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Tryptophan-Derived Microbial Metabolite Indole-3-Acetic Acid Ameliorates Periodontitis Through AhR/CYP1A1-Mediated Macrophage Polarisation

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ABSTRACT

Aim: To investigate the association between tryptophan-derived microbial metabolites and periodontitis, and to assess whether the differential tryptophan metabolite indole-3-acetic acid (IAA) could ameliorate periodontitis and elucidate its potential mechanism.

Materials and Methods: The association between tryptophan-derived metabolites and periodontitis was evaluated using targeted metabolomics analysis, 16S rRNA gene amplicon sequencing and correlation analysis. The impact of the differential metabolite IAA on periodontitis was assessed with micro-computed tomography (micro-CT), histological staining and real-time quantitative polymerase chain reaction (RT-qPCR). The effects of IAA on macrophages under an inflammatory microenvironment, along with its potential mechanism, were explored using RT-qPCR, enzyme-linked immunosorbent assay (ELISA), immunofluorescence staining and Western blot.

Results: IAA, a differential tryptophan metabolite, was identified in the saliva of patients with periodontitis and periodontally healthy individuals. Local application of IAA in experimental mice with periodontitis alleviated the immune-inflammatory response and reduced periodontal tissue destruction. Mechanistically, IAA induced M2 macrophage polarisation by activating the AhR/CYP1A1 axis, thereby contributing to the amelioration of periodontal destruction.

Conclusions: These findings highlight the potential role of the microbial metabolite IAA in the development and treatment of periodontitis, offering a new perspective for intervention strategies based on microbial metabolites for periodontitis.

1 | Introduction

Periodontitis, primarily initiated by dysbiotic plaque microbiota, is a chronic inflammatory disease that affects the

periodontal supporting tissue. This pathological condition can lead to gingival inflammation, periodontal pocket formation and progressive alveolar bone resorption, eventually resulting in tooth mobility and loss. These outcomes significantly impair

Shuyu Cheng and Shiyuan Song contributed equally to the work.

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patients' quality of life (Kinane et al. 2017). Therefore, there is an urgent need for more effective therapeutic strategies to manage periodontitis.

The imbalance between periodontal microbiota and host immunity mediates excessive immune-inflammatory responses, thereby exacerbating periodontal tissue destruction (Freire et al. 2021; Hajishengallis et al. 2020). Beyond microbial structural components, microbial metabolites including short-chain fatty acids play pivotal roles in modulating host immunity (Ding et al. 2023; Lun et al. 2024). Recent research has increasingly focused on amino acid-derived bacterial metabolites and their physiological impacts (Roager and Licht 2018). Notably, elevated salivary tryptophan (Trp) level was identified in patients with periodontitis (Balci et al. 2021; Onder et al. 2023), suggesting that Trp metabolism may participate in the pathophysiology of periodontitis.

Trp, an essential aromatic amino acid, is primarily metabolised through three distinct pathways: the serotonin pathway (5-HT pathway); the kynurenine pathway (Kyn pathway); and the indole pathway (microbial pathway) (Xue et al. 2023). Microbiota can directly metabolise Trp into indole and various indole derivatives, including indole-3-acetic acid (IAA), indolelactic acid (ILA), indole-3-acetaldehyde (3-IAld), indole acrylic acid (IA), indole-3-propionic acid (IPA), tryptamine and skatole (Jia et al. 2019). Accumulating evidence shows that these microbial metabolites regulate critical physiological processes, including gastrointestinal function, antioxidant defence and immunoinflammatory responses (Ji et al. 2019; Ji et al. 2020; Zhang et al. 2023).

The aryl hydrocarbon receptor (AhR) is a ligand-dependent transcription factor. Many indole derivatives have been reported to be ligands of AhR (Chen et al. 2024; Sun et al. 2022). Upon ligand binding, AhR translocates from the cytoplasm to the nucleus and forms a heterodimer with the aryl hydrocarbon receptor nuclear translocation factor. This complex then binds to xenobiotic response elements, activating the transcription of target genes such as *CYP1A1*, *CYP1B1* and *Ahrr* (Rothhammer and Quintana 2019). Modulation of AhR signalling has been shown to influence the outcomes of periodontitis. For instance, Huang et al. demonstrated that an AhR agonist attenuated alveolar bone loss and inflammation in experimental periodontitis (Huang et al. 2019). However, the potential linkage between these protective effects and microbial Trp metabolism requires further elucidation.

Macrophages are key immune cells within periodontal tissues. Induced by various factors, macrophages can polarise into M1 or M2 types, which are involved in the process of destruction or repair in periodontitis (Sun et al. 2021). Numerous studies have reported that AhR modulates immune inflammation by inducing macrophage polarisation. For example, Yang et al. reported that AhR activation suppresses M1 macrophage polarisation while promoting M2 polarisation, thereby attenuating disease development (X. Yang et al. 2020). However, it remains unclear whether indole derivatives can induce macrophage polarisation by regulating AhR in the context of periodontitis.

In this study, we assessed the differences in Trp metabolites in saliva between patients with periodontitis and periodontally healthy individuals. The correlation between differential microbiota and Trp metabolites was investigated. Subsequently, a differential Trp metabolite, IAA, was applied in experimental mice with periodontitis to evaluate its potential to ameliorate the disease. Further, the underlying mechanism by which IAA alleviated periodontal inflammation was explored in vitro.

2 | Materials and Methods

2.1 | Clinical Saliva Sample Collection

Saliva samples were collected from 12 patients with periodontitis and 8 periodontally healthy individuals (Table S1), according to preset inclusion and exclusion criteria (Table S2), following approval from the Ethics Committee at Nanjing Stomatological Hospital, Medical School of Nanjing University (NJSJH-2023NL-081). Informed consent was obtained from each participant. The study procedures were carried out in compliance with the Helsinki Declaration.

2.2 | Targeted Metabolomics Analysis

A 100- μ L saliva sample was carefully added to an Eppendorf tube, followed by 400 μ L of extract solution. The samples underwent two rounds of centrifugation at $13,860 \times g$ at 4°C for 15 min. The resulting supernatant was analysed using ultrahigh-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS). Chromatographic separation was performed on an EXIONLC System ultra-performance liquid chromatograph (SCIEX, Shanghai, China). Mass spectrometry was conducted using a 6500 QTRAP+ triple quadrupole mass spectrometer (SCIEX, Shanghai, China) in the multiple reaction monitoring (MRM) mode.

