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Systematic Review

Ligature-induced Periodontitis in Rats and Mice: A Systematic Review on Therapeutic Targets

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ABSTRACT

Introduction: *Periodontitis is currently managed through plague removal, both mechanically and chemically. However, limitation to reach the base of deep pockets often results in incomplete protection from periodontal pathogens. Therefore, new treatment modalities are emerging with the aim of controlling the inflammation-associated surrounding tissue damage. There have been strong evidences of preclinical studies on the mechanisms of protection, which may bring insights into the potential therapeutic target(s). We performed a systematic review to identify the therapeutic target(s) in rodent model of periodontitis.* **Method:** *The study protocol was registered in the International Prospective Register of Systematic Reviews (PROSPERO), ID: CRD42022355578. We searched PubMed, Scopus, and Wiley databases from 2017 to 2021. This systematic review was conducted in accordance with the PRISMA guidelines and studies were selected based on predefined inclusion criteria. We developed a diagram to show the cellular location of the targets. These targets were summarized based on their effects on the outcome determinants such as inflammatory response and the extend of alveolar bone and/or periodontal attachment loss. Decision matrix analysis was used to identify which target(s) hold the most potential.* **Result:** *Eleven studies met the inclusion criteria. Result showed that protection in early and late phases of periodontitis involve targets such as transcription factor, protein and lipid kinases, transmembrane receptor, and extracellular protein. Further analysis showed that transcription factor is the most investigated target with the most reported protective outcomes.* **Conclusion:** *Protective targets in periodontitis involve different cellular levels. Transcription factor appears as the most potential therapeutic target in rodent model of periodontitis.*

Keywords: *Periodontitis, Systematic review, Protective target, Rodent model.*

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INTRODUCTION

Periodontitis is a chronic inflammatory disease of oral cavity. It is characterized by both inflammation and bone loss (Graves et al. 2011). The bone loss may be due to the direct effect of inflammation (Hardy and Cooper, 2009), where activation of inflammatory cascade is believed to affect the integrity of the periodontium, resulting in progressive loss of gingival tissue, periodontal ligament, and alveolar bone (Könönen et al. 2019).

Clinical features of periodontitis include periodontal probing depth of more than 3 mm, loss of attachment, furcation involvement, bleeding on probing, recession, bone loss of more than one third of root length and tooth mobility (Serio, 2014). The key etiological element for these conditions is the bacterial biofilm (Sima and Van Dyke, 2016). Colonization of the bacteria within the complex dental biofilm triggers the host innate immune response, followed by signs of acute inflammation (Mehrotra and Singh, 2020). The persistent insults then activate adaptive immunity, causing inevitable destruction to the periodontium (Mehrotra and Singh, 2020; Silva et al. 2015). Clinically, periodontitis is managed through plague removal, both mechanically and chemically. The standard treatment approach is scaling and root planning, and the efficiency of mechanical debridement is enhanced with the prescription of antibiotics, antiseptic mouthwash, and anti-inflammatory agents (Krayer et al. 2010; Bogdanovska et al. 2012). However, limitation such as inability to reach the base of deep pockets often results in incomplete protection from the periodontal pathogen activities (Krayer et al. 2010).

In preclinical research of periodontitis, new treatment modalities are emerging with the aim of controlling the inflammation-associated surrounding tissue damage. Researchers are investigating novel strategies that may provide the next generation of adjunctive therapeutics that protect the periodontium. In rat model of ligature-induced periodontitis, treatment with magnolol for example, showed significant reduction of inflammatory markers in inflamed gingival tissue, which was further corroborated with restoration of the structure of affected bone (Lu et al. 2013). The preclinical studies of periodontitis in animal model are done either on rats or mice, primarily due to their periodontal anatomy that shares some similarities with humans, and well-described biological mechanisms (Oz and Puleo, 2011; Jeong-Hyon et al. 2020). The periodontitis are induced either by intragingival injection of lipopolysaccharides derived from P.gingivalis (Oz and Puleo, 2011), or by placing ligature around the molar teeth (Jeong-Hyon et al. 2020). The placement of ligature causes bacteria to build up around it, resulting in rapid periodontitis (Jeong-Hyon et al. 2020).

In research using rats and mice model of periodontitis, there have been strong evidence on the protective effects by potential agents. This data has led to a better understanding on the pathophysiology of periodontitis. Among these, there are several studies which reported on the mechanisms of protection, which may bring insights into the potential therapeutic targets of periodontitis. The ideal therapeutic target may crucially depend on the ability of those agents to attenuate inflammation and minimize the extend of alveolar bone and/or periodontal attachment loss. However, the degree of protection may significantly depend on the size of the affected area, and the success of protection may critically depend on the accuracy of the time point of treatment based on periodontitis pathophysiology, with focus to the host response that are activated in the early and late phases of periodontitis. A systematic review is therefore needed to define the appropriate target(s), in order to design and develop targeted therapy for periodontitis. The aims of this systematic review are: (i) to identify the effects of agents based on evidence on the structural outcomes of the alveolar bone and/or periodontal attachment, (ii) to identify the potential therapeutic target(s) based on evidence on the protective outcomes.

