Use of Analgesics and Nonsteroidal Anti-inflammatory Drugs, Genetic Predisposition, and Bladder Cancer Risk in Spain

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Abstract

Background: We assessed use of nonaspirin nonsteroidal anti-inflammatory drugs (NSAID), aspirin, paracetamol (acetaminophen), phenacetin, and metamizol (dipyrone) and risk of bladder cancer and their interaction with polymorphisms in drug-metabolizing genes.

Methods: We analyzed personal interview data from 958 incident bladder cancer cases and 1,029 hospital controls from a multicenter case-control study in Spain. A drug matrix was developed to estimate cumulative lifetime dose of active ingredients. Polymorphisms in GSTP1, SULT1A1, CYP2E1, CYP2C9, and NAT2 were examined.

Results: A significant reduction in bladder cancer risk [adjusted odds ratio (OR), 0.4; 95% confidence interval (95% CI), 0.2-0.9] was observed for regular users of nonaspirin NSAIDs compared with never users. Regular users of aspirin experienced no reduction in risk (OR, 1.0; 95% CI, 0.7-1.5). Regular users of paracetamol had no overall increased risk of bladder cancer (OR, 0.8; 95% CI, 0.4-1.3), but our data suggested a qualitative interaction with the GSTP1 I105V genotype. Subjects with at least one copy of the 359L or 144C variant alleles in the NSAID-metabolizing gene CYP2C9 had a slightly decreased risk of bladder cancer (OR, 0.8; 95% CI, 0.7-1.0; P = 0.037); however, having at least one copy of the 359L or 144C variant alleles did not significantly modify the protective effect of nonaspirin NSAID use.

Conclusion: Regular use of nonaspirin NSAIDs was associated with a reduced risk of bladder cancer, which was not modified by polymorphisms in the NSAID-metabolizing gene CYP2C9. We found no evidence of an overall effect for paracetamol or aspirin use. (Cancer Epidemiol Biomarkers Prev 2006;15(9):1696–702)

Introduction

The long-term health effect of certain medications has become an important public health issue because of the high prevalence of chronic use of many drugs. Analgesics and nonsteroidal anti-inflammatory drugs (NSAID) are among the most frequently used drugs worldwide and their molecular targets are thought to be involved in cancer development/progression based on epidemiologic associations, clinical trials in humans, and experimental studies in animals [e.g., cyclooxygenase (COX) inhibition in colorectal cancer or estrogenic modulation in breast cancer].

Increased bladder cancer risk has been associated with the use of cyclophosphamide; some studies have suggested that the over-the-counter analgesic, phenacetin, may also increase bladder cancer risk (1, 2). Phenacetin commonly occurred in drug mixtures until it was withdrawn from the market in Spain during the late 1980s because it was associated with an elevated risk of interstitial nephritis. Most epidemiologic studies have found phenacetin to be a risk factor for renal cancer (3-9), whereas the relation with bladder cancer is less conclusive (9-13).

Paracetamol (acetaminophen), a phenacetin metabolite, is currently one of the most frequently used drugs worldwide, and concerns have been raised regarding the possibility that paracetamol may have the urological carcinogenicity potential of its parent compound. The available evidence to date, although inconclusive, suggests that paracetamol causes interstitial nephritis (14), slightly increases renal cancer risk (5-9, 14-17), but does not increase bladder cancer risk (9-19).

Aspirin and other NSAIDs have been associated with reduced risks of several cancers mainly of the gastrointestinal tract (20, 21). The effect of NSAIDs on bladder cancer has been less studied and results are discrepant (11, 22-26).

Metamizol (dipyrone) is a pyrazolone derivative analgesic that was banned in the United States by the Food and Drug Administration in 1977 because agranulocytosis was associated with its use. It is still widely used in Spain, Italy, France, India, and Central/South America. There is some evidence that metamizol might be carcinogenic in both humans (27, 28) and animals (29-32). One study (11) suggested an increased risk of bladder cancer in chronic users of metamizol and similar drugs [odds ratio (OR), 2.03; 95% confidence interval (95% CI), 0.68-6.07].
Interindividual variation in the ability to metabolize certain classes of drugs might modify the effect of NSAIDs and other analgesics. The potential interaction between the use of analgesics or NSAIDs and genetic polymorphisms in metabolic enzymes has not been evaluated in bladder cancer studies but may be useful in identifying subpopulations particularly sensitive to the effects of these drugs, both deleterious and protective. The a priori hypothesis is that those subjects carrying polymorphism(s) leading to an increase in the concentration of the drug or its metabolites would tend to present more marked effects than those without such polymorphisms. A priori, we selected the main enzymes implicated in the metabolism of the drugs of interest and tested genetic polymorphisms that have been shown to have a functional effect (i.e., decreasing the activity of the enzymes). For paracetamol, 90% of the absorbed drug is inactivated by SULT1A1, UGT1A1, and UGT1A6 in the liver and kidney, 5% is excreted without being metabolized in urine, and another 5% is bioactivated by CYP2E1 generating N-acetyl-p-benzoquinoneimine (NAPQI), a highly reactive metabolite responsible for liver and kidney toxicity in acute paracetamol intoxications (33). NAPQI has been shown to form protein and DNA adducts (34). NAPQI is inactivated by GSTP1. The main enzyme involved in the metabolism of most NSAIDs is CYP2C9 (35). The metabolism of metamizol generates a toxic metabolite (4-aminoantipyrine) that is inactivated by NAT2 (36).

