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Original Research Article

Effect of acute and chronic DSS induced colitis on plasma eicosanoid and oxylipin levels in the rat

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ABSTRACT

Eicosanoids and oxylipins are potent lipid mediators involved in the regulation of inflammation. In order to evaluate their role and suitability as biomarkers in colitis, we analyzed their systemic levels in the acute and chronic phase of dextran sulfate sodium (DSS) induced colitis. Male Fischer 344 rats were treated in three cycles with 4% DSS in the drinking water (4 days followed by 10 days recovery) and blood was drawn 3 days prior to the first DSS treatment and on days 4, 11, 32 and 39. Histopathological evaluation of the colon tissue after 42 days showed that the animals developed a mild to severe chronic colitis. Consistently, prostaglandin levels were massively (twofold) elevated in the colonic tissue. LC–MS based targeted metabolomics was used to determine plasma oxylipin levels at the different time points. In the acute phase of inflammation directly after DSS treatment, epoxy-fatty acid (FA), dihydroxy-FA and hydroxy-FA plasma concentrations were uniformly elevated. With each treatment cycle the increase in these oxylipin levels was more pronounced. Our data suggest that in the acute phase of colitis release of polyunsaturated FAs from membranes in the inflamed tissue is reflected by a uniform increase of oylipins formed in different branches of the arachidonic acid cascade. However, during the recovery phases the systemic oxylipin pattern is not or only moderately altered and does not allow to evaluate the onset of chronic inflammation in the colon.

1. Introduction

Ulcerative colitis (UC) is a chronic, tissue-destructive disease with unknown etiology and high prevalence in Europe with up to 500 affected persons per 100 000 [1]. Recent research indicates that UC derives from excessive inflammation [2,3]. The arachidonic acid (AA) cascade is a central pathway in the paracrine and autocrine regulation of inflammation [4,5]. The conversion of AA (20:4n6) by cyclooxygenases (COXs) and lipoxygenases (LOXs) leads to highly potent lipid mediators such as prostaglandins (PG) and leuktorienes (LT). Recent years have shown that products derived from other polyunsaturated fatty acids (PUFA), particularly docosahexaenoic acid (22:6n3, DHA) and eicosapentaenoic acid (20:5n3, EPA) – generally named oxylipins – also play key roles in the regulation of inflammation [6]. Another class of lipid mediators, the anti-inflammatory epoxy-FAs, is formed by cytochrome P450 monooxygenases (CYP). They are inactivated by hydrolysis to dihydroxy-FAs by soluble epoxide hydrolase (sEH) [7]. A large number of currently sold anti-inflammatory drugs target PG formation by COX inhibition. The first line treatment of mild and moderate UC is 5-aminosalicylic acid (5-ASA, mesalazine) [8]. One mode of action of 5-ASA is a competitive inhibition of COXs [9]. However, the use of other COX inhibitors, i.e. non-steroidal anti-inflammatory drugs (NSAID), is controversial because of the central role of PGs in epithelia protection and homeostasis [10–12]. New studies in rodents

Abbreviations: AA, arachidonic acid; COX, cyclooxygenase; DAI, disease activity index; DiHETE, dihydroxy eicosatetraenoic acid; DiHETrE, dihydroxy eicosatrienoic acid; DiHODE, dihydroxy octadecadienoic acid; DiHOME, dihydroxy octadecenoic acid; DHA, docosahexaenoic acid; DSS, dextran sulfate sodium; EpETrE, epoxy eicosatrienoic acid; EpODE, epoxy octadecadienoic acid; EpOME, epoxy octadecenoic acid; EDTA, ethylenediaminetetraacetic acid; EPA, eicosapentaenoic acid; EpDPE, epoxy docosapentaenoic acid; HEPE, hydroxy eicosapentaenoic acid; HETE, hydroxy eicosatetraenoic acid; HETrE, hydroxyl eicosatrienoic acid; HODE, hydroxy octadecadienoic acid; HOTrE, hydroxy octadecatrienoic acid; IS, internal standard; LC–MS, liquid chromatography mass spectrometry; LOX, lipoxygenase; LT, leukotriene; MeOH, methanol; NSAID, non-steroidal anti-inflammatory drug; PBS, phosphate buffered saline; PG, prostaglandin; PUFA, polyunsaturated fatty acid; SPE, solid phase extraction; SRM, selected reaction monitoring; Tx, thromboxane; UC, ulcerative colitis.

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suggest that inhibiting sEH and stabilizing epoxy-FAs in the CYP branch of the AA cascade could be a promising treatment of UC [13,14]. Moreover, studies in animals and humans suffering from UC demonstrate that n3-PUFAs, which are believed to act mainly based on a modulation of the endogenous oxylipin pattern, act in a preventive/curative way on colitis [15–17]. However, only few data regarding the changes in oxylipin pattern occurring during UC are available. Particularly, information on the effect of colonic inflammation on systemic markers, i.e. oxylipins circulating in plasma, is scarce. Therefore, the aim of the present study was to characterize the effects of dextran sulfate sodium (DSS) induced colitis on oxylipin plasma levels in acute inflammation, healing/regeneration and onset of chronic colitis.

2. Materials and methods

2.1. Chemicals

DSS (36–50 kDa) was purchased from MP Biomedicals (Heidelberg, Germany). LC–MS grade acetonitrile (ACN), acetic acid (HAc) and methanol (MeOH) were from Fisher Scientific (Nidderau, Germany). Oxylipin standards and internal standards were obtained from Cayman Chemicals (local distributor: Biomol, Hamburg, Germany). Further standards such as EpODEs and DiHODEs were a kind gift from the laboratory of Bruce Hammock (UC Davis, California, USA). 1-(1-(Ethyl-sulfonyl)piperidin-4-yl)-3-(4-(trifluoromethoxy)phenyl)urea synthesized as described [18] was used as internal standard 2 (IS 2). All other chemicals were from Sigma–Aldrich (Schnelldorf, Germany).