MATERIALS AND METHODS

The study protocol was registered in the International Prospective Register of Systematic Reviews (PROSPERO), ID: CRD42022355578.

Search strategy

The articles were systematically searched in accordance with the PRISMA guidelines. It was conducted in four stages involving article i. identification, ii. screening, iii. eligibility and iv. included, in parallel with the predefined inclusion criteria (Table 1). In identification stage, articles were searched through PubMed, Scopus and Wiley databases, from the year 2017 to 2021, based on Booleans ‘Periodontitis’ AND ‘signalling pathways’.

At the end of the identification stage, the duplicates were removed. In screening stage, articles were chosen based on the language, and the types of study, intervention, and treatment. At the end of the screening stage, only research articles with full text available were chosen. In eligibility stage, only research articles which studied ligature-induced periodontitis model in rats and mice were chosen, while in the included stage, only research articles which concluded with protective signalling pathways were chosen. The chosen articles underwent inter-examiner calibration, followed by validation process, where the selected articles were ensured as non-predatory based on List of Journals not recognized by Ministry of Higher Education, Malaysia. All data were recorded in the PRISMA 2009 flow diagram, and articles were finally selected for the present systematic review.

Selection schedule

The search strategy is based on Boolean 'Periodontitis' AND 'signaling pathways'. The selection criteria include (a) The restrictions of publication date and language, and (b) The types of study, animal, model, intervention, and treatment.

The inclusion and exclusion criteria used to select studies is illustrated in Table 1

Table 1: Inclusion and exclusion criteria

	Inclusion criteria	**Exclusion criteria**
(a) Restriction		
i. Publication date	Articles between 2017 - 2021 (5 years)	Articles before 2017, and articles published after December 2021
ii. Language	Articles in English language	Articles in other languages
(b) Type		
i. Study	Experimental studies using animals	Review of experimental studies
ii. Animal	Rodent model (rats and mice)	Other animals
iii. Model	Periodontitis induced by ligature placement	Periodontitis induced by lipopolysaccharide injection
iv. Intervention	Adjunctive (natural product, essential oil)	Antibiotics, steroids
v. Treatment	Use of agents only	Root debridement, bone grafting

Details of model production

The details of periodontitis induction in selected studies involving species, weight/age, type of ligature, location of ligature placement and induction period were extracted.

Determination of outcome determinant

The outcome parameters investigated in selected studies were described based on the effects by the agents. The outcomes were analysed based on improvement or unimproved and were summarized in table based on authors and their findings.

Determination of protective target(s)

The protective target(s) reported in selected studies was drawn in a diagram to show its cellular location. The protective targets were then summarized based on their mechanisms of protection.

Identification of the potential protective target(s)

The potential protective target(s) was identified using the decision matrix analysis. The analysis began with a simple decision matrix, followed by weighted decision matrix, as described by Chang (2015). In simple decision matrix, the target(s) were scored against each criteria (outcome), based on the number of articles which reported protective outcomes. This was followed by the weighted decision matrix, where weighting was done by having 1 – 4 points from the least mentioned outcome to the most mentioned. The target with the highest score represents the most investigated target with the most protective outcome.

RESULTS

Articles selection

3040 articles were obtained following searching through the databases. At the end of the identification stage, the duplicates were removed, where 2391 articles were obtained. 104 articles were chosen at the end of the screening stage, 40 research articles were chosen at the end of the eligibility stage, while 11 research articles were finally selected for the present systematic review at the end of the included stage (Figure 1).