The purpose of this study was to assess the association of lifetime use of analgesics and NSAIDs with the risk of bladder cancer in a large, multicenter case-control study known as the Spanish Bladder Cancer Study. In addition, we evaluated the effects of polymorphisms in the genes coding for the enzymes involved in the metabolism of certain analgesics or NSAIDs. Finally, we assessed whether these effects varied according to the stage and grade of the initial tumor at diagnosis.

Materials and Methods

Study Population. We conducted a hospital-based case-control study in 18 hospitals from five regions in Spain (Barcelona, Vallés/Bages, Alacant, Tenerife, and Asturias). All newly diagnosed, histologically confirmed bladder cancer cases between ages 20 and 80 years old, who resided in the catchment area of each hospital, were included from May 1997 to December 2000. Controls were selected from in-patients at the same hospital at the same time as the cases and matched to the cases by gender, age at interview (±5 years), hospital, and ethnicity. Special care was taken to include only patients admitted to the hospital with a diagnosis thought to be unrelated to the exposures under study, including the chronic use of analgesics and NSAIDs, as eligible for control selection. Eighty-four percent of the cases and 88% of the controls eligible for study were interviewed. For cases, reasons for nonparticipation were patient refusal to be interviewed (2%), interview break-off (0.5%), and other (1%); for controls, reasons for nonparticipation were subject refusal to be interviewed (2%), interview break-off (0.5%), and other (1%). The main diagnostic categories for controls were hernia (36%), other abdominal surgery (12%), fracture (23%), other orthopedic problems (6%), hydrocele (12%), vascular diseases (4%), dermatologic problems (2%), eye surgery (1%), and other (4%). This restrictive control selection criterion was followed to prevent bias in prevalence of lifetime, regular drug use among hospital controls. In addition, we a priori selected a group of chronic and potentially painful admission diagnoses potentially associated to a greater prevalence of analgesic intake to compare the prevalence of drug use across different diagnostic categories of hospital controls [i.e., hydrocele (n = 125), hernia surgery (n = 358), varicose veins (n = 20), and fractures (n = 235), a diagnosis that might include subjects with chronic rheumatic diseases].

Cases and controls were interviewed in the hospital using a computer-assisted personal interview to obtain information on the following exposures: medication use, tobacco consumption, occupational and environmental exposures, medical and family history, and diet. Of the 1,219 cases and 1,271 controls recruited, 261 (21%) cases and 239 (19%) controls were excluded from the present analysis because they did not provide any information on medication use. In addition, three controls with a diagnosis of hip fracture who reported use of analgesics for osteoporosis were excluded.

The study protocol was approved by Institut Municipal d’Investigació Médica (Barcelona, Spain), National Cancer Institute, and local institutional review boards/ethics committees and written informed consent for study participation was obtained from all study subjects.

Drug Information. Participants were asked to report lifetime use of any anti-inflammatory or analgesic drug. A hand-card with the names of 43 commonly used analgesics was provided to the subjects to enhance recall. Drugs were recorded if they had been used at least 20 times. If used on a regular basis (i.e., twice or more weekly for ≥1 month), detailed information on dose and time periods of use was collected. Subjects who had never used the drug or had used it <20 times in their life were considered nonusers.

We evaluated the effect of consumption of commonly used drugs (i.e., aspirin, paracetamol, and metamizol) as well as a broader but homogeneous category of acetic acid NSAIDs that included diclofenac, indomethacin, and sulindac.

Determination of Exposure to Active Ingredients. Information on drugs was collected by brand names during the interview. To recode this information into active ingredients, we designed a drug matrix that included the dose of each active ingredient contained in every regularly used brand name. The matrix also included information on dose and active constituents over time because brand names were often retained after compositional changes had been introduced. This practice was particularly common in the Spanish pharmaceutical market before the 1980s. Information on brand name ingredients was essentially complete from 1960 to 2001. Official publications of the Spanish Pharmacists’ Association provided complete information on all registered drugs used after 1970 (37). A variety of sources were used to assess the ingredients of brand names before 1970, including publications of the pharmaceutical industry, personal communications, and historical sources, such as museums and advertising brochures. Each subject’s cumulative dose for each regularly taken drug was derived from the annihilation of the drug matrix.

