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THE TISSUE RENIN-ANGIOTENSIN SYSTEM (tRAS) AND THE IMPACT OF ITS INHIBITION ON INFLAMMATION AND BONE LOSS IN THE PERIODONTAL TISSUE

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Abstract

Recently, the existence of the tissue renin-angiotensin system (tRAS) has been described for multiple tissues in humans, suggesting its fundamental role in the progression of inflammation and fibrosis. Evidence arises that tRAS might have an impact on the progression of periodontitis and bone loss. However, neither the role of tRAS nor its impact as a therapeutic target have been systematically evaluated for periodontal tissue. The present study sought to characterise tRAS in the periodontal tissue and the effect of its inhibition on periodontal inflammation and bone loss. This systematic review was performed according to the preferred reporting items for systematic reviews and meta analyses (PRISMA) statement. Literature was searched using Web of Science core collection (Web of Science), Medline (Ovid), Cochrane central register of controlled trials (Ovid), Cochrane database of systematic reviews (Ovid), Google Scholar databases and the references of the retrieved studies in March 2020. Information on study design, sample size, population, procedure, type of intervention, observation time, as well as information on sources of bias, was extracted and evaluated. From 455 identified articles, 17 were included in the qualitative synthesis and 11 were included in the quantitative synthesis. Outcomes of studies indicated that the inhibition of tRAS components led to a reduction of periodontal bone loss and inflammation, dependent on the inhibitor used. The findings suggested an important role of tRAS in the periodontal tissue and indicate a potential therapeutic approach for periodontal diseases.

Keywords: Periodontal bone loss, periodontitis, inflammation, renin-angiotensin system, degeneration, regeneration, osteogenesis.

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Introduction

Severe periodontitis is one of the most prevalent human diseases and the major cause of tooth loss in adults worldwide, with an overall prevalence of approximately 11 % (Dye, 2012; Frencken *et al.*, 2017; Kassebaum *et al.*, 2014; Petersen and Ogawa, 2012). The global cost of lost productivity from periodontitis has been estimated to be 54 billion $US annually (Marcenes *et al.*, 2013; Tonetti *et al.*, 2017). The disease is characterised by progressive inflammation, loss of periodontal ligament, and alveolar bone loss (Pihlstrom *et al.*, 2005). The infection of the periodontal tissue is known as the main aetiological factor of this inflammation process (Darveau, 2010). However, the dysregulated cellular

pathways promoting the inflammatory vicious circle are host mediated (Darveau, 2010; Kantarci *et al*., 2006). Current treatment strategies include pharmacological approaches, such as systemic and local antibiotic therapy and antiseptic therapy, mechanical anti-infective therapy, and surgical interventions (Krayer *et al*., 2010). These approaches focus on the primary aetiology of periodontal disease: bacterial infection. Another therapeutical approach is the host modulation therapy (HMT), based on the management of periodontal disease through the control of host immune and inflammatory response (Greenwell, 2001; Krayer *et al*., 2010; Reddy *et al*., 2003). Non-steroidal anti-inflammatory drugs (NSAIDs), anti-cytokine, and biological therapies and inhibition of matrix metalloproteinase (MMP) activity are some described host modulation therapeutics for periodontal diseases (Preshaw, 2018). Furthermore, diet is an important factor in host modulation and progression of periodontal diseases (Woelber and Tennert, 2020).

Taking account of the socioeconomic relevance of periodontal diseases, new biomolecular therapies targeting the inflammatory and destructive processes are crucial when other less invasive therapeutic approaches fail. As inflammation induces and triggers these degenerative pathways, profound knowledge on the proinflammatory interactions helps to find optimally targeted therapeutics and control critical host response reactions.

Nearly a hundred years after the first description of the renin-angiotensin system (RAS) by Tigerstedt and Bergmann, the concept of a local or tissue renin-angiotensin system (tRAS) emerged in the scientific world (Dzau and Re, 1994; Lindpaintner and Ganten, 1991; Paul *et al*., 1992; Tigerstedt and Bergman, 1898). Hence, an intracellular-renin-angiotensin system (iRAS) and an intercellular-renin-angiotensin system (intRAS) were introduced interacting with the classic circulating humoral RAS (Abadir *et al*., 2011; Alzayadneh and Chappell, 2015; Filipeanu *et al*., 2001; Gwathmey *et al*., 2012). Angiotensin II (ATII), as the most important effector of the tRAS, is involved in multiple intracellular pathways including mitochondrial and nuclear signalling (Filipeanu *et al*., 2001; Re, 2018). Furthermore, the iRAS – as the regulatory arm of the tRAS – determines in which way the cell will react, dependent on extracellular signals and the intracellular ATII concentration (Filipeanu *et al*., 2001). Moreover, the angiotensin II type 1 receptor (AT1R) has been described in multiple intracellular compartments – such as mitochondria, nucleus and lysosomes – and is responsible for many intracellular processes, such as aging, cell proliferation, and increase of mRNA expression (Valenzuela *et al*., 2016; Villar-Cheda *et al*., 2017). Further, anti-proliferative and anti-inflammatory, or proliferative and pro-inflammatory, cell responses are reported to be directly related to the cellular ATII concentration (Filipeanu *et al*., 2001; Villar-Cheda *et al*., 2017). Additionally, studies indicated a central trend of the tRAS signalling: the dominance of the AT1R mediated pathway leads to inflammation and oxidative stress, while the intracellular AT1R signalling processes and the angiotensin II type 2 receptor (AT2R) pathway seem to have modulating roles (Chabrashvili *et al*., 2003; Valenzuela *et al*., 2016; Villar-Cheda *et al*., 2017). The existence and importance of a tRAS have been described for multiple human tissues such as the brain, heart, liver, pancreas, musculoskeletal and adipose tissue (Karlsson *et al*., 1998; van Kats *et al*., 1998; Lu *et al*., 2007; Moulik *et al*., 2002; Zhao *et al*., 2019) (also congress abstract: Lang *et al*., 2018. The tissue-renin-angiotensin-system of the human intervertebral disc. Deutscher Kongress für Orthopädie und Unfallchirurgie. DOI: 10.3205/18DKOU478).

Recent studies implicate a fundamental role of the tRAS in metabolic diseases and host-mediated inflammation reactions. For example, a link between the tRAS and diabetes, obesity, atherosclerosis, Alzheimer's disease, kidney, vascular and heart diseases have been described (Biancardi *et al*., 2017; Gebre *et al*., 2018; Montecucco *et al*., 2009; Ramalingam *et al*., 2017; Remuzzi *et al*., 2005; Ruiz-Ortega *et al*., 2001). The complex interactions of tRAS with the circulatory renin-angiotensin system could be one explanation for the association between periodontitis and cardiovascular diseases (Martin-Cabezas *et al*., 2016; Santos *et al*., 2015; Viafara-Garcia *et al*., 2019).

The presence of a tRAS has also been recently described for bone and connective tissues (Gebru *et al*., 2013; Morimoto *et al*., 2013) (also congress abstract: Lang *et al*., 2018. See earlier paragraph). Additionally, the existence of a tRAS in the periodontal and gingival tissue has been described by numerous studies (Berggreen and Heyeraas, 2003; Ohuchi *et al*., 2002; Ohuchi *et al*., 2004; Santos *et al*., 2009). It is suggested that this system is a key player in bone remodelling and modulation of host immunoreactions (Gebru *et al*., 2013). Fig. 1 illustrates potential tRAS mechanisms in the presence of periodontitis-associated bacteria. It is well known that stimulation of toll-like receptors (TLRs) in periodontal fibroblasts, through pathogens, leads to an activation of the transcription factor nuclear factor-kappa B (NF-kB), an increase of reactive oxygen species (ROS) and the expression of pro-inflammatory molecules, such as interleukin (IL)-1β (Gabriele *et al*., 2017; Miggin, 2006; Nadlonek *et al*., 2013; Wu, 2006). A recent study showed cross-talks between TLRs and ATII with an upregulation of TLRs and an increase of NF-kB, monocyte chemoattractant protein 1 (MCP1), and IL-6, all are important factors of the host-mediated immune-response (Lv *et al*., 2015). The induction of adhesion molecules and proinflammatory mediators, such as tumour necrosis factor-alpha (TNF-α) and IL-1β, promotes the host-mediated process, leading to bone resorption and tissue destruction (Graves and Cochran, 2003). Shimizu *et al*. showed that ATII induces the expression of NF-kB ligand (RANKL)

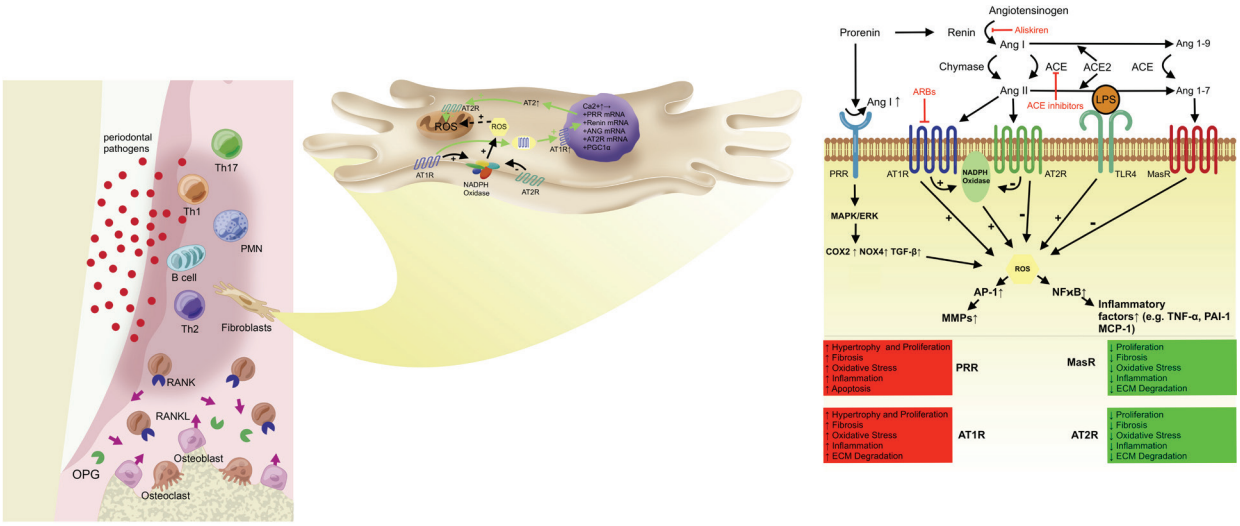

Fig. 1. Illustration of possible tRAS mechanisms modulating the host response in periodontal tissue after periodontal pathogen invasion. Pathogen invasion leads to a host-mediated cascade of inflammatory response involving an increase of AT1R activity, production of ROS, upregulation of NF-kB, and secretion of inflammatory- and tissue degrading molecules. Proinflammatory molecules lead to an upregulation of RANKL and an increase of the RANKL/OPG ratio, promoting periodontal bone loss. This upregulation of the proinflammatory pathway by the prorenin receptor (PRR), AT1R and TLR4 (stimulated by LPS of periodontal pathogens) is controlled by the AT2R, mas receptor (MasR) and intracellular RAS signalling (Nakamura *et al.*, 2011; Villar-Cheda *et al.*, 2017; Zhao *et al.*, 2019).

in osteoblasts, leading to activation of osteoclasts, whereas the effect was blocked by an AT1R inhibitor (Olmesartan) (Shimizu *et al.*, 2008). Furthermore, ATII has mitogenic properties in periodontal cells and that stimulation could lead to cell proliferation, generation of ROS, an increase of NF-kB and subsequent stimulation of various proinflammatory molecules, such as Prostaglandin E2 and IL-1β, promoting the periodontal inflammation (Gabriele *et al.*, 2017; Lundergan *et al.*, 1999; Nakamura *et al.*, 2011; Segawa *et al.*, 2003). A knockdown of the AT1R in periodontal fibroblasts impaired the secretion of IL-1β, IL-6 and IL-8, all important mediators of periodontal inflammation (Gabriele *et al.*, 2017). Finally, studies revealed that inhibition of tRAS pathways lead to an increase in bone mass and a reduction of fracture risks (Lynn *et al.*, 2006; Nakagami and Morishita, 2009; Shimizu *et al.*, 2008; Solomon *et al.*, 2011; Zhang *et al.*, 2014). These observations indicate that a tRAS exists in the periodontal tissue and might be an important target approach.

Based on these findings, this systematic review aimed to answer the following question: What is the impact of the tRAS inhibition on inflammation and bone loss in the periodontal tissue?

Materials and Methods

Review protocol

This review was conducted based on the preferred reporting items for systematic reviews and meta-analyses (PRISMA) Statement and our systematic review protocol was specified using SYRCLE's systematic review protocol for animal intervention studies (Tricco *et al.*, 2018; de Vries *et al.*, 2015). The review protocol was registered in PROSPERO (international prospective register of systematic reviews) hosted by the UK's National Institute for Health Research (NHS), University of York, Centre for Reviews and Dissemination, under the code CRD42020178423.

Search strategy

Literature was searched up to March 2020, focusing on animal studies, tRAS and its inhibitors [angiotensin II type 1 receptor inhibitors (ARBs)], angiotensin-converting-enzyme inhibitors (ACE-inhibitors), renin inhibitors, periodontitis, and alveolar bone loss. Initially, a combined medical-subject headings (MeSH) and free-text term electronic search of the literature was performed using Medline (Ovid), Cochrane central register of controlled trials (CENTRAL, Trials) (Ovid), and Cochrane database of systematic reviews (CDSR) (Ovid). Subsequently, the search strategy used in Medline was translated into an appropriate format for searching the Web of Science core collection (Web of Science) with a free-text term search. Text-word truncation was applied to retrieve all forms of the search terms and Boolean logical operators were used to combine the search results (Table 1). Furthermore, a supplementary free-text term search was performed using Google Scholar as well as hand searches of the references of the selected studies. All studies published before March 25th, 2020 were considered. No language restrictions were applied and retrieved articles in foreign languages were translated.

