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Review

Anticarcinogenic effects of diet-related apoptosis in the colorectal mucosa

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Summary

The crypt is the fundamental unit of epithelial proliferation in the intestinal mucosa. The progeny of the pluripotent stem cells located near the base of the crypt migrate towards the crypt orifice, divide once or twice more, and then undergo differentiation, senescence and exfoliation. Programmed cell death (apoptosis) also occurs deep in the proliferative zone. Various lines of evidence suggest that apoptosis provides a protective mechanism against neoplasia by removing genetically damaged stem cells from the epithelium before they can undergo clonal expansion. Several different classes of food constituents, including certain polyunsaturated fatty acids, the short-chain fatty acid butyrate, and some phytochemicals including flavonoids and glucosinolates breakdown products, can modulate both cellular proliferation and programmed death. Each of these food components has also been shown to suppress the emergence of aberrant crypt foci in animal models of carcinogenesis. Further mechanistic and clinical studies are required to establish whether such dietary effects can be exploited to achieve preventive or therapeutic effects in humans.

Keywords: Gastrointestinal tract; Cancer; Apoptosis; Diet; PUFA; Phytochemicals

1. Introduction

Although the cellular events corresponding to what we now describe as apoptosis were first described in the 19th century (Virchow and Chandler, 1859), the existence of an orderly, energy-dependent programme for the selective destruction of individual cells has only been widely recognized for only a decade or two. The modern concept of apoptosis, again based largely on histological data, was introduced in the 1970s, by Kerr and colleagues (Kerr et al., 1972). Morphological assessment remains a key technique for the study of apoptosis in vivo, but recent advances in biochemistry and molecular genetics have revolutionized both the investigation and our basic understanding of the process. These developments owe much to the growing realization that apoptosis plays a vital role in many fundamental biological processes, including development, the response to infection and the maintenance of tissue homeostasis.

It is widely accepted that rapidly proliferating cells are particularly vulnerable to mutational events. Intestinal epithelia are self-renewing tissues in which exceptionally high rates of mitosis are combined with chronic exposure to potentially mutagenic food residues and bacterial metabolites. Carcinomas of the human digestive tract are common, and the risk of disease increases with age. Nevertheless there is good evidence that mechanisms to preserve the integrity of the intestinal epithelial genome do exist, and that their effectiveness can be improved by dietary manipulation. This paper is concerned with dietary influences on proliferation and apoptosis among the epithelial cells of the intestinal mucosa. The structure of the intestinal crypt and the organization of epithelial cytokinetics, are first described. The cellular physiology of apoptosis is briefly reviewed, and the evidence that modulation of apoptosis provides a protective mechanism against intestinal neoplasia is discussed in the context of the epidemiological and experimental evidence for protective effects of NSAIDS against colorectal carcinoma. Finally, the influence of dietary factors on colorectal apoptosis and mitosis, and the possible implications for the prevention of colorectal neoplasia, are briefly discussed.

Abbreviations: ACF, aberrant crypt foci; DHA, docosahexaenoic acid; DMH, 1,2-dimethylhydrazine; EPA, eicosapentaenoic acid; NSAIDS, non-steroidal anti-inflammatory drugs; PUFA, polyunsaturated fatty acid; SCFA, short-chain fatty acidTNF, tumour necrosis factor.

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2. Cell birth and death in the intestinal crypt

Both the surfaces of the small intestinal villi, and the essentially flat mucosa of the large intestine, consist of a single layer of columnar epithelial cells. Senescent cells are shed into the colonic lumen at a rate of around 200 cells per square millimeter per hour in humans, but the integrity of the mucosal surface is maintained by new cells that emerge from an array of blind-ending glandular crypts and migrate to the villi, or to the gently ridged inter-cryptal zones of the colon and rectum. Every crypt is a self-contained proliferating unit (Fig. 1).

2.1. Crypt cell mitosis

Mitosis is normally at its peak near the base of the crypt, and virtually absent from the uppermost third, nearest the gut lumen. In the basal zone, a small population of putative *stem cells* is thought to undergo asymmetric divisions, each yielding one new stem cell and one new *dividing transit cell*. The latter undergoes several further symmetrical divisions as it migrates upwards through the lower half of the crypt (Potten and Morris, 1988). In the upper part of the crypt, the maturing cells cease to divide, and then undergo terminal differentiation before emerging in to the mucosal surface. It has been proposed that the existence of stem cells, which have a cycle time roughly twice as long as dividing transit cells (Potten, 1986), may be an adaptation to minimize the exposure of DNA to mutagenic events during mitosis, and increase the time available for detection of unrepaired mutations, and the initiation of apoptosis (Heddle et al., 1996).

