Curcumin alleviates IMQ-induced dermatitis and regulates gut microbiota of mice

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Abstract

Background: As a polyphenolic compound originated from the food spice turmeric, curcumin (CUR) has various pharmacological effects, such as anti-inflammatory, anti-oxidation, anti-proliferative and anti-angiogenic activities. Psoriasis is centered on the overproduction of Th1- and Th2-related cytokines (e. g. IL-23, IL-17, TNF-α, IL-22), which is involved in the occurrence and development of its pathogenesis. However, whether CUR is involved in the treatment of psoriasis and its specific mechanisms are not fully understood. Methods: In this study, we detected the therapeutic effect of CUR (100mg/kg· d) on IMQ-induced dermatitis in mice, analyzed by PASI scores, ELISA, HE staining, immunofluorescence. Moreover, we further confirmed the alteration in the relative abundance of the gut microbiota through 16sRNA to explore whether CUR could regulate the gut microbiota of IMQ-induced mice. Result: Through intragastric administration, CUR can alleviate psoriasis-like lesions of mice by decreasing PASI scores, reducing the level of IL-6, IL-17A, IL-22, IL-23, TNF-α and TGF-β1, promoting the expression of IL-10. Moreover, 16 sRNA sequencing revealed that CUR could regulate the alteration in the abundance alteration of gut microbiota related to inflammation, such as Alistipes, Mucispirillum and Rikenella at genus level. The correlation analysis further confirmed the close association between important microflora and psoriasis-like inflammation indicators. Conclusions: CUR exerts the effect of alleviating dermatitis of psoriatic mice by regulating Th-17 related inflammatory factors. Moreover, the changes in gut microbiota via CUR may be another factor of relieving IMQ-induced lesions in mice. Therefore, CUR may be a highly promising candidate for the treatment of psoriasis.

Introduction

Psoriasis is a chronic, immune-mediated skin disease that can be accompanied by joint involvement. For its high prevalence and the complexity of causative mechanisms, psoriasis has caused great concern worldwide [1]. Its typical clinical manifestations are scales, erythema, and epidermal thickening. Currently, it is well recognized that psoriasis results from the complex interaction between keratinocytes and immune cells [2]. Many inflammatory factors (e. g. TNF- α, IL-1 β, IL-23, and IL-12) secreted by Th17 cells, Th1 cells, and keratinocytes played crucial effects in their pathogenesis [3]. A growing body of evidence suggests that psoriasis is closely linked to multiple co-morbidities, such as systemic inflammation and cardiovascular comorbidity [4]. Therefore, psoriasis has gained significant attention worldwide. However, there are no effective measures to achieve a cure for psoriasis.

Curcumin is a polyphenolic compound purified and isolated from Curcuma longa. Since ancient times, turmeric had been considered an herbal remedy for the treatment of skin and gastrointestinal disorders [5]. Now, it is clear that its widespread use in medicine stems from its many properties, such as antioxidant, anti-inflammatory, anti-proliferative, anti-cancer and anti-bacterial effects [6]. It had been demonstrated that curcumin can reduce various inflammatory factors such as TNF-α, IFN-γ, IL-22 and IL-23 in mouse

serum, which are closely involved in the pathogenic mechanism of psoriasis [7]. Meanwhile, curcumin was also considered as a protective compound that can regulate the intestinal microflora. Currently, increasing evidence had found that gut microbes were potentially involved in regulating the progression of a variety of diseases closely related to inflammation, such as psoriasis, psoriatic arthritis, inflammatory bowel diseases (IBD) [8, 9].

To further confirm our speculation, we found that curcumin could significantly improve the IMQ-induced mouse model, and reduce the expression of multiple pathogenic factors in its skin lesions, such as TNF-α, IL-17, IL-1 β, etc. Furthermore, we analyzed the changes of intestinal microbiota in mice after curcumin treatment. By using 16s rRNA technology, this study investigated whether curcumin could exert its therapeutic effect in psoriasis, which was in relation to the regulation of intestinal flora.

