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Fluoxetine reduces periodontal disease progression in a conditioned fear stress model in rats

J. C. A. Aguiar[1,2]*, E. P. P. Gomes [1]*, T. Fonseca-Silva[1], N. A. Velloso[3], L. T. Vieira[1], M. F. Fernandes[4] S. H. S. Santos[5,6], J. F. R. Neto[5] A. M. B. De-Paula[1], A. L. S. Guimarães[1]

[1]Department of Dentistry, Universidade Estadual de Montes Claros, Montes Claros, Brazil, [2]School of Psychology, Faculdades Integradas Pitágoras, Montes Claros, Brazil, [3]School of Pharmacy, Faculdades Santo Agostinho, Montes Claros, Brazil, [4]Department of Mathematics, Universidade Estadual de Montes Claros, Montes Claros, Brazil, [5]Department of Medicine, Universidade Estadual de Montes Claros, Montes Claros, Brazil and [6]Department of Pharmacology, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil

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Background and Objective: Recent evidence suggests that the use of fluoxetine could reduce periodontal disease severity. However, the effect of fluoxetine on periodontal disease has not been tested in the context of conditioned fear stress (CFS). We hypothesized that inhibition of chronic stress by fluoxetine might decrease the levels of bone loss in periodontal disease. The aim of the present study was to analyze the effect of fluoxetine on bone loss in chronic periodontitis.

Material and Methods: Fourteen Wistar rats were submitted to ligature-induced periodontal disease and divided into four groups (A–D). Groups A (n = 3) and B (n = 4) were not stressed, while Groups C (n = 3) and D (n = 4) were submitted to a CFS paradigm for 38 d. Daily fluoxetine (20 mg/kg) was administered to Groups B and D from day 20 to day 39, at which point the rats were submitted to an open field test and killed on day 40. Mandibles were removed for histological and immunohistochemical analyses.

Results: Stress was associated with a higher level of bone loss in Group C compared with Group A. Additionally, no differences in bone loss were observed among Groups A, B and D.

Conclusion: We showed that stress is associated with the progression of bone loss in a CFS model in rats and that fluoxetine treatment reduces the bone loss.

André Luiz Sena Guimarães, DDS, PhD, Universidade Estadual de Montes Claros, Hospital Universitário Clemente de Faria, Laboratório de Pesquisa em Saúde, Avenida Cula Mangabeira, 562, Montes Claros, cep 39401-001, Minas Gerais, Brasil
Tel/ Fax: 55 38 32248382
e-mail: andreluizguimaraes@gmail.com

*Dr J. C. A. Aguiar and Dr E. P. P. Gomes contributed equally to this work.

Key words: chronic stress; experimental periodontitis; antidepressant; fluoxetine; stress

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Inflammatory periodontal disease is an important condition that affects a vast number of people worldwide (1). The pathological hallmarks of periodontal disease are inflammation and destruction of tooth-supporting tissues as a result of the immunological response to bacterial challenge (2). Inflammatory cytokines, particularly interleukin (IL)-1 and IL-6, are prominently involved in periodontal bone resorption (1–4). Animals treated with inhibitors of IL-1 exhibit less periodontal bone loss compared with control animals (5). Moreover, IL-6-deficient mice infected with *Porphyromonas gingivalis* experience less periodontal bone loss compared with wild-type mice (6,7). These findings, together with classical studies of periodontal disease (8), suggest that elimination of the agents provoking inflammatory responses in periodontal tissue is the treatment of choice for chronic periodontitis (9).

Microbial biofilms are major contributors to the development of periodontal disease. However, emerging evidence supports a role for systemic factors in the progression of periodontal disease (3). Several studies suggest that mental disorders (2) such as depression, stress and anxiety might contribute to the development

of periodontal disease (4–7). This association may be related to the bidirectional communication between the immune system and the nervous system. However, the pathways by which stressors may affect the local immune response and thus lead to the development of periodontal disease remain largely uncharacterized (8,9).