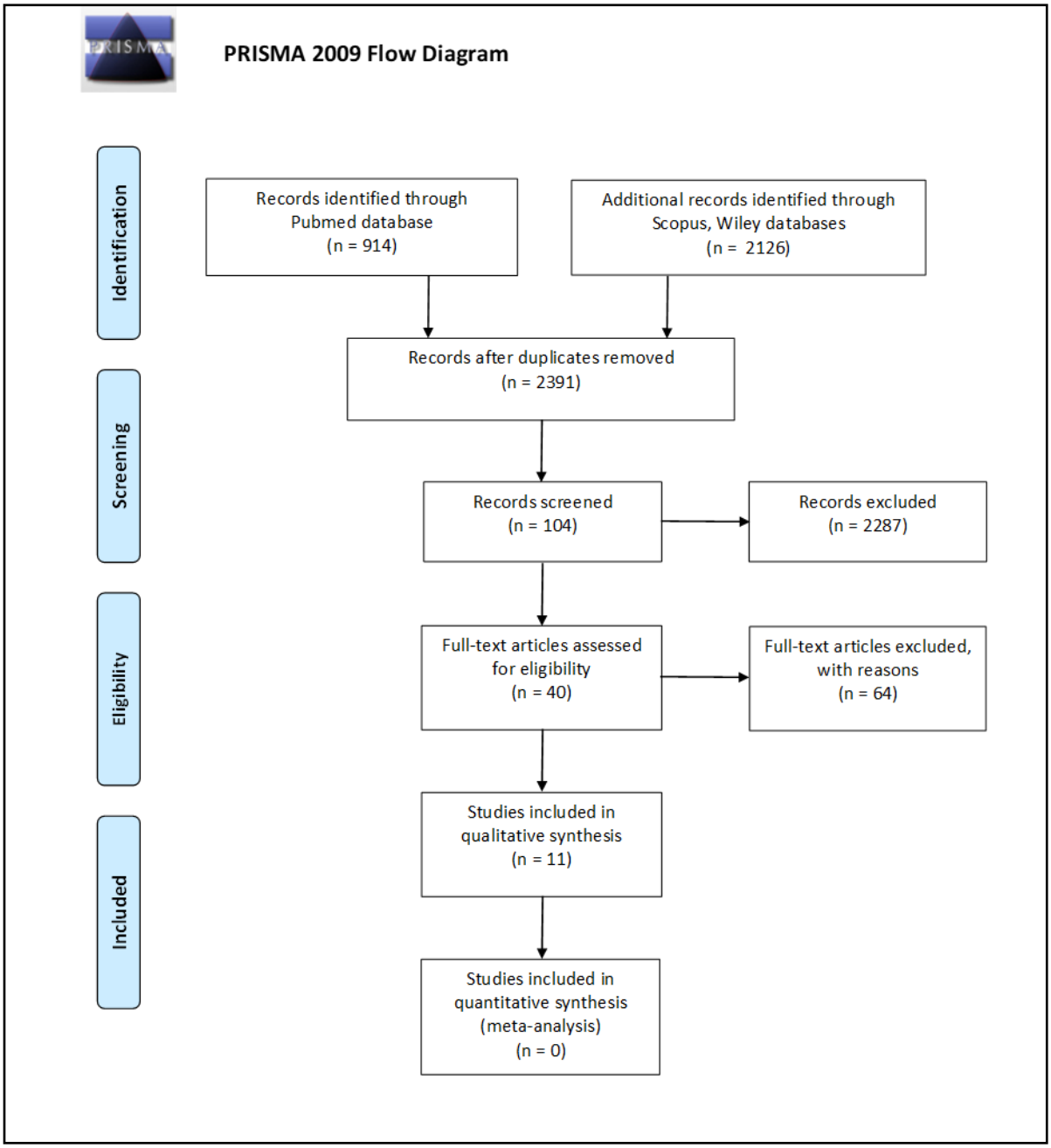

Figure 1: PRISMA 2009 flow diagram

The search and selection of the articles were carried out in three stages. In the first stage (Identification), the articles were searched based on Booleans, through databases. In the second stage (Screening), the duplicates were removed, and research articles in language of interest with full text available which studied the animal model of interest were chosen. In the third stage (Eligibility), only research articles which reported on protective signaling pathways were chosen. At the end of the third stage, inter-examiner calibration was done to finalize selected articles for the present review.

Periodontitis induction

The details of periodontitis induction include the species/ gender and weight/ age of animals, the type and location of ligature, and the period of induction. 63.6% of studies used rats, while 36.4% of studies used mice. In studies that used rats, 57.1% of studies used Sprague Dawley (SD) and 42.9% used Wistar species. In studies that used mice, all studies used C57BL/6 species.

72.7% of studies placed ligature on maxillary tooth, while 27.3% of studies placed ligature on mandibular tooth. In studies that placed ligature on maxillary tooth, 75.0% placed the ligature on the second molar, while 25.0% placed the ligature on the first molar. In studies that placed ligature on mandibular tooth, all studies placed the ligature on the first molar. 63.6% of studies used silk ligature, while 36.4% of studies used stainless steel wire, cotton ligature, braided thread, and nylon thread with 9.1% for each type of ligature respectively. 72.7% of studies induced periodontitis during the early phase (within 1 - 14 days), while 27.3% of studies induced periodontitis during the late phase (more than 14 days). The details of periodontitis induction in the eleven studies are summarized in Table 2.

