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Original article

Advancing nutritional therapy: A novel polymeric formulation attenuates intestinal inflammation in a murine colitis model and suppresses pro-inflammatory cytokine production in ex-vivo cultured inflamed colonic biopsies

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SUMMARY

Background & aims: Nutritional therapy is a viable therapeutic option for the treatment of Crohn disease (CD). Therefore improving nutritional therapy would greatly benefit CD patients. The aim of this study was to define the anti-inflammatory properties of a novel nutritional polymeric formula (PF) in comparison to a currently available standard PF.

Methods: Dextran sodium sulfate (DSS) was utilized to induce colitis in C57BL/6 mice with mice randomized to receive either standard PF or novel PF in addition to control groups. Changes in body weight were recorded and colonic damage was assessed histologically and biochemically. Additional experiments were also included where the cytokine response of colonic biopsies from pediatric CD patients was measured following exposure to standard PF or novel PF.

Results: DSS induced significant body weight loss, morphological changes in the colon, increased myeloperoxidase (MPO) activity and up-regulated colonic mRNA expression of tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-12 and monocyte chemoattractant protein (MCP)-1, as well as associated histological changes. Other than histological damage, these inflammatory changes were reversed by both novel and standard PF. However, the novel PF, but not standard PF, completely suppressed TNF-α, IL-6 and IL-8 levels from cultured biopsies.

Conclusions: Newly developed nutritional formula reproducibly ameliorated DSS-induced colitis in a murine model, although this response was not measurably different to standard PF. However, the novel PF was significantly superior in suppressing inflammatory cytokine release from cultured colonic biopsies. Collectively, these findings support a possible role for novel PF in advancing nutritional therapy for CD patients.

1. Introduction

CD is one entity of inflammatory bowel disease (IBD) that is characterized by chronic involvement of any part of the gastrointestinal tract [1]. CD is an increasingly global health concern; recent reports indicate that the disease is no longer confined to Western countries with a rapid rise in incidence and prevalence rates worldwide [2]. CD is most commonly diagnosed in young adults [3] and recent data show rapid increases in the pediatric population [1]. Despite recent medical advances that can limit disease flares, CD remains without cure [4]. Further, nutritional complications and side effects can arise secondary to the current available pharmacological treatments [5]. The need for safe and more effective therapies is therefore desirable.

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Exclusive enteral nutrition (EEN) utilizing PF is a viable therapeutic option in young patients with active CD [6]. However, this therapy is implemented variably in clinical practice [7], and felt to be less effective in adults [8]. Importantly, PF as used for EEN therapy was designed primarily as a nutritional formula to support ill and hospitalized patients rather than as an induction therapy specifically for CD [9]. Thus, designing a specific nutritional therapy characterized by enhanced efficacy for induction of remission is warranted. Pre-clinical studies have shown that manipulating concentrations of the constituents of the enteral diet is one potential avenue to develop a specific nutritional formulation for the treatment of IBD [10].

In previous work utilizing an *in vitro* cell culture model with TNF-α stimulated colonic epithelial cell we developed a novel PF as a potential treatment for CD [11]. This novel formula is composed of standard PF with altered glutamine and arginine concentrations together with addition of curcumin to the formula. It exhibited superior anti-inflammatory activity compared to standard PF in suppressing pro-inflammatory cytokine production from the colonic epithelial cells without influencing cell viability or activity [11]. However, in order to validate these preliminary observations, further investigation of the novel PF in pre-clinical studies is required.

Murine models of colitis including the DSS induced murine colitis model, remains one of the most popular protocols to investigate potential therapeutics [12]. Organ culture involving human gut mucosa can also offer a realistic model of intestinal inflammation [13]. Colonic biopsies can be successfully maintained *in vitro* for short periods of time [14], and those collected from IBD patients are also capable of the spontaneous release of pro-inflammatory cytokines and other mediators [15]. Therefore the aims of this study were to investigate the anti-inflammatory activity of the novel PF with comparison to a standard PF in the DSS murine colitis model, and an *ex vivo* system involving colonic biopsies collected from pediatric CD patients.

