The Therapeutic Potential of Time-Restricted Fasting on Experimental Ulcerative Colitis

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ABSTRACT

Aim: Intermittent fasting have been reported to have beneficial effect, in that it improves gut microbiota and lowers inflammation. This research is, however, targeted at evaluating the healing effects of fasting on ulcerative colitis in rats.

Study Design: A total of eighteen Wistar rats were used for this study, and were divided into three major groups; animals that were neither induced with colitis nor fasted (group 1), animals with colitis and were allowed to fast (group 2), animals with colitis but were not allowed to fast (group 3).

Place and duration of study: Department of Physiology, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, between March, 2020 and July, 2020.

Methodology: The weight of the 18 animals used was 180 ± 20 g. Colitis was induced by a single dose of intra-rectal administration of 1mL/100g body weight of 6% acetic acid. Animals in group 1 served as control animals. Animals in group 2 were only given access to food between 4:00 p.m and 6:30 p.m, whereas animals in group 3 were given food and water ad libitum. Animals were sacrificed ten days post colitis induction. Colonic levels of Tumor necrotic factor-alpha (TNFα), Glutathione (GSH) concentration, Superoxide dismutase (SOD), Catalase (CAT) and Myeloperoxidase (MPO)
activities were measured. The blood glucose level in the animals was also recorded by the use of a glucometer.

**Results:** There was a reduction in the concentration of TNFα and GSH, an increase in CAT and SOD activities in the colitis animals that were allowed to fast when compared with colitis animals that were not allowed to fast, ten days post colitis induction. There was also the lowering of blood sugar level, all signifying the beneficial effect of fasting on chemically-induced colitis. Thus, intermittent fasting helped the animals to heal from chemically-induced colitis.

**Keywords:** Ulcerative colitis; intermittent fasting; inflammation; antioxidants.

1. INTRODUCTION

Inflammatory bowel disease (IBD), which includes Crohn's disease (CD) and Ulcerative colitis (UC), is a group of chronic inflammatory diseases of the gastrointestinal tract (GIT) [1]. The global prevalence of IBD has been on the increase in the last few decades [2] and this is the morale behind this study, as there are still many grey areas regarding the subject of IBD, taking colitis as a case study. Ulcerative Colitis, as an intestinal disorder, is characterized with mucosa damages, inflammation and ulceration in the colon and rectum [3].

Gut microbiota diversity is critical for linking the diet (which includes nutrition as well as fasting) and host physiology and pathology, and are influenced by dietary composition and feeding patterns [4]. Intermittent fasting (IF) are a group of periodic energy restriction dietary patterns, including Alternate-day Fasting (ADF), Time-Restricted Fasting (TRF), and Intermittent Energy Restriction (IER) [5,6]. Intermittent Fasting (IF) and Fasting-Mimicking Diets (FMDs) have been effective in increasing healthy lifespan or as therapies in mouse models for a variety of diseases [7]. Fasting Modified Diets (FMDs) can reduce cancer incidence and aging-associated immunosuppression or immunosenescence, a process aided by hematopoietic stem-cell-based regeneration [8].

Previous researches have reported that ADF, TRF, and IER had beneficial regulatory effects on the compositions of gut microbes in various animal models and human trials [9–11]. Intermittent fasting (IF) can improve function and health during aging in laboratory model organisms, but the mechanisms at work await elucidation. More recent, pilot, clinical trials used a fasting mimicking diet (FMD) (consisting of monthly cycles of a 5-day fast during which daily food intake was reduced to 50% normal caloric intake), which reduced multiple health risk factors during the post-fast recovery period, including lowered blood pressure, and reduced blood glucose and insulin-like growth factor-1 (IGF-1) levels [12,13]. However, systematic reviews of the clinical benefits of fasting regimens in humans found that study designs were heterogeneous and compliance data limited, making it difficult to draw definitive conclusions [14,15]. This present research is concerned with demonstrating that TRF significantly improves the gut function in colitis rat models by accessing the colonic levels of myeloperoxidase (MPO), tissue necrotic factor-alpha (TNFα) and superoxide dismutase (SOD).

2. METHODOLOGY

2.1 Experimental Animals

Eighteen healthy female Wistar rats that have not being previously used for any experimental procedure, with weight of 180±20g were used. Animals were grouped into three groups which include animals that were neither induced with colitis nor fasted (group 1), animals with colitis and were allowed to fast (group 2), animals with colitis but were not allowed to fast (group 3), each consisting of 6 animals. Group 2 animals were only fed between 4:00 pm - 6:30 pm for 10 consecutive days, group 3 animals were given food and water *ad libitum* after colitis induction.