Eligibility criteria

The following inclusion criteria were adopted, based on the PICOS process: (P) Population where animals in which experimental periodontitis or similar methods were used to investigate the desired tRAS inhibition outcomes. The intervention (I) was an inhibition of tRAS using ARBs, ACE-inhibitors, or renin inhibitors. The comparator (C) had no tRAS inhibition. The primary outcome (O) was (histo-) morphometric measurements of bone loss and bone volume. The secondary outcomes were the number of osteoclasts and immunoinflammatory/oxidative stress markers (*e.g.* CRP, TNF-α, MPO) and bone remodelling markers (*e.g.* RANKL, OPG). Study designs (S), with an experimental examination of tRAS inhibition in the periodontal tissue in animals, were included without restrictions to retrieve all available evidence.

The following exclusion criteria were applied:

1. human studies and *in vitro* studies
2. review articles, case studies, method-comparison studies
3. Comparator and intervention group are not similar, *e.g.* one of the groups has study characteristics which could affect tRAS outcomes (*e.g.* hypertension or other cardiovascular diseases))
4. studies which focussed on the examination of adjacent structures (*e.g.* tooth) or related diseases (*e.g.* pulpitis) there was no matched control group.

To do justice to the nature of preclinical animal studies (*e.g.* often multiple experiments in one animal study, multiple intervention arms) and to retrieve the maximum information from the current evidence, the desired data corresponding to a PICOS research question was extracted – instead of excluding the studies relating to multiple intervention groups or experimental settings, regardless of the fact that groups or experimental settings that are part of the study would not meet our inclusion criteria. In the studies reporting data from multiple groups with multiple interventions, baseline characteristics, or experimental setups, only the intervention group was considered and its matched comparator that met our inclusion and exclusion criteria.

Study selection and data extraction

The selection of studies was performed in two-steps. Firstly, two reviewers (B.S. and S.U.) independently screened the titles and abstracts for eligibility. Subsequently, an independent full-text analysis was performed by the two reviewers. The reasons for exclusion were recorded in the second step. Any disagreement between reviewers was solved by consensus with a third reviewer (T.B.). Agreement between reviewers was assessed using κ statistics (Cohen, 1960).

Data extraction was performed by the two independent reviewers using a data extraction form which contained the following data extraction parameters:

- article and study identifiers: author, country, year of publication, objective
- setting and population: sample size of comparator and intervention groups, animal species, weight,

Table 1. Search strategy. exp: explode function; TS: Topic; NEAR: proximity operator; OR: boolean operator; AND: boolean operator; *: truncation symbol; .mp: multi-purpose field search.

Search date	Database	Search strategy	Results
25/03/2020	Ovid Medline® and Epub ahead of print, In-process & other non-indexed citations, Daily and versions® <1946 to present> EBM reviews - Cochrane central register of controlled trials (CENTRAL, Trials) *via* Ovid <inception to present> EBM reviews - Cochrane database of systematic reviews (CDSR) *via* OVID <inception to present>	1. exp periodontitis/ 2. exp periodontal diseases/ 3. exp gingiva/ 4. exp periodontium/ 5. exp alveolar bone loss/ 6. periodont*.mp 7. exp alveolar process/ 8. marginal bone loss*.mp 9. alveolar bone loss*.mp 10. exp oral health/ 11. mandib*.mp 12. maxill*.mp 13. attachment loss*.mp 14. exp Tooth/ 15. or/1-14 16. exp angiotensin-converting enzyme inhibitors/ 17. exp angiotensin II/ 18. exp angiotensin II type 1 receptor blockers/ 19. exp angiotensin receptor antagonists/ 20. angiotensin*.mp. 21. ARBs.mp 22. ACE.mp 23. RAAS.mp 24. or/16-23 25. 15 and 24	33.041 92.750 18.515 46.601 11.326 105.613 14.406 2.108 12.516 16.571 123.883 121.926 6.748 91.554 398.501 49.491 37.797 19.915 26.137 145.504 4.197 40.267 3.227 168.047 178
25/03/2020	Web of Science core collection (Web of Science)	#1 TS = ((periodontitis) OR (gingiva) OR (periodontal NEAR/5 disease*) OR (periodontium) OR (periodont*) OR (oral NEAR/5 health) OR (tooth) OR ((bone* OR osteo* OR alveolar) NEAR/5 (alveolar OR maxill* OR mandib* OR jaw OR bucc* OR marginal OR process OR ridge OR attachment) NEAR/5 (loss OR resorption OR defect* OR density OR atroph* OR lyses OR osteolys* OR volume))) #2 TS = ((angiotensin-converting enzyme inhibitors) OR (angiotensin II) OR (angiotensin II type 1 receptor blockers) OR (angiotensin receptor antagonists) OR (ARBs) OR (*sartan) OR (ACE) OR (RAAS) OR (angiotens*) OR ((angiotensin* OR AT1 OR AT-1) NEAR/5 (receptor* OR antagonist* OR type* OR convert* OR inhibit* OR blocker*))) #3 #1 AND #2	239.022 183.501 261
25/03/2020	Google Scholar and hand searching of retrieved studies		16

age, sex, and experimental setting to examine tRAS inhibition, observation period, ethic statement (yes/no)
- intervention: name, dose, type of tRAS inhibition and duration of the inhibition
- method: method of outcome measurement
- outcome and results: reported outcomes (intervention *vs.* comparator)
- author's conclusion
- reviewer comments.

The extraction of data to assess the risk of bias was performed separately and is shown in the risk of bias section.

Synthesis of results

Methods for direct treatment comparisons to assess secondary outcomes

Initially, a pairwise meta-analysis was performed to assess study outcomes quantitatively for every outcome with at least three reporting studies. Pairwise meta-analysis was conducted using Review Manager (RevMan, version 5.3, Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2011). Because of the heterogeneity of the included studies, especially the different duration of tRAS inhibition, different doses of inhibitors, different inhibitors, different species, and different methodological settings, it was decided to use a random-effects model meta-analysis (Higgins J, Green S (editors) (2011) Cochrane handbook for systematic reviews of interventions Version 5.1.0 [updated March 2011]; The Cochrane Collaboration (2003) Review Manager (RevMan) version 4.2.10 [computer program]. The Nordic Cochrane Centre, The Cochrane Collaboration; 2003; The Cochrane Collaboration, 2011: Available from http://handbook.cochrane.org.). Hedge's g was used to calculate the standardised mean difference (SMD). Confidence intervals (CI) were used to measure the degree of unncertainty or certainty using a confidence level of 95 % (95 % CI). To evaluate the heterogeneity of the included studies I^2 statistics were used. Values of I^2 more than 25 %, 50 % and 75 % were specified as low, moderate and high heterogeneity, respectively (Higgins *et al*., 2003). No subgroup or sensitivity analysis was performed due to the limited number of animal studies, but a qualitative interpretation of possible heterogeneity sources was provided if the heterogeneity was judged high. Cohen's D was used to evaluate the effect size, and a D between 0.2 and 0.5 was specified as a small effect, a D between 0.5 and 0.8 as a medium effect and a D greater than 0.8 as a large effect. To visualise differences between intervention arm and comparator for secondary outcomes with regards to the tRAS inhibitor class and the duration of intervention, values were calculated for the secondary outcomes as a percentage of the reference value (untreated control group) and a heatmap was produced using GraphPadPrism version 8.4.2 (GraphPad Software, LLC.).

Methods for mixed and network comparisons of multiple intervention arms for the primary outcome

Network geometries were presented as spider-like web-charts, to show the connections between the different pharmacological intervention arms regarding the periodontal bone loss. A network meta-analysis was conducted for all treatment arms, including the different doses and subgroups of the tRAS inhibitors, as well as network meta-analysis of pooled tRAS inhibitor treatments (Control *vs.* ARB *vs.* ACE-inhibitor *vs.* renin-inhibitor). Because of the heterogeneity of animal studies and the comparison of multiple treatment arms, the approach based on random-effects multivariate meta-regression "mvmeta" was used as presented by White *et al*. that applies the frequentist method for estimation in the network meta-analysis (White *et al*., 2012). The network analysis was performed using Stata Statistical Software Release 15 (StataCorp. 2011, College Station, TX, USA). Furthermore, the network package, the "mvmeta" command and self-programmed Stata routines (Web ref. 1) were used (Chaimani *et al*., 2013; White, 2015). To assess the relative treatment rankings, the "surface under the cumulative ranking" (SUCRA) curve was used as well as mean ranks (Salanti *et al*., 2011). From the three assumptions: similarity, transitivity and consistency, to be satisfied a priori for the network meta-analysis similarity was satisfied by the PICOS procedure, consistency by statistical methods using global ("design-by treatment approach") and local approaches ("loop-specific approach") to assess inconsistency, and transitivity by logical interpretation of outcome interferences and the statistical consistency tests (Cipriani *et al*., 2013).

Data management and assessment of treatment effects

If studies contained multiple experimental groups with the same tRAS inhibitor compared to a single control, the experimental groups were combined to create a single pair-wise comparison to avoid unit-of-analysis error due to double-counts in the shared-control groups. If studies contained multiple experimental groups, with different tRAS inhibitors compared to a single control group, the control group was split (Higgins J, Green S (editors) (2011) Cochrane handbook for systematic reviews of interventions Version 5.1.0). If studies contained multiple experimental groups, with matched control groups, the experimental groups were considered as separate experiments. Multiple animal subgroups within a single study (*e.g.* different species or strains) were included as independent experimental groups with independent SMDs. Because the focused outcomes were presented as continuous data and measures of outcomes were presented in a variety of ways (*i.e.* outcomes measured using different scales and methods) the SMD was used as an effect measure. When the included studies only reported the standard error of the mean, the standard deviation

was calculated first (Altman and Bland, 2005). When only bone volume measurements were shown in the manuscripts, the bone loss was calculated from the baseline value of untreated study groups, where applicable. Where necessary, means and standard deviations (or standard errors of the mean) were extracted from figures of the included manuscripts using WebPlotDigitizer (Web ref. 2).

Assessment of the risk of bias

SYRCLE's Risk of Bias tool was used to assess the risk of bias for all included studies (Hooijmans *et al*., 2014). This tool was adapted from the Cochrane risk of bias tool for randomised controlled trials with human participants (Higgins J, Green S (editors) (2011) Cochrane handbook for systematic reviews of interventions Version 5.1.0). The following ten methodological domains were examined:

1. Selection bias:
   a) Was the allocation sequence adequately generated and applied?"
   b) Were the groups similar at baseline or were they adjusted for confounders in the analysis?
   c) Was the allocation adequately concealed?
2. Performance bias:
   a) Were the animals randomly housed during the experiment?"
   b) Were the caregivers and/or investigators blinded from knowledge which intervention each animal received during the experiment?
3. Detection bias:
   a) Were animals selected at random for outcome assessment?
   b) Was the outcome assessor-blinded?
4. Attrition bias: Were incomplete outcome data adequately addressed?
5. Reporting bias: Are reports of the study free of selective outcome reporting?
6. Other sources of bias: Was the study free of other problems that could result in high risk of bias?

The items in the RoB tool were scored with "high" (high risk of bias), "low" (low risk of bias), and "unclear" (the item was not reported and the risk of bias could not be examined). Two independent investigators (B.S. and S.U.) performed a quality assessment of all included studies. Disagreements were resolved by discussion with a third reviewer (T.B.).

Results

Study selection and study characteristics

A total of 455 articles were identified and assessed for eligibility. After removing duplicates and screening abstracts and titles, 423 studies were excluded. In the following list, 15 studies were excluded based on the full-text analysis. Overall, 17 studies (Araujo *et al*., 2013a; Araujo *et al*., 2013b; Araújo *et al*., 2014; Dionisio *et al*., 2019; Goncalves-Zillo *et al*., 2013; Li *et al*., 2019; Matos *et al*., 2013; Matos *et al*., 2014; Matos *et al*., 2015; Matos *et al*., 2016; Matos *et al*., 2019; Moura *et al*., 2016; Mulinari-Santos *et al*., 2019; Oliveira *et al*., 2019; Queiroz-Junior *et al*., 2015; Santos *et al*., 2015; Suda *et al*., 2013) were included in the qualitative synthesis (Fig. 2). Of these, 11 studies reported the primary outcome with extractable quantitative data and were included in the network meta-analysis (Araujo *et al*., 2013a; Araujo *et al*., 2013b; Araújo *et al*., 2014; Dionisio *et al*., 2019; Goncalves-Zillo *et al*., 2013; Li *et al*., 2019; Mulinari-Santos *et al*., 2019; Oliveira *et al*., 2019; Queiroz-Junior *et al*., 2015; Santos *et al*., 2015; Suda *et al*., 2013). In the secondary outcome, quantitative synthesis was assessed – based on at least three reports – and included studies with extractable quantitative data: malondialdehyde (MDA), myeloperoxidase (MPO), glutathione (GSH), IL-1β, IL-10, TNF-α, RANKL and number of tartrate-resistant acid phosphatase positive (TRAP+) cells/osteoclasts. Based on these data, 11 studies were included in the pair-wise meta-analysis (Araujo *et al*., 2013b; Araújo *et al*., 2014; Li *et al*., 2019; Matos *et al*., 2013; Matos *et al*., 2014; Matos *et al*., 2015; Moura *et al*., 2016; Oliveira *et al*., 2019; Queiroz-Junior *et al*., 2015; Suda *et al*., 2013). Two studies did not report on the sample sizes (Matos *et al*., 2016; Matos *et al*., 2019). No answer was received on contacting the authors, thus these two studies were only included in the qualitative synthesis. The resulted value of κ statistic test to evaluate the agreement between the reviewers was 0.92, indicating an excellent agreement.

