Free Rad. Res., Vol. 22, No. 2, pp. 131–143
Reprints available directly from the publisher
Photocopying permitted by license only

© 1995 Harwood Academic Publishers GmbH
Printed in the United States of America

ALTERED ASCORBIC ACID STATUS IN THE MUCOSA FROM INFLAMMATORY BOWEL DISEASE PATIENTS

G.D. BUFFINTON, and W.F. DOE

Division of Clinical Science,
John Curtin School of Medical Research,
Australian National University

(Received March 31, 1994; in revised form, July 15, 1994)

Attempts to establish the presence of oxidant stress and tissue damage in inflammatory bowel disease (IBD) have relied on determining the capacity of peripheral blood inflammatory cells to produce reactive oxygen species (ROS) and other indirect indices. These approaches have failed to address whether or not there are adequate chemical antioxidant defences to prevent oxidative injury in the inflamed mucosa. In this investigation we have determined the mucosal concentrations of reduced and total ascorbic acid and the redox status in paired non-inflamed and inflamed mucosa using colonic biopsies from IBD patients. In inflamed mucosa from Crohn's disease (CD) patients, reduced and total ascorbic acid content decreased by 35% ($p = 0.014$ and $p = 0.009$, respectively). In ulcerative colitis (UC) patients, mucosal total ascorbic acid content decreased by 73% ($p = 0.069$) and reduced ascorbic acid by 41% ($p = 0.014$). The proportion of total ascorbic acid present in its reduced form in histologically normal mucosa from CD patients was unusually low at ~30%. In the paired-inflamed mucosa from CD patients, the redox ratio was also ~30% despite the loss of 35% of total ascorbate. In UC patients, the ascorbate redox ratio in the non-inflamed mucosa was 23% which increased to 51% in paired inflamed mucosa. This increase reflected the loss (73%) of total ascorbate. Reduction of dehydroascorbic acid by GSH/NADPH dependent dehydroascorbic acid reductase decreased significantly ($p = 0.046$) in inflamed mucosa from UC patients, suggesting that the capacity of the inflamed mucosa to maintain the concentration of reduced ascorbic acid is also diminished. HPLC analysis of mucosal preparations for diketogulonic acid, the decomposition product of dehydroascorbic acid, did not account for the loss of total ascorbate in the inflamed mucosa suggesting that ascorbate equivalents underwent further decomposition reactions or were excreted to the colonic lumen.

We conclude that the normal luminal environment is strongly oxidising in character and that oxidant stress derived from inflammatory cells contributes to the loss of 35–73% total and reduced ascorbate. In absolute terms, the overall loss of this antioxidant buffering capacity would decrease the capacity of the inflamed mucosa to prevent oxidative tissue damage and hinder recovery of the inflamed mucosa.

KEY WORDS: Inflammatory bowel diesease, ascorbic acid, 2,3-diketogulonic acid, redox status, mucosal inflammation.

INTRODUCTION

Inflammatory bowel disease (IBD), a collective term for Crohn's disease (CD) and ulcerative colitis (UC), is characterised by a dense inflammatory cell infiltrate composed primarily of recently recruited monocytes and polymorphonuclear leukocytes

Address for Correspondence: G.D. Buffinton, Division of Clinical Science, John Curtin School of Medical Research, Australian National University, P.O. Box 334, Canberra City, A.C.T., 2601, Australia.

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/13/11
For personal use only.

RIGHTSLINK

(PMNs) to the active mucosal lesion. Among the mediators produced by these inflammatory cells upon stimulation are reactive oxygen and nitrogen species (ROS), $O_2^{-\cdot}$, H_2O_2, $\cdot NO$ and, in the presence of myeloperoxidase, HOCl. These species have been implicated in the tissue injury seen in numerous diseases[1,2] such as adult respiratory disease syndrome, ischaemia/reperfusion and components of IBD pathology including mucosal erosions, haemorrhagic changes and alterations in microvascular permeability.[3,4] Under physiological conditions chemical and enzymatic antioxidant defence mechanisms protect tissues from the potentially damaging effects of ROS. The antioxidant defences of different tissues vary and those with precariously low defences may become preferential targets of oxidative injury in the presence of the increased oxidative stress[5] as found at sites of inflammation.

The chemical and enzymatic antioxidant defences of the human colonic mucosa are poorly defined. The activities of catalase, superoxide dismutase and glutathione peroxidase have been reported to be only 4, 8, and 45% of that found in the liver.[6] Specific activities of these enzymes within the subfractionated mucosa suggest that the colonic epithelium is relatively well protected compared to the lamina propria. The colonic mucosal content of reduced glutathione (GSH), a co-substrate for GSH-peroxidase which catabolises H_2O_2 and can interact with ROS directly, is decreased by 27% in inflamed mucosa from IBD patients.[7] Such a decrease in GSH is suggestive of increased oxidative stress, but it is insufficient to establish the linkage definitively. Studies on plasma vitamin status in patients with IBD have found that plasma concentrations of vitamins A, C, E and β-carotene are decreased in IBD patients with 40–50% of patients at risk of developing hypovitaminosis.[8,9] Indeed, one report documents a CD patient with scurvy.[10] Among the chemical antioxidants, vitamin C or ascorbic acid is regarded as one of the most important in the prevention of lipid peroxidation.[11,12] Ascorbic acid acts as a strong reducing agent capable of reducing oxygen, nitrogen and sulphur-centred radicals.[13] In addition to scavenging radicals directly, ascorbic acid is a key reductant which acts in concert with other chain-breaking antioxidants such as vitamin E, ubiquinol-10, β-carotene, uric acid, thiols and bilirubin in the formulation of the overall antioxidant defence capacity.[14]