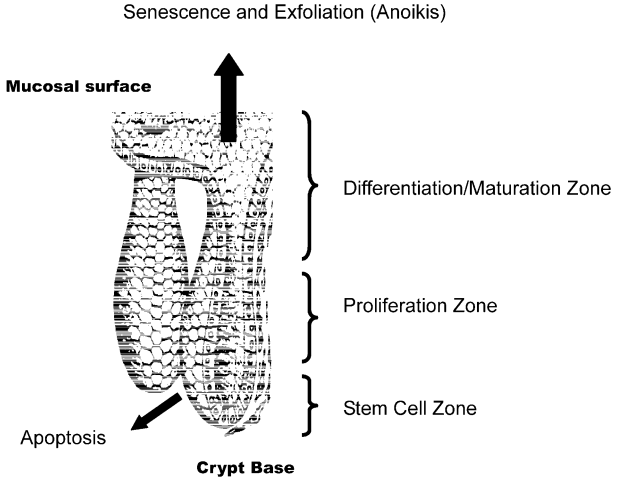

Fig. 1. Morphology and cytokinetics of the colorectal crypts. The stem cell zone is located near the base of the crypt. Pluripotent stem cells undergo symmetric divisions yielding single daughter cells. These undergo further divisions in the stem cell zone, and then differentiate as they migrate towards the mucosal surface. Senescent cells are lost from the surface epithelium by a form of programmed cell death sometimes called *anoikis*, in which exfoliation is thought to precede apoptosis. A smaller component of cell loss from the crypt compartment occurs as a result of apoptosis in the stem cell and proliferation zones.

2.2. Mechanisms of apoptosis

In contrast to necrotic cell death, which tends to affect many adjacent cells simultaneously, apoptosis is typically confined to individual cells dispersed throughout a tissue. Among epithelial cells, the earliest morphological signs include shrinkage and convolution of the nucleus, aggregation of the chromatin to the nuclear membrane, and a loss of contact and communication with neighbouring cells. The microvilli disappear and the plasma membrane acquires large irregular protuberances or "blebs". Meanwhile, the cytoplasm undergoes condensation and the endoplasmic reticulum becomes dilated, crowding the organelles within the cell. The nucleus then disintegrates into several densely-staining, membrane-bound bodies within the cytoplasm. In due course the irregular blebbing of the plasma membrane facilitates the disintegration of the cell into discrete membranous packages containing the degraded nuclear fragments called *apoptotic bodies* (Wyllie et al., 1980).

The *caspases*, a family of cysteine proteases, mediate much of the cleavage of intracellular components (Fig. 2). The caspases exist in cells as inactive zymogens requiring cleavage for their activation. This often occurs through the proteolytic activity of other caspases in the series, thus forming a signaling cascade that transmits and amplifies death signals arising from a variety of intra- and extracellular sources to the downstream events of cell death. Caspase 8 is an initiator caspase that reacts to extracellular signals acting through the tumour necrosis factor (TNF) receptor superfamily. Activation of a receptor by an extracellular ligand (e.g. Fas) causes an intracellular region, the death effector domain, to form a cytosolic complex with procaspase-8, followed by release of active caspase-8, which initiates downstream activation of effector caspases 3, 6 and 7. The second main pathway of caspase activation is mediated via the mitochondria and triggered by various types of cellular stress, including DNA damage. The key signaling event is release of cytochrome-c from the mitochondria into the cytosol, where it forms a complex with Apaf-1 and procaspase-9, followed by activation of caspase-9 and the effector caspases. These pathways are regulated at many points by proteins such as the Bcl-2 family, that can stimulate or retard the release of cytochrome-c, the IAP family that directly inhibit caspase activity, and Smac/DIABLO, which is a negative regulator of IAP (Fig. 2).

Orderly removal of apoptotic debris by adjacent epithelial cells, or by specialized cells of the immune system, is the final stage of the process. It is vital to the preservation of tissue integrity because it prevents leakage of pro-inflammatory cellular debris into the extracellular environment (Afford and Randhawa, 2000).