Baseline characteristics

All studies included in this systematic review were published after 2013, each reporting data from between 10 and 40 animals. Data from an overall sample of 390 animals were assessed, but noting also that two of the studies did not report on sample sizes. 6 animal model studies reported data from mice and 12 from rats. One study reported data from both mice and rats. The baseline characteristics of the included studies are shown in Table 2.

Qualitative synthesis of study characteristics

Seven studies used the application of a ligature to induce periodontitis (ligature induced experimental periodontitis), 2 studies used an infection with *Porphyromonas gingivalis*, 1 study used an infection with *Aggregatibacter actinomycetemcomitans*, 5 studies used lipopolysaccharide (LPS) application, 1 study compared physiological conditions and 1 study used orthodontic force application as the examination method (Table 3). The ligature induced periodontitis model was based on the placement of a sterile nylon thread or silk ligature around the submarginal position of the maxillary or mandibular molars. For the bacteria-induced periodontitis model with *Porphyromonas gingivalis*, the strain was grown under anaerobic conditions at 37 °C. Li *et al*. then added sterile 2 % (w/v) carboxymethylcellulose to the bacteria, mixed the suspension, and administered

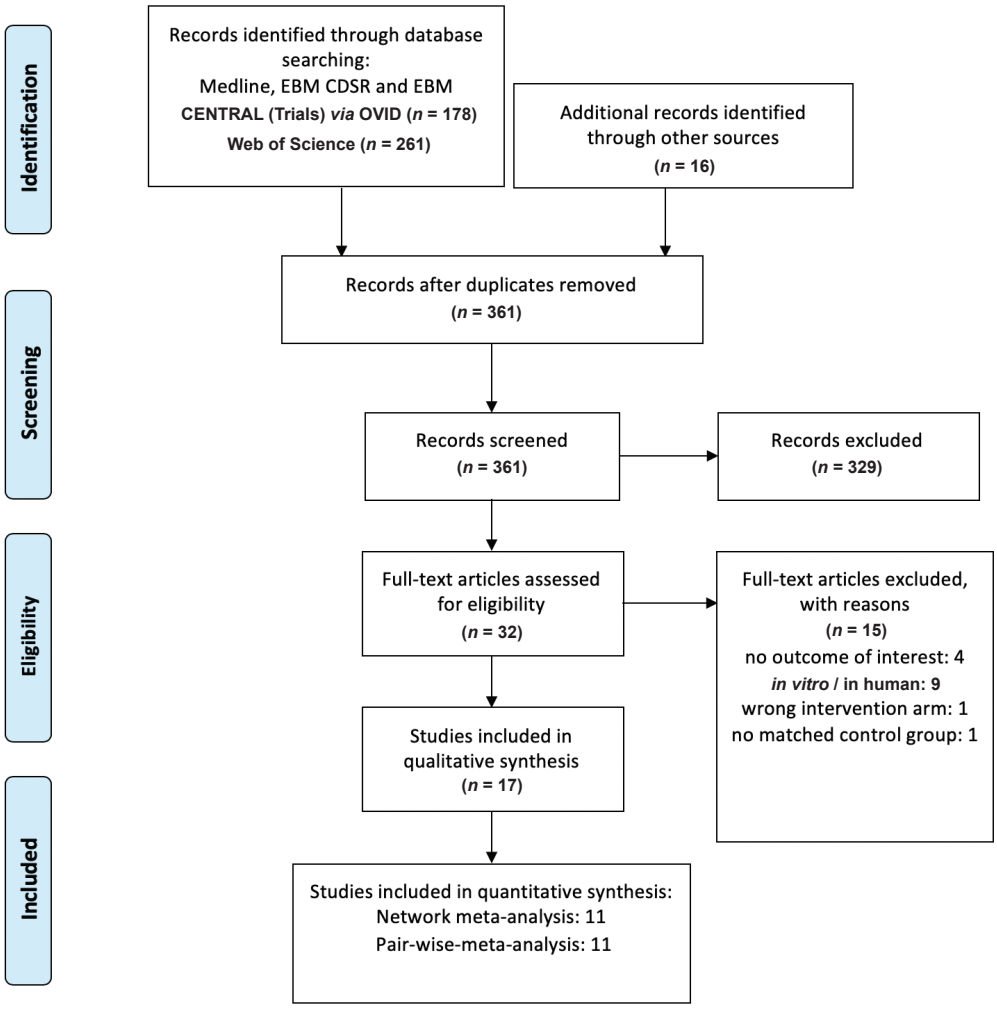

Fig. 2. PRISMA flow diagram of the selection process.

Table 2. Baseline characteristics of the included studies.

Animal model: Rat							
Reference	**Country**	**Strain/animal**	**Sex**	**Age**	**Weight**	**Total number: control and intervention**	**Ethics committee**
Araújo *et al.*, 2013	BRA	Wistar	M	NA	180–220 g	40	Yes
Araújo *et al.*, 2013	BRA	Wistar	M	NA	180-220 g	40	Yes
Araújo *et al.*, 2014	BRA	Wistar	M	NA	180-220 g	40	Yes
Dionísio *et al.*, 2019	BRA	Wistar	M	60-90 d	320-400 g	15	Yes
Gonçalves-Zillo *et al.*, 2013	BRA	Wistar	M	12 weeks	NA	21	Yes
Matos *et al.*, 2013	VEN	Sprague-Dawley	M	NA	280–300 g	10	Yes
Matos *et al.*, 2014	VEN	SpragueDawley	NA	NA	280–300 g	27	Yes
Matos *et al.*, 2015	VEN	SpragueDawley	M	NA	280–300 g	27	Yes
Matos *et al.*, 2016	VEN	SpragueDawley	M	NA	280–300 g	NA	Yes
Matos *et al.*, 2019	VEN	Sprague-Dawley	M	NA	280–300 g	NA	Yes
Mulinari-Santos *et al.*, 2016	BRA	Wistar	M	16 weeks	250-300 g	16	Yes
Santos *et al.*, 2015	BRA	Wistar	M	50-64 d	196-270 g	20	Yes
Animal model: Mouse							
Reference	**Country**	**Strain/animal**	**Sex**	**Age**	**Weight**	**Total number: control and intervention**	**Ethics committee**
Gonçalves-Zillo *et al.*, 2013	BRA	C57BL/6	M	12 weeks	NA	16	Yes
Li *et al.*, 2019	CHN	$Nos3^{-/-}$	F	7-8 weeks	NA	30	Yes
Moura *et al.*, 2016	BRA	C57BL/6J	M	10 weeks	NA	40	Yes
Oliveira *et al.*, 2019	BRA	Balb/c	M	NA	30 ± 5 g	12	Yes
Queiroz-Junior *et al.*, 2015	BRA	C57BL/6J and Balb/c	M	NA	NA	20	Yes
Suda *et al.*, 2013	JPN	NA	M	6 weeks	NA	16	Yes

Table 3. Qualitative synthesis of study characteristics and outcomes. ?: only significant results are presented. *: InterventionA: treatment started on the same day as EP induction; InterventionB: animals previously treated with Losartan for 30 d followed by induction of EP for 14 d. #: control and intervention with primary hypertension as baseline characteristic; i.d.w. = in drinking water. %: compression and tension sites were investigated separately after 6 d and 12 d for histopathologic assessment and after 0 h and 12 h for molecular assessment; outcomes reported are shown when appeared in either compression or tension site. +: mice and rats with matches control groups; i.d.w. = in drinking water.

Author (year)	Study Groups	Experimental method	Intervention arm	Outcome	Results[?]	Observation time
Araújo (2013)	Control: 10 InterventionA (1 mg/kg): 10 InterventionB (6 mg/kg): 10 InterventionC (10 mg/kg): 10	Experimental periodontitis (ligature induced)	Olmesartan	Alveolar bone loss, histological analysis of the inflammatory response, inflammation markers and bone-remodelling markers	InterventionB: COX-2↓, MMP-2↓, MMP-9↓, RANKL↓, RANK-L↓, MPO↓, IL-1β↓, Alveolar bone loss↓, OPG↑ InterventionA-C: TNF-α↓, MDA↓	11 d
Araújo (2013)	Control: 10 InterventionA (1 mg/kg/d): 10 InterventionB (5 mg/kg/d): 10 InterventionC (10 mg/kg/d): 10	Experimental periodontitis (ligature induced)	Telmisartan	Alveolar bone loss histological analysis of the inflammatory response, Inflammation markers and bone-remodelling proteins	InterventionC: Alveolar Bone loss↓, COX-2↓, MMP-2↓, MMP-9↓, RANK-L↓, RANKL↓, OPG↑, MPO↓, IL-1β↓, MDA↓ InterventionA-C: TNF-α↓	11 d
Araújo (2014)	Control: 10 InterventionA(1 mg/kg/d): 10 InterventionB(5 mg/kg/d): 10 InterventionC(10 mg/kg/d): 10	Experimental periodontitis (ligature induced)	Azilsartan	Alveolar bone loss histological analysis of the inflammatory response inflammation markers and bone-remodelling proteins	InterventionA-C: Alveolar bone loss↓; InterventionB: MMP-2↓, MMP-9↓, COX-2↓, RANK-L↓, RANK↓, CathepsinK↓, OPG↑, MPO↓, IL-1β↓, IL-10↑	11 d
Dionísio (2019)[*]	Control: 5 InterventionA: 5 (50 mg/kg/d) InterventionB: 5 (50 mg/kg/d)	Experimental periodontitis (ligature induced)	Losartan	Alveolar bone loss, histological analysis of inflammatory response, number of TRAP+ cells, inflammation markers and tRAS components	InterventionA-B: AT1b receptor↑, TNF-α↓, IL-1-β↓, MMP-2↓, MMP-9↓, IL-6↓, IL-17↓, MCP-1↓, MIP-1-α↓, IFN-γ↓, COX-2↓, VEGF↓, VEGFR1↓, RANK-L↓, TRAP+ cells↓, InterventionB: -increase in bone volume -reduction of the inflammatory score -blocked increase of AGT, ACE -blocked reduction of SOD, GPx, Cat	Intervention A: 14 d B: 44 d
Gonçalves-Zillo (2013)[+]	Control Rats: 7 Control Mice: 8 Intervention-Rats-A: 7 (12 mg/L i.d.w.) Intervention-Rats-B: 7 (60 mg/L i.d.w) Intervention-Mice: 8 (12 mg/L i.d.w)	Experimental periodontitis (ligature induced)	Enalapril	Periodontal bone loss (histometric measurement of furcation region)	Less bone losses in all intervention groups	21 d
Li (2019)[#]	Control: 15 Intervention*: 15 (0.6 g/L i.d.w.)	Experimental periodontitis (by bacterial infection with *Porphyromonas gingivalis*)	Losartan	Periodontal bone loss, number of immune-related cells, inflammatory markers	Periodontal bone loss↓, TRAP+ cells↓, CD11c-positive-cells↓, TLR4-positive-cells↓, TNF-α-mRNA↓, TLR4-mRNA↓, Ang II↓	8 weeks
Matos (2013)	Control: 6 Intervention: 4 (10 mg/kg/d)	Experimental periodontitis (by LPS administration)	Valsartan	Inflammatory marker, marker for oxidative stress	MDA↓	7 d
Matos (2014)	Control: 14 Intervention: 13	Experimental periodontitis (by LPS administration)	Valsartan	Number of osteoclasts/area (TRAP+ cell counting), bone remodelling proteins	Number of osteoclasts/area↓, RANK-L↓	7 d
Matos (2015)	Control: 14 Intervention: 13 (10 mg/kg/d)	Experimental periodontitis (by LPS administration)	Valsartan	Number of osteoclasts/area (TRAP+ cell counting), alveolar bone loss (histological examination)	Number of osteoclasts/area↓, alveolar bone loss↓	7 d
Matos (2016)	Control: NR Intervention: NR (10 mg/kg/d)	Experimental periodontitis (by LPS administration)	Valsartan	Inflammatory markers	CRP↓, IL-1α↓	7 d
Matos (2019)	Control: NR Intervention: NR (10 mg/kg/d)	Experimental periodontitis (by LPS administration)	Valsartan	Inflammatory markers (CRP, IL-17, IL-4, MIP-3α, RANTES)	CRP↓	7 d
Moura (2016)[%]	ControlA: 5 ControlB: 5 ControlC: 5 ControlD: 5 InterventionA: 5 (10 mg/kg/d) InterventionB: 5 (10 mg/kg/d) InterventionC: 5 (10 mg/kg/d) InterventionD: 5 (10 mg/kg/d)	Orthodontic force application	Losartan	Number of osteoclasts/area (TRAP+ cell counting), bone remodelling proteins	Number of osteoclasts/area↓,RANK↓, Rank-L↓, Cathepsin-K↓, MMP-13↓, MMP-2↑, periostin↑, ALP↑, SEMA3A↑, OPG↑,	6 or 12 d for histopathologic assays 0 or 12 h for molecular analysis
Mulinari-Santos (2016)	Control: 8 intervention: 8 (30 mg/kg/d)	Physiological condition	Losartan	Alveolar bone dynamics (bone volume formed, mineralized surface, active surface of mineralization, bone formation rate, mineral apposition rate)	Alveolar bone dynamics↑	67 d
Oliveira (2019)	Control: 6 Intervention: 6 (50 mg/kg/d)	Experimental periodontitis (ligature induced)	Aliskiren	Inflammatory markers (CRP, Il-1β, CXCL2, CCL8), tRAS components (AGT, ACE, AT1R, AT2R, Mas1), gene expression of tissue components (Col1a1, Col3a1, Col1a2, Fn1, tgfb1), periodontal bone loss (histometric measurement of the furcation region)	Periodontal bone loss↓, CCL8↓, AGT↓, AT1R↓, ACE↓, AT2R↓, Mas1↓, Col1a1↓, Col3a1↓, Igfb1↓, Fn1↓	15 d
Queiroz-Junior (2015)	Control: 10 Intervention: 10 (10 mg/kg/d)	Experimental periodontitis (by infection with *A. actinomycetemcomitans*)	Losartan	Alveolar bone loss (quantitative morphometric analysis; cementoenamel junction to alveolar bone crest in mm²), number of osteoclasts (TRAP+ cell counting), MPO-activity, inflammatory markers (TNF-α, IFN-γ, IL-17, IL-10, CXCL-1) bone remodelling markers (RANK-L, OPG)	Alveolar bone loss↓, number of osteoclasts↓, MPO↓, IFN-γ↓, IL-17↓, CXCL-1↓, RANK-L↓, OPG↓, RANK-L/OPG↓	30 d
Santos (2015)	Control: 5 Aliskiren-Group: 5 (30 mg/kg/d) Enalapril-Group: 5 (10 mg/kg/d) Losartan-Group: 5 (50 mg/kg/d)	Experimental periodontitis (ligature induced)	Enalapril, Losartan, Aliskiren	Periodontal Bone loss (quantitative morphometric analysis; cemento-enamel junction to alveolar bone crest in mm²), inflammatory markers, tRAS components (AGT, AT1aR, AT1bR, AT2R, Renin, ACE, ACE2, MasR)	Losartan-Group: AT2R↑, periodontal bone loss↓ Aliskiren-Group: periodontal bone loss↓	14 d

