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Westernized high-fat diet accelerates weight loss in dextran sulfate sodium-induced colitis in mice, which is further aggravated by supplementation of heme☆

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Abstract

The Western diet, rich in fat and red meat, predisposes for inflammatory bowel disease (IBD); however, little is known about mechanisms involved. Red meat contains high levels of heme, a well-known inducer of the cytoprotective enzyme heme oxygenase-1 (HO-1). Pharmacological induction of HO-1 ameliorates experimental colitis. We analyzed the effect of a westernized high-fat (HF) diet supplemented with heme on intestinal HO-1 expression and dextran sulfate sodium (DSS)-induced colitis.

Mice were fed chow or HF diets for 2 weeks. In the second week, the HF diet was supplemented with or without 0.5 μmol/g heme. Subsequently, the 3 diet groups were given drinking water with or without 4% DSS to induce colitis.

Significant body weight reduction was first observed after 4 days in the chow/DSS mice (−5±3%), whereas this was evident already after 2 days (−6±2%) in HF/DSS mice, showing increased weight loss compared to chow/DSS mice in the following days. Heme supplementation further aggravated DSS-induced weight loss in HF mice (−18±4% vs. −7±5% for HF+heme/DSS vs. HF/DSS, $P<.01$). Heme increased HO-1 expression in the colon epithelium but decreased villin messenger RNA levels, indicating epithelial damage. In contrast, heme did not affect DSS-induced colon shortening and histological scores of epithelial damage and inflammation.

A westernized diet accelerates DSS-induced weight loss in mice, which is further aggravated by heme, despite the induction of HO-1 in the colon epithelium. Our data warrant a detailed analysis of the association of (red) meat-containing diets and the development of IBD.

Keywords: Heme; Heme oxygenase-1; High-fat diet; DSS-induced colitis; Ulcerative colitis; Red meat

1. Introduction

The inflammatory bowel diseases (IBDs) Crohn's disease (CD) and ulcerative colitis (UC) are characterized by recurrent inflammation of the colon (=UC) or any part of the gastrointestinal tract but predominantly the terminal ileum and colon (=CD). The etiology of these inflammatory processes is only partially understood. Genetic predisposition only explains about 20% of disease development [1]. The predominant triggers leading to IBD seem to be environmental factors and lifestyle. The general concept is that these factors trigger the recurrent inflammation in genetically susceptible persons. The most prominent controllable environmental factors that are implicated in the development of IBD are the Western/high-fat (HF) diet [2] and cigarette smoke [3].

Most evidence linking dietary factors to IBD comes from epidemiological studies. The incidence of IBD is rising in populations that consume a Western-style diet, rich in fat and protein but low in fruits and vegetables [4]. For instance, in the past two decades, the incidence of IBD increased remarkably in Eastern Europe, Asia and Central America, where the lifestyle became more 'westernized' [5]. Studies with a focus on specific compounds of the diet revealed that high meat intake, in particular, red and processed meat, increases the likelihood of relapse for colitis patients [6].

An HF intake is also linked to IBD. The introduction of margarine in Europe coincides with the first reports of granulomatous ileitis (CD involving the ileum with typical signs of granuloma formation [7]). Several studies have demonstrated an increased risk of developing IBD with high intake of total fat, monosaturated and polysaturated fat and fatty acids [2]. The high intake of fat may result in obesity, a condition that is associated with constant low-grade inflammation and characterized by increased expression of pro-inflammatory cytokines. Notably, elevated levels of these cytokines are also observed in mesenteric adipose tissue of CD patients [8].

In patients with IBD, chronic inflammation results in severe damage of the intestinal wall. To reduce the damage, endogenous defense systems such as heme oxygenase-1 (HO-1) become activated

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[9]. HO-1 converts its natural substrate heme into carbon monoxide, iron and biliverdin, which is subsequently converted to bilirubin by biliverdin reductase [10]. CO has antiinflammatory properties [11] and induces vasodilatation. Bilirubin is an antioxidant and provides cellular protection against free-radical-mediated injury [12].