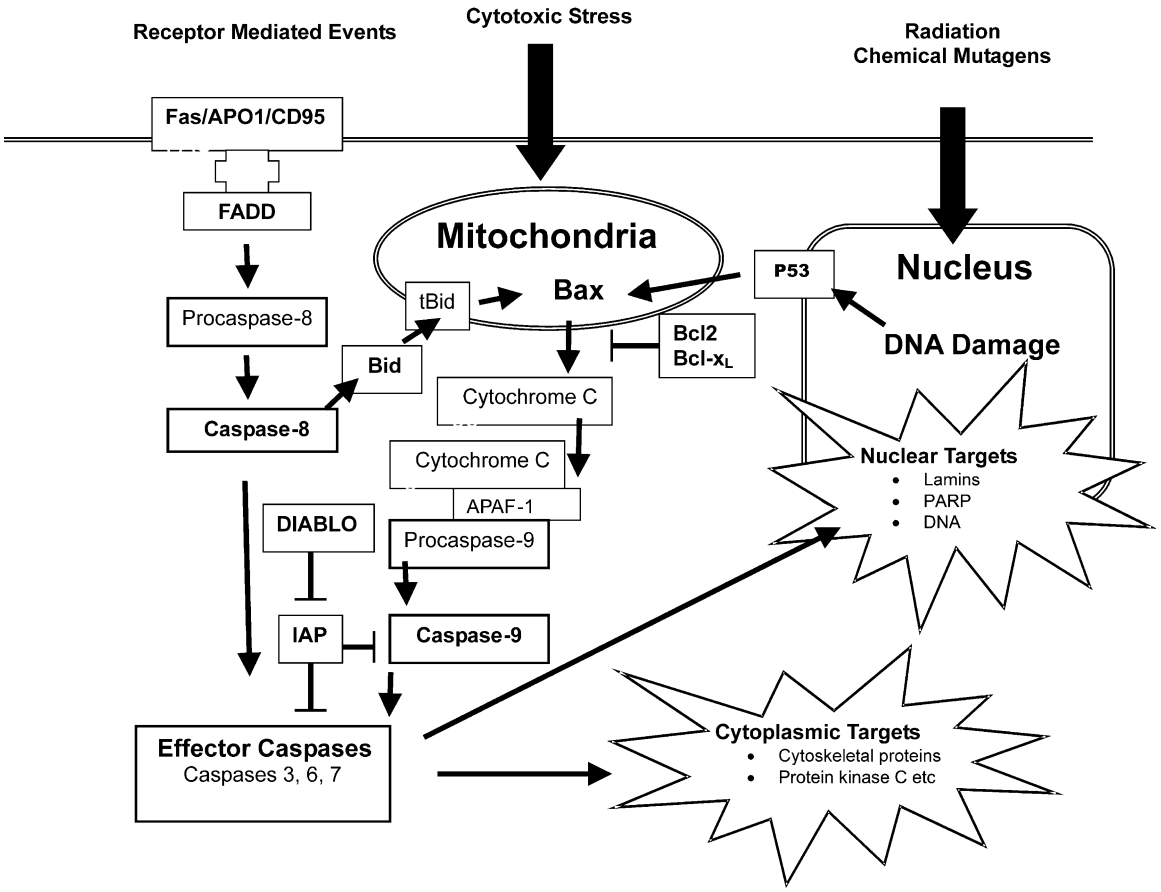

Fig. 2. Initiation and regulation of apoptosis. The two principal known pathways of apoptosis are mediated via cell surface receptors (e.g. Fas/APO1/CD95 and the TNF family), and the mitochondria respectively. Binding of extracellular death signals to receptors of the tumour necrosis superfamily initiates the formation of an intracellular complex comprising a *death domain* (e.g. FADD) and procaspase-8. This initiates activation of caspase-8, which in turn activates the effector caspases 3, 6 and 7. The mitochondrial pathway is thought to be activated by a variety of stimuli, including DNA-damaging agents, cytotoxic stresses, or the absence of essential growth factors. The pro-apoptotic protein Bax facilitates release of cytochrome-c from the mitochondrial inner membrane. An association of cytochrome-c, APAF-1 and procaspase-9 within the cytosol forms an *apoptosome*, which activates of caspase-9 and the effector caspases. In some cell types caspase-8 is thought to activate the mitochondrial pathway via cleavage of Bid. Inhibitory regulators of apoptosis include Bcl-2 and Bcl-X_L, which suppress release of cytochrome-c. The IAP family are direct inhibitors of both initiator and effector caspases. They are themselves inhibited by the protein DIABLO/smac, which thus functions as promoter of apoptosis.

