Aspirin, NSAIDs, and Colorectal Cancer: Possible Involvement in an Insulin-Related Pathway

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Abstract

Introduction: Aspirin and nonsteroidal anti-inflammatory drugs (NSAIDs) have been shown to reduce risk of colorectal cancer. Although inhibition of cyclooxygenase (COX)-2 is generally thought to be the relevant mechanism, aspirin-like drugs apparently are involved in other pathways and mechanisms. We explore the associations between aspirin/NSAIDs, the insulin-related pathway, and the risk of colorectal cancer. Methods: Genetic polymorphisms of five genes identified as being involved in an insulin-related pathway were genotyped using data collected in a case-control study of 1346 incident colon cancer cases and 1544 population-based controls and 952 incident rectal cancer cases and 1205 controls. Genotypes assessed were the 3′ untranslated region poly(A) and the intron 8 *Bsm*I polymorphisms of the *VDR* gene, a CA repeat polymorphism of the *IGF1* gene, the A/C polymorphism at nucleotide −202 of the *IGFBP3*, the Gly972Arg polymorphism of the *IRS1* gene, and the Gly1057Asp polymorphism of the *IRS2* gene. Results: Use of aspirin and NSAIDs was associated with a decreased risk of colorectal cancer, with slightly greater protection from NSAIDs than aspirin for rectal cancer. We observed a significant interaction between *IRS1* genotype and aspirin/NSAIDs use and risk of colorectal cancer. Relative to the *GR/RR IRS1* genotype, a protective effect from the *GG IRS1* genotype was seen in those who did not use NSAIDs; use of NSAIDs was protective for all genotypes. These associations were especially strong for those diagnosed prior to age 65 (*P* interaction = 0.0006). We also observed a significant interaction between aspirin/NSAIDs use and the *VDR* gene. Having the *SS* or *BB VDR* genotypes reduced risk of colorectal cancer among non-aspirin/NSAID users; however, aspirin/NSAIDs reduced risk for all *VDR* genotypes. Conclusions: These data support the protective effect of aspirin and NSAIDs on colorectal cancer risk. In addition, the observed interactions for aspirin/NSAIDs and *IRS1* and *VDR* genotypes suggest that mechanisms other than COX-2 inhibition may be contributing to the protective effect of aspirin and NSAIDs on colorectal cancer risk. (Cancer Epidemiol Biomarkers Prev 2004;13(4):538–545)

Introduction

Use of aspirin and nonsteroidal anti-inflammatory drugs (NSAIDs) have been associated with reduced risk of colorectal cancer in both case-control and cohort studies (1–8). Although fewer studies of rectal cancer and aspirin and NSAIDs have been reported, there are reports of these agents having chemopreventive properties for rectal cancer as well (4, 9, 10). Studies of colorectal adenomas provide additional support for the protective effect of aspirin/NSAIDs on reducing the likelihood of colorectal polyp recurrence (11, 12). The mechanism of protection has been proposed to be inhibition of a cyclooxygenase (COX)-2 pathway, a pathway that involves the regulation of prostaglandin synthesis and related cell growth (7, 13). Other studies, although not directly related to colorectal cancer, suggest possible biological mechanisms for the action of aspirin and NSAIDs that do not involve COX-2 pathway (14–16). One proposed mechanism involves aspirin/NSAID effects on an insulin-related pathway (17). Because inflammation is one condition that is thought to promote insulin resistance (17), aspirin/NSAIDs may reduce inflammation and therefore impact colorectal cancer risk by altering insulin resistance.

The hypothesis that an insulin-related pathway and insulin resistance is involved in the etiology of colon cancer has been constructed through a variety of research results (17). Diet and life-style factors, such as physical activity and body mass index (BMI), have been associated with insulin levels as well as with colon cancer (18, 19). A growing body of research suggests that insulin and insulin-like growth factors may be associated with colon cancer (20–22).

The insulin-like growth factor 1 (*IGF1*), insulin-like growth factor binding protein 3 (*IGFBP3*), insulin receptor substrate 1 (*IRS1*), insulin receptor substrate 2 (*IRS2*), and the vitamin D receptor (*VDR*) genes have been proposed as being directly or indirectly involved in insulin-related pathways. Polymorphisms of these genes have been identified, some of which have been shown

Received 8/8/03; revised 11/26/03; accepted 12/9/03.

