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The involvement of heme oxygenase-1 activity in the therapeutic actions of 5-aminosalicylic acid in rat colitis

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Abstract

The mechanism of action of 5-aminosalicylic acid (5-ASA), the active therapeutic moiety of a number of clinically used anti-colitic agents, is unclear. The present study investigates whether the beneficial effects *in vivo* could involve induction of the heat shock protein, heme oxygenase-1 (HO-1), known to provide endogenous anti-oxidant and anti-inflammatory moieties which can modulate colonic inflammation. The effects of 5-ASA on the colonic expression and activity of HO-1 along with its effect on the inflammatory damage have been evaluated in the colitis provoked by instillation of trinitrobenzene sulphonic acid (TNBS) over 48 h in the rat. Intracolonic administration of 5-ASA (8, 25 and 75 mg/kg/day) dose-dependently reduced the TNBS-provoked macroscopic colonic inflammatory injury, myeloperoxidase (MPO) activity and TNF-α levels, while also dose-dependently increasing colonic heme oxygenase enzyme activity. Colonic HO-1 protein expression, determined by Western blot analysis in this colitis model, was likewise further induced by 5-ASA. Intracolonic administration of 5-ASA alone under unchallenged conditions also induced colonic HO-1 protein expression and stimulated heme oxygenase enzyme activity. Administration of zinc protoporphyrin (50 μmol/kg/day, s.c.), which prevented the increase in colonic heme oxygenase activity, abolished the anti-colitic effect of 5-ASA. These results suggest that 5-ASA may exert its colonic anti-oxidant and anti-inflammatory effects *in vivo* in part through the up-regulation of HO-1 enzyme expression and activity.

Keywords: Heme oxygenase-1 (HO-1); Aminosalicylates; 5-Aminosalicylic acid (5-ASA); Mesalamine; Zinc protoporphyrin (ZnPP); Inflammatory bowel diseases; Model of colitis; Trinitrobenzene sulphonic acid; TNBS colitis

1. Introduction

Preparations of aminosalicylates, exemplified by 5-aminosalicylic acid (5-ASA), for oral or colonic administration have been used over many decades for the treatment of the inflammatory bowel diseases, ulcerative colitis and Crohn's disease (van Bodegraven and Mulder, 2006). 5-ASA, known as mesalamine or mesalazine, is also considered to be the active therapeutic moiety of the other classical anti-colitic agent, sulphasalazine. Hence, 5-ASA has been formulated in a range of clinically used preparations that are enteric coated for controlled and delayed release, combined with an inert carrier or prepared as an azo-linked dimer for oral ingestion or as enemas for rectal administration to yield high colonic levels (Sandborn and Hanauer, 2003; Quershi and Cohen, 2005). Newer preparations of mesalamine that allow high daily sustained dosing are still being developed, approved and launched (Kamm et al., 2007).

Despite its long-term and wide-spread use, the mechanisms underlying the anti-colitic actions of 5-ASA have not been fully identified. Its complex pharmacological profile includes inhibition of intestinal macrophage chemotaxis (Nielsen et al., 1988) and mononuclear cell antibody secretion (MacDermott et al.,

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1989), inhibition of pro-inflammatory cytokine release (Galvez et al., 2003) and inhibition of the arachidonate lipoxygenase and cycloxygenase pathways (Hawkey et al., 1985). More recent molecular studies have identified actions on the nuclear factor, NFκB (Egan et al., 1999; Song et al., 2006) and on the peroxisome proliferator-activated receptor-γ (PPAR-γ), a nuclear receptor (Rousseaux et al., 2005). However, an enduring concept is that at least part of the beneficial activity of 5-ASA reflects its actions as an anti-oxidant and free radical scavenger (Aruoma et al., 1987; Allgayer et al., 1992; Simmonds et al., 1999; Reifen et al., 2004). Thus, the generation and release of local reactive oxygen species have long been considered to be involved in the vascular, epithelial and mucosal inflammatory injury in colitis and hence scavenging these moieties offers a potential mechanism of action of 5-ASA (Grisham et al., 1990; McKenzie et al., 1996; Pavlick et al., 2002; Dryden et al., 2005).

The direct anti-oxidant and radical scavenging properties of 5-ASA, which are attributed to its phenolic structure, can be demonstrated in cell-free systems and in inflamed colonic tissue *in vitro*, but the concentrations required for such activity are often in the millimolar range (Aruoma et al., 1987; Allgayer et al., 1992; Simmonds et al., 1999). Although high, these concentrations can be achieved in the colonic lumen after therapeutic dosing of 5-ASA preparations and anti-oxidant effects are observed following treatment with 5-ASA *in vivo* (Ahnfelt-Ronne et al., 1990; Galvez et al, 2000; Reifen et al., 2004). However, it is also possible that such properties of 5-ASA *in vivo* and indeed the therapeutic activity of the preparations in colitis may also reflect additional pharmacological actions to up-regulate endogenous anti-oxidant and ant-inflammatory systems.

