Estrogen Plus Progestin Use, Microsatellite Instability, and the Risk of Colorectal Cancer in Women

Polly A. Newcomb, Yingye Zheng, Victoria M. Chia, Libby M. Morimoto, V. Paul Doria-Rose, Allyson Templeton, Stephen N. Thibodeau, and John D. Potter

Abstract

Current users of postmenopausal hormones (PMH) have ~30% to 40% lower risk of colorectal cancer (CRC), although associations with specific types of hormones have been inconsistent. Further, it is not clear whether some tumor types have a different risk. We conducted a case-control study to examine the relationship between PMH and CRC. Cases (n = 1,004), ages 50 to 74 years, were identified from the Surveillance Epidemiology and End Results registry in Washington from 1998 to 2002; controls (n = 1,062) were randomly selected from population lists. Case tissue samples were obtained for microsatellite instability (MSI) analyses. Interviews collected risk-factor data for CRC, including detailed information on PMH. Multivariable logistic regression models estimated odds ratios (OR) and 95% confidence intervals (95% CI). Current use of any PMH was associated with a 20% reduction in CRC risk (95% CI 0.6–0.9). This reduction in risk was limited to women who had taken estrogen plus progestin (EP) preparations only (OR = 0.6, 95% CI 0.5–0.9); there was no association with estrogen-only (E alone) use (OR = 0.9, 95% CI 0.7–1.1). For women with MSI-low or MSI-stable tumors, there was a statistically significant 40% reduction in CRC risk associated with EP use (95% CI 0.4–0.9); there was no clear association with MSI-high tumors. EP use was associated with a decreased risk of CRC; however, there seemed to be no association with E alone data that are consistent with the recent Women's Health Initiative findings. Progestin may enhance the estrogenic effect of conjugated estrogen so the combination may be more biologically active in the colon than E alone. [Cancer Res 2007;67(15):7534–9]

Introduction

Observational evidence regarding the association between exogenous hormones and colorectal cancer (CRC) in women has been remarkably consistent. In a metaanalysis, Grodstein et al. reported a statistically significant inverse relative risk of 0.66 for current users of postmenopausal hormones (PMH) compared with nonusers (1). We observed over 10 years ago that it seemed the two types of treatments [unopposed estrogen and estrogen plus progestin (EP)] yielded similar inverse effects, suggesting that estrogen was the active agent (2). A similar reduction was observed in studies of PMH and colorectal adenomas (3–8). Nonetheless, some have questioned whether PMH users simply represented a population that was healthier than women not using PMH (9, 10) and that the inverse association was, instead, due to the attributes of the users rather than the preparations themselves (9, 11). Any doubts about bias, however, were resolved by the recent results of the randomized controlled trial in the Women’s Health Initiative (WHI) which found that PMH was indeed associated with a reduction in CRC risk (12). In these two studies, an important difference in effect was observed according to preparation: there was a 40% reduction in risk associated with EP use, whereas estrogen only (E alone) was not related to incidence (13). However, small numbers of CRC cases limited the interpretation.

Thus, in aggregate, epidemiologic evidence supports a protective relationship of PMH in the colorectum, yet there are many outstanding issues. Perhaps foremost is the question raised by WHI regarding the role of progestin in the colon and rectum. Further questions remain regarding specific patterns of use and subgroups, including user characteristics and tumor types, such as microsatellite unstable lesions, that may be more strongly associated with PMH use (14). We conducted a population-based case-control study to specifically evaluate the relationship between PMH type and CRC incidence.

Materials and Methods

The study was approved by the Institutional Review Board of the Fred Hutchinson Cancer Research Center in accordance with assurances filed with and approved by the U.S. Department of Health and Human Services. Informed consent was obtained from all participants. Eligible case patients included all female residents, ages 50 to 74 years, residing in the 13 counties reporting to the Cancer Surveillance System [a population-based registry that is part of the National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) program], who were newly diagnosed with invasive colorectal adenocarcinoma (International Classification of Diseases for Oncology codes C18.0, C18.2–9, C19.9, C20.0–9; ref. 15) between October 1998 and February 2002. Eligibility for all individuals was limited to those who were English-speaking with available telephone numbers, in which they could be contacted. On average, cases were identified within 4 months of diagnosis.

