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Colon Cancer Chemoprevention With Ginseng and Other Botanicals

Colorectal cancer is becoming increasingly common in Asian countries and still remains the second leading cause of cancer deaths in the United States. Efforts to prevent colon cancer have targeted early detection through screening and chemoprevention. For the last ten years our laboratory has utilized an in vivo screening assay for the testing of potential cancer preventives for colon cancer. We have conducted investigations on over 150 compounds including many with botanical or herbal origins. As part of our program on natural products we have examined a number of herbal and botanical products in the aberrant crypt foci (ACF) assay including Korean red ginseng powder, green tea catechins, curcumin from the Indian culinary spice, tumeric, compounds from garlic and onion, resveratrol from red grapes, among others. In the ginseng experiments groups of 10 F344 rats were fed ginseng powder at a dose of 0.5 g/kg or 2 mg/kg for 5 weeks. During weeks 2 and 3 rats were injected with 10 mg/kg azoxymethane to induce ACF. Controls (n=10) did not receive azoxymethane (AOM). Rats were killed by CO_2 overdose and ACF counted in the rat colon. In 8 week post-initiation experiments ginseng powder inhibited the progression of established ACF, indicating a cytostatic effect. This may be due to an anti-inflammatory effect. There is a body of literature that suggests that compounds in wine, tumeric, and tea inhibit cyclooxygenases, thus reducing prostaglandin-mediated effects on the colon. As colon tumors have been shown to highly express COX-2 protein, and given, that many NSAID drugs also suppress COX-1, it is tempting to speculate that herbal products that inhibit one or both forms of the COX enzyme will be effective agents for the prevention of cancer in man.

Key Words : *Ginseng; Colonic Neoplasms; Aberrant Crypt foci; Chemoprevention; Antineoplastic Agents, Phytoqenic Botanicals*

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INTRODUCTION

Colorectal cancer is still a leading cause of cancer deaths in the United States and is increasing at an alarming rate in Asia (1, 2). Cancer chemoprevention is a discipline of cancer research emerging from its infancy 20 yr ago to now gain center stage in the armamentarium against cancer (3). Yet it has not abandoned its founding principles of discovery of natural and man-made agents that inhibit the initiation, promotion, or progression of cancer. The pioneering efforts of Wattenberg (4, 5) first focused the search for chemopreventive agents by examining the diet for them. Dietary patterns may account for wide differences in the risk for leading cancers across the world. It was logical to propose that dietary factors, in countries with populations at low risk for certain cancers could be identified and exploited for use in man as cancer inhibitors (6). From the origins of this discipline it was found that compounds in cruciferous vegetables, alliums, citrus, soy, and tea had the ability to inhibit experimentally-induced cancers in animal models (7). In the early 1980s the National Cancer Institute (U.S.A.) established within its structure a chemoprevention screening program to identify promising agents for rapid development into clinical trial agents. This program encompassed a tier of strategies to first select candidate agents, evaluate these agents in in vitro based test systems (often involving human tumor cell lines), then test the most promising agents in short-term animal models for chemoprevention efficacy (8). Finally, the program attempted to confirm the anti-tumor or cancer preventive aspects of these agents in long-term tumor studies in mice and rats. The animal models employed are designed to test the same agents across tumor type, and these were targeted to the most common cancers: lung, breast, colon and prostate. During this effort a list of hundreds of potential compounds were distilled down to a dozen or so per year with the remaining goal of progressing them to clinical trials (9). As a discipline we are finally at the point in cancer chemoprevention where we are able to see the fruits of this long process, as studies in high risk individuals for cancer have begun. As a laboratory associated with this effort we

have evaluated over 150 potential agents for the chemoprevention of colon cancer (10-12). While many of these included drugs created for other medical purposes, we also investigated a number of naturally occurring agents. Currently, the laboratory has focused on herbal and botanical sources of chemopreventive agents because of the increasing use of complementary and alternative medicines among cancer patients and the general public (13). The primary assay for efficacy screening is the aberrant crypt assay in the rat colon.

CHEMOPREVENTION EFFICACY STUDIES IN ANIMAL MODELS FOR COLON CANCER

The most widely used animal model sytem for the evaluation of chemopreventive activity has been azoxymethane-induced (AOM) colon cancer in the Sprague-Dawley or F344 rat, although more recent efforts have employed the $APC^{min+/-}$ mouse (14, 15). The AOM model is widely appreciated for its histological similarity to human colon cancer, although tumorigenesis assays can take up to a year to complete. Many of the same molecular and biochemical defects in human cancer are also observed in the AOM model, although some differences in the role of tumor suppressor genes such as *p53* are notable (16). For this reason, many investigators have turned to intermediate biomarkers that economize in terms of duration of the experiment. An ideal compromise in the AOM model is the aberrant crypt foci (ACF) assay.

