Selenium and inflammatory bowel disease

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Kudva AK, Shay AE, Prabhu KS. Selenium and inflammatory bowel disease. Am J Physiol Gastrointest Liver Physiol 309: G71–G77, 2015.—Dietary intake of the micronutrient selenium is essential for normal immune functions. Selenium is cotranslationally incorporated as the 21st amino acid, selenocysteine, into selenoproteins that function to modulate pathways involved in inflammation. Epidemiological studies have suggested an inverse association between selenium levels and inflammatory bowel disease (IBD), which includes Crohn’s disease and ulcerative colitis that can potentially progress to colon cancer. However, the underlying mechanisms are not well understood. Here we summarize the current literature on the pathophysiology of IBD, which is multifactorial in origin with unknown etiology. We have focused on a few selenoproteins that mediate gastrointestinal inflammation and activate the host immune response, wherein macrophages play a pivotal role. Changes in cellular oxidative state coupled with altered expression of selenoproteins in macrophages drive the switch from a proinflammatory phenotype to an anti-inflammatory phenotype to efficiently resolve inflammation in the gut and restore epithelial barrier integrity. Such a phenotypic plasticity is accompanied by changes in cytokines, chemokines, and bioactive metabolites, including eicosanoids that not only mitigate inflammation but also partake in restoring gut homeostasis through diverse pathways involving differential regulation of transcription factors such as nuclear factor-κB and peroxisome proliferator-activated receptor-γ. The role of the intestinal microbiome in modulating inflammation and aiding in selenium-dependent resolution of gut injury is highlighted to provide novel insights into the beneficial effects of selenium in IBD.

Glutathione peroxidase; prostaglandin; selenoproteins; cyclooxygenase

Selenium (Se) is an essential trace element that exists in both inorganic and organic forms. Selenium is incorporated into proteins (25 in human and 24 in mouse) as selenocysteine (Sec), recognized as the 21st amino acid, and encoded by the UGA codon (20). Selenium, in the form of selenite and selenate, is reduced to hydrogen selenide (H₂Se) by glutathione as well as thioredoxin and glutaredoxin (8). H₂Se can also be derived from Sec by the action of β-lyase while selenomethionine is metabolized to methylselenol (CH₃SeH) by γ-lyase and subsequently converted to H₂Se. H₂Se is then converted to selenophosphate by the selenoprotein selenophosphate synthetase (SPS)-2 and is further charged to seryl-tRNA²Sec to form Sec-tRNA²Sec (20). The selenoprotein synthesis machinery is comprised of Sec-tRNA²Sec, a specialized elongation factor (EF²sec), Sec insertion sequence-binding protein-2 (SBP2), SECp43, ribosomal protein L30, SP51, and soluble liver antigen protein that recognize a unique secondary structure in the 3’-untranslated region of the mRNA to cotranslationally incorporate Sec in the growing polypeptide chain (7, 30). Schoenmakers et al. have reported a rare heterozygous mutation within SBP2, suggesting a plausible link for selenoprotein deficiency and multisystem dysfunction, including ileocolonic inflammation in one of the subjects (designated as Proband 2) (50). Likewise, studies in mouse models of azoxymethane-induced colon cancer and selenoprotein gene deletion studies further suggest that selenoproteins play a pivotal role in the maintenance of gut homeostasis (23, 45). Among selenoproteins, glutathione peroxidases (GPx), selenoprotein S (SelS), and selenoprotein P (SePP1) have been extensively studied for their redox regulation, antioxidative, or anti-inflammatory roles in preventing chronic intestinal inflammation (45, 56). In this review the importance of selenoproteins in modulating gastrointestinal inflammation will be discussed with a specific focus on selenium-dependent regulation of arachidonic acid metabolism and the role of macrophages in supporting resolution.