In this investigation, the mucosal concentrations of ascorbic acid and its redox status have been determined in paired biopsies from both inflamed and non-inflamed sites from IBD patients. When compared to the non-inflamed mucosa of the same patient, the content of reduced and total ascorbic acid was markedly decreased in the inflamed mucosa. The magnitude of the loss of ascorbic acid is such that it may seriously impair the antioxidant defences of the inflamed mucosa and place oxidative pressure on other antioxidant compounds. These findings provide further evidence for the consideration of antioxidant strategies for the treatment of IBD.

MATERIALS AND METHODS

Specimens

Paired mucosal tissue specimens were obtained by either biopsy of histologically normal (non-inflamed) and inflamed sites in patients with inflammatory bowel disease undergoing colonoscopy or from freshly resected colon material. Routine hospital histopathology confirmed the diagnosis of IBD in tissue samples taken adjacent to

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/13/11
For personal use only.

RIGHTSLINK

those used for experimental analysis. Material from 30 IBD patients, 17 Crohn's disease and 13 ulcerative colitis patients were examined. Disease, gender and age data are given in appropriate figure legends. Biopsy or resection samples were either processed immediately on collection or snap frozen on dry ice, stored at −70°C and analysed as soon as possible. Protocols for obtaining tissue samples used in this study were in accordance with protocols approved by the Ethics Committees from both ACT Health Institutions Committee and the John Curtin School of Medical Research.

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/13/11
For personal use only.

Sample Processing and Analysis

Tissue samples (usually 2 biopsies or a similar sized piece of mucosa from resected material) were analysed for ascorbic acid content as modified by Hennet *et al.* (1992).[15] Samples were homogenized in 0.5 ml Hank's Balanced Salt Solution (HBSS, without phenol red) on ice using a pre-chilled teflon/glass homogenizer (Wheaton, 2 ml, 20 passes). Several 50 μl aliquots were stored at −70°C for subsequent DNA and protein determinations to normalize the paired data. The remainder was microfuged (10 000 × g, 4°C, 10 min), an aliquot of the supernatant added to an equal volume 5% metaphosphoric acid (MPA), vortexed and microfuged (10 000 × g, 4°C, 10 min) to precipitate mucosal proteins. From each supernatant, 2 × 50 μl aliquots were analysed for reduced and total ascorbic acid content. To the first aliquot 14.4 μl 2.58 M K_2HPO_4 and to the second 14.4 μl 1% (w/v) homocysteine in 2.58 M K_2HPO_2 were added. Both reactions were incubated at 37°C, 15 min and returned to ice followed by addition of 436 μl mobile phase before analysis. Samples were analysed using a Millipore-Waters HPLC system with a 600 MS quaternary pump with SILK pulse dampening, 717 refrigerated autosampler C18 GuardPak module and NovaPak C18 (3.9 × 300 mm) column. Electrochemical detection was by a dual series glassy carbon electrode (CC4) and two amperometric detectors (LC4) modules verses a Ag/AgCl reference electrode, 500 mV, 50 nA FS. Mobile phase consisted of 40 mM Na acetate, 540 μM Na_2EDTA, 1.5 mM Q12 ion pair reagent and 7.5% CH_3OH. The pH was adjusted to 4.75 with glacial acetic acid. The mobile phase was sparged continuously with analytical grade helium at 100 ml/min. The mobile phase flow rate was maintained at 0.5 ml/min, 50 μl samples injected and compared to a standard curve. The retention time was Rt ~ 17 min.

Determination of Dehydroascorbic Acid Reductase Activity

Dehydroascorbic acid reductase activity (DHAR) was determined spectrophotometrically as described previously with minor modifications.[16] The final reaction being carried out in quartz microcurvettes in a final volume of 150 μl.

Determination of 2,3-diketogulonic acid

2,3-Diketogulonic acid standard was prepared as described by Kagawa[17] and analysed as described by Doner and Hicks[18] with minor modifications. The analytical conditions were: mobile phase: 25% 50 mM KH_2PO_4:75% CH_3CN, pH 4.0 at 3 ml/min. Samples in 5% MPA were separated using a μBondapak C-NH_2 cartridge (8 × 100 mm) and Radial Compression Module (Millipore-Waters) maintained at 40°C by immersion in a water bath to prevent precipitation of buffer salts. The 990

RIGHTSLINK

Diode Array Detector (Waters) was set at 3nm resolution and spectra from 190–300 nm were acquired at 1 sec intervals for the duration of the run. Additional HPLC hardware was as described above for ascorbic acid analysis.

DNA Determinations

Paired data (i.e., non-inflamed and inflamed from the same patient) were normalized against DNA content as described.[19] A 50 μl aliquot of sample homogenate was diluted and analysed for DNA content using the Hoechest H33258 stain with each analysis calibrated against a calf thymus DNA internal standand.

