

Relationship between alcoholism addiction and periodontitis. An *in vivo* study using drinking-in-darkness protocol in rats.

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Abstract: Periodontal disease (PD) has been considered a probable risk factor for several systemic diseases. Among them, PD is presumed to be one of the possible etiologies of chronic illness of the central nervous system. In this context, poor oral health and PD is associated with substance abuse in humans. However, if periodontal lesions can produce addiction is unknown. This paper aims to evaluate the possibility that chronic periodontal injury (CPL) can cause ethanol binge intake in drink-in-darkness (DID) protocol in rats. In CPL group (n=10) experimental damage was done to the periodontal tissue of the second maxillary molar, the control group (n=9) received sham injury. Forty-three days after CPL the intake of ethanol was assessed using several concentrations in DID experiment. During the DID experiment, we observed significant differences between the binge-type consumption of ethanol at the lowest concentration of 10% ($p=0.01$). Differences in consumption of 20% ethanol are observed during a few days ($p=0.04$), and there are no differences in consumption at 40% concentration of ethanol ($p=0.2$). It is concluded that chronic periodontal lesion leads to alcoholism in Wistar rats.

Keywords: ethanol, periodontal lesion, addiction, alveolar bone loss, drink-in-darkness.

INTRODUCTION.

Periodontitis is a chronic, infectious and inflammatory disease that destroys the teeth's supporting tissues and leads to the loss of teeth. The Global Burden of Disease Study, which evaluated several chronic diseases during 1990, 2005 and 2010, cataloged severe periodontitis as the sixth most common head and neck pathology and the second oral illness with the highest prevalence among adults.^{1,2}

Periodontitis is a silent disease, often subclinical. It has become a global public health problem that negatively affects different aspects of people's lives. In advanced stages it leads to the loss of the dental organs, reducing the masticatory function and harming the aesthetics. Periodontitis is one of the leading causes of edentulism and alterations in occlusion that, due to their complexity, imply a higher cost for rehabilitation. On the other hand, its extensive relationship with diverse systemic diseases has been demonstrated.³

Also, comorbidities associated with chronic periodontitis have been reported, such as type 2 diabetes mellitus in bidirectionally:⁴ Chronic periodontitis worsens diabetes mellitus and *vice versa*. It is believed

that both diseases negatively influence the patient's metabolic balance and overall inflammatory burden.^{5,6} The associations between chronic periodontitis and cardiovascular disease, stress, and obesity have also been supported in the literature, but these relationships have not yet been established.⁷

Recent studies have reported an association between alcoholism and alveolar bone loss,⁸ and ethanol consumption has been shown to enhance periodontal inflammatory markers.^{9,10}

To assess the relationship between alcoholism addiction and periodontitis, this paper aims to evaluate the possibility that chronic periodontal injury (CPL) can cause ethanol binge intake in drink-in-darkness (DID) protocol in rats

MATERIALS AND METHODS.

Animals

Nineteen male *Wistar* rats obtained from the FES-I bioterium, weighing 270-350 g/13-15 weeks old at the time of periodontal lesion were used. The rats were randomly divided into two groups: 1) group with chronic periodontal lesion [CPL; n=10] and 2) group with sham surgery for periodontal lesion [Sham; n=9]. The rats were individually housed in polycarbonate boxes with metal grille and had access to water and food (Laboratory Autoclavable Rodent Diet 5010) ad libitum. The rats adapted to the photoperiod inverted cycle 12:12 L: D.

All procedures were carried out according to the rules of the Ethics Committee of the Faculty of Higher Studies Iztacala of the National Autonomous University of Mexico (Office: CE/FESI/022017/1105).

Chronic periodontal lesion (CPL)

We used a technique previously described.¹¹ Animals were anesthetized with ketamine (80-100mg/kg IP) and xylazine. (10-12.5mg/kg IP). 5-0 silk suture was attached around the right second maxillary molar and was tied gently to avoid damage to the periodontal tissue.

The ligature was checked every two days to ensure subgingival placement. The second contralateral maxillary molar was left intact. For the control group with sham surgery (Sham), the rats were anesthetized, but no ligation

was performed. The rats were left during recovery in the boxes in which they were housed.