Selective serotonin reuptake inhibitors (SSRIs) potentiate and prolong the action of the neurotransmitter 5-hydroxytryptamine (5-HT) and are widely used to treat depression. The effects of 5-HT-active drugs, such as SSRIs, on anxiety and depressive disorders strongly suggest a role for 5-HT in the neurochemical basis of these disorders (10). The SSRIs fluoxetine and citalopram have been suggested to have anti-inflammatory effects also, as they selectively inhibit endosomal toll-like receptor signaling and inflammatory cytokine production in human rheumatoid arthritis tissue (11). In agreement with these observations, tianeptine, a selective serotonin reuptake enhancer used to treat major depression, significantly inhibited periodontal bone loss in a rat model of depression (12). In addition, recent evidence suggests that the use of fluoxetine could reduce periodontal disease severity (13). However, the effect of fluoxetine on periodontal disease has not been tested in a conditioned fear stress (CFS) context. We hypothesized that inhibition of chronic stress by fluoxetine might decrease the levels of bone loss in periodontal disease. Therefore, the aim of the present study was to analyze the effect of fluoxetine on the severity of chronic periodontitis.

Material and methods

Experimental design

Fourteen Wistar rats were submitted to ligature-induced periodontal disease and divided into four groups (A–D). Groups A ($n = 3$) and B ($n = 4$) were not stressed, whereas Groups C ($n = 3$) and D ($n = 4$) were submitted to a CFS paradigm for 38 d. Daily fluoxetine (20 mg/kg) was administered to Groups B and D from day 20 to day 39, at which point the rats were submitted to an open field test and killed on day 40. Mandibles were removed for histological and immunohistochemical analyses.

Animals

The experiments were performed on male Wistar rats weighing approximately 300 g at the beginning of the experiment. The animals were housed in groups of three or four and allowed free access to standard rat pellets and tap water. They were maintained under a 12-h light/12-h dark cycle (lights on from 07:00 h to 19:00 h) with the temperature and humidity maintained at 22°C and 40–60%, respectively. The animals were acclimated for 2 wk before the beginning of the study. The experiments were registered with and approved by the local Experimental Animal Board (process 151/2008).

Induction of inflammatory periodontal disease

Experimental periodontal disease was induced in rats under ketamine (60 mg/kg, intraperitoneally) and xylazine (10 mg/kg, intraperitoneally) anesthesia by placement of a sterile cotton (000) thread ligature around the neck of the maxillary left first-molar tooth. The ligatures retained oral microorganisms and remained fixed until the end of the experiment (day 40) when the rats were killed. The contralateral right side was used as the unligated control.

CFS protocol

CFS-induced freezing behavior has been proposed as an animal model of anxiety, as previously described (14,15). One day after placement of the ligature, rats were subjected to brief CFS sessions for 38 d. Initially, rats were individually placed for 3 min in a chamber to habituate to the apparatus (37 cm × 25 cm × 21 cm, Skinner Box, ELT-02; Eltrones, Joinvile, Santa Catarina, Brazil). During this period, the number of crossings across an imaginary line that divided the box floor into two equal segments was counted. Thereafter, rats received one presentation of a neutral conditioned stimulus (CS; 90-dB sound at 1000 Hz) for 5 s followed immediately by a noxious unconditioned stimulus (US; 1.10-mA foot shock) for 5 s × 6 with an intershock interval of 20 s, totaling 3 min. Animals in the non-CFS groups were also placed individually in the chamber and submitted to the same experimental conditions but did not receive the shocks. An inescapable electric foot shock through a stainless-steel grid floor was delivered to the rats via a shock generator. Stimulus strength and number of training CS/US pairings were chosen based on a pilot experiment. After the last CS/US pairing, the rats were maintained in the chamber for 3 min before being returned to their home cages. The number of freezing behaviors (yes/no) was recorded by an observer and the sum of freezing was used for comparison during the analyses. CFS tends to increase the levels of freezing behavior in rats. The chamber was cleaned with 70% ethanol before and after each rat. Freezing was defined as the absence of all observable movements, except for those related to respiration, and was measured by an observer who was blind to the experimental groups of the animals. On day 39, the open-field test was performed to evaluate anxiety and stress in the rats. The observers counted the number of squares that each rat completely crossed in the open-field apparatus.

Fluoxetine treatment

Starting on day 20, in order for the animal to develop stress before the beginning of the treatment, fluoxetine (Compaz; Laboratório Cristália, Itapira, São Paulo, Brazil), suspended in 12% bidistilled glycerin, was administered once daily by intragastric gavage (20 mg/kg), as described previously (13), to the rats in Groups B and D.