Grant support: National Cancer Institute grants CA48998 and CA85846 (M. S.), Utah Cancer Registry (contract N01-PC-67000 from the National Cancer Institute), State of Utah Department of Health, Northern California Cancer Registry, and Sacramento Tumor Registry.

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to have effects on insulin resistance and/or colon cancer risk. High *IGF1* levels have been associated with an increased risk of colorectal cancer, and variation in serum *IGF1* levels is associated with a polymorphism 1 kb upstream of the transcription start site (23). This is a CA repeat polymorphism, and the most common allele contains 19 CA repeats and is denoted ''192'' for the size of the PCR product (23). In men, serum *IGF1* concentrations were lower with the 192/192 genotype than for other *IGF1* genotypes, so one could predict that this genotype might be associated with a decreased risk of colorectal cancer. High levels of *IGFBP3* have been associated with a reduced risk of colorectal cancer (24). An A/C polymorphism at nucleotide −202 is associated with different levels of *IGFBP3* in a dose response fashion (*i.e.*, AA > AC > CC). The AC or CC genotypes would thus be predicted to be associated with an increased risk of colorectal cancer. Polymorphisms of the *VDR* gene have been associated with colorectal cancer and adenomas (25–28); in particular, an intron 8 *Bsm*I polymorphism (denoted B for absence of restriction site) and a relatively short 3′ untranslated region poly(A) (donated S for short), which are in linkage disequilibrium, have been reported to be protective against colonic adenomas and prostate cancer, respectively (25, 29). In a previous study of a relatively small number of colon cancer cases, we saw a mild protective effect of these *VDR* genotypes (28). There are also data showing an association between *VDR* and insulin, *IGF1*, and *IGFBP3* levels (30–32). A recent report also showed that mice lacking a *VDR* receptor had lower serum insulin levels than mice with a functional receptor (33). A Gly972Arg (G972R) polymorphism in the *IRS1* gene has been associated with insulin resistance and type 2 diabetes and might therefore be associated with an increased risk of colorectal cancer. The Gly1057Asp *IRS2* polymorphism has been associated with obesity; therefore, a plausible link to insulin resistance and colorectal cancer exists (34). Interaction between aspirin/NSAIDs and these genes may provide insight into the hypothesis that use of aspirin/NSAIDs alters colorectal cancer risk through an insulin-related pathway.

In this study, we report associations between aspirin/NSAIDs and rectal cancer; we have previously reported similar associations for colon cancer (8). We evaluate genetic polymorphisms involved in the insulin pathway and their interaction with aspirin/NSAIDs in affecting the risk of developing colon and rectal cancer. We report associations for men and women as well as by age at time of diagnosis. We evaluate these factors because many risk factors for colorectal cancer have been shown to differ by age and sex.

Methods

Study Population. Participants in the study were from the northern California Kaiser Permanente Medical Care Program (KPMCP) and Utah. All eligible cases within these defined geographic areas were identified as possible study participants. Two study populations are included in these analyses. The first study includes cases and controls from a population-based case-control study of first primary colon cancer (*International Classification of Diseases, Second Edition* codes 18.0 and 18.2–18.9) diagnosed between October 1, 1991 and September 30, 1994. The second study includes cases with a first primary tumor in the rectosigmoid junction or rectum, who were identified between May 1997 and May 2001 in Utah and KPMCP. Case eligibility was verified by the Surveillance Epidemiology and End Results cancer registries in northern California and in Utah. In both studies, cases were identified using rapid-reporting systems and eligibility included being between 30 and 79 years old at time of diagnosis, English speaking, mentally competent to complete the interview, no previous history of colorectal cancer (35), and no known (as indicated on the pathology report) familial adenomatous polyposis, ulcerative colitis, or Crohn's disease.

Controls were matched to cases by sex and by 5-year age groups. At the KPMCP, controls were randomly selected from membership lists; in Utah, controls 65 years and older were randomly selected from the Health Care Financing Administration lists and controls younger than 65 years were randomly selected from driver's license lists. The analysis includes 1346 colon cancer cases and 1544 controls interviewed between February 1991 and May 1994 and 952 rectal cancer cases and 1205 controls interviewed between October 1997 and January 2002. For the colon study, 80.8% of cases and 71.6% of controls whom we were able to contact were interviewed; for the rectal study, we interviewed 73.2% of cases and 68.8% of controls contacted. Response rates, or the number interviewed over all persons identified, were 71.8% for colon cancer cases and 68.0% for controls selected for the colon cancer study and 65.2% of cases and 65.3% of controls for the rectal cancer study.