Table 2: Summary of the details of periodontitis induction by ligation technique

No	References	Species, gender	Weight/age	Ligature type	Ligature placement	Induction period
1	Hu et al. (2021)	SD rat, male	10 weeks	Stainless steel wire, 0.25 mm	Maxillary, subgingival, first molar	8 weeks
2	Wei et al. (2021)	C57BL/6 mice, male	N/A	Silk, 6-0	Maxillary, second molar	10 days
3	Gu et al. (2021)	Wistar rat, male	10-12 weeks	Silk, 3-0	Mandibular, first molar	7 days
4	Li et al. (2019)	SD rat, male	200-220 g	Cotton, 3-0	Mandibular, subgingival, first molar	7 days
5	Huang et al. (2019)	C57BL/6 mice, N/A	6 weeks	Silk, 5-0	Maxillary, second molar	7 days
6	Wang et al. (2019)	SD rat, male	3 months	Silk, 3-0	Maxillary, subgingival, first molar	4 weeks
7	Qiao et al. (2018)	SD rat, male	180-200 g	Braided thread, 3-0	Mandibular, cervical, first molar	8 weeks
8	Renn et al. (2018)	Wistar rat, male	250-300 g	Silk, 3-0	Maxillary, subgingival, second molar	14 days
9	Ihn et al. (2018)	C57BL/6 mice, N/A	6 weeks	Silk, 5-0	Maxillary, second molar	6 days
10	Kure et al. (2018)	C57BL/6J mice, N/A	8 weeks	Silk, 6-0	Maxillary, second molar	8 days
11	Lima et al. (2017)	Wistar rat, male	200 g	Nylon thread	Maxillary, cervical, second molar	11 days

Outcome determinant

The efficacy of therapeutic agents used in the eleven studies were described based on their effects on the alveolar bone/ osteoclast differentiation. All studies assessed alveolar bone, while 45.5% of studies assessed osteoclast differentiation as primary outcome with alveolar bone assessment as secondary outcome. The description of the effects of the agents and their outcomes is summarized in Table 3.

Table 3: Summary of the efficacy of therapeutic agents against ligature-induced periodontitis

11 studies assessed alveolar bone loss / osteoclast differentiation	1. Hu et al. (2021), CTS increases bone volume and mineral density. 2. Wei et al. (2021), Quercetin slows alveolar bone resorption. 3. Li et al. (2019), Paeonol decreases alveolar bone resorption. 4. Ihn et al. (2018), OCLI-070 inhibits osteoclast differentiation and resorbing activity, attenuates alveolar bone resorption. 5. Huang et al. (2019), FICZ reduces osteoclast count and prevents alveolar bone loss. 6. Qiao et al. (2018), RGZ in short term reduces osteoclast count, bone resorption area and CEJ-ABC distance. 7. Kure et al. (2018), IMD-0354 reduces osteoclast count and suppresses volumetric bone resorption. 8. Wang et al. (2019), AZD8835 reduces osteoclast activity and suppresses alveolar bone destruction. 9. Renn et al. (2019), Melatonin increases trabecular thickness normalizes RANKL/OPG ratio and reduces alveolar bone loss. 10. Gu et al. (2021), Berberine attenuates alveolar bone loss and reduces CEJ-AC distance. 11. Lima et al. (2017), CLO decreases alveolar bone loss.

Protective target(s)

The findings of the eleven studies were described, and further summarized in Figure 2. The protective targets involve different cellular levels.

1. Transcription factor

I. STAT3

Signal transducer and activator of transcription 3 (STAT3) is a transcription factor that mediates cellular responses to a variety of cytokines and growth factors. STAT3 regulates microglia and astrocyte activation in the cortex and hippocampus after induction of periodontitis for 8 weeks (Hu et al. 2021). Periodontitis causes activation of STAT3 at periodontal tissue and promote the processing of amyloid precursor protein (APP) by β and γ secretases, and increase of inflammatory cytokines (IL-1β, IL-6, IL-8, IL-21) in peripheral blood. Inhibition of STAT3 by Cryptotanshinone (CTS) reduces local inflammatory cell infiltration, decreases inflammatory cytokines level and alveolar bone resorption. In addition, STAT3 inhibitor protects from systemic inflammation and neuroinflammation, which eventually protects from cognitive impairment in rats with periodontitis.

II. Nrf2/ NF-κB/ NFATc1

The nuclear factor erythroid factor 2 (Nrf2) is a transcription factor that regulates the expressions of genes under normal and stressed conditions. It regulates oxidative stress level activation after induction of periodontitis for 10 days (Wei et al. 2021). Quercetin prevents oxidative stress by decreasing malondialdehyde (MDA) and increasing superoxide dismutase (SOD) activities and upregulate the expression of Nrf2 in the periodontal ligaments, which eventually alleviate alveolar bone resorption.

The nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) is a protein complex in a family of transcription factors that controls the transcription of DNA in the regulation of cellular immunity, cell proliferation, differentiation, and survival. It regulates the development of osteoclast after induction of periodontitis for 7 days (Li et al. 2019). Paeonol downregulates NF-κB, while upregulates osteoprotegerin and inhibits osteoclast formation. Paeonol also reduces local inflammatory cell infiltration, and inflammatory factors (IL-1β, IL-6 and TNF-α) which leads to suppression of oxidative stress in gingival tissue, which eventually ameliorate alveolar bone destruction.

The nuclear factor of activated T cells 1 (NFATc1) plays role in the expression of cytokine genes in T-cells. It regulates osteoclast differentiation after induction of periodontitis for 16 days (Ihn et al. 2018). Its suppression by OCLI-070 downregulates NFATc1 protein expression and the number of cells with NFATc1 nuclear localization, inhibit osteoclastogenesis, and subsequently reduces osteoclastic bone erosion.

