Electronic Physician (ISSN: 2008-5842) http://www.ephysician.ir
September 2015, Volume: 7, Issue: 5, Pages: 1290-1295, DOI: 10.14661/1290

# Diacerein: A potential therapeutic drug for the management of experimental periodontitis in rats

Basma Mostafa Zaki[1], Enji Ahmed Mahmoud[2], Azza Ahmed Aly[3]

[1] Surgery and Oral Medicine Department, Oral and Dental Research Division, National Research Centre, Cairo, Egypt
[2] Oral Medicine and Periodontology Department, Faculty of Oral and Dental Medicine, Cairo University, Cairo, Egypt
[3] Clinical & Chemical Pathology Department, Medical Division, National Research Centre, Cairo, Egypt

**Type of article:** Original

**Abstract**

**Introduction:** Knowledge about the pathogenic process in the progression of periodontal disease indicates that the central cause of periodontal disease is the loss of a healthy balance between microbial virulence factors and the host's inflammatory response. The aim of this study was to evaluate the potential effectiveness of diacerein as an anti-inflammatory drug in the management of experimental periodontitis in rats.

**Methods:** The study included 60 albino rats that were divided into two groups. Periodontitis was induced in both groups. The drug group received systemic administration of diacerein, and the control group received a placebo. IL-1ß was measured two weeks after the induction of periodontitis and before the administration of the drug (baseline measurement), and it was measured again at the end of two and end of four weeks after scaling and root planning and diacerein administration.

**Results:** The results indicated that there was a significant decrease in IL-1ß level in both groups. For the control group, there were significant decreases of the IL-1ß values from the baseline to two weeks and also from the baseline to four weeks, with p-values of 0.0001 for both comparisons. The same results were obtained for the drug group.

**Conclusion:** It was concluded that it is likely that diacerein may play a therapeutic role as a potent anti-inflammatory drug in the management of periodontitis.

**Keywords:** diacerein, experimental, periodontitis, rats

## 1. Introduction

Periodontal diseases are chronic inflammatory diseases that destroy the tooth-supporting structures, including bone, cementum, and periodontal ligament. They are one of the most prevalent forms of pathological bone conditions in humans, and they can adversely affect the systemic health of patients (1). Pathogenic bacteria initiate the disease by accumulating in the plaque biofilm, and periodontal tissues are destroyed by the host's inflammatory immune response. This issue has been addressed is previous studies (2). The host's responses to these microbial challenges initiate a local immune response that results in the recruitment of inflammatory cells and the release of various inflammatory mediators, including pro-inflammatory cytokines (i.e., IL-1, IL-6, TNFα) and the liberation of lytic enzymes, i.e., matrix metalloproteinases (MMPs) and prostaglandins (PG). These responses ultimately result in the loss of connective tissue and resorption of bone due to the activation of osteoclasts (3). Inflammatory cell infiltration, the production of enzymes that destroy connective tissue, and the resorption of alveolar bone occur when primary mediators, such as interleukin-1 (IL-1), are produced. Then, these primary mediators, in turn, stimulate the production of secondary mediators that amplify the inflammatory response (1, 2). IL-1 is considered to be involved in the pathogenic mechanisms that lead to the breakdown of periodontal tissues and structures (4), resulting in

**Corresponding author:**
Assistant Professor Dr. Basma Mostafa Zaki, Oral and Dental Research Division, National Research Center, 33 El Bohouth Street, 12311 Dokki, Cairo, Egypt. Tel: +20.237623537, Mobile: +20.1224901019, Fax: +20.233387803, E-mail: boshta@hotmail.com

Received: July 04, 2015, Accepted: September 01, 2015, Published: September 16, 2015
iThenticate screening: August 04, 2015, English editing: August 26, 2015, Quality control: September 02, 2015

several adverse biological effects, including inflammatory, metabolic, physiologic, hematopoietic, and immunologic effects (5, 6). In addition to surgical therapy for periodontal disease, an approach that potentially can be used to prevent and/or treat its occurrence is the regulation of the interactions between the immune and inflammatory responses to the disease (2). The destruction of periodontal tissue is initiated by various host factors, and these factors have been investigated to determine their potential for arresting the progression of the disease (7). Consequently, the use of the anti-inflammatory mediator therapy, in conjunction with anti-biofilm treatments, may prove to be advantageous in counteracting and attenuating the progression of the disease (8). Diacerein, a purified derivative of anthraquinone, was first identified as a constituent of plants that have anti-inflammatory and analgesic activities (9). The diverse functions of diacerein were investigated several years ago, but its beneficial impact has been related predominantly to the treatment of osteoarthritis (10-12). In many aspects, the nature of periodontal disease is quite similar to arthritis. Thus, it is likely that diacerein could serve a therapeutic role in the prevention and treatment of periodontal disease (3). To date, there no studies have been conducted to investigate the potential role of diacerein in the treatment of periodontal disease. So, the aim of this study was to evaluate the effect of diacerein in the management of ligature-induced experimental periodontitis in rats.

