Human colonocyte detoxification

Detoxification or biotransformation of drugs and xenobiotics are usually linked with liver metabolism, yet colonocytes of the gastrointestinal tract have an equal capacity to mediate these processes. This brief overview specifically discusses the ability of human colonocytes, but not other tissues, to detoxify chemical agents and relates pertinent findings to ulcerative colitis and some aspects of colon cancer. Failure to detoxify, leading to epithelial cell damage, or an exaggerated capacity to biotransform, leading to carcinogen formation in colonocytes, have been the main implications in disease processes.

In general, two categories of detoxification processes are recognised (table 1): phase I reactions concern oxidation, reduction and hydrolysis within the cytosol, and phase 2 reactions require ATP and concern conjugation with a donor substrate synthesised in the cell. Both reactions need enzymes such as oxidoreductases, hydrolases, transferases, and lyases. Amongst these may be subclasses, genetic polymorphism and variability of enzyme activity in organs and along the gastrointestinal tract. Particularly, differences in enzyme activity in the proximal and distal colon may occur.

Biochemists, pharmacologists, toxicologists, molecular biologists, geneticists, oncologists, and gastroenterologists are involved in this field of study, from each of which information is now drawn together. Many new toxicological advances made with liver and lung tissues still have to be applied to colonocytes and would be a fruitful area of future research. The subject of clinical gastrointestinal toxicology7 makes it possible to bridge a gap between these processes. The P-450 superfamily of enzymes is composed of families and subfamilies of enzymes based on amino acid differences. P-450 are designated by CYP followed by a number designating the family (1–27) and a letter for the subfamily (A–C). Subfamilies may occur in one or two forms.

The activity and expression of cytochrome P-450 in human colonocytes is generally low. The low levels of cytochrome P-450 found in human colonocytes have been attributed to methodological difficulties, but observations in the rat suggest that colonic P-450 activity is equivalent to that in the liver.

The activity of CYP1A2 is directed by substrates such as caffeine and ethanol, but certain cytochrome P-450 concentrations are greater in human adenocarcinomas, leading to the proposal that “activated” mono-oxygenases can convert pro-carcinogens to carcinogens; however, other studies found low levels of CYP1A1, CYP2B1 in colon cancer. Correlation of mutations of CYP1A1, normally active against polycyclic aromatic hydrocarbons acting on colonocytes, have been found in colorectal cancers of Japanese and Hawaiians but not Caucasians. These mutations make colonocytes vulnerable to the carcinogenic activity of polycyclic aromatic hydrocarbons. In general, polymorphic phenotypes of the oxidation of various drugs by cytochrome P-450 have been established, but no relation was found in patients with colon cancer.

Oxidant damage control

Reactive oxygen metabolites have been implicated in the development of radiation colitis and ulcerative colitis, yet defences against such radicals are strongly present in colonocytes. The oxidant defence enzymes superoxide dismutase, catalase and glutathione peroxidase, are mainly present in colonocytes rather than in submucosal structures. Distribution along the length of the gastrointestinal tract is variable, but colonic levels are lower than those found in the stomach. Colonic cells seem to have adequate control against oxidants such as tertiary butyl hydroperoxide with lesser effect against oxidants such as menadione. In ulcerative colitis antioxidant enzymes such as catalase and glutathione peroxidase are not significantly impaired despite the imputation of oxygen free radicals as damaging agents.

Glutathione: redox control and transfer reactions

The effect of cellular and circulating glutathione against oxidants is considerable and plays an important role in redox control. The concentrations of glutathione in human colonocytes are roughly half those found in the liver. Both transport into colonocytes and synthesis of glutathione occurs in colonocytes where levels can be diminished by paracetamol or specific inhibitors such as a buthionine sulfoximine. Inter-individual variation in glutathione concentrations in the colonic mucosa can be 16-fold, with similar variability found for glutathione peroxidase, which is involved in redox control. Genetic factors probably account for such variability.

In animals, bodily depletion of glutathione leads to colitis and low mucosal concentrations of glutathione have been found in quiescent and active ulcerative colitis, suggesting that redox control by glutathione is impaired in colonocytes in this disease.