One such endogenous system could be the inducible enzyme and heat shock protein 32, heme oxygenase-1 (HO-1), which is capable of producing anti-oxidant and anti-inflammatory products, biliverdin and bilirubin, as well as carbon monoxide (Willis et al., 2000; Maines 2005; Ryter et al., 2006). Colonic HO-1 expression and enzyme activity has been demonstrated to be up-regulated in models of colitis (Wang et al., 2001; Berberat et al., 2005; Varga et al., 2007), as well in samples of human colitic tissue (Barton et al., 2003; Paul et al., 2005) and this may reflect a stress-gene related defence mechanism.

Further up-regulation of HO-1 is associated with amelioration of experimental colitis (Berberat et al., 2005; Paul et al., 2005; Hegazi et al., 2005; Varga et al., 2007). Thus, in order to explore any link between HO-1 induction and the therapeutic actions of 5-ASA, the effects of the compound administered directly into the rat colon *in vivo*, on colonic heme oxygenase enzyme activity and HO-1 protein expression have been evaluated in a hapten-induced model of colitis provoked by instillation of trinitrobenzene sulphonic acid (TNBS). In addition, the effects of 5-ASA on HO-1 expression and enzyme activity in the unchallenged rat colon have been investigated. Furthermore, the actions of the heme oxygenase enzyme inhibitor, zinc protoporphyrin (ZnPP), in doses previously shown to reduce heme oxygenase activity in the rat colon (Varga et al., 2007), have also been evaluated on the anti-colitic activity of 5-ASA in this model.

2. Methods

2.1. Induction of colonic inflammation

The animal care and research protocols were in accordance with the guidelines of the University of Szeged and with the United Kingdom Home Office guidance on the operation of the Animals (Scientific Procedures) Act 1986. Male Wistar rats (200–240 g) were randomised before commencement of the study, housed at a temperature of 21–25 °C with a 12 h light cycle in groups of 5 animals, and inspected and weighed every day. Food was only withdrawn overnight for 12 h prior to TNBS administration, and the rats were allowed free access to drinking water during all periods. Under transient ether-induced anaesthesia, 2,4,6 trinitrobenzene sulphonic acid (TNBS; 10 mg in 0.25 ml of 50% ethanol, v/v) was administered intra-rectally through an 8 cm long soft plastic catheter (Boughton-Smith et al., 1988; Morris et al., 1989). The dose of TNBS used produced a reproducible yet not unduly severe colitis. After 48 h, the distal colon was dissected, photographed, weighed, processed and stored appropriately for subsequent analyses. This time period has been used by others in the TNBS model for the evaluation of the actions of 5-ASA (Galvez et al., 2000) and is the time when HO-1 expression and enzyme activity reaches plateau levels following TNBS challenge in this model (Varga et al., 2007).

2.2. Experimental design

5-Aminosalicylic acid (5-ASA) prepared freshly in 1% carboxymethylcellulose (CMC), was administered once daily in a volume of 0.25 ml into the colon, 24 h before, immediately following TNBS challenge and 24 h after TNBS challenge. The TNBS-challenged group received also intracolonic administration of the vehicle (1% CMC, 0.25 ml), while a baseline control group received no treatment. All such intracolonic treatments were conducted under very transient ether-induced anaesthesia.

In studies on the effects of a heme oxygenase inhibitor, zinc protoporphyrin (ZnPP) was administered daily at a dose of 50 μmol/kg, s.c., at the same times as the administration of 5-ASA, these being 24 h before, immediately following challenge and 24 h after TNBS challenge. ZnPP was dissolved in sodium hydroxide (0.1 M) and sodium chloride (0.9% w/v) and pH adjusted to pH 7.4, with the concentration of the stock solution being 0.5 mg/ml. This dose and dosing schedule of ZnPP were taken from previous studies in the TNBS colitis model (Varga et al., 2007).

2.3. Measurement of macroscopic colonic inflammatory damage

In all experimental groups, the distal 8 cm portion of the colon (measured from the rectum) was removed, opened longitudinally and gently rinsed with ice-cold phosphate buffer (pH 7.4), blotted, weighed (Scaltec, Germany) and photographed (Samsung, Digimax 340, digital camera). The increase in colon weight of the segment after challenge was taken as an