100 µL (5×10^9 cells/mL) by oral and anal topical application (Li *et al*., 2019). In contrast, all animals in the study conducted by Suda *et al*. were treated with antibiotics for 10 d (sulphamethoxazole trimethoprim at 0.08 - 0.016 % in the drinking water *ad libitum*) and were infected with the *Porphyromonas gingivalis* strain (10^9 colony-forming units in 0.2 mL of phosphate-buffered saline and 2 % carboxymethylcellulose) by gavage on the maxillary gingiva (Suda *et al*., 2013). The orthodontic force application method was conducted by bonding a nickel-titanium coil spring by light-cured resin between the maxillary first molar and both maxillary incisors. The apparatus was then calibrated to exert a force of approximately 0.34 N (Moura *et al*., 2016). Endotoxin-induced periodontitis was accomplished by injections of 10 µL (1 mg/mL) chromatographically purified *Escherichia coli* LPS into the gingiva. The pharmacological intervention arm ARB was used by 15 studies, both ACE-inhibitors and renin-inhibitors were used by 2 studies. One study compared all tRAS inhibitors in the experimental setup. Aliskiren was examined solely as a renin-inhibitor, whereas Enalapril was investigated as ACE-inhibitor. For the ARB class, Olmesartan, Telmisartan, Azilsartan, Valsartan and Losartan were examined. Three studies (Araujo *et al*., 2013b; Araújo *et al*., 2014) investigated the dose-dependent effects of the ARBs and 1 study (Goncalves-Zillo *et al*., 2013) the dose-dependent effect of ACE-inhibitor on the outcomes of interest. The duration of the experiments ranged from 12 h to 67 d, with 11 studies reporting results between 12 h and 15 d and 6 studies reporting results between 21 d and 67 d.

One of the 2 intervention groups examined by Dionisio *et al*. had previously been treated with Losartan for 30 d before induction of experimental periodontitis. This was also included in our synthesis to examine the effects on the outcomes of interest (Dionisio *et al*., 2019). Li *et al*. examined these outcomes in primary hypertensive mice, in both the intervention and matched control group (Li *et al*., 2019). Moura *et al*. examined them separately at tension and compression sites, after orthodontic force application in separate intervention groups and matched control groups, the intervention groups being evaluated as separate experiments (Moura *et al*., 2016).

Qualitative synthesis of the primary outcome

ARBs and periodontal bone loss

Three studies conducted by Araujo *et al*. examined the impact of different ARBs (Olmesartan, Telmisartan and Azilsartan), with different doses in the experimental ligature induced periodontitis setup, on periodontal bone loss as measured by digital photography and image analysis software (Araujo *et al*., 2013a; Araujo *et al*., 2013b; Araújo *et al*., 2014). Bone loss in the Olmesartan group, with doses of 6 mg/kg/d, was significantly reduced compared to the untreated ligated group (4.03 ± 0.17 *vs.* 7.02 ± 0.17 mm). The Olmesartan groups – with doses of 1 mg/kg/d and 10 mg/kg/d, respectively – also showed lower bone losses; however, these findings were not significant. With 10 mg/kg/d Telmisartan, bone loss was significantly lower compared to the untreated ligated group (4.1 ± 0.8 *vs.* 7.02 ± 0.17 mm). The Telmisartan groups with doses of 1 mg/kg/d and 5 mg/kg/d also showed lower bone losses; however, these findings were not significant. With 5 mg/kg/d Azilsartan, bone loss was significantly lower compared to the untreated ligated group (2.5 ± 1.9 *vs.* 4.6 ± 1.4 mm). The Azilsartan groups with doses of 1 mg/kg/d and 10 mg/kg/d, respectively, also showed lower bone losses; however, these findings were not significant. Dionisio *et al*. examined the impact of Losartan on bone volume, after experimental ligature induced periodontitis in two intervention groups, as measured by computer tomography (Dionisio *et al*., 2019). One of the intervention groups was pre-treated with Losartan (50 mg/kg/d) for 30 d before and for a further 14 d after induction of experimental periodontitis, and one group was treated with Losartan (50 mg/kg/d) simultaneously with experimental periodontitis induction, lasting 14 d from then on. The intervention group with Losartan treatment, simultaneous to experimental periodontitis induction, showed higher bone volumes after 14 d; however, this finding was not significant. The intervention group with Losartan pre-treatment showed significantly higher bone volumes after 14 d compared to the untreated group (65.08 ± 6.92 *vs.* 37.4 ± 8.31 cm^3). Li *et al*. examined the impact of Losartan on periodontal bone loss, as measured by digital microscopy and an image analysis software, in an experimental periodontitis model induced by bacterial infection with *Porphyromonas gingivalis* (Li *et al*., 2019). Both intervention and matched control group had primary hypertension as a shared baseline characteristic. Treatment with Losartan resulted in significantly lower bone loss measurements when compared to the untreated group (0.82 ± 0.18 *vs.* 1.83 ± 0.1 mm^2). In the same study, primary hypertensive mice without experimental periodontitis showed higher, but not significant, bone resorption compared to the non-hypertensive mice and this could also not be reduced through Losartan application. Thus, the lower bone losses through Losartan applications were only seen in the experimental periodontitis groups. Matos *et al*. examined the impact of Valsartan in rats with periodontitis, induced by four injections of LPS with *Escherichia coli*, within the experimental period of 7 d (Matos *et al*., 2015). Periodontal bone loss was measured by digital microscopy and quantification of density histograms. The Valsartan treated group showed lower bone loss compared to the untreated group. Interestingly, the Valsartan treated group also showed higher bone densities when compared to the non-periodontitis group. However, no data regarding statistical significance was provided; therefore, the provided data could not be included in the quantitative synthesis and only the qualitative results of the findings were included. Mulinari-

Santos *et al*. examined the impact of Losartan on bone volumes in normal and spontaneously hypertensive rats with each having matched control groups (Mulinari-Santos *et al*., 2019). Quantitative measurements were made with confocal laser scanning microscopy and image analysis software. The bone volume formed increased significantly in the Losartan treated group as compared to the untreated normal rat group (0.071 ± 0.01 *vs.* 0.039 ± 0.003 mm^3/d). The increase in bone volume in the spontaneously hypertensive rats treated with Losartan, when compared to the untreated, was not significant. However, Losartan treated groups also showed a statistically significant increase in the mineralised surface, active surface of mineralisation, bone formation rate, and mineral apposition rate in the alveolar bone, as measured by biomarking of fluorochromes. Queiroz-Junior *et al*. examined the impact of Losartan on periodontal bone loss in an experimental periodontitis mouse model induced by palatal injection of *A. actinomycetemcomitans* suspension (Queiroz-Junior *et al*., 2015). Bone loss was quantified by digital photography and morphometric analysis with image analysis software. The Losartan treated group showed significantly lower bone losses after 30 d of experimental periodontitis as compared to the untreated group (0.297 ± 0.134 *vs.* 0.494 ± 0.249 mm^2).

ACE-inhibitor and periodontal bone loss

Goncalves-Zillo *et al*. examined the impact of Enalapril, an ACE-inhibitor, on periodontal bone loss, as measured by histometric evaluation of the furcation region after 21 d, in an experimental ligature induced periodontitis model, in both rats and mice with matched control groups (Goncalves-Zillo *et al*., 2013). The rat intervention group, with an application of 12 mg/L Enalapril in drinking water for 21 d, showed significantly lower bone losses compared to the untreated control group (0.23 ± 0.12 *vs.* 0.4 ± 0.02 mm^2). The rat intervention group, with 16 mg/L, also showed significantly lower bone losses compared to the control group (0.25 ± 0.09 *vs.* 0.4 ± 0.02 mm^2). However, the group with 16 mg/L Enalapril, but not the group with 12 mg/L in drinking water, showed a significant reduction in systolic blood pressure; thus, comparability to the matched group is only given for the 12 mg/L group. In mice, only 12 mg/L Enalapril was added to the drinking water and resulted in significantly lower bone losses after 21 d as compared to the untreated group (101.66 ± 15.33 *vs.* 129.67 ± 15.33 mm^2).

Renin-inhibitor and periodontal bone loss

Oliveira *et al*. examined the impact of Aliskiren on periodontal bone loss in an experimental ligature induced periodontitis mouse model (Oliveira *et al*., 2019). Bone loss was measured by morphometric measurements of the furcation region using image analysis software. The experiments were conducted in two intervention groups, normal and diabetic mice, with matched control groups. Aliskiren treated mice group showed a significantly higher percentage of bone area *per* μm^2 (%/μm^2) compared to the untreated normal control group (35.08 ± 3.31 *vs.* 24.24 ± 1.05 %/μm^2). This significant increase of alveolar bone area was also seen in the Aliskiren group of diabetic mice compared to the matched but untreated control group.

Comparison of different tRAS inhibitors and periodontal bone loss

The study conducted by Santos *et al*. was the only included study comparing the effects of different tRAS inhibitors, Aliskiren, Enalapril and Losartan, regarding periodontal bone loss (Santos *et al*., 2015). Experimental periodontitis was induced by ligature and the experimental observation period lasted 14 d. Aliskiren, Enalapril and Losartan were administered daily over the experimental period with doses of 10, 30 and 50 mg/kg, respectively. Bone loss measurements were conducted using digital photography and image analysis software. Bone loss in the Aliskiren treated group was significantly reduced as compared to the shared control group (2.08 ± 0.36 *vs.* 3.29 ± 0.55 mm^2). The Losartan group also showed significantly lower bone losses compared to the shared control group (2.22 ± 0.33 *vs.* 3.29 ± 0.55 mm^2). Bone loss in the Enalapril group was also lower compared to the shared control group (2.86 ± 0.38 *vs.* 3.29 ± 0.55 mm^2); however, this finding failed to reach statistical significance.

Qualitative synthesis of the secondary outcomes

Bone remodelling markers

The impact of tRAS inhibition on bone remodelling markers was analysed by 3 studies (Matos *et al*., 2014; Moura *et al*., 2016; Queiroz-Junior *et al*., 2015) (Table 3 and Fig. 3). All of these studies used ARBs as the pharmacological intervention arm and the time until examination ranged from 12 h to 30 d. Valsartan non-significantly decreased the optical immunofluorescence density (arbitrary unit (AU) as measured by confocal microscopy and image analysis software) of RANK (5391.38 ± 181.25 *vs.* 6157.99 ± 188.09 AU), RANKL (6188.41 ± 679.31 *vs.* 7333.33 ± 488.04 AU), the ratio of RANKL/OPG (1.01 ± 0.05 *vs.* 7.35 ± 1.07) and increased OPG (5632.65 ± 401.36 *vs.* 990.72 ± 208.26 AU). The study conducted by Moura *et al*. (2016) with Losartan application in a mice model of orthodontic force application and separate examination of tension and compression sites with matched control groups showed a decrease of RANK mRNA expression (AU calculated as relative fold gene expression, normalised to β-actin as the internal control; $2^{-\Delta\Delta ct}$ method) after 12 h for the tension (0.88 ± 0.09 *vs.* 1.27 ± 0.07 AU) and compression sites (1.24 ± 0.09 *vs.* 2.27 ± 0.26 AU), respectively. RANKL mRNA in tension (0.71 ± 0.05 *vs.* 0.74 ± 0.09 AU) and compression sites (1.87 ± 0.09 *vs.* 2.76 ± 0.23 AU) also decreased; however, only the decrease at the compression sites was significant.