Apoptotic bodies derived from the epithelial cell lining the crypts of the intestinal mucosa can be identified in sections of wax-embedded intestinal mucosa (Potten, 1992), and in microdissected whole-crypt preparations (Latham et al., 1999). In normal tissue, such apoptotic nuclei are rare in comparison to mitotic profiles because their removal is rapid, but a few apoptotic remnants reflect a relatively high level of cell death (Bellamy et al., 1995).

2.3. Apoptosis in the crypt

Potten and colleagues have proposed that "background" apoptosis in the stem cell region of mouse small intestinal crypts serves to delete extra stem cells that may be formed from time to time by the symmetrical division of a parent stem cell (Potten, 1992; Bach et al., 2000). In the mouse colon, the basal level of apoptosis is lower than in the small intestine, and this appears to be associated with greater expression of regulatory proteins that suppress apoptosis, including Bcl-2 (Merritt et al., 1995) and Bcl-w (Pritchard et al., 2000). Exposure to low doses of radiation or cytotoxic drugs induces a dose-dependent increase in apoptosis in the stem cell region of both small and large intestinal crypts in the mouse. (Potten et al., 1992), but the small intestine appears to be more radio-sensitive than the colon, and the apoptotic zone coincides more precisely with the stem cell zone. This may facilitate deletion of mutated stem cells and help to account for the rarity of cancers of the small bowel (Merritt et al., 1995; Bach et al., 2000).

Suppression of apoptosis may favour progression at every stage of the adenoma-carcinoma sequence. Down-regulation of apoptosis can be caused by loss of functional pro-apoptotic genes, or by adaptive changes associated with a chronic state of mucosal inflammation and repair (Kinzler and Vogelstein, 1996). Suppression of cell death is also modulated by endocrine mechanisms.

For example, the gastrointestinal regulatory peptide GLP-2 stimulates epithelial proliferation and has been shown to inhibit apoptosis via a G-protein coupled receptor pathway (Yusta et al., 2000). The possible significance of this mechanism, in relation to nutrition and the pathogenesis of intestinal neoplasia, has yet to be explored.

An increased tendency to undergo apoptosis, whether as a field-effect involving normal crypts, or as a localized mechanism affecting focal lesions at a later stage in the sequence, can be expected to inhibit carcinogenesis. Increased rates of cell death may suppress the appearance of initiated cells, slow their clonal expansion, or perhaps even cause the regression of established lesions. Striking evidence for this has recently emerged from studies on the anticarcinogenic effects of non-steroidal anti-inflammatory drugs (NSAIDS), and this is reviewed briefly below.

3. NSAIDS, apoptosis and colorectal neoplasia

Waddell and colleagues (1989) showed that treatment with the NSAID sulindac substantially reduced, and in some cases entirely eliminated polyps in patients suffering from familial adenomatous polyposis. Takayama et al. (1998) used sequential endoscopies to study the effects of the NSAID sulindac on aberrant crypt foci ACF in human subjects, and showed marked reductions, and in some cases complete eradication of these lesions in patients treated with sulindac. A reduction of around 50% in the incidence or mortality from colorectal cancer has been observed in several epidemiological studies conducted on regular users of aspirin (Lancaster and Silagy, 1994), and it has recently been shown that the specific COX-2 inhibitor Celicoxib reduces the number of polyps in patients with familial adenomatous polyposis (Steinbach et al., 2000).

The regression of colorectal lesions induced by treatment with NSAIDS provides strong circumstantial evidence that these drugs can arrest cell growth, or stimulate apoptosis, and this is supported by experimental studies. Aspirin and other NSAIDS can arrest the cell cycle and induce apoptosis in colorectal carcinoma cell lines (Elder et al., 2000), and there is growing evidence that this is the principal mechanism whereby they cause regression of polyps and ACF in the human colon (Reinacher-Schick et al., 2000).