III. AhR

Aryl hydrocarbon receptor (AhR) is a cytosolic ligand-activated transcription factor that regulates gene expression in cellular homeostasis and immune responses. Periodontitis suppresses the expression of AhR target gene cytochrome P450 subfamily B member 1 (CYP1B1) and upregulates the expression of proinflammatory cytokines (IL-1β and TNF-α). The Ahr ligand 6 formylindolo[3,2-b] carbazole (FICZ) mitigate the inflammatory cytokines after induction of periodontitis for 7 days, and eventually rescued alveolar bone loss (Huang et al. 2019).

IV. PPARγ

Peroxisome proliferator-activated receptor gamma (PPAR-γ) is a nuclear ligand-activated transcription factor that regulates cellular defense mechanisms. PPAR-γ modulates inflammation and bone remodelling in a time dependent manner following periodontitis by regulating receptor activator of NF-κB ligand (RANKL) (Qiao et al. 2018). Periodontitis causes activation of inflammatory cytokines in periodontal tissues. Activation of PPAR-γ by PPAR-γ agonist (Rosiglitazone (RGZ)) decrease inflammatory cytokines expression and osteoclast, and eventually protects from alveolar bone loss after short term activation (1 and 4 weeks). However, long term PPAR-γ activation (8 weeks) by RGZ increase the RANKL/OPG ratio and promote alveolar bone loss, while PPAR-γ antagonist (bisphenol-A-diglycidyl ether (BADGE)) enhance alveolar bone formation.

2. Protein kinase

I. IKK

I kappa-B kinase (IKK) is a type of protein kinase that is involved in the regulation of cell functions including proliferation, gene expression, cell survival and apoptosis. IKK is involved in cellular protection against alveolar bone loss after induction of periodontitis for 8 days (Kure et al. 2019). Inhibition of IKK by IKK inhibitor (IMD-0354) reduces the number of osteoclasts and the level of inflammatory cytokines (IL-1β, TNF-α), and downregulates the expression of RANKL and NF-κB in gingival tissue, which eventually suppresses linear bone loss.

3. Lipid kinase

I. PI3K

Phosphatidylinositol 3-kinase (PI3K) is a family of enzymes with oncogenic potential including cellular growth, proliferation, and differentiation. PI3K is involved in cellular protection against alveolar bone loss after induction of periodontitis for 4 weeks (Wang et al. 2019). Inhibition of PI3K by PI3K inhibitor (AZD8835) reduces the number of osteoclast and periodontal membrane width, which eventually suppress alveolar bone destruction.

4. Transmembrane receptor

I. TLR4

Toll-like receptor 4 (TLR4) is a cell surface pattern recognition receptor which functions in innate immune response. Periodontitis stimulates host immune response and inflammation. Activation of TLR4 enhances the expression of its downstream target protein (myeloid differentiation primary response 88 (MyD88)), and production of proinflammatory cytokines (TNF-α, IL-1β and IL-6) in the affected gingival regions after induction of periodontitis for 14 days (Renn et al. 2018). It causes lower level of osteoprotegerin (OPG), higher level of RANKL, higher number of osteoclast and lower number of osteoblasts in the periodontal tissue, which results in marked alveolar bone loss in the affected regions. Melatonin depresses TLR/MyD88-mediated proinflammatory cytokine activation, thus normalizes RANKL/OPG signaling and subsequently counteract periodontitis-induced alveolar bone destruction.

II. GPR30

G protein-coupled estrogen receptor (GPR30) is a cell surface receptor which is able to detect extracellular changes and activate intracellular signaling pathways. Periodontitis causes a cellular stress response after induction of periodontitis for 7 days. It causes downregulation of GPR30 expression and increase of inflammatory cytokines (TNF-α, IL-1β and IL-10) in gingival tissues (Gu et al. 2021). Berberine upregulates GPR30 protein level, downregulates the activity of stress-activated protein kinase P38 (MAPKp38) and NF-κB, and inhibit alveolar bone loss. Inhibition of GPR30 by GPR antagonist (G15) reverse the protective effects of Berberine, by reducing inflammatory cell infiltration, suppressing inflammatory response, and eventually suppresses alveolar bone loss.

5. Extracellular protein

I. Dkk-1

Dickkopf1 (Dkk-1) is an extracellular inhibitor of Wnt/β-catenin signaling. The Wnt/β-catenin pathway regulates cellular functions including the activity of bone-forming osteoblasts and bone resorbing osteoclasts. Induction of periodontitis for 11 days reduces the antioxidant capacity and increases oxidative damage in rats. Periodontitis increases Dkk-1 and reduces Wnt10b and β-catenin proteins expression, and alveolar bone loss (Lima et al. 2017). Inhibition of Dkk-1 by herb Calendula officinalis (CLO) activates the Wnt/β-catenin pathway, increases antioxidant enzymes (glutathione (GSH), superoxide dismutase (SOD), catalase (CAT)) activity, while decreases the marker of oxidative stress (malondialdehyde (MDA)) in gingival tissue, and eventually preserve collagen fibers in periodontium and alveolar bone.