Aberrant crypt foci are precursor lesions for colon cancer (17). Arising out of the normal colonic epithelium they are dilated, often protruding above the surface of neighboring normal crypts. This protrusion is a "signature" for ACFs and they are easily detected in carcinogen-treated rodent colon by immersing the tissue briefly in a solution of methylene blue and viewing the tissue under a dissecting microscope (Fig. 1). ACFs show many abnormal features histologically. They are wider then normal crypts and exhibit a slit-like opening when viewed from above compared to the circular appearance of normal crypts (18, 19). They often are hyperplastic although dysplasia is also common. ACFs are more proliferative than normal cryptal glands and are more mitotic. At the molecular level ACFs often are mutated in the *apc* gene and have a mutated *ras* oncogene, although these are more likely to occur in the more dysplastic ACFs (20-22). Defects in cell adhesion molecules have been found (23, 24). While ACFs are considered to be precursors to adenomas, and are found in humans at risk for colon cancer, not all ACFs become adenomas, just as only a few adenomas become colon cancers (25). Still, as a proxy for colon cancer, ACFs have been extensively used as an intermediate biomarker for colon cancer prevention research.

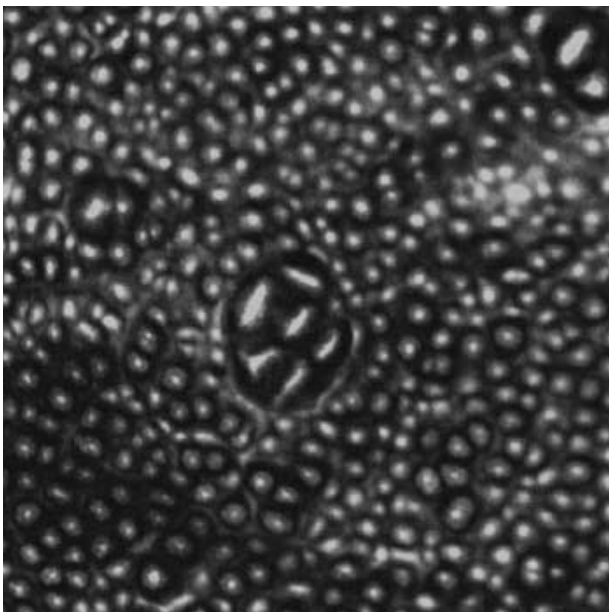

Fig. 1. Aberrant crypt foci in methylene blue stained colon. Note large multicrypt cluster in ACF (× 50).

ACFs are induced in rodents by all known carcinogens, thus making them an attractive model for chemoprevention. In our laboratory we have developed two protocols for the evaluation of potential chemopreventive agents. In the first protocol, F344 rats are injected twice at 15 mg/kg with AOM during the first two weeks of a five week experimental period. The test compound is fed in the diet during the four week period (2 weeks following the last injection of AOM). This protocol evaluates the effects of the test compound during the initiation phase, when interactions with the carcinogen's metabolism can occur. In the second protocol we repeat the same course of treatment but do not expose the animals to the test compounds until ACFs have grown in the rat colon for 4 weeks. During a second four week period we introduce the test agent, thus evaluating the effects of the agent on outgrowth and progression of ACFs. This fea-

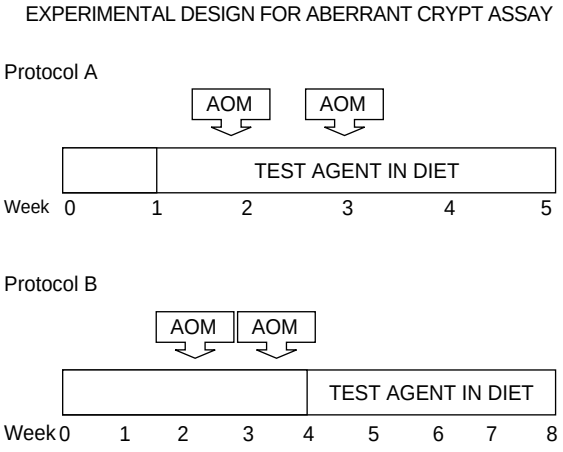

Fig. 2. Experimental design for chemoprevention studies.