The principle pharmacological effect of aspirin and other NSAIDS is the inhibition of cyclooxygenase (prostaglandin H synthase) activity, leading to a reduction in prostaglandin synthesis (Vane and Botting, 1998). It might be inferred from this that the ability of NSAIDS to inhibit proliferation and induce apoptosis in tumour cells is a consequence of COX-2 inhibition, but this is not necessarily the case. In vitro studies have shown that the pro-apoptotic effects of NSAIDS are independent of COX-2 expression in various systems (Smith et al., 2000a), and alternative models for the effects of sulindac have been proposed, including direct induction of apoptosis by disruption of the mitochondrial inner membrane (Waddell, 1998), modification of arachidonic acid metabolism (Chan et al., 1998), or direct modulation of apoptosis via the Bcl-2 family of regulatory proteins (Marx, 2001).

4. Dietary components and intestinal apoptosis

There is growing evidence that a number of different classes of food constituents, including some polyunsaturated fatty acids (PUFA), the short-chain fatty acid butyrate, and certain flavonoids and glucosinolate breakdown products, can regulate the processes of cell proliferation and death in vitro, and in colorectal crypts in vivo (Smith et al., 1998). Many of these dietary factors also suppress the emergence of ACF in animal models of carcinogenesis, and may protect human populations against colorectal cancer. Some examples of these potentially anticarcinogenic food constituents are discussed below.

4.1. Long-chain fatty acids

There is growing interest in the significance of specific fatty acids in relation to the growth and metabolism of tumour cells. Certain essential fatty acids, notably gamma-linolenic acid, arachidonic acid, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are selectively toxic to tumour cells. Das (1991) demonstrated that the inhibition of tumour cell proliferation in the presence of gamma-linolenic acid, arachidonic acid and EPA was caused by selective cytotoxicity, and that cell death was blocked by antioxidants, enhanced by pro-oxidants and proportional to the degree of peroxidation induced in the cells. More recent studies show that apoptosis plays an important role in the cytotoxicity induced by PUFA. In the human colorectal adenocarcinoma cell line HT29, incubation with EPA leads first to detachment of the cells from the substratum, followed by apoptosis, which can be enhanced by depletion of intracellular glutathione levels (Latham et al., 1998) and blocked with antioxidants and caspase inhibitors (Clarke et al., 1999).

Long-chain fatty acids also modulate crypt cytokinetics in vivo. Consumption of fish oil, which is rich in EPA and DHA, leads to a reduction in crypt cell mitosis (Pell et al., 1994). Latham et al. (1999) fed rats on a semi-synthetic basal diet containing corn oil (80 g/kg) prior to treatment with 1,2-dimethylhydrazine (DMH). Immediately after DMH or a sham injection the rats were subdivided to receive the control diet or a diet in

which the corn oil was replaced with fish oil. In rats fed fish oil with no DMH treatment there were statistically significant increases in apoptosis and reductions in mitosis after 48 h. In the rats treated with both fish oil and DMH, the apoptotic response was more than doubled compared to those fed corn oil, and after 18 weeks the frequency of ACF was significantly lower in the animals fed fish oil. These results are consistent with the hypothesis that dietary exposure to fish oil during the phase of DNA damage following exposure to DMH enhances the level of apoptosis and increases the deletion of cells otherwise destined to form precancerous lesions. The precise mechanism by which n-3 PUFA enhances apoptosis in intestinal epithelial cells is not clear, but the probable involvement of lipid peroxidation is indicated by the observation that the induction of apoptosis by fish oil is significantly enhanced in the intact rat intestine by depletion of epithelial glutathione levels (Latham et al., 2001). There is increasing evidence that both reactive oxygen species, and the intracellular redox potential play a role in the regulation of apoptosis (Carmody and Cotter, 2001).

4.2. Short-chain fatty acids

The short-chain fatty acids (SCFA) are the major products of carbohydrate fermentation in both the rumen and the non-ruminant colon. Acetate, propionate and butyrate account for about 90% of SCFA in the human large bowel. Of these, butyrate provides around 25% of the total, and it is usually present at a concentration of approximately 25 mm/kg of faecal material in the proximal human colon (Cummings et al., 1987). It is estimated that 95% of the SCFA produced in the human large bowel is absorbed and metabolised, and it is well established that butyrate in particular functions as a metabolic substrate for the colonic epithelial cells in vivo (Roediger, 1990).