Histopathological analysis

The resected tissue specimens were fixed in formalin, demineralized in

40% ethylenediaminetetraacetic acid (EDTA), embedded in paraffin, cut into serial sections of 3-μm thickness and mounted on organosilane-coated slides. The sections were stained with hematoxylin and eosin and evaluated for bone loss, especially in the area of ligature placement between the first and second molars, by light microscopy. The bone loss was quantified by measuring the distance from the cemento–enamel junction to the alveolar bone crest. All morphological measurements were made using Image J software (National Institutes of Health, Bethesda, MD, USA).

Immunohistochemical staining for IL-1β and IL-6

For antigen retrieval, tissue sections were heated in a pressure cooker for 5 min at 125°C in Tris–EDTA buffer (1 mM Tris base, pH 9.0, 1 mM EDTA and 0.05% Tween 20). The sections were incubated for 18 h at 4°C with rabbit polyclonal anti-IL-1β (1:100 dilution, H-153; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) or goat polyclonal anti-IL-6 (1:100 dilution, M-19; Santa Cruz Biotechnology, Inc.) as the primary antibody. Endogenous peroxidase activity was blocked by incubation with 0.03% H_2O_2 in ethanol for 30 min. The primary antibodies were detected using the LSAB kit (Dako, Glostrup, Denmark). The sections were incubated with 3,3′-diaminobenzidine tetrahydrochloride for 5 min to develop the signals and then counterstained with Mayer's hematoxylin for 30 s. Samples of oral fibrous hyperplasia at the University Dental Clinic and samples of mouse heart served as positive controls for IL-1β and IL-6, respectively. Negative-control staining was performed by replacing the primary antibody with Universal Negative Control (Dako, Carpinteria, CA, USA).

Quantification of immunostaining

The expression levels of IL-1β and IL-6 in the immunostained sections were evaluated using an Olympus® BH2 microscope (Center Valley, PA, USA; fitted with a 10 × ocular lens and a 40 × objective lens). An ocular lattice grid (area = 0.092 mm^2), composed of 10 horizontal and 10 vertical test lines to make 100 points, was superimposed on the test field, and a total area of 1.84 mm^2 of each sample was evaluated. The percentages of cells that stained positive for IL-β and IL-6 were counted (10 fields per specimen). The immunohistochemical expression data are shown as mean ± standard error.

Statistical analysis

Initial 2 × 2 comparisons were performed using the Mann–Whitney *U*-test. Subsequently, analysis of variance tests were used for intergroup comparisons. As a post-hoc test, Fisher's least significant difference was used. *p*-Values of <0.05 were considered statistically significant. All analyses were performed using the Statistical Package for Social Sciences (SPSS, Inc., Chicago, IL, USA) version 13.0 for Windows.

Results

Isolated analyses demonstrated that the number of freezings per animal are increased after CFS sessions (Table S1). Similar results were also observed in intergroup comparisons of the number of freezings per animal; CFS was significantly higher in Groups C ($p < 0.01$) and D ($p < 0.01$) that were submitted to CFS compared with control Groups A and B (Table 1), confirming that the CFS method successfully stressed the animals.

To demonstrate the effect of stress on locomotor activity, we treated rats in Groups B and D with fluoxetine, daily, from day 20 to day 39; the rats in Groups A and C were treated with saline over the same time-period. We then compared the groups by measuring the crossing parameters in the open-field test (Table 1). We did not observe a difference in movement between the fluoxetine-treated groups (B and D). Rats in Groups B and D performed fewer center passes than rats in Group A (Table 1). These data demonstrate that rats treated with fluoxetine behave similarly independently of stress (Table S1), suggesting that fluoxetine was associated with decreased locomotor activity in the open field test. On the other hand, the number of crossings was significantly higher in Group A (no CFS, saline-treated) compared with Group C (CFS, saline-treated).

Stress was associated with a higher level of bone loss in Group C ($p = 0.006$) compared with Group A (Fig. 1a). Figure 1(b) shows the representative histological images of alveolar bone loss among groups. Additionally, no differences in bone loss were observed among Groups A, B and D (Fig. 1a). These facts indicate that even in the presence of stress, fluoxetine treatment decreases bone-loss levels.

Immunohistochemical staining for IL-1β and IL-6 are presented in Figure 2. There was no effect of stress or fluoxetine treatment on the expression of IL-1β or IL-6 proteins at the ligature site (Fig. 2a,b; $p = 0.681$ and $p = 0.500$, respectively). These data suggest that neither stress nor fluoxetine affect the levels of these cytokines.

