Allyl isothiocyanate selectively kills undifferentiated HT29 cells in vitro and suppresses aberrant crypt foci in the colonic mucosa of rats.

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A negative association between the risk of colorectal cancer and the consumption of fruit and vegetables is a strong and consistent finding of many population based ecological studies [1,2]. One possible explanation for this association is that such diets are rich in the antioxidant vitamins A, C and E, which are thought to protect tissues against free-radical mediated genotoxicity, but this hypothesis is not supported by recent epidemiological data [3], nor by intervention trials [4]. Attention has therefore turned to the many other secondary plant metabolites which display biological activity toward mammalian cells [5]. The glucosinolates are a large group of sulphur containing compounds found in many vegetables of the genus Brassica [6]. When the plant tissue is disrupted by preparation for cooking or by chewing, the enzyme thioglucosidase is released, and the glucosinolates undergo hydrolysis to yield a variety of breakdown products. Among the most important of these are the isothiocyanates, which impart both flavour and biological activity to condiments such as mustard, and to vegetables such as radishes, sprouts and cauliflower [6]. Isothiocyanates are already recognised as potential protective factors because of their ability to modify carcinogen metabolism [7] but here we present evidence for a suppressive effect on the development of pre-cancerous lesions, after initiation with a chemical carcinogen. In previous work we have shown that some isothiocyanates are selectively toxic against undifferentiated phenotype of the human colorectal cancer cell line HT29 [8]. In the present study we explored the effect of the glucosinolate sinigrin and its hydrolysis product, allyl isothiocyanate, on cell proliferation, apoptosis and induction of aberrant crypt foci in rats treated with dimethylhydrazine (DMH).

Four groups of 20 male Wistar rats were fed a basal semisynthetic powdered diet containing casein (200g/Kg), corn starch (310g/Kg), sucrose (310g/Kg), cellulose (40g/Kg) and appropriate levels of micronutrients. Two groups of rats were given DMH (ca. 30mg/Kg body weight) by sub-cutaneous injection at days 1 and 5. The third and fourth groups were controls receiving sham injections. On day 6, one control group and one DMH-treated group were given basal diet supplemented with sinigrin (400 μg/g diet). On day 7, ten rats from each group were killed for measurements of cytokinetics and apoptosis in colonic crypts. The remaining ten rats in each group were killed after 42d and numbers of colonic aberrant crypt foci were compared.

Samples of whole mucosa were obtained from 5 sites along the entire length of the colon, fixed in acetic acid ethanol (25:75) and bulk-stained in Feulgen's reagent. The numbers and positions of mitotic and apoptotic nuclei in 10 whole-crypt mounts from each site were determined by light microscopy, and an estimate of the total number of apoptotic cells per crypt was obtained for each animal. The total numbers of aberrant crypt foci for each animal were determined by light microscopy, and an estimate of the total number of apoptotic cells per crypt was obtained. The significance of differences between means was assessed by one-way ANOVA combined with Tukey's test for individual comparisons or, in the case of aberrant crypt foci, two-way ANOVA with body weight as a covariate.

Thus oral administration of the glucosinolate sinigrin caused increased apoptotic cell death in the colonic mucosal crypts of DMH-treated rats. This was associated with a significantly smaller number of aberrant crypt foci, 42 days after treatment with DMH. A crucial aspect of this experiment is that the glucosinolate was given 24h after the final dose of DMH, and therefore could not have modified the metabolism of the carcinogen. One interpretation of these results is that in this experimental model, sinigrin, or more probably its degradation product allyl isothiocyanate, enhances the deletion of initiated cells from the damaged intestinal crypts, thus reducing the induction of aberrant crypts.

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