Materials and methods

2.1 Materials

Imiquimod cream (5%) was gained from Sichuan Mingxin Pharmaceutical Corporate, methotrexate was obtained from Chromabio (Chengdu, China). Anti-PCNA mouse mAb were obtained from Proteintech. All ELISA kits used in this experiment were purchased from Beyotime.

2.2 Experimental sketch

BALB/c mice (8-10 weeks, 20-22 g) used in this study were gained from SPF (Beijing) Biotechnology Co., Ltd. All animal experiments were in accordance with the relevant provisions and obtained the approvement from The Animal Experiment Committee of Fudan University (Shanghai, China). The mice were shaved a smooth area of 2 x 3 cm at the back. Meanwhile, every mouse could acquire enough food and water during experiment. Their living environment was also dry and comfortable. We randomly divided the mice into four groups: 1) control (CON, N=4); 2) imiquimod (IMQ, n=4) treated; 3) methotrexate (MTX, a clinical drug to treat psoriasis, n=4,) treated; 4) curcumin treated group (CUR, n=4). Except the CTR group mice, other groups mice were treated topically with a daily dose of 62.5 mg imiquimod cream every day. The psoriasis-like model was built after continuous 6 days. MTX treated mice and CUR-treated mice were treated for the intragastric application with MTX (1.0 mg/kg· d) and curcumin (100 mg/kg· d), applied after 1 day of imiquimod treatment. MTX and CUR were dissolved and diluted by clean and distilled water. After killed all the mice, the back skin and intestinal feces were collected for further processing.

2.3 H&E staining and immunofluorescence

The acquired mice skin tissues were fixed in 4% paraformaldehyde solution, and paraffin blocks were treated and stained by hematoxylin/eosin (HE). For immunofluorescence staining (IFC), main steps were performed as described previously [10]. All sections were examined with laser confocal microsopes (AOSVI, M303-HK830).

2.4 ELISA detection of skin tissues inflammatory factors

After adding the lysate, each collected skin tissue was broken through a crusher to form a solution. The levels of IL-17, TNF-α, IL-1β in mice skin tissues were assessed by ELISA kits (SAIPEISEN BIOLOGY). The main steps followed the manufacturer's instructions.

2.5 DNA extraction and polymerase chain reaction amplification

Total DNA was extracted from mouse feces using the PowerSoil® DNA Isolation kit according to the manufacturer's instructions. After extraction, the 16S full-length primer was designed according to the conservative region 27F-1492R, for polymerase chain reaction (PCR) amplification of the target area (10μL system, Solexa PCR). The synthesized specific primers (27F-1492R) were designed as follows: Forward primer 27F: AGRGTTTGATYNTGGCTCAG; Reverse primer 1492R: TASGGHTACCTTGTTASGACTT.

2.6 Sequencing and data analysis

Quality inspection was performed on the formed sequencing library, and processing, including barcode recognition, was performed on the high-quality circular consensus sequencing (CCS) sequence obtained. The generated optimization CCS was clustered at the level of 97% similarity (USEARCH, version 10.0), and its species classification was obtained based on the sequence composition of the operational taxonomic unit (OTU). The platforms 16S: Silva database and RDP Classifier were used to analyze species annotation and taxonomy as well as the diversity of gut microbiota.

2.7 Statistical analysis

All data in this study were processed with the GraphPad Prism 7.0, which has been showed as the mean ± SD. Statistical significance of all data was examined by one-way analysis of variance (ANOVA).

3.Results

3.1 Curcumin inhibits psoriasis-like skin lesions induced by IMQ

To further clarify the regulatory effect of CUR on psoriasis, we constructed psoriasis-like skin lesions induced by IMQ (Figure. 1A). As shown in Figure.1B-D, PASI scores were largely increased in IMQ-induced mice than in control mice. CUR could significantly decrease PASI scores and epidermal thickness in affected lesions of IMQ-induced mice (Figure. 1B-E). Therefore, CUR could relieve the IMQ-induced mouse skin lesions and reduce the PASI scores in the affected areas.