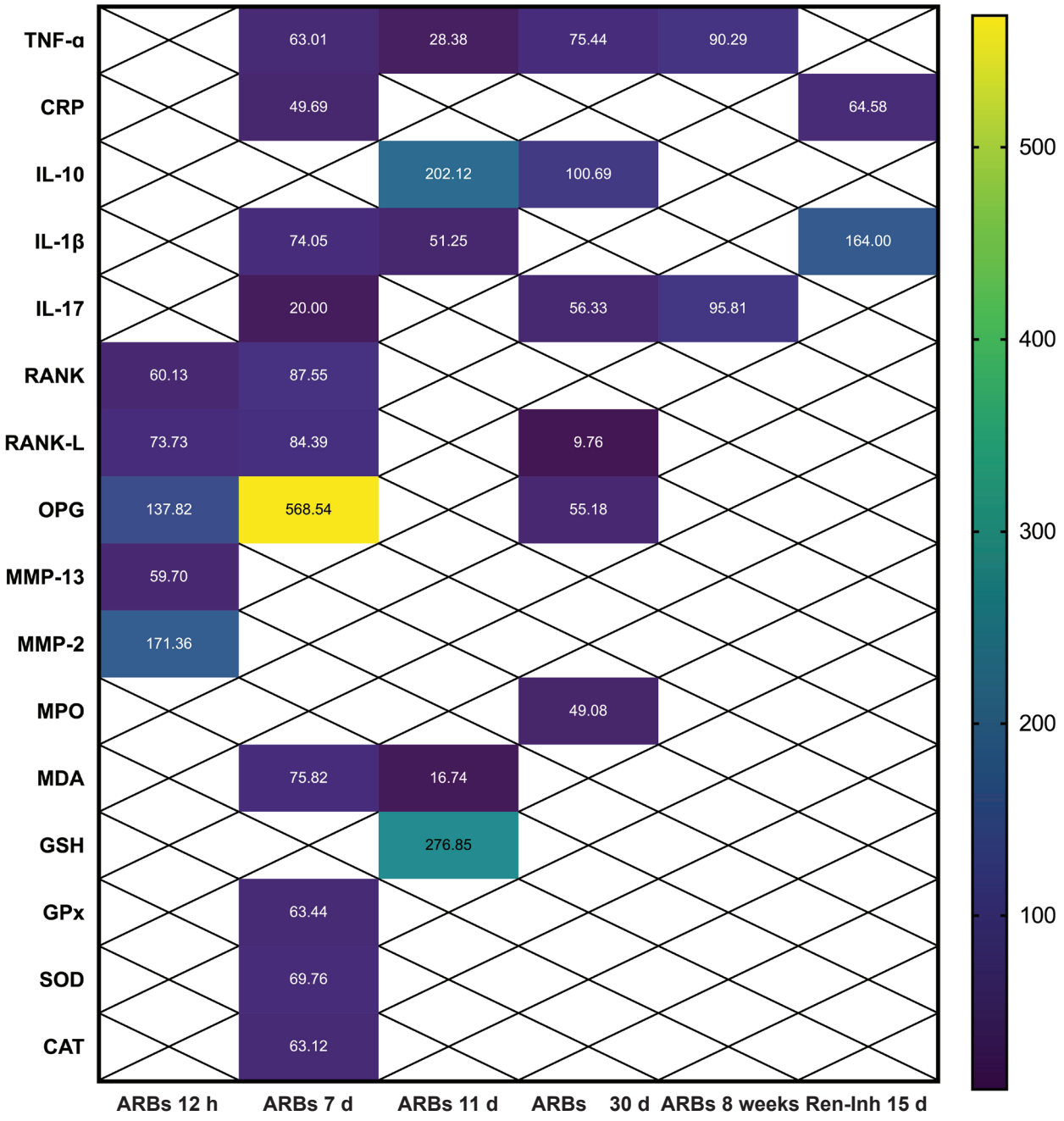

Fig. 3. Heatmap illustration of secondary outcomes. Outcomes after intervention are shown as a percentage (%) of the reference value (untreated control group). CRP = c-reactive protein; MPO = myeloperoxidase; MDA = malondialdehyde; GSH = glutathione; GPx = glutathione peroxidase; SOD = superoxide dismutase; CAT = catalase.

Furthermore, Losartan decreased mRNA expression of cathepsin K for the tension (1.17 ± 0.11 *vs.* 1.25 ± 0.19 AU) and compression (2.15 ± 0.17 *vs.* 3.5 ± 0.29 AU) sites and increased OPG mRNA expression in the tension (2.05 ± 0.1 *vs.* 1.5 ± 0.14 AU) and compression (2.92 ± 0.09 *vs.* 2.1 ± 0.15 AU) sites, respectively. However, for cathepsin K only the tension site and for OPG only the compression site showed statistical significance. The application of Losartan for 30 d in the study conducted by Queiroz *et al.* (2015) resulted in a significant decrease of RANKL (103.00 ± 213.76 *vs.* 1055.79 ± 967.01 pg/100 mg tissue) and the RANKL/OPG ratio (0.06 ± 0.09 *vs.* 0.37 ± 0.30) and a significant increase of OPG (1676.59 ± 1534.04 *vs.* 3038.29 ± 915.04 pg/100 mg tissue).

Number of osteoclasts

The impact of ARBs on the number of tartrate-resistant acid phosphatase positive (TRAP+) cells was examined by 5 studies (Li *et al.*, 2019; Matos *et al.*, 2014; Matos *et al.*, 2015; Moura *et al.*, 2016; Queiroz-Junior *et al.*, 2015). The study conducted by Matos *et al.* reported a significantly lower number of osteoclasts/area, measured as the number of osteoclasts *per* oil-immersion field (OCs/field), after Valsartan intervention and examination after 7 d compared to

the untreated group (2.54 ± 0.51 *vs.* 5.21 ± 1.11 OCs/field). The study published by Matos *et al*. (2015), with the same experimental setup, showed similar results confirming previous data. Losartan application for 8 weeks in the study conducted by Li *et al*. showed a significantly lower number of osteoclasts/area (OCs/mm^2), compared to the untreated group (30.79 ± 5.73 *vs.* 87.83 ± 6.92 OCs/mm^2) (Li *et al*., 2019). Losartan application also resulted in a significantly lower number of osteoclasts/area (shown as osteoclasts *per* histologically examined 5 μm-thick sagittal sections of the periodontal tissue; OCs/section) after 6 d (3.58 ± 0.59 *vs.* 8.18 ± 0.54) and 12 d (4.97 ± 1.77 *vs.* 13.74 ± 1.39) in the study conducted by Moura *et al*. (Moura *et al*., 2016). Queiroz *et al*. reported a significantly lower number of osteoclasts/area (measured as osteoclasts in five consecutive microscopic fields (400×) *per* section; OCs/section) after 30 d of Losartan application compared to the untreated control group (0.55 ± 0.53 *vs.* 1.85 ± 1.00) (Queiroz-Junior *et al*., 2015). In contrast to these findings, Suda *et al*. examined the appearance of osteoclasts by immunostaining of cathepsin K and reported a non-significant decrease of osteoclast surface/bone surface (%) compared to the untreated group (4.95 ± 2.78 % *vs.* 5.55 ± 2.45 %) (Suda *et al*., 2013).

(Anti-) inflammatory markers

Ten of the included studies (Araujo *et al*., 2013a; Araujo *et al*., 2013b; Araújo *et al*., 2014; Dionisio *et al*., 2019; Li *et al*., 2019; Matos *et al*., 2014; Matos *et al*., 2016; Matos *et al*., 2019; Oliveira *et al*., 2019; Queiroz-Junior *et al*., 2015) reported at least one of the following inflammatory markers with dysregulated expression level after tRAS inhibition: TNF-α, IL-10, IL-1β, IL-17, CRP. Intervention with Telmisartan showed a dose-dependent reduction of TNF-α in the study conducted by Araujo *et al*. (Araujo *et al*., 2013a). Doses of 1, 5 and 10 mg/kg/d Telmisartan for a period of of 7 d resulted in significantly lower TNF-α concentrations (pg/mL) of 1883.66 ± 1051.17 *vs.* 5052.63 ± 2662.97, 1174.52 ± 1051.17 *vs.* 5052.63 ± 2662.97 and 686.98 ± 560.63 *vs.* 5052 ± 2662.97, respectively, compared to the untreated comparator. Suda *et al*. provided results for Telmisartan treatment with 5 mg/kg/d for 8 weeks and reported non-significant lower TNF-α concentrations compared to the untreated group (25.54 ± 1.53 *vs.* 28.29 ± 3.56 pg/mL). Similar observations were seen after Olmesartan application with doses of 1, 6 and 10mg/kg which resulted in significantly lower TNF-α concentrations (pg/mL) of 1104.06 ± 722.35 *vs.* 3629.44 ± 3090.04, 241.12 ± 321.04 *vs.* 3629.44 ± 3090.03, and 482.23 ± 963.13 *vs.* 3629.44 ± 3090.04, respectively (Araujo *et al*., 2013b). However, the lowest concentrations of TNF-α were seen in the group treated with 6 mg/kg Olmesartan. For Azilsartan the lowest TNF-α concentrations were seen in the Azilsartan group treated with 5 mg/kg (755.04 ± 674.38 *vs.* 1262.25 ± 637.92 pg/mL) (Araújo *et al*., 2014). Furthermore, the Azilsartan groups treated with 1 mg/kg (933.72 ± 947.77 *vs.* 1262.25 ± 637.92 pg/mL) and 10 mg/kg (1204.61 ± 856.64 *vs.* 1262.25 ± 637.92 pg/mL) also showed lower TNF-α concentrations, but none of these findings reached statistical significance. In the study conducted by Li *et al*., the Losartan treated group showed significantly lower TNF-α concentrations after 8 weeks compared to the untreated group (18.57 ± 1.92 *vs.* 49.53 ± 5.75 pg/mL) (Li *et al*., 2019). Similar to this, Queiroz-Junior *et al*. reported significantly lower TNF-α concentrations for the Losartan group after 30 d (307.77 ± 219.87 *vs.* 407.98 ± 148.74 pg/100 mg tissue) (Queiroz-Junior *et al*., 2015). For Valsartan, data were available from Matos *et al*. who reported non-significant lower TNF-α concentrations after 7 d of Valsartan treatment compared to the untreated group.

Data regarding IL-1β concentrations after ARB treatment with Olmesartan, Telmisartan and Azilsartan for 7 d were provided by Araujo *et al*. (Araujo *et al*., 2013a; Araujo *et al*., 2013b; Araújo *et al*., 2014). For Olmesartan, the lowest concentrations were seen in the group treated with 6 mg/kg Olmesartan (56.45 ± 32.26 *vs.* 786.29 ± 177.42 pg/mL). The Olmesartan groups treated with 1 and 6 mg/kg, respectively, also showed lower IL-1β concentrations. However, only the findings for 6 mg/kg Olmesartan reached statistical significance. For Telmisartan, the 10 mg/kg treated Telmisartan group showed the significantly lowest IL-1β concentrations (137.54 ± 203.87 *vs.* 876.79 ± 448.52 pg/mL). Telmisartan groups with 1 and 6 mg/kg also showed lower IL-1β levels, but these findings did not reach statistical significance. For Azilsartan, lower IL-1β concentrations were only seen in the 1 mg/kg (666.67 ± 121.11 *vs.* 1217.63 ± 165.29 pg/mL) and the 5 mg/kg Azilsartan group (512.4 ± 104.68 *vs.* 1217.63 ± 165.29 pg/mL), with only the latter reaching statistical significance. The IL-1β concentration of the 10 mg/kg Azilsartan group was not significantly different from the untreated control group. Matos *et al*. reported non-significant lower IL-1β concentrations after 7 d of treatment with Valsartan (Matos *et al*., 2016). Oliveira *et al*. was the only study reporting that IL-1β concentration after Aliskiren treatment, and IL-1β concentration after Aliskiren treatment for 14 d, was not significantly different compared to the untreated control group (Oliveira *et al*., 2019).

For IL-10, an anti-inflammatory marker, data were provided by 4 studies (Araujo *et al*., 2013a; Araujo *et al*., 2013b; Araújo *et al*., 2014; Queiroz-Junior *et al*., 2015). For Olmesartan and Telmisartan, treatment for 7 d with concentrations of 1, 6 and 10 mg/kg increased IL-10 concentrations in all treated groups (Araujo *et al*., 2013a; Araujo *et al*., 2013b). However, none of the findings reached statistical significance. For Azilsartan, the group treated with 5 mg/kg Azilsartan showed significantly higher IL-10 levels compared to the untreated control group (3207.76 ± 3311.19 *vs.* 814.40 ± 473.03 pg/mL) (Araújo *et al*., 2014). The groups treated with 1 and 10 mg/kg, respectively, were not significantly different from the untreated

control group. Treatment with Losartan (10 mg/kg/d) for 30 d resulted in no significant difference in IL-10 levels compared to the control group (Queiroz-Junior *et al.*, 2015).

Data regarding IL-17 concentrations after inhibition with ARBs were provided by 3 studies (Matos *et al.*, 2019; Oliveira *et al.*, 2019; Suda *et al.*, 2013). Treatment with Losartan for 30 d resulted in a significant reduction of IL-17 levels compared to the untreated control group (87.63 ± 54.36 *vs.* 155.56 ± 45.08 pg/100 mg tissue). Valsartan treatment for 7 d showed no significant reduction of IL-17 levels compared to the control group (Matos *et al.*, 2019). Further, treatment with 5 mg/kg Telmisartan for 8 weeks showed no significant differences compared to the untreated control group (Suda *et al.*, 2013).

CRP concentrations were examined after 15 d of treatment with Aliskiren (Oliveira *et al.*, 2019). CRP concentrations were non-significantly lower in the treatment group, compared to the control group (1957.49 ± 447.43 *vs.* 3031.32 ± 380.31 pg/mL). For ARBs, data was available for Valsartan which led to significantly lower CRP concentrations after 7 d compared to the untreated control group (Matos *et al.*, 2016; Matos *et al.*, 2019).

