported model of periodontitis in rats induced by the presence of a ligature around a tooth [25]. Our study demonstrated that cotton ligatures around the inferior right first molar resulted in progressive bone loss in the furcation region after 7 days.

The measurement of bone loss area was performed by optical coherence tomography, which demonstrated to be a good apparatus for this type of analysis, since it did not cause damage to the samples and provides better image quality and resolution when compared to the traditional imaging methods. This was the first time that OCT was used in experimental models of periodontitis in rats. Previously, the analysis of bone loss was performed with radiographs [14,26] and histometric analysis [15,16,27,28]. The OCT data demonstrated significantly reduced bone loss in the aPDT group compared to the SRP group at 7 days. Several animal studies and clinical trials that investigate the aPDT as adjuvant of periodontal therapy use the PS methylene blue (MB) [9–11,14,16]. Animal studies have shown good results with this combination [16,27], but in clinical trials the efficacy of aPDT using MB is still inconclusive [18–20]. In attempt to improve the results obtained by aPDT, we proposed the use of PpNetNI as photosensitizer. The PpNetNI was used for the first time in animal model associated with irradiation with LED.

The results obtained in the present study showed that the association of PpNetNI with LED irradiation at the settings mentioned before revealed more satisfactory results than isolated utilization of SRP. Significant bone gain was achieved in the aPDT group following 7 days from the treatment, and this might be due to effects of low level laser therapy on osteogenic differentiation and proliferation [29]. Similar results were also verified in other animal studies [16,27]. The results suggested that aPDT reduced periodontal tissue destruction and may be useful as an adjuvant to SRP. However, further studies should be conducted to elucidate questions about the efficacy of aPDT on periodontal disease and to establish effective protocols for the use of aPDT in clinical procedures.5. Conclusions

In conclusion, within the parameters used in this study, aPDT was an effective alternative to held periodontal health after treatment, and it was able to improve regeneration and prevent further tissue loss.

Acknowledgement

The authors thanks to UNINOVE to research support and FAPESP (grant number 2016/10269-5). The authors also thanks Danielle Vieira for the technical support with the experimental animals.