Measurement of alveolar bone loss

For evaluation of alveolar bone loss, the removed jaws were boiled in water for 10 min. After dissection of the soft tissues, the jaws were brushed and bleached. The jaws were stained with 0.5% eosin and 1% methylene blue in order to visually distinguish between the alveolar bone and the tooth. The image of the alveolar bone height was captured using a stereomicroscope (Nikon SMZ-745T, Minato, Tokyo, Japan) at a magnification of 30x.

For the evaluation of alveolar bone loss, the distance from the cemento-enamel junction to the alveolar bone crest was measured at the points described by Abe *et al.*¹² Three sites were measured in the lingual part and three points in the buccal part. The mesio-palatine (MP) or mesio-buccal (MB) cusp, the palatal (GP) or buccal groove (GB), and the disto-palatine (DP) or disto-buccal (DB) cusp to the alveolar crest (Figure 1 B and C). Bone measurements were performed three times by two evaluators in a random and blind protocol way.

Drink in Darkness

For alcohol consumption, the DID model previously described was employed.¹³ Each animal was individually housed. This model consists in habituating the rats to a reverse light cycle 12:12 L:D and replacing the water bottle by a container with ethanol. Exposure to ethanol was carried out for 3 hours, starting at 3 hours of the dark cycle.

The experiment was started at the post-surgical day 52 and was reported after 3 to 5 days of an experimental baseline with water, in order to condition the rats to the DID model. After the baseline days, a solution of 10% ethanol (Sigma- Aldrich, St Louis, MO) *v/v* in water was initially used, after that, the concentration of ethanol was raised to 20 and 40% *v/v* in water. Each concentration was maintained for 4 to 5 days. Rats were never deprived of water or food, with the exception of the three hours during which the experiment was carried out.

Both the intake and the weight of the animals was recorded daily. Volume was calculated using $V_c = ((W_i - W_f) - W_i) / \delta$ where V_c is volume consumed. W_i is the bottle weight at the beginning of the session. W_f is the bottle weight at the end of the session. W_i is the average weight of

the leaked ethanol or water solution, and δ is the density for water (1 g/ml) or 20% ethanol solution (0.97336 g/ml) at 25–30 °C. The ethanol intake in g/kg was calculated according to $I=(V_c \times \delta_c)/W_m$; where I is ethanol intake in g/kg. V_c is the volume consumed of 10, 20 and 40% ethanol, δ_c is the density of ethanol (0.789 g/ml), and W_m is the weight of the mouse in kg.

For every session, the volume leaked was measured using a pair of dummy bottles placed on empty cages. The average leak volumes for the ethanol and water solutions were subtracted from each ethanol and water volume measurement, respectively (see Figure 1 A for experimental protocol).

Data analysis

The mean±SE values were used for statistical comparison. An unpaired *t*-test for alveolar bone loss and two-way-ANOVA for the drinking-in-darkness protocol was made. The significance for the statistical test was $p<0.05$.

RESULTS.

Figure 1 (D, E, and F) shows the evaluation of bone loss for both groups. As previously described in the methods section, we took six measures for determining bone loss (Figure 1 C: DB, BG, MB, DP, PG, and MP). In several tests, the CPL group showed significant bone loss ($p<0.05$, see table 1).

Figure 1. A. Experimental chronogram since day 0 (surgery day). B. Periodontal injury (PI). To induce bone loss, we used the technique described by Abe & Hajishenga 2013. Periodontal ligation was made in the second maxillary molar. C and D. Measurements of alveolar bone loss. For the evaluation of alveolar bone loss, the points described by Abe & Hajishenga were used. Three sites were measured in the lingual part and three points in the buccal part: the mesio-palatine (MP) or mesio-buccal cusp (MB), the palatal (PG) or buccal groove (BG), and the disto-palatine (DP) or distobuccal cusp (DB) to the alveolar crest. E. Measurements of alveolar bone loss. Bone loss is significantly higher, at all points of reference, in the PI group than in the sham group ($*p<0.05$). F. Comparison photographs between the alveolar bone of a jaw of the PI group and the sham group, seen from buccal and lingual side. It is noticeable that PI produces a higher alveolar bone loss (black arrows).