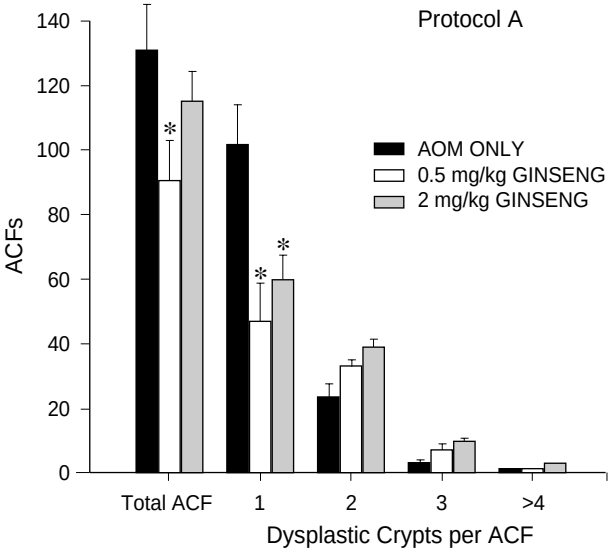

Fig. 3. Effect of Korean red ginseng on induction of ACF by azoxymethane in the rat colon during initiation of carcinogenesis. Data significantly different from AOM only group at $p<.05$ indicated by*.

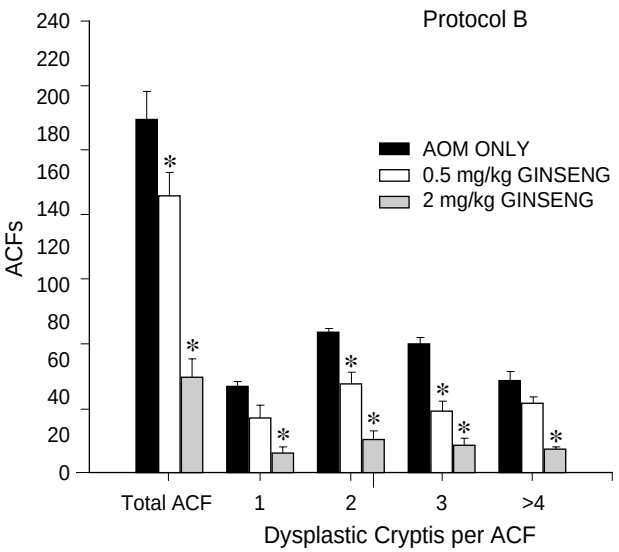

Fig. 4. Effect of Korean red ginseng on induction of ACF by azoxymethane in the rat colon during the post-initiation phase of carcinogenesis. Data significantly different from AOM only group at $p<.05$ indicated by*.

ture allows the more facile testing of an agent on the outgrowth of multicrypt-ACFs which are more likely to acquire subsequent mutations and progress to cancer. The protocols we have used are shown in Fig. 2. As described above, we have tested a significant number of compounds in this assay and chemical class/activity patterns have begun to emerge. Recently we have engaged the hypothesis that botanical and herbal supplements may indeed be sources of cancer chemopreventive agents. To illustrate one series of investigations we have recently tested Korean red ginseng for chemopreventive activity in the ACF assay.

EVALUATION OF KOREAN RED GINSENG FOR CHEMOPREVENTION OF COLON CANCER

A wealth of evidence links elements of the Asian diet to reduced risk for certain cancers, hence the recent interest in herbals and botanicals of Asian origin as potential sources of cancer inhibitors (26-28). Korean red ginseng (*Panax ginseng* C.A. Meyer cultivated in Korea) is valued for its human-shaped storage root that has a long history of purported medicinal uses. Ginseng has been widely reported to be an effective tonic or has adaptogenic activity which effectively increases the ability to withstand stressors (26, 29, 30). Many other medical effects are attributed to ginseng use, mainly associated with increased energy levels and feelings of well-being.

The major agents in ginseng root thought to be responsible for medicinal activity are the triterpene saponins, also known as ginsenosides, and many of these appear in extracts of red ginseng (31-33). Red ginseng has been tested for anti-carcinogenicity in a variety of tumor models. Yun et al. (34) found that aged red ginseng powder was more effective than fresh ginseng powder in reducing benzo(a)pyrene induced lung adenoma in mice. Inhibition was also found for lung tumors induced by dimethylbenz(a)anthracene in mice (33). While over 30 ginsenosides have now been identified in ginseng only a few have been isolated and tested for cancer preventive effects.