Several studies support the antiinflammatory properties of HO-1. HO-1-deficient mice develop chronic inflammation [13], and a 6-year-old boy, who was deficient for HO-1, died because of severe endothelial damage [14]. In a rat model of trinitrobenzene sulfonic acid (TNBS)-induced colitis, pharmaceutical inhibition of HO-1 by tin mesoporphyrin led to increased epithelial damage of the colon [15]. Similar results were obtained after inhibition of HO-1 by zinc protoporphyrin [16] or tin protoporphyrin [17] in mice models of dextran sulfate sodium (DSS)-induced colitis. This suggests that HO-1 expression may ameliorate the disease course of IBD. Varga et al. [18] supported this hypothesis in an animal model for CD. Daily subcutaneous administration of heme before and after TNBS challenge caused induction of HO-1, and this was accompanied by a decrease in mucosal damage of the colon of rats. Similarly, pharmaceutical induction of HO-1 by ip injections of cobalt protoporphyrin ameliorated colonic inflammation in mice with colitis induced by DSS [17,19,20], as well as following interleukin 10 gene deletion [21].

The protective effect of heme-induced HO-1 in experimental colitis is in apparent contradiction with the epidemiological studies that indicate that red-meat-enriched diets, which contain high levels of heme (e.g., beef contains 0.5 μmol/g wet weight heme [22,23]), are a risk factor for colitis. Inducers of HO-1 in the aforementioned studies were administrated via injection (sc, ip or iv), and most compounds are nonphysiological substrates/inducers of HO-1. Obviously, dietary heme may have completely different physiological effects and trigger inflammatory responses in the gut. Therefore, the present study was undertaken to examine the effect of heme supplementation to a 'westernized high-fat' diet on DSS-induced colitis.

2. Methods and materials

DSS (molecular weight, 36,000–50,000) salt was from MP Biochemicals (Illkirch, France), and heme was from Sigma-Aldrich (St. Louis, MO, USA). The mouse myeloperoxidase (MPO) enzyme-linked immunosorbent assay (ELISA) kit was obtained from Hycult Biotechnology (HK210; Uden, The Netherlands). Primers were from Invitrogen (Breda, The Netherlands), while probes were from Eurogentec (Maastricht, The Netherlands).

2.1. Animals, diet and colitis induction

Female Balb/c mice (20–22 g; Harlan, Horst, The Netherlands) were housed at random in groups, kept in a temperature- and humidity-controlled environment in a 12-h light–dark cycle and had free access to food and tap water. The animal welfare committee of the University Medical Center Groningen approved the study.

We investigated the effects of a westernized HF diet supplemented with or without heme on DSS-induced colitis in mice. Three different diets (all in powder form) were used in the study: a control diet, a westernized diet and a westernized diet supplemented with heme (0.5 μmol/g diet). The control diet consisted of standard laboratory chow (RHM-B; Arie Blok, Woerden, The Netherlands) with 20% (energy) fat, whereas the westernized diet contained 40% (energy) fat and was prepared as described earlier. In short, the low-calcium westernized diet contained (per kilogram) 160 g of palm oil and 40 g of corn oil, exactly as described [23]. Heme was added to the diet and mixed. The fatty acid composition of the blend of palm oil and corn oil was 37 wt.% 16:0, 5% 18:0, 37% 18:1 and 20% 18:2 [24]. This mimics the ratio of saturated:monounsaturated:polyunsaturated fatty acids in a typical Western human diet [25]. During an acclimatization period of 1 week, mice were fed either a control or a westernized diet. This was followed by 1 week of pre-treatment with control, westernized or westernized diet supplemented with heme. To prevent possible degradation of heme by light, food was supplied to the mice just before dark. After pre-treatment, all three groups were subdivided in a group that was exposed to 4% DSS in the drinking water for 4 days and another group that was maintained on normal drinking water. Body weight was recorded daily. Colon length, colon histology, HO-1 staining, inflammation markers [MPO, inducible NO synthase (iNOS), cytokines] and epithelial markers (villin, caspase-3 activity) were studied.

2.2. Histology

Colon tissue was fixed in 4% formaldehyde, embedded in paraffin and cut into slides of 4 μm. To stain for morphology, a standard hematoxylin–eosin staining was performed. Slides were scored by a pathologist for epithelial damage (E) and inflammation (I) as was previously described by Obermeier et al. [26].