Data were acquired and analysed using the SCIEX Analyst Work Station Software (v1.6.3) and SCIEX MultiQuant software (v3.0.3). Orthogonal projections to latent structures-discriminant analysis (OPLS-DA) (Trygg and Wold 2002) and hierarchical clustering analysis (Kolde 2012) were employed to analyse differences in Trp metabolism between the groups.

2.3 | 16S rRNA Gene Amplicon Sequencing

Genomic DNA from human saliva was isolated using the CTAB/SDS method by Novogene Bioinformatics Technology (Beijing, China). Specific primers 341F and 785R (5'-CCTAYGGGRBGCASCAG-3' and 5'-GACTACHVGGGTATCTAATCC-3') were used for amplification of the V3-V4 region of the 16S ribosomal RNA gene. The library was built using the TruSeq DNA PCR-Free Sample Preparation Kit, and on-machine sequencing was performed on NovaSeq 6000 in the paired-end (PE) 250 mode.

After pre-processing, the PE sequences were merged by FLASH (v1.2.7) (Magoc and Salzberg 2011). The joined reads were subsequently imported into QIIME 2 (v2020.2) for

further analysis (Bolyen et al. 2019). The data were denoised via q2-deblur to generate amplicon sequence variants (ASVs) (Amir et al. 2017), followed by analysis of species diversity (Chao 1987; Faith 1992; Lozupone et al. 2011). The naive Bayes classifier (NBC) was used to annotate species and calculate the relative abundance of species based on the SILVA 132 database (Bokulich et al. 2018; Yilmaz et al. 2014). Linear discriminant analysis effect size (LEfSe) was employed to identify the differential microbiota (Segata et al. 2011).

2.4 | Metabolite–Metabolite and Metabolite–Microbiota Correlation Analyses

Spearman's correlation analyses were conducted to evaluate the relationships between differential metabolites within each group. Additionally, correlations between metabolites and differentially abundant genera identified by the LEfSe method were evaluated. For these analyses, metabolite concentration data and genus-level relative abundance were employed as variables. The corr.test function in the R package psych (v2.2.9) was employed to perform Spearman's correlation analysis. The metabolite–metabolite associations within each group were visualised using Cytoscape (Shannon et al. 2003), while metabolite–microbiota correlations were displayed as a heatmap.

2.5 | Animal Experiment

Seven-week-old male C57BL/6 mice, raised in a specific pathogen-free environment, were provided by Beijing Vital River Laboratory Animal Technology Co. Ltd. All animal experiments were approved by the Experimental Animal Welfare and Ethics Committee of Nanjing Agricultural University (PZW2023039). All animal experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. After 1 week of accommodation, mice were divided into four groups: (1) NC group ($n = 5$); (2) P group ($n = 6$); (3) P + IAA group ($n = 6$); (4) P + CH-223191 group ($n = 6$). Fourteen days after the establishment of the experimental periodontitis model, 25 μ L of the prepared solution was gently injected into the gingival sulci or periodontal pockets of the second molars of each mouse daily for the next 7 days. Subsequently, all mice were euthanised, and the maxillary samples were harvested for micro-CT and histological examinations.

2.6 | Additional Experimental Details

Details of saliva sample collection, targeted metabolomics analysis, animal experiment, micro-CT, haematoxylin and eosin (H&E) staining, immunohistochemical staining, cell culture, RT-qPCR, ELISA, immunofluorescence staining and Western blot analysis are given in [Supporting Information](#).

2.7 | Statistical Analyses

Apart from the bioinformatics analyses described above, the unpaired Student's *t*-test was used for comparison between two

groups, and one-way ANOVA was employed for comparison among multiple groups. Data were presented as mean $\pm$ SD. GraphPad Prism software (GraphPad Software Inc., USA) was used for data analysis.

3 | Results

3.1 | Salivary Trp Metabolites Altered in Patients With Periodontitis

Tryptophan-targeted metabolomics analysis was performed to investigate the differences in salivary tryptophan metabolites between groups. In the analysis, quantitative data for 16 target metabolites, including Trp, were obtained; of these, 5 metabolites were in the Kyn pathway and 10 in the indole pathway. The OPLS-DA model separated the saliva samples into two distinct clusters (Figure 1A), indicating that Trp metabolism in saliva varied between the periodontitis group and the healthy group. Hierarchical clustering analysis of the identified metabolites between the two groups was visualised (Figure 1B). Specifically, the Trp concentration in the saliva of patients with periodontitis was nearly two-fold higher than that of healthy individuals (Figure 1C). Among the metabolites from the indole pathway, IAA trended to be lower in the periodontitis group than in the healthy group ($p = 0.0516$), while the ratio of IAA/Trp was significantly reduced ($p = 0.0028$). Other metabolites from the indole pathway exhibited no significant differences between groups. For metabolites from the Kyn pathway, only the concentration of kynurenic acid (KYNA) in the periodontitis group showed a significant increase, while the ratio KYNA/Trp showed no difference between groups (Figure S1). These results indicated a relative reduction in the conversion of Trp to IAA in periodontitis compared to healthy controls, which may be attributed to the disturbed ability of the oral microbiota to metabolise Trp into IAA.

3.2 | Salivary Microbiota Altered in Patients With Periodontitis

We used 16S rRNA sequencing to observe the differences in salivary microbial composition between the groups. The results revealed that microbial alpha diversity was significantly higher in the saliva of patients with periodontitis (Figure 2A). Beta diversity analysis showed that samples from the periodontitis group clustered away from those of the healthy group (Figure 2B), indicating that the structure of the saliva microbial community varied between the two groups.

Periodontitis altered the relative abundance of bacteria at different taxonomic levels (Figure 2C,D). At the genus level, higher relative abundances of *Fusobacterium*, *Porphyromonas*, *Aggregatibacter* and *Treponema* 2 were observed in the periodontitis group, while relative abundances of *Neisseria*, *Prevotella* 7, *Haemophilus*, *Prevotella* and *Campylobacter* displayed a reduction in the periodontitis group (Figure 2D). Furthermore, LEfSe analysis showed that the most

