Oxidative stress in colon tissue induced by vitamin E depletion


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Abstract
Inflammatory disorders of the bowel and colon cancer are associated with elevated indices of oxidative stress. Analogous elevations in markers of oxidative stress and loss of cell-membrane integrity are also observed in the colons of rats deficient in vitamin E (α-tocopherol), the major lipid-soluble antioxidant in biological systems. The causal relationship between colon pathologies associated with oxidative stress and dietary deficiency in antioxidant vitamins such as vitamin E is still uncertain. Investigation of potential mechanisms by which lack of dietary vitamin E may lead to clinically relevant pathological changes in colon tissue was conducted using gene expression profiling strategies on vitamin E-sufficient and -deficient rats. Morphological changes and increased indices of lipid peroxidation were linked to vitamin E deficiency. These changes in colon tissue are potentially important in disease pathogenesis of the colon linked with oxidative stress or other direct consequences of inadequate levels of vitamin E.

Introduction
Vitamin E deficiency and elevated indices of lipid peroxidation are associated with several disorders of the colon including inflammatory bowel disease [1,2] and colon cancer [3]. Moreover, colons from vitamin E-depleted rats exhibit greater fragility and have severe damage to the underlying muscle layer as suggested by consistent elevation of the muscle-specific gene, calponin, in mucosal scrapings [4]. The role of vitamin E in maintaining cellular redox status and normal cellular metabolism may not exclusively invoke its antioxidant function [5]. Alternative mechanisms involving anti-inflammatory effects and intracellular signalling are the focus of intensive investigation [6]. Enhanced lipid peroxidation products, a consequence of vitamin E insufficiency, may impact on cellular homeostasis, initiating concomitant changes in gene expression (see reviews [7,8]) and potential pathologies. Combined expression-profiling techniques, biochemical and microanatomical analysis were used here to investigate further mechanisms whereby a micronutrient deficiency may modulate gene expression and potential consequences for colon pathogenesis.

Methods
Vitamin E concentrations in plasma, liver and colon from Hooded Lister male rats (n = 8) fed with vitamin E-supplemented or -deficient diets for 12 weeks were determined by reverse-phase HPLC with fluorimetric detection [9]. Lipid peroxidation was estimated in tissue homogenates as thiobarbituric acid-reactive substances using HPLC [10]. Plasma pyruvate kinase activity was determined using a test kit (Boehringer Mannheim, Sussex, U.K.). Crypt height, sub-mucosa, muscularis externa and serosa depth (subserosa and serosa) were measured (Leica DM IRB microscope, Leica QFluro imaging software; Leica Microsystems Imaging Solutions Ltd., Cambridge, U.K.). RNA was extracted (RNeasy Midi Kit; Qiagen, Crawley, U.K.) from colon segments adjacent to that used for microanatomical analysis and subjected to Agilent analysis (Agilent Technologies, Bracknell, U.K.). Atlas rat stress macroarray analysis of distal colon was performed according to the manufacturer’s instructions (Clontech, Basingstoke, U.K.; see http://www.clontech.com). Complete data set and analysis information are available at the National Center for Biotechnology Information’s Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/ Accession no. GSE1063). Semi-quantitative PCR was performed on colon regions from rats fed with vitamin E-sufficient or -deficient diets using a High Fidelity PCR System (Roche Applied

Key words: collagen α1(I), colon tissue, lipid peroxidation, lysyl oxidase deregulation, vitamin E.

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Table 1 | Gene expression changes in distal colon of rats fed a vitamin E-deficient diet

<table>
<thead>
<tr>
<th>GenBank® accession no.</th>
<th>Gene</th>
<th>Vitamin E deficiency (fold change)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>L24804</td>
<td>TEBP</td>
<td>↓(0.11)</td>
<td>0.06</td>
</tr>
<tr>
<td>J04791</td>
<td>Odc1</td>
<td>↓(0.06)</td>
<td>0.10</td>
</tr>
<tr>
<td>X02904</td>
<td>GSTpi</td>
<td>↑(0.21)</td>
<td>0.10</td>
</tr>
<tr>
<td>D10864</td>
<td>Id3</td>
<td>↑(0.14)</td>
<td>0.14</td>
</tr>
</tbody>
</table>

Science, Lewes, East Sussex, U.K.) as described previously [11]. In situ hybridization of colon tissue was performed essentially as described previously [12].

