Celecoxib Inhibits N-Butyl-N-(4-hydroxybutyl)-nitrosamine-induced Urinary Bladder Cancers in Male B6D2F1 Mice and Female Fischer-344 Rats


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Abstract

Epidemiological studies have shown that nonsteroidal anti-inflammatory drugs (NSAIDs) may have a role in the prevention of human cancers. A number of preclinical studies have also suggested that inhibition of cyclooxygenase (COX) with NSAIDs has an anticancer effect in animal models of colon, urinary bladder, skin, and breast. In these studies, we evaluated the COX-2 inhibitor celecoxib in two rodent models of urinary bladder cancer. Male B6D2F1 mice treated with N-butyl-N-(4-hydroxybutyl)-nitrosamine (OH-BBN) developed transitional and squamous cell urinary bladder cancers, many of which grew rapidly and caused substantial morbidity that required sacrifice of the mice. Groups of mice received various daily doses of celecoxib in the diet (1250, 500, or 200 mg/kg of diet) beginning 7 days before the initiation of 12 weekly doses of OH-BBN. Mice were checked weekly for the presence of palpable urinary bladder masses. The study was terminated at 8 months following the initial treatment with OH-BBN. The percentage of mice with large palpable bladder lesions, which necessitated sacrifice of the mice, was 40% in the OH-BBN control group. In contrast, only 10% of all celecoxib-treated mice required sacrifice before the scheduled termination of the experiment, implying that all three doses of celecoxib inhibited the formation of large palpable lesions. Celecoxib did not significantly alter the incidence of preneoplastic bladder lesions, but did dose-dependently decrease the total number of urinary bladder cancers/mouse, palpable plus microscopic, by 77, 57, and 43% at dosages of 1250, 500, and 200 mg of celecoxib/kg of diet, respectively. In the second model, female Fischer-344 rats were administered OH-BBN twice/week for a period of 8 weeks. After 8 months, all rats developed preneoplastic lesions, whereas roughly 60% of the rats developed relatively small urinary bladder cancers. Rats were treated continually with celecoxib in the diet (500 or 1000 mg/kg of diet) beginning either 1 week prior to the initial OH-BBN treatment or beginning 1 week following the last OH-BBN treatment. Neither celecoxib treatment regimen significantly altered the number of preneoplastic lesions. Whereas celecoxib treatment initiated prior to OH-BBN administration decreased cancer incidence roughly 65%, celecoxib treatment initiated beginning 1 week after the last dose of OH-BBN profoundly decreased cancer incidence (>95%). Celecoxib did not alter the body weights of the mice or rats, or cause other signs of toxicity at any of the doses studied. Taken together these results demonstrate that: (a) celecoxib effectively inhibits tumor growth and enhances survival in the mouse model of urinary bladder cancer; and (b) celecoxib profoundly inhibits development of urinary bladder cancers in the rat model even when administered following the last dose of OH-BBN. Clinical trials will be necessary to determine whether COX-2 inhibitors will provide a clinical benefit in human bladder cancer.

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The abbreviations used are: NSAID, nonsteroidal anti-inflammatory drug; OH-BBN, N-butyl-N-(4-hydroxybutyl)-nitrosamine; COX, cyclooxygenase.

Conventional NSAIDs inhibit both COX-1 and -2 and therefore inhibit the conversion of arachidonic acid to prostaglandins. COX-1 is constitutively expressed and plays an important role in gastrointestinal cytoprotection and maintenance of platelet and kidney function. In contrast to the housekeeping function of COX-1, COX-2 is primarily expressed in response to inflammatory stimuli and cellular activation (1, 2). Conventional NSAIDs have been shown to effectively inhibit inflammation. More recently, epidemiological data demonstrate that regular NSAID use can significantly reduce the risk for colon (3) and urinary bladder (4) cancers and inhibit tumor growth in animal models of cancer. Conventional NSAIDs block both forms of COX, hence their anti-inflammatory activity is linked mechanistically and intimately to gastrointestinal toxicity and altered platelet function. Molecular-based targeting for the COX-2 isofrom has led to the development of COX-2 selective inhibitors such as celecoxib (5) and rofecoxib (6), which potently inhibit COX-2-dependent inflammation while avoiding typical NSAID-associated side effects (7). Although the initial premise for developing these drugs was their potential for inhibiting the inflammation and pain of adult osteo- and rheumatoid arthritis, use of these agents appears to be a promising strategy for chemoprevention and chemotherapy of various epithelial cancers.