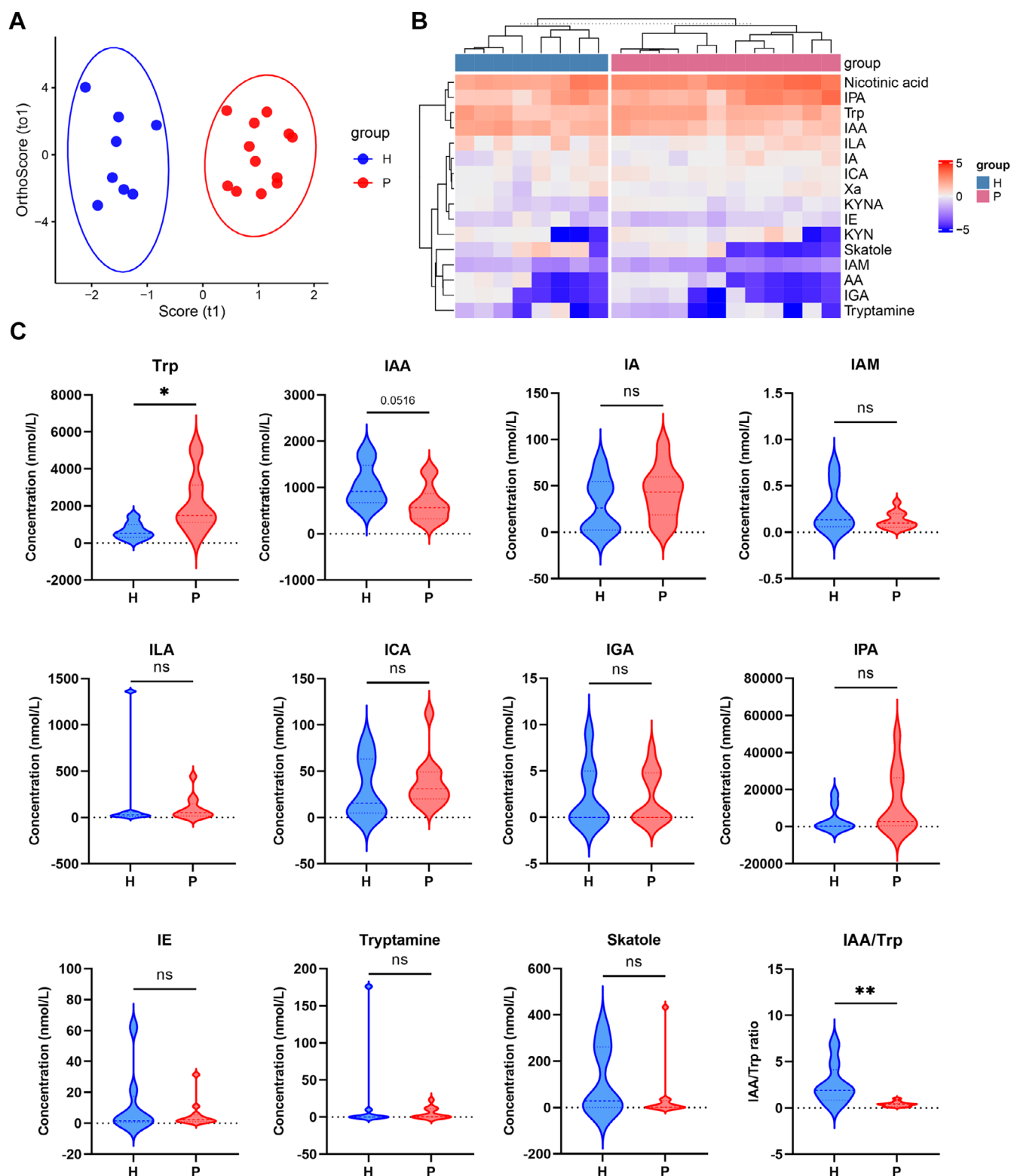


FIGURE 1 | Levels of Trp metabolites in saliva of periodontitis and periodontally healthy individuals. (A) Score scatter plot of OPLS-DA model. Each scatter represents a sample. (B) Heatmap of hierarchical clustering analysis for identified Trp metabolites. (C) Differences in Trp and indole pathway metabolites in saliva from two groups. H indicates periodontally healthy individuals and P patients with periodontitis. Differences were determined by unpaired Student's *t*-test (in C). * $p < 0.05$, ** $p < 0.01$.

representative differential microbiota at the genus level between the two groups were *Fusobacterium*, *Porphyromonas*, *Aggregatibacter*, *Treponema_2* and *Prevotella_1* (Figure 2E). These results collectively indicated that the salivary microbiota were altered in patients with periodontitis.

3.3 | Trp Metabolite IAA Was Negatively Correlated With Periodontitis Differential Microbiota

To identify the key metabolites among the targeted Trp metabolites, Spearman correlation analysis was conducted separately