For HO-1 detection, antigen retrieval was performed in EDTA buffer at 300 W for 15 min in a microwave oven. Endogenous peroxidase was blocked with 0.3% hydrogen peroxide. Slides were incubated with an anti-HO-1 polyclonal antibody (1:1000, #SPA-896; Stress-Gen). Peroxidase-conjugated goat anti-rabbit Ig and peroxidase-conjugated rabbit anti-goat were used as secondary and tertiary antibodies (both 1:50 in 1% bovine serum albumin/phosphate-buffered saline containing 1% human serum; Dako). Color was developed using 3-3-diaminobenzidine tetrahydrochloridehydrate. Slides were counterstained with hematoxylin. Negative control stainings were performed by omitting the anti-HO-1 antibody. Images were taken with a Leica DM LB microscope (Leica, Wetzlar, Germany).

2.3. MPO ELISA kit

MPO activity in colon tissue was measured using a mouse MPO ELISA kit (HK210; Hycult Biotechnology). Colon tissue was homogenized in MPO lysis buffer (200 mM NaCl, 5 mM EDTA, 10 mM Tris–HCl, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml leupeptide, 28 μg/ml aprotinine, pH 7.4; 20 μl/mg) by 25 strokes with a plastic pestle. Supernatant was collected after two centrifugation steps of 10 min, 1500g, at 4°C, and stored at −80°C until use. Each sample was diluted 10 times in dilution buffer. The ELISA was performed according to the suppliers' protocol, except that, after addition of the substrate, the reaction was stopped after 15 min.

2.4. Protein assay

Protein concentration of colon tissue lysates (see MPO activity assay) were determined using the BioRad D_C protein assay (BioRad, Hercules, CA, USA). Absorbance was measured at 750 nm on an EL800 universal microplate reader (Bio-Tek instruments).

2.5. Caspase-3 activity

Caspase-3 activity was measured as described previously [27]. For each sample, we used 20 μg of protein in the assay. Excitation and emission were measured at 380 and 460 nm, respectively, on an FL600 microplate fluorescence reader (Bio-Tek instruments).

2.6. Reverse transcription polymerase chain reaction and quantitative PCR

RNA isolation was done using Trizol (Sigma-Aldrich). RNA concentrations were determined using the NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). Reverse transcription polymerase chain reaction (PCR) was carried out as described by Blokzijl et al. [28], but on 12.5 ng RNA in the presence of 0.5 μg random nanomers. We performed quantitative PCR (qPCR) for villin, iNOS and tumor necrosis factor α (TNF-α). Primers (Invitrogen) and probes (Eurogentec) were designed using Primer Express 2.0 software (Applied Biosystems). Details of primers and probes are listed in Table 1. The qPCR conditions were according to Blokzijl et al. [28], except that we used 1 ng undiluted complementary DNA. Fluorescence was measured using 7900 HT Fast Real-Time PCR system (Applied Biosystems). Each sample was analyzed in duplicate by ABI PRISM Sequence Detector software, version 2.1. Finally, the gene of interest was normalized to 18S ($2^{-\delta Ct}$ method).

2.7. Statistics

Data obtained for each experimental group were depicted as a Box–Whisker plot (minimum–lower quartile–median–upper quartile–maximum) or described as median (range). To verify statistically significant differences among experimental groups, a Kruskal–Wallis test was performed. When this resulted in a $P<.05$, data were analyzed

Table 1
Mice primer and probe sequences for qPCR

Villin	Forward	5′-CGC CTC ACC TCA TGT CTA TCT TC-3′
	Reverse	5′-CAG GCT CCA AGT TGT TCT TTC G-3′
	Probe	5′-FAM-CCT GAT AAA CCA CCA TGC GGC CCT-TAMRA-3′
iNOS	Forward	5′-CTA TCT CCA TTC TAC TAC TAC CAG ATC GA-3′
	Reverse	5′-CCT GGG CCT CAG CTT CTC AT-3′
	Probe	5′-FAM-CCC TGG AAG ACC CAC ATC TGG CAG-TAMRA-3′
TNF-α	Forward	5′-CAT CTT CTC AAA ATT CGA GTG ACA A-3′
	Reverse	5′-CCA GCT GCT CCT CCA CTT G-3′
	Probe	5′-FAM-CCT GTA GCC CAC GTC GTA GCA AAC CA-TAMRA-3′
18S	Forward	5′-CGG CTA CCA CAT CCA AGG A-3′
	Reverse	5′-CCA ATT ACA GGG CCT CGA AA-3′
	Probe	5′-FAM-CGC GCA AAT TAC CCA CTC CCG A-TAMRA-3′