Glutathione peroxidases. GPx belong to the antioxidant family of enzymes that use glutathione to efficiently reduce peroxides. There are four isoforms of GPx that are expressed in the gut (11, 39). In general, GPx plays a crucial role in the reduction of reactive oxygen species (ROS), in the form of hydroperoxides that include hydrogen peroxide and lipid hydroperoxides, to mitigate inflammatory pathways that set an antioxidant tone in the gastrointestinal mucosa. This may, in part, suppress chronic inflammation in the colon that is known

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to initiate tumorigenesis. GPx1 is expressed in all cell types of the gut, whereas GPx2 is predominantly expressed in the epithelial cells, including the paneth cells, of the gastrointestinal mucosa (10, 16, 18). GPx3 is secreted and found in plasma, and GPx4 is expressed in epithelial cells and the lamina propria of the intestine (53, 59).

Both GPx1 and GPx2 are upregulated by cellular stress pathways mediated by transcription factors such as β3, nuclear factor (erythroid-derived) 2-like 2 (Nrf-2), and p63, respectively (4, 60, 66). In addition, GPx2 has been identified as a target of the Wnt pathway that controls the expression of genes required for proliferation of stem cells at crypt bases that maintain continuous self-renewal of intestinal epithelium and mucosal homeostasis (28, 63). Previous reports suggest that prostaglandin E2 (PGE2), a downstream bioactive product of cyclooxygenase-2 (COX2), can activate the canonical Wnt signaling pathway, contributing to tumorigenesis (49). However, GPx2 reportedly dampened the COX2-dependent PGE2 production, signifying its potential anti-inflammatory role within the gastrointestinal tract (5).

A sequence comparison study conducted by Esworthy et al. using mouse GPx1 and GPx2 genes revealed that the coding region and 3'-untranslated regions corresponding to the SECIS element were conserved (16). Such dissimilarity in GPx1 and GPx2 sequences may partly explain their differential regulation. For example, GPx2 mRNA is consistently upregulated in chemically induced experimental colitis [dextran sodium sulfate (DSS)] in mice and in human inflammatory bowel disease (IBD) colonic biopsies (61). Upregulation of GPx2 could be part of a compensatory response for protection against oxidative damage during inflammation (61). Conversely, Florian et al. reported an increase in GPx1 activity within the colon and ileum crypts in selenium-fed GPx2 knockout mice, which further supports the notion that these proteins may have a partial compensatory role (17). However, lack of both GPx1 and GPx2 led to a severe inflammatory phenotype in the form of spontaneous ileocolitis (14, 15).

The other two isoforms, GPx3 and GPx4, have also been implicated for their role in modulating oxidative stress and inflammation within the gut. Barrett et al., using an azoxymethane/DSS-induced experimental colitis model, reported that loss of GPx3 induced severe colitis and enhanced tumorigenesis (6). This study suggests that plasma GPx3 has a potent tumor suppressor role in colitis-associated carcinoma through abrogation of ROS and changes in tumor microenvironment (6). However, as in other tumor suppressor genes, hypermethylation of the human GPx3 promoter in primary colorectal and pancreatic tumor xenografts and cell lines was associated with decreased expression of GPx3 and increased sensitivity to the chemotherapeutic agent cisplatin (40). Thus, epigenetically silenced GPx3 may serve as a predictive biomarker for platinum sensitivity in gastrointestinal malignancies.

GPx4 has been shown to play a pivotal role in detoxification of lipid hydroperoxides thereby preventing oxidative membrane damage and maintaining cellular integrity (13). GPx4 is shown to uniformly express in colonic crypts, but differentially expressed along the crypt-villus axis, where it prevents oxidative damage within the gastrointestinal tract (53). Meta-analysis of functional single nucleotide polymorphisms in selenoprotein genes revealed that genetic variants of GPx4, namely rs713041 and rs8178974, were correlated with increased colorectal cancer risk in Czech Republic and United States populations, respectively (34, 35). However, the direct link between the two remains elusive.