All animals were kept in the Animal House of LadokeAkintola University of Technology, Ogbomosho. All through the course of the research, animals in group 1 and group 3 were fed with standard pelletized rat feed and water *ad libitum* after colitis induction. All animals were kept in the Animal House of LadokeAkintola University of Technology, Ogbomosho. All through the course of the research, animals in group 1 and group 3 were fed with standard pelletized rat feed and water *ad libitum*, with 12 hours night/day cycle.

2.1.1 Animal Housing

Animals were kept in large plastic cages with dried wood shaving as bedding to provide the needed comfort for the rats. The animal bedding, in each cage, was changed 2 times per week.
2.2 Induction of Colitis

The animals were fasted for 24 hours prior to the administration of 6% acetic acid, while water was made available ad libitum. Colitis was induced by a single instillation of 1mL/100g body weight of 6% acetic acid intra-rectally. The control animals were only given an equivalent quantity of distilled water.

2.3 Method of Animal Sacrifice

Animals were sacrificed using the cervical dislocation method, 10 days post colitis induction. The animals were then dissected and the colon of each animal was cut 6cm proximal to the anal opening to assess the severity of colonic tissue damage.

2.4 Assessment of Colitis

After animals were sacrificed and the colon excised, the colonic tissues were cut open longitudinally and washed in chilled normal saline solution and then weighed. A large portion of each rat’s colonic tissue was homogenized in phosphate buffer solution (PBS) and then centrifuged at a speed of 16,000RPM at 4°C for 20mins. The supernatant was kept between 20°C-4°C for biochemical assay.

2.5 Blood Glucose Level Assessment

By the use of a glucometer, the concentration of blood glucose level of individual animals were checked by cutting the rat tails 1cm to the end of the tail and collecting blood from there.

2.6 Biochemical Assays

Tumor Necrosis Factor-alpha (TNFα) and Myeloperoxidase (MPO) was done by the use of an Enzyme-Linked Immunosorbent Assay (ELISA) kit, following the instruction of usage by Elabscience Biotechnology Inc., U.S.A.

2.6.1 Catalase (CAT)

This assay method is based on the measurement of the hydrogen peroxide substrate remaining after the action of catalase. First, the catalase converts hydrogen peroxide to water and oxygen (catalytic pathway) and then this enzymatic reaction is stopped with sodium azide. An aliquot of the reaction mix is then assayed for the amount of hydrogen peroxide remaining by a colorimetric method. In the colorimetric method [16], a substituted phenol is used (3,5-dichloro-2-hydroxybenzenesulfonic acid), which couples oxidatively to 4-aminantipyrine in the presence of hydrogen peroxide and horseradish peroxidase (HRP) to give a red quinoneimine dye (N-(4-antipyril)-3-chloro-5-sulfonate- benzoquinone-monoimine) that absorbs at 520 nm.

2.6.2 Glutathione (GSH)

The spectrophotometric procedures used to measure the level of GSH are based on the method of Ellman, who reported that 5,5'-dithiobis- (2,-nitrobenzoic acid) is reduced by SH groups to form 1 mole of 2-nitro-5-mercaptopbenzoic acid per mole of SH. The nitromercaptobenzoic acid anion has an intense yellow color which when measured at a wavelength of 412 nm can be used to measure SH groups [17].

2.6.3 Superoxide dismutase (SOD)

The ability of Superoxide dismutase enzyme to inhibit the autoxidation of pyrogallol was used. The autoxidation of pyrogallol in the pH 8.2 is 50% in the presence of EDTA. This method is based on competition between the pyrogallol autoxidation by O2•− and the dismutation of this radical by SOD principle [18].

2.7 Statistical Analysis

Data were expressed as mean ± standard error of mean (Mean ± S.E.M). Data were analyzed with Graph Pad Prism version 5 software. Level of significance among the groups was compared by one-way analysis of variance (ANOVA). Mann-Whitney test was used to compare the difference between two groups. Statistical significance was set at P <0.05.