Drug use that occurred during the year before the inclusion in the study was disregarded in both cases and controls. Cumulative dose and duration of use were recoded in terciles or values above and below the median according to the distribution of the drug exposure variable in the control group. We did not compute a cumulative dose for acetic acid NSAIDs because drugs in this category are not equipotent.

Histopathology. For all cases, the study pathologists reviewed a section from all blocks corresponding to the tumor(s) obtained at the time of the initial diagnosis to confirm diagnosis, histologic classification, and tumor stage and grade of all cases. Stage was established based on the histopathologic assessment as well as on information from clinical records, including ancillary procedures. Histologic classification and grading were done according to the WHO/International Society of Urological Pathology 1998 classification (38).

Genotype Assays. Germ-line DNA was obtained from 97% of the cases (1,125 subjects donated blood and 43 buccal cell samples) and 91% of the controls (1,042 subjects donated blood and 109 buccal cell samples). Eight hundred thirty-nine cases...
and 791 controls with valid drug and genetic information were included in the interaction analysis of drug use and genetic predisposition. Genotype assays were done at the Core Genotyping Facility, Division of Cancer Epidemiology and Genetics, National Cancer Institute. Description of genotype assays can be found at http://snp500cancer.ncc.nih.gov. We determined polymorphisms in the CYP2C9 (I593L and R144C), GSTP1 (I105V and A114V), CYP2E1 (−1054C>T), SULT1A1 (E73Q), and NAT2 (G286E, R64Q, Y94Y, I114T, L161L, and R197Q) genes. The polymorphisms were selected based on published evidence of having an effect on the functionality of the protein and also on assay availability. CYP2C9 I593L and R144C polymorphisms have been shown to affect the capacity of the enzyme to metabolize NSAIDs, among other drugs (35). GSTP1 I105V polymorphism has been reported to affect the capacity of this enzyme to metabolize carcinogens (39). CYP2E1 −1054C>T polymorphism has been related to a modification of the risk of lung cancer (40). Subjects were classified as rapid, intermediate, and slow acetylators based on the six NAT2 single nucleotide polymorphisms as described previously (41).

All genotypes under study were in Hardy-Weinberg equilibrium among the controls included in the analysis. A quality control among 101 duplicate quality control samples showed high genotype agreement: 98% for CYP2C9 R144C, 100% for CYP2C9 I593L, 100% for CYP2E1 −1054C>T, and 100% for SULT1A1 E73Q. Agreement for GSTP1 and NAT2 polymorphisms was also high as published elsewhere (41). NAT2, GSTP1, and CYP2C9 R144C assays were done in both blood and buccal cells; the other assays were done exclusively in blood samples.

**Statistical Analysis.** ORs and 95% CIs were used to estimate the relative risk and were computed from logistic regression models adjusting for matching variables (age at diagnosis/interview, gender, and region), smoking status, and use of drugs other than the exposure of interest. Current smokers were defined as subjects that reported smoking regularly within a year of the reference date and former smokers as subjects that quit regular smoking >1 year before participation in the study. Never smokers were those subjects who never smoked ≥100 cigarettes in their life. In an alternative analysis (data not shown), we further assessed possible confounding by smoking adjusting for the number of cigarettes smoked and the number of years of smoking. Point estimates remained largely unchanged, but 95% CIs were wider. We also created a variable summarizing the average lifetime levels of trihalomethanes in drinking water in residence area of the study subjects as an indicator of lifelong exposure to water chlorination byproducts (42). When we adjusted our models for lifelong exposure to water chlorination byproducts, our risk estimates remained unchanged, indicating that water chlorination byproducts did not significantly confound the relation between analgesics and NSAIDs and bladder cancer risk.

Linear trends were tested by including a single term for categorical exposures with weights specified as terciles of dose or duration of use in the controls in logistic regression models. Multiplicative interactions were tested by adding an interaction term to the logistic regression models. Because of small numbers, we assessed the effect of CYP2C9 and CYP2E1 by creating variables with only two categories: homozygous for the wild-type allele and heterozygous or homozygous for the mutated alleles. GSTP1 polymorphisms were evaluated using three categories: homozygous wild-type (Ile/Ile), heterozygous (Ile/Val), and mutated homozygous (Val/Val). All analyses were done using the statistical package Stata 8.2 (Stata Corp., College Station, TX).