Selenoprotein S. SeLS is predominantly known to localize to the plasma membrane and endoplasmic reticulum (ER). High SeLS expression has been found in paneth cells and macrophages in the gut. Speckmann et al. have recently shown that ER stress induces the expression of SeLS in vivo and in vitro (54). Furthermore, SeLS and GRP78, an ER stress marker protein, were elevated in inflamed ileal tissues of patients with Crohn’s disease (54). Interestingly, genetic knockdown of SeLS failed to cause an ER stress response in colon-derived enterocytes and goblet-like cell lines.

Selenoprotein P. SePP1 is one of the major selenoproteins in plasma and consists of up to ten Sec residues, hence making it sensitive to changes in selenium levels (1). The main function of SePP1 is to deliver selenium to various tissues (1, 51). SePP1 has been reported to have two additional activities in the form of a phospholipid hydroperoxide glutathione peroxidase and a peroxynitrite reductase (2, 47). However, its phospholipid hydroperoxide glutathione peroxidase activity is ~100-fold lower than that of GPx4 (47).

SePP1 levels are inversely associated with the development of IBD and colorectal cancer (1, 55, 57). Although the reason for the decrease in SePP1 is not clear, it is thought that decreased absorption of selenium may be a contributing factor. Due to its peroxidase and reductase functions, SePP1 might protect the intestinal epithelium from oxidative damage and therefore reduce the risk of IBD or colorectal cancer. However, little is currently known about the exact function of SePP1 in the gut. Nevertheless, it is likely that a decrease in plasma SePP1 impacts selenoprotein expression in target cells such as macrophages, which are of great interest to our laboratory.

Proresolving functions of macrophages and arachidonic acid metabolites. Macrophages are versatile innate immune cells endowed with the ability to enhance either inflammation or resolution based on various stimuli. The most popular classification divides macrophages into two groups: M1, classically activated macrophages, and M2, alternatively activated macrophages, which represents two ends of a spectrum comprising intermediary phenotypes that are poorly described (33). M1 macrophages are considered proinflammatory due to their production of ROS and are activated by interferon (IFN)−γ, tumor necrosis factor (TNF)−α, granulocyte macrophage colony-stimulating factor, lipopolysaccharide, and other Toll-like receptor (TLR) ligands (52). M2 macrophages are considered anti-inflammatory due to their increased expression of arginase-1, among other factors, that competes for L-arginine, a substrate for inducible nitric oxide synthase, to produce L-ornithine and urea instead of nitric oxide (38). M2 macrophages are activated by IL-4, IL-13, and IL-10 (38, 52). Studies from our laboratory have shown that selenium supplementation increases the polarization of macrophages from a M1- to M2-like phenotype, thereby decreasing inflammation and increasing resolution (27, 38).

Macrophages express various receptors, not necessarily exclusive to this cell type, including Fc, mannose, and TLRs, for the detection of pathogens in the environment. Activation of these receptors leads to the production of lipid mediators, specifically eicosanoids, through the mobilization of arachidonic acid from the phospholipid bilayer (3). In a resting cell,
arachidonic acid is rarely found as a free fatty acid. During an immune response, arachidonic acid is first mobilized by phospholipase A2 and then acted upon by cyclooxygenase (COX) enzymes in addition to cytochrome P-450, and lipoxygenases. There are two isozymes of cyclooxygenase: COX1, which is constitutively expressed, and COX2, which is inducible by diverse stimuli. In this review, we will focus mainly on the selenium-dependent metabolism of arachidonic acid via the COX pathway to form PGH2 and its downstream metabolism by specific synthase enzymes.