3. RESULTS

3.1 Effects of Fasting on Weight Gain in Experimental Acetic Acid-Induced Colitis

Colitis caused a significant decrease in percentage weight gain of colitis + food ad libitum and colitis + fasting animals groups when compared with control group. There was no significant change when weight of fasted animals was compared with that of animals that have free
Table 1. Effects of fasting on weight gain in experimental acetic acid-induced colitis

<table>
<thead>
<tr>
<th>Weight / Group</th>
<th>Control</th>
<th>Colitis + fasting</th>
<th>Colitis + food ad libitum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial weight (g)</td>
<td>170.83 ± 6.51</td>
<td>203.50 ± 5.63</td>
<td>187.16 ± 6.89</td>
</tr>
<tr>
<td>Final Weight (g)</td>
<td>196.67 ± 6.65</td>
<td>176.83 ± 5.84</td>
<td>179.67 ± 7.96</td>
</tr>
<tr>
<td>Weight gain (g)</td>
<td>25.83 ± 1.57</td>
<td>-26.67 ± 5.25*</td>
<td>-7.50 ± 9.73*</td>
</tr>
<tr>
<td>% weight gain (%)</td>
<td>15.24 ± 1.17</td>
<td>-12.99 ± 2.41*</td>
<td>-3.51 ± 4.69*</td>
</tr>
</tbody>
</table>

*P < 0.05 vs control

access to food after colitis induction, though there was non-significant decrease in fasted animals weight when compared with the food ad libitum animals weight, Table 1.

3.2 Effects of Fasting on Colon Weight of Experimental Acetic Acid-Induced Colitis

Weight of colon was significantly increased as a result of colitis induction. Fasting of the animals has no significant effect on the weight of the colon, Fig. 1.

3.3 Fasting Decrease Blood Glucose Level in Experimental Acetic-Acid-Induced Colitis

Fasting of the animals after colitis induction caused a significant reduction in blood glucose level. There was no significant difference in blood glucose level of fasted animals and food ad libitum animals, likewise the control and the food ad libitum animals Fig. 2.

3.4 Effect of Fasting on Blood Total Protein of Experimental Acetic Acid-Induced Colitis

Fasting the animals for ten days after colitis induction did not alter blood total protein, Fig. 3.

3.5 Colonic Tumor Necrotic Alpha of Rats on Day 10 Post Colitis Induction

Tumor necrotic alpha (TNF-α) was significantly increased in colitis animals. When comparing the TNF-α of fasted and food ad libitum animals, there was a significant increase in the TNF-α of food ad libitum group, Fig. 4.

3.6 Effect of Fasting on Colonic Myeloperoxidase Activity on Day 10 Post Colitis Induction

Myeloperoxidase was not significant across the groups. Non significant decrease in myeloperoxidase activities was observed in colitis + fasting group, while myeloperoxidase activities of the colitis + food ad libitum group was also non significantly increased when compared with both control and Colitis + fasting group, Fig. 5.

3.7 Colonic Antioxidant Status on Post Colitis Induction

Glutathione (GSH) was significantly increased in colitis + food ad libitum group when compared with both control and colitis + fasting groups. There was no significant difference in GSH of colitis + fasting group when compared with control, Table 2. There were no significant differences in colonic total protein, superoxide dismutase (SOD) and catalase (CAT) activities across the groups, Table 2.
Fig. 2. Effect of fasting on blood glucose level

Blood glucose (mg/dL)

Control  Colitis + fasting  Colitis + food ad libitum

a P = 0.02 vs control

Fig. 3. Effect of fasting on blood total protein level of colitis rats

Total protein (g/dL)

Control  Colitis + fasting  Colitis + food ad libitum
Fig. 4. Colonic TNFα of rats on day 10 post acetic acid-induced colitis

Fig. 5. Colonic myeloperoxidase level in the colon of rats on day 10 post acetic acid-induced rats

Table 2. Colonic antioxidant status of rats on post acetic acid-induced colitis

<table>
<thead>
<tr>
<th>Parameter / Group</th>
<th>Control</th>
<th>Colitis + fasting</th>
<th>Colitis + food ad libitum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein (g/dl)</td>
<td>5.52 ± 0.19</td>
<td>5.79 ± 0.23</td>
<td>5.72 ± 0.12</td>
</tr>
<tr>
<td>CAT (umol/mL/mins)</td>
<td>16.97 ± 1.18</td>
<td>16.61 ± 2.41</td>
<td>14.46 ± 0.95</td>
</tr>
<tr>
<td>SOD (U/mL)</td>
<td>0.60 ± 0.08</td>
<td>0.45 ± 0.11</td>
<td>0.44 ± 0.06</td>
</tr>
<tr>
<td>GSH (mM)</td>
<td>0.56 ± 0.03</td>
<td>0.65 ± 0.06</td>
<td>0.82 ± 0.04^*</td>
</tr>
</tbody>
</table>
4. DISCUSSION

As seen in the result of this study, the blood glucose level of fasted animals was significantly lower when compared to the other groups. This implies that TRF helps to control the sugar level; this is beneficial in colitis because treating colitis in the presence of high sugar level can be particularly challenging [19].