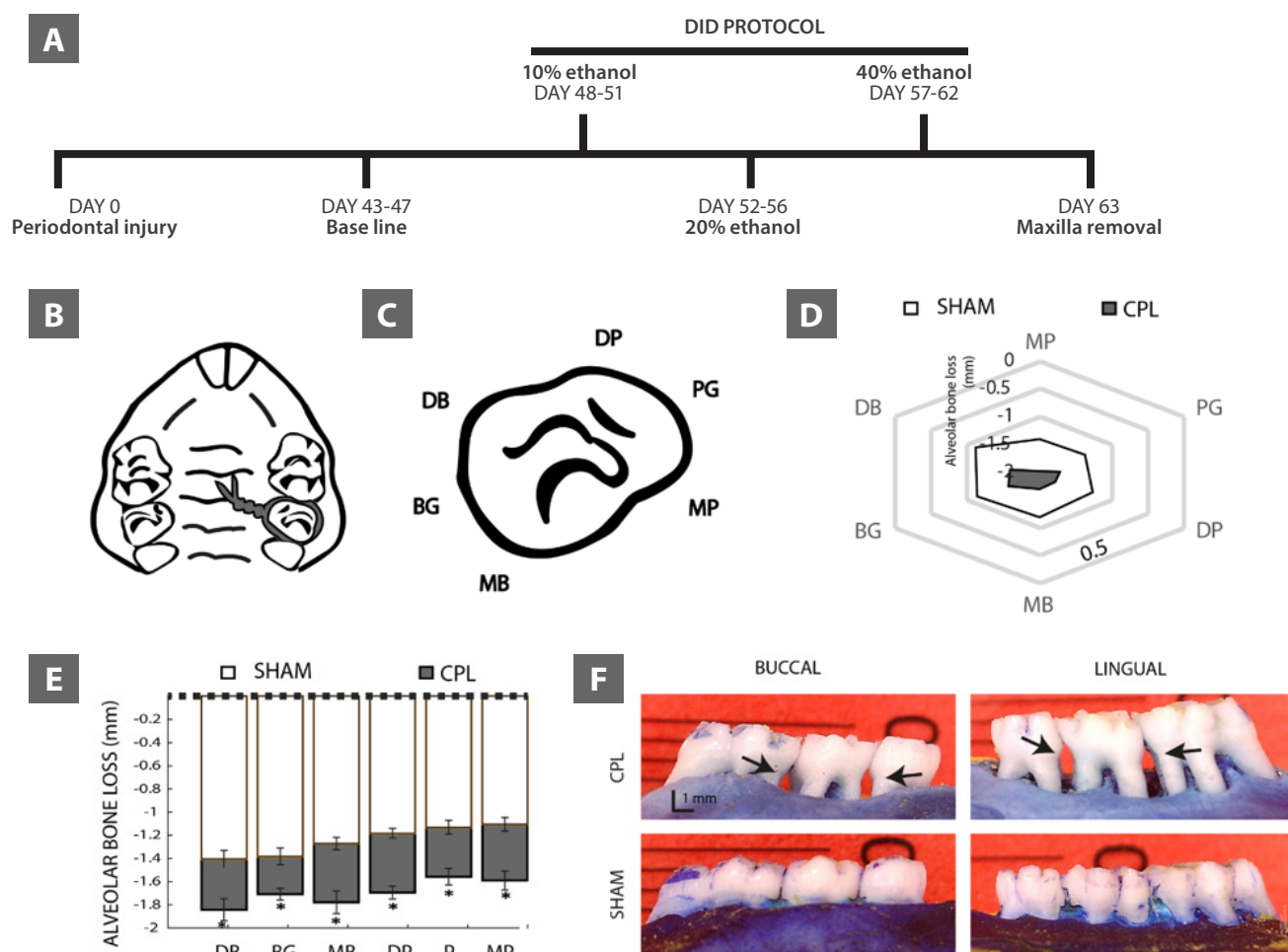