Through the action of glutathione transferases (γ, µ and π), glutathione undergoes transfer to electrophilic substrates.
such as benzyl chloride or diethyl maleate, resulting in soluble complexes that are more hydrophilic and less cytotoxic. As a result of the hypothesis that chemical selection processes can cause overexpression of detoxification enzymes, glutathione transferase activity has been measured in the human colon, 22 30 in both health and disease. High activities of glutathione transferases (GST π) have been found in colon cancer, 31 32 which is in support of the hypothesis. A correlation in levels of GST (α, μ, and π) between colonic mucosa and circulating lymphocytes has been found, 33 enabling the detection of cancer prone subjects. Lowered glutathione transferase activity has been observed in ulcerative colitis, 22 34 particularly in early onset disease and more severe forms of colitis. The importance of these observations has yet to be evaluated, but proposals are that failure of detoxification may be seen as a potential factor in the development of colitis.

Acetylation
N-acetylation in colonocytes is implicated in the biotransformation of chemical agents such as arylamine to carcinogens 35 36 or inactivation (detoxification) of therapeutic agents, such as isoniazid, hydralazine, 4-aminosalicylic acid (ASA) 37 and 5-aminosalicylic acid. 38 Acetylation of drugs masks functional chemical groups and renders the drugs less water soluble. Acetylation is recognised in two genetic phenotypes in terms of slow and fast acetylators. 39

In humans there are two N-acetyl transferase genes (NAT1, NAT2) located on chromosome 8. NAT1 has a monomorphic pattern and NAT2 polymorphic activity, which is mainly found in liver. 40

The N-acetyl transferase activity in human colonocytes 41 is as high as in the liver. Previous studies associating NAT genes in colonocytes with colorectal cancer had only shown a slightly increased odds ratio of 1.29 in association with colonic adenomas 42 43 though a combination of CYP1A2 and NAT2 have a higher odds ratio related to potential mutagen transformation in colonic epithelial cells. 44 The presence of a fast acetylator phenotype in colonocytes in conjunction with high meat intake seems to predispose to colonic carcinogenesis. 45 46

In ulcerative colitis acetylation of 5-ASA is prominent, 47 yet acetylation of 5-ASA does not produce a therapeutic gain. 48 Acetylation renders 5-ASA less water soluble and diminishes uptake by colonocytes. 49 Acetylation of 5-ASA by colonocytes is biochemically preserved as the reaction proceeds even when mitochondrial oxidation has been reduced by more than 75%. 50 The bacterial amine content of the colonic lumen is high and presumably acetylation of amines protects against their entry into the circulation and thereby potential adverse reactions on organ metabolism.

Sulphation and sulphotransferases
Sulphotransferases in epithelial cells require ATP and “monocarboxylate” for sulphation of bile salts, mucopolysaccharides, catecholamines, phenols, steroids, and xenobiotics, in the process of which they alter the activity or function of each agent. Sulphotransferases for steroids 50 and bile salts 51 52 are not found in mammalian colonocytes; however, phenols, such as napthol or paracetamol, 52 53 catecholamines, 54 and mucin 55 are extensively sulphated in human colonocytes. Sulphation in colonocytes is six to eight times greater than glucuronidation of phenols in human colonocytes 55 56 and there is “compartmentalisation” of sulphation depending upon luminal or contraluminal application of xenobiotics. 56

In ulcerative colitis sulphation of phenols, whether measured by dialysis in vivo 57 or in vitro with isolated cells, 58 is significantly diminished. Such impairment could result from diminished formation of activated sulphate, diminished sulphotransferase activity or diminished supply of ATP. The latter seems to be the most likely explanation that would lead to diminished formation of activated sulphate, which also depends upon the availability of ATP. Diminished phenol detoxification may lead to continuing damage to colonocytes and perpetuation of the disease process.

Sulphation of mucin is diminished in ulcerative colitis 59 60 and colon cancer. 61 62 Both biochemical and cytochemical evidence 63 reveal significantly reduced ability of colonocytes to sulphate mucin in colitis. The activity of sulphotransferases has been measured in the human colon 64 65 in the cancerous state, but not in ulcerative colitis. Sulphation of mucin 66 and phenols 67 are also diminished in cancer tissue. The mechanisms of diminished sulphation, respectively, in ulcerative colitis and colon cancer have not been compared further. Colonic sulphotransferases have the ability to bioactivate potential harmful agents leading to carcinogen formation. 68 Both genetic and biochemical factors of either over-regulation or under-regulation of enzymes in colonocytes seem to be part of the disease process in colon cancer and ulcerative colitis, but biochemical details are lacking.