**Results**

Table 1 shows the sociodemographic characteristics of the cases and controls. Subjects showed no significant differences in gender, age, region, or educational level. Cases were more prone to be current smokers than controls. Table 2 shows the prevalence of regular use of phenacetin, paracetamol, aspirin, nonaspin NSAIDs, and metamizol, overall, and for specific diagnostic subgroups of controls. Among total controls, the proportion of regular users was 9% for aspirin, 6% for paracetamol, 3% for nonaspin NSAIDs, 2% for metamizol, and 1% for phenacetin, which was similar to that among controls without these conditions.

### Table 1. Sociodemographic characteristics of study population

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total, N</td>
<td>1,029</td>
<td>958</td>
</tr>
<tr>
<td>Gender, n (%) males</td>
<td>881 (86)</td>
<td>829 (86)</td>
</tr>
<tr>
<td>Age, mean (SD)</td>
<td>64.2 (10)</td>
<td>65.4 (10)</td>
</tr>
<tr>
<td>Ethnicity, n (%)</td>
<td>1,028 (100)</td>
<td>1,028 (100)</td>
</tr>
<tr>
<td>Caucasians</td>
<td>1 (0)</td>
<td>1 (0)</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Region, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Barcelona</td>
<td>188 (18)</td>
<td>160 (17)</td>
</tr>
<tr>
<td>Valles</td>
<td>116 (12)</td>
<td>116 (11)</td>
</tr>
<tr>
<td>Alacant</td>
<td>73 (7)</td>
<td>76 (8)</td>
</tr>
<tr>
<td>Asturias</td>
<td>465 (47)</td>
<td>438 (46)</td>
</tr>
<tr>
<td>Tenerife</td>
<td>185 (18)</td>
<td>172 (18)</td>
</tr>
<tr>
<td>Education, n (%)</td>
<td>472 (46)</td>
<td>414 (43)</td>
</tr>
<tr>
<td>Less than primary school</td>
<td>400 (39)</td>
<td>386 (40)</td>
</tr>
<tr>
<td>Primary to high school</td>
<td>140 (14)</td>
<td>148 (15)</td>
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<tr>
<td>High school or more</td>
<td>13 (1)</td>
<td>12 (1)</td>
</tr>
<tr>
<td>Other</td>
<td>4 (0.4)</td>
<td>0.1 (0.1)</td>
</tr>
<tr>
<td>Unknown</td>
<td>6 (0.6)</td>
<td>6 (0.6)</td>
</tr>
<tr>
<td>Smoking status, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>401 (39)</td>
<td>185 (19)</td>
</tr>
<tr>
<td>Former</td>
<td>396 (38)</td>
<td>382 (40)</td>
</tr>
<tr>
<td>Current</td>
<td>226 (22)</td>
<td>389 (40)</td>
</tr>
<tr>
<td>Unknown</td>
<td>26 (2.5)</td>
<td>6 (0.6)</td>
</tr>
</tbody>
</table>

We found neither an overall association nor a dose-response relation between regular use of aspirin or other salicylates and bladder cancer risk. Regular use of metamizol was slightly more prevalent among cases than controls. Table 2 shows the prevalence of regular use of phenacetin, paracetamol, aspirin, nonaspin NSAIDs, and metamizol, overall, and for specific diagnostic subgroups of controls. Among total controls, the proportion of regular users was 9% for aspirin, 6% for paracetamol, 3% for nonaspin NSAIDs, 2% for metamizol, and 1% for phenacetin, which was similar to that among controls without these conditions.

### Table 2. Use of analgesics and NSAIDs

<table>
<thead>
<tr>
<th>Drug</th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenacetin</td>
<td>1.3%</td>
<td>1%</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>6%</td>
<td>2%</td>
</tr>
<tr>
<td>Aspirin</td>
<td>9%</td>
<td>6%</td>
</tr>
<tr>
<td>Nonaspin NSAIDs</td>
<td>2%</td>
<td>1%</td>
</tr>
<tr>
<td>Metamizol</td>
<td>9%</td>
<td>6%</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>4%</td>
<td>2%</td>
</tr>
<tr>
<td>Aspirin</td>
<td>9%</td>
<td>6%</td>
</tr>
<tr>
<td>Nonaspin NSAIDs</td>
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<tr>
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</tr>
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<td>9%</td>
<td>6%</td>
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<td>1%</td>
</tr>
<tr>
<td>Metamizol</td>
<td>9%</td>
<td>6%</td>
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</tbody>
</table>
Tumor Stage and Grade. No significant modification by tumor stage or grade was seen on the effects of analgesic and nonaspirin NSAID use on bladder cancer risk. There was a suggestion of a small inverse association with aspirin use (OR, 0.8; 95% CI, 0.6-1.0) and paracetamol use (OR, 0.8; 95% CI, 0.6-1.1) among cases with high-grade bladder tumors.