PGH2 is acted upon by a variety of synthases to form specific prostanoids that impart various biological activities. For instance, selenium, in the form of selenoproteins, has been shown to upregulate the expression of hematopoietic PGD2 synthase (HPGDS), a sigma-class glutathione S-transferase (19). The increased expression of HPGDS effects the production of PGD2 in immune cells, such as macrophages and T cells. PGD2 undergoes spontaneous dehydration followed by an isomerization to form prostaglandin J2 (Δ13-PGJ2) and Δ12-PGJ2, respectively. A second thermodynamically less favored dehydration converts Δ12-PGJ2 to 15-deoxy-Δ12,14-prostaglandin J2 (15d-PGJ2). These metabolites of PGD2 belong to the cyclopentenone prostaglandin family (CyPGs) and serve as ligands for the transcription factor peroxisome proliferator-activated receptor-γ (PPARγ), which in turn binds to the PPAR response element present in the Hpgds promoter and upregulates its expression (19). Our long-standing hypothesis is that there exists a selenoprotein or a group of selenoproteins that can metabolize PGH2 to PGD2 or CyPGs to activate PPARγ-dependent expression of Hpgds to create a feedforward loop. Studies are currently underway to identify these selenoproteins.

CyPGs have been shown to have multiple anti-inflammatory effects in vitro and in vivo. First, the utilization of PGH2 for the production of PGD2 leaves little to no substrate for the production of PGE2, which is essential for initiation of inflammation (41, 46). Second, PGD2 and its cyclopentenone derivatives, Δ12-PGJ2 and 15d-PGJ2, resolve inflammation by controlling leukocyte trafficking and macrophage efflux to draining lymphatics (41). Third, recent studies from our laboratory have shown that selenoprotein expression is required to upregulate the expression of 15-prostaglandin dehydrogenase (15-PGDH), an enzyme that oxidizes lipid mediators with an enhanced preference toward PGE2, specifically in the gut (27, 46, 58). Preliminary studies suggest that PPARγ mediates this process. Furthermore, our studies also suggest a positive correlation between selenoprotein status and increased oxidation of PGE2 to 15-keto-PGE2, which has been reported to lack proinflammatory activity (27). 15-keto-PGE2 can be further converted to 13,14-dihydro-15-keto-PGE2 and subsequently to 13,14-dihydro-15-keto-PGAs that could potentially serve as a ligand for PPARγ activation (27). Such a metabolic inactivation of PGE2 may, in part, be mediated by PPARγ to facilitate an anti-inflammatory response and promote resolution in a murine model of ulcerative colitis (27, 32). Finally, recent studies in our laboratory have shown that pharmacological inhibition of 15-PGDH blocked the protective effect of selenium supplementation in a DSS model of experimental colitis (27).

The ability of selenium to downregulate nuclear factor-κB (NF-κB)-dependent pathways, including microsomal PGE2 synthase (mPGES-1), which catalyzes the conversion of PGH2 to PGE2, also aids in the eicosanoid class-switching phenomenon to differentially regulate pathways of inflammation and resolution (19). The chemically reactive electrophilic nature of CyPGs enables these bioactive molecules to form Michael adducts with specific protein thiols to impact various pathways of inflammation, including the NF-κB pathway. Previous studies in our laboratory have demonstrated such a covalent adduct formation of IkB kinase-2 with 15d-PGJ2 to inhibit the NF-κB pathway was dependent on selenium and selenoprotein expression in macrophages (65). A recent report from our laboratory demonstrated the ability of selenium to inhibit the acetylation of nonhistone and histone proteins by histone acetyltransferase p300 and therefore affect the expression of proinflammatory genes, including NF-κB member p65, in macrophages (37, 44). Such an epigenetic modulation of inflammatory gene expression was, in part, dependent on the selenoprotein-mediated shunting of arachidonic acid (37, 44). Taken together, the ability of selenoproteins to effectively shunt the eicosanoid pathway may represent one of the many anti-inflammatory and proresolving functions of selenium (Fig. 1).

A study conducted by Vong et al. found that patients with active colitis had increased levels of proinflammatory cytokines, such as TNF-α and IFN-γ, as well as PGE2. In contrast, patients in remission had higher levels of PGD2 (64). Even though the prolonged elevation of PGD2 has beneficial effects, as in the promotion of resolution, studies from rodent models suggest that PGD2 might potentiate elevated epithelial proliferation and susceptibility to colon cancer (41, 67, 68). Further experiments are needed to determine the exact role of PGD2 in the remission of colitis and colon cancer. Based on the above studies, it appears that selenium, through various selenoproteins, effectively resolves inflammation by driving the production of PGD2 and its CyPG metabolites that potentially modulate NF-κB- and PPARγ-dependent pathways, in addition to influencing other pathways that are currently unknown. It is important to determine which of the selenoproteins are specifically involved in these pathways.