Statistical Analysis

The data from paired non-inflamed and inflamed samples from the same patient were analysed using the Wilcoxon Signed Rank analysis for paired samples. Significance was accepted at $p < 0.05$.

Analytical Reagents

HPLC grade solvents were from Millipore-Waters, Ma, USA or Ajax Chemicals, Sydney. The Q12 (dodecyltriethylammonium phosphate) ion pair reagent was from Regis Chemicals, Il, USA. Ascorbic acid standard (highest quality) was from Aldrich. Other biochemicals were of analytical grade from Boehringer Mannheim, Sigma Chemical Co., Aldrich or Ajax Chemicals.

RESULTS

Ascorbic Acid Content in Inflamed and Non-inflamed IBD Mucosa

The levels of both reduced and total (reduced plus dehydroascorbic acid) ascorbic acid were determined in paired biopsies from histologically non-inflamed and inflamed mucosa from patients with active Crohn's disease and ulcerative colitis by

TABLE 1

Summary of Total and Reduced Ascorbic Acid Content of IBD Mucosa

Tissue source	Total Ascorbic	Reduced Ascorbic	Ratio (Reduced/Total, %)
Crohn's disease			
Non-inflamed	619 ± 119	188 ± 51	30.4
Inflamed	402 ± 78	123 ± 42	30.3
% Decrease	34.7	34.8	
Statistic	0.014	0.009	
Ulcerative colitis			
Non-inflamed	897 ± 337	208 ± 67	23.2
Inflamed	240 ± 87	122 ± 51	50.7
% Decrease	73.2	41.3	
Statistic	0.069	0.014	

Mean ± SEM, CD n = 17, UC n = 10.

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/13/11
For personal use only.

RIGHTSLINK

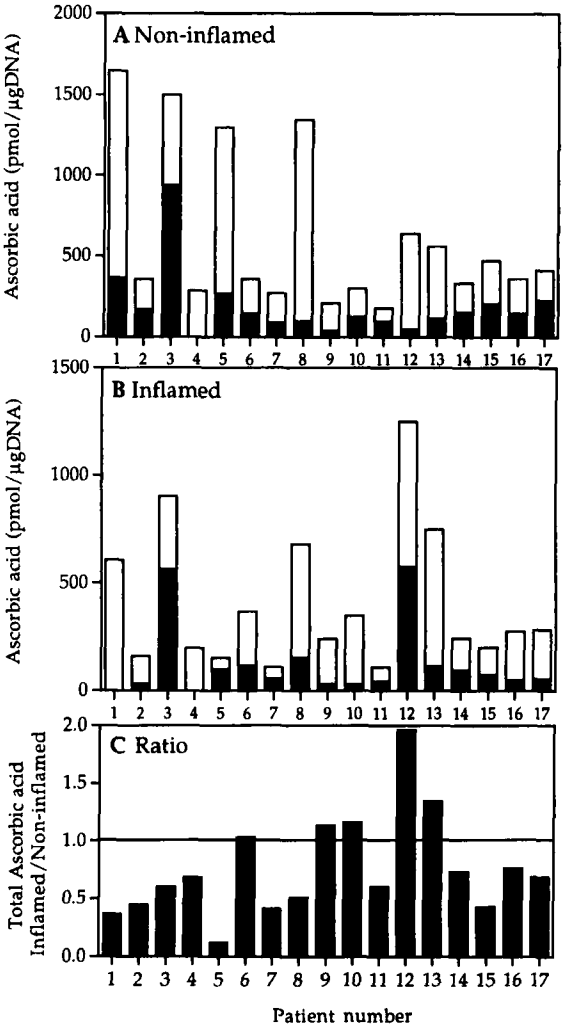

FIGURE 1 *Ascorbic acid content of non-inflamed and inflamed colonic mucosa from Crohn's disease patients.* Total ascorbic acid was defined as the amount of ascorbic acid detectable after reaction with homocysteine. The difference between reduced and total ascorbic acid was defined as dehydroascorbic acid. (A). Non-inflamed mucosa, (B) Inflamed mucosa, (C) Ratio of Inflamed/non-inflamed total ascorbic acid content of paired biopsies. Patient numbers for parts A, B and C refer to the same patient. The patient cohort comprised 8 males, 9 females; mean age 28.5 years, range 18–43. Solid bars, reduced ascorbic acid; open bars, dehydroascorbic acid.

HPLC using electrochemical detection. Determination of the ascorbic acid redox status of 17 CD patients showed that in the non-inflamed mucosa, total ascorbic acid concentrations ranged widely (Figure 1a) with a mean value of 618.9 pmol/μgDNA (Table 1). In the involved mucosa of the same patients (Figure 1b), the total ascorbic acid content decreased in 12/17 patients and increased in 4/17 patients (Figure 1c).

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/13/11
For personal use only.