Polymorphisms in Drug-Metabolizing Genes. Presence of at least one variant allele for the CYP2C9 I359L genotype conferred a 20% bladder cancer risk reduction compared with homozygous wild-types, and the OR for CYP2C9 R144C variant alleles was also 0.8. In Table 5, we show the significant association with a small overall decreased risk of bladder cancer (OR, 0.8; 95% CI, 0.7-1.0; P = 0.037) of the presence of at least one minor allele of the CYP2C9 I359L or R144C polymorphisms when compared with the homozygous wild-type for both I359L and R144C polymorphisms. Nonaspirin NSAIDs seemed to be more protective among subjects with at least one minor allele of the two studied polymorphisms (I359L or R144C) compared with at least one variant allele for the CYP2C9 I359L genotype. Nonaspirin Use of paracetamol and GSTP1 I105V was significant (P for interaction between ever use of paracetamol with GSTP1 Ile/Val genotype is 0.04 and P for interaction between ever use of paracetamol with GSTP1 Val/Val is 0.008). Subjects who regularly used paracetamol for periods longer than 4 years (median length of use in controls) tended to display an increased risk of bladder cancer if they were carriers of two GSTP1 I105V variant alleles (Val/Val; OR, 2.5; 95% CI, 0.4-15.6). There was no increase in risk associated with duration of use among individuals with a GSTP1 Ile/Ile genotype (OR, 0.5; 95% CI, 0.1-1.6). Estimates for cumulative dose were similar. The CYP2E1 –1054C>T polymorphism did not modify risk associated to paracetamol use. However, users of paracetamol who were –1054C>T wild-type (i.e., with a full capacity of transforming paracetamol into its toxic metabolite, NAPQI) and who were also GSTP1 Val/Val (i.e., poor capacity of eliminating NAPQI) had a significantly higher risk of bladder cancer (OR, 2.5; 95% CI, 1.1-5.6) when compared with nonuser carriers of the same genotype.

Although the NAT2 slow acetylator phenotype was found to be associated with an increased bladder cancer risk (OR, 1.4; 95% CI, 1.2-1.7) in the Spanish Bladder Cancer Study (41), it did not significantly modify the association between bladder cancer and metimizol and other pyrazolone derivatives regular use [bladder cancer risk for regular users of pyrazolones was 1.4 (0.1-13.3) among slow acetylators, 0.9 (0.3-2.3) among intermediate acetylators, and 0.8 (0.4-1.5) among rapid acetylators].

Discussion

In this case-control study, we found an inverse association between regular use of NSAIDs and the risk of bladder cancer, particularly among regular users who used NSAIDs recently (i.e., within 1-5 years before interview). In addition, presence of at least one minor allele of the I593L or R144C polymorphisms in the CYP2C9 gene, involved in NSAID metabolism, was also associated with a small decreased bladder cancer risk. Regular users of paracetamol had no overall increased risk of bladder cancer, but our data suggested a qualitative interaction with the GSTP1 I105V genotype. Use of aspirin was not associated with an overall reduced risk.

There is some previous evidence supporting a protective effect of NSAIDs for bladder cancer. One report based on a large, population-based case-control study in southern California showed a reduced risk of bladder cancer among nonaspirin NSAID users (11), with a suggestion of a dose

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**Table 2. Prevalence of regular drug use in controls by selected admission diagnoses**

<table>
<thead>
<tr>
<th>Drug</th>
<th>All controls (N = 1,029)</th>
<th>Controls with fractures (n = 235)</th>
<th>Controls with hernia surgery (n = 358)</th>
<th>Controls with hydrole (n = 125)</th>
<th>Controls with varicose veins (n = 20)</th>
<th>Controls without these conditions (n = 291)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenacetin, n (%)</td>
<td>12 (1)</td>
<td>3 (1)</td>
<td>1 (0.3)</td>
<td>4 (3)</td>
<td>0 (0)</td>
<td>4 (1)</td>
</tr>
<tr>
<td>Paracetamol, n (%)</td>
<td>53 (6)</td>
<td>14 (6)</td>
<td>14 (4)</td>
<td>5 (4)</td>
<td>2 (10)</td>
<td>18 (7)</td>
</tr>
<tr>
<td>Aspirin, n (%)</td>
<td>85 (9)</td>
<td>14 (6)</td>
<td>30 (8)</td>
<td>11 (9)</td>
<td>1 (5)</td>
<td>29 (10)</td>
</tr>
<tr>
<td>Nonaspirin NSAIDs, n (%)</td>
<td>28 (3)</td>
<td>9 (4)</td>
<td>6 (2)</td>
<td>3 (2)</td>
<td>1 (5)</td>
<td>9 (3)</td>
</tr>
<tr>
<td>Metamizol, n (%)</td>
<td>21 (2)</td>
<td>9 (4)</td>
<td>3 (1)</td>
<td>2 (2)</td>
<td>2 (10)</td>
<td>5 (2)</td>
</tr>
</tbody>
</table>

