CANDIDA ALBICANS IN GUT ENHANCED THE SEVERITY OF DEXTRAN SULFATE SOLUTION-INDUCED COLITIS MOUSE MODEL

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ABSTRACT

The influence of gut fungi against acute colitis is intriguing. While, dextran sulfate sodium (DSS)-induced colitis mouse model is a representative model for colitis in patients, *Candida albicans*, are detectable only in human intestine, but not in mouse. Thus, 10^6 cells/ml of *C. albicans* were administered in 3% DSS-colitis mouse model to explore the influence of the fungi toward the model.

DSS-*C. albicans* administration enhanced the severity of colitis model as determined by the increased serum IL-6 cytokine level (a pro-inflammatory cytokine).The additive effect of heat-kill *C. albicans* and *Escherichia coli* toward HT 29 cell, a human colon adenocarcinoma cell line, in the production of IL-8 cytokine, neutrophil chemotactic factor, compared to the cells activated with *E. coli* or *C. albicans* alone were also demonstrated.

In conclusion, *C. albicans* enhanced the severity and the inflammatory response in DSS induced colitis mouse model.

Keywords: Colitis, *Candida albicans*, Gut translocation, Dextran sulfate sodium

1. Introduction

Dextran sulfate sodium (DSS) induced colitis mouse model is a model that frequently used to represent inflammatory bowel disease (IBD) in human. IBD consists of Crohn's disease (CD) and Ulcerative colitis (UC) that are the relapsing systemic inflammatory bowel diseases (Ashton, Harden, & Beattie, 2017; Baumgart & Sandborn, 2012; Hoarau et al., 2016; Kaser & Blumberg, 2017). IBD is a worldwide health care problem (Dillman et al., 2015; Yang & Jobin, 2014) with the increasing trend in the several regions of the world including Asia (Asakura, Suzuki, Kitahora, & Morizane, 2008; Baumgart & Sandborn, 2012; Van Leeuwen et al., 1994).

DSS induces intestinal epithelium cells (IECs) damage and the loss of epithelium tight junction (TJs), they are decrease of probiotic and increases enterophathogens bacteria such as *E. coli*, *Sallmonella* spp. and *Pseudomonas aeruginosa*. Which, *E. coli* might be a "mildly pathogenic", they can adhesion and secretion of proteases that the causing of inflammation (Rhodes, 2007). The translocation of intestinal pathogen associated molecules (PAMPs) through the injured epithelium cells into the gut circulation or serum is possible. This character

leads to the responses of immune system such as the engulfment of pathogens by macrophages and dendritic cells, a professional antigen presenting cell (APC). In addition, cytokines and chemokines such as tumor necrosis factor (TNF)-α, interferon-γ (IFN- γ), interleukins (IL-1β, IL-6, IL-8, IL-12) are released to recruit and activate another immune cells (neutrophils, NK cells, T cells and B cells) at the site of inflection, resulting in tissue damage or chronic inflammation (Hooper, 2009; Johansson et al., 2010).

The predominant fungi in human Gastrointestinal (GI) tract are *C. albicans* which could be an opportunistic fungal pathogen of candidiasis especially in the immunocompromised host (Kumamoto, 2011). As such, (1, 3)-β-D-glucan (BG), the major polysaccharide component in cell wall of *C. albicans*, (Sherrington et al., 2017) possibly be recognized through Dectin-1 of innate immune cells (Zheng, Wang, Hu, Yan, & Jiang, 2015) and could enhance the severity of DSS induce colitis mouse model. Hence, we tested this hypothesis in a mouse model.

2. Objectives of the study

To investigate if *C. albicans* enhance the severity in DSS induced colitis mouse model.

3. Materials and methods

***C. albicans* preparation**

C. albicans ATCC 90028 (Microbiologics, Saint Cloud, Minnesota, USA), a Fluconazole susceptible strain (minimal inhibitory concentration 0.25-1 μl/ml) was used. *C. albicans* were cultured in Sabouraud dextrose broth (SDB) overnight and counted the cells in hemocytometer, $1x10^6$ cells/ml, live cells for gavages mice model and was counted $5x10^5$ cells/ml heat-kill before used to induced local inflammation. Heat-kill *C. albicans* were prepared by immersion in a water bath at 60 °C for 1 h.