RIGHTSLINK

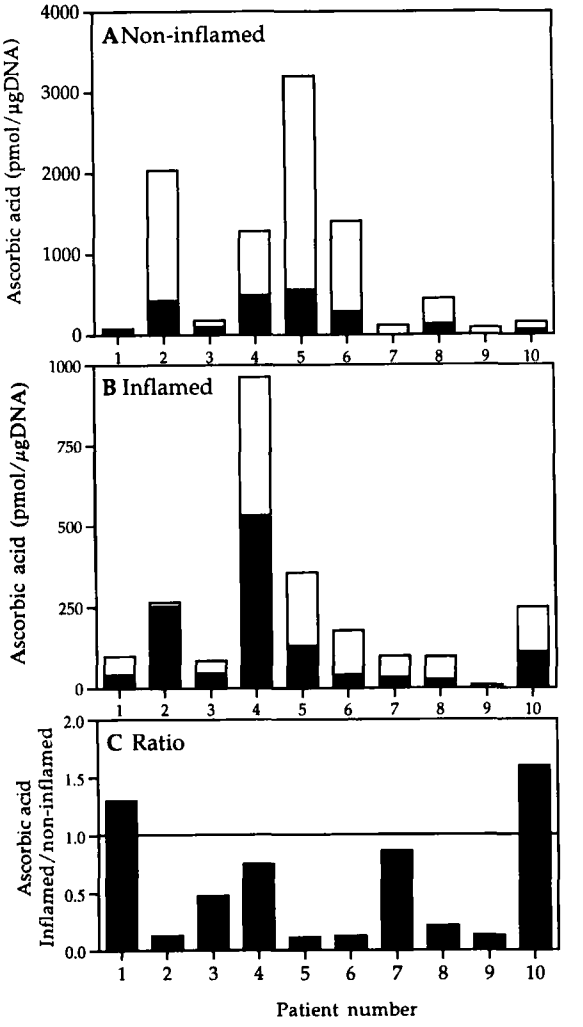

FIGURE 2 *Ascorbic acid content of non-inflamed and inflamed colonic mucosa from Ulcerative colitis patients.* Total and reduced ascorbic acid are defined in the legend for Figure 1. (A). Non-inflamed mucosa, (B) Inflamed mucosa, (C) Ratio of Inflamed/non-inflamed total ascorbic acid content of paired biopsies. Patient numbers for parts A, B and C refer to the same patient. The patient sample cohort comprised 5 males, 5 females; mean age 40.1 years, range 22–54. Solid bars, reduced ascorbic acid; open bars, dehydroascorbic acid.

The mean decrease was by 34.7% to 404.2 pmol/μgDNA ($p = 0.014$). While such a marked decrease in the total content of ascorbic acid would potentially hinder the antioxidant capacity of ascorbic acid, the proportion of the total ascorbate pool present in the reduced form is also important. In non-inflamed IBD mucosa, the proportion of the total ascorbate pool present as reduced ascorbic acid was 187.9 pmol/μgDNA which decreased significantly ($p = 0.009$) to 122 pmol/μgDNA

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/13/11
For personal use only.

RIGHTSLINK

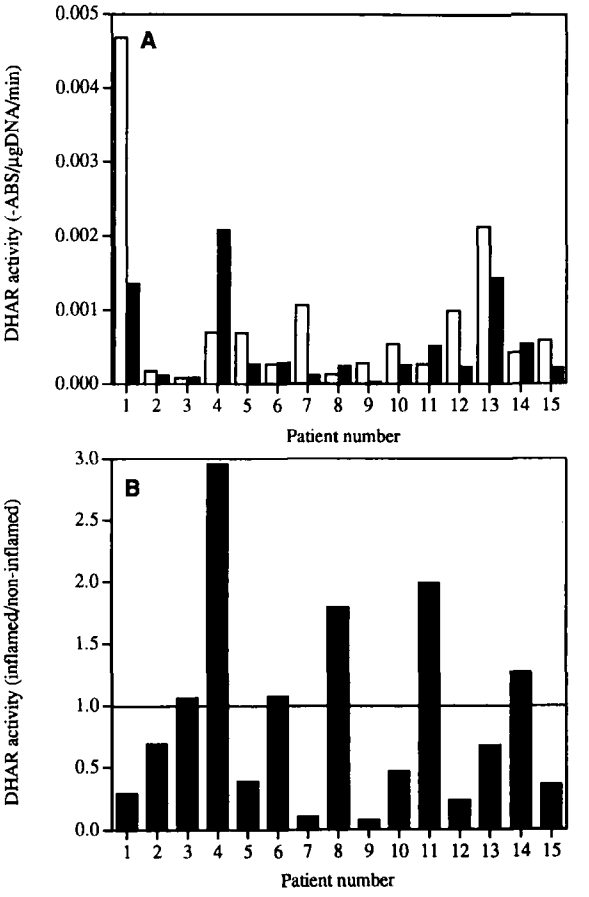

FIGURE 3 *Dehydroascorbic acid reductase (DHAR) activity in non-inflamed and inflamed mucosa specimens from Crohn's disease and ulcerative colitis patients.* (A) DHAR activity in paired non-inflamed and inflamed specimens from Crohn's disease (patients 1–7) and ulcerative colitis (patients 8–15) patients. (B) Ratio of inflamed/non-inflamed paired specimens. The Crohn's disease cohort comprised 1 male, 6 females; mean age 31.3 years, range 21–43. The ulcerative colitis cohort comprised 5 males, 3 females; mean age 42.6 years, range 27–69.

in paired inflamed mucosa, while the proportion of the total ascorbic acid present as reduced ascorbic acid remained ~30% (Table 1). Similar observations were seen when the ascorbic acid redox status of mucosa from 10 ulcerative colitis patients was determined (Figures 2a,b,c, Table 1). The large decrease in total ascorbic acid in UC inflamed mucosa relative to the paired reduced ascorbic acid content increased the redox ratio from 23.7% to 50.7%. This decrease was a result of the marked loss (73%) of total ascorbic acid from these patients rather than a specific response by the inflamed mucosa since the inflamed levels approximated those detected in CD patients (Table 1). No significant difference was observed in the total or reduced ascorbic acid content between the UC and CD mucosal specimens.