2.2. DSS induced colitis model

Male Fischer 344 rats (95–115 g, 6 weeks) were obtained from Charles River Laboratories International Inc. (Sulzfeld, Germany) and kept in type IV polycarbonate cages (EHRET, Emmendingen, Germany). The bedding consisted of poplar granules (Lignocel select, Rosenberg, Germany), which were changed once a week. Before starting the DSS treatment the animals were allowed to acclimatize in our laboratory for 2 weeks. The animals had access to standard chow (#1324 Altromin, Lage, Germany) and water ad libitum and were kept at a 12 h light/dark cycle.

Colitis was induced by three cycles of a 4-day treatment with DSS in drinking water (4%, w/v) followed by 10 days of recovery (Fig. 1) as described [19]. Five hundred microliters EDTA-blood were collected from the retrobulbar venous plexus 3 days prior to the first DSS treatment as well as on days 4, 11, 32 and 39. The obtained blood was centrifuged (1500 × *g*, 10 min, 4 °C) and the plasma was stored at −80 °C until it was analyzed. On day 42 the animals were killed by cardiac puncture after anesthesia with xylazine/ketamine (66/5 mg/kg BW). The gut was transferred to ice cold phosphate buffered saline and after measuring the colon length, the proximal colon, distal colon and rectum sections [20] were sampled. The intestine was opened longitudinally, cleaned with ice-cold buffer and cut into pieces. One piece of the distal colon (50–100 mg) was immediately frozen at −80 °C for oxylipin analysis. The other piece and a sample of the proximal colon and rectum were fixed in 4% formalin at room temperature for the histopathological examination. Samples were prepared and scored as described [21] regarding the severity of inflammation (0 = none, 1 = minimal, 2 = mild, 3 = moderate, 4 = severe inflammation) and the extent of inflammation (0 = none, 1 = mucosal, 2 = mucosal and submucosal, 3 = mucosal, submucosal and muscular, 4 = mucosal, submucosal, muscular and serosal layers involved). The health status of the animals was daily inspected, and the disease activity index (DAI) was determined based on body weight loss, feces consistency and macroscopically visible blood in feces [17,22]. In the present study, which was approved by the animal welfare service of the state of Lower Saxony (Oldenburg, Germany), a subset of samples from another study [23] was used.

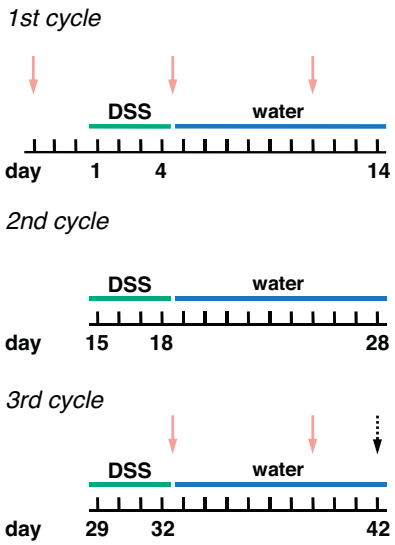

Fig. 1. DSS treatment regime of rats to induce acute and chronic colitis. Animals received 4% DSS in the drinking water for 4 days followed by a recovery phase with tap water for 10 days. This cycle was repeated thrice. Animals of the control group received tap water over the whole period of time. Red arrows indicate blood sampling time points. At day 42 animals were sacrificed and colon tissue was sampled.

2.3. LC–MS analysis of oxylipins

Quantification of oxylipins in plasma and colon tissue was carried out by LC–MS as described [24]. In brief, following addition of internal standards, 250 μL plasma were extracted utilizing Oasis HLB solid phase cartridges (Waters, Eschborn, Germany). Colon tissue (75 ± 25 mg) was homogenized in 500 μL MeOH/water 50/50 (v/v) with a ball mill (Retsch, Haan, Germany) for 10 min at 30 Hz. After centrifugation the supernatant was transferred to a preconditioned SPE column; the column was filled with wash solution and further processed like plasma samples. The residue of the evaporated solid phase extract was dissolved in 50 μL methanolic solution of IS2 and 5 μL were separated on an Agilent Zorbax Eclipse Plus C-18 reversed phase column (2.1 mm × 150 mm, particle size 1.8 μm) with a gradient of 0.1% aqueous HAc as solvent A and ACN/MeOH/HAc (800/150/1, v/v/v) as solvent B. Mass spectrometric detection was carried out on an AB Sciex 6500 QTRAP instrument (AB Sciex, Darmstadt, Germany) in scheduled selected reaction monitoring mode following negative electrospray ionization (ESI). Instrument controlling was performed with Analyst 1.6.2 and data analysis was carried out with Multiquant 2.1.1 (AB Sciex).

3. Results

The time course of the DAI (weight loss, stool consistency and blood in feces) during the DSS treatment and the results of the histopathological analysis of the colon are shown in Fig. 2. In treated rats, the DAI increased after the first 4-day treatment period with DSS, reaching a maximum at day 7 during the recovery phase. Following the second DSS treatment cycle (days 15–18) the DAI raised more rapidly to a maximum of 1.5 at days 18 and 19. Thereafter, during the second recovery phase the DAI decreased and reached a plateau at 0.7–0.8. Following the third treatment cycle the DAI remained at an even higher level (0.8–1.0), an indication of chronic inflammation. The DAI of the control animals was always below