2. Materials and methods

2.1. Mouse model of colitis

All experiments and related procedures were approved by the Animal Research Ethics of UNSW (approval ID; 12/149B). Female C57BL/6 mice aged 6–8 weeks old were obtained from the Animal Resource Centre (Perth, Australia) and housed with no more than 6 mice per cage in the UNSW animal facility. After acclimatisation, colitis was induced in three-quarters of the mice by adding 3% DSS (w/v) (M.W 40 KDa; Sigma–Aldrich, NSW, Australia) dissolved in drinking water. Colitis was induced over a five-day period with mice having access to mouse pellets and DDS water *ad libitum*. DSS mice were then randomly assigned to three experimental groups (positive control and two treatment groups) with 12 mice per group, while the remaining quarter of mice that did not receive DSS became the negative control group with access to mouse pellets and water *ad libitum*, for the duration of the experiment (19 days). Following DSS treatment, positive control mice received normal drinking water and access to mouse pellets *ad libitum* for the duration of the experiment. In the two treatment groups, following DSS exposure, mice received either standard PF (Osmolite, Abbott, NSW, Australia); or Novel PF given in liquid form through the drinking bottle *ad libitum* for the duration of the experiment. PF was prepared fresh and replaced daily. Mice that received the formulas did not have access to mouse pellets, replicating an EEN regimen in clinical practice. Novel PF was comprised of standard PF with the addition of glutamine and arginine to final concentrations of 250 and 100 mM, respectively, plus curcumin added to a final concentration of 250 μM (Table 1), as previously established [11].

2.2. Evaluation of colitis and response to treatment

2.2.1. Body weight

Body weight was recorded at the initiation of the experiment and then daily until end of the experiments. Change in body weight of mice was calculated as percentage of weight drop and/or gain relative to the original weight measured at the beginning of experiment.

2.2.2. PCR analysis of colonic cytokines mRNA expression

At completion of the experiments, mice were euthanized using CO_2 inhalation before the entire colon was removed and gently cleared of feces. As previously utilized [16], colons were divided into 3 sections, proximally to distally as follows: first section for myeloperoxidase (MPO) assay; the second section for RNA isolation and the third section for histological examination. RNA was extracted from the colon using the TRIzol method. Turbo DNA-free kit (Ambion, Austin, TX, USA) was used to remove DNA from extracted RNA. Extracted RNA of high quality (OD 260/280 >1.8 and 260/230 >2) was reverse-transcribed to complementary DNA (cDNA) using SuperScript® VILO™ cDNA Synthesis Kit (Life Technologies, USA). Gene expression of TNF-α, IL-6, IL-12, and MCP-1 was quantified relative to expression of the housekeeping gene GAPDH using specific mice primers sense and anti-sense sequences (Invitrogen; Table 2). cDNA was amplified using the Realplex master cycler and SYPR-Green fluorescent dye. The PCR reaction volume was 20 μl, including 10 μl of Fast SYBR® Green Master Mix (Fast SYBR® Green Master Mix, Life Technologies), 3 μl of cDNA (10 ng), 3 μl forward primer (300 nM), 3 μl reverse primer (300 nM) and 1 μl nuclear-free water. Tubes were heated at 95 °C for 20 s and followed by 40 cycles of 95 °C for 3 s and 60 °C for 30 s. Melting curve analysis was included to check homogeneity of PCR products. Average threshold cycle (C_T) was measured and gene expression quantified. Fold induction was calculated by using comparative C_T method.

2.2.3. Histology examination

Colonic tissue was fixed in 10% formalin (Sigma–Aldrich), embedded in paraffin and cut into cross sections. The sections were stained with hematoxylin and eosin, and examined by light microscopy. Histology was assessed by an experienced histologist who was blind to treatments, using an established score system as follows [17]. Inflammation severity score (0 – none, 1 – slight, 2 – moderate, and 3 – severe), the extent of injury score (0-none, 1-mucosal, 2-mucosal + submucosal, 3-transmural), and crypt damage score (0 – none, 1 – basal 1/3 damaged, 2 – basal 2/3 damaged, 3 – only surface epithelium intact, 4 – loss of entire crypt and epithelium). Each score was multiplied by an equivalent with the percentage of tissue involved (×1: 0–25%, ×2: 26–50%, ×3: 51–75%, ×4: 76–100%). A maximum histopathological score is the sum of the individual scores and ranged from 0 (no damage) to 40 (maximal damage).

Table 1
Concentrations of glutamine, arginine and curcumin components per 1 ml standard PF to create the novel PF.

Supplement	Concentration (mM)	Concentration per 1 ml (g)
Glutamine	250	0.03645
Arginine	100	0.0166
Curcumin	0.250	0.00009

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