Increased myeloperoxidase levels in the colon have been implicated as an index for the confirmation of colitis in animal models [20]. This claim is evident in this present study (Fig. 5) as the level of MPO became higher in the group of animals that were induced with colitis but were given food ad libitum after colitis induction. In consonance with a study conducted by Ige and Adio where animals that were allowed to heal naturally had lower level of MPO [21], we see that the levels of MPO was significantly lower in the animals that were fasted for 10 days post colitis induction when compared to animals in the control group and animals that were given feed after colitis induction. This implies that fasting is generally a good agent in reducing the reactive oxygen metabolites (ROM) in the colon, thus, is helpful in the healing of colitis.

It has been found out that hemorrhagic necrosis is usually caused by tumor-necrosis factor (TNFa), thus, the increased expression of TNFa in animals induced with colitis as seen in Fig. 4. This is similar to the work of Takizawa et al. [22], where Dextran Sulfate Sodium (DSS) increased the expression of TNFa as an indication of colitis formation. The report given by Lars et al. completely agrees with the results of this study, in that the level of TNFa in the animals that were induced with colitis and then fasted was lower than in the animals that were not fasted, hence, the inference, that fasting helps to heal colitis [23].

Previous researches have confirmed catalase (CAT) as an antioxidant enzyme potentially responsible for the treatment of oxidative stress and IBD since it scavenges free radicals and convert them into hydrogen peroxide molecule which can be further hydrolyzed to the non-toxic molecule of water and hydrogen on the presence of superoxide dismutase (SOD) enzyme [24]. Thus, an increase in the level of CAT in the colon directly speaks of the anti-oxidative status of the colon. There was significantly higher level of CAT in the fasted group of colitis animals than in the group of animals that were not fasted, hence fasting improves gut function.

Superoxide dismutase (SOD) level was reported, by Yildiz et al. [25], to be elevated in the colon of animals that were induced with colitis by intra-rectal instillation of acetic acid since the elevation of this antioxidant is an indication that inflammation and oxidative stress are present [22]. However, in animals that TNBS was used to induce the colitis the level of colonic SOD was seen to be reduced which typically infers an elevation of the oxidative stress [26]. The later report agrees with the findings of this study where the level of SOD reduced in the animals that were induced with colitis but were not fasted while in animals that were fasted, the level of SOD was slightly higher, signifying the healing effect of TRF on acute colitis.

There was a significant increase in the colonic weight of colitis animals when compared with the control animals as observed in Fig. 1. Whereas there was slight increase in the colonic weight of fasted animals when compared with the group of animals that were not fasted but were induced with colitis. According to Toshihiko et al., fasting-refeeding plan (intermittent fasting) helped to arrest epithelial cell division which was followed by a phase of hyper-proliferation in colonic tissue [27]. Essentially, this report agrees with the result of this study where the weight of colonic tissue was higher in fasted animals than the rest of other animals in other groups.

5. CONCLUSION

A well-established model for acute colitis induction (single dose of intra-rectal administration of 6% acetic acid) was employed in this study, and we have also shown, as confirmed by various previous studies, that inflammation and damage in the colon are associated with increased expression of MPO, TNFa, and GSH. We, in this study, found out that there exists a curative (healing) effect of fasting, especially, time restricted fasting (TRF), which we employed, on ulcerative colitis caused by induction of acetic acid, by the significant reduction in the expression MPO, TNFa as well as increased expression of SOD in fasted animals when compared with animals that were allowed to feed after colitis.

ACKNOWLEDGMENTS

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CONSENT

It is not applicable.

ETHICAL APPROVAL

The experimental procedures were examined and approved by ethical committee, Faculty of Basic Medical Sciences, LAUTECH, Omomoso, Oyo State, Nigeria. Principles of laboratory animal care (NIH publication No. 8523, revised 1985) were also followed.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES