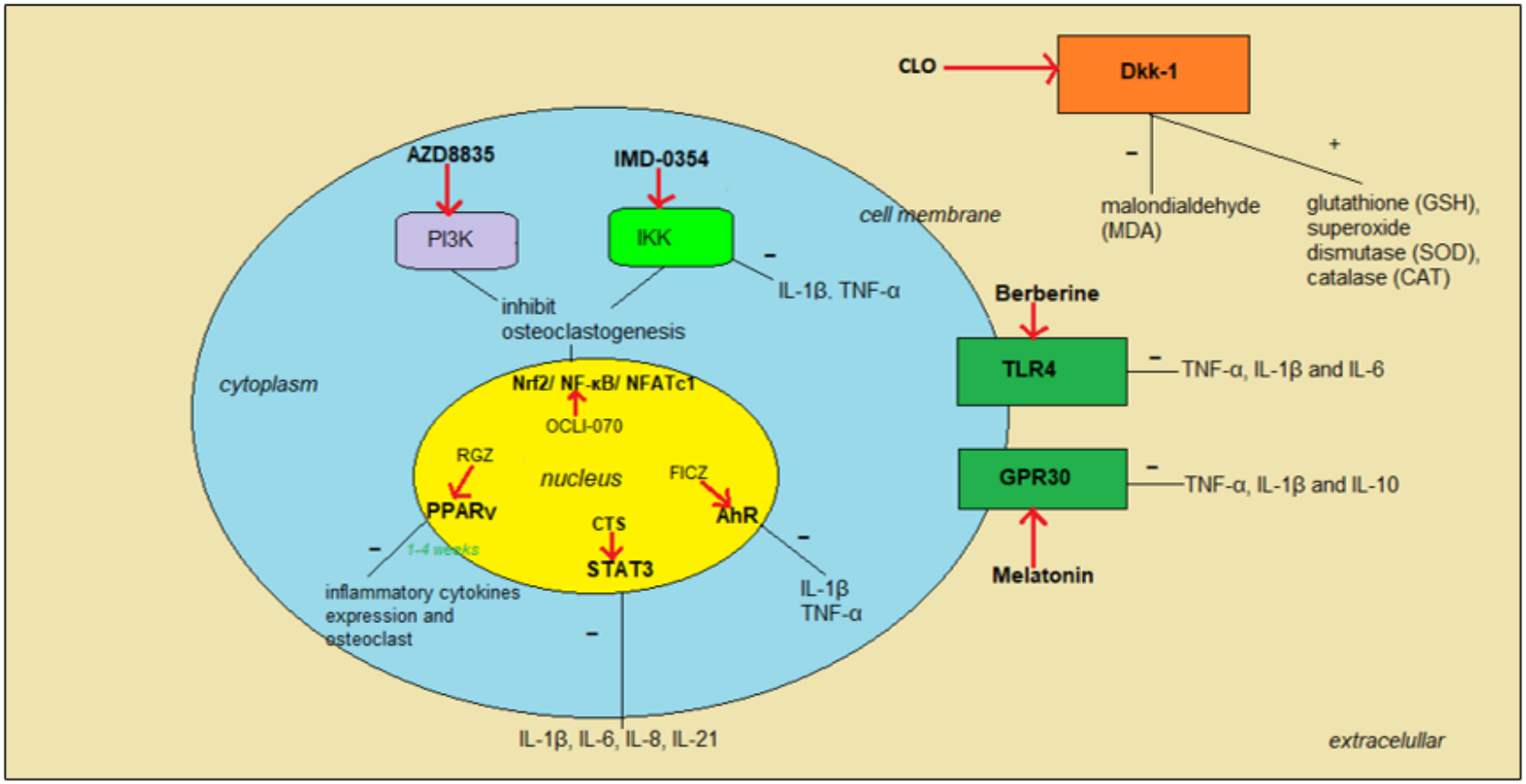

Figure 2: Protective targets in periodontitis, involving different cellular levels.

The protective targets at different cellular levels (intracellular and extracellular). The targets at intracellular include transcription factors, protein kinase, lipid kinase and transmembrane receptor, while target at extracellular include extracellular protein.