Oxidative stress markers

Myeloperoxidase (MPO), malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione (GSH) as markers for oxidative stress were only examined after inhibition with ARBs (Araujo *et al.*, 2013a; Araujo *et al.*, 2013b; Araújo *et al.*, 2014; Matos *et al.*, 2013; Queiroz-Junior *et al.*, 2015). Olmesartan treatment resulted in a significant reduction of MDA, regardless of the dose (Araujo *et al.*, 2013b). For MPO, all doses also resulted in lower levels, but only the 6 mg/kg Olmesartan group reached statistical significance (0.8 ± 1.61 *vs.* 23.74 ± 11.28 nmol/g tissue). GSH levels in all Olmesartan groups were not significantly different from the control group. Similar findings were found for Telmisartan and levels of MPO, MDA, and GSH (Araujo *et al.*, 2013a). However, only the 10 mg/kg resulted in significantly lower MDA and MPO levels. Higher levels of GSH were found for the different Telmisartan groups, but none of these findings reached statistical significance. Also, all doses of Azilsartan resulted in lower MPO levels, but only the 5 mg/kg Azilsartan group reached statistical significance (3.01 ± 1.32 *vs.* 7.54 ± 11.44 nmol/g tissue) (Araújo *et al.*, 2014). For GSH levels after Azilsartan treatment, non-significant higher GSH levels were found in the different Azilsartan groups. Valsartan treatment for 7 d resulted in significantly lower MDA levels compared to the control group (31.29 ± 1.04 *vs.* 41.27 ± 2.04 nmol/mL). Furthermore, Valsartan resulted in non-significant lower levels of SOD, CAT and GPx compared to the untreated control group. Queiroz *et al.* reported significantly lower MPO levels for the Losartan treated group, compared to the control group (Queiroz-Junior *et al.*, 2015).

Quantitative synthesis of the primary outcome

Presentation of the network geometry

Network geometry of the control group and the tRAS inhibitor classes are illustrated in Fig. 4. Four interventions were compared in this network geometry (control, ARB, ACE-inhibitor, and renin-inhibitor). Sixteen comparisons between ARBs and control, 4 comparisons between ACE-inhibitors and control, 2 comparisons between renin-inhibitor and control, 1 comparison between ARB and renin-inhibitor, 1 comparison between ARB and ACE-inhibitor and 1 comparison between renin-inhibitor and ACE inhibitor were considered. The network geometry for the different doses of tRAS inhibitors is shown in Fig. 6. Closed loops were present for Olmesartan, Telmisartan and Azilsartan (each with three different doses), Losartan and "PreLosartan" (treatment of Losartan before induction of experimental periodontitis), Enalapril (with 2 different doses) and the cross-comparison between Aliskiren, Losartan and Enalapril. *Enalapril12mg*, *Enalapril60mg* and *Losartan600mg* were given as mg/L in drinking water for the observation time. Because the authors did not provide the weight of the animals, no estimation of the values in mg/kg/d could be performed. All other doses are given in mg/kg/d during the period of the experiments.

Network interval plot and treatment ranks

Network interval plot and treatment ranks are shown in Fig. 5. There was a statistically significant difference between ARB *vs.* control (SMD = − 1.26, 95 % CI − 1.93 to − 0.58), ACE-inhibitor *vs.* control (SMD = − 1.43, 95 % CI − 2.74 to − 0.12) and renin-inhibitor *vs.* control (SMD = − 2.11, 95 % CI − 3.85 to − 0.37). Thus, all tRAS inhibitor classes resulted in lower periodontal bone loss compared to the control group in this analysis. Furthermore, no significant differences were found when comparing the different tRAS inhibitors on periodontal bone loss. According to the SUCRA ranking, the most effective tRAS inhibitor with regards to preventing periodontal bone loss was the renin-inhibitor (Aliskiren). ARBs and ACE-inhibitor (Enalapril) performed similarly and the worst was the untreated control group.

Network forest plot for the different tRAS doses

Network forest plot for the different tRAS doses is shown in Fig. 7. Statistically significant differences in SMD were seen for *Aliskren30mg vs.* control (SMD = − 2.13, 95 % CI − 3.74 to − 0.52), *Aliskren50mg vs.* control (SMD = − 1.64, 95 % CI − 3.21 to − 0.58), *Enalapril12mg vs.* control (SMD = − 1.98, 95 % CI − 3.02 to − 0.94), *Enalapril60mg vs.* control (SMD = 1.71, 95 % CI − 3.01 to − 0.42), *Losartan30mg vs.* control (SMD = − 3.88, 95 % CI − 5.86 to − 1.91), *Losartan50mg vs.* control (SMD = − 1.5, 95 % CI − 2.65 to − 0.35), *Losartan600mg vs.* control (SMD = − 6.75, 95 % CI − 8.53 to − 4.64), *PreLosartan50mg vs.* control (SMD = − 1.59, 95 % CI − 3.03 to − 0.14). Overall, most of the intervention groups performed better than the

control group and *Losartan600mg* group performed significantly better than all other comparators. In addition, *PreLosartan50mg* performed better than the control group but SMD was not significantly different when comparing to other tRAS inhibitor groups. Furthermore, significant differences were found comparing *Azilsartan10mg vs. Aliskren30mg* (SMD = 2.27, 95 % CI 0.28 to 4.26), *Losartan600mg vs. Aliskren30mg* (SMD = − 4.62, 95 % CI − 7.27 to − 1.97), *Losartan600mg vs. Aliskren50mg* (SMD = − 5.11, 95 % CI − 7.74 to − 2.48), *Azilsartan6mg vs. Azilsartan10mg* (SMD = − 1.25, 95 % CI − 2.45 to − 0.44), *Enalapril12mg vs. Azilsartan10mg* (SMD = − 2.13, 95 % CI − 3.69 to − 0.56), *Enalapril 60mg vs. Azilsartan10mg* (SMD = − 1.86, 95 % CI − 3.59 to − 0.12), *Losartan30mg vs. Azilsartan10mg* (SMD = − 4.03, 95 % CI − 6.32 to − 1.73), *Losartan50mg vs.*

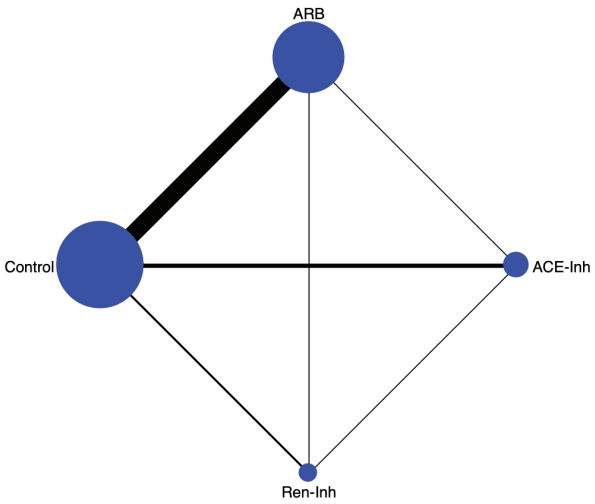

Fig. 4. Network geometry for inhibitor types and the outcome periodontal bone loss. Nodes represent the inhibitor types and edges represent the comparisons. The thickness of lines and nodes represent the number of reporting studies. ARB = Angiotensin II type 1 receptor blocker; ACE-Inh = Angiotensin-Converting-Enzyme inhibitor; Ren-Inh = Renin inhibitor.

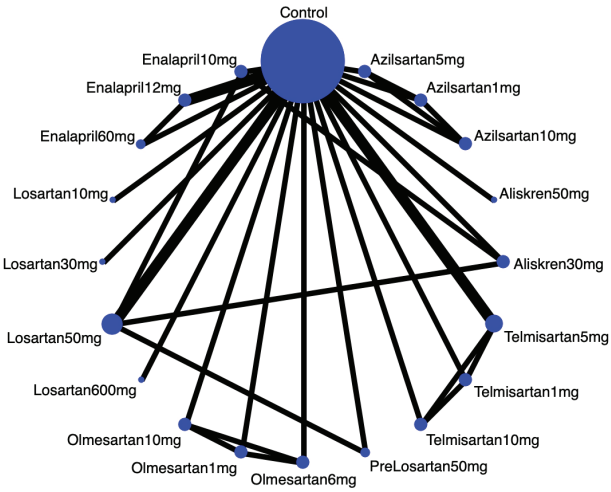

Fig. 6. Network geometry for inhibitor doses and the outcome periodontal bone loss. Nodes represent the inhibitor types and doses and edges represent the comparisons. The thickness of lines and nodes represent the number of reporting studies. *Losartan600mg*, *Enalapril12mg*, and *Enalapril60mg* are given as mg/L in drinking water for the duration of the experiment. All other values are shown as mg/kg/d for the duration of the experiment.

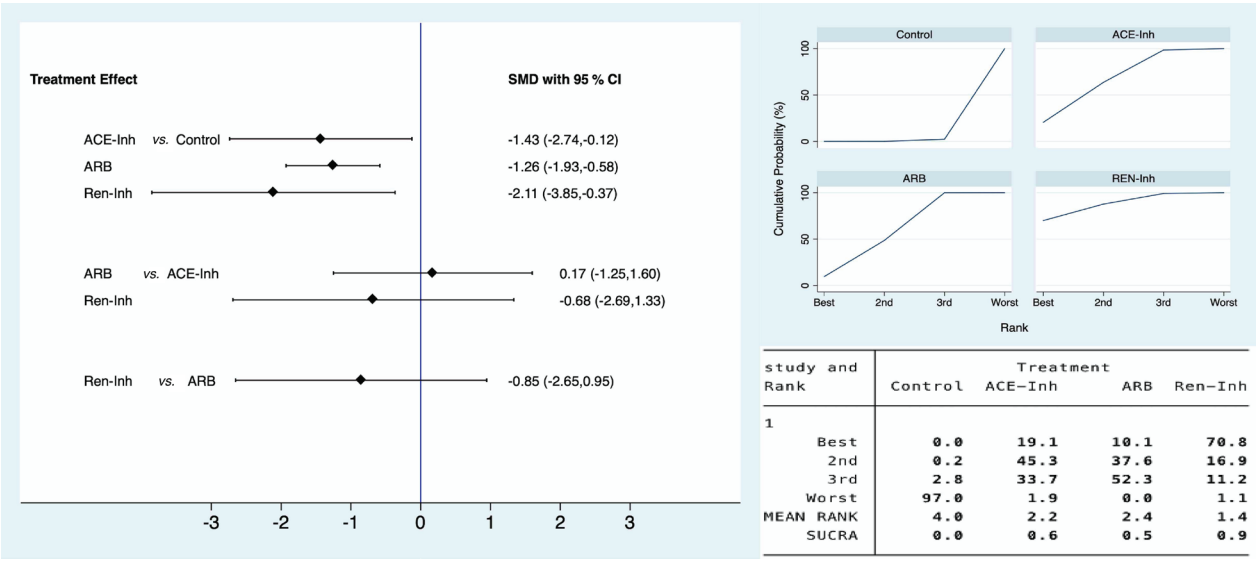

Fig. 5. Network interval plot showing the treatment effect of tRAS inhibitors and the comparator. The effect size (SMD) is shown with its 95 % confidence interval. Angiotensin II type 1 receptor blockers (ARB), angiotensin-converting-enzyme inhibitors (ACE-Inh) and renin inhibitors (Ren-Inh) performed significantly better than the untreated comparator (SMD lower than 0 ("line of null effect") favours the intervention arm), resulting in lower periodontal bone losses. SUCRA plot and treatment rankings are showing the probability of a given treatment (or control) to be best, second, third, and worst in preventing periodontal bone loss. (x-axis: ranking of treatment; y-axis: probability of the given treatment (or control); SUCRA = surface under the cumulative ranking curve).

Azilsartan10mg (SMD = − 1.64, 95 % CI − 3.28 to − 0.01), *Losartan600mg vs. Azilsartan10mg* (SMD = − 6.89, 95 % CI − 9.29 to − 4.49), *Losartan30mg vs. Azilsartan1mg* (SMD = − 3.09, 95 % CI − 5.39 to − 0.79), *Losartan600mg vs. Azilsartan1mg* (SMD = − 5.96, 95 % CI − 8.37 to − 3.55), *Losartan30mg vs. Azilsartan6mg* (SMD = − 2.78, 95 % CI − 5.09 to − 0.47), *Losartan600mg vs. Azilsartan6mg* (SMD = − 5.64, 95 % CI − 8.07 to − 3.23), *Losartan30mg vs. Enalapril10mg* (SMD = − 3.35, 95 % CI − 5.76 to − 0.94), *Losartan600mg vs. Enalapril110mg* (SMD = − 6.24, 95 % CI − 8.73 to − 3.70), *Losartan600mg vs. Enalapril12mg* (SMD = − 4.77, 95 % CI − 7.12 to − 2.42), *Olmesartan10mg vs. Enalapril12mg* (SMD = 1.61, 95 % CI 0.04 to 3.17), *Telmisartan10mg vs. Enalapril12mg* (SMD = 1.64, 95 % CI 0.08 to 3.21), *Telmisartan5mg vs. Enalapril12mg* (SMD = 1.85, 95 % CI 0.23 to 3.47), *Losartan600mg vs. Enalapril60mg* (SMD = −5.04, 95 % CI 7.51 to -2.57), *Losartan 30mg vs. Losartan10mg* (SMD=-2.95, 95 % CI − 5.26 to − 0.63), *Losartan600mg vs. Losartan10mg* (SMD = − 5.81, 95 % CI − 8.24 to − 3.38), and *Losartan50mg vs. Losartan30mg* (SMD = 2.38, 95 % CI 0.97 to 4.67).