Gastrointestinal inflammation. In clinical studies, patients with Crohn’s disease have been reported to be selenium deficient compared with controls (1, 43). In particular, patients who have undergone bowel resection surgery have a decreased ability to absorb nutrients, including selenium (43). Many patients with Crohn’s disease receive enteral nutrition, not all formulations of which contain selenium, therefore potentially contributing to selenium deficiency (24, 31). Further studies are required to examine the association between Crohn’s disease and selenium deficiency.

A recent study from our laboratory showed that selenium supplementation increased body weight, colon length, and survival of mice treated with DSS compared with selenium-deficient mice (27). In addition, selenium supplementation of mice [0.4 parts/million (ppm) sodium selenite] treated with DSS led to suppression of M1 markers and an upregulation of M2 markers such as IL-10, Fizz1, and Arg-1 in colonic tissue. These studies clearly indicated that supplementation of mice with selenium at supraphysiological levels (0.4 ppm) alleviated inflammation while promoting resolution of gut epithelial damage. Interestingly, the use of macrophage-specific Sec-tRNA<sup>Sec</sup> (Trsp) conditional knockout mice indicated that selenoproteins in macrophages were key in protection from severe gastroin-
testinal injury and efficient resolution. Mice lacking macrophage-specific Trnp, despite being on high selenium diets, demonstrated increased PGE\(_2\) and decreased 15-keto-PGE\(_2\), which corroborated with increased mPGES-1 expression and decreased 15-PGDH expression in the colonic extracts of DSS-treated selenium-deficient mice (27). In a related study, high selenium (2 \(\mu\)g sodium selenite/g body wt) effectively protected rats from 2,4,6-trinitrobenzenesulfonic acid-induced experimental colitis (62). However, a recent report by Hiller et al. suggested that short-term (1 wk) supplementation with sodium selenite (at 0.6 ppm) had little or no effect on DSS colitis while supplementation with selenomethionine (at 0.6 ppm) was relatively more effective in suppressing inflammation (21). Taken together these studies suggest a crucial role for long-term (~8 wk or more) selenium supplementation in suppressing gastrointestinal inflammation-based tissue damage, particularly through mechanisms where host immune cells play a crucial role. In addition to examining host responses, there is an increased interest in understanding the cross talk between the host immune system and the gut microbiota, which also has the ability to incorporate selenium into selenoproteins. Due to the complexity of these interactions, it is likely that multiple factors affect the development of disease in the gastrointestinal tract.

Gut microbiota. The intestinal tract is colonized by a broad range of diverse microorganisms (collectively referred to as the gut microbiota) that are important for defense against invasive microbes and nutrient absorption. The composition of the gut microbiota can be altered by factors such as infections, antibiotics, and diet that affect the gut mucosal immune response. A previous report demonstrated that the composition of the gut microbiota, in mice, affected host selenium levels, and therefore selenoprotein expression in the host (22). It is likely that the intestinal microbes compete with the host for available selenium, exacerbating host selenium deficiency therefore making the gut more vulnerable to disease pathologies. On the other hand, it has been shown that selenium levels also altered the composition of the gut microbiota in mice (26). In humans, it has been shown that patients with IBD have an altered microbiota, compared with controls (48). Studies suggest that the microbial community present in the intestine of an IBD patient may play a role in the initiation or maintenance of the disease, which determines the severity of the disease (48). Therefore, it would be of interest to study the underlying mechanisms of selenium on the gut microbiota and how those correlate with incidences of IBD and ultimately reduce severity of the disease. Specifically, it would be interesting to examine if the protective effect of selenium supplementation is mediated through microbial metabolite(s) that may not only impact the species selection but also assist in mitigating inflammation or enhancing resolution through the modulation of the host immune response.

