Aspects Of Colonic Sulphur Metabolism In Health And Disease

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Summary

The aetiology of ulcerative colitis remains unclear although most authors would now accept that events occurring in the colonic lumenal environment, the colonic epithelium and the mucosal immune compartment are of importance. A number of possible contributing lumenal factors have been proposed in the past such as infectious agents, dietary antigens and proinflammatory substances of bacterial origin. More recently, the role of colonic luminal sulphur metabolism in the aetiology of ulcerative colitis has received some attention. Reduced sulphur compounds impair oxidation of n-butyrate in colonocytes in a manner analogous to that seen in ulcerative colitis and the levels stool sulphide are increased in one animal model of colitis in which the delivery of sulphate to the colon and the presence of an intact anaerobic microflora are required. Organisms capable of sulphide production are found in the human colon and are present in increased numbers in ulcerative colitis. Sulphatase activity, which may increase the availability of sulphate in the colonic lumen, is increased in the stool of patients with ulcerative colitis. Finally, levels of sulphide in stool may be elevated in patients with ulcerative colitis, although this has been disputed. Such evidence has led to the general hypothesis that luminal sulphides are aetiologically important in ulcerative colitis and is reviewed in Chapter 2.

As studies indicating a deleterious effect of sulphide on colonic epithelial metabolic performance have been performed in vitro, an in vivo model to test this hypothesis was established (Chapter Three). Adult Sprague Dawley rats had ante-grade Roux-en-Y colostomies fashioned to allow "in-flow" access to the bowel. Animals were treated with 2 ml sodium hydrosulphide (10, 20, 30 mM) or saline control twice daily via the stoma for four or ninety days and then sacrificed. Isolated colonic epithelial cell suspensions were prepared and incubated at 37°C in the presence of [1-14C] labelled n-butyrate (5mM) or [6-14C] glucose (5 mM). Metabolic performance of cell suspensions was measured radiometrically (14CO2 production) and enzymatically (ketone body production and lactogenesis). Mucosal specimens were assessed histologically and scored for acute inflammatory changes.
Sulphide treatment produced a highly significant reduction in $^{14}$CO$_2$ production from n-butyrate in all groups compared to control in both four and ninety day experiments. Ketone body formation from n-butyrate was significantly reduced in treated animals compared to controls. Lactate production was not significantly reduced between control and treated animals. There was no difference between groups with respect to histologic appearance in either four or ninety day experiments. These results suggest that luminal sulphide, while capable of producing a reduction in n-butyrate oxidation of a degree similar to that observed in quiescent ulcerative colitis, is not of itself sufficient to produce mucosal inflammation.

The cause of diminished n-butyrate oxidation by the colonic epithelial cell in patients with ulcerative colitis is not clear. Short chain acyl-dehydrogenase (SCAD) is an important regulatory enzyme in beta oxidation and SCAD prepared from non colonic sources can be inhibited by sulphides. Studies of acyl-CoA ester profiles in human colonic epithelial cells in health or ulcerative colitis have not been performed. Should an abnormal pattern of acyl-CoA ester concentrations, consistent with SCAD inhibition, be shared by human ulcerative colitis and an animal model utilising sulphide exposure, then this might be regarded as further supportive evidence for the role of sulphides in the pathogenesis of colitis.

The studies described in Chapter 4 examine the acyl-CoA ester profiles in isolated rat colonic epithelial cells treated in vitro with sodium hydrosulphide (NaHS). Isolated rat colonic epithelial cell suspensions were incubated for ten minutes in the presence of [1-$^{14}$C] n-butyrate (5 mM), with and without NaHS (1.5 mM). Incubations were carried out both in the presence and the absence of exogenous CoA and ATP. Metabolic performance was assessed by $^{14}$CO$_2$ production and by acyl CoA ester production measured by HPLC with UV detection.