## 2. Material and Methods

### *2.1. Research design*

The research design was an experimental study. The study protocol was approved by the Medical Research Ethical Committee of the National Research Center, Cairo, Egypt.

### *2.2. Animals*

This study was conducted on 60 male albino rats (100-120 g). The animals were kept in plastic cages with access to food and water ad libitium in a temperature-controlled room with a standard of a 12/12 hour light/dark illumination cycle. The cages were kept under hygienic conditions and away from any source of chemical contamination according to the ethical protocol for housing animals. Before the induction of periodontitis, all of the animals were allowed to acclimatize to the laboratory environment for a period of five days.

### *2.3. Experimental Design*

The animals were divided randomly into two groups, each composed of thirty rats: 1) Group one (n = 30) was the control group in which experimental periodontitis was induced, and a placebo was administered systemically, 2) Group two (n = 30) was the drug group in which experimental periodontitis was induced, and diacerein was administered systemically.

### *2.4. Protocol of the Induction of Experimental Periodontal Disease*

The rats in the control and drug groups were anesthetized via intra-muscular injection of ketamine© (0.4 ml/kg) and xylazine© (0.2 ml/kg). Ligatures (4/0 sterile silk©) were tied on the necks of the mandibular first molars on both sides in the sub-marginal area in all of the animals in both groups. The ligatures were kept in position to promote the accumulation of microbial dental plaque and inflammation, and they were inspected often to make sure that they had not become loose or disconnected (13, 14). These conditions eventually resulted in damaging the periodontal tissues and structures, thereby inducing experimental periodontitis. The appearance of signs of inflammation, such as redness, edema, and bleeding occurred after seven days. After two weeks, in addition to the signs of inflammation, examinations using a periodontal probe indicated the loss of gingival tissue, denoting the establishment of periodontal disease. Then, the ligatures were removed from all of the animals, and a blood sample was taken from all animals in both groups to measure the level of IL-1ß. These measurements were used later as the baseline measurements for both groups. After the removal of the ligatures, scaling and root planing (SRP) was initiated in both groups for the mandibular molars using manual #13–14 mini five curettes (HuFriedy Co. Inc., Chicago, IL, USA) by performing ten distal–mesial traction movements in the buccal and lingual aspects. The interproximal areas were scaled with the same curettes using cervico-occlusal traction movements. After SRP, the submarginal areas were irrigated with 1 ml of saline solution. Note that SRP was performed by the same experienced operator in both groups (15). After experimental periodontitis was established, diacerein (100 mg/kg/day) was administered orally to the drug group (3). A computer-generated table was used to allocate the animals in group one and two to the SRP, drug treatment, and the measurement of IL- 1ß. For better standardization, animal one was the first choice, followed by two and three, respectively. Blood samples for IL-1ß measurement were taken at three time intervals from both groups, i.e., 1) two weeks after the induction of periodontitis before SRP and the administration of diacerein (baseline samples), 2) at two weeks after SRP and diacerein administration, and 3) at four weeks after SRP and diacerein administration.

*2.5. Laboratory Investigations*

After the blood samples collected, the samples were centrifuged to separate the serum, which was stored at -20 0C until the assay was to be conducted. A Rat IL-1ß ELISA kit (Enzyme linked immunosorbent assay kit) was obtained from Glory Science Co., Ltd., (Del Rio, TX, USA), and the kit was used to measure IL-1ß according to the manufacturer's recommendations. The concentrations of IL-1ß were reported as µg/L, and the animals were euthanized by ether anesthesia followed by cervical dislocation.

*2.6. Statistical Analysis*

Data management and analysis were performed using SAS version 8.2 and SPSS version 17. Changes of the level of IL-1ß within the test animals were determined using repeated measure ANOVA test. The Bonferroni post hoc test was conducted to determine the differences between the levels of IL-1ß for the different time periods. The independent t-test was performed to determine the difference in the level of IL-1ß during the different time periods in the study. All p-values are two-sided, and p-values < 0.05 were considered significant (16).