3.2 CUR ameliorate the morphology changes of psoriasis-like dermatitis in mice

We further processed the skin tissues collected from all groups of mice for HE staining and IFC staining. The results showed that oral curcumin alleviated IMQ-induced dermatitis in mice, such as parakeratosis, erythema, and infiltrating inflammatory cells (Figure. 2A). Moreover, PCNA was a highly proliferative marker of keratinocytes at psoriatic lesions [11]. The IFC results showed that CUR significantly decreased the expression of PCNA in the back skin of model mice (Figure. 2B). Thus, the data revealed that CUR can markedly inhibit over-proliferating keratinocytes in IMQ-induced lesions of mice.

3.3 CUR regulates the inflammatory indicators associated with psoriasis-like lesions in mice

To further explore whether CUR regulates the inflammatory responses induced by IMQ, we explored the expression levels of multiple inflammatory indicators in mouse skin lesions by ELISA kit. As shown in Figure. 2C-D, CUR can significantly the epidermal expression levels of IL-6, IL-17A, IL-22, IL-23, TNF-α, TGF-β1 compared with IMQ group. In addition, the expression levels of IL-10 in CUR group were higher than in the IMQ group (Figure. 2C). The results demonstrated that CUR can repress the expression of inflammation factors related to psoriasis onset and development.

3.4 CUR reshapes the intestinal microflora composition of mice

Next, we further investigated the effect of CUR on the intestinal microbiota of mice. We examined the V3 and V4 regions within the 16sRNA of all mouse gut microbiota, and the rarefaction curves showed extremely high coverage in every sample (Figure. 3A). From the principal coordinate analysis (PCoA) results, we found a significant separation of the microflora distribution between groups CON, IMQ and CUR (Figure. 3B). After analyzing the flora diversity among the CON, IMQ and CUR groups, Simpson and Shannon index showed that the CON and CUR groups were significantly higher than the IMQ group (Figure. 3C-D). Moreover, the analysis of the microflora diversity results showed that it mainly included 10 classes: *Bacilli* , *Clostridia* ,*Bacteroidia* , *Desulfovibrionia* , *Deferribacteria* ,*Campylobacteria* , *Saccharimonadia* , *Coriobacteriia* ,*Gammaproteobacteria* , *Alphaproteobacteria* (Figure. 4A).

For further exploring these data, we performed high dimensional analysis by line discriminant analysis effect size (LEfSe) that determined significant differences in the main flora between CON, IMQ and CUR groups. The dominant flora was Lactobacillus at the genus level IMQ group, which we speculated is due to reduced diversity of microflora. The CUR group showed the dominant flora were*Mucispirillum* 、*unclassified_desulfovibrionaceae* at the genus level, while *Deferribacters* , *Desulfovibrionia* at the class level. The CON group

revealed that the predominant flora was*Ruminococcaceae* at the family level (Supplementary Figure. 1A-B).

Subsequently, we performed Kruskal-Wallis test to detect the microflora differences between the three groups. Compared with CON and CUR groups, the relative abundance of *Bacilli* at the class level was marked increased in IMQ group, while the result of *Desulfovibrionia* was contrary (Figure. 4B-C). Moreover, compared with IMQ group, the relative abundances of clostridia, *Deferribacteres* at the class level were significantly promoted in the CUR group, while the result of*Saccharimonadia* was opposite (Figure. 4B-C). Ultimately, at the genus level, the abundance of *Alistipes* , *Mucispirillum* ,*Rikenella* in CON and CUR groups was markedly higher than in the IMQ group (Figure. 5A-D).

3.5 Changed intestinal microflora by CUR was associated with psoriasis-related factors

To further understand the role of altered gut microbiota by CUR and its connections with psoriasis, we treated psoriasis-related inflammation indicators as a variable by spearman correlation analysis at the genus level based on OTU abundance, presented with a heatmap. As shown in Figure.6A, *Ligilactobacillus* and *Anaeroplasma* (inhibited in CUR mice) were positively correlated with multiple psoriasis-related factors, such as IL-6, IL-17A, IL-22, Il-23, while *Rikenella, Alistipes,* and *Mucispirillum* (promoted in CUR mice) were negatively correlated with them. These results indicated that the gut flora changes induced by CUR are associated with the inflammatory factors in psoriasis.