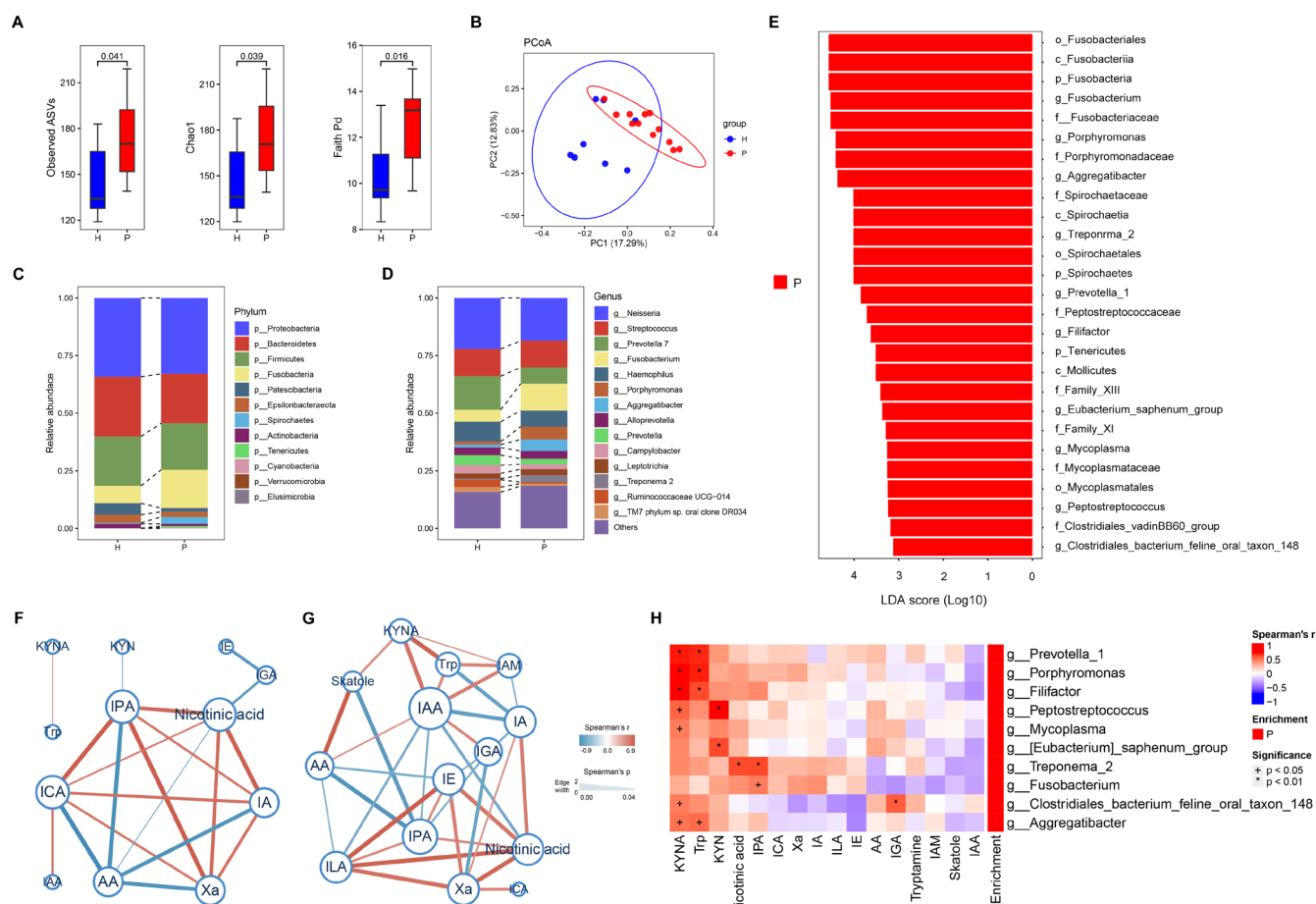


FIGURE 2 | Alterations of microbiota structure in patients with periodontitis and correlation analyses of Trp metabolites and microbiota composition in saliva samples from two groups. (A) Alpha diversity of bacteria species, including Observed ASVs, Chao1 and Faith Pd. (B) PCoA of unweighted unfracs analysis. (C,D) Relative abundance of microbiota at phylum (C) and genus (D) levels between the periodontitis group and the healthy group. (E) LEfSe analysis indicating discriminative bacteria of the two groups (LDA > 3). (F,G) Networks indicating correlation of differential metabolites in (F) the healthy group and (G) the periodontitis group. Metabolite concentration was used as the variable for correlation analysis in the two groups separately. The size of a node reflects the number of metabolites which are significantly correlated with the metabolite represented by that node. The edge colour aligns with Spearman's r value, where red indicates positive correlation and blue indicates negative correlation. The thickness of the edge reflects the significance of the correlation. (H) Heatmap showing the correlation between Trp metabolites and LDA differential microbiota at the genus level. Metabolite concentration and genus level relative abundance were used as the variables for correlation analysis. PCoA, principal coordinate analysis; LDA, linear discriminant analysis; LEfSe, linear discriminant analysis effect size. H indicates periodontally healthy individuals and P patients with periodontitis. Differences were determined by unpaired Student's t -test (in A). + $p < 0.05$. * $p < 0.01$.

in two groups (Figure 2F,G). Notably, in the periodontitis group, IAA was significantly correlated with nine other metabolites, making it the most connected metabolite; of these, five were positively correlated and four were negatively correlated (Figure 2G). This suggested that IAA might be playing a key role in Trp metabolism in the saliva of patients with periodontitis. According to the correlation analysis between differential microbes and metabolites, IAA was negatively related to *Prevotella_1*, *Porphyromonas*, *Filifactor*, *Peptostreptococcus*, *Mycoplasma*, *Eubacterium_saphenum_group*, *Treponema_2*, *Fusobacterium* and *Aggregatibacter*, which were LDA differential microbiota from the saliva of patients with periodontitis (Figure 2H). Most of these microbes have been reported to be associated with periodontitis. These results indicated that the Trp metabolite IAA was negatively correlated with the differential microbiota of periodontitis.

3.4 | IAA Alleviated Periodontal Inflammation and Tissue Destruction in Experimental Periodontitis Mice

To investigate whether this metabolite can ameliorate periodontitis, we applied IAA locally to mice with periodontitis and observed changes in periodontal tissue. Micro-CT analysis showed attenuated alveolar bone destruction in group P+IAA versus group P (Figure 3A), along with significantly reduced CEJ-ABC distances, particularly at mesial sites (Figure 3B, Figure S2). Concomitantly, H&E staining demonstrated decreased attachment loss in group P+IAA (Figure 3C). These results suggested that IAA could alleviate the pathological phenotype of periodontitis. IAA treatment suppressed periodontal inflammation, as evidenced by reduced TNF- α and IL-6 and elevated IL-10 in immunohistochemistry (Figure 3D,E) and