References

[1] American Academy of Periodontology, Treatment of plaque-induced gingivitis, chronic periodontitis, and other clinical conditions, J. Periodontol. 72 (December (12)) (2001) 1790–1800.
[2] G.C. Armitage, Periodontal diagnoses and classification of periodontal diseases, Periodontology 2000 34 (2004) 9–21.
[3] S.S. Socransky, A.D. Haffajee, Microbial mechanisms in the pathogenesis of destructive periodontal diseases: a critical assessment, J. Periodontal Res. 26 (May (3 Pt. 2)) (1991) 195–212.
[4] S.S. Socransky, A.D. Haffajee, Dental biofilms: difficult therapeutic targets, Periodontology 2000 28 (2002) 12–55.
[5] D.L. Tagge, T.J. O'Leary, A.H. El-Kafrawy, The clinical and histological response of periodontal pockets to root planing and oral hygiene, J. Periodontol. 46 (September (9)) (1975) 527–533.
[6] J. Slots, Selection of antimicrobial agents in periodontal therapy, J. Periodontol. Res. 37 (October (5)) (2002) 389–398.
[7] J. Slots, M. Ting, Systemic antibiotics in the treatment of periodontal disease, Periodontology 2000 28 (January (1)) (2002) 106–176.
[8] C.B. Walker, The acquisition of antibiotic resistance in the periodontal microflora, Periodontology 2000 10 (February) (1996) 79–88.
[9] L. Ge, R. Shu, Y. Li, C. Li, L. Luo, Z. Song, Y. Xie, D. Liu, Adjunctive effect of photodynamic therapy to scaling and root planing in the treatment of chronic periodontitis, Photomed. Laser Surg. 29 (January (1)) (2011) 33–37.
[10] J. Betsy, C.S. Prasanth, K.V. Baiju, J. Prasanthila, N. Subhash, Efficacy of antimicrobial photodynamic therapy in the management of chronic periodontitis: a randomized controlled clinical trial, J. Clin. Periodontol. 41 (2014) 573–581.
[11] M.F. Kolbe, F.V. Ribeiro, V.H. Luchesi, R.C. Casarin, E.A. Sallum, F.H. Nociti Jr, G.M. Ambrosano, F.R. Cirano, S.P. Pimentel, M.Z. Casati, Photodynamic therapy during supportive periodontal care: clinical, microbiologic, immunoinflammatory, and patient-centered performance in a split-mouth randomized clinical trial, J. Periodontol. 85 (August (8)) (2014) e277–e286.
[12] M.R. Hamblin, T. Hasan, Photodynamic therapy: a new antimicrobial approach to infectious disease? Photochem. Photobiol. Sci. 3 (May (5)) (2004) 436–450.
[13] L. Huang, Y. Xuan, Y. Koide, T. Zhiyentayev, M. Tanaka, M.R. Hamblin, Type I and Type II mechanisms of antimicrobial photodynamic therapy: an in vitro study on gram-negative and gram-positive bacteria, Lasers Surg. Med. 44 (August (6)) (2012) 490–499.
[14] J.M. de Almeida, L.H. Theodoro, A.F. Bosco, M.J.H. Nagata, M. Oshiiwa, V.G. Garcia, Influence of photodynamic therapy on the development of ligature-induced periodontitis in rats, J. Periodontol. 78 (March (3)) (2007) 566–575.
[15] A.S. Carvalho, M.H. Napimoga, J. Coelho-Campos, V. Silva-Filho, G. Thedei, Photodynamic therapy reduces bone resorption and decreases inflammatory response in an experimental rat periodontal disease model, Photomed. Laser Surg. 29 (November (11)) (2011) 735–740.
[16] R.A. Prates, A.M. Yamada Jr, L.C. Suzuki, C.M. França, S. Cai, M.P. Mayer, A.C. Ribeiro, M.S. Ribeiro, Histomorphometric and microbiological assessment of photodynamic therapy as an adjuvant treatment for periodontitis: a short-term evaluation of inflamatory periodontal conditions and bacterial reduction in a rat model, Photomed. Laser Surg. 29 (December (12)) (2011) 835–844.
[17] D.T. Graves, D. Fine, Y.T. Teng, T.E. Van Dyke, G. Hajishengallis, The use of rodent models to investigate host-bacteria interactions related to periodontal diseases, J. Clin. Periodontol. 35 (February (2)) (2008) 89–105.
[18] A. Azarpazhooh, P.S. Shah, H.C. Tenenbaum, M.B. Goldberg, The effect of photodynamic therapy for periodontitis: a systematic review and meta-analysis, J. Periodontol. 81 (January (1)) (2010) 4–14.
[19] F. Sgolastra, A. Petrucci, M. Severino, F. Graziani, R. Gatto, A. Monaco, Adjunctive photodynamic therapy to non-surgical treatment of chronic periodontitis: a systematic review and meta-analysis, J. Clin. Periodontol. 40 (2013) 514–526.
[20] V.F. Carvalho, P.V. Andrade, M.F. Rodrigues, M.H. Hirata, R.D. Hirata, C.M. Pannuti, G. De Micheli, M.C. Conde, Antimicrobial photodynamic effect to treat residual pockets in periodontal patients: a randomized controlled clinical trial, J. Clin. Periodontol. 42 (May (5)) (2015) 440–447.
[21] A.F. Uchoa, C.S. Oliveira, M.S. Baptista, Relationship between structure and photoactivity of porphirins derived from protoporphyrin IX, J. Porphyr. Phthalocyanines 14 (2010) 833–845.
[22] E. Alves, L. Costa, C.M. Carvalho, J.P. Tomé, M.A. Faustino, M.G. Neves, A.C. Tomé, J.A.S. Cavaleiro, A. Cunha, A. Almeida, Charge effect on the photoinactivation of gram-negative and gram-positive bactéria by cationic meso-substituted porphyrins, BMC Micorbiol. 9 (April) (2009) 70.
[23] R. Dosselli, M. Gobbo, E. Bolognini, S. Campesrtrini, E. Reddi, Porphyrin-apidaecin conjugate as a new broad spectrum antibacterial agente, ACS Med. Chem. Lett. 1 (February (1)) (2010) 35–38.
[24] F.F. Sperandio, Y.Y. Huang, M.R. Hamblin, Antimicrobial photodynamic therapy to kill gram-negative bacteria, Recent Pat. Antiinfect. Drug Discov. 8 (August (2)) (2013) 108–120.
[25] S. Rovin, E.R. Costich, H.A. Gordon, The influence of bacteria and irritation in the initiation of periodontal disease in germfree and conventional rats, J. Periodontal Res. 1 (1966) 193–204.
[26] L.A. Fernandes, J.M. de Almeida, L.H. Theodoro, A.F. Bosco, M.J. Nagata, T.M. Martins, T. Okamoto, V.G. Garcia, Treatment of experimental periodontal disease by photodynamic therapy in immunosuppressed rats, J. Clin. Periodontol. 36 (March (3)) (2009) 219–228.
[27] J.M. de Almeida, L.H. Theodoro, A.F. Bosco, M.J. Nagata, M. Oshiiwa, V.G. Garcia, In vivo effect of photodynamic therapy on periodontal bone loss on dental furcations, J. Periodontol. 79 (June (6)) (2008) 1081–1108.
[28] J.M. de Almeida, L.H. Theodoro, A.F. Bosco, M.J. Nagata, S. Bonfante, V.G. Garcia, Treatment of experimental periodontal disease by photodynamic therapy in rats with diabetes, J. Periodontol. 79 (November (11)) (2008) 2156–2165.
[29] Y. Wang, Y.Y. Huang, Y. Wang, P. Lyu, M.R. Hamblin, Photobiomodulation (blue and green light) encourages osteoblastic-differentiation of human adipose-derived stem cells: role of intracellular calcium and light-gated ion channels, Sci. Rep. 21 (September (6)) (2016) 33719.