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Short communication

Filtered Kombucha Tea Rings the Bell for TLR2, TLR4, MYD88, and Dectin-1 in Mice Model of Colitis

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Abstract

Background and objectives: TLR2, TLR4, and Dectin-1 (Clec7) are pattern recognition receptors (PRRs) expressed by intestinal epithelia cells and MYD88 is a signaling molecule of TLR2 and TLR4. They warn immune system about the presence of invading pathogens promoting initiation of inflammatory response. Because of colonic cancer risk, therapy of intestinal inflammation is of high importance. Natural products are suitable candidates among which Kombucha tea has shown healing effect on mice model of colitis. **Methods**: Filtered Kombucha tea was prepared from black tea and sucrose plus tea fungus and previously fermented Kombucha tea. The collection was fermented by incubation at 28 °C for 14 days and filtered. Colitis was induced in young and old mice by administration of 3.5% (w/v) dextran sodium sulfate in drinking water during 7 days; filtrated Kombucha tea was given orally to animals with colitis, at a dose volume of 10 mL/kg daily for 21 days. Dectin-1, f toll-like receptor (TLR)-2, 4, as PRRs, and MyD88, as PRR signaling molecule were measured and compared with the age-matched normal and colitis model. **Results**: Treatment with filtrated Kombucha tea significantly affected TLR2/TLR4 pathway and its downstream signaling molecules, MYD88 & dectin-1expression and subsequently inflammatory condition in dextran sodium sulfate-induced colitis. **Conclusion:** The results of the present study may indicate possible implication of Kombucha tea with TLRS which consequently produces the anti-colitis effects.

Keywords: Colitis; Dectin-1; Kombucha tea; MYD88; TLR

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Introduction

Intestinal epithelium detects and senses antigens by pattern recognition receptors (PRRs) such as toll-like receptors (TLRs) [1,2]. Failure in the balance of PRRs' expression and function in the intestine can contribute to the development of "leaky gut syndrome" [3], maintenance of IBD and ulcerative colitis. Because of colonic cancer risk, therapy or amelioration of colitis is of high importance [4]. TLR-2/TLR-4 drug inhibition has been a new promising strategy to prevent inflammation in IBD. Some therapeutic approaches that may mediate IBD have been suggested, but none of the proposed methods were so far proven to be effective. In addition, the available synthetic anti-colitis drugs have side effects and are expensive [5,6]. Kombucha tea has previously shown healing effects on aging skin, intestinal ulcer and animal model of colitis [7-9]. We previously showed the therapeutic effects in a mice model of colitis [9]. This study aimed to investigate if filtrated Kombucha tea effect on colitis was due to alteration in intestinal PRRs such as TLR2 &TLR4, MYD88, and Dectin-1 level.

Material and Methods

Ethical considerations

The animals were treated in accordance with

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Alborz University guideline for animal use and the NIH Guide for Care and Use of Laboratory Animals (NIH Publications No. 80-23). Ethics Committee of Alborz University of Medical Sciences approved the study with the code of 1394/37 on 2016-11-07

Preparation of filtrated Kombucha tea

Filtered Kombucha tea was prepared from black tea (Golestan, Iran) in boiling water (1.2 %w/v) and sucrose (10%) plus 3% w/v tea fungus and 10 %v/v previously fermented Kombucha tea by incubating at 28 °C for 14 days. The resultant fermented tea was centrifuged at 5000 rpm for 20 min and filtered using a 0.45 µm cellulose filter equipped with a vacuum pump [8].

Study design and induction of colitis

Forty eight male NMRI mice were provided from Pasteur Institute, Tehran, Iran. According to the age, the mice were equally divided into two groups: young (2 months) and old (16 months) animals. Each group was divided into three subgroups (eight mice in each group) including diseased animals without treatment or treated with filtrated Kombucha tea and healthy control animals. Dextran sodium sulfate salt-induced colitis was established according to previous studies [9] by administration of drinking water containing 3.5% (w/v) dextran sodium sulfate (40000 kDa, MP Biomedical Co., Germany) per mouse per day. The filtrated Kombucha tea was given orally to animals with colitis, at a dose volume of 10 mL/kg for a period of 21 days.