Discussion

Psoriasis is well recognized as a chronic inflammatory disease prone to recurrent attacks. Its typical symptoms are well-demarcated erythema and skin scales accompanied with systemic symptoms [12]. Curcumin, as a polyphenolic compound stemmed from turmeric, owns multiple pharmacological effects, comprising anti-inflammatory, anti-proliferative, antioxidant, and anti-angiogenic activities [13].

It is well known that the typical histological changes of psoriasis show excessive proliferation of keratinocytes and infiltration of inflammatory cells, increased PCNA in skin lesions, and elevation of various inflammatory factors, such as IL-6, IL-17A, IL-22, IL-23, IL-1β [14, 15]. We found that CUR could significantly alleviate IMQ-induced psoriasis-like dermatitis, decreasing lesion thickness of mice, inflammatory cell infiltration, dyskeratosis, and spinous layer hypertrophy. In additions, CUR could inhibit the levels of PCNA in the epidermis of IMQ-induced mice, and the epidermal expression of IL-6, IL-17A, IL-22, IL-23, TNF-α, TGF-β1. Thus, the results revealed a possible effective mechanism of CUR against psoriasis-like dermatitis induced by IMQ.

Recently, reports had shown that the occurrence and development of psoriasis were closely associated with intestinal microbiota, and some pro-inflammatory gut microbes promoted Th1/Th17 activation in psoriasis, which then could cause the expression of various inflammatory factors in the epidermis, such as IL-17 and TNF-α [16]. Therefore, the intestinal microbiota can be regarded as a potential therapeutic target for alleviating psoriasis. Meanwhile, it had been shown that CUR can participate in the regulation of intestinal microbiota [17]. Taken together, we reasoned that CUR reshaped the diversity of the mouse gut microbiota.

To further investigate the supposition, we detected the effect of CUR on intestinal flora by using 16S rRNA sequencing. Remarkably, we found that the abundances of genus *Alistipes* , *Rikenella* and*Mucispirillum* were promoted in CUR group. The researchers found that *Alistipes* and *Odorbacter* were positively correlated with intestinal acetate and propionate production, which inhibits the inflammatory activity of inflammatory bowel diseases (IBD) [18].*Rikenella* had been shown to be associated with the formation of short-chain fatty acids (SCFAs). It had been reported that the intestinal microflora and its metabolites can catabolize polysaccharides to produce SCFAs. SCFAs can maintain the intestinal barrier function, which is important for intestinal homeostasis. SCFAs not only maintained the cellular barrier but also prevented the transfer of LPS from the intestinal barrier [19]. Moreover, the abundance of*Mucispirillum* positively correlated with the concentration of the inflammatory suppressor IL-10 in breast cancer model mice [20]. Interestingly, *Lactobacillus* at the genus level was increased, which was probably due to the decreased diversity of gut microbiota of mice in the IMQ group.

Taken together, although there was no direct evidence to demonstrate that the gut flora changes induced by CUR linked with psoriasis, these bacteria were associated with inflammation. Furthermore, Moreover, the correlation analysis further showed a significant association between the three picked gut microflora at genus level regulated by CUR and inflammatory factors. We thought that there was a close connection between them. Therefore, inflammation served as an important role to connect CUR, psoriasis, and intestinal microbiota together. To be brief, CUR could relief psoriasis-like lesions of mice by repressing Th-17 related inflammatory factors, and regulate intestinal microbiota associated with inflammation, showing CUR may be a promising drug for alleviating psoriasis.

Conflict of Interest

The authors have no any conflicts of interest to declare.

Author Contributions

ZC, WW, and YZ designed and guaranteed the whole experiment studies; ZC, WW carried out all the experiments, ZC and YZ analyzed the statistical data, ZC, WW and YZ drafted the manuscript; ZC, YZ edited and revised the whole manuscript; all authors read and approved the final manuscript.

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