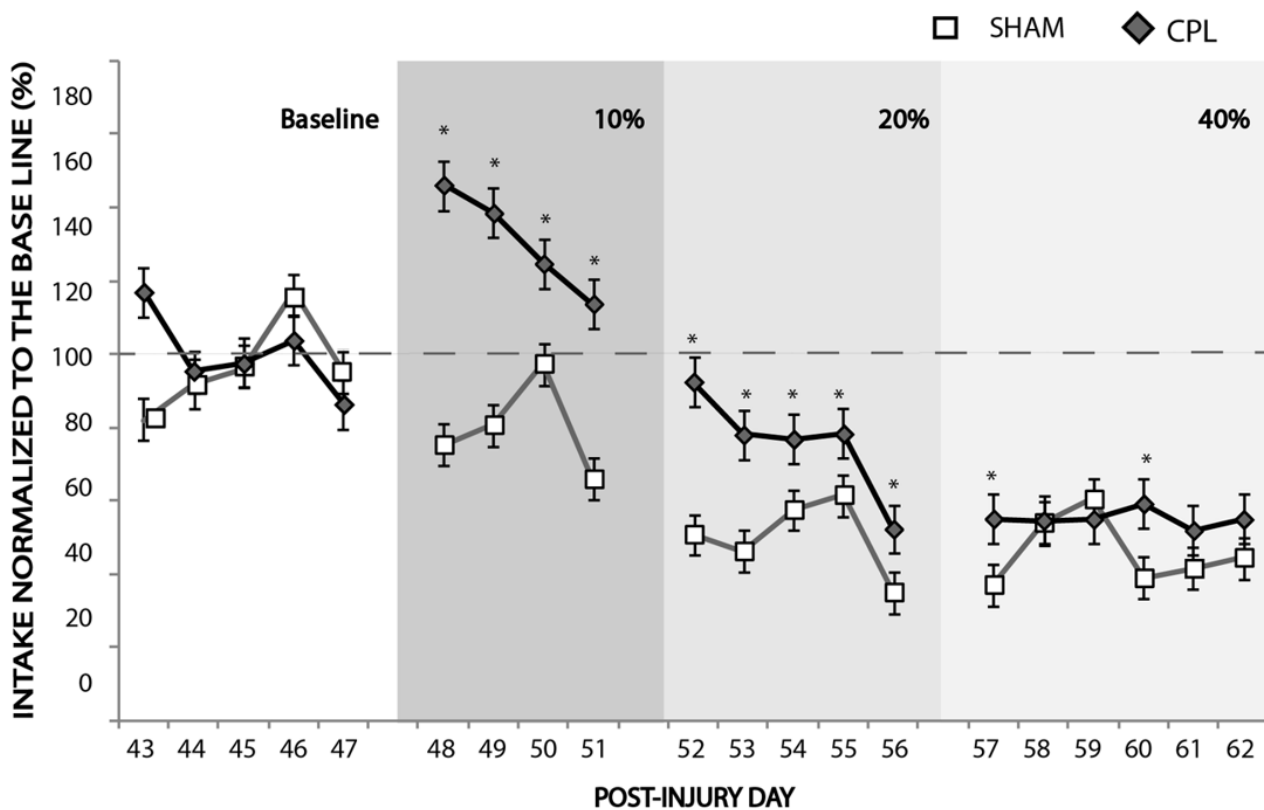