Animal models

Male C57BL/6 mice, 8 weeks old, National Laboratory Animal Center (ICR), Nakhornpathom, Thailand were used. The US National Institutes of Health (NIH) animal care and use protocols were followed.

Induction of colitis and gavages *C. albicans*

Animals were randomly divided into four groups (n=7 each group). Colitis was induced with DSS, molecular weight 40 KDa (SIGMA-ALDRICH, Co., 3050 spruce Street, St. Louis, MO 63103 USA). The DSS group was starting given 3.0% DSS at the first day. The DSS and *C. albicans* group was gave 3.0% DSS followed by gavages $1x10^6$ cells/ml live *C. albicans* (0.5 ml) or water control (placebo group) administer in the individual mouse at the first day. Body weight were measured before experiment and every twice diary after induced colitis. Blood collection was performed at indicated time point by tail vein and at sacrifice with cardiac puncture under isofurane anesthesia, 7 days post-DSS, respectively (Fig 1A.).

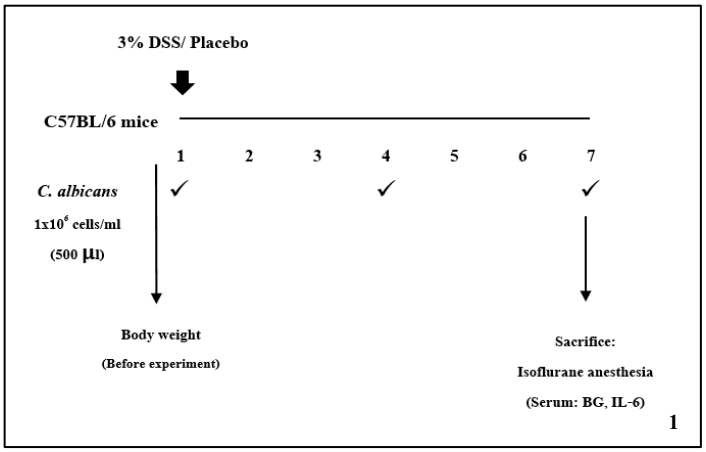

Fig 1. *C. albicans* enhanced DSS induce colitis severity design.

Mouse blood sample analysis

Blood samples were collected, centrifugation at 3000x g, 4 °C, 15 min, the serum were collected and stored at -80 °C until used (Zhang et al., 2017). Serum IL-6 cytokine was measured with ELISA assay (BioLegend).

***E. coli* preparation**

E. coli ATCC 25922 were cultured in Nutrient broth (NB) overnight and then counted the cells by spectrophotometer at a concentration $1x10^9$ cells/ml before used. Prepared heat-kill by immersion in a water bath at 60 °C for 1 h (Schmied, Rupa, Garvie, & Wilkie, 2012).

Cell lysate preparation

C. albicans and *E. coli* were prepared by vigorous sonication of heat-kill cells (Sonics Vibra Cell, VCX 750, Sonics & Materials Inc., Newtown, CT, USA) until a homogenous solution was form. Mix complete homogenate and the supernatant after centrifugation of the preparation were used as heat-kill *C. albicans* or *E. coli* and lysate, respectively (Panpetch et al., 2017).

Induction of human colon adenocarcinoma cell line HT29 cytokine production

Homogenous solution of *C. albicans* and *E. coli* were co-incubated with human colon adenocarcinoma cell line HT29 (HT 29 cell line) ($1x10^5$ cells/well) in culture plate for 24 h. Then, collected the supernatant and measured for IL-8 cytokine release by ELISA assay (BioLegend).

Statistical analysis:

Data was analyzed as mean ± standard error (SE) and the difference between groups were examined for statistical significance by one-way analysis of variance (ANOVA) followed by Bonferroni analysis for the multiple groups comparison. Which, analyses were performed with GraphPad Prism 6 software (Suite 230 La Jolla, CA 92037 USA). A P-value < 0.05 was interpreted as a statistical significant.