Genetic Variants. DNA was extracted from peripheral blood leukocytes. Of the colon cancer cases and controls who were interviewed, 1048 cases and 1194 controls had DNA available for analysis. Of the 952 rectal cancer cases and 1205 controls interviewed, 827 cases and 1031 controls had DNA extracted. Of these, genotypic data were available for 793 rectal cancer cases and 994 controls for *VDR*, 792 rectal cancer cases and 985 controls for *IGF1*, 794 rectal cancer cases and 989 controls for *IGFBP3*, 796 rectal cancer cases and 988 controls for *IRS1*, and 775 cases and 984 controls for *IRS2*.

VDR. Intron 8 *Bsm*I polymorphism: Genomic DNA was amplified and digested as described previously (28). Presence of the restriction site was scored as allele ''b'' and absence of the restriction site was scored as allele ''B.'' 3′ Untranslated region poly(A) repeat: Genomic DNA was amplified and allele length was determined as described previously (28). Repeat length was classified as short (14–17 repeats) or S or long (18–22 repeats) or L or as described by Ingles *et al.* (29).

IRS1. The G972R polymorphism was detected using PCR amplification with primers 5′-CTTCTGTCAGGTGTCCATCC and 5′-TGGCGAGGTGTCCACGTAGC and subsequent *Bst*NI digestion (36). PCR cycling consisted of an initial denaturation at 94C for 2 min, 10 cycles at 94C, 60C, and 72C for 10 s each followed by 30 cycles at 94C, 55C, and 72C for 10 s each. Genotypes were scored as either G for glycine or R for arginine (absence or presence of the restriction site, respectively).

IRS2. The G1057D (G>A) polymorphism was detected using a previously described TaqMan assay (Ehrmann *et al*.) with minor modifications. Primer and probe sequences for the TaqMan assay were as follows: primer IRS2-F 5′GGA GCT GTA CCG CCT GCC3′, primer IRS2-R 5′ACC AAA AGC CAT CTC GGT GT3′, G-probe FAM-CCG GGC GCC GCC TCA T-Trauma, and A-probe VIC-CCG ACG CCG CCT CAT CGT T-Tamra. Each 17 μl PCR reaction contained 20ng genomic DNA, 900 nM of each primer, 130 nM of each TaqMan probe, and 8.5 μl TaqMan 2x Universal PCR Master Mix (contains AmpErase UNG and AMpliTaq Gold enzymes, dNTPs, and reaction buffer). PCR was carried out under the following conditions: 95°C for 10 min, followed by 40 cycles of 95° for 15 sec, and 62°C for 1 minute using the BIO-RAD IQ detection system. The fluorescence of each sample was collected and analyzed version 3.0 of the iCycler IQ Real-Time detection software.

IGF1. The *IGF1* CA repeat was PCR amplified using primers *IGF1*-F 5′-GCTAGCCAGCTGGTGTTATT and *IGF1*-R 5′-ACCACTCTGGGAGAAGGGTA (37). PCR conditions consisted of a 2-min denaturation at 94°C followed by 30 cycles of 94°C for 10 s, 57°C for 10 s, and 72°C for 15 s. The *IGF1* products were electrophoresed on 6% denaturing polyacrylamide gels at 70 W for 3 h. The gels were dried and exposed to X-ray film. Alleles were assigned by size of fragment (in bp) and classified as ''192'' or ''no 192.'' ''192'' is the PCR product size of the most common allele, which contains 19 CA repeats.

IGFBP3. The A/C single nucleotide polymorphism at nucleotide −202 was evaluated by PCR amplification using primers F 5′-CCACGAGGTACACACGAATG and R3 5′-TGAGCAGCCGGGGCCGAG and *Alw*21I digestion (38). Amplitaq gold (0.5 units) and 5% DMSO were used to increase efficiency of amplification. PCR conditions were 9-min initial denaturation at 95°C followed by 40 cycles at 95°C for 10 s and 66°C for 20 s. The resulting PCR product was digested with *Alw*21I (4 units) at 37°C overnight. Digested products were separated on a 2% Nusieve gel stained with ethidium bromide and visualized with UV light. Genotypes were scored by the presence of the A/C allele; band sizes of 242 and 162 corresponded to the A allele, while band sizes of 288 and 162 corresponded to the C allele.