*The nonaspirin NSAIDs considered in our study that had at least one regular user were acetaminophen, diclofenac, indomethacin, ketoprofen, dexketoprofen, ibuprofen, naproxen, piroxicam, and tenoxicam.

**Table 3. Use of phenacetin and paracetamol and risk of bladder cancer**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Cases/controls (n)</th>
<th>OR* (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenacetin Nonusers*</td>
<td>848/893</td>
<td>1.0 (Reference)</td>
</tr>
<tr>
<td>Ever users</td>
<td>79/67</td>
<td>1.1 (0.7-2.0)</td>
</tr>
<tr>
<td>Nonregular</td>
<td>50/25</td>
<td>1.1 (0.6-2.0)</td>
</tr>
<tr>
<td>Regular</td>
<td>7/12</td>
<td>1.3 (0.3-4.5)</td>
</tr>
<tr>
<td>Paracetamol Nonusers*</td>
<td>664/670</td>
<td>1.0 (Reference)</td>
</tr>
<tr>
<td>Ever users</td>
<td>243/295</td>
<td>0.8 (0.6-1.0)</td>
</tr>
<tr>
<td>Nonregular</td>
<td>204/242</td>
<td>0.8 (0.6-1.1)</td>
</tr>
<tr>
<td>Regular</td>
<td>39/53</td>
<td>0.8 (0.4-1.3)</td>
</tr>
<tr>
<td>Duration of use (y)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never users &lt;2.5</td>
<td>664/670</td>
<td>1.0 (Reference)</td>
</tr>
<tr>
<td></td>
<td>9/16</td>
<td>0.6 (0.3-1.5)</td>
</tr>
<tr>
<td></td>
<td>14/13</td>
<td>0.4 (0.2-3.2)</td>
</tr>
<tr>
<td></td>
<td>11/12</td>
<td>1.0 (0.4-2.7)</td>
</tr>
<tr>
<td>Linear trend</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td>Cumulative dose (g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never users &lt;2.5</td>
<td>664/670</td>
<td>1.0 (Reference)</td>
</tr>
<tr>
<td></td>
<td>10/14</td>
<td>0.7 (0.3-1.7)</td>
</tr>
<tr>
<td></td>
<td>8/11</td>
<td>0.7 (0.3-2.1)</td>
</tr>
<tr>
<td></td>
<td>14/12</td>
<td>1.1 (0.5-2.5)</td>
</tr>
<tr>
<td>Linear trend</td>
<td>0.13</td>
<td></td>
</tr>
</tbody>
</table>

*Adjusted by age, gender, region, and smoking status and also mutually adjusted for use of other NSAID/analgesics.

†Never use the drug <20 times lifelong.

‡Use of the drug >20 times lifelong and less than twice weekly during 1 month.

§Use of the drug twice or more weekly for ≥1 month.
response (OR, 0.46; 95% CI, 0.21-1.03 for heaviest users). By contrast, in a prescription database study in Denmark, regular use of nonaspirin NSAIDs was associated to a slight increase in bladder cancer risk (OR, 1.2; 95% CI, 1.0-1.3), although no dose-response relationship was observed (24). Another study based on a general practitioner database in the United Kingdom found no relation between NSAID use and bladder or colon cancer (43). Experimental studies provide support for a protective effect of NSAIDs in bladder carcinogenesis; these drugs have been shown to act as antitumoral agents in chemically induced bladder cancer (23, 44, 45).

### Table 4. Risk of bladder cancer and use of nonaspirin NSAIDs, aspirin, and metamizol

<table>
<thead>
<tr>
<th>Duration of use (y)</th>
<th>Cases/controls (n)</th>
<th>OR* (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Never use the drug or use &lt;20 times lifelong</td>
<td>866/893</td>
<td>1.0 (Reference)</td>
</tr>
<tr>
<td>Ever users</td>
<td>41/72</td>
<td>0.7 (0.4-1.0)</td>
</tr>
<tr>
<td>Regular*</td>
<td>30/44</td>
<td>0.8 (0.5-1.4)</td>
</tr>
<tr>
<td>Recency of use</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within 1-5 y before interview</td>
<td>4/17</td>
<td>0.3 (0.1-0.9)</td>
</tr>
<tr>
<td>Stopped &gt;5 y ago</td>
<td>5/7</td>
<td>0.7 (0.2-2.3)</td>
</tr>
<tr>
<td>P_linear trend</td>
<td>&gt;0.05</td>
<td></td>
</tr>
</tbody>
</table>