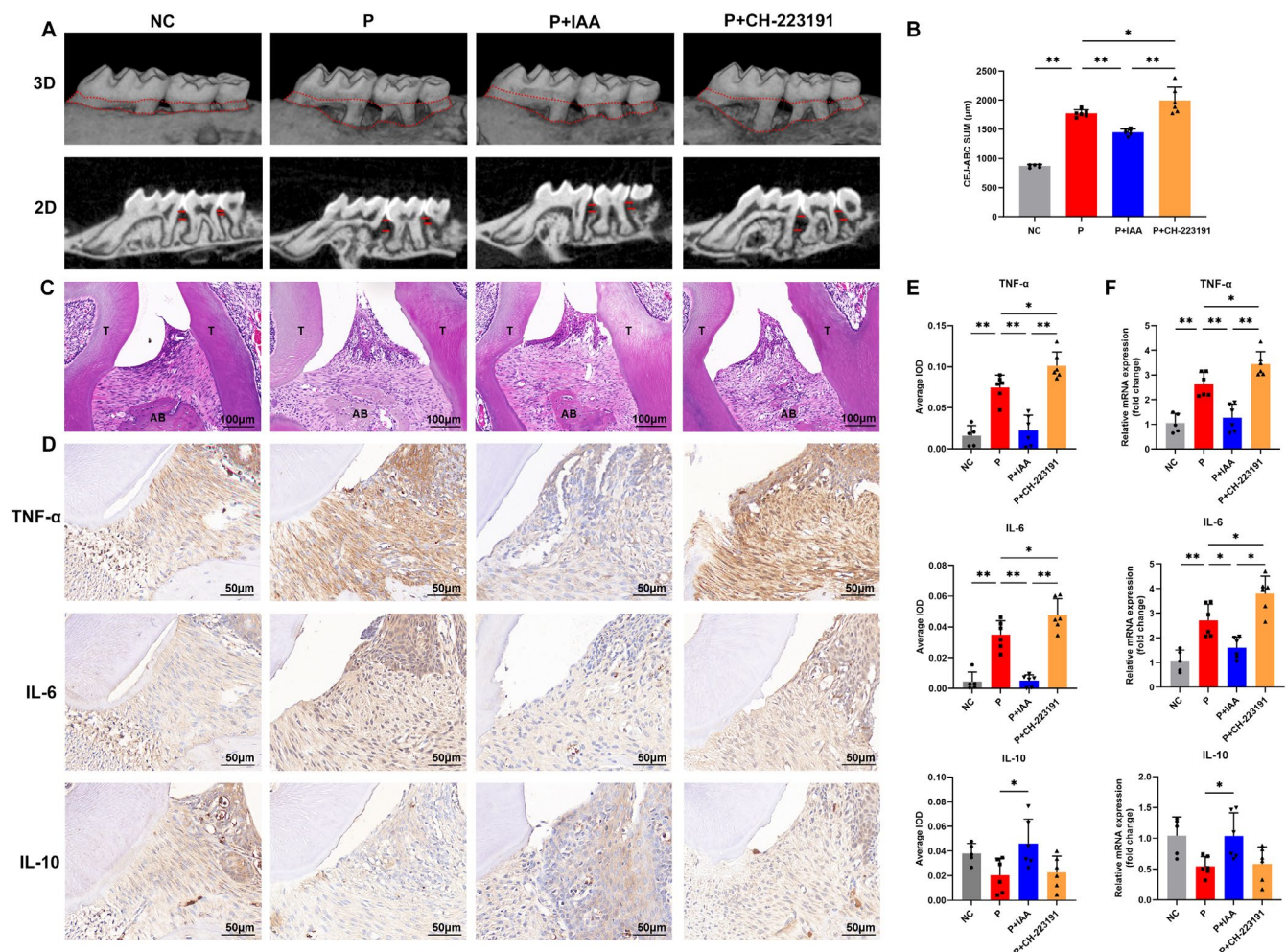


FIGURE 3 | Effect of IAA on alveolar bone loss and periodontal inflammation. (A) 3D reconstruction images of maxillary alveolar bone and 2D images of mesio-distal section of maxillary second molars. Alveolar bone loss was demarcated using red-dashed contours (3D reconstructions) and continuous red lines (2D sections). (B) Sum distance of CEJ–ABC at mesial, mesial buccal, mesial palatal, distal, distal buccal and distal palatal sites. (C) Representative H&E staining images of maxillae. (D) Representative immunohistochemical images of periodontal tissues (TNF- α , IL-6 and IL-10). (E) Immunohistochemical semi-quantitative analysis results. (F) Relative mRNA expression of TNF- α , IL-6 and IL-10 in periodontal tissues. NC, negative control group. P indicates the periodontitis group; P + IAA, the periodontitis + IAA group; P + CH-223191, the periodontitis + AhR inhibitor CH-223191 group. T, tooth. AB, alveolar bone. CEJ, cemento-enamel junction. ABC, alveolar bone crest. Differences were determined by one-way ANOVA. * $p < 0.05$, ** $p < 0.01$.

corroborated by RT-qPCR (Figure 3F). Critically, AhR inhibition by CH-223191 exacerbated bone loss, attachment degradation (Figure 3A–C) and pro-inflammatory cytokine expression (Figure 3D–F), indicating AhR mediation in IAA's therapeutic effects on periodontitis.

3.5 | IAA Induced Macrophage Polarisation Through Regulating the AhR/CYP1A1 Axis in Periodontal Tissues of Mice

Immunohistochemical staining results revealed AhR and CYP1A1 up-regulation in group P + IAA (Figure S3), suggesting that activation of the AhR/CYP1A1 axis might mediate IAA's role in alleviating periodontal tissue destruction. Concurrently, decreased iNOS and elevated Arg-1 levels indicated that the macrophage tended to present as the M2 phenotype in group P + IAA (Figure S3).

To directly assess macrophage-related AhR/CYP1A1 activation, dual immunofluorescence co-staining was performed. The results showed that IAA treatment significantly reduced M1 macrophage infiltration (iNOS⁺F4/80⁺ cells decreased by 16.47%, $p < 0.01$) and promoted M2 polarisation (Arg-1⁺F4/80⁺ cells increased by 18.88%, $p < 0.01$), corroborating its therapeutic effect on periodontitis (Figure 4A–D). Co-staining of F4/80 with AhR/CYP1A1 revealed an increase in double-positive cells following IAA treatment (Figure 5A–D), confirming pathway activation specifically within macrophages. IAA likely alleviates periodontitis by influencing the AhR/CYP1A1 axis and macrophage polarisation.

Local application of the AhR inhibitor CH-223191 further supported these findings. The expression of AhR and CYP1A1 significantly decreased after treatment with CH-223191 (Figure 5A–D). Compared to group P + IAA, group P + CH-223191 showed higher iNOS and lower Arg-1 levels

