Quercetin, a Rat Intestinal and Bladder Carcinogen Present in Bracken Fern (Pteridium aquilinum)

A. Mahir Pamukcu, Șenay Yalçiner, James F. Hatcher, and George T. Bryan

ABSTRACT

Albino noninbred weanling male and female rats were fed a basic grain diet (Group 1) or a basic diet supplemented with 33% bracken fern [BF (Group 2)] or 0.1% quercetin [purity, >99% (Group 3)] for 58 weeks. The quantities of quercetin and kaempferol (a close structural analog) in BF as glycosides were determined to be 0.57 and 1.1 g, respectively, per kg of dried BF. Estimated mean total cumulative doses (mmol) per rat were: Group 1, quercetin, males and females < 0.03; kaempferol, males and females < 0.03; Group 2, quercetin, males 5.8, females 5.2; kaempferol, males 11.9, females 10.8; and Group 3, quercetin, males 27.8, females 25.3; kaempferol, males and females < 0.03. Growth of rats fed BF or quercetin was comparable but significantly (p < 0.01) slower after 24 weeks than that of Group 1. Mean survivals (weeks) of rats of all groups were: Group 1, 58 ± 7 (S.D.); Group 2, 51 ± 13; and Group 3, 56 ± 8. They were not significantly different, although rats fed BF tended to die earlier secondary to intestinal tumor-induced intussusception and obstruction. The following incidences of intestinal or bladder neoplasms in male or female rats, respectively, were observed: Group 1, intestinal and bladder, males, 0 of 9; females, 0 of 10; Group 2, intestinal, males, 7 of 8; females, 10 of 11; bladder, males, 6 of 8, females, 8 of 11; Group 3, intestinal, males, 6 of 7, females, 14 of 18; bladder, males, 2 of 7, females, 3 of 18. The histopathology of neoplasms of the 2 target organs was identical for rats of Groups 2 and 3. Multiple ileal intestinal neoplasms of rats fed quercetin included: adenoma, 4; fibroadenoma, 7; and adenocarcinoma, 9 (with mesenteric metastases, 3). The 5 bladder tumors were papillary or sessile transitional cell carcinomas.

INTRODUCTION

Quercetin (meleitin, flavin, sophoretin, 3,3',4',5,7-pentahydroxyflavone) occurs in conjugated or free forms in many edible plant products, including fruits, vegetables, tea (8), sumac (18), and BF (11, 12, 21, 22). The chemical and biological activities of many flavonoids, including quercetin, have been the subject of extensive study for many years (3, 7, 17, 25).

In view of the widespread occurrence of quercetin in foods, it is surprising that relatively little work has been done on the toxicity of this compound. Ambrose et al. (1) reported a low toxicity of quercetin for rats and rabbits in short- and long-term (410 days) studies. We reported (23) that quercetin was mutagenic in Salmonella typhimurium TA 98 and TA 100 strains. This observation was confirmed later by many workers (2, 4, 5, 9, 18-20). Quercetin showed mutagenic activity in the absence of liver-mediated metabolism (23); however, mutagenic activity was approximately tripled in the presence of liver microsomes (2, 4, 5, 9, 18-20). The mutagenicity of quercetin and kaempferol (a homologous flavonol) was of the same order of magnitude as that of o-aminooazotoluene and 4-aminobiphenyl with S. typhimurium TA 98 and 3'-methyl-4-dimethylaminoazobenzene with S. typhimurium TA 100 (20).

Although quercetin and several other flavonoid compounds have been reported to be noncarcinogenic (7, 19, 21), the carcinogenic potential of a mutagen, such as quercetin which is present in BF, must not be overlooked. BF contains carcinogens which induce, naturally or experimentally, intestinal and urinary bladder cancer in different species of animals (6, 12). In view of the recently reported mutagenic activity of quercetin, we have deemed it important to assess the carcinogenic activity of this compound in rats through a comparative feeding study with BF. The results of this study are presented here.