Discussion

It is well known that fluoxetine is used to treat depression (16). On the other hand, evidence has suggested that fluoxetine administration results in decreasing locomotor activity during the multiple-administration period relative to the saline control (17). In the current study we observed that fluoxetine reduced the number of crossings in the open field test. The fact that fluoxetine did not induce hyperlocomotion, but in some cases decreased motor activity, in the open field test in rats that were exposed to CFS, could be related to the fact that fluoxetine may also produce sedative effects, as demonstrated previously (18). The decrease in locomotor activity in consequence to predominant effects in the dorsal raphe reducing serotoninergic transmission in the forebrain (19). In addition, this fact was observed in other studies (20). Moreover, it was demonstrated

Table 1. Comparison of behavioral parameters among Groups A–D

Treatment (Group)	Parameter	Mean	Standard deviation	*p*-Value
No shock and vehicle (A)	Freezing	2.26	1.89	Referent
No shock and fluoxetine (B)	Freezing	4.54	1.41	0.14
Shock and vehicle (C)	Freezing	27.07	2.37	**< 0.01***
Shock and fluoxetine (D)	Freezing	22.33	5.72	**< 0.01***
No shock and vehicle (A)	Crossing	36.67	3.79	Referent
No shock and fluoxetine (B)	Crossing	16.00	3.83	**< 0.01***
Shock and vehicle (C)	Crossing	20.19	9.42	**< 0.01***
Shock and fluoxetine (D)	Crossing	16.75	5.56	**< 0.01***

Freezing: the effect of fluoxetine treatment (20 mg/kg/d, starting on day 20) was studied on freezing behavior induced by conditioned fear stress (CFS). Data are expressed as mean ± standard deviation. The values shown in bold with an asterisk (*) represent $p < 0.05$ compared with the control group (A) (no CFS/vehicle). Crossing: the effect of fluoxetine treatment (20 mg/kg/d, starting on day 20) on the number of crossings was evaluated on day 39 after starting the stress sessions. Data are expressed as mean ± SD. The values shown in bold with an asterisk (*) represent $p < 0.05$ compared with the control group (A) (no CFS/vehicle). Values were analyzed using analysis of variance followed by correction with Fisher's least significant difference.

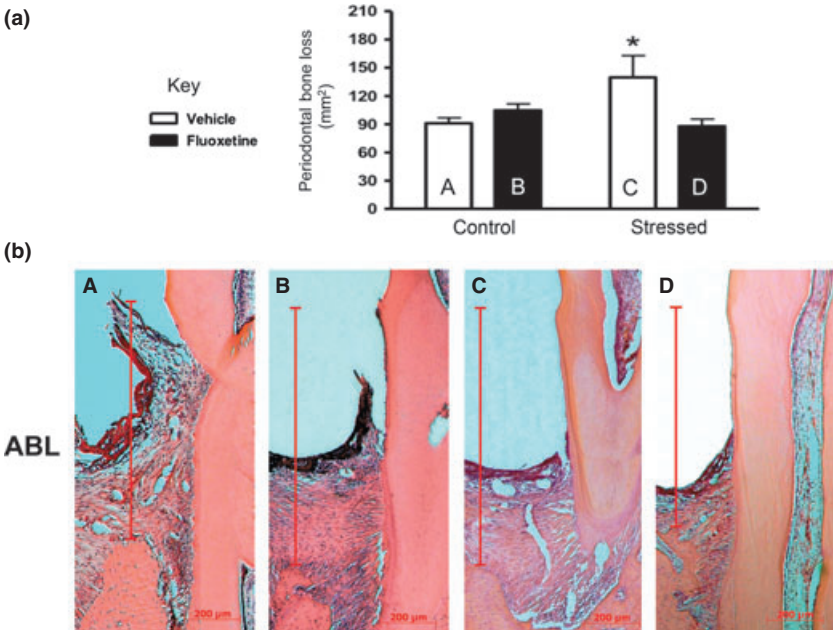

Fig. 1. Effect of fluoxetine treatment on aveolar bone loss. (a) Effect of fluoxetine treatment (20 mg/kg/d, at 20 d) on periodontal bone loss induced by stress. Data are expressed as mean ± standard error of the mean. Group C presented more bone loss than Group D [*$p < 0.05$ compared with the control group (conditioned fear stress/vehicle)]. (b) Representative histological images of alveolar bone loss (ABL) among Groups A–D.

that relative to sham controls, olfactory bulbectomized rats displayed decreased activity in the same conditions of the open field test (21). It is important to highlight that, in the current study, the number of freezings per animal after stress induction was significantly higher in groups submitted to CFS compared with control groups, demonstrating that animals were stressed.