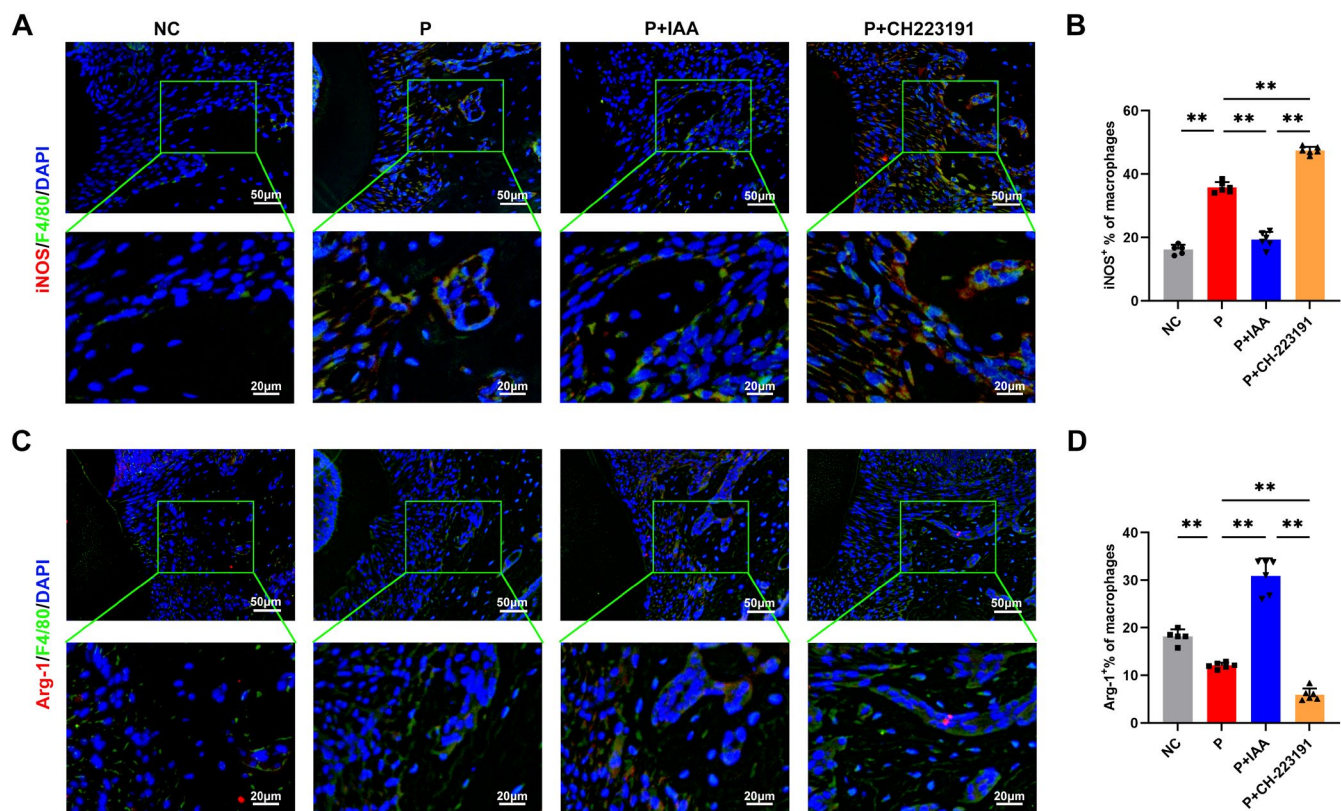


FIGURE 4 | Effect of IAA on macrophage polarisation in periodontal tissue. (A,C) Representative immunofluorescent images of iNOS⁺ and Arg-1⁺ macrophages. (B,D) Relative quantitative analysis of the percentages of iNOS⁺ and Arg-1⁺ positive cells in macrophages. Differences were determined by one-way ANOVA. ** $p < 0.01$.

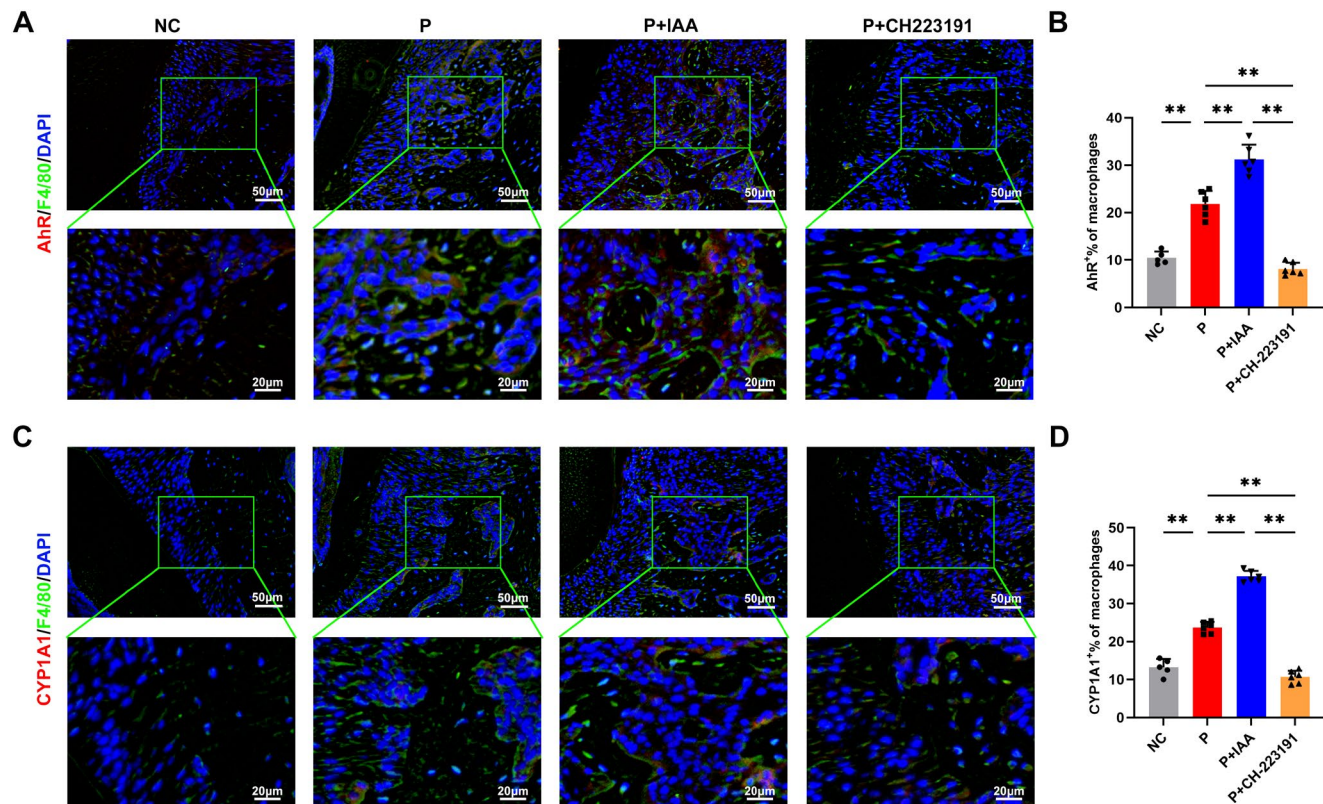


FIGURE 5 | Effect of IAA on the AhR/CYP1A1 axis in macrophages in periodontal tissue. (A,C) Representative immunofluorescent images of AhR⁺ and CYP1A1⁺ macrophages. (B,D) Relative quantitative analysis of the percentages of AhR⁺ and CYP1A1⁺ positive cells in macrophages. Differences were determined by one-way ANOVA. ** $p < 0.01$.

(Figure 4A–D), suggesting a shift towards the M1 macrophage phenotype upon AhR/CYP1A1 axis inhibition.

3.6 | IAA Activated the AhR/CYP1A1 Axis and Promoted M2 Polarisation in Macrophages Under an Inflammatory Microenvironment

We further investigated the anti-inflammatory role of IAA and its mechanism in RAW264.7. Cell activity assay indicated that IAA concentrations below 1000 μ M were non-toxic to macrophages, with or without lipopolysaccharide (LPS) (Figure S4).

