W1969

Colorectal Cancer Despite Colonoscopy: Estimated Size of the Truly Missed Lesions

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Background: Colorectal cancer (**CC**) discovered within 3 years after a normal or clearing colonoscopy (**C**) is considered to be the result of a missed polyp or cancer at the time of the preceding C. Multiple studies comparing sequential images of colon lesions over time have calculated lesion volume doubling rates varying from 4 months for very large lesions (~35 mm) to more than 2 years for medium size polyps (~10 mm). **Aim**: To estimate the size of CC considered missed (i.e., diagnosed within 3 years of a prior C) using the tumor size at the time of CC diagnosis. **Methods**: A large dataset was created containing (1) patients who had undergone C at Mayo Clinic Rochester (**MCR**) between 1992 and 2002 (138,318 Cs in 98,960 patients), and (2) patients who had a tissue diagnosis of CC between 1992 and 2004 (N=10,136). We defined a missed CC (**CCdespiteC**) as a CC occurring from 90 to 1095 days after C. The estimated size of the tumor at the time of the C preceding the date of CC diagnosis was estimated using the exponential growth function $y=xe^{\beta t}$ where t is the interval between date of the preceding C and the date of CC diagnosis; y and x are the tumor size (diameter of the greatest dimension) at the time of the CC diagnosis and the preceding C respectively. Despite the fact that most lesions missed during preceding C were expected to be medium sized, we elected an aggressive tumor volume doubling rate of 6 months (i.e., β=0.0013). **Results**: Among the 10,136 patients with either primary or metastatic colon cancer 2692 patients had undergone a total of 4743 colonoscopies at MCR. From this cohort, we identified 187 Cs in 145 patients who developed CCdespiteC. There was a subset in which the CC was truly missed: no lesion was seen or treated in the colonic segment during the preceding C: 120 Cs in 100 patients. Of these 120 truly missed tumors, we were able to retrieve the tumor size at the time of CC diagnosis of 96 tumors using the pathology and surgical reports. Out of 96 CCdespiteC tumors, 67 lesions have an estimated maximal diameter (obtained using the exponential growth model) of more than 10 mm at the time of preceding C. Nearly all lesions (94 out of 96) have an estimated maximal diameter of more than 5 mm at the time of the preceding C. **Conclusions**: Almost all CC diagnosed within 3 years after a negative or clearing colonoscopy have an estimated maximal tumor diameter greater than 5 mm using an exponential growth model and a very aggressive tumor volume doubling time. Our results strongly suggest that CCdespiteC is the result of a lesion truly being missed at the time of the preceding C and not the result of a *de novo* rapidly growing neoplasm.

W1970

Systematic Administration of 5-ASA Derivatives During Remissions for More Than 9 Months/Year Seems to Protect Patients with Longstanding Ulcerative Colitis Against Colorectal Cancer

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The aim of this retrospective study was to estimate if administration of 5-aminosalicylic acid derivatives (5-ASA) on a regularly base during remissions has any benefit in prevention of colorectal carcinoma (CRC) in patients with longstanding ulcerative colitis (i.e. more than 10 years after initial diagnosis has been made). Patients and methods. The study was performed between april 2000 and may 2008 and evaluated 406 patients with longstanding UC enrolled in our regional registry. Data collection showed that 219 of 406 patients (53.94 +/- 3.15%) were taking 5-ASA derivatives every day on a regularly base for more than 9 months/year. The mean duration of therapy was 12.34+/-3.47 years in this group and the extension of colitis has been stratified as follows: proctosigmoiditis: 43.12+/-3.54%, left-side colitis 34.23+/-1.25% and pancolitis (22.25%+/-5.73%). In average, each patient received a dose of 1.645+/-0.213 grams of 5-ASA per day. Results. At the end of study we found an overall incidence of CRC of 3.02+/-0.23%. Lower incidences of 1.44+/-0.13% were observed in the 5-ASA treated group, while in the non-5-ASA treated group a significantly higher incidence of 3.23+/-0.45% has been noticed ($p<0.05$). The risk for CRC was higher in patients with pancolitis in both groups but those from the 5-ASA group has a significantly lower risk with an odds ratio of 0.51 (95% CI, 0.37-0.69) compared with 0.86 (95% CI, 0.71-0.92) for patients which did not followed chemoprevention. Conclusion. Chemoprevention with 5-ASA derivatives seems to protect patients with UC from CRC, but the results are influenced by the extension and perhaps the duration of disease.