Questionnaire Data. Data were collected by trained interviewers; quality control procedures have been described previously (39). The referent period was the calendar year 2 years prior to date of diagnosis or interview. Regular use of aspirin and NSAID use was collected. Regular use was defined as using at least thrice a week for 1 month or more. Year started and stopped using aspirin and NSAIDs were collected. Other questionnaire data included information on physical activity, diet, family history of cancer, and recalled height and weight 2 years prior to interview.

Statistical Methods. SAS Statistical Package, Version 8.2 was used to conduct the analysis. The B and b *Bsm*I *VDR* alleles are highly associated with the S and L poly(A) alleles, respectively. Because we did not have data on both polymorphisms for all cases, we combined the polymorphism results and report the genotypes as BB or SS, bb or LL, or other (most of which are Bb/SL). Separate analyses of *Bsm*I and poly(A) polymorphisms showed no additional associations (data not shown). Among those that had data for both genotypes, 97% of those with the LL genotype also had the bb genotype; between SS and BB, there was 95% agreement; and between LS and Bb, there was 96% agreement. The *IRS1* polymorphism was designated as GG or GR/RR. *IGF1* genotypes were homozygous 192/192, heterozygote 192/no 192 alleles, or homozygous with no 192 alleles. *IGFBP3* was categorized as CC, CA, or AA genotypes. *IRS2* was genotyped as GG, GD, or DD. Tumor site was defined as proximal (cecum through transverse colon), distal (splenic flexure, descending, and sigmoid colon), and rectal (rectosigmoid junction and rectum).

Nonconditional logistic regression analysis was done to generate odds ratios (OR) and 95% confidence intervals (CI). In these models, the following factors were adjusted: age at diagnosis or selection; gender (for combined analysis); long-term, vigorous, leisure time physical activity; BMI (kg/m^2); energy intake; dietary fiber; calcium; and usual number of cigarettes smoked. Age-specific analyses were done assessing those diagnosed after age 65 and 65 or younger. Sex-specific analysis for men and women was done. Statistical differences in effect were tested by determining the relative excess risk from interaction (RERI) and corresponding 95% CI as well as by testing for significant differences in slopes using the Wald χ^2 test. The RERI (40) provides insight into differences that might be expected on an additive scale of interaction, while the test for differences in slopes is related to multiplicative interaction.

Results

Colon cancer cases were slightly older than rectal cancer cases (Table 1). For both colon and rectal cancer, cases were more likely to be male than female. Thirty percent of the population reported taking aspirin on a regular basis, while about 25% reported using NSAIDs drugs regularly. Colon cancer cases were slightly less likely to have the BB *Bsm*I or SS poly(A) *VDR* genotype while they were slightly more likely to have the GR/RR genotypes of *IRS1*.

Using aspirin reduced risk of rectal cancer in women but not in men (P for gender-aspirin interaction < 0.01; Table 2). However, NSAIDs were associated inversely with rectal cancer risk in both men and women. Among women, ORs were similar to those observed for aspirin. Use of either aspirin or NSAIDs resulted in slightly higher ORs (less protective effect) for men than for NSAIDs only; among women, associations were similar for aspirin, NSAIDs, or combination of aspirin/NSAIDs. The association between aspirin and colon cancer for men and women has been reported previously with data from an additional medical center not evaluated for rectal cancer in the current study and are not included in Table 2 (8). For purposes of comparison with rectal cancer, the ORs for colon cancer from the two institutions, which were evaluated for rectal cancer, are as follows: for aspirin

Ehrmann DA, Xu T, Issei Y, Cox NJ, Bell GI. Relationships of Insulin Receptor Substrate-1 and 2 genotypes to phenotypic features of polycystic ovary syndrome. J Clin Endocrinol Metab, 2002;87:4297–300.