When animals with a conventional colonic microflora are fed fermentable forms of dietary fibre they have a faster crypt cell proliferation rate than animals fed a fibre-free diet, but this effect is absent in germ-free animals that cannot produce butyrate by fermentation (Pell et al., 1995). Perfusion of the intact colon with a butyrate solution close to physiological concentrations stimulates crypt cell proliferation in rats (Kripke et al., 1989). Installation of SCFA into the human rectum also exerts a trophic effect on the mucosa (Mortensen et al., 1991). Conversely, exposure to butyrate inhibits growth and induces a more differentiated phenotype in a variety of different tumour cells in vitro (Augeron and Laboisse, 1984; Barnard and Warwick, 1993). It is also well established that exposure to butyrate, at physiologically relevant concentrations, induces apoptosis in human colorectal adenoma and carcinoma cell lines (Hague et al., 1993). Butyrate induces apoptosis in human colorectal tumour cells despite the absence of a functional p53 pathway (Hague et al., 1993). The mechanism is caspase dependent (Chai et al., 2000) and involves signal transduction via the Fas-ligand death receptor (Chapkin et al., 2000). Transcriptional activation of the Bax gene via the c-Jun N-terminal kinase, (JNK) signal pathway may also be involved, at least in some tumour cells (Mandal et al., 2001).

There is intense interest in the possibility that intracolonic butyrate associated with colorectal fermentation of polysaccharides may cause apoptosis of neoplastic epithelial cells in vivo, and recent reports provide some support for this hypothesis. Caderni and co-workers (2001) used slow-release pellets to expose the rat colonic epithelium to butyrate and reported increased apoptosis in colonic crypts, but no protective effect against azoxymethane induced neoplasia. However, Perrin et al. (2001) have recently reported that resistant starch or fructo-oligosaccharides generate a high stable concentration of butyrate and suppress the appearance of ACF in a rat model. Similarly Avivi-Green et al. (2000) have described increased expression of pro-apoptotic signal proteins, an increased crypt apoptotic index, and suppression of chemically induced neoplasia in rats fed a fermentable type of fibre, and in those treated directly with butyrate by intra-colonic installation.

4.3. Flavonoids

The flavonoids are a large and complex group of phenolic compounds found in beverages such as tea, coffee and wine, and in fruits and vegetables, where they provide much of the flavour and colour (Hollman and Arts, 2000). Their generic three-ring structure contains two aromatic centres and a central oxygenated heterocycle. Flavonoids exhibit a range of biological activities including both pro- and antioxidant effects, and inhibition of key intracellular enzymes and signalling cascades. Quercetin, myricetin and kaempferol are consumed predominantly in the form of water-soluble glycosides, and are partially absorbed from the diet (Hollman and Arts, 2000). Humans consume flavonoids as water-soluble glycosides. Flavonoid metabolites appear in the circulation soon after consumption, but the epithelial tissues of the gastrointestinal tract are probably exposed to much higher local concentrations (Halliwell et al., 2000).

Quercetin and other flavonoids inhibit proliferation of human gastrointestinal carcinoma cells (Musk et al., 1995), often by blocking the G1/S transition of the cell cycle and inducing apoptosis (Yoshida et al., 1992). To explore the effects of quercetin and its glycoside rutin on colorectal carcinogenesis, Deschner et al. (1991) gave supplements of quercetin (1, 5 or 20 g/kg diet) or rutin (20 or 40 g/kg diet) to mice and measured induction of crypt hyperproliferation, dysplasia and tumours after treatment with azoxymethanol. Both quercetin and rutin caused suppression

and spatial relocalisation of crypt cell hyperproliferation induced by the carcinogen, and a reduction in tumours. In contrast, Pereira et al. (1996) reported a dose-dependent enhancement of tumours by exposure to dietary quercetin (16.8 and 33.6 g/kg diet) in a rat model. Mahmoud et al. (2000) studied the effect of quercetin and rutin on the development of tumours in an *APC* knockout mouse model, but observed no protective effect at a dietary supplementation level of (20 g/kg).

Recently Yang et al. (2000) reported that dietary supplementation with quercetin and rutin increased apoptosis in the colonic crypts of mice treated with azoxymethanol. This effect was associated with a reduction in focal areas of dysplasia, but quercetin itself induced these lesions in some animals in the absence of azoxymethanol. Hara et al. (1999) explored the relationship between dietary quercetin level and crypt cell proliferation and apoptosis in the small bowel and colon of the rat and showed that quercetin inhibited crypt cell mitosis at low concentrations. However, the effect diminishes as the level of exposure increased. Dietary supplementation with quercetin at a level that caused inhibition of crypt cell mitosis suppressed the induction of ACF by DMH.