**3. Results**

After one week of inducing experimental periodontitis, there were clinical signs of inflammation, including edema, redness, and bleeding. By the end of the second week, the loss of clinical attachment had occurred. Other than the deaths of one rat in each of the two groups before SRP and drug therapy were initiated, there were no other losses in the animals in the experiment. Increased urination and a change in the color of the rats' urine (to yellowish brown) were observed. The samples were analyzed to determine the baseline levels of IL-1ß (two weeks after the induction of periodontitis and before SRP and drug therapy), and they were analyzed again two and four weeks after SRP and the administration of the drug therapy. The means and standard deviation (SD) values of IL-1ß levels for the two groups at baseline, after two and four weeks are shown in Table 1. Repeated ANOVA measurements were conducted to determine the within-subject changes of the IL-1ß levels at baseline, after two and four weeks, as shown in Table 2. For both the control and drug groups, the repeated ANOVA measurements showed a statistically significant decrease within subjects, since the p-value was 0.0001 for both groups.

**Table 1.** Mean ± SD of IL-1ß levels at baseline, after two weeks, and after four weeks for both groups (control and drug groups)

| Study Time Periods | Control group | Drug group |
|---|---|---|
| | Mean ± SD | Mean ± SD |
| Baseline | 10.22 ± 0.34 | 10.27 ± 0.34 |
| Two weeks | 8.81 ± 0.6 | 8.36 ± 0.55 |
| Four weeks | 7.68 ± 0.64 | 6.95 ± 0.35 |

**Table 2.** Repeated measurement ANOVA of IL-1ß levels at baseline, two weeks, and four weeks for control and drug groups

| Group | p-value | Significance |
|---|---|---|
| Control group | 0.0001 | S* |
| Drug group | 0.0001 | S |

*significant

The Bonferroni post hoc test was conducted to determine the differences between the IL-1ß values at baseline, after two and four weeks. For the control group, there was a significant decrease of the IL-1ß values from the baseline to the end of two weeks and to the end of four weeks since the p-value was 0.0001 for the comparisons. In addition, there was a significant decrease of IL-1ß values from two to four weeks (p = 0.0001) (Table 3). For the drug group, there was a significant decrease of IL-1ß levels from baseline to two weeks (p = 0.0001) and also from baseline to four weeks with the same p-value as before. In addition, there was a significant decrease of IL-1ß levels from two to four weeks (p = 0.0001) (Table 3). The independent t-test was performed to determine the difference in IL-1ß levels at baseline, at two and four weeks between the control and drug groups. There was no significant difference in either group in IL-1ß levels at baseline, where the t-value was 0.31, and p-value was 0.76. Also, there was no significant difference between the groups in IL-1ß levels at two weeks (t = 1.84, p = 0.08). However, there was a significant difference between the groups in IL-1ß levels at four weeks (t = 3.29, p = 0.004) (Table 4).

**Table 3.** Bonferroni post hoc test of the of IL-1ß values at baseline, at two weeks, and at four weeks for the control and drug group

| Comparison | | Mean Difference | p-value | Significance |
|---|---|---|---|---|
| Control group | At baseline vs. at two weeks | 1.4 | 0.0001 | S* |
| | At baseline vs. at four weeks | 2.54 | 0.0001 | S |
| | At 2 weeks vs. at four weeks | 1.13 | 0.0001 | S |
| Drug group | At baseline vs. at two weeks | 1.9 | 0.0001 | S* |
| | At baseline vs. at four weeks | 3.31 | 0.0001 | S |
| | At 2 weeks vs. at four weeks | 1.4 | 0.0001 | S |

*significant

**Table 4.** Independent t-test of IL- 1ß levels at baseline, at two weeks, and at one month between the control and drug groups

| IL- 1ß levels | Baseline | Two weeks | Four weeks |
|---|---|---|---|
| Mean difference | 0.04 | 0.45 | 0.72 |
| t-value | 0.31 | 1.84 | 3.29 |
| p-value | 0.76 | 0.08 | 0.004 |
| Significance | NS* | NS | S** |