Table 1. Description of population

	Colon		Rectal	
	Cases [*n* (%)]	Controls [*n* (%)]	Cases [*n* (%)]	Controls [*n* (%)]
Age				
<50	108 (8.0)	163 (10.6)	147 (15.4)	178 (14.8)
50–59	265 (19.7)	267 (17.3)	248 (26.1)	304 (25.2)
60–69	475 (35.0)	546 (35.4)	319 (33.5)	392 (32.5)
70–79	498 (37.0)	568 (36.8)	238 (25.0)	331 (27.5)
Gender				
Male	756 (56.2)	845 (54.7)	559 (58.7)	673 (55.9)
Female	590 (43.8)	699 (45.3)	393 (41.3)	532 (44.2)
Education level				
Below high school	217 (16.1)	193 (12.5)	104 (10.9)	127 (10.6)
High school	372 (27.6)	431 (27.9)	226 (23.7)	270 (22.4)
College	607 (45.1)	708 (45.9)	473 (49.7)	578 (48.0)
Graduate school	150 (11.1)	211 (13.7)	149 (15.7)	229 (19.0)
Aspirin use (ever on regular basis)				
Yes	362 (26.90)	514 (33.3)	267 (28.1)	398 (33.1)
No	984 (73.1)	1029 (66.7)	684 (71.9)	804 (66.9)
Ibuprofen use (ever on regular basis)				
Yes	259 (19.3)	383 (24.9)	189 (19.9)	333 (27.7)
No	1082 (80.7)	1156 (75.1)	761 (80.1)	870 (72.3)
*VDR Bsm*I				
bb	433 (37.1)	410 (35.6)	308 (40.0)	368 (37.7)
Bb	568 (48.7)	538 (46.7)	348 (45.2)	465 (47.6)
BB	165 (14.2)	205 (17.8)	114 (14.8)	144 (14.7)
VDR poly(A)				
LL	373 (37.8)	406 (35.0)	310 (39.9)	373 (37.5)
LS	479 (48.6)	545 (47.0)	351 (45.1)	466 (46.9)
SS	134 (13.6)	208 (18.0)	117 (15.0)	155 (15.6)
IRS1				
GG	998 (84.5)	1031 (88.4)	681 (87.2)	873 (88.4)
GR/RR	183 (15.5)	135 (11.6)	100 (12.8)	115 (11.6)
IRS2				
GG	467 (46.5)	481 (41.2)	325 (42.4)	421 (42.8)
GD	409 (40.7)	552 (47.3)	343 (44.8)	423 (43.0)
DD	128 (12.8)	134 (11.5)	98 (12.8)	139 (14.1)
IGF				
192/192	433 (37.0)	479 (40.9)	327 (42.1)	399 (40.5)
192/no 192	567 (48.5)	533 (45.5)	348 (44.8)	450 (45.7)
no 192	170 (14.5)	159 (13.6)	102 (13.1)	136 (13.8)
IGFBP3				
CC	303 (25.9)	340 (29.2)	221 (28.4)	267 (27.0)
CA	613 (52.4)	565 (48.6)	384 (49.3)	517 (52.3)
AA	253 (21.6)	258 (22.2)	174 (22.3)	205 (20.7)

use, OR = 0.7, 95% CI = 0.5–0.8 for women and OR = 0.8, 95% CI = 0.6–0.9 for men. The association for NSAIDs and colon cancer for women and men were OR = 0.8, 95% CI = 0.6–1.1 and OR = 0.6, 95% CI = 0.4–0.8, respectively. The associations for use of either aspirin or NSAIDs on a regular basis and colon cancer were OR = 0.7, 95% CI = 0.6–0.9 and OR = 0.7, 95% CI = 0.6–0.8, respectively, for women and men. There were no significant differences in risk according to age at diagnosis for either colon or rectal cancer (data not shown in table).