Quantitative synthesis of the secondary outcomes

Results of the pairwise meta-analysis of the secondary outcomes reported by at least 3 studies are shown in Fig. 8. The Intervention groups reporting outcomes from different tissue sites in the study conducted by Moura *et al.* were included separately ("Moura2016a" and "Moura2016b"). Overall, 11 studies (Araujo *et al.*, 2013a; Araujo *et al.*, 2013b; Araújo *et al.*, 2014; Li *et al.*, 2019; Matos *et al.*, 2013; Matos *et al.*, 2014; Matos *et al.*, 2015; Moura *et al.*, 2016; Oliveira *et al.*, 2019; Queiroz-Junior *et al.*, 2015; Suda *et al.*, 2013), 12 experimental intervention groups and 8 outcomes (IL-10, IL-1β, TNF-α, MDA, MPO, GSH, RANKL and number of TRAP+ cells/osteoclasts) were included in the pairwise meta-analysis. For the oxidative marker MDA, a significant difference was found for pooled Olmesartan (SMD = − 4.71, 95 % CI − 6.01 to − 3.41) and Telmisartan (SMD = − 5.11, 95 % CI − 6.49 to − 3.73) doses, for Valsartan (SMD = − 5.20, 95 % CI − 8.39 to − 2.01) and for the combined ARBs (SMD = − 4.92, 95 % CI − 5.83 to − 4.02). Only the pooled Telmisartan groups (SMD = 0.89, 95 % CI 0.15 to 1.63) and the combined ARBs (SMD = 0.62, 95 % CI 0.19 to 1.04) increased the antioxidative marker GSH. For MPO, all but the pooled Azilsartan groups resulted in a significantly lower MPO level leading to a significant overall effect (SMD = − 1.18, 95 % CI − 1.81 to − 0.54). Regarding IL-1β, pooled Olmesartan and Telmisartan groups, respectively, were significantly different from

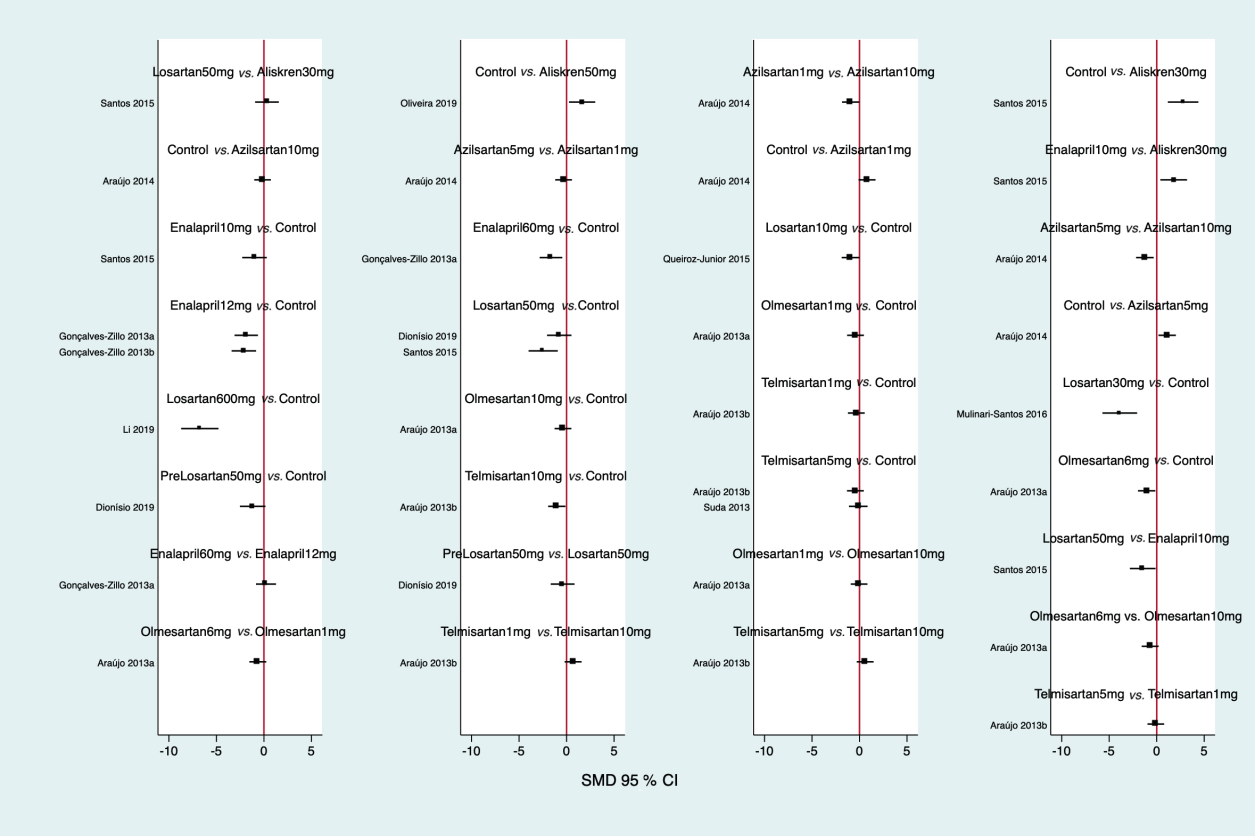

Fig. 7. Network forest plot of the network meta-analysis for the different doses of tRAS inhibitors and the comparator. The effect size (SMD) is shown with its 95 % confidence interval. An SMD under 0 ("line of null effect") favours the intervention for the prevention of periodontal bone loss (How to read: A *vs.* B; A = Intervention, B = Comparator). *Losartan600mg*, *Enalapril12mg*, and *Enalapril60mg* are given as mg/L in drinking water for the duration of the experiment. All other values are shown as mg/kg/d for the duration of the experiment.

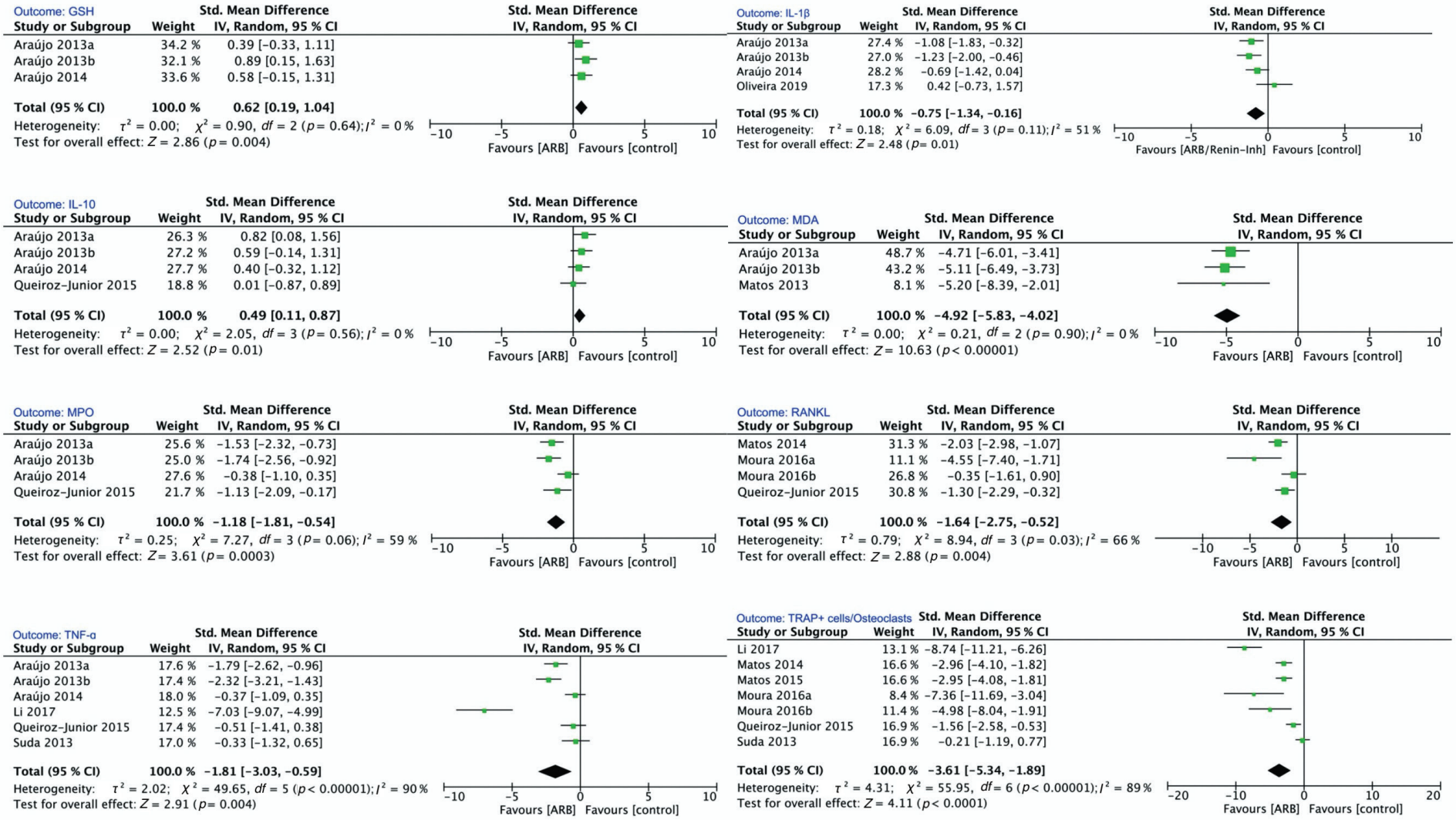

Fig. 8. Forest plots showing the SMD and 95 % CI for tRAS inhibitors *versus* comparators for the secondary outcomes included in the pairwise meta-analysis. The SMDs and 95 % CI for each study, pooled SMD for each outcome, weights of the studies in the pairwise meta-analysis, and results for the heterogeneity tests are shown. An SMD under 0 ("line of null effect") favours the intervention (lower values for the intervention group compared to the control group for the respective secondary outcome). GSH = glutathione; MDA = malondialdehyde; MPO = myeloperoxidase; TRAP+cells = tartrate-resistant acid phosphatase positive cells (osteoclasts); ARB =angiotensin II type 1 receptor inhibitor; Renin-Inh = renin inhibitor.

the control group. Further, the combined groups of tRAS inhibitors were significantly different from the control group (SMD = − 0.75, 95 % CI − 1.34 to − 0.16), but Aliskiren alone was not significantly different from the control group. For IL-10 only the pooled Olmesartan group and the combined ARBs reached statistical significance (SMD = 0.49, 95 % CI 0.11 to 0.87). The Losartan group reported by Li *et al.* showed the strongest significant difference regarding TNF-α levels (SMD = − 7.03, 95 % CI − 9.07 to - 4.99). Additionally, pooled Olmesartan and Telmisartan groups showed significant differences compared to the control group, leading to a significant overall effect of the ARBs (SMD = − 1.81, 95 % CI − 3.03 to − 0.59). All, but the Telmisartan group resulted in a significantly lower TRAP+ cell/osteoclast count compared to the untreated control group. The overall effect of the pooled ARBs was significantly different from that of the control group (SMD = − 3.61, 95 % CI − 5.34 to − 1.89). Finally, data for RANKL presented a significant difference for Valsartan (10 mg/kg/d for 7 d), Losartan (10 mg/kg/d for 12 h) and Losartan (10 mg/kg/d for 30 d). Only one of the intervention groups ("compression site") reported by Moura *et al.* led to significantly lower RANKL levels. The combined effect showed statistical significance (SMD = − 1.64, 95 % CI − 2.75 to − 0.52). Significantly high between-study heterogeneity was found in the pairwise meta-analysis of the secondary outcomes TNF-α and TRAP + cells/osteoclasts. Heterogeneity was incorporated in a random-effects model as an analytical approach. Between reviewer explanations for the high heterogeneities in these 2 cases were the hypertension baseline characteristic in the study conducted by Li *et al.* (Li *et al.*, 2019), for the outcome TNF-α, and the experimental method of orthodontic force application in the study conducted by Moura *et al.* (Moura *et al.*, 2016) for the outcome TRAP+cells/osteoclasts, respectively. When excluding these studies from the pairwise meta-analysis, heterogeneity was judged moderate with an $I^2 < 75$ %.

Risk of bias assessment

The assessment of the risk of bias according to the items included in SYRCLE's tool is provided in Fig. 9. Overall, only a few experiments adequately reported the items included in SYRCLE's tool, leading to a high percentage of the judgment "unclear risk of bias". The best-reported items were "random group allocation (selection)" (8/17, 47 %) and "group similar at baseline (selection)" (8/17, 47 %). Santos *et al.* were reporting most of the Items (7/9) (Santos *et al.*, 2015). The 5 studies conducted by Matos *et al.* were the studies reporting the fewest items and with the most positive judgments in the item category "other sources of bias" due to not reporting the sample sizes overall (Matos *et al.*, 2016; Matos *et al.*, 2019) and not adequately reporting sample sizes before the result section to evaluate drop-outs (Matos *et al.*, 2013; Matos *et al.*, 2014; Matos *et al.*, 2015). The items

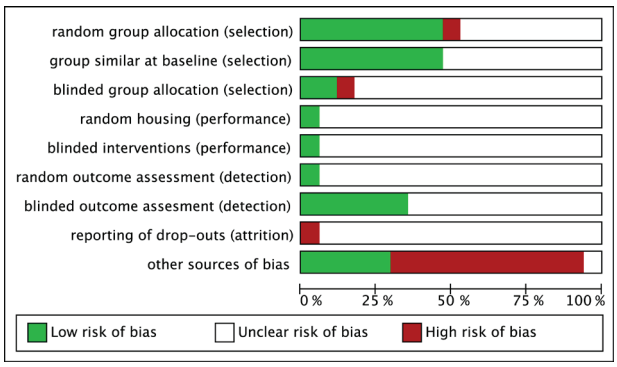

Fig. 9. Assessment of the risk of bias using the SYRCLE's tool.