*Not significant, **Significant

**4. Discussion**

Periodontal disease is one of the most prevalent diseases associated with alveolar bone resorption and frequent loss of teeth. The process of periodontal disease is initiated mainly by bacteria. It has been proposed that bacterial stimulation induces a host response that leads to the formation of periodontal pockets, the loss of clinical attachment, differentiation of the osteoclasts, and resorption of bone. It was documented that IL-1ß has an essential role in the process that leads to periodontal disease process in that it is an important factor in the recruitment of inflammatory cells and the loss of bone (3). Much attention has been focused on drugs that are not primarily aimed at the palliation of disease symptoms, but modulate the host immune response to the causative factor. These host modifying agents are aimed at modifying the pathobiologic and pathoanatomic changes in tissues/cells, either through inhibition of different cytokines or by stimulating anabolic activities (17). Diacerein is one of the drugs that have IL-1 inhibitory activity, and it was developed mainly for the treatment of osteoarthritis. It has been reported that it also has exhibited analgesic effects and antipyretic activities in animal models. Cytokines, mainly IL-1ß, and matrix metalloproteinases (collagenase and stromelysin) are involved in the degradation of cartilage, and both diacerein and its active metabolite rhein effectively inhibit the synthesis of cytokines in vitro. In vitro, diacerein has been shown to inhibit the activity of IL-1ß by inhibiting the synthesis of cytokines in synovium and chondrocytes and by reducing the level of the bioactivity of the IL-1 receptor in these cells (18). The dose of diacerein (100 mg/kg/day) that was selected for use in our study was based on the medical hypothesis proposed by Ren-Yeoung et al. (3). The results indicated that there was a significant decrease in the level of IL-1ß in both groups during the different study periods. Its reduction in the drug group was explained on the basis that diacerein reduced the association of IL-1 receptors to form heterodimer complexes, thereby repressing IL-1 and its related downstream events, including inducing the synthesis of nitric oxide synthase (iNOS), stromelysin-1, collagenase, matrix metalloproteinases-1, -3, -9, and -13, and their activities (17, 19). In addition, diacerein impairs the activation of IL-1 due to the inhibition of the IL-1-converting enzyme (19, 20). The significant decrease in the levels of IL-1ß was more obvious in the drug group than in the control group through all study periods. Pelletier et al. (18) reported similar findings, except the change that occurred between two-week and four-week periods in the drug group. However, they also concluded that diacerein is a slow-acting agent, the effects of which appeared four weeks after the treatment began, which also was the case in our study. This finding also has been documented by several other studies (18, 20).

**5. Conclusions**

Even with the limitations of the small sample size and short study periods in this study, it was evident that diacerein, as a potent anti-inflammatory drug, can provide a therapeutic role in the management of periodontal disease. Based on our findings and observations, we concluded that diacerein inhibited the production of inflammatory cytokines/mediators and regulated the signaling pathways, thereby ameliorating inflammation-induced periodontal destruction. Thus, the results indicated that diacerein could be of significant value in treating periodontal disease because of its proven ability to reduce periodontal inflammation.

**Acknowledgments:**
The authors deeply thanks the National Research Centre for supporting the research.

**Conflict of Interest:**
There is no conflict of interest to be declared.

**Authors' contributions:**
All authors contributed to this project and article equally. All authors read and approved the final manuscript.