Tissue preparation and analysis of gene expression by Real-Time PCR

Animals were sacrificed [9] and 50 mg of the colon tissue was separated and, the total RNA was extracted using RNeasy kits (Qiagen) following the manufacturer's protocol. Reverse transcription of 1µg RNA to DNA was performed using Aid First Strand cDNA Synthesis Kit (Munich, Germany), on a BIA real time PCR machine. PCR amplification was performed in a final volume made up to 10 µL using 5 µL of cDNA, 0.3 µM forward and reverse primers, and SYBR Green Supermix (Bio-Rad). The sequences of primers, designed by Integrated DNA Technologies, were forward 5′-AGCATCCGAATCGCATCACC-3′ and reverse 5′-ACCCCAGAAGCATCACATGA-3′ for TLR2 (NM_011905.3), forward 5′-TCTAACTTCCCTCCTGCGAC-3′ and reverse R: 5′-ACGATCTGTAACTGGTGGCA-3′ for TLR4 (NM_021297.3), forward 5′-GTCTCCAGGTGTCCAACAG-3′ and reverse 5′-CATCTTCCCCTCTGCCCT-3′ for MYD88 (NM_010851.2), and forward 5′-CAGCATTCTTCCCCAACTCG -3′ and reverse R: 5′-CACACACACACACACACCAT -3′ for Dectin-1 (NM_020008.3). The GAPDH gene was chosen as an internal control. The resultant gene expression level was presented as $2^{-\Delta Ct}$, in which ΔCt was the difference between Ct values of target gene and GAPDH [10].

Statistical analysis

One-way ANOVA followed by the Tukey's post hoc procedure and Graph Pad Prism 7.01 software were used and data were presented as means± SEM. P-value less than 0.05.was considered statistically significant.

Results and Discussion

Our previous results demonstrated therapeutic effect of filtrated Kombucha tea on dextran sodium sulfate-induced mice model of colitis [9]. To assess the effect on changes in the expression of TLR2, TLR4, MYD88, and dectin-1, mRNA, the tissue sections of colon were evaluated. Figures 1 and 2 depict that, TLR2 and TLR4 mRNA levels were markedly down-regulated in both dextran sodium sulfate -induced colitis young and old animals compared to the age-matched healthy animals and markedly increased following filtrated Kombucha tea treatment. TLR2 mRNA expression was significantly different between the young and old healthy animals with more expression in the young animals. Inversely, TLR4 mRNA level was significantly increased in old healthy animals compared to the young healthy group which is consistent with the previous reports [11]. In addition, the rate of decrease in TLR2 level in the young animals after colitis induction was more than that of the old group. This may indicate a more severe disease in the young animals after treatment with dextran sodium sulfate compared to older animals as observed and reported before [9]. This event was not observed in TLR4. According to previous reports, this receptor and its signaling molecule show homeostatic role in the intestine [12], and presumably, there are optimum levels to make TLR4 with restorative effect and beyond that levels make TLR4 with disruptive effects on epithelial tight junctions. Given the downstream signaling association of

MYD88 with TLR2/TLR4 [13], we further examined the effects of filtrated Kombucha tea treatment on MYD88 expression in dextran sodium sulfate-induced colitis young and old mice.

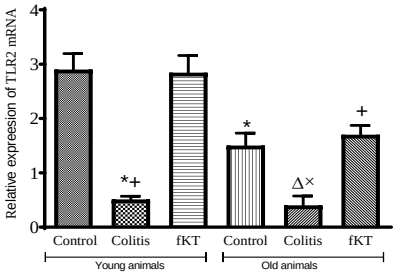

Figure 1. TLR2 mRNA expression in the colon of young and old healthy, DSS-induced colitis, DSS-induced colitis treated with fKT. Data are mean ±SD; *, +, ×, Δ: significant difference with young healthy animals (p<0.0001), young DSS -induced colitis treated with fKT (p<0.0001), old healthy animals (p<0.01), old DSS -induced colitis treated with fKT (p<0.01), respectively; fKT: filtrated Kombucha tea; DSS: dextran sodium sulfate