The associations of the insulin-related pathway genotypes and use of aspirin or NSAIDs on colorectal cancer risk are shown in Table 3. Although associations were slightly stronger for rectal cancer than for colon cancer, there were no statistically significant site differences; therefore, data are presented for colorectal cancer. Analysis of aspirin and NSAIDs separately showed similar results, although associations were slightly stronger for NSAIDs (data not shown in table). There was a statistically significant interaction between both *VDR* and *IRS1* genotypes and aspirin/NSAIDs. For the *VDR* gene, having the SS or BB genotypes reduced risk of colorectal cancer among nonusers of aspirin/NSAIDs to a similar extent as that observed for using aspirin/NSAIDs regardless of genotype. The *IRS1* polymorphism modified the effect of NSAIDs. Among those taking NSAIDs with the *GG/RR,* the OR was 0.52, while among those not taking NSAIDs with the *GG* genotype, the OR was 0.67. Aspirin/NSAID use reduced risk of colorectal cancer irrespective of genotype. Results were slightly stronger for women than for men in that the inverse associations with both aspirin/NSAIDs and *SS/BB VDR* and *GG IRS1* genotypes were greater (data not shown in table). The only gender-related unique finding was among women where we observed a significant interaction between aspirin/NSAIDs and *IGFBP3* for colon cancer risk (P = 0.03). Women taking aspirin/NSAIDs with the CC genotype were at a 60% decreased risk of colon cancer (95% CI = 0.3–0.7) relative to women not taking aspirin/NSAIDs with the CC genotype; a 30–40% reduction in risk

Table 2. Associations between aspirin, NSAIDs, and rectal cancer

	Men			Women			Everyone
	Cases	Controls	OR (95% CI)	Cases	Controls	OR (95% CI)	OR (95% CI)
Aspirin							
Never	378	444	1.0	306	360	1.0	1.0
Current/ever	181	227	0.9 (0.7–1.2)	86	171	0.6 (0.4–0.8)	0.8 (0.6–0.9)
Never	378	444	1.0	306	360	1.0	1.0
Current	151	190	0.9 (0.7–1.2)	62	132	0.6 (0.4–0.8)	0.8 (0.6–1.0)
Ever use	29	35	0.9 (0.5–1.6)	20	36	0.7 (0.4–1.2)	0.8 (0.5–1.2)
NSAIDs							
Never	467	512	1.0	294	358	1.0	1.0
Current/ever	91	159	0.6 (0.5–0.8)	98	174	0.6 (0.5–0.9)	0.6 (0.5–0.8)
Never	467	512	1.0	294	358	1.0	1.0
Current	67	119	0.6 (0.4–0.8)	71	127	0.6 (0.4–0.9)	0.6 (0.5–0.8)
Ever use	24	38	0.7 (0.4–1.2)	27	42	0.8 (0.5–1.4)	0.8 (0.5–1.1)
Either aspirin or NSAIDs							
Never	318	346	1.0	231	254	1.0	1.0
Current/ever	241	347	0.8 (0.6–1.0)	162	278	0.6 (0.5–0.8)	0.7 (0.6–0.8)
Never	317	343	1.0	230	353	1.0	1.0
Current	200	269	0.8 (0.6–1.0)	119	226	0.6 (0.4–0.7)	0.7 (0.6–0.8)
Ever use	42	61	0.7 (0.5–1.1)	44	53	0.9 (0.5–1.4)	0.8 (0.6–1.1)

Note: ORs and 95% CIs are adjusted for age, BMI, physical activity, cigarette smoking, energy intake, dietary fiber, and calcium.

of colon cancer was observed for the AC/AA genotypes when taking aspirin/NSAIDs.

Assessment of associations by age at diagnosis showed significantly stronger associations between *IRS1* genotype and aspirin/NSAIDs among those diagnosed prior to age 65 (P interaction = 0.0006; Table 4). The pattern of association was similar to that described overall for younger people. There were no significant age-related interactions between aspirin/NSAIDs and other genotypes.

Discussion

Aspirin-like drugs have been identified as factors that reduce the risk of colorectal cancer, with increasing support for their protective effect coming from clinical trials (11, 41, 42). The major mechanism of action of these anti-inflammatory agents has been thought to be their inhibitory effect on the COX-2 pathway (43). COX-independent actions also have been proposed, stimulated in part by the observations that NSAIDs inhibit tumor formation and growth in COX-deficient cell lines (14). It has been proposed that aspirin and other NSAIDs reduce inflammation and that a reduction in inflammation decreases insulin resistance (17).