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/13/11
For personal use only.

RIGHTSLINK

Dehydroascorbic Acid Reductase Activity in Inflamed and Non-inflamed IBD Mucosa

Dehydroascorbic acid can be reduced to ascorbic acid either chemically, by reaction with reduced glutathione, or by a glutathione/NADPH reductase activity (dehydroascorbate reductase, DHAR).[20] DHAR activity was determined in 15 IBD patients (7 CD and 8 UC). When the enzyme rates were compared between paired inflamed and non-inflamed mucosa the rates decreased significantly in inflamed mucosa from UC patients (non-inflamed 0.00067 ± 0.00023, inflamed 0.00043 ± 0.00002 ABS min/ug DNA, mean ± SEM, $p = 0.046$) (Figure 3). The mean decrease in paired inflamed mucosa from CD patients proved not to be significant (non-inflamed 0.00109 ± 0.00061, inflamed 0.00062 ± 0.0003 ABS/min/ug DNA, mean ± SEM, $p = 0.199$). A patient specimen diagnosed as "quiescent colitis" showed no difference between non-inflamed and inflamed mucosal DHAR activity (not shown).

Determination of 2,3-diketogulonic Acid in Inflamed and Non-inflamed Mucosa from IBD Mucosa

The absolute loss of ascorbate from the involved mucosa which could not be recovered by treatment of the sample with a reducing agent such as homocysteine, as is the case for dehydroascorbic acid, raises the possibility that a significant proportion of the dehydroascorbic acid had undergone additional chemically or enzymically. Analysis of eight paired samples from patients with active IBD for the presence of 2,3-DKG did not reveal a peak that coincided with that of authentic standard. When a biological sample was spiked with 10nmol 2,3-DKG however, the spiked standard eluted with a new apparent retention time of 8.3 min. In several samples a small peak was detected with a retention time of about 8.4 min. (Figure 4). and analysis of subtraction absorbance spectrums of the 8.4 min peak compared favourably with that of 2,3-DKG with an absorption maxima of 192 nm (Figure 4, insert). While this peak was detectable in several samples, its presence was variable and the levels detected did not account for the loss of total ascorbate from the mucosa.

DISCUSSION

The inflamed intestinal mucosa appears to be subjected to increased oxidative stress but the evidence is largely indirect. This includes increased capacity of peripheral blood inflammatory cells to undergo a respiratory burst in response to a variety of stimuli such as PMA and fMLP,[21] a decrease in the content of GSH in the mucosa,[7] efficacy of antioxidant compounds in ameliorating animal models of acute colitis,[22] beneficial responses in limited open trials of encapsulated superoxide dismutase in CD patients[23] and the detection of oxidized 5-aminosalicylic acid in faecal extracts from IBD patients.[24] Direct evidence of oxidative injury to epithelial cells from inflamed mucosa has been demonstrated by the detection of the oxidation of the active site thiol of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and inhibition of enzyme activity.[25] Here we present the first analysis of the redox status of ascorbic acid – the prime chain-breaking antioxidant – in mucosal biopsies from IBD patients.

There were two main observations arising from this investigation. The first was that the redox status of ascorbate in apparently normal mucosa was

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/13/11
For personal use only.