Cellular level

Transcription factor = PPAR-γ: Peroxisome proliferator-activated receptor gamma AhR: Aryl hydrocarbon receptor, STAT3: Signal transducer and activator of transcription 3 Nrf2: nuclear factor erythroid factor 2 NF-Kb: nuclear factor kappa-light-chain-enhancer of activated B cells NFATc1: nuclear factor of activated T cells 1

Protein kinase = IKK: I kappa-B kinase

Lipid kinase = PI3K: Phosphatidylinositol 3-kinase

Transmembrane receptor = GPR30: G protein-coupled estrogen receptor, TLR4: Toll-like receptor 4
Extracellular protein = Dkk-1: Dickkopf1

CTS: Cryptotanshinone, FICZ: 6 formylindolo[3,2-b] carbazole, RGZ: Rosiglitazone, CLO: Calendula officinalis, TNF: Tumor necrosis factor, IL: interleukin

The outcome determinants include reduction of inflammation and oxidative stress of periodontal tissue, and the improvement on alveolar bone and osteoclast differentiation. The therapeutic targets are summarized in Table 4 based on their mechanisms of protection.

Table 4: The findings of the eleven studies on their therapeutic targets in the treatment of ligature-induced periodontitis in rats and mice.

Therapeutic target		Agent	Effect	Outcome	Reference
Transcription factor	STAT3	*STAT3 inhibitor* Cryptotanshinone (CTS)	Anti-inflammatory	(-) alveolar bone loss	Hu et al. (2021)
	Nrf2	*Flavonoid compound* Quercetin	Antioxidant	(-) alveolar bone resorption	Wei et al. (2021)
	Nrf2/ NF-kB/ NFATc1	*Phenolic compound* Paeonol	Anti-inflammatory, antioxidant, inhibit osteoclastogenesis	(-) alveolar bone lesion	Li et al. (2019)
	NFATc1	*Benzamide-linked molecule* OCLI-070	Inhibit osteoclast differentiation	(-) alveolar bone erosion	Ihn et al. (2018)
	AhR	*AhR ligand* 6 formylindolo[3,2-b] carbazole (FICZ)	Anti-inflammatory	(-) alveolar bone loss	Huang et al. (2019)
	PPARγ	*PPARγ agonist* Rosiglitazone (RGZ)	Anti-inflammatory	(-) alveolar bone loss	Qiao et al. (2018)
Protein kinase	IKK	*IKK inhibitor* IMD-0354	Anti-inflammatory	(-) linear bone loss	Kure et al. (2018)
Lipid kinase	PI3K	*PI3K inhibitor* AZD8835	Anti-inflammatory	(-) alveolar bone loss	Wang et al. (2019)

Transmembrane receptor	TLR4	*Anti-inflammatory agent* Melatonin	Anti-inflammatory	(-) alveolar bone loss	Renn et al. (2018)
	GPR30	*Active alkaloid* Berberine	Anti-inflammatory	(-) alveolar bone loss	Gu et al. (2021)
Extracellular protein	Dkk-1	*Herb* Calendula officinalis (CLO)	Antioxidant	(-) alveolar bone loss	Lima et al. (2017)

STAT3: Signal transducer and activator of transcription 3, Nrf2: Nuclear factor erythroid- related factor 2, NF-kB:Nuclear factor-kappa B, NFATc1: Nuclear factor of activated T-cells 1, AhR: Aryl hydrocarbon receptor, PPARγ: Peroxisome proliferator-activated receptor gamma, IKK: IkappaB kinase, PI3K: Phosphatidylinositol 3-kinase, TLR4: Toll-like receptor 4, GPR30: G protein-coupled estrogen receptor, Dkk-1: Dickkopf-1, (-): reduce, (+): increase, (-/+): no

Identification of the potential protective target(s)

Analysis from the simple decision matrix (Table 5a) and further analysis from the weighted decision matrix (Table 5b) show that transcription factor is the most investigated protective target with most reported protective outcomes, followed by transmembrane receptor, while protein kinase, lipid kinase and extracellular protein share the same score as the least investigated targets.

Table 5: Identification of the potential protective target(s)

A) **Simple decision matrix of the effect of protective targets in periodontitis, based on their outcomes.**

Outcome / Target	**Anti-inflammatory**	**Antioxidant**	**Inhibit osteoclastogenesis/ osteoclast differentiation**	**Inhibit alveolar bone loss/resorption/ lesion/erosion**
Transcription factor				
1. STAT3	/			/
2. Nrf2		/		/
3. Nrf2/NF-κB/NFATc1	/ /	/	/	/ /
4. AhR	/			/
5. PPARγ				
Protein kinase				
IKK	/			/
Lipid kinase				
PI3K	/			/
Transmembrane receptor				
1. TLR4	/			/
2. GPR30	/			/
Extracellular protein				
Dkk-1	/			/

STAT3: Signal transducer and activator of transcription 3, Nrf2: Nuclear factor erythroid- related factor 2, NF-kB:Nuclear factor-kappa B, NFATc1: Nuclear factor of activated T-cells 1, AhR: Aryl hydrocarbon receptor, PPARγ: Peroxisome proliferator-activated receptor gamma, IKK: IkappaB kinase, PI3K: Phosphatidylinositol 3-kinase, TLR4: Toll-like receptor 4, GPR30: G protein-coupled estrogen receptor, Dkk-1: Dickkopf-1

B) **Weighted decision matrix of the effect of protective targets in periodontitis, based on their outcomes.**