Several laboratories have recently demonstrated that celecoxib can exert potent anticancer activity in both carcinogen-induced and genetically determined rodent models of colon (8, 9) and UV-induced skin (10) carcinogenesis. In addition, COX-2 has been shown to be overexpressed in human colon (11), breast (12), skin (13), and urinary bladder cancers (14). Taken together, these data are consistent with the hypothesis that the anticancer activity of NSAIDs observed in humans may be dependent upon their ability to inhibit COX-2.

To test this hypothesis, we examined the chemopreventive efficacy of celecoxib on the induction of urinary bladder cancer by N-butyl-N-(4-hydroxybutyl)-nitrosamine (OH-BBN) in male B6D2F1 mice and female Fischer-344 rats. Treatment of mice or rats with OH-BBN results in the development of transitional and squamous cell urinary bladder cancers (15–18) that bear significant histopathological similarities to the human disease and tend to be invasive. For these reasons, this model has been used previously to characterize the tumorigenic process for urinary bladder cancer and to assess the efficacy of potential chemopreventive agents to inhibit the development of carcinogen-induced bladder cancers (16, 17). Among the compounds which have shown promise previously in the OH-BBN urinary bladder cancer models have been a number of specific or nonspecific inhibitors of the COX isozymes (19, 20). Furthermore, the NSAID piroxicam has shown efficacy in treating spontaneous urinary bladder tumours in dogs (21). On the basis of these preclinical findings,
200 mg/kg of diet; and
F
incubated with antisera in the presence of 100-fold excess COX-2 protein, or
were diluted to 1:500 in TNB-BB for all tissues. Control sections were
specific COX-2 polyclonal (PG-27; Oxford Biomedical Research) antisera
saponin), and incubated with primary antibody overnight at 4°C. The isoform-
(0.1M Tris (pH 7.5)/0.15 M NaCl/0.5% blocking agent/0.3% Triton-X, 0.2%
biotin (Vector Blocking Kit). The sections were permeabilized in TNB-BB
materials and Methods
OH-BBN-induced urinary bladder cancer models in rodents.
bladder cancer (14), we examined the efficacy of celecoxib in two
epidemiological data in humans (4), and COX-2 staining of human
were:
E
and blocked for endogenous peroxidase (3% H2 O 2 in methanol) and avidin/
buffered formalin, removed, and observed under a high-intensity light for gross
months after the initial OH-BBN treatment.
were palpated for urinary bladder lesions weekly. The study was terminated 8
by Kaplan-Meier analysis (P > 0.001 for all groups). In fact, the numbers sacrificed in the
control group exceeded that in all celecoxib groups combined. This
result clearly demonstrates that celecoxib can profoundly suppress the
epidemiological data in humans (4), and COX-2 staining of human
bladder cancer (14), we examined the efficacy of celecoxib in two
OH-BBN-induced urinary bladder cancer models in rodents.

Materials and Methods

Male B6D2F1 (C57BL/6 × DBA/2F1) mice and female Fisher-344 rats
were obtained from Harlan Sprague Dawley, Inc. (Indianapolis, IN; virus-free
colony 202) at 28 days of age and housed in polycarbonate cages (5/cage). The
animals were kept in a room lighted 12 h each day and maintained at
22 ± 0.5°C. Teklad (4%) diet (Harlan Teklad, Madison, WI) and tap water
were provided ad libitum. Celecoxib (SC-635) was provided by Monsanto/ Searle
(St. Louis, MO).