"random housing (performance)" and "blinded interventions (performance)" were only reported by Santos *et al.* (Santos *et al.*, 2015). Overall, the results of the assessment of bias are as expected for animal studies, which often lack adequate techniques to avoid the risk of bias or simply do not adequately report items to assess the risk of bias in animal studies.

Discussion

Severe periodontitis is still a global health problem leading to a huge socio-economic burden for the affected individuals and the healthcare system. New treatment approaches require intense and specified preclinical research and therefore this topic has been intensely researched in preclinical settings in recent years. There is a lack of information regarding the role of tRAS in the periodontal tissue. A summary of the evidence was required to pave the way for more specialised research and to transfer the findings into the clinical setting in humans. To the authors' best knowledge, this is the first systematic review evaluating the impact of periodontal tRAS inhibition on important surrogate and clinical endpoints in animal studies. We used a combination of pairwise- and network meta-analysis techniques to assess the outcomes after inhibition with different pharmacological intervention arms.

The data show, that tRAS inhibition with ARBs, renin-inhibitors, and ACE-inhibitors resulted in significantly lower periodontal bone loss when compared to the untreated experimental groups. We could not find a significant difference when comparing the different tRAS inhibitors with regards to bone loss in the network meta-analysis. Nevertheless, the treatment ranking suggests, that the renin-inhibitor Aliskiren was most effective in preventing periodontal bone loss. When comparing the different ARBs, the Losartan group performed significantly better than other ARBs. Furthermore, the group treated with 0.6 g/L in drinking water for 8 weeks performed significantly better than all other tRAS inhibitor groups. Additionally, inhibition

with tRAS inhibitors led to lower osteoclast counts, lower RANK and RANK-L levels, and higher OPG levels. Further, these findings for RANK, RANKL and OPG were present at 12 h of tRAS inhibition and were more pronounced after 30 d of tRAS inhibition. The oxidative markers were also affected by tRAS inhibition with ARBs, leading to a small increase in the antioxidative marker GSH, suggesting a lower oxidative stress level in the ARB treated groups, and a decrease of MPO and MDA levels – both markers which are usually increased during oxidative stress. From the surrogate markers related to inflammation, TNF-α was decreased in the pooled ARB group compared to the untreated control, and IL-1β was decreased in the pooled ARB and renin-inhibitor group compared to the untreated control. However, lower SMD was observed when excluding Aliskiren from the analysis, which alone showed no significant impact on IL-1β levels, thus this effect was only evident in the pooled ARB treated group. IL-10, an anti-inflammatory cytokine, seems to be higher in the combined ARB treated group; however, this finding failed to reach statistical significance.

Several limitations need to be addressed when interpreting these findings:

1. All studies included in this systematic review were conducted in rats and mice, thus translating the results to other animal species or humans should be performed carefully. Although rodent anatomy and physiology is not identical to that of humans, they are well established and reliable models for research on periodontal microbiology and immune response (Baker *et al*., 2000; Graves *et al*., 2008; Kantarci *et al*., 2015; Marchesan *et al*., 2018; Struillou *et al*., 2010). Moreover, several recent studies provided related findings for human gingival and periodontal tissues. For example, the presence of tRAS components in human gingival and periodontal ligament fibroblasts was recently confirmed (Monnouchi *et al*., 2011; Santos *et al*., 2015). Further, AT1R knockdown resulted in higher OPG levels (an inhibitor of bone resorption) and lower IL-1β, IL-8 and IL-6 concentration (major inflammatory cytokines involved in periodontal diseases) in human gingival fibroblasts(Gabriele *et al*., 2017). Moreover, Angiotensin II was shown to induce prostaglandin E2 (which has an important role in the periodontal inflammatory process) release in human gingival fibroblasts, whereas the effect was inhibited by the AT1R antagonist FR-13,739 (Segawa *et al*., 2003).

2. Different methodological approaches were used by the authors to assess the impact of RAS-inhibition on primary and secondary outcomes, which limits the comparability. The use of ligatures is considered to not significantly induce inflammation and the effect is mainly dependent on accumulating bacteria. In comparison, direct injections of LPS and infection with periodontal pathogens have different mechanisms, such as inflammation triggering *via* toll-like receptors or modulation of the host subgingival biofilm (Marchesan *et al*., 2018; Struillou *et al*., 2010). Nevertheless, the effect measure was overall similarly distributed, as assessed in the random-effect paired meta-analysis. Interestingly, high heterogeneity was seen between the studies of Li *et al*. and Suda *et al*. for the outcome TNF-α, although both used the periodontitis model based on an infection with *Porphyromonas gingivalis*. In contrast, the between-study heterogeneity, especially for the studies using ligature-induced periodontitis models and infection with *Porphyromonas gingivalis* can be considered comparable with the focus on the results of the heterogeneity test. The study conducted by Moura *et al*. showed a relatively high heterogeneity compared to the other studies for RANKL and the number of TRAP+ cells/osteoclasts. The orthodontic force application, which could affect bone remodelling processes in a different way from the other methodological examination methods could be one explanation.

3. Different tRAS inhibitors with different doses and experiment durations were compared and thus results should not be generalised for all tRAS inhibitors. It is important to know that within the group of ARBs there are also differences in the effective mechanism of the inhibitors. For example, it is stated that Losartan is not only blocking AT1R on the cell surface but also intracellular AT1Rs while Candesartan seems to remain surface-bound (Cook *et al*., 2001). Nevertheless, it is not recommendable for this systematic review to only focus on one specific tRAS inhibitor, as there is not enough evidence and it would possibly overlook other important relationships between tRAS inhibition and outcomes of interest. Furthermore, a subgroup or sensitivity analysis with consideration of duration, dose, and one specific tRAS inhibitor class was not possible with regards to the available evidence. With the network meta-analysis, the different intervention arms could be compared regardless of the methods used or duration of tRAS inhibition to present an overview, possible relationships for future comparison studies, and help to prevent duplicate reporting in prospective studies.

4. Multiple studies conducted by 2 research groups were included in this systematic review which may lead to bias and should be considered when interpreting the results. Especially, the studies conducted by the research group of Matos *et al*. were rated low with regards to the assessment of bias using the SYRCLE's tool. However, these studies were excluded from the quantitative syntheses, when no sample size was present. In this case, a qualitative synthesis was provided of the results and only included the significant findings. The other included studies can be considered as adequate with regards to the risk of bias in animal studies and are in accordance with the majority of animal studies, which often lack in reporting of important items for the assessment of the risk of bias ("unclear risk of bias") as the assessment of the risk of bias in systematic reviews of animal studies is far less

common (Hooijmans *et al*., 2014; Sandercock and Roberts, 2002).

5. The exclusion criteria led to the exclusion of possibly important preclinical studies in human and animal cell cultures, which could also be of interest to the research topic and might lead to different results when analysed quantitatively. However, it would be not possible to include these cell culture studies in the network analysis as the presumption (similarity, transitivity, consistency) which should be confirmed *a priori* would not be satisfied. Thus, only a systematic literature review with a qualitative synthesis of the results would have been possible. Furthermore, as far as is known, there are no clinical studies in humans listed in the peer-reviewed literature evaluating RAS-inhibitors' impact on periodontal outcome parameters. Therefore, a systematic review focusing on human participants was not feasible. As a compromise, all available studies on this topic were considered in the discussion section, as part of the available evidence, regardless of the study type. Lastly, it must be mentioned that the task of this evidence-based systematic review was to summarise preclinical studies regarding the desired topic, to pave the way for more clinically oriented research. Definitive conclusions cannot be drawn regarding the clinical applicability in humans. Drug therapeutics mostly have side effects and limitations; and should therefore be considered to be an invasive therapeutical approach. Thus, intervention in this important host modulating system should be reserved for severe periodontitis and in cases where other non-invasive host modulating therapies (*e.g.* diet) fail.

To interpret the information retrieved in this systematic review properly, recent *ex vivo* studies regarding tRAS in the periodontal tissue and inflammation should also be considered. The study conducted by Choe *et al*. in 2019 evaluated the influence of Telmisartan on inflammatory mediators in murine macrophages stimulated with LPS from *Prevotella intermedia*, an important periodontitis-associated species (Choe *et al*., 2019). They found out, that Telmisartan led to significant inhibition of LPS-induced generation of inflammatory mediators, such as IL-1β, which is in accordance with the presented data. In contrast, the study conducted by Gabriele *et al*. (2017) found an impairment of IL-1β induced secretion of proinflammatory mediators when silencing AT1R but not after Losartan treatment in human gingival fibroblasts and human periodontal fibroblast, suggesting a different control of inflammatory cytokines after AT1R knockdown and pharmacologic blockade by Losartan. One explanation for this finding could be the multiple regulatory mechanisms Losartan can affect. Ang II binding on AT1R is known to result in translocation of the Ang II/AT1R complex to the nuclear membrane and Losartan is known to block not only surface located AT1Rs, but also intracellular AT1Rs (Cook *et al*., 2001; Villar-Cheda *et al*., 2017). Further, the Losartan application for 14 d was shown to upregulate AT2R in the gingival tissue of rats with experimental induced periodontitis (Santos *et al*., 2015). This could promote the anti-inflammatory pathways of the cell *via* an alternative route (Dandona *et al*., 2007). Another explanation could be, that Telmisartan is also known to downregulate AT1R through the activation of peroxisome proliferator-activated receptor-gamma (PPAR-γ), resulting in stronger inhibition of proinflammatory mechanisms (Imayama *et al*., 2006). In this case, Telmisartan, as a partial agonist of PPAR-γ, was the only ARB to show relevant activation of PPAR-γ that can be achieved in plasma with conventional oral dosing (Benson *et al*., 2004).

Candesartan, as another widely used ARB, has been shown to inhibit LPS induced TLR4 upregulation (Dasu *et al*., 2009). AT1R and TLR4 seem to work synergistically and upregulation of TLR4 by Angiotensin 2 *via* AT1R has been stated in multiple studies (Biancardi *et al*., 2016; De Batista *et al*., 2014; Goel *et al*., 2018; Shirai *et al*., 2013; Wolf *et al*., 2006; Wu *et al*., 2009). An inhibition with Candesartan inhibited the TLR4/angiotensin II-induced NF-kB inflammatory pathway in a recent study (Goel *et al*., 2018). Moreover, LPS has been shown to increase the expression of AT1R (Li *et al*., 2015; Xianwei *et al*., 2012), whereas the LPS response was prevented with the ARB Candesartan (Sanchez-Lemus *et al*., 2008). There is further evidence, that AT1R in the periodontal tissue is upregulated (Gabriele *et al*., 2017; Nakamura *et al*., 2011) or more pronounced than AT2R (Santos *et al*., 2015) in the inflammation setting, whereas it is lower expressed in healthy periodontal tissue. In a recent study, bacteria-induced periodontitis resulted in a significant upregulation of Ang II concentrations and TLR4 mRNA, while the effect was inhibited with the ARB Losartan (Li *et al*., 2019). This finding was also found for Valsartan (Matos *et al*., 2014).

The expression of matrix metalloproteinases, such as MMP-1 and MMP-2, and the osteoclastogenic factor RANKL was shown to be correlated with the expression of the proinflammatory markers IL-1β and TNF-α in the course of experimental periodontal disease (Garlet *et al*., 2006). IL-1β upregulated RANKL, but not OPG and induced osteoclastogenesis in human cementoblasts (Huynh *et al*., 2017). An increase in the RANKL/OPG ratio is known to be one main feature of periodontitis (Belibasakis and Bostanci, 2012). The ARBs evaluated in this systematic review were shown to decrease RANKL, increase OPG, and thus resulting in a higher RANKL/OPG ratio and a decrease in the number of osteoclasts. However, it was not possible to evaluate the pathway that led to these results. It could be that the decrease in proinflammatory cytokines led to lower RANKL secretion, as stated above. Another hypothesis would be, that the ARBs directly inhibit the Ang II-mediated induction of RANKL expression in osteoblasts, leading to the lesser activation of osteoclasts, as described for Olmesartan (Shimizu *et al*., 2008). This

would suggest that the AT1R pathway is directly involved in bone remodelling processes and might be an important therapeutic target as described recently by Zhao *et al*. in 2019 (Zhao *et al*., 2019).

Conclusions

Outcomes of this systematic review suggested an important role for the tRAS in periodontal tissue. The inhibition of its components in animal models led to a reduction of periodontal bone loss and reduced inflammation with different intensity, depending on the type of inhibitor used. Thus, the inhibition of tRAS components could be a new target approach for treating periodontal diseases in humans. Future research should, therefore, consider different mechanisms of tRAS inhibitors. However, some of the included studies were associated with certain limitations and a high risk of bias. More well-designed randomised preclinical and clinical studies are needed to adequately translate the present findings into clinical practice.

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TB, VW, SP, ZL, and SG: Substantial contributions to study design, revising the article critically, and final approval.
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Conflict of interest

The authors of the present manuscript declare that they have nothing to disclose.

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Discussion with Reviewers

Piefrancesco Pagella: The authors only briefly discuss some studies concerning tRAS in human gingival and periodontal tissues. Are there reliable clinical studies concerning the association between tRAS and periodontitis? If yes, do their results go in the same direction of the animal studies discussed in this systematic review?

Authors: To the best of our knowledge, there are currently no clinical studies investigating the interaction between tRAS and periodontal diseases. We have recently started the first clinical study to assess and prove available preclinical evidence. The present study demonstrates that clinical trials are warranted to assess the impact of tRAS inhibition on periodontal diseases.

Editor's note: The Scientific Editor responsible for this paper was Thimios Mitsiadis.