Target / Outcome	Lipid kinase		Transcription factor		Protein kinase		Transmembrane receptor		Extracellular protein	
	Score	Total	Score	Total	Score	Total	Score	Total	Score	Total
Inhibit osteoclastogenesis/ osteoclast differentiation (score=1)	-	-	1	1	-	-	-	-	-	-
Antioxidant (score=2)	-	-	2	4	-	-	-	-	-	-
Anti-Inflammatory (score=3)	1	3	4	12	1	3	2	6	1	3
Inhibit alveolar bone loss/resorption/ lesion/erosion (score=4)	1	4	5	20	1	4	2	8	1	4
Total		**7**		**37**		**7**		**14**		**7**

DISCUSSION

In the current preclinical studies, the outcome determinants for periodontitis are generally anti-inflammatory, antioxidant, osteoclast differentiation, and alveolar bone loss, resorption, and lesion. Estimation of inflammatory factors is considered as the main parameter indicating biochemical outcome, while alveolar bone loss is the main parameter indicating preservation of the gingival tissue.

The degree of anti-inflammatory effect is determined by the activity of pro-inflammatory cytokines including TNF-α and interleukins (Jeong-Hyon et al. 2020). TNF-α and IL-1β are the key players in the pathophysiology of early phase of periodontitis (Gomes et al. 2016). Therefore, host modulation following periodontitis is an important outcome to be measured thoroughly. This is usually done through specific biochemical tests including ELISA and RT-qPCR. The changes of the inflammatory factors, indeed, have been evaluated by three-fourth of the studies. It is, however, noteworthy, that in periodontitis, downstream effector cytokine that regulates osteoclast formation is now recognized as an important outcome for bone remodelling (Kitaura et al. 2020). The changes in RANKL/OPG axis, however, have been evaluated by less than one-third of the studies.

Periodontal attachment loss is a prominent feature associated with periodontitis (Hienz et al. 2015). The evaluation of alveolar bone loss is determined radiographically using the microcomputed tomography (micro-CT). Alveolar bone loss leading to periodontal attachment loss develop due to inflammatory response of the gingival tissue supporting the teeth (Cekici et al. 2014). Subsequently, some crucial external parameters could be evaluated including bone volume and mineral density, trabeculae thickness, and cementoenamel junction (CEJ)-alveolar bone crest (ABC) distance (Liu et al. 2010). In ligature-induced periodontitis model, some crucial internal parameters relating to cellular substances could be observed, where histological analyses show

osteoclast activity (Ihn et al. 2018; Qiao et al. 2018) and arrangement of collagen fibers (Adhikari et al. 2019; Xu et al. 2020).

The protective targets in periodontitis that have been the focus of investigations include transcription factor, protein kinase, lipid kinase, transmembrane receptor, and extracellular protein. Transcription factors are able to control the transcription of DNA in the regulation of cellular immunity and survival (Jurdziński et al. 2020). Protein and lipid kinases are able to modify its substrate, leading to either initiation or inhibition of cytoplasmic proteins (Groeger and Meyle, 2019). Transmembrane receptors are able to detect extracellular changes and activate intracellular signalling pathways (Jang et al. 2015). Extracellular proteins are able to control receptors and subsequently, intracellular activities (de Andrade et al. 2019). Of these protective targets, transcription factors have been evaluated by more than half of the studies. This may be due to the role of transcription factor in the modulation of signalling pathways leading to protection. Transcription factor mediates cellular inflammatory response to a variety of cytokines, while oxidative stress response may be attributed to the alteration of the transcription activities. The recent preclinical studies also show that transcription factors are useful targets at both early and late phases of periodontitis. This shows that transcription factors undergo significant alteration throughout the pathophysiology of periodontitis including both the early and late phases.

CONCLUSION

The present systematic review shows that protective mechanisms in periodontitis involve targets at different cellular levels. Transcription factors appear as the most potential protective target in rat/mice model of ligature-induced periodontitis. Interaction between these transcription factors and other molecules in the modulation of host immune response in periodontitis may provide important insights with regards to targeted therapy of periodontitis.

SCOPE AND LIMITATIONS OF STUDY

This study focused on reviewing the protective signaling pathways/targets following ligature-induced periodontitis. Despite closest resemblance of ligature-induced periodontitis model to human periodontitis, this model does not accurately mimic periodontitis in human.

A number of current studies using (i) combination of ligation and lipopolysaccharide injection in the induction procedure, and (ii) combination of assessment of signaling pathways using cell lines in vitro and the outcome on alveolar bone using rats/mice in vivo, may produce more significant effects. Thus, evaluation on the findings from those articles may provide a better insight on the protective targets in periodontitis.

RECOMMENDATION FOR FUTURE RESEARCH

Further reviews need to be done to summarize preclinical evidences, focusing on the interaction between transcription factors and other molecules in the modulation of host immune response, to provide a better insight on targeted therapy of periodontitis.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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