RIGHTSLINK

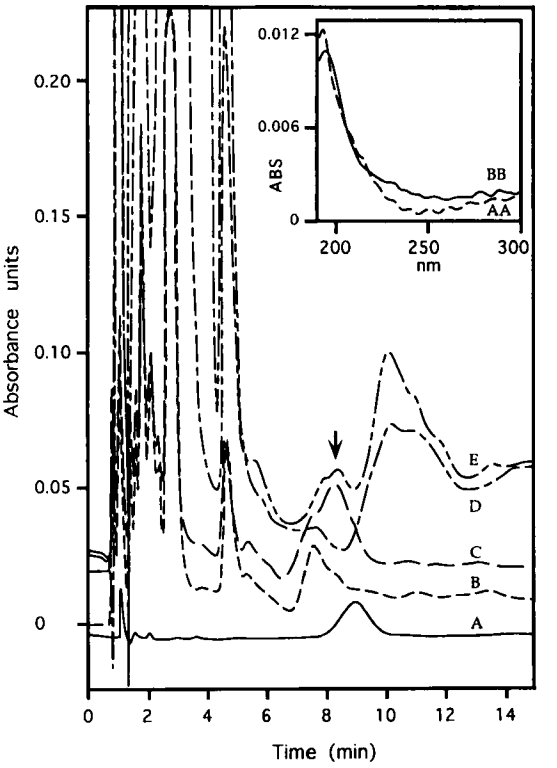

FIGURE 4 *Detection of 2,3-diketogulonic acid (2,3-DKG) in inflamed IBD colonic mucosa.* (A) 10 nmol 2,3-DKG, (B) normal mucosal specimen derived from cancer-bearing resection, (C) Specimen (B) spiked with 10 nmol DKG, (D) non-inflamed IBD mucosal specimen, (E) paired inflamed IBD mucosal specimen. Insert. (AA) Spectral analysis of 2,3-DKG (Trace A, Rt 9.5 min), (BB) Unidentified peak in inflamed IBD mucosa (Trace E, Rt 8.4 min). The retention times for ascorbic acid and dehydroascorbic acid were 3.0 and 1.5 min., respectively.

unusually low (about 30%) where as in other tissues and in blood the redox ratio is typically >80–95%. The cause of the low redox status of ascorbate from apparently normal mucosa may be due to oxidative stress derived from faecal material containing multiple forms of metal ion catalysts, potential carcinogens and oxidising agents.[3,26,27] Indeed, ascorbic acid has been proposed to play an important role in the protection of the mucosa from ROS.[28] Compounding this, the mucosal ascorbate redox status would also be influenced by dietary ascorbate intake. It is not known how much ascorbate reaches the colonic lumen or its lumenal redox status,[29] however, given the faecal content of potentially oxidising agents, it is likely that a substantial proportion is present as dehydroascorbic acid (DHAA). Studies using guinea pig jejunum have demonstrated that active transport of DHAA occurs on the brush border and basolateral surfaces of small intestine epithelial cells which was saturable by excess DHAA.[28] Intracellular enzymatic reduction of the transported DHAA was also saturable though at a higher Km ensuring efficent reduction of the intracellular DHAA. However, when mucosal to sersoal transepithelial

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/13/11
For personal use only.

RIGHTSLINK

DHAA transport was measured in the presence of excess DHAA, the proportion of [^{14}C]-label detectable as reduced ascorbate decreased suggesting that there also existed a non-saturable component of DHAA transport possibly involving diffusion through inter-epithelial cell tight junctions.[28] The mechanisms of ascorbate transport which operate in the human colonic mucosa remain to be explored in detail, however, the high proportion of total ascorbate present as DHAA may well be due to the combined effects of luminal oxidant stress and uptake of DHAA at a rate which exceeds either the capacity of the DHAR or the supply of essential reducing equivalents.

The second striking observation of these studies was the decrease in the absolute concentrations of both reduced and total ascorbic acid in the inflamed mucosa compared to that detected in paired specimens of non-inflamed mucosa from the same patient. The decrease in ascorbate content may be a direct result of overwhelming oxidative stress in the form of activated inflammatory cells resulting in depletion of the total ascorbate pool. Reduced ascorbic acid reduces the tocopherol radical at the lipid/aqueous interface, the formed ascorbyl radical decomposes to dehydroascorbic acid, which under normal homeostasis, is recycled or reduced to ascorbic acid either directly by GSH or by a GSH-dependent DHAR.[20] DHAR activity has been reported in rat colon[30] but there is no report in human colonic mucosa. In the present study, 9/15 IBD patients had decreased DHAR activity in the inflamed mucosa suggesting that recycling of dehydroascorbic acid may be hindered. Moreover, a constant supply of reducing equivalents in the form of NADPH would also be required to maintain GSH in its reduced form *via* glutathione reductase. Few details are available on the status of reducing equivalents in the mucosa, although the inflamed epithelial cell is known to be metabolically impaired by the oxidative inhibition of GAPDH[25] and the decrease in the inflamed mucosa GSH content.[7] The oxidation of GAPDH and the decrease in mucosal GSH levels may result from the decomposition of H_2O_2 by GSH peroxidase or insufficient NADPH to reduce the GSSG catalysed by GSH reductase. The outcome of either mechanism would be a decrease in the reduced ascorbic acid pool and an accumulation of dehydroascorbic acid, which if not reduced efficiently, hydrolyses to 2,3-DKG. The inability to account for the loss of total ascorbate by the formation of 2,3-DKG is probably due to the high reactivity of 2,3-DKG with biomolecules[31] and may play a role in the increased risk of colorectal cancer in UC patients.

A proportion of the decrease in total ascorbic acid and in ascorbate recycling in the inflamed mucosa may result from inhibition of mucosal uptake of ascorbate by the inflamed mucosa mediated by inflammatory cell-derived oxidative stress on transport mechanisms. Uptake of dietary ascorbate occurs against a concentration gradient in the ileum of both humans[32] and guinea pigs,[33] is metabolically demanding and sensitive to Na^+/K^+ ATPase and mitochondrial inhibitors.[29] While the mechanism of oxidation-mediated inhibition of ascorbate transport was not investigated here, ascorbate accumulation in guinea pig ciliary body/iris preparations is dependent upon on glucose metabolism and is sensitive to inhibition by iodoacetate.[34] In IBD, inhibition of glycolysis mediated by oxidation of GAPDH[25] would restrict the flux of reducing equivalents required for cellular transport/reduction processes and potentially adversely affect ascorbate uptake by the inflamed mucosa.[28] Indeed, the plasma concentrations of ascorbic acid, vitamin E and β-carotene in IBD patients have been reported to be significantly lower than those in controls and despite dietary multivitamin (including ascorbic acid) supplementation, ascorbic acid concentrations remained unchanged.[8,9]

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/13/11
For personal use only.