There is a great deal of evidence that aspirin/NSAIDs have effects on insulin resistance. It has been long known that salicylates have a hypoglycemic effect and that they reduce fasting blood glucose in diabetic persons (44–48). High doses of salicylates have been shown to reverse hyperglycemia, hyperinsulinemia, and dyslipidemia in obese rodents by sensitizing insulin signaling (49). In patients with type 2 diabetes, aspirin treatment has been shown to reduce fasting plasma glucose, total cholesterol, C-reactive protein, triglycerides, and insulin clearance; aspirin reduced hepatic glucose production and improved insulin-stimulated peripheral glucose uptake by 20% (50). Aspirin/NSAID influence on insulin resistance appears to be independent of COX-2 inhibition, instead involving inhibition of nuclear factor-κB and IκB and/or activation of peroxisome proliferator-activated receptors (49). An interaction between aspirin and *IRS1* in antagonizing effects of tumor necrosis factor-α (TNF-α) has also been reported. TNF-α, a major cause of insulin resistance in obesity and inflammation, has been reported to inhibit insulin-induced glucose uptake by targeting components of the insulin signaling cascade, one of which is insulin receptor substrate (51–55). *IRS1* is the major cytoplasmic substrate of the insulin receptor in most insulin-sensitive tissues and is necessary for maintenance of metabolic homeostasis. Aspirin has been shown to inhibit the TNF-α-induced serine phosphorylation of *IRS1* through inhibition of multiple serine kinases, including IB kinase (50).

The results of our current study support the notion that aspirin/NSAIDs may be altering colorectal cancer risk at least in part through effects on insulin resistance, as we saw interactions between aspirin/NSAID use and polymorphisms in genes in an insulin-related pathway. The nature of this interaction was that certain genotypes (*i.e.*, *SS* or *BB* of *VDR* and *GG* of *IRS1*) showed a protective effect only in those who were not taking aspirin/NSAIDs; those taking aspirin/NSAIDs were protected against colorectal cancer regardless of genotype (Tables 3 and 4). Effects of these polymorphisms are only seen in those whose insulin-related pathway and insulin resistance have not been already down-regulated by aspirin/NSAIDs. These genes and their respective polymorphisms are plausible candidates for effects on colon cancer risk and/or insulin resistance. The G972R *IRS1* polymorphism has been associated with a 50% reduction in insulin sensitivity (55), so the *GG* genotype would be predicted to decrease insulin resistance and the risk of colorectal cancer. *VDR* is involved in many pathways in addition to insulin, so the observed interaction between *VDR* and aspirin/NSAIDs could suggest the possibility of other pathways and mechanisms. However, vitamin D-related

Table 3. Combined effects of NSAID use and genotype on colorectal cancer risk

Aspirin/NSAID use	Everyone	
	Never	Current/ever
VDR		
LL or bb (*n*)	473/395	303/414
LS or Bb	498/471	368/498
SS or BB	171/206	130/181
LL or bb [OR (95% CI)]	1.00	0.59 (0.48–0.72)
LS or Bb	0.91 (0.75–1.09)	0.61 (0.50–0.74)
SS or BB	0.70 (0.54–0.89)	0.59 (0.45–0.77)
P interaction[a]	0.01	
IRS1		
GR/RR (*n*)	171/116	111/134
GG	971/943	693/952
GR/RR [OR (95% CI)]	1.00	0.52 (0.37–0.74)
GG	0.67 (0.52–0.87)	0.46 (0.35–0.60)
P interaction	0.02	
IRS2		
GG (*n*)	456/445	332/453
GD	431/475	314/497
DD	130/137	93/134
GG [OR (95% CI)]	1.00	0.70 (0.58–0.85)
GD	0.91 (0.75–1.09)	0.60 (0.50–0.74)
DD	0.93 (0.71–1.23)	0.65 (0.48–0.88)
P interaction	0.93	
IGF1		
192/192 (*n*)	439/434	314/441
192/no 192	536/470	370/508
no 192	159/157	113/117
192/192 [OR (95% CI)]	1.00	0.68 (0.56–0.83)
192/no 192	1.15 (0.96–1.39)	0.71 (0.58–0.86)
no 192	0.98 (0.76–1.28)	0.78 (0.58–1.03)
P interaction	0.35	
IGFBP3		
CC (*n*)	315/295	204/309
CA	572/541	417/537
AA	250/221	174/240
CC [OR (95% CI)]	1.00	0.60 (0.47–0.77)
CA	0.97 (0.79–1.19)	0.68 (0.56–0.84)
AA	1.04 (0.82–1.33)	0.64 (0.50–0.83)
P interaction	0.54	