To confirm the effect of Korean red ginseng on experimentally induced colon cancer we tested a spray dried extract powder in the ACF assay. The experimental design is shown in Fig. 2. Forty male F344 male rats (Harlan Sprague Dawley) were randomized into 4 treatment groups of 10 rats each for protocol A and B. Group 1 did not receive ginseng. Group 2 received red ginseng powder dissolved in tap water to a concentration of 0.5 mg/kg body weight whereas Groups 3 and 4 received ginseng at a concentration of 2 mg/kg body weight. Groups 1-3 were injected with azoxymethane (Sigma) at a concentration of 15 mg/kg at weeks 2 and 3 of the experiments. Protocol A tested the effects of red ginseng during weeks 2-5, whereas the ginseng was given only during weeks 4-8 in protocol B. All animals were fed the AIN93G semi-purified diet (Dyets) throughout the experimental periods. At the end of 5 weeks (Protocol A) or 8 weeks (protocol B) the rats were killed by CO_2 overdose. Their colons were removed, flushed free with PBS, then stained briefly in 0.3% methylene blue. The colons were fixed flatly in buffered formalin for 24 hr, then viewed under a dissecting microscope for the evaluation of ACF. Only dysplastic ACFs were scored. Both hyperplastic and dysplastic ACFs were present in the rat colons.

The results of these experiments are shown in Fig. 3 and

4. When red ginseng powder was delivered during the initiation phase of carcinogenesis, that is, during the same time with exposure to AOM only a modest inhibition of ACF was noted and only at the 0.5 mg/kg dose of ginseng. Thus, the effect of ginseng during the initiation of ACFs from the normal mucosa is relatively weak. However, after ACFs have been established in the colon during a four week outgrowth period, an additional four week exposure to red ginseng at a dose of 2 mg/kg significantly reduced the incidence of ACFs. We also noted that the ginseng powder during the post-initiation time limited the number of multiple crypt containing foci.

These results suggest that some factors in red ginseng powder are inhibitory toward the growth of preneoplastic lesions in the rat colon treated with azoxymethane. The mechanisms by which this occurred are currently unknown but at least two possibilities may explain the effect. Ginseng has been shown to inhibit the proliferation of a number of tumor cells in culture and could exert a general antiproliferative effect on the colonic epithelium (35, 36). The second possibility is that ginseng could, through inhibition of anti-inflammatory pathways preferentially inhibit the growth of ACFs. Previous work in our laboratory has established that non-steroidal anti-inflammatory drugs such as aspirin, ibuprofen, and indomethacin are potent suppressors of colon carcinogenesis and ACFs. It is tempting to speculate that specific ginsenosides might inhibit either COX-1 or COX-2 as inhibition of either of these isoforms results in chemoprevention of colon cancer (37-39). COX-2 is specifically elevated in colon tumors and at least some COX-2 selective drugs are chemopreventive in the AOM model (40). These results also verify the report of Li et al. (41) who examined the effect of ginseng on 1, 2 dimethylhyrazine-induced ACF in the rat colon, however, their study did not detect an chemopreventive effect of ginseng in the post-initiation phase of carcinogenesis. Dimethylhydrazine is the metabolic precursor to AOM used in the present study and it cannot be ruled out that ginseng may affect carcinogen metabolism. In any case, it is clear that extended studies should be performed to further confirm the chemopreventive activity of red ginseng powder on ACFs and colon tumors.

CHEMOPREVENTION OF COLON CANCER BY OTHER BOTANICALS

As part of a research program focused on the identification of naturally occurring inhibitors of cancer our laboratory has also investigated a number of botanicals with chemopreventive activity. Substances that were active either in protocol A or B include: epigallocatechin gallate from green tea, curcumin from the spice turmeric, several compounds from garlic (s-allyl cysteine, diallyl sulfide), quercetin from onions, and indole 3 carbinol from cruciferous vegetables, all elements found in traditional Asian diets (11, 12, 42). The very promising potency of non-steroidal anti-inflammatory drugs (NSAIDs) yields the prospect of searching for botanical sources of anti-inflammatories (43-45). As alluded to earlier, most NSAIDs have been shown to inhibit ACF formation and colon cancer in the AOM model, but widespread use of NSAIDs is limited by their most common and significant side effect: ulceration with intestinal bleeding. One of the primary screens for biological activity when new ethnopharmacological compounds are discovered is for anti-inflammatory activity in the paw edema assay. Literally scores of plants have anti-inflammatory effects and the results are in the ethnopharmacological literature. Among those we are interested in testing include between ginger, tumeric, cat's claw, gingko, and grapeseed and we are in the process of comparing their effects with the common NSAIDs that we have shown to be preventive in the rat model.

SUMMARY

The field of cancer chemoprevention has found fertile ground in its applicability of colon cancer and other neoplastic diseases. Factors in the Asian diet such as tea, spices, and medicinal herbs are now being screened for potential use as cancer chemopreventives in man. There is interest in systematically testing ancient medicinal herbs for this activity.

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