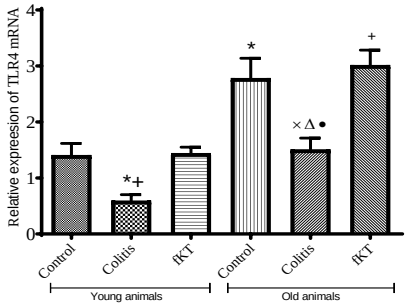

Figure 2. Changes of TLR4 mRNA level in the colon of young and old healthy and treatment animals. Data are mean ±SD; *, +, ×, Δ, ●: significant difference with young healthy animals (p<0.0001), young DSS -induced colitis treated with fKT (p<0.0001), old healthy animals (p<0.01), old DSS -induced colitis treated with FKT (p<0.01), young DSS -induced colitis (p<0.01), respectively; fKT: filtrated Kombucha tea; DSS: dextran sodium sulfate

MYD88 significantly decreased in the young and old animals with colitis compared with the age-matched healthy animals. Figure 3 shows that treatment with filtrated Kombucha tea was successful in increasing MYD88 mRNA level in the young animals with colitis, compared with the age-matched control group. Nevertheless, such an effect was not observed in the old group. MYD88 in the young healthy and dextran sodium sulfate -induced colitis animals was significantly more than that of the corresponding old animals. It may be somehow similar to the previous studies showing that the young colitis animals respond better to filtrated Kombucha tea treatment than older mice [9]. Since absence of MYD88 increases Dectin-1 inflammatory activity [14], we also measured Dectin-1 mRNA level. Figure 4, displays that the expression increased in dextran sodium sulfate -induced young colitis group and returned back to the normal level following treatment with filtrated Kombucha tea.

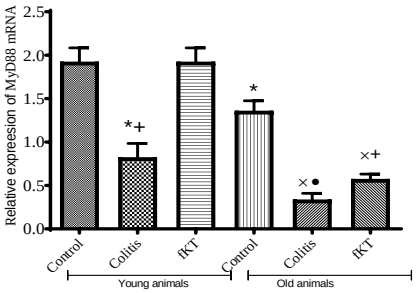

Figure 3. MYD88 expression level in the colon of young and old healthy and treatment animals. Data are mean ±SD; *, +, ×, Δ, ●: significant difference with young healthy animals (p<0.0001), young DSS -induced colitis treated with fKT (p<0.0001), old healthy animals (p<0.01), old DSS -induced colitis treated with FKT (p<0.01), young DSS -induced colitis (p<0.01), respectively; fKT: filtrated Kombucha tea; DSS: dextran sodium sulfate

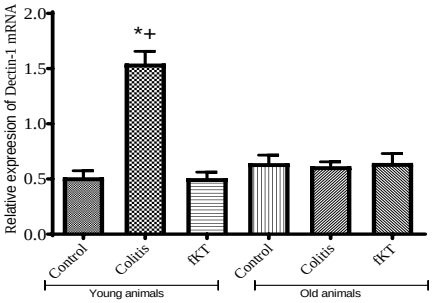

Figure 4. Relative change of dectin-1 mRNA level in the colon of young and old healthy and treatment animals. Data are mean ±SD; *, +; significant difference with young healthy animals (p<0.0001), young DSS -induced colitis treated with fKT (p<0.0001), respectively; fKT: filtrated Kombucha tea; DSS: dextran sodium sulfate

There was no change in the old group after treatment with dextran sodium sulfate or filtrated Kombucha tea. Furthermore, there was no significant difference between the young and old healthy animals in Dectin-1 mRNA level. Dectin-1 is another member of PRRs family by which intestinal epithelial cells respond to β-glucans of fungi [14]. The present study demonstrated that dectin-1 was not affected by age in the healthy animals. Nevertheless, there was obvious difference in using filtrated Kombucha tea and response to colitis induction. This may be due to the changes in the fungal flora of the gut by aging [15]. More severe colitis due to dextran sodium sulfate in the young animals may be explained by the higher level of dectin-1 compared to older animals. This is because inhibition of dectin-1 ameliorates colitis by inducing anti-inflammatory

response in the intestine [16].
Collectively, these novel findings demonstrate that treatment with filtrated Kombucha tea affects TLR2/TLR4 pathway and its downstream signaling molecules, MYD88 & dectin-1 expression, and subsequently inflammatory condition in dextran sodium sulfate-induced colitis.

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Author contributions
Elaheh Mahmoudi designed the experiments; Mansoureh Yazdkhasti wrote the first draft of the paper and contributed in molecular testing; Amin Gharanfoli contributed in the molecular experiments.

Declaration of interest
The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

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Abbreviations
IBD: inflammatory bowel disease; fKT: filtrated Kombucha tea