4. Results

Oral administration of live *C. albicans* induced colitis severity in mouse model

The severity of colitis, *C. albicans* administration at 1x10^6 cells/ml with 3% DSS, was demonstrated by weight loss. Mice should be monitored twice daily for body weight and present occult blood in stool, showed onset of disease in 3 days after 3% DSS treatment, continuously decreased, 28% were showed occult blood in 5 days and present in all mice 7 days after treatment. While, the administration ether 1x10^6 cells/ml per mouse 3% DSS alone was showed only weight loss 6.25 % (Fig 2.).

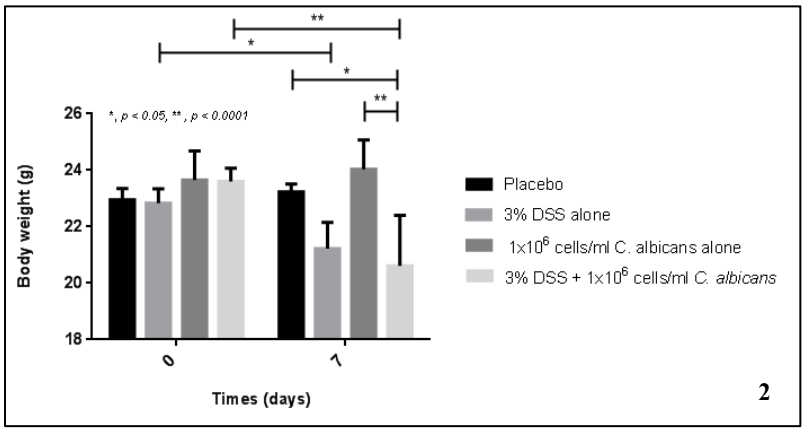

Fig 2. Body weight analysis of mice with *C. albicans* 1x10^6 cells/ml, 3% DSS induced colitis, 3% DSS induced colitis with *C. albicans* 1x10^6 cells/ml compared with placebo group (A), compared with *C. albicans* 1x10^6 cells/ml group (B) was analyzed (n=7/group); *, $p < 0.05$; #, $p < 0.0001$.

Serum IL-6 cytokine was increased in *C. albicans*-DSS induced colitis mouse model

The level of serum IL-6 cytokine represent to systemic inflammatory response in mouse model, showed higher in 1x10^6 cells/ml *C. albicans* administration with DSS induced colitis group (257.7 ± 42.93 pg/ml) followed by 1x10^6 cells/ml *C. albicans* alone (139.6 ± 26.1 pg/ml), 3% DSS alone (104.43 ± 45.53 pg/ml) and placebo (78.8 ± 26.5 pg/ml), respectively (Fig 3.).

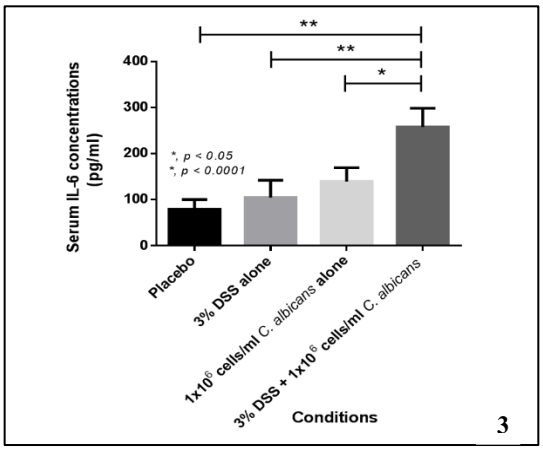

Fig 3. Serum IL-6 level (n=7/group); *, $p < 0.05$; **, $p < 0.0001$.