IAA treatment mitigated macrophage-mediated inflammation by reducing TNF- α and IL-6 and enhancing IL-10 expression, as evidenced by RT-qPCR and ELISA (Figure 6A,B). Furthermore, IAA treatment elevated CD206 and reduced CD86 expression, along with decreased iNOS levels (M1 marker) and increased Arg-1 levels (M2 marker) in immunofluorescence staining (Figure 6C,D). These findings indicate the role of IAA in promoting macrophage M2 polarisation. Moreover, IAA up-regulated both gene and protein expression of AhR and CYP1A1 in LPS-stimulated macrophages (Figure 6E–G, Figure S5), suggesting a potential connection between the anti-inflammatory effect and macrophage phenotype alteration with the activation of the AhR/CYP1A1 signalling pathway. To further validate the above observations, we conducted experiments in bone marrow-derived macrophages from mice and obtained comparable results (Figure S6).

4 | Discussion

Microbiota-derived Trp metabolites play an important role in intestinal homeostasis and other systemic diseases (Ji et al. 2019; Zhang et al. 2023). However, the role of salivary microbiota-derived Trp metabolites in periodontitis remains poorly understood. In this study, we detected a reduced relative content of IAA in the saliva of patients with periodontitis. Oral local application of IAA in experimental periodontitis alleviated the inflammation and reduced periodontal tissue destruction. IAA may contribute to M2 polarisation by activating the AhR/CYP1A1 axis in macrophages. This study explored the regulatory role of IAA in periodontitis and its potential mechanism.

Alterations in salivary Trp metabolism were observed in patients with periodontitis. Trp concentration in the periodontitis group was approximately two-fold higher than that in the healthy group. Patients with periodontitis typically exhibit poor oral hygiene, leading to plaque accumulation and food debris retention. These protein-rich residues are degraded by proteolytic bacteria, releasing free Trp into the saliva (Duran-Pinedo and Frias-Lopez 2015). Among the three metabolic pathways, the majority of tryptophan is metabolised via the Kyn pathway (Xue et al. 2023). Our data showed significantly increased KYNA in the periodontitis group, consistent with Trp accumulation.

A very small portion of tryptophan is metabolised through the indole pathway. In the periodontitis group, the levels of individual indole derivatives did not reach statistical significance. This may be because the periodontitis group in this study exclusively

included patients with stage III–IV periodontitis, whereas alterations in this metabolic pathway might be more pronounced in the early stages of the disease. Additionally, given the convenience, non-invasiveness and repeatability of salivary testing, this study opted to evaluate changes in tryptophan levels in the saliva (Kim et al. 2021; Wang, Hao, et al. 2025). Compared to the saliva, gingival crevicular fluid or gingival tissue lysates might provide a more direct reflection of local metabolite concentrations in the periodontal microenvironment. The dysbiosis and sustained inflammation in periodontitis likely impair microbial metabolic capacity. For instance, disordered microorganisms may lack key enzymes (e.g., tryptophanase) required for Trp to indole conversion, mirroring observations in inflammatory bowel disease (Gao et al. 2025) where microbial dysfunction led to Trp accumulation without proportional increase in indole derivatives. Supporting this, we found a reduced IAA/Trp ratio in the periodontitis group, indicating insufficient microbial conversion of Trp to IAA despite substrate abundance. The metabolite–metabolite correlation analysis highlights the key role of IAA in the saliva of the periodontitis group.

To explore potential correlations between dysbiotic taxa and metabolic perturbations, 16S rRNA analysis of saliva was performed, the results of which were consistent with those of previous studies (Ji et al. 2023; Ma et al. 2024). The metabolite–microbiota correlation analysis revealed that IAA was negatively related to most differential bacteria enriched in the periodontitis group, suggesting that IAA may be a metabolite associated with a healthy periodontal status. However, it should be noted that Spearman correlation analysis cannot infer causality between these factors. Analogous relationships may also exist with other clinical parameters, such as bone loss. To clarify direct microbial contributions to Trp metabolism, further metagenomic and in vitro validation models are necessary.

The interventional efficacy of IAA in periodontitis was next investigated. Most of the indole derivatives are specific AhR ligands and can induce AhR-dependent gene expression (Agus et al. 2021; Cannon et al. 2021; Hezaveh et al. 2022). We observed that IAA increased the overall AhR expression, potentially due to its promotion of AhR nuclear translocation from the cytoplasm, which concurrently induced compensatory up-regulation of cytoplasmic AhR expression (Jiang et al. 2023; Palrasu et al. 2025; Uberoi et al. 2021). Activation of AhR has shown anti-inflammatory effects in a variety of previous studies. For instance, Sun et al. reported that microbiota-derived indoles could alleviate neuroinflammation of Alzheimer's disease through up-regulating the AhR pathway (Sun et al. 2022). Our study also found that the local application of the AhR-specific inhibitor CH-223191 in experimental periodontitis mice yielded the opposite results. These supported the hypothesis that the activation of the AhR/CYP1A1 pathway by IAA could alleviate periodontal inflammation and reduce tissue destruction.

Currently, intestinal flora-derived IAA is reported to alleviate inflammation via AhR-mediated macrophage polarisation in systemic disease research (Krishnan et al. 2018; Liu et al. 2025), yet its role and mechanism in plaque-induced periodontitis remain less clear. In the experimental periodontitis mouse model, IAA successfully activated the AhR/CYP1A1 axis and promoted macrophage M2 polarisation. Besides, in LPS-stimulated