In incubations carried out in the presence of exogenous CoA and ATP, treatment with NaHS significantly diminished $^{14}$CO$_2$ production and was associated with
increased levels of butyryl-CoA and a reduction in levels of crotonyl-CoA. The mean concentration of acetyl CoA in the reaction mix at ten minutes was not significantly different between control and sulphide treated incubations. These results support the suggestion that sulphides inhibit n-butyrate oxidation in colonic epithelial cells by inhibiting short chain acyl dehydrogenation of activated fatty acids.

The demonstration of elevated concentrations of sulphide in the stool of patients with ulcerative colitis would provide support for the hypothesis of sulphide induced damage in this disease. Furthermore, if such a hypothesis were to be true, it might be expected that stool sulphide would be elevated in patients with quiescent colitis, as the metabolic abnormality is observed in this group also. Evidence concerning luminal sulphide in ulcerative colitis has been conflicting and it seemed worthwhile to re-examine stool sulphides and sulphate in control and colitic subjects (Chapter 5). Fifteen control and nineteen patients with ulcerative colitis were enrolled for study. Eleven colitic subjects had disease limited to the left colon and thirteen had inactive disease. Six patients were not taking salicylate therapy. Stool sulphide was measured using a direct spectrophotometric method and by reversed phase high performance liquid chromatography (RPHPLC) with absorbance detection. Mean free and total stool sulphide levels did not differ significantly between control and colitic subjects. Neither disease extent nor disease activity was observed to influence stool sulphide. The level of free stool sulphide in colitic patients who were taking salicylate medication was not significantly different from either control patients or colitic patients not on salicylates. Similarly, free and bound stool sulphate, measured by RPHPLC with conductivity detection, was not influenced by presence of disease, disease extent, disease activity or salicylate medication. Breath methane was measured in all subjects using gas chromatography. Methane was detectable in 32% of subjects studied. The mean free stool sulphide was higher in non-methanogenic individuals but this was not significantly different.
The ability of the colonic mucosa to detoxify sulphide may be an equally important determinant leading to sulphide induced damage of the colonic mucosa.

Detoxification of sulphide by the colonic mucosa may be by oxidation, cationic and protein binding, sulphane sulphur transferase reactions or methylation. Of these, methylation by the enzyme thiol methyltransferase (TMT) was considered worthy of study. It may be hypothesised that the degree of sulphide effect on the metabolic performance of the colonic epithelium depends on the activity of mucosal TMT.

The experiments described in Chapter 6 assessed the role mucosal detoxification of sulphide by thiol methyltransferase (TMT) mediated methylation may play in protecting the healthy human colonic mucosa from the adverse effects of luminal sulphide. Colonic epithelial cell suspensions from healthy human proximal (n = 9) and distal colon (n = 10) were incubated in the presence of [1-¹⁴C] labelled n-butyrate (5 mM) alone, butyrate plus NaHS (1.5 mM), or butyrate plus NaHS plus S adenosyl-[¹⁴C methyl]-methionine (SAMe) (5 mM). The end points of the study were ¹⁴CO₂ production and mucosal TMT activity. Incubation with NaHS produced a significant decrease of ¹⁴CO₂ production compared to control incubations which was similar for cell suspensions from the proximal and distal colon. SAMe reversed this effect completely in cell suspensions from the proximal colon but not the distal colon, suggesting a greater susceptibility of the distal colon to sulphide damage. Median whole mucosal TMT values did not differ between proximal and distal colonic mucosa, but a bimodal distribution of TMT values was observed in the distal colon. However, neither the degree of inhibition of ¹⁴CO₂ production due to sulphide nor the degree to which SAMe reversed this inhibition correlated with whole mucosal TMT activity. Regional differences in TMT activity mediating S-methylation may help to explain the predilection for ulcerative colitis to occur in the distal colon but this cannot be explained solely on the basis of TMT activity.

The studies described provide only qualified support for the general hypothesis as previously stated. While luminal sulphide produces a metabolic abnormality in rat colonic mucosal tissue consistent with the selective inhibition of n-butyrate oxidation seen in human ulcerative colitis, stool levels of sulphide are not elevated
in patients with ulcerative colitis, even when allowing for possible effects of drug therapy. Future studies should perhaps focus on the role of sulphide detoxification by the colonic mucosa in both health and disease.