The knowledge that psychological stress is associated with concurrent activation of the hypothalamic–pituitary–adrenal axis has prompted several studies investigating the possible role of stress in periodontal disease (22–26). However, the relationship between mental disorders (2) and periodontal disease remains unclear. For example, it has been shown that induced depression did not alter ligature-induced bone loss in Lewis rats (27). On the other hand, in a different study model (28), the non-specific antidepressant tianeptine significantly inhibited periodontal bone loss in rats (29). Recently we demonstrated that diazepam could also reverse bone loss in the same study model (15). In the same way, it was demonstrated that fluoxetine reduces bone loss in the experimental periodontal disease model, but the animals were not stressed (13). To date, no study has investigated the effect of fluoxetine on periodontal disease under stress conditions, and, given the clinical relevance of fluoxetine, we studied whether fluoxetine could reduce bone loss in a CFS experimental periodontal disease model. Here, and in agreement with previous studies, we demonstrated that fluoxetine reduces periodontal disease severity (13). In addition, it was observed that Group C presented significantly more bone loss than the Group A animals. These data indicate that chronic stress is associated with bone loss, and are in agreement with previous studies (25,30). On the other hand, fluoxetine did not affect local expression of the proinflammatory cytokines IL-1β and IL-6. In the same way, diazepam also did not affect local expression of the proinflammatory cytokines IL-1β and IL-6 (15). Recently, it was demonstrated that fluctuations in mood can influence inflammation by affecting cytokine production (31). This and other observations (12) suggest that there may be clinical benefit of the use of antidepressant drugs in the treatment of periodontal disease. Branco-de-Almeida *et al.* (13) observed that fluoxetine administration reduced the expression of *IL1β* and cyclooxygenase-2 (*COX2*) mRNAs. The difference between this result and our results could be attributed to the methods used to detect IL-1β and IL-6 in the current study. It is important to highlight an impressive study (12) that observed a reduction in bone loss after treatment with an atypical

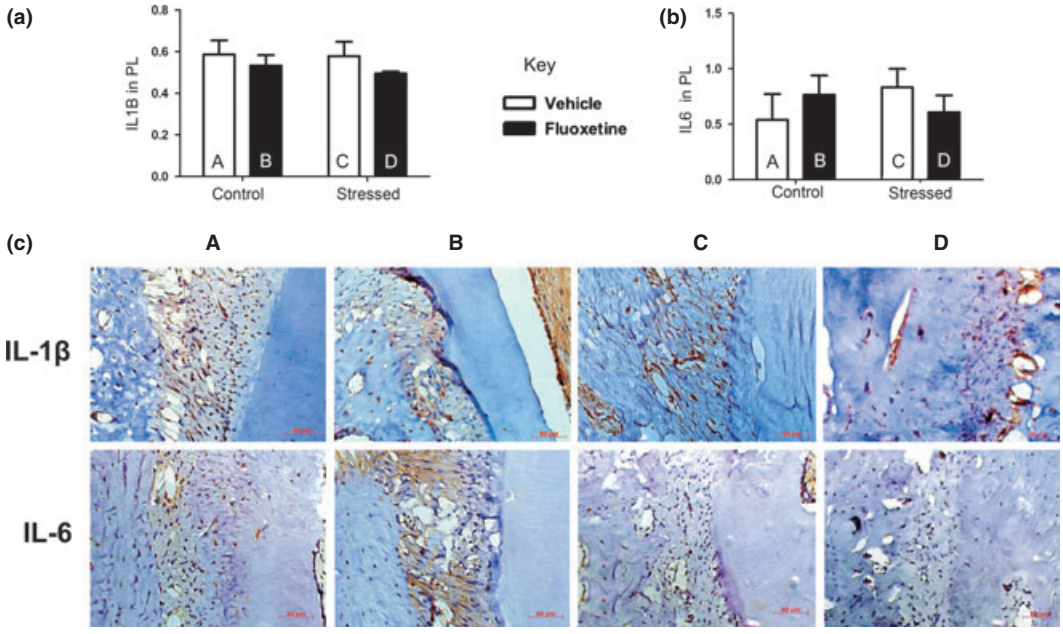

Fig. 2. Effect of fluoxetine treatment on alveolar cytokine expression Effect of fluoxetine treatment (20 mg/kg/d, at 20 d) on interleukin (IL)-1β (a) and IL-6 (b) in periodontal ligament. Data are expressed as mean ± standard error of the mean. $p < 0.05$, compared with the control group [no conditioned fear stress (CFS)/vehicle]. (c) Representative immunohistochemical images of IL-1β and IL-6 among Groups A–D.