In the mouse study, celecoxib was administered at 200, 500, and 1250
mg/kg of diet when the animals were 49 days of age and was continued throughout
the study. At 56 days of age, carcinogen-treated mice received the first of 12 weekly intragastric doses of OH-BBN (TCI America, Portland, OR).
Each 7.5-mg dose was dissolved in 0.1 ml ethanol:water (20:80). The mice
were weighed weekly and checked daily. Some animals were lost during the
first two weeks of the study due to gavage errors. These mice were excluded
from the final analysis. After the last dose of carcinogen, the animals were
palpated weekly for urinary bladder masses. Animals which developed large
palpable tumors masses, bloody urinary, and weight loss became rapidly mori-
bund and were sacrificed. Mice not sacrificed specifically because of the
presence of large lesions were sacrificed 8 months after the initial OH-BBN
treatment.

Diet supplementation of female Fischer-344 rats with celecoxib was initi-
ated at 43 days of age (1 week prior to the initial OH-BBN treatment) or at 107
days of age (1 week after the last OH-BBN treatment). OH-BBN (150
mg/gavage, 2×/week) was started when the rats were 49 days of age and
continued for 8 weeks. The carcinogen vehicle was ethanol:water (20:80);
volume was 0.5 ml. The rats were observed daily, weighed weekly, and
palpated for urinary bladder lesions weekly. The study was terminated 8
months after the initial OH-BBN treatment.

At necropsy, urinary bladders of both mice and rats were inflated with 10%
buffered formalin, removed, and observed under a high-intensity light for gross
lesions. After fixation, each lesion was dissected, processed for routine paraffin
embedding, cut into 4-μm sections, and mounted onto polylysine-coated slides. Sections were dewaxed in xylene, rehydrated in descending alcohols,
and blocked for endogenous peroxidase (3% H2O2 in methanol) and avidin/
biotin (Vector Blocking Kit). The sections were permeabilized in TNB-BB
(0.1 M Tris (pH 7.5)/0.15 M NaCl/0.5% blocking agent/0.3% Triton-X, 0.2%
saponin), and incubated with primary antibody overnight at 4°C. The isoform-
specific COX-2 polyclonal (PG-27; Oxford Biomedical Research) antisera
were diluted to 1:500 in TNB-BB for all tissues. Control sections were
incubated with antisera in the presence of 100-fold excess COX-2 protein, or
with an isotype-matched IgG normal rabbit serum. Immunoreactive complexes
were detected using tyramide signal and amplification (TSA-indirect; NEN
Life Science) and visualized with the peroxidase substrate, AEC. Slides were
then counter-stained with aqueous hematoxylin (Biomedia), mounted in crystal
solution (Biomedia), and coverslipped in 50:50 xylene/Permount.

The Kaplan-Meier test (22) was used to analyze survival data. Urinary
bladder cancer incidence and multiplicity were analyzed by the Fisher’s exact
and Wilcoxon’s rank-sum tests, respectively.

Results and Discussion

We used two different rodent models of urinary bladder cancer
induced by the organ-specific nitrosamine OH-BBN to examine the
efficacy of celecoxib. In the first model, male B6D2F1 mice develop-
ed relatively aggressive bladder cancers of both transitional cell and
squamous cell phenotypes. These tumors grew rapidly and, because of
bleeding and obstruction of the urinary bladder, caused morbidity in a
high percentage of mice (40%). The 40% incidence of palpable
lesions in OH-BBN-treated mice represents almost 80% of the 51% of
mice which developed urinary bladder cancers at all. Interestingly,
although female Fischer-344 rats treated with OH-BBN also develop
both transitional and squamous cell carcinoma, these tumors grew
slowly, and virtually no rats were lost due to development of large
palpable tumors. Therefore, we used these models to answer two
separate questions. In the rat model, we wanted to address the efficacy
of celecoxib when administration was initiated either before or after
OH-BBN treatment. This was done to determine the stage in tumor
development when celecoxib was effective. In the mouse model, we
wished to determine whether celecoxib could substantially decrease
the growth of these rapidly growing cancers when administered
throughout the duration of the study.