After cases were identified, physicians were contacted about their patients' eligibility for this study. If the physicians approved, the individual was approached with an introductory letter and then by a telephone call. Of the 1,414 eligible case patients identified, 141 (10%) were deceased, 66 (4%) were refused by physicians to contact, 22 (2%) could not be located, and 155 (11%) declined to participate, resulting in a final sample size of 1,030 case patients and an overall response proportion of 73%.

Community-based controls were randomly selected according to age distribution (in 5-year age intervals) of the eligible cases by using lists of licensed drivers from the Washington State Department of Licensing for individuals, ages 50 to 64 years, and rosters from the Health Care Financing
Administration (now the Centers for Medicare and Medicaid) for individuals older than 64 years. Of the 1,617 potential control subjects identified, 44 (3%) were deceased, 46 (3%) could not be located, and 453 (28%) declined to participate. The final study sample included 1,074 control individuals older than 64 years. Of the 1,617 potential control subjects, 44 (3%) were deceased, 46 (3%) could not be located, and 453 (28%) declined to participate. The final study sample included 1,074 control individuals older than 64 years.

We used a structured 60-min telephone interview to obtain information from all study participants on known or suspected risk factors for CRC, including use of PMH 2 years before the interview date for cases and controls. Women were asked about their use of estrogen and/or progestin, the date of use it was started, the date of use it was stopped, and total duration of use. The interview also elicited other medication use, reproductive experiences, physical activity, smoking history, selected dietary elements, height and weight, and medical history, including use of screening, family history of cancer, and demographic factors.

SEER registry reports were used to obtain information on cancer characteristics, including site, stage (local, regional, distant), and histology; along with additional demographic information. Histologic confirmation was reported for 97% of cases. For consenting cases, we collected pathology reports for a standardized review of cancer diagnoses, including site, stage, histology, grade, and distant spread.

Pathology materials. Paraffin-embedded colorectal tumors and diagnostic pathology reports were requested from a sequential sample of 757 CRC cases. Consent to acquire this material was obtained from 95% of cases, and treating institutions provided specimens for 90% of these consenting cases. In general, sections were cut from the most representative tumor block and normal tissue block and stained with H&E. Stained sections were reviewed by site pathologist(s) and a colorectal tumor block consisting of 70% to 80% tumor cells, and a block of normal tissue were selected for further sectioning. For microsatellite instability (MSI) testing (and other future tumor DNA-based studies), ten 5-µg tumor sections were prepared. DNA was extracted from tumor and normal tissue using tissue DNA extraction kits from Qiagen, Inc.

**MSI analysis.** MSI testing was completed on 590 tumors (9% had insufficient tissue for analysis) using nine markers: four mononucleotide markers (BAT25, BAT26, BAT40, and BAT234C4), four dinucleotide repeats (ACTC, D5S346, D18S55, and D10197), and one complex marker (MYCL). These markers include the five recommended markers in the panel proposed during the National Cancer Institute workshop on MSI for cancer detection. PCR fragments were tagged with a fluorescent dye and analyzed on an ABI3100 generic analyzer using a previously described protocol. For all of the cases, we corroborated the MSI results with IHC testing for hMLH1, hMSH2, and hMSH6. In a round-robin reading, this approach and interpretation has been highly reproducible.

**Definitions and statistical analysis.** PMH use was defined as any use for 6 months or more, and then was further divided, if possible, by type of preparation. Women were classified as E alone users if they had used estrogen exclusively for 6 months or more and had never used progestin, and as EP users if they had taken a combination preparation exclusively for 6 months or more. Women who had used both an E alone regimen and an EP regimen were included as a separate category, because the interpretation of