Methylation and methyltransferases
Methyltransferases subserve detoxification of xenobiotics and a number of cellular synthetic functions. Methylation depends on S-adenosylmethionine (SAM), 69 all of which require the high energy cofactor S-adenosylmethionine. The function of DNA methylation 69 and methylation of phospholipids in membranes 70 play a part in tumorigenesis and colonic absorption 71 in roles other than detoxification: these physiological functions are not discussed further.

The S-methyltransferases in colonocytes, which subserve detoxification processes, are of two functional types: those that methylate aromatic or heterocyclic sulphhydrils, such as mercaptopurine (tiotopurine methyltransferase, TPMT), 72 and those that methylate aliphatic sulphhydrils, such as mercaptopropionic acid or potassium sulphide (methylmercaptans). 73 All of these catalyse one or more functional reactions. TPMT activity is highest in the colon, exceeding values in the liver with an aboral change between stomach and colon. 74 75 In humans, however, TPMT activity, although high in the large intestine, does not exceed values in the liver. 76 A large number of sulphur containing xenobiotics are acted upon by TPMT’s. 77

The activity of TPMT in red blood cells is diminished in Parkinson’s disease 78 and rheumatoid arthritis, 79 in contrast with erythrocyte values of TPMT activity in ulcerative colitis which are very high compared with control cases. 80 Colonocyte values of TPMT activity in ulcerative colitis are unknown, but healthy colonocytes show a 10-fold variation in activity. 81 As sulphides are toxic to colonocytes, 82 the activity of TPMT in ulcerative colitis is an important factor in maintaining epithelial integrity. Distinction between erythrocyte, colonocyte and inflammatory cell activity of TPMT in the colon is important and needs to be considered in the disease process of ulcerative colitis. The concentration of the high energy cofactor S-adenosyl methionine, needed for TPMT activity in colonocytes, is reduced in ulcerative colitis, 83 suggesting a failure of capacity to detoxify sulphide by methylation. DNA hypomethylation has also been observed in ulcerative colitis. 84

Variability of colonic TPMT activity suggests a strong genetic control mechanism for this enzyme—genetic polymorphism for TPMT activity in red blood cells has already been established. 85 86 Apart from genetic factors, competition...
between N-methylation and S-methylation in colonocytes needs to be evaluated, particularly in colon cancer and ulcerative colitis.

Hydrogen ion and pH control

Partially to protect against organic acid production by bacteria, bicarbonate secretion by colonocytes is high, and regulated by mucosal CO₂ concentrations that depend on cell metabolism or general respiratory control. Hydration of CO₂ is determined by carbonic anhydrase in apical epithelial cells of the entire colon, but with greater activity in the proximal colon. Bicarbonate secretion is closely linked with absorption of sodium and chloride.  

In ulcerative colitis luminal acidification is associated with diminished bicarbonate secretion, either due to diminished metabolic supply of CO₂ or diminished carbonic anhydrase activity. None of the established metabolic inhibitors of carbonic anhydrase are known to induce colitis, but most animal models of experimental colitis produced by acetic acid, propionic acid, TNBS, or hydrochloric acid require extreme acidic conditions to damage colonocytes. Certain volatile sulphur compounds which may occur in the colon, may act on carbonic anhydrase to raise the intracellular concentration of hydrogen sulphide. The role of pH control and the place of carbonic anhydrase for detoxification in the colon deserves further investigation, particularly with regard to ulcerative colitis.

Conclusions

Many avenues of investigation indicate that colonic epithelial cells have diverse mechanisms to detoxify luminal agents of dietary, bacterial or fermentative origin. Colonocytes are equal to hepatocytes in their capacity to carry out detoxification processes or transformation of chemical agents. The failure of detoxification in ulcerative colitis and exaggerated biotransformation in colon cancer suggest disease mechanisms worthy of further exploration.

Even though the pharmacological substances mentioned earlier are foreign to the colon, experimentation has revealed a substantial genetic diversity in the capacity of colonocytes to detoxify these agents. Pharmacogenetic findings with regard to colonic mucosal metabolism may, in the future, unravel disease processes, particularly in colonic carcinogenesis and ulcerative colitis.

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