Review

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.[1 2] 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)[3 4]: 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.[5 6]

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 toxicology[7] makes it possible to bridge a gap between colonic disease, genetics and the ability to detect initiating or promoting factors in ulcerative colitis and colon cancer.

Cytoplasmic oxidases and reductases

Cytochrome P-450 are a superfamily of haem containing mono-oxygenases[8] acting in the metabolism of foreign compounds, as well as synthesis of steroids and bile components. 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.[9 10]

TABLE 1 *Detoxification and biotransformation reactions found in human colonocytes*

Action	*Enzyme*	*Substrate/cofactor*
Phase I		
Oxidation Hydroxylation Sulphoxidation Dealkylation	Cytochrome P-450	O_2
Azoreduction Nitroreduction	Cytochrome P-450 reductase	H^+
Co-oxidation	Peroxidases Catalases	H_2O_2
Hydrolysis	Esterases Epoxide hydrolases	H_2O
Hydration	Carbonic anhydrase	$CO_2 + H_2O$
Phase II		
Sulphation	Sulphotransferases	PAPS + ATP
Glucuronidation	Glucuronyl transferase	UDP-GA + ATP
Acetylation	Acetyl transferase	Acetyl-CoA + ATP
Methylation	Methyl transferase	SAM + ATP
Glutathione conjugation	Glutathione transferase	Glutathione + ATP

The activity and expression of cytochrome P-450 in human colonocytes is generally low.[10–12] 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.[13]

The activity of CYP1A2 is directed by substrates such as caffeine[14] and ethanol, but certain cytochrome P-450 concentrations are greater in human adenocarcinomas,[15] leading to the proposal that "activated" mono-oxygenases can convert pro-carcinogens to carcinogens[16]; however, other studies[17] 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.[18] 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,[8] 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.[19 20] The oxidant defence enzymes superoxide dismutase, catalase and glutathione peroxidase, are mainly present in colonocytes rather than in submucosal structures.[6 19] Distribution along the length of the gastrointestinal tract is variable, but colonic levels are lower than those found in the stomach.[20] Colonocytes seem to have adequate control against oxidants such as tertiary butyl hydroperoxide with lesser effect against oxidants such as menadione.[21] In ulcerative colitis antioxidant enzymes such as catalase and glutathione peroxidase are not significantly impaired[22] 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[23 24] are roughly half those found in the liver. Both transport into colonocytes and synthesis of glutathione[25 26] occurs in colonocytes where levels can be diminished by paracetamol[24] or specific inhibitors such as a buthionine sulphoxime.[25] Inter-individual variation in glutathione concentrations in the colonic mucosa can be 16-fold,[27] with similar variability found for glutathione peroxidase, which is involved in redox control.[28] Genetic factors probably account for such variability.

In animals, bodily depletion of glutathione leads to colitis[25] and low mucosal concentrations of glutathione have been found in quiescent and active ulcerative colitis,[29] suggesting that redox control by glutathione is impaired in colonocytes in this disease.

Through the action of glutathione transferases (α, 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 CYPIA2 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.[36 39]

In ulcerative colitis acetylation of 5-ASA is prominent,[45 46] yet acetylation of 5-ASA does not produce a therapeutic gain.[38] Acetylation renders 5-ASA less water soluble and diminishes uptake by colonocytes.[47] Acetylation of 5-ASA by colonocytes is biochemically preserved as the reaction proceeds even when mitochondrial oxidation has been reduced by more than 75%.[48] 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 "activated sulphate" 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[49] and bile salts[50 51] 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[52 53] 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[6 61] in the cancerous state, but not in ulcerative colitis. Sulphation of mucin[61] and phenols[52] 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.[64] 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 upon O-N- and S-methyltransferases,[65] all of which require the high energy cofactor S-adenosylmethionine. The function of DNA methylation[66] and methylation of phospholipids in membranes[67] play a part in tumorigenesis and colonic absorption[68] 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 sulphydryls, such as mercaptopurine (thiopurine methyltransferase, TPMT),[69] and those that methylate aliphatic sulphydryls, such as mercaptopropionic acid or potassium sulphide (thiol-methyltransferase, TMT).[70] Of all tissues in animals, TMT activity is highest in the colon, exceeding values in the liver with an aboral change between stomach and colon.[71 72] In humans, however, TMT activity, although high in the large intestine, does not exceed values in the liver.[73 74] A large number of sulphur containing xenobiotics are acted upon by TMTs.[75]

The activity of TMT in red blood cells is diminished in Parkinson's disease[76] and rheumatoid arthritis,[77] in contrast with erythrocyte values of TMT activity in ulcerative colitis which are very high compared with control cases.[78] Colonocyte values of TMT activity in ulcerative colitis are unknown, but healthy colonocytes show a 10-fold variation in activity.[79] As sulphides are toxic to colonocytes,[80] the activity of TMT in ulcerative colitis is an important factor in maintaining epithelial integrity. Distinction between erythrocyte, colonocyte and inflammatory cell activity of TMT in the colonic mucosa 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 TMT activity in colonocytes, is reduced in ulcerative colitis,[81] suggesting a failure of capacity to detoxify sulphide by methylation. DNA hypomethylation has also been observed in ulcerative colitis.[82]

Variability of colonic TMT activity suggests a strong genetic control mechanism for this enzyme—genetic polymorphism for TMT activity in red blood cells has already been established.[83–85] 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[86 87] and regulated by mucosal CO_2 concentrations that depend on cell metabolism or general respiratory control. Hydration of CO_2 is determined by carbonic anhydrase found in apical epithelial cells of the entire colon,[88 89] but with greater activity in the proximal colon.[90] Bicarbonate secretion is closely linked with absorption of sodium and chloride.[91 92]

In ulcerative colitis luminal acidification is associated with diminished bicarbonate secretion,[92 93] either due to diminished metabolic supply of CO_2[94] or diminished carbonic anhydrase activity.[95] None of the established metabolic inhibitors of carbonic anhydrase[96] are known to induce colitis, but most animal models of experimental colitis produced by acetic acid,[97] propionic acid,[98] TNBS,[99] or hydrochloric acid[100] require extreme acidic conditions to damage colonocytes. Certain volatile sulphur compounds which may occur in the colon, may act on carbonic anhydrase[101] 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 pathways, particularly in colonic carcinogenesis and ulcerative colitis.

WILLIAM E W ROEDIGER

WENDY BABIDGE

Colorectal Unit and Cell Physiology Laboratory,
Department of Surgery of the University of Adelaide at The Queen Elizabeth Hospital, Adelaide, SA 5011, Australia

Correspondence to: Associate Professor Roediger.

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