<table>
<thead>
<tr>
<th>Table 1. Age-standardized characteristics (%) of women with CRC</th>
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<tr>
<td><strong>BMI (kg/m²)</strong></td>
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<tr>
<td>&lt;22.9</td>
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<tr>
<td>22.9-25.6</td>
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<tr>
<td>25.7-29.2</td>
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<tr>
<td>&gt;29.2</td>
</tr>
<tr>
<td>Adult diabetes</td>
</tr>
<tr>
<td>Absent</td>
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<tr>
<td>Present</td>
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<tr>
<td>Smoking status</td>
</tr>
<tr>
<td>Never</td>
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<tr>
<td>Former</td>
</tr>
<tr>
<td>Current</td>
</tr>
<tr>
<td>NSAID use</td>
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<tr>
<td>Never</td>
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<td>Former</td>
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<tr>
<td>Current</td>
</tr>
<tr>
<td>Screening sigmoidoscopy</td>
</tr>
<tr>
<td>None</td>
</tr>
<tr>
<td>Within 2 y</td>
</tr>
<tr>
<td>More than 2 y, distal only</td>
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<tr>
<td>Family history</td>
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<tr>
<td>No</td>
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<tr>
<td>Yes</td>
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<td>NOTE: Case frequencies were adjusted to the age distribution of controls.</td>
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<tr>
<th>Table 2. Risk of CRC associated with use of PMH</th>
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<tr>
<td><strong>All PMH</strong></td>
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<tr>
<td>-------------</td>
</tr>
<tr>
<td><strong>Cases</strong></td>
</tr>
<tr>
<td>Never †</td>
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<tr>
<td>Ever</td>
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<tr>
<td>Current</td>
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<td>&lt;5 y</td>
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<td>≥5 y</td>
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<td><strong>P trend</strong></td>
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<td>Former</td>
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<td>≥5 y</td>
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<td><strong>P trend</strong></td>
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†Adjusted for age at diagnosis, BMI, adult onset diabetes, smoking status, regular NSAID use, colorectal screening sigmoidoscopy history within the past 10 y, physical activity, and family history of colorectal cancer.

Reference for each category of PMH use and PMH preparation.
The exposure was unclear. Current use of PMH was defined as use of hormones 2 years before the interview date to ascertain patterns of use before diagnosis (reference date). Duration of PMH use was defined as the cumulative duration of all periods of use. Women were considered nonusers if they had never used PMH or had used PMH for <6 months. Tumors were classified as MSI-stable (MSS, 0% of loci unstable), MSI-low (0% to <30% of loci unstable), or MSI-high (>30% of loci unstable); unequivocal results for at least five markers were required to classify a tumor's MSI status.

Odds ratios (OR) and 95% confidence intervals (95% CI) for the association between PMH use and CRC incidence were estimated using logistic regression models, adjusting for age (in 5-year intervals), body mass index (BMI, kg/m² in quartiles), adult onset diabetes (present, absent, or unknown), smoking status (never, former, current), regular NSAID use of 3 months or more (yes, no), colorectal screening sigmoidoscopy history within the past 10 years (yes, no, unknown), physical activity (in quartiles of hours per week), and family history of CRC (present, absent, unknown). These covariates were chosen based on a priori knowledge about risk factors for CRC. Tests of trend were conducted by including the variable in the model as an ordinal variable. To evaluate whether there were differences in the OR for E alone use versus EP use, we tested the significance of the OR for E alone use versus EP use in the logistic model. Covariates, such as age at diagnosis, BMI, and tumor characteristics, were selected as effect modifiers because they may also influence hormone levels. Tests for interactions were assessed by a change in the log-likelihood ratio after the addition of a cross-product term between the exposure and effect modifier. Hormone use was unknown for 26 cases and 12 controls; after these exclusions, 1,004 cases and 1,062 controls remained for analysis. All statistical analyses were done using SAS v8.2 (SAS Institute, Inc.); all statistical significance tests were two-sided.

**Results**

The mean age was 64.2 years (range, 50–74 years) for cases and 64.5 years (range, 50–74 years) for controls. Age-adjusted selected characteristics of cases and controls are presented in Table 1. Cases were more likely than controls to have higher BMIs (mean BMIs, 28.1 kg/m² versus 26.7 kg/m²), adult onset diabetes, ever smoked, and to have had a family history of CRC. Among cases, MSI-high status was observed in 23.7% of tumors.

