

The Effects of Kombucha Tea on Intestinal Integrity in Mice

Elaheh Mahmoudi

Abstract

Objective(s): Oxidative stress is implicated within the pathogenesis of hyperpiesia, a risk factor for cardiovascular morbidity and mortality. Several human studies have shown that tomato carotenoids can affect various aspects of human health. During this presentation the author will address two issues: a) balancing the response of skin cells to UV irradiation and b) the reduction of elevated vital signs. Several human studies have shown that tomato carotenoids can reduce UV-induced damage by reducing erythema and improving the balance between collagen production and breakdown. We hypothesized that a mixture of tomato carotenoids alongside polyphenols might yield better skin protection than that expected from summation of their activity. Truly, we understood that mixtures of tomato nutrient complex (containing lycopene) with rosemary extract (containing the polyphenol carnosic acid) synergistically reduced inflammatory markers and induced antioxidant activity in skin cells foremost to decrease of Matrix Metalloproteinase (MMPs) and thus may lessen collagen breakdown and delay skin ageing. b) Hyperpiesia may be a risk factor for cardiovascular morbidity and mortality. We performed a dose-response analysis to uncover the optimal effective dose of a tomato nutrient complex supplement in maintaining normal signs among hypertensive individuals.

Materials and Methods: Sieve-like gut was persuaded in two collections of young and adulthood using dextran sodium sulphate in beverage for seven days. Formerly, fKT was managed to the mice pretentiously by colitis and related to the age-matched normal and untreated animals with colitis.

Kombucha Tea (KT) preparation: Black tea (Golestan, Tehran, Iran) was added to boiling water (1.2%w/v), mixed, and left to brew for 10 min. The tea was then filtered through a sterile sieve, and sucrose (10%) was dissolved within the tea. To organize KT, 200 ml of the cooled tea was inoculated with 3%w/v tea fungus plus 10%v/v KT liquid that was previously fermented. The gathering was then left to ferment by

incubating at 28 °C for 14 days. to form filtered KT (fKT), the resultant fermented tea was centrifuged at 5000 rpm for 20 min and filtered employing a 0.45 µm cellulose filter equipped with an air pump.

Experimental groups and study design: Male NMR mice were purchased from the Pasteur Institute Experimental Animal Center, Tehran, Iran. All animals were housed for one week before the experiments began in light- and temperature-regulated rooms at the traditional animal department of Alborz University of Medical Sciences. All experimentations were permitted by the native ethical group (reference No Abzums.Rec.1395.51) and performed consistent with Animal Care and Use Protocol of Alborz University of Medical Sciences.

The animals were divided into two groups of young and old. Each group was then further subdivided into two groups (8 in each) including normal and colitis-induced. because the figure indicates, each of colitis-induced old or young groups was further subdivided into two subgroups, including colitis-induced with no treatment and colitis-induced treated with fKT. The study was performed in three phases. within the initiative, DSS-induced colitis was found out in young (2 months) and old (16 months) mice during a period of 21 days during which weight loss and therefore the clinical score were evaluated and compared with the age-matched healthy animals. within the second phase, the effect of fKT administration on survival analysis and therefore the clinical score were evaluated during a period of 21 days. After completion of phase I clinical trial and II of the study, molecular and histological evaluations were performed on young and old healthy controls, DSS-induced colitis, and DSS-induced colitis treated with fKT animals in phase III clinical trial. Considering the death rate and clinical signs that occurred within the animals with colitis, animals at this phase of the study were sacrificed on day 14 after the start of the study.

Colitis induction

Colitis was induced on day 0 using administration of drinking water containing 3.5% (w/v) dextran sodium sulfate salt (DSS) (40000 kDa, MP Biomedical, Eschwege, Germany) per mouse per day. The animals and clinical signs of disease were daily monitored, indicated by weight loss, occurrence of blood in the stool or around the rectum, and diarrhea until day seven after the colitis induction. Weight loss was determined by comparing the body weight for each mouse to the baseline body weight and expressed as a percentage of weight loss. Other symptoms were scored according to the previously suggested system by Siegmund et al. Briefly, the different signs for stool consistency were scored as follows: score 0, well-formed pellets; score 2, pasty and semi-formed stools that did not adhere to the anus; score 4, liquid stools that did adhere to the anus. The different signs for bleeding were scored as follows: score 0, no blood measured using the Hemocult system (Beckman Coulter); score 2, positive Hemocult; score 4, gross bleeding. Animals with borderline scores were given a one-half score.

Histological and histopathological analysis

To perform histological evaluation of the colon, the animals were sacrificed under ether anesthesia after the last treatment with beverage or fKT. Colon was initially flushed with 1x ice-cold phosphate buffered saline (PBS) to get rid of feces completely. Tissue samples of the colon were then removed, fixed in 10% buffered formalin, and processed for paraffin sectioning. Sections of about 5 μ m thickness were taken and stained with Hematoxylin and Eosin (H&E). The stained sections were examined with an Olympus cX41 microscope and photographed using an Olympus D330 camera. Damage score ranged from 0 to 4 scale was judged based on: inflammation represented by number and extent of leukocyte infiltration, epithelial defects represented by the severity of injury to the somatic cell layer, crypt atrophy estimated visually for the percent of atrophy within the crypts, edema, polymorphonuclear cells (PMNs) infiltration, and mucosal disruption.