RIGHTSLINK

The potential for ascorbic acid to participate in pro-oxidant reactions in the presence of metal ions[35] represents a further mechanism for the decreased ascorbic acid content in IBD mucosa. The lumen of the colon, particularly the inflamed colon in patients with bloody diarrhoea, and aberrant ascorbate metabolism, may be exposed to potentially catalytic metal ions in multiple forms.[3] A prime candidate would be haem proteins, particularly degraded haemoglobin. In these circumstances, the antioxidant capacity of ascorbic acid may then become pro-oxidant thereby promoting the formation of $\cdot OH$, $O_2^{-\cdot}$, H_2O_2 and ferryl iron species.[3] In chemical systems, these pro-oxidant reactions of ascorbic acid inactivate glucose-6-phosphate dehydrogenase (G6PDH),[36] the key enzyme in the hexose monophosphate shunt and one critical for the generation of NADPH. Interestingly, in the diabetic liver, decreased G6PDH activity and therefore decreased reducing equivalents, was implicated in the observed decreased recycling of dehydroascorbic acid.[37] The potential exists in IBD for such reactions to occur but to date they have not been properly defined.

This paper reports that the antioxidant defence capacity associated with ascorbic acid is compromised in the inflamed mucosa of IBD patients. The cause of this deficiency remains unknown and specific mucosal ascorbate transport studies are required to address this. Never-the-less, the decreases in ascorbic acid and in DHAR reported here may seriously undermine the capacity of the inflamed mucosa to protect itself against sustained oxidative stress during recurrent disease. As a consequence, this deficit would place an oxidative pressure on the other antioxidant defences such as vitamin E, ubiquinol-10, β-carotene and uric acid and gives further support to the development of therapeutic strategies aimed at bolstering the overall antioxidant capacity of the mucosa to protect it against the potential damage caused by ROS in IBD.

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/13/11
For personal use only.

Acknowledgements

We would like to acknowledge Dr Paul Pavli, Dr Ibraham Abdool and Staff Gastroenterologists and their registrars at Woden Valley Hospital and Calvary Hospital who kindly provided mucosal specimens for this study.

References

1. H. Sies Ed. (1985) *Oxidative Stress* (1985) Academic Press, London.
2. B. Halliwell and J.M.C. Gutteridge (1989) *Free Radicals in Biology and Medicine*. (2nd edition) Clarendon Press, Oxford.
3. C.F. Babbs (1992) Oxygen radicals in ulcerative colitis. *Free Radicals in Biology and Medicine*, **13**, 169–181.
4. M.B. Grisham and D.N. Granger (1988) Neutrophil-mediated mucosal injury. Role of reactive oxygen metabolites. *Digestive Diseases and Sciences*, **33**, S6–S15.
5. J.H. Doroshow, G.Y. Locker and C.E. Meyers (1980) Enzymatic defence of the mouse heart against reactive oxygen metabolites. *Journal of Clinical Investigation*, **65**, 128–135.
6. M.B. Grisham, R.P. MacDermott and E.A. Deitch (1990) Oxidant defense mechanisms in the human colon. *Inflammation*, **14**, 669–680.
7. B.H. Lauterburg, Bilzer, M.E. Rowedder and R.W. Inauen (1988) Decreased glutathione in inflamed colonic mucosa in man. Possible role of hypochlorous acid and prevention by 5-aminosalicylic acid. In *Inflammatory Bowel Disease: Current Status and Future Approach* (Ed. R.P. MacDermott) Elservier Science Publishers B.V. 273–277.
8. A. Abad-Lacruz, F. Fernandez-Banares, E. Cabre, A. Gil, M. Esteve, F. Gonzalez-Huix, X. Xiol and M.A. Gassull (1988) The effect of total enteral tube feeding on the vitamin status of malnourished patients with inflammatory bowel disease. *International Journal of Vitamin and Nutrition Research*, **58**, 428–435.