Results

Concentrations of α-tocopherol in plasma, liver and colon of rats consuming a vitamin E-deficient ration were significantly decreased and approached the lower levels of detection compared with rats maintained on sufficient ration. By 12 weeks, there were no overt effects on the final weight, coat condition or colon length. However, there was a marked increase in cell-membrane damage as indicated by enhanced plasma pyruvate kinase activity and increased indices of lipid peroxidation in plasma and colon. Regional concentrations of vitamin E measured in the colon of rats consuming a vitamin E-sufficient ration revealed significantly greater levels in the proximal region. Significant increases in crypt heights were recorded in transverse and distal colon of vitamin E-depleted rats. The transverse region of vitamin E-deficient rats also revealed a significant increase in muscularis mucosa depth. No significant differences were observed in measurements of sub-mucosa and serosa (subserosa and serosa) depth in transverse or distal colon tissue in response to vitamin E depletion. No significant differences were measured in any of the parameters measured in proximal colon. Macroarray analysis of comparable regions of the distal colon of vitamin E-sufficient and -deficient rats (n = 5) predicted four differentially regulated transcripts at low significance and low fold changes (Table 1). Northern-blot experiments supported a trend towards down-regulation of telomerase binding protein and ornithine decarboxylase in the vitamin E-deficient distal colon samples used for macroarray (normalized against glyceraldehyde-3-phosphate dehydrogenase). Northern blots did not verify variation in glutathione S-transferase π1 and inhibitor of DNA binding 3 transcripts. A further set of three independent replicates (not subjected to array analysis) failed to corroborate the array analysis for expression of telomerase binding protein or ornithine decarboxylase in vitamin E-deficient distal colon, perhaps reflecting inter-individual variation and the heterogeneous nature of the tissue samples. Considering the lack of robust changes in the Atlas rat stress array gene set, focus on the biochemical and microanatomical changes recorded prompted investigation of target genes influenced by lipid peroxidation. Consequently, collagen α1(I), lysyl oxidase and telopeptide lysyl hydroxylase gene expression was assessed in rat colon tissue. Deregulation of these transcripts was not significant when normalized against glyceraldehyde-3-phosphate dehydrogenase. Collagen α1(I) and lysyl oxidase transcripts were predominantly localized in the lamina propria underlying the epithelial cells at the luminal surface at the crypt apex in all three regions of the colon that were examined. Collagen α1(I) transcripts were also prominent over the serosa and in discrete areas interspersed throughout the submucosa and muscle layers. Additional sites of lysyl oxidase transcripts were observed in discrete regions, possibly blood vessels, in the submucosa. The variable location in expression observed confounds further attempts to establish differential expression of these transcripts in colon tissue.

Conclusions

Perturbations in antioxidant status, oxidative stress and aberrant collagen production are associated with colon pathologies [1–3,14,15]. Oxidative stress induced by dietary vitamin E deficiency in this study was not reflected by major changes in the expression of the 207 genes screened using the stress gene array. The array incorporated transcripts associated with the cell cycle, nucleotide metabolism, basic transcription factors, intracellular kinase networks, membrane channels and transporters, heat-shock transcripts and chaperones and transcripts related to DNA damage, repair and recombination, oncogenes and tumour suppressors. Fischer et al. [13] also observed a lack of significant changes in array analysis of vitamin E-depleted rat liver. Low-level responses to a major nutritional insult highlight the difficulty in detecting gene expression changes in heterogeneous tissue. Evidence presented here of oxidative stress induced by dietary vitamin E depletion, coupled with observation of colon remodelling, suggests that lack of vitamin E may contribute to the development of clinical pathologies of the colon.

References


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Received 10 June 2004