MATERIALS AND METHODS

Materials. Quercetin, kaempferol (3,4',5,7-tetrahydroxylavone), and rutin (quercetin 3-rutinoside) were purchased from Sigma Chemical Co., St. Louis, Mo. Quercetin was recrystallized 3 times from methanol:H2O. Its identity and purity (>99%) were ascertained by melting point (314° with decomposition), IR and UV spectrophotometry, and TLC. BF collection and preparation (14, 15) and the basic grain diet (16) (Yem Sanayii An. Sirketi, Ankara, Turkey) used have been described previously. Three diets were prepared: (a) basic grain diet without any additions; (b) basic diet containing BF (basic diet:dried, powdered BF, 2:1) (16); and (c) basic diet containing 0.1% quercetin. The dose level of quercetin fed was based on data from the toxicity studies of Ambrose et al. (1).

Determination of Quercetin and Kaempferol Content of BF. Procedures described by Wildanger and Herrmann (24) were used with slight modifications for the quantitative analyses of quercetin and kaempferol in BF. Briefly, dried BF (200 g) was suspended in a 2% solution of aqueous NaHSO3, heated (95°) with stirring for 20 min, cooled, and centrifuged. The BF was extracted 3 times with 1-liter aliquots of hot methanol, and the methanol was filtered and concentrated in a vacuum with a rotary evaporator at 37° (yield, 60 g). This concentrate was extracted with hexane; the aqueous layer was concentrated and applied to a 5- x 31-cm polyamide SC6 (Macherey-Nagel & Co., Düren, W. Germany) column; and the column was...
washed successively with 1 liter of $H_2O$, 5 liters of methanol, and 2 liters of ammoniacal methanol. Flavonol glycosides were eluted with the final 3.4 liters of methanol. This fraction was concentrated in a vacuum and restored to a final volume of 100 ml with methanol. Ten-m1 aliquots of this were refluxed for 1 hr in 1% $H_2SO_4$ in methanol, and the aglycones formed (24), identified by TLC on cellulose plates, were developed with chloroform:acetic acid:$H_2O$ (1:3:3) (Rf: quercetin, 0.37; kaempferol, 0.61). After removal from the TLC plates, quercetin and kaempferol were quantitated by UV spectrophotometry (24).

Animal Selection and Care. Male and female albino rats, bred from local stock derived from the Norwegian strain about 1930 and weighing 100 to 105 and 90 to 95 g, respectively, were housed in screen-bottomed metal cages with 4 rats/cage or less. They were fed their diets and water ad libitum. Animal care and inspection procedures have been described previously (15). A total of 63 rats, 35 days of age, were divided into 3 groups. The rat sexes, initial numbers in each group, and treatment schedules are presented in Table 1. No thiamine supplement was administered to rats of Group 3 (16). Rats in each group received the prescribed diets until termination of the experiment after 58 weeks of observation.

Carcinogenicity Studies. Rats that died or were killed were necropsied as described previously (14, 15). Urinary bladders were distended at postmortem examination with Bouin’s fixative injected into the urethra. Representative histological sections from all animals of stomach, intestine, kidney, urinary bladder, lungs, liver, heart, brain, and other organs exhibiting gross abnormalities were prepared and studied by light microscopy (14–16). The incidences of intestinal and urinary bladder neoplasms were used to assess carcinogenicity. Probabilities of statistical significance were computed as described previously (16, 21).

RESULTS

Quercetin and Kaempferol Content of BF. Dried BF contained 0.57 g of quercetin and 1.1 g of kaempferol per kg. Rutin, a constituent of BF, was quantitatively recovered using the isolation and hydrolysis procedures described by Wildanger and Herrmann (24). Quercetin and kaempferol were stable when subjected to hydrolysis (recoveries, >98%). The basic grain diet (Group 1) contained less than 1 mg of quercetin and kaempferol per kg (8).

Diet and Chemical Consumption and Effects on