For all types of PMH use, there was a statistically significant reduction in CRC risk only among current users of PMH (OR = 0.8, 95% CI 0.6–0.9), particularly among those who had used PMH for 5 years or more (Table 2). When stratified by type of preparation, the analysis showed no clear associations between E alone use and CRC risk regardless of recency or duration. There was even a suggestion of a slightly increased risk associated with former use of E alone (OR = 1.5, 95% CI 1.0–2.2). For women who used EP, there was no association between former use and CRC; however, current users had a 40% reduction in risk (95% CI 0.5–0.9), although this association seemed to be only present among women who used EP for 5 years or more. There was a statistically significant difference in the risk estimates for women who took E alone preparations and women who took EP (P = 0.01). When examining time since last use
A differential effect of PMH was observed in this study: EP use was associated with a statistically significant inverse risk, but E alone use was not associated with CRC. Neither MSI status nor stage at diagnosis seemed to modify the associations between PMH use and CRC risk. Our confidence in these findings is enhanced by the large size of the case and control groups, the population-based nature of ascertainment, and the standardized evaluation of hormone use and other CRC risk factors. Our MSI results were further confirmed with immunohistochemistry testing, which examined expression for the most commonly known mismatch repair genes (hMLH1, hMSH2, hMSH6, hPMS2; ref. 18).

Discussion

A differential effect of PMH was observed in this study: EP use was associated with a statistically significant inverse risk, but
known; data for whether the inverse association would be stronger among long-term users and whether it is independent of the reduction associated with recent use will not be available. It is also not known whether alternative regimens confer similar reductions in risk of CRC. To our knowledge, the only other study that has examined PMH use in relation to MSI colorectal tumors found that recent users of hormones had a reduced risk of MSI-high tumors, which we did not find in the present study; that study, however, used a different set of markers for determining MSI status (14).

The major risk factors for large bowel cancer—obesity, physical activity, and smoking—do seem to differ by sex; indeed, there is substantial evidence that these factors have a hormonal basis (23, 24). McMichael and Potter first suggested a role for estrogens and progestins in preventing colon cancer (25). Although the mechanism proposed at that time was based on changes in bile acid metabolism synthesis, the biological actions of hormones are myriad and are regulated by, among other things, their circulating concentrations, the conversion to more active or less active derivatives, and relevant receptor concentrations in the target tissue (26). In mammary tissue, provocative studies have shown that progesterone receptor-β elicits a response to progesterone similar to that of estrogen. The biological mechanisms underlying an effect of progestins in the colon are less clear, although they may be synergistically amplifying estrogen’s effects. Also, progesterone induces the isozyme of 17β-hydroxy steroid dehydrogenase to catalyze the conversion of the less potent estrone to the more potent estradiol (27, 28). This effect has been hypothesized to explain the increased risk of breast cancer associated with EP beyond that of E alone (29). The biological mechanisms underlying an effect of progestins in the colon are less clear, although they may be synergistically amplifying estrogen’s effects.

Epigenetic events not involving changes in DNA nucleotide sequences may also play a role. Estrogen and perhaps progestins may be key factors in the pathway leading to hypermethylation [CpG island methylator phenotype (CIMP); ref. 30], a central feature of CRC (31) in which many genes can be silenced. In small studies of CRC cases, an estimated 28% to 58% were CIMP+ (32–34). In vitro and animal studies have also suggested that estrogen intervention reduces DNA methylation of specific genes and restores protective methylation patterns (31).

Despite the consistency of this study with other observational and clinical trials, there are limitations that should be considered in interpreting our results. We relied, as have most observational studies, on self-reports of hormone use. We were unable to validate hormone use but other studies report good agreement of self-reported use of PMH and information in medical records (35–37). Not all eligible subjects were interviewed because of death, refusal, and other reasons; for laboratory analyses, we were not able to obtain tissue or have MSI testing completed on all individuals. We also have a higher proportion of MSI-high cases than that found in previous studies (18, 38, 39), which may indicate differences in survival by molecular subtype, an outcome that has been associated with MSI-high tumor status (40, 41).

The results of this large study support a role for exogenous hormones in the genesis of CRC in women. However, they modify the previously held belief, first postulated over 30 years ago by McMichael and Potter, that estrogen per se is the active agent in endogenous and exogenous hormone’s effects (25). In future investigations the mechanisms whereby estrogens and progestins reduce risk of CRC should be explored.

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References


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