The effect of celecoxib on survival due to bladder cancer is shown in
Figure 1. Mice were sacrificed during the study if they developed
large palpable tumors which caused bloody urine and weight loss (Fig.
2). The number of mice sacrificed in the vehicle-treated group
(n = 27) significantly exceeded the number sacrificed in any of the
celecoxib-treated groups (n < 6) by Kaplan-Meier analysis
(P > 0.001 for all groups). In fact, the numbers sacrificed in the
control group exceeded that in all celecoxib groups combined. This
result clearly demonstrates that celecoxib can profoundly suppress the

Fig. 1. Celecoxib increases survival of OH-BBN-treated male B6D2F1 mice. The mice
received the first of 12 weekly doses of OH-BBN beginning at 56 days of age. The groups
were: ○, celecoxib, 1250 mg/kg of diet; □, celecoxib, 500 mg/kg of diet; △, celecoxib,
200 mg/kg of diet; and ●, diet only.

Fig. 2. Celecoxib inhibits urinary bladder cancer growth in the OH-BBN-treated
mouse. All bladders were excised, inflated with 10% buffered formalin, and observed
under high-intensity light for gross lesions. Pictured are representative urinary bladders
from celecoxib- (A) and vehicle- (B) treated animals 8 months after initiation of OH-BBN
treatment.

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Table 1  Effect of celecoxib on urinary bladder cancers induced in male B6D2F1 mice

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of mice</th>
<th>Carcinogen</th>
<th>Treatmenta</th>
<th>Incidence (%)</th>
<th>Average no./animal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>67</td>
<td>OH-BBN</td>
<td>Celecoxib, 1250 mg/kg of diet</td>
<td>12(^{a})</td>
<td>0.13(^{a})</td>
</tr>
<tr>
<td>2</td>
<td>72</td>
<td>OH-BBN</td>
<td>Celecoxib, 500 mg/kg of diet</td>
<td>24(^{a})</td>
<td>0.24(^{a})</td>
</tr>
<tr>
<td>3</td>
<td>68</td>
<td>OH-BBN</td>
<td>Celecoxib, 200 mg/kg of diet</td>
<td>29(^{a})</td>
<td>0.32(^{a})</td>
</tr>
<tr>
<td>4</td>
<td>68</td>
<td>OH-BBN</td>
<td>Celecoxib (1000 mg/kg of diet), started 1 week prior to carcinogen</td>
<td>51</td>
<td>0.56</td>
</tr>
</tbody>
</table>

\(^{a}\) Diets of male B6D2F1 mice were supplemented with celecoxib beginning when the mice were 49 days of age.

\(^{a}\) Study was terminated 8 months after the initial administration of the carcinogen at 56 days of age.

\(^{a}\) Significantly different from group 4.

Table 2  Effect of celecoxib on urinary bladder lesions induced in female Fischer-344 rats

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of rats</th>
<th>Carcinogena</th>
<th>Treatmentb</th>
<th>Preneoplastic lesions</th>
<th>Incidence (%)</th>
<th>Average no./animal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>29</td>
<td>OH-BBN</td>
<td>Celecoxib (1000 mg/kg of diet), started 1 week prior to carcinogen</td>
<td>93</td>
<td>2.66</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>28</td>
<td>OH-BBN</td>
<td>Celecoxib (500 mg/kg of diet)</td>
<td>100</td>
<td>3.11</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>29</td>
<td>OH-BBN</td>
<td>Celecoxib (1000 mg/kg of diet), started 1 week prior to carcinogen</td>
<td>90</td>
<td>3.59</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>26</td>
<td>OH-BBN</td>
<td>Celecoxib (500 mg/kg of diet), started 1 week after carcinogen</td>
<td>100</td>
<td>3.50</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>30</td>
<td>OH-BBN</td>
<td>Diet only</td>
<td>77</td>
<td>2.93</td>
<td></td>
</tr>
</tbody>
</table>

\(^{a}\) OH-BBN was administered to female Fischer-344 rats for 8 weeks beginning at 49 days of age.

\(^{a}\) Diets were supplemented with celecoxib at either 43 days of age (1 week before the initial OH-BBN treatment) or at 107 days of age (1 week after the last OH-BBN treatment).

\(^{a}\) Significantly different from group 5.
These data demonstrate that celecoxib is highly effective in preventing OH-BBN-induced urinary bladder cancers in both mice and rats with no associated toxicity. The results in the rat, and more indirectly the results in mice, on the growth of large tumors, support the potential therapeutic utility for urinary bladder cancer patients.

References


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References


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