Table 1. Statistical comparison of bone loss between groups.

Anatomical Reference	CPL group	Sham	t-test
DB	M=-1.59 SD±0.06	M=-1.10 SD±0.03	t(16)=1.74; p=0.0001
BG	M=-1.15 SD±0.05	M=-1.3 SD±0.03	t(17)=1.73; p=0.0002
MB	M=-1.6 SD±0.03	M=-1.18 SD±0.01	t(16)=1.74; p<0.001
DP	M=-1.7 SD±0.09	M=-1.2 SD±0.02	t(14)=1.76; p=0.0004
PG	M=-1.7 SD±0.04	M=-1.3 SD±0.02	t(16)=1.73; p=0.002
MP	M=-1.8 SD±0.09	M=-1.4 SD±0.05	t(16)=1.7; p=0.0023

Figure 2. Intake of ethanol normalized to the baseline (BL), in concentrations of 10%, 20% and 40% using the DID model. The dashed line determines the water consumption during the BL period. It is clear that the CPL group had a higher consumption of ethanol at both 10% and 20%. There is a marked increase of 10% ethanol consumption in CPL group (two-way ANOVA: F (1,3) = 6.009; p=0.01), reaching consumption values even higher than those obtained on baseline days.



During the DID experiment, we observed significant differences in the binge-type consumption of ethanol at the lowest concentration (10%). (Figure 2) (two-way ANOVA: F (1,3) = 6, p=0.01).

Differences in consumption of 20% ethanol are observed during a few days (two-way ANOVA: F (1,4) = 2.5, p=0.04), and there are no differences in the consumption of 40% ethanol (two-way ANOVA: F (1,5) = 1.13, p=0.2).

DISCUSSION.

Our results show for the first time that a chronic periodontal injury can induce the development of an addictive behavior such as alcoholism. Recent studies have suggested a close relationship between periodontal disease and systemic diseases such as cardiovascular disease, type 2 diabetes, rheumatoid arthritis, osteoporosis, Parkinson's disease, Alzheimer's disease, psoriasis, and respiratory infections.¹⁴

Various studies have suggested a relationship between addictions and periodontal disease.¹⁴⁻¹⁸ However, it has not been previously shown that CPL leads to addiction-like behavior. Much is still unknown about the mechanisms behind this correlation. It is likely that the increase in ethanol consumption after periodontal injury is due to changes in ethanol metabolism since changes in liver tissue occur due to CPL.^{19,20}

Periodontal disease has also been linked to neurodegenerative diseases such as Alzheimer's and Parkinson's disease based on the inflammatory response in microglia and leptomeningeal cells.^{14,21} However, there is still much to be clarified about the molecular mechanisms that induce changes at the level of the central nervous system.

A bidirectional and robust interaction between the inflammatory process and different comorbidities has been established.^{14,22} The increase of neuropeptides in the trigeminal ganglion after CPL could explain changes in the nervous system that induce binge intake behavior. Specifically, the activation of the so-called "neuro-immune axis" results in neurogenic responses that lead to neuroadaptations at a different level in the central nervous system.²³

There is no previous evidence, before the present study, for the induction of binge-type ethanol consumption. This result is highly innovative as it relates orofacial lesions to ailments in the central nervous system. It is exceptionally novel that during the first day of consumption of 10% ethanol, the CPL group shows a higher consumption compared to the control. Consumption decreases with time due to post-ingestion effects, and differences are not observed for 40% ethanol.

These results are comparable to those obtained when there is chronic neuropathic pain.²⁴ However, our results show a more radical difference between groups than in chronic neuropathic pain. Therefore, this finding is a

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precedent to many experiments that must be performed in order to understand this relationship.

Changes in endocannabinoid signaling occur in drug abuse due to inflammation.²⁵ Are these changes causally or coincidentally associated with addiction? There is a need to elucidate the contribution of neuroinflammation to the behavioral and neuroprotective effects of cannabinoids on drug addiction. The increased ethanol intake after CPL is the first step in this approach.

Alcohol and other drugs of abuse have significant impacts on the neuroimmune system. Neuroimmune factors mediate neuroinflammation and modulate a wide range of brain function including neuronal activity, endocrine function, and the development of nervous system.

These neuromodulator properties of the neuroimmune system, have an essential role in neuroinflammation, mediating functional and behavioral brain changes contributing to addiction. Consumption of alcohol and other drugs, like opiates, marijuana, methamphetamine, and cocaine, induces neuroimmune signaling that increases the dependence-like behavior, but this modulation is bidirectional.²⁶

The binge intake of ethanol is a challenging behavior in experimental conditions. The relationship between CPL and higher ethanol consumption indicates both bilateral and a robust interaction between pathologies.

However, there still remain many experiments to be done in order to establish the relationship between CPL and drug abuse. This article shows behavioral evidence of the development of ethanol binge intake induced by CPL as a starting point for research in this matter.

CONCLUSION.

Chronic periodontal lesion leads to alcoholism in Wistar rats.

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