antidepressant (28). Tianeptine did not attenuate the increased corticosterone response or the decreased expression of hippocampal glucocorticoid receptor (28). These findings suggest the possibility that the effect of tianeptine on bone loss may have been associated with nonspecific actions of the drug. Several studies in humans have suggested an association between mental disorders and periodontal disease (25,32), but those study populations had not received dental care. In a recent study of our group, with a police population, we did not observe an association between periodontal disease and the General Health Questionnaire 12, a questionnaire used to assess MD (26). It is important to highlight that to date there is no clinical evidence that antidepressants have a beneficial effect on oral health. In contrast, it is well known that some antidepressants are associated with xerostomia and poor oral health (33). Given the contradictions in the literature, it is clear that more studies are necessary to elucidate a possible benefit of antidepressants in periodontal disease.

In conclusion, we showed here that CFS was associated with periodontal disease severity in a CFS model in rats and that fluoxetine treatment reduces the bone loss.

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Conflict of interests

None declared.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1 Isolated comparison of parameters by Mann–Whitney test.

References

1. Graves DT, Li J, Cochran DL. Inflammation and uncoupling as mechanisms of periodontal bone loss. *J Dent Res* 2011;**90**:143–153.
2. Moreira P, Filho PM, Silva EA *et al.* Effect of periodontal treatment on oral anticoagulation in patients with heart disease. *Rev Port Cardiol* 2007;**26**: 977–989.
3. Moreira PR, Costa JE, Gomez RS, Gollob KJ, Dutra WO. The IL1A (-889) gene polymorphism is associated with chronic periodontal disease in a sample of Brazilian individuals. *J Periodontal Res* 2007;**42**:23–30.
4. Moreira PR, Lima PM, Sathler KO *et al.* Interleukin-6 expression and gene polymorphism are associated with severity of periodontal disease in a sample of Brazilian individuals. *Clin Exp Immunol* 2007;**148**:119–126.
5. Delima AJ, Karatzas S, Amar S, Graves DT. Inflammation and tissue loss caused by periodontal pathogens is reduced by interleukin-1 antagonists. *J Infect Dis* 2002;**186**:511–516.
6. Gao Y, Grassi F, Ryan MR *et al.* IFN-gamma stimulates osteoclast formation and bone loss *in vivo* via antigen-driven