Note: ORs and 95% CIs are adjusted for age, BMI, energy intake, long-term vigorous physical activity, calcium, dietary fiber, and usual number of cigarettes smoked.
[a]Interactions based on RERI.

compounds can inhibit cancer cell growth possibly through interference with insulin-like growth factors or by altering insulin levels (56, 57) and *VDR* also is thought to be involved in *IGFBP3* regulation (58). There are also data showing an association between *VDR* and insulin, *IGF1*, and *IGFBP3* levels (30–32) as well as a report showing that mice lacking functional *VDR* have lower insulin levels (33). The particular genotypes we identify as protective have also been shown to be protective against colonic adenomas and prostate cancer (25, 29, 59) and in our previous smaller study of colonic adenocarcinoma (28).

Among women (data not shown in tables), we also observed a significant interaction between aspirin/NSAIDs and an *IGFBP3* polymorphism such that aspirin use was of greatest benefit to those with the CC genotype. High levels of *IGFBP3* have been associated with a reduced risk of colorectal cancer (60) and an A-to-C substitution in nucleotide −202 has been shown to result in different serum levels of *IGFBP3* in a dose response fashion (*i.e.*, AA > AC > CC; Ref. 38). Given the lower levels of *IGFBP3* among those with the CC *IGFBP3* genotype, it is reasonable that this group would indeed benefit the most from using aspirin/NSAIDs.

These data provide support for NSAIDs reducing the risk of rectal cancer for both men and women as well as for the reduced risk detected previously for colon cancer (8). Of interest is the observation that although aspirin reduced the risk of rectal cancer among women, it was not associated with reduced risk of rectal cancer among men. On the other hand, NSAIDs were associated with reduced risk of rectal cancer in both men and women. Although most studies show inverse associations between current use of aspirin/NSAIDs for colorectal cancer in both men and women, some have shown stronger associations for more proximal tumors or for women (2, 9, 10, 41).

There are limitations to the study. First, only five polymorphisms along a complex pathway have been examined. Second, we could not directly observe the effect of aspirin on the insulin pathway but can only infer that this might be important given the interaction of *IRS1* with colorectal cancer. Although our sample was large, we were limited in power to precisely assess interactions, especially for rare genotypes. Associations detected with aspirin and NSAIDs are comparable with those detected in other case-control and cohort studies. This is one of the largest, if not the largest, study to evaluate risk factors for rectal cancer. Because data were collected in the same manner for both colon and rectal studies, we are able to compare associations from measurements of exposure collected in the same manner for both cancer sites.

Table 4. Combined effects of NSAID use and *IRS1* genotype on colorectal cancer risk by age at diagnosis

Aspirin/NSAID use	<65 yr		>65 yr	
	Never	Current/ever	Never	Current/ever
IRS1				
GR/RR (*n*)	97/58	47/59	74/58	64/75
GG	465/465	329/419	506/478	364/533
GR/RR [OR (95% CI)]	1.00	0.41 (0.24–0.68)	1.00	0.65 (0.40–1.06)
GG	0.57 (0.40–0.81)	0.43 (0.30–0.62)	0.80 (0.55–1.16)	0.50 (0.34–0.73)
P interaction[a]		0.0006		0.82

Note: ORs and 95% CIs are adjusted for age, BMI, energy intake, long-term vigorous physical activity, calcium, dietary fiber, and usual number of cigarettes smoked.
[a]Interactions based on RERI.

In summary, we have demonstrated that use of NSAIDs reduce risk of rectal cancer. The *IRS1* homozygous *GG* genotype and the *VDR* BB or SS genotypes significantly reduced risk of cancer in the absence of using NSAIDs, while those who used aspirin/NSAIDs did not experience reduction in risk beyond that from the drugs themselves. These data provide some support for an aspirin/NSAIDs effect on a non-COX-2 disease pathway. Replication of these results by others will provide support for the hypothesized role of aspirin/NSAIDs in an insulin-related pathway.

Acknowledgments

We thank Thao Tran and Kazuko Yakumo for contributions in genotyping and Sandra Edwards, Karen Curtin, Roger Edwards, Leslie Palmer, Donna Schaffer, and Judy Morse for contributions in data collection and analysis.

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Cancer Epidemiol Biomarkers Prev 2004;13:538-545.