**References**

1) Bartold, P.M., Cantley, M.D. and Haynes, D.R. Mechanisms and control of pathologic bone loss in periodontitis. Periodontol 2000, 2000, 53, 55–69. doi: 10.1111/j.1600-0757.2010.00347.x, PMid: 20403105
2) Amano, A. Host-parasite interactions in periodontitis: subgingival infection and host sensing. Periodontal 2000, 2000, 52, 7–11. doi: 10.1111/j.1600-0757.2009.00328.x, PMid: 20017792
3) Ren-Yeong, H., Sheng-Hua Lu, Kwan-Wei Su, et al. Diacerein: A potential therapeutic drug for periodontal disease. Medical Hypotheses, 2012, 79, 165–77. doi: 10.1016/j.mehy.2012.04.024, PMid: 22583561
4) Dinarello, C.A. IL-1: discoveries, controversies and future directions. Eur. J. Immunol, 2010, 40, 599–606. doi: 10.1002/eji.201040319, PMid: 20201008
5) Graves, D.T. and Cochran, D. The contribution of interleukin-1 and tumor necrosis factor to periodontal tissue destruction. J. Periodontol, 2003, 74, 391–401. doi: 10.1902/jop.2003.74.3.391, PMid: 12710761
6) Cochran, D.L. Inflammation and bone loss in periodontal disease. J. Periodontol, 2008, 79, 1569–76. doi: 10.1902/jop.2008.080233, PMid: 18673012
7) Gaspersic, R., Stiblar-Martincic, D., Osredkar, J. and Skaleric, U. Influence of subcutaneous administration of recombinant TNF-alpha on ligature-induced periodontitis in rats. J. Periodontal Res, 2003, 8, 198–203. doi: 10.1034/j.1600-0765.2003.01395.x
8) Waykole, Y.P., Doiphode, S.S., Rakhewar, P.S. and Mhaske, M. Anticytokine therapy for periodontal diseases: where are we now? J. Indian Soc. Periodontol, 2009, 13, 64–8. doi: 10.4103/0972-124X.55837, PMid: 20407652 PMCid: PMC2847126
9) Hunter, D.J. and Wise, B. Review diacerein is more effective than placebo and is as effective as NSAIDs for knee and hip osteoarthritis. Evid Based Med, 2007, 12, 74. doi: 10.1136/ebm.12.3.74, PMid: 17537882
10) Pujol, J.P., Felisaz, N., Boumediene, K., et al. Effects of diacerein on biosynthesis activities of chondrocytes in culture. Biorheology, 2000, 37, 177–84. PMid: 10912190
11) Brandt, K.D. Studies in animal models of osteoarthritis as predictors of a structure-modifying effect of diacerin in humans with osteoarthritis. Biorheology, 2008, 43,589–594.
12) Alvarez-Soria, M.A., Herrero-Beaumont, G., Sanchez-Pernaute, O., Bellido, M. and Largo, R. Diacerein has a weak effect on the catabolic pathway of human osteoarthritis synovial fibroblast – comparison to its effects on osteoarthritic chondrocytes. Rheumatology (Oxford), 2008, 47, 627–33. doi: 10.1093/rheumatology/ken116, PMid: 18375401
13) Page, R.C. and Schroeder, H.E. Periodontitis in Man and Other Animals. New York: Karger, 1982, 58,106.
14) Nociti, F.H., Nogueira-Filho, M.T., Primo, M.A., Machado, V., Tramontina, S.P., Barros, A. and Sallum, E.A. The influence of nicotine on the bone loss rate in ligature-induced periodontitis. A histometric study in rats. J. Periodontol, 2000, 71, 1460-4. doi: 10.1902/jop.2000.71.9.1460, PMid: 11022776
15) Nasry, S.A., Zaki, B.M., Elkarargy, A., Aly, A.A. and Omer, M.G. IL-17 Levels in Diabetic and Non-diabetic Experimental Periodontitis: a Pilot Study. Australian Journal of Basic and Applied Sciences, 2011, 5, 1466-1474.
16) Dawson, B. and Trapp, R.G. Basic and Clinical Biostatistics, New York: Lange Medical Books-McGraw Hill, 2001.
17) Tamura, T., Kosaka, N., Ishiwa, J., Sato, T., Nagase, H. and Rhein, Ito A. An active metabolite of diacerein, down-regulates the production of pro-matrix metalloproteinases-1, -3, -9 and -13 and up-regulates the production of tissue inhibitor of metalloproteinase-1 in cultured rabbit articular chondrocytes. Osteoarthritis Cartilage, 2001, 9, 257–63. doi: 10.1053/joca.2000.0383, PMid: 11300749
18) Pelletier, J., Yaron, M., Haraoui, B., Cohen, P., Nahir, M.A., Choquette, D., Wigler, I., Rosner, I.A. and Beaulieu, A.D. Efficacy and safety of diacerein in osteoarthritis of the knee: A double-blind, placebo-

controlled trial. Arthritis & Rheumatism, 2000, 43, 2339–48. doi: 10.1002/1529-0131(200010)43:10<2339::AID-ANR23>3.0.CO;2-P
19) Verbruggen, G. Chondro-protective drugs in degenerative joint diseases. Rheumatology (Oxford), 2006, 45, 129–38. doi: 10.1093/rheumatology/kei171, PMid: 16278282
20) Moldovan, F., Pelletier, J.P., Jolicoeur, F.C., Cloutier, J.M. and Martel-Pelletier, J. Diacerein and rhein reduce the ICE-induced IL-1beta and IL-18 activation in human osteoarthritic cartilage. Osteoarthritis Cartilage, 2000, 8, 186–96. doi: 10.1053/joca.1999.0289, PMid: 10806046