W1971

The Novel Compound GWG-120817 Affects Cell Cycle Progression and Replication Fidelity in Colon Epithelial Cells

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Background/Aim: Individuals with IBD are at increased risk of developing colorectal cancer (CRC). 5-ASA is widely used in the treatment of IBD and prevention of IBD-associated CRC due to its anti-inflammatory properties but also by installing a replication checkpoint, by reducing the mutation rate and by inducing apoptosis. Newly developed and optimized derivatives of 5-ASA are of special interest in the field of chemoprevention. In this study, we tested the effects of GWG-120817, a new compound, with regard to its effect on superoxide scavenging, cell cycle progression, inhibition of cell growth, and improvement of replication fidelity in colon epithelial cells. Methods: Freshly isolated neutrophils were activated with PMA in the presence of 5mM GWG-120817 and superoxide was measured by lucigenin-amplified chemiluminescence. HT29 and HCT116 were treated with up to 10mM for 72 hours. Inhibition of cell growth was analyzed by MTT. Cell cycle progression of HT29 and HCT116 cells was analyzed by PI staining and mutation rates at a (CA)13-repeat were analyzed as previously described (Gasche C, PNAS 2003). Results: At 5mM GWG-120817 acts as a strong scavenger of superoxide (5% of control). GWG-120817 reduces cell growth above 1.25mM in a dose-dependent fashion. Upon 72 hours treatment (at 10mM), GWG-120817 induces a significant G2/M arrest in HCT116 cells. Also >G2/M polyploid cells increase from 2.9% to 4.5% suggesting interference of GWG-120817 with the mitotic checkpoint. Interestingly, in HT29 cells GWG-120817 induced a G1 arrest ($p=0.001$). Similar to 5-ASA, GWG-120817 led to a 50% reduction of intermediate mutant cells (M1 population) at 5mM and a 30% reduction of definitive mutant cells (M2 population) at concentrations between 2.5 and 5mM (Fig. 1). Conclusions: Our data demonstrate that GWG-120817 causes cells to accumulate in the G1- or G2/M-phase of the cell cycle depending on the type of cell (G2/M in mismatch repair-deficient HCT116 or G1 in p53-deficient and chromosomally unstable HT29). Furthermore, GWG-120817 acts as a potent superoxide scavenger and most importantly increases replication fidelity. These findings suggest that GWG-120817 is a potential candidate for colorectal cancer chemoprevention.

W1972

Both High and Low-Dose Aspirin Inhibits the Growth of Human Esophageal Adenocarcinoma in Nude Mice