RIGHTSLINK

9. F. Fernandez-Banares, A. Abad-Lacruz, X. Xiol, J.J. Gine, C. Dolz, E. Cabre, M. Esteve, F. Gonzalez-Huix and M.A. Gassull (1989) Vitamin status in patients with inflammatory bowel disease. *American Journal of Gastroenterology*, **84**, 744–748.
10. B.D. Linaker (1979) Scurvy and vitamin C deficiency in Crohn's disease. *Postgraduate Medical Journal*, **55**, 26–29.
11. B. Frei, R. Stocker and B.N. Ames (1988) Antioxidant defenses and lipid peroxidation in human blood plasma. *Proceedings of the National Academy of Sciences (USA)*, **85**, 9748–9752.
12. B. Frei, L. England and B.N. Ames (1989) Ascorbate is an outstanding antioxidant in human blood plasm. *Proceedings of the National Academy of Sciences (USA)*, **86**, 6377–6381.
13. E. Niki (1991) Action of ascorbic acid as a scavenger of active and stable oxygen radicals. *American Journal of Clinical Nutrition*, **54**, 1119S–1124S.
14. B. Halliwell and J.M.C. Gutteridge (1990) How to characterise a biological antioxidant. *Free Radical Research Communications*, **9**, 1–32.
15. T. Hennet, E. Peterhans and R. Stocker (1992) Alterations in antioxidant defences in lung and liver of mice infected with influenza A virus. *Journal of General Virology*, **73**, 39–46.
16. M. Coassin, A. Tomasi, V. Vannini and F. Ursini (1991) Enzymatic recycling of oxidized ascorbate in pig heart: One-electron vs two-electron pathway. *Archives of Biochemistry and Biophysics*, **290**, 458–462.
17. Y. Kagawa (1962) Enzymatic studies on ascorbic acid catabolism in animals. (I) Catabolism of 2, 3-diketogulonic acid. *Journal of Biochemistry*, **51**, 134–144.
18. L.W. Doner and K.B. Hicks (1981) High-performance liquid chromatographic separation of ascorbic acid, erythrorbic acid, dehydroascorbic acid, dehydroerythrobic acid, diketogulonic acid and diketogluconic acid. *Analytical Biochemistry*, **115**, 225–230.
19. C.F. Brunk, K.C. Jones and T.W. James (1979) Assay for nanogram quantities of DNA in cellular homogenates. *Analytical Biochemistry*, **92**, 497–500.
20. R.C. Rose and A.M. Bode (1992) Tissue-mediated regeneration of ascorbic acid: Is the process enzymatic? *Enzyme*, **46**, 196–203.
21. J.G. Williams, L.E. Hughes and M.B. Hallett (1990) Toxic oxygen metabolite production by circulating phagocytic cells in inflammatory bowel disease. *Gut*, **31**, 187–193.
22. C. von Ritter, M.B. Grisham, Hollwarth, W. Inauen and D.N. Granger (1989) Neutrophil-derived oxidants mediate formyl-methionyl-leucyl-phenyl- alanine-induced increases in mucosal permeability in rats. *Gastroenterology*, **97**, 778–780.
23. J. Emerit, J. Loeper and G. Chomette (1981) Superoxide dismutatase in the treatment of post-radiotherapeutic necrosis and of Crohn's disease. *Bulletin of Européen Physiopathologie Respiratoire*, **17**, 287–288.
24. I. Ahnfelt-Rønne, O. Haagen Nielsen, A. Christensen, E. Langholz, V. Binder and P. Reis (1990) Clinical evidence supporting the radical scavenger mechanism of 5-aminosalicylic acid. *Gastroenterology*, **98**, 1162–1169.
25. S.J. McKenzie, G.D. Buffinton, M.S. Baker and W.F. Doe (1993) Colonic epithelial cell glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a target of oxidative injury in inflammatory bowel disease. *Gastroenterology*, **104**, A741.
26. B.N. Ames (1983) Dietary carcinogens and anticarcinogens. *Science*, **223**, 1256–1264.
27. J.M. McCord (1987) Radical explanations for old observations. *Gastroenterology*, **92**, 2026–2037.
28. R.C. Rose, J.-L. Choi and M.J. Koch (1988) Intestinal transport and metabolism of oxidized ascorbic acid (dehydroascorbic acid). American Journal of Physiology, **254**, G824–G828.
29. R.C. Rose (1988) Transport of ascorbic acid and other water-soluble vitamins. *Biochimica et Biophysica Acta*, **947**, 335–366.
30. J.-L. Choi and R.C. Rose (1989) Regeneration of ascorbic acid by rat colon. *Proceedings of the Society for Experimental Biology and Medicine*, **190**, 369–374.
31. W.H. Kalus, W.G. Filby and R. Miinzner (1982) Chemical aspects of the mutagenic activity of the ascorbic acid autoxidation system. *Zeitung für Naturforschung*, **37C**, 40–45.
32. N.R. Stevenson (1974) Active transport of L-ascorbic acid in the human ileum. *Gastroenterology*, **67**, 952–956.
33. N.R. Stevenson and M.K. Brush (1969) Existence and characteristics of Na^+dependent active transport of ascorbic acid in guinea pig. *American Journal of Clinical Nutrition*, **22**, 318–326.
34. B. Becker (1967) Ascorbate transport in guinea pig eyes. *Investigative Opthalmology*, **6**, 410–415.
35. E.R. Stadtman (1991) Ascorbic acid and oxidative inactivation of proteins. *American Journal of Clinical Nutrition*, **54**, 1125S–1128S.
36. C.N. Oliver, B.-W. Ahn, E.J. Moerman, S. Goldstein and E.R. Stadtman (1987) Age-related changes in oxidized proteins. *Journal of Biological Chemistry*, **262**, 5488–5491.

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/13/11
For personal use only.

RIGHTSLINK

37. A.M. Bode, C.R. Yavarow, D.A. Fry and T. Vargas (1993) Enzymatic basis for altered ascorbic acid and dehydroascorbic acid levels in diabetes. *Biochemical and Biophysical Research Communications*, **191**, 1347–1353.

Accepted by Professor H. Sies

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/13/11
For personal use only.

RIGHTSLINK