### Table 5. Main effect of CYP2C9 polymorphisms and nonaspirin NSAID use in relation to risk of bladder cancer

<table>
<thead>
<tr>
<th>CYP2C9 Polymorphism</th>
<th>Cases/controls (n)</th>
<th>OR* (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I359L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>359 Ile/Ile</td>
<td>788/730</td>
<td>1.0 (Reference)</td>
</tr>
<tr>
<td>359 Ile/Leu or 359 Leu/Leu</td>
<td>306/114</td>
<td>0.8 (0.6-1.1)</td>
</tr>
<tr>
<td>P</td>
<td>0.27</td>
<td></td>
</tr>
<tr>
<td>R144C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>144 Arg/Arg</td>
<td>673/650</td>
<td>1.0 (Reference)</td>
</tr>
<tr>
<td>144 Arg/Cys or 144 Cys/Cys</td>
<td>243/278</td>
<td>0.8 (0.7-1.0)</td>
</tr>
<tr>
<td>P</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>NSAID-CYP2C9 interaction</td>
<td>564/488</td>
<td>1.0 (Reference)</td>
</tr>
<tr>
<td>359 Ile/Ile and 144 Arg/Arg + nonusers of nonaspirin NSAIDs</td>
<td>326/351</td>
<td>0.8 (0.7-1.0)</td>
</tr>
<tr>
<td>359 variant† or 144 variant† or nonaspirin NSAIDs regular user</td>
<td>292/306</td>
<td>0.8 (0.6-1.0)</td>
</tr>
<tr>
<td>359 variant† or 144 variant† or nonaspirin NSAIDs never user</td>
<td>5/13</td>
<td>0.3 (0.1-0.9)</td>
</tr>
</tbody>
</table>

On the other hand, there is published evidence suggesting that aspirin may have a weaker protective effect on bladder cancer than nonaspirin NSAIDs. In the study by Castelao et al., aspirin seemed to be protective only in heavy users (OR, 0.63; 95% CI, 0.43-0.92 for the highest tertile of cumulative dose). Several other epidemiologic studies have found no relation between the use of aspirin and the risk of bladder cancer (9, 22-26). One recent prospective cohort study found an increased risk of death from bladder cancer associated to aspirin use, especially among women (relative risk, 12.31; 95% CI, 2.98-50.80; ref. 25). Finally, one experimental study provided evidence of a weaker protective effect of aspirin when compared with that of sulindac and ketoprofen NSAIDs (23).

Bladder tumors often overexpress COX-2 (46) and nonaspirin NSAIDs and aspirin are potent inhibitors of COX enzymes, with different affinities for the two main isoenzymes (COX-1 and COX-2) depending on the specific drug. Additionally, aspirin and other NSAIDs exert part of their actions through other mechanisms that are complementary and interrelated with the classic COX pathway. For instance, some analgesics and NSAIDs have been shown to inhibit the antiapoptotic and COX-2-stimulating nuclear factor-κB pathway and also to induce the peroxisome proliferator-activated receptor tumor-suppressing enzymatic cascade (47-50). These effects would tend to limit chronic inflammation, to restrict the severity of acute inflammatory symptoms, and to restore apoptosis in tumoral cells. Some differences in the mechanisms of action of nonaspirin NSAIDs and aspirin have been described. One refers to the lack of peroxisome proliferator-activated receptor activation by aspirin and other salicylates (51), whereas peroxisome proliferator-activated receptor is activated by acetic acid NSAIDs, such as indomethacin. This failure to activate one proapoptotic pathway may partially account for the observed disparate effects between aspirin and other NSAIDs.

A decreased risk of bladder cancer associated with the heterozygous or homozygous variant genotypes for CYP2C9

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*aAdjusted by age, gender, region, and smoking status and also mutually adjusted for use of other NSAID/analgesics.
†D’ = 1.0 and r² = 0.014. Both single nucleotide polymorphisms are genetically linked but have a low correlation.
‡359 variant includes CYP2C9 359 Ile/Leu and 359 Leu/Leu genotypes.
§144 variant includes CYP2C9 144 Arg/Arg and 144 Cys/Cys genotypes.

Never use the drug or use ≤20 times lifelong.
*Use of the drug twice or more weekly for ≥1 month.