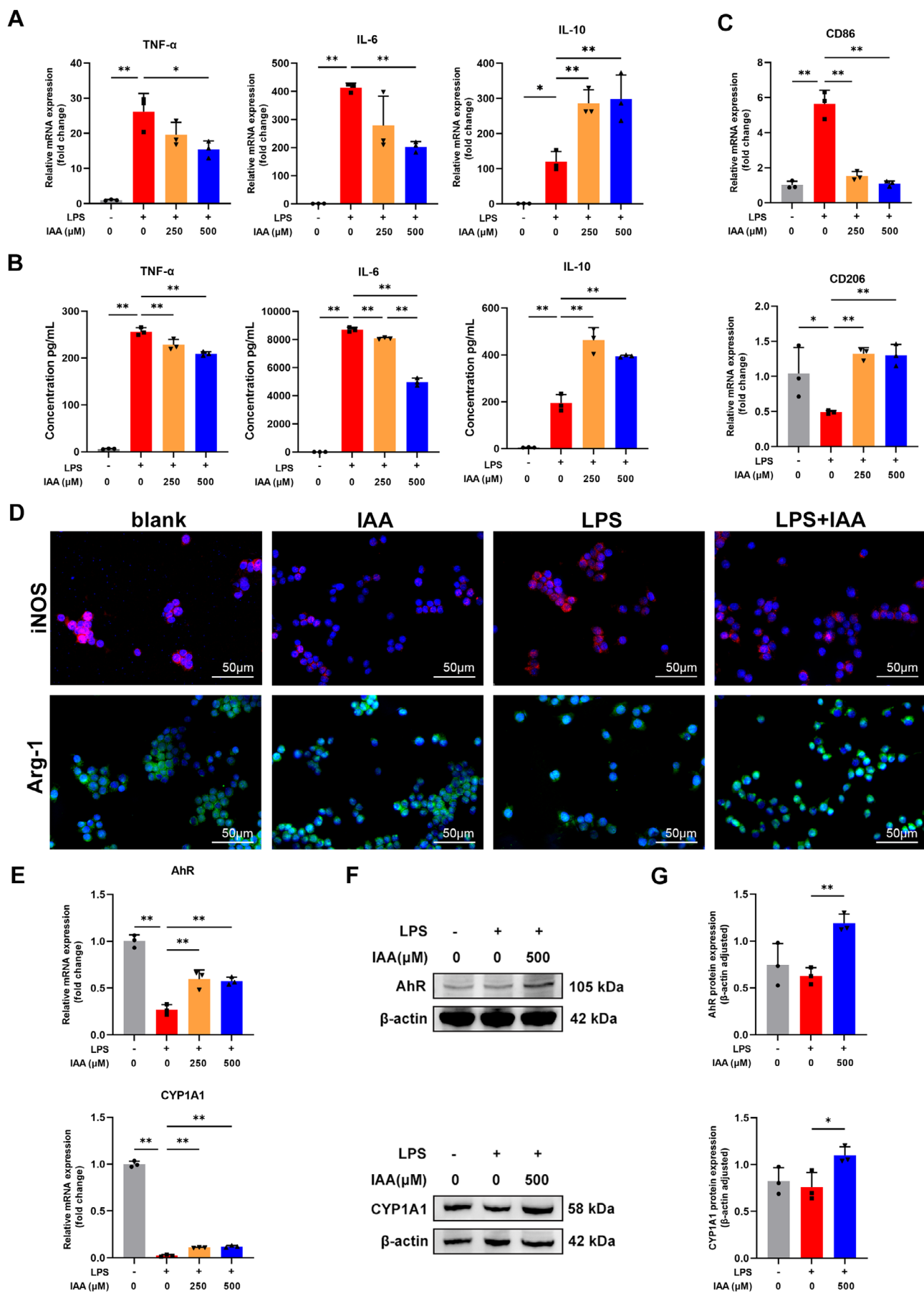


FIGURE 6 | Legend on next page.

FIGURE 6 | Effect of IAA on AhR/CYP1A1 expression and polarisation of RAW264.7 in vitro. (A,C,E) Relative mRNA expression levels of TNF- α , IL-6, IL-10, CD86, CD206, AhR and CYP1A1. (B) ELISA analysis of TNF- α , IL-6, IL-10 in cell culture supernatant. (D) Cell immunofluorescence staining of iNOS and Arg-1. (F,G) Representative Western blot images and relative protein expression of AhR and CYP1A1. $N=3$, experiments performed in triplicate. Red fluorescence, iNOS. Green fluorescence, Arg-1. Blue fluorescence, DAPI. Differences were determined by one-way ANOVA. * $p<0.05$, ** $p<0.01$.

macrophages, IAA attenuated pro-inflammatory factors, enhanced anti-inflammatory factors, induced M2 polarisation and up-regulated the AhR/CYP1A1 axis. These findings demonstrate that IAA ameliorates periodontitis by activating the AhR/CYP1A1 axis to regulate macrophage polarisation, specifically promoting an anti-inflammatory M2 phenotype. Further, a full dose-response analysis of IAA treatment would be valuable for its potential therapeutic application.

AhR, a transcription factor, cross-talks with several signalling pathways to modulate immune responses. AhR was found to suppress NF- κ B-mediated gene expression, thereby inhibiting the M1 macrophage polarisation (Xie et al. 2022). In addition, AhR activation was reported to reduce nephritis damage by down-regulating IRF1 and HIF-1 α and inhibiting M1 polarisation (X. Yang et al. 2020). Furthermore, the AhR-CaMKII pathway was shown to play a critical role in protecting against periodontitis via regulating macrophage polarisation (Zeng et al. 2024). These mechanisms may contribute to the therapeutic effects of IAA in periodontitis by promoting M2-like macrophage polarisation.

Our study has some limitations. First, as a preliminary observational investigation, we analysed salivary Trp metabolism using a relatively small clinical sample size. Validation in larger scale prospective cohorts is imperative. Second, potential confounding variables may influence Trp metabolism and oral microbiome composition, such as dietary patterns (Lin et al. 2022), medication interventions and metabolic diseases. Implementing specific exclusion criteria would minimise confounding effects. Third, this study exclusively focused on macrophages, but IAA likely regulates other immunologically relevant cells such as T cells and gingival epithelial cells (Su et al. 2024; Wang, Lu, et al. 2025). Systematic investigation of IAA-mediated regulatory mechanisms across multiple cell types would provide a more comprehensive understanding of its biological effects. Finally, in addition to local actions in periodontal tissues, IAA likely modulates multiple physiological systems, which merit further study.

5 | Conclusion

The differential Trp metabolite IAA was identified in saliva samples from patients with periodontitis and periodontally healthy individuals. We verified that IAA activated the AhR/CYP1A1 signalling pathway, subsequently promoting macrophage M2 polarisation, which inhibited inflammation and ameliorated periodontal destruction. The study revealed the potential role of the microbial metabolite IAA in the development and treatment of periodontitis, offering a new perspective for intervention strategies based on microbial metabolites for periodontitis.

Author Contributions

S.C. and S.S. contributed equally to this work. S.C. contributed to the conception, design, data acquisition and interpretation, and drafted and critically revised the manuscript. S.S. contributed to the design, data acquisition and analysis, and critically revised the manuscript. W.Z. contributed to data acquisition and interpretation, and critically revised the manuscript. D.Q. and M.W. contributed to data acquisition, and critically revised the manuscript. Z.W. and J.P. contributed to data analysis, and critically revised the manuscript. W.Y. contributed to the design, and critically revised the manuscript. F.Y. and Y.Z. contributed to the conception and design, and critically revised the manuscript. All authors gave their final approval and agree to be accountable for all aspects of the work.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Data S1:** Supporting Information.