It is becoming clear that flavonoids exert a complex variety of biological effects on mammalian cells. The modulation of mitosis and apoptosis previously observed in vitro appears to be reproducible in mucosal epithelial cells but the implications of this are not yet clear. Much of the work that has been carried out so far involves dietary exposure to flavonoids at levels far in excess of those achievable from conventional foods and further studies are needed before their use at enhanced levels in functional products can be recommended.

4.4. Glucosinolate breakdown products

There are more than 100 glucosinolates, found in both wild and domestic *Brassica* species, including cabbages, broccoli and Brussels sprouts. Their common structure contains both a β-d-thioglucose and a sulphonated oxime group, while the variable side-chain contains methionine, tryptophan, phenylalanine or a variety of branched-chain amino acids (Mithen et al., 2000). Glucosinolates remain sequestered within the plant tissues until cellular disruption brings them into contact with the endogenous enzyme myrosinase (thioglucoside glycohydrolase). Rapid hydrolysis of the glucosidic bond releases an unstable aglycone, which degrades further to form a variety of end-products including nitriles and isothiocyanates or *mustard oils*. The precise course of the reaction depends on the ambient conditions. Substantial quantities of isothiocyanates are formed during food preparation, mastication and digestion (Fenwick et al., 1983).

There is good evidence that consumption of *Brassica* vegetables, rich in their precursor glucosinolates, is protective against cancers of the lung and alimentary tract (van Poppel et al., 1999). Isothiocyanates have been shown to to block the action of mutagens in model systems by modulating the activities of Phase I and Phase II biotransformation enzymes, including glutathione *S*-transferase and UDP-glucuronyl transferase (Hecht, 1999). There is also good evidence that they can induce apoptosis in previously initiated cells, and may thereby suppress the progress of established carcinogenesis. For example, Musk et al. (1995) reported a selective toxic effect of allyl isothiocyanate, phenethyl isothiocyanate and benzyl isothiocyanate against undifferentiated HT29 cells. Human leukaemia cells (HL60) and human myeloblastic leukaemia 1 cells have also been reported to undergo caspase-dependent apoptosis following exposure to phenethyl isothiocyanate (Xu and Thornalley, 2000).

In the rat, oral administration of the glucosinolate sinigrin induces apoptosis and suppresses mitosis in the colorectal crypts, but only during the wave of cell death following treatment with DMH. These effects are associated with a suppression of ACF, 6–8 weeks after exposure to the carcinogen (Smith et al., 1998). A similar induction of apoptosis is induced by oral administration of juice derived from Brussels sprouts, which are one of the main sources of sinigrin in the human diet. (Smith et al., 2000b).

These pro-apoptotic effects of glucosinolate breakdown products in vitro, and in animal models, may provide a mechanism to explain the epidemiological evidence showing protective effects of *Brassica* vegetables against cancers of the alimentary tract. The compounds undergo complex changes both before and after their passage through the digestive tract, and it is not yet clear whether the intestinal epithelial cells become exposed to isothiocyanates released in the gut lumen, or whether blood-borne metabolites are of primary importance. Further studies are needed to explore these aspects of bioavailability and biological activity in human beings.

5. Conclusion

The wide geographical variation in incidence of cancers of the alimentary tract indicates that these diseases are potentially avoidable. Although several food-borne colorectal carcinogens have been identified and are currently the subject of intensive study, there is good reason to believe that protective factors in the diet are at least as important as determinants of human disease. Some of these seem likely act via effects on crypt cell cytokinetics. The unique accessibility of the gut to clinical endoscopy, and the recent rapid advances in tumour

biology, provide exciting opportunities for the development of strategies for cancer chemoprevention. The protective effects of NSAIDS against colorectal cancer are extremely encouraging and the evidence presented here suggests that food constituents may also exert significant and potentially beneficial effects on epithelial cell proliferation and apoptosis in the gut. However, human intervention studies coupled with a deeper understanding of epithelial cell biology will be essential if we are to establish whether dietary effects on apoptosis can be manipulated for preventive or therapeutic effect.

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