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I359L and R144C has not been reported previously. CYP2C9 participates in the generation of several endogenous compounds related to inflammation and angiogenesis (i.e., 9,10-epoxy-12-octadecanoate and 12,13-epoxy-9-octadecanoate) and participates in the metabolism and inactivation of most NSAIDs (35). An impaired activation of these compounds and the increased half-life of NSAIDs, caused by the presence of the studied polymorphisms on CYP2C9, may confer some protection against inflammation- and angiogenesis-related diseases, such as bladder cancer (35). Our data were consistent with a stronger protection from nonaspirin NSAID use among subjects with the variant CYP2C9 alleles, although the interaction was not statistically significant. Although CYP2C9 is responsible for the metabolism of most NSAIDs, it may not be the most relevant enzyme in the metabolism of diclofenac (52), an acetic acid NSAID that accounted for the majority of nonaspirin NSAIDs used in our study.

The urological carcinogenic potential of paracetamol has been assessed in several epidemiological studies in humans. Some studies have found a slight, nonsignificant increased risk of <50% among heavy users (10-12, 15, 19). Most animal experiments have failed to show any urological carcinogenic potential for paracetamol, although one study in rats found heavily paracetamol-treated animals to be at an increased risk for bladder cancer (53). In our study, paracetamol was protective for bladder cancer among individuals who were GSTP1 I105V wild-type homozygous and was nonsignificantly associated to an increased risk of bladder cancer among subjects homozygous for GSTP1 minor allele. If these findings are replicated, they may suggest that GSTP1 is responsible for the inactivation of the toxic metabolite (NAPQI) of paracetamol and that the inability to eliminate this DNA adduct-forming compound may override the chemoprotective properties of paracetamol described elsewhere for bladder cancer (9, 11) and breast cancer (54). The Spanish Bladder Cancer Study has several strengths: high participation rates, large sample size, high quality exposure and genotype information, and detailed assessment of drug composition and dose. Our analysis has also several potential limitations, including possible misclassification of exposure, limited statistical power particularly for stratified analyses, and possible selection bias. Misclassification of drug use is possible because it was evaluated retrospectively and was based on self-report during in-person interviews. Any misclassification is unlikely, however, to be differential for cases and controls because both were groups of hospital patients and bladder cancer is not generally perceived as a disease related to analgesics. Thus, any misclassification would have resulted in a dilution of the point estimates, underestimating the true effect of drugs. Although this is one of the largest studies on bladder cancer and medication use, our analysis has limited power particularly in analyses assessing interactions between drug use and genetic polymorphisms. Hospital controls may have a higher consumption of drugs than the general population, thus resulting in selection bias. It is unlikely, however, that this potential bias has substantially affected our results. First, the diagnoses of controls were mostly acute conditions that are not associated with heavy use of analgesics and NSAIDs. Second, a sensitivity analysis using different a priori defined control subgroups (i.e., varicose vein surgery, hydrocele surgery, fractures, and hernia surgery) gave very similar results (data not shown). Third, the prevalence of drug use in our control population was similar to that observed in other studies in southern Europe (55, 56) and there were no systematic differences of drug use prevalence across diagnostic categories.

In conclusion, we provide evidence supporting a protective effect of regular use of nonaspirin NSAIDs against bladder cancer. In addition, our data suggested that the variant alleles for the I359L and R144C genotypes in the CYP2C9 gene involved in NSAID metabolism might reduce bladder cancer risk. We found no evidence of an overall increase in risk associated with paracetamol use as has been suggested previously, and we found an indication that the effect of paracetamol on bladder cancer risk could be affected by the GSTP1 I105V genotype. Finally, we found no reduction in risk in regular users of aspirin. Further research on larger populations, which may require pooling data across comparable studies, is needed to confirm our findings.

Acknowledgments

We thank Robert C. Saal (Westat, Rockville, MD), Leslie Carroll and Eric Boyd (IMS, Silver Spring, MD), and Paco Fernández (Institut Municipal d’Investigació Médica) for support in study and data management; Dr. María Sala (Institut Municipal d’Investigació Médica) for work in data collection; Jane Wang (IMS) for genotype data management; Isabel López (Institut Municipal d’Investigació Médica) for secretarial assistance; and the physicians, nurses, interviewers, and study participants for effort during field work.

References


Correction

In the May and September 2006 issues, two articles were published (1, 2) without an author’s full affiliation. Joan Fortuny is a PhD student at the Autonomous University of Barcelona (UAB), and these two articles were part of a PhD thesis.

References

Use of Analgesics and Nonsteroidal Anti-inflammatory Drugs, Genetic Predisposition, and Bladder Cancer Risk in Spain

Joan Fortuny, Manolis Kogevinas, Montserrat Garcia-Closas, et al.


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