T cell activation. *J Clin Invest* 2007; **117**:122–132.
7. Yang S, Madyastha P, Ries W, Key LL. Characterization of interferon gamma receptors on osteoclasts: effect of interferon gamma on osteoclastic superoxide generation. *J Cell Biochem* 2002;**84**: 645–654.
8. Loe H, Theilade E, Jensen SB. Experimental gingivitis in man. *J Periodontol* 1965;**36**:177–187.
9. Slots J. Primer for antimicrobial periodontal therapy. *J Periodontal Res* 2000;**35**:108–114.
10. Guillaume S, Jaussent I, Jollant F, Rihmer Z, Malafosse A, Courtet P. Suicide attempt characteristics may orientate toward a bipolar disorder in attempters with recurrent depression. *J Affect Disord* 2010;**122**:53–59.
11. Sacre S, Medghalchi M, Gregory B, Brennan F, Williams R. Fluoxetine and citalopram exhibit potent antiinflammatory activity in human and murine models of rheumatoid arthritis and inhibit toll-like receptors. *Arthritis Rheum* 2010;**62**:683–693.
12. Breivik T, Gundersen Y, Myhrer T *et al*. Enhanced susceptibility to periodontitis in an animal model of depression: reversed by chronic treatment with the anti-depressant tianeptine. *J Clin Periodontol* 2006;**33**:469–477.
13. Branco-de-Almeida LS, Franco GC, Castro ML *et al*. Fluoxetine Inhibits Inflammatory Response and Bone Loss in a Rat Model of Ligature-Induced Periodontitis. *J Periodontol* 2011; **83**: 664–671.
14. Yoshioka M, Matsumoto M, Togashi H, Saito H. Effect of conditioned fear stress on dopamine release in the rat prefrontal cortex. *Neurosci Lett* 1996;**209**:201–203.
15. Gomes EP, Aguiar JC, Fonseca-Silva T *et al*. Diazepam reverses the alveolar bone loss and hippocampal interleukin-1beta and interleukin-6 enhanced by conditioned fear stress in ligature-induced periodontal disease in rats. *J Periodontal Res* 2012; doi: 10.1111/j.1600-0765.2012.01515.x
16. Cheer SM, Goa KL. Fluoxetine: a review of its therapeutic potential in the treatment of depression associated with physical illness. *Drugs* 2001;**61**:81–110.
17. Bjork JM, Gaytan O, Patt N, Swann AC, Dafny N. Behavioral tolerance to and withdrawal from multiple fluoxetine administration. *Int J Neurosci* 1998;**93**:163–179.
18. Molina-Hernandez M, Tellez-Alcantara NP, Olivera-Lopez JI, Jaramillo MT. The folic acid combined with 17-beta estradiol produces antidepressant-like actions in ovariectomized rats forced to swim. *Prog Neuropsychopharmacol Biol Psychiatry* 2011;**35**:60–66.
19. Artigas F. 5-HT and antidepressants: new views from microdialysis studies. *Trends Pharmacol Sci* 1993; **14**: 262.
20. Takeuchi H, Yatsugi S, Hatanaka K *et al*. Pharmacological studies on YM992, a novel antidepressant with selective serotonin re-uptake inhibitory and 5-HT2A receptor antagonistic activity. *Eur J Pharmacol* 1997;**329**:27–35.
21. Mar A, Spreekmeester E, Rochford J. Fluoxetine-induced increases in open-field habituation in the olfactory bulbectomized rat depend on test aversiveness but not on anxiety. *Pharmacol Biochem Behav* 2002;**73**:703–712.
22. Hilgert JB, Hugo FN, Bandeira DR, Bozzetti MC. Stress, cortisol, and periodontitis in a population aged 50 years and over. *J Dent Res* 2006;**85**: 324–328.
23. Takada T, Yoshinari N, Sugiishi S, Kawase H, Yamane T, Noguchi T. Effect of restraint stress on the progression of experimental periodontitis in rats. *J Periodontol* 2004;**75**:306–315.
24. Ng SK, Leung WK. A community study on the relationship of dental anxiety with oral health status and oral health-related quality of life. *Community Dent Oral Epidemiol* 2008;**36**:347–356.
25. Rosania AE, Low KG, McCormick CM, Rosania DA. Stress, depression, cortisol, and periodontal disease. *J Periodontol* 2009;**80**:260–266.
26. Godinho EL, Farias LC, Aguiar JC *et al*. No association between periodontal disease and GHQ-12 in a Brazilian Police population. *Medicina Oral, Patología Oral y Cirugía Bucal* 2011;**16**: e857–863.
27. Soletti AC, Gaio EJ, Rosing CK. Effect of neonatal clomipramine in the pathogenesis of ligature-induced periodontitis in Lewis rats. *Acta Odontol Scand* 2009;**67**:94–98.
28. Uzbay TI. Tianeptine: potential influences on neuroplasticity and novel pharmacological effects. *Prog Neuropsychopharmacol Biol Psychiatry* 2008;**32**:915–924.
29. Fanselow MS, Kim JJ. Acquisition of contextual Pavlovian fear conditioning is blocked by application of an NMDA receptor antagonist D,L-2-amino-5-phosphonovaleric acid to the basolateral amygdala. *Behav Neurosci* 1994;**108**: 210–212.
30. Nakajima K, Hamada N, Takahashi Y *et al*. Restraint stress enhances alveolar bone loss in an experimental rat model. *J Periodontal Res* 2006;**41**:527–534.
31. Matsunaga M, Isowa T, Yamakawa K *et al*. Association between perceived happiness levels and peripheral circulating pro-inflammatory cytokine levels in middle-aged adults in Japan. *Neuro Endocrinol Lett* 2011;**32**:458–463.
32. Chiou LJ, Yang YH, Hung HC *et al*. The association of psychosocial factors and smoking with periodontal health in a community population. *J Periodontal Res* 2010;**45**:16–22.
33. Baumann P. Pharmacology and pharmacokinetics of citalopram and other SSRIs. Int Clin. *Psychopharmacol* 1996;**11**(suppl 1):5–11.

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