Immunofluorescence studies of ZO-1 and ZO-2 expression

Sections of 5 μ m paraffin-embedded colon tissues were prepared from each sample and then dewaxed, hydrated, and

incubated in a protein block solution. Subsequently, the sections were incubated with the primary rabbit monoclonal ZO-1 or ZO-2 antibody (diluted 1:100 in 0.01 mol/L PBS; Zo-1: ab214228, Zo-2: ab2273, UK) followed by incubation with a goat anti-rabbit Alexa flour 488 (ab150077, Abcam, Cambridge shire, UK). The images were captured using a DeltaPix fluorescent microscope (Smorum, Denmark) and evaluated independently by two expert pathologists.

Analysis of gene expression by real-time PCR

Total RNA was extracted from ~50 mg of frozen colon tissue using guanidine/phenol solution (reagent lysis Qiazol-USA) consistent with the manufacturer's instruction. The standard and quantity of RNA concentrations were monitored employing a NanoDrop 2000c (Eppendorf, Germany). Then, 1 μ g RNA was reversely transcribed to DNA using Thermo Scientific Revert Aid First Strand cDNA Synthesis Kit (Munich, Germany), consistent with the manufacturer's instructions. The relative expression of mRNA for GAPDH, ZO-1, and ZO-2 decided by preparing reaction mixer with PCR Master Mix (2X) (Amplicon-Denmark) and gene-specific primers with diluted cDNA and final volume made up to 10 μ l with nuclease-free water. Quantification and analysis were administered in ABI real-time PCR. The sequences of primers, designed by Integrated DNA Technologies, were forward 5'-TGTCCTCACTTGAATCCCC-3' and reverse 5'-TGTTTCCTCCATTGCTGTG-3' for ZO-1 and forward 5'-CTCCCTCTTCACATCTGCTTC-3' and reverse R: 5'-CTGTTACTTGCCTTTGGTCTGG-3' for ZO-2. The efficiencies for primers utilized in the study varied between 95% and 105%. Primer pairs were validated to make sure the right size of the PCR product and therefore the absence of primer dimers. The GAPDH gene was chosen as an indoor control against which mRNA expression of the target gene was normalized. The resultant organic phenomenon level was presented as $2^{-\Delta\text{Ct}}$, during which ΔCt was the difference between Ct values of the target gene and GAPDH.

Statistical analysis

Statistical analysis was performed using Graph Pad Prism 7.01. Data are presented as means \pm SD. ANOVA was used to indicate any significant difference between the groups. Survival

rates were illustrated using Kaplan–Meier plots and compared using the log-rank test. Value of P was considered statistically significant when it had been but 0.05.

Results:

Characteristics and clinical course of DSS-induced colitis

The DSS-induced colitis in mice is that the common animal model to deal with the pathogenesis of colitis and to gauge therapeutic approaches. This model was found out in our laboratory and monitored for a period of 21 days (Phase I). To do so, male NMR mice were administered 3.5% DSS in beverage for seven days. The animals were checked daily during the amount of the study for survival rate, weight loss, and clinical signs of colitis, including bleeding and diarrhea and compared with the age-matched healthy animals. Survival analysis of the young DSS-treated animals demonstrated that 66% and 33% of the animals were alive on days 7 and 14, respectively, and every one were dead on day 2. just in case of the old DSS-treated animals, survival analysis revealed that 66% and 50% of the animals were alive on days 7 and 14, respectively, and every one were dead on day 21. There was a big weight loss within the DSS-treated young and old groups compared to the age-matched healthy animals. The DSS-treated young mice had lost roughly 10% and 46% of their weight on days 7 and 14, respectively. The DSS-treated old mice had lost roughly 13.5% and 15% of their weight on days 7 and 14, respectively. As for digestive disorder signs, bleeding and diarrhea were observed in DSS-treated young and old mice on day 2 and three after DSS administration, respectively. These results demonstrate that DSS-treated young mice show more severe clinical signs and lower survival rate than the DSS-treated old mice.

Histological observations

Histological analysis of H&E-stained tissue sections (Figure 6) of all DSS-challenged young and old mice showed increased infiltration of PMNs, cryptic loss, epithelial defect, mucosal disruption, apoptosis, edema, and mucosal thinness compared with the age-matched healthy mice. Treatment with fKT decreased the extent of injury, though it didn't cause an entire reversal to the healthy status by the regimen administered during this study. Notably, old healthy mice had more infiltration of PMNs, cryptic loss, and edema than the young,

healthy animals. As depicted in Figure 6c, the mucosal thickness was decreased by DSS administration because the clinical score increased in both young and old with DSS-induced colitis.