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BACKGROUND AND AIMS: Epidemiological studies have shown that regular use of aspirin (ASA) is associated with a reduced risk of esophageal cancer. However there has been no direct evidence that ASA prevents esophageal adenocarcinoma (EAC). We examined whether treatment with ASA affects the growth of human EAC in a nude mice xenograft model. METHODS: The effect of ASA was evaluated both *In Vitro* and *In Vivo*. For *In Vitro* studies, human EAC cells (OE33 cell line) were treated with ASA (0-5mM) to evaluate proliferation (BrdU), apoptosis (ELISA) and migration (Transwell chambers). *In Vivo* model: OE33 cells were subcutaneously inoculated into two 6-week-old athymic nude mice. OE33-derived tumours were excised and cut up. Xenografts from primary tumours were subcutaneously re-implanted into new mice which were randomized to different treatments (6 animals/group): low-dose ASA (5 mg/Kg/day) or high-dose ASA (50 mg/Kg/day). For each treatment, a control group of animals (vehicle) carrying xenografts derived from the same primary tumour was included. Tumour growth was assessed twice a week and tumour volume was estimated by the formula LxW2x /6 (L=largest diameter, W=smallest diameter). After 2 months mice were sacrified. Both ASA and salicylic acid (SA) levels in mice plasma were assessed by HPLC. Histological and immunohistochemical (Ki67) analysis of tumours were performed. Data analysis was carried out using a repeated measures variance analysis and anova test. RESULTS: Cell proliferation and migration was significantly inhibited ($p< 0.05$), while apoptosis was significantly increased ($p< 0.05$) by ASA (0-5 mM, dose-dependent effects). Histological analysis of OE33-derived tumours showed that all of them were poorly differenciated adenocarcinomas, which invaded blood, linfatic vessels and perineural capsule. All animals receiving ASA but none of the control mice showed detectable plasmatic levels of ASA and SA. Although ASA did not induce tumour remission at any dose, tumour progression was significantly lower in ASA-treated mice when compared to non-treated animals (614% low-dose ASA vs 5505% CONTROL,$p< 0.01$; 427% high-dose ASA vs 2124% CONTROL,$p< 0.01$). This effect was associated with a significant decrease of proliferation index (ki67 positive cells/total cells) in tumours (47.35±6.74% in ASA 50 vs 56.10±4.17% in control group, $p=0.022$ and 46.58±3.58% in ASA 5 vs 55.84 ±1.82% in control group, $p<0.01$). Tumoral inhibition was 85% and 78.3% in ASA 5 and ASA 50 groups respectively. CONCLUSIONS: Both low and high-dose ASA inhibits the growth of human EAC xenografts in nude mice, which suggests a potential role for ASA in the treatment of this type of tumours.

W1973

Sodium Butyrate Enhances Growth Inhibitory Effect of TGF-Beta On Gut Epithelial Cells

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The molecular mechanisms by which dietary fiber decreases the risk of colorectal cancers are not known. Previously, we have shown that sodium butyrate (NaB), a short-chain fatty acid produced in the human gut by bacterial fermentation of dietary fiber, enhances tumor suppressor TGF-beta signaling in normal gut epithelial cells. Therefore, we hypothesize that the chemo-preventive effects of NaB is mediated in part by enhancing the growth inhibitory effect of TGF-beta on gut epithelia. To test our hypothesis, we used rat intestinal epithelial (RIE-1) cells as a model of normal gut epithelia. Treatment with NaB (5 mM) and TGF-beta1 (40 pM) reduced cell number by 86% on day 2 compared to either treatment alone (40% for TGF-beta1 and 66% for NaB). This synergistic effect on growth inhibition was observed from day 2 to day 7 after treatment. To further investigate the underlying mechanisms for growth inhibition, we analyzed cell cycle progression, apoptosis induction and differentiation. We found that (1) TGF-beta1, NaB, and the combination of NaB and TGF-beta1 respectively increased G0/G1 phase fraction by 1.46-fold, 1.16-fold, and 1.73-fold and correspondingly reduced S phase fraction by 1.67-fold, 1.35-fold, and 2.58-fold, compared to control. Therefore there was no synergistic effect observed on cell cycle arrest; (2) TGF-beta1 and NaB alone did not induce apoptosis; however, the combination of NaB and TGF-beta1 treatment induced apoptosis 2.01-fold compared to control; (3) NaB alone induced alkaline phosphatase (ALP) gene expression, a marker of differentiation, by 176-fold, TGF-beta1 alone had no effect on ALP expression, while the addition of TGF-beta1 did not further enhance NaB-induced ALP expression. Taken together, our data demonstrate that NaB enhances the growth inhibitory effect of TGF-beta and that this effect may be mediated through the induction of apoptosis. Our data provide a novel mechanism by which NaB decreases the risk of colorectal cancers.

W1974

Dietary Sulforaphane Enhances Sulindac-Induced Chemoprotection Against Colon Tumor in Azoxymethane + Dextran Sodium Sulfate-Treated Mice

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Background and Aims: NSAIDs induce chemoprevention against colon cancer. However, long term use of NSAIDs frequently causes serious problems, such as GI bleeding and cerebral vascular events. Thus, it is important to minimize adverse effects of NSAIDs in