IL-8 cytokine was increased in human colon adenocarcinoma cell line (HT-29) against bacteria and fungus

The *in vivo* study were showed loss of body weight (Fig 2.) and increased serum IL-6 (Fig 3), suggested that *C. albicans* enhanced severity in DSS induced colitis mouse model. To support the important role of IECs response to *C. albicans*, we incubated heat-kill and lysate of *C. albicans* with or without *E. coli*, as the representative of fungi and pathogenic bacteria in colon, respectively. As such, fungi with bacteria induced the higher level of IL-8, an important inflammatory cytokine, in comparison with fungi or *E. coli* alone (Fig 4.).

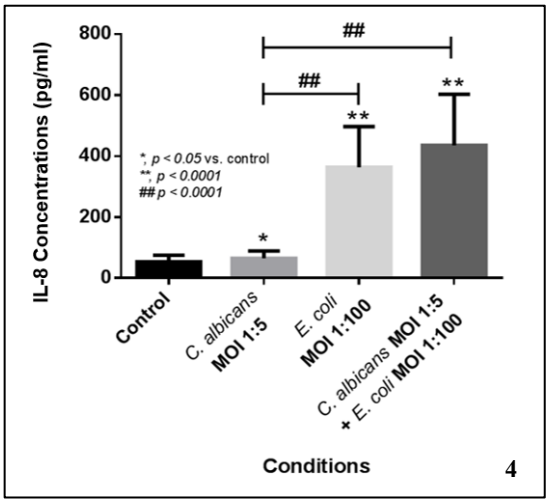

Fig 4. Heat-killed lysate showed additive effect to enhanced HT29 cell line to secreted IL-8 cytokine level (n=12/group); *, $p < 0.05$; *, $p < 0.0001$; ##, $p < 0.0001$.

5. Discussion

Inflammatory bowel disease (IBD) is a chronic colitis of colon. The actual causes of these diseases are remained unknown (Baumgart & Sandborn, 2012), but the current hypothesis implies epithelial barrier defects with the presentation of gut micro-organisms. As such, 3% DSS is the chemical agent that causes intestinal epithelial damage (Perse & Cerar, 2012)which is commonly used as DSS induced colitis mouse model (Johansson et al., 2010; Qiu et al., 2015; Zhang et al., 2017).

Interestingly, *C. albicans* is the predominant fungal species in human intestine but not in mouse. Thus, the administration of fungi in DSS induced colitis mouse model might be more closely to the colitis in human. *C. albicans* at $1x10^6$ cells/ml was used because of the induction of positive fecal culture without candidemia. It is possible that *C. albicans* in gut enhances the severity of DSS induced colitis mouse model. Indeed, colitis with *C. albicans* administration induced the more severe colitis as determined by the higher serum IL-6 level compare to DSS induced colitis alone but non-difference in weight loss. Moreover, in the *in vitro* study, we observed that the production of IL-8 cytokine, neutrophils chemokine in inflammatory response, from HT-29, an intestinal cell line,

was higher with the induction by heat-kill *E. coli* plus heat-kill *C. albicans* in comparison with the induction by each molecules alone. This probably because the additional induction of TLR with Dectin-1 by the induction of molecules from *E. coli* and fungi, respectively. For an example, TLR-5 and TLR-4 in epithelial cells are important for the recognition of flagella endotoxin (Kawai & Akira, 2010), respectively, from *E. coli*. Dectin-1 is responsible for the recognition of (1→3)-β-D-glucan, a molecule of fungal cell wall structure. Thus, the combination *E. coli* and heat-kill *C. albicans* demonstrated an additive effected on intestinal epithelium compare with the induction with each molecule alone. The fungal and bacteria molecules were activated immune responses that probably lead to the more severity in mouse model.

6. Conclusion

In conclusion, *C. albicans* enhanced the severity and the inflammatory response in DSS induced colitis mouse model as determined by the higher serum IL-6 level that probably due to the additive activation of the cell wall molecules from *E. coli* and fungi.

Acknowledgements

The scholarship from the graduate school, Chulalongkorn University to commemorate the 72nd anniversary of his Majesty King Bhumibala Aduladeja is gratefully acknowledged. Interdisciplinary Program of Medical Microbiology, Graduate school, Chulalongkorn University.

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