Colon cancer. Inflammation is a key underlying event in the development of all stages of cancer. Epidemiological, histopathological, and pharmacological studies suggest a correlation between inflammation and the development of cancer, with chronic inflammation leading to dysplasia (42). The risk of cancer is sixfold greater in IBD patients than non-IBD subjects (6). Clinical studies have also indicated an inverse relationship between selenium levels and severity and risk of colon cancer (12). As an additional indicator of the relationship between selenium and colon cancer, aberrant expression of GPx1, GPx3, and GPx4 in colorectal cancers has been reported. For instance, GPx2 expression is increased in patients with colorectal cancer, potentially to compensate for the loss of other GPx isoforms (9, 36).

In addition to the direct functions of selenoproteins, low-molecular-weight seleno-compounds have the ability to reduce preneoplastic lesions independent of the selenoprotein genotype (23). Kipp et al. demonstrated that selenium deficiency downregulated Wnt-inhibitory factors and upregulated Wnt-signaling factors, suggesting that selenium deficiency in sen-

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**Fig. 1.** Summary of the effects of selenium on gut inflammation. Left: dietary selenium affects the composition of the gut microbiota (dots) and regulates various prostaglandins that promote resolution and reduce reactive oxygen species. Right: in the absence of selenium, nuclear factor-κB signaling increases inflammation that is perpetuated by reactive oxygen species, which inflicts tissue damage affecting the integrity of the epithelial barrier. The composition of the gut microbiota is also altered by selenium deficiency.
sitized tissues could lead to carcinogenesis (29). Selenium deficiency also decreased the expression of Smad4, which is involved in the transduction of extracellular signals from the transforming growth factor (TGF)-β receptor upon ligation to TGF-β. Because TGF-β acts as a tumor suppressor in the normal intestinal epithelium, the downregulation of Smad4 might contribute to higher cancer risk in selenium-deficient patients (29). Therefore, selenium may mediate its beneficial effects directly through low-molecular-weight seleno-compounds as well as selenoproteins by regulating diverse pathways involved in tumorigenesis.

Conclusions and future directions. Selenium is an essential micronutrient that is uniquely incorporated into a variety of selenoproteins to impart its beneficial functions. Even though the pathophysiology of IBD is multifactorial in origin, dietary selenium (and selenoprotein) deficiency exacerbates experimental colitis by affecting various signaling pathways involved in inflammation and oxidative stress as well as by altering the gut microbiota. This supports the outcome of several epidemiological studies that suggest selenium status to be inversely associated with IBD severity and colon cancer risk. Such an effect may be partially due to selenium’s ability to polarize macrophages from a M1- to M2-like phenotype, therefore alleviating inflammation and enhancing resolution of epithelial damage in the gut. Among the various cellular pathways, the ability of selenoproteins to effectively shunt the eicosanoid damage in the gut. However, a recent study by Hiller et al. suggested that the form of selenium and the duration of supplemental therapy may be important for imparting the beneficial effects of selenium (21). Regardless of the form of selenium used, it is evident that inflammation is a key factor during selenium deficiency that leads to pathophysiology in the gut, which can be alleviated by increased expression of selenoproteins.

A key lingering question of much debate is whether selenium deficiency is the cause or effect of IBD. Although an array of data support the notion that selenium deficiency is the primary cause for the disease, it is necessary to exercise some caution, particularly when data from genetic ablation models are used, since they seldom represent selenoprotein status during deficiency (25). Therefore, the cause and effect relationship between selenium deficiency and IBD needs to be examined further. From this review it is clear that the development of appropriate animal models is critical for characterization of selenoprotein functions in gut homeostasis that take into account many factors, including changes in microbiota and environmental cues in addition to understanding the molecular basis of initiation and progression of inflammation that are suppressed by selenium. Such studies may ultimately provide a strong mechanistic basis and better biomarkers to identify patient populations that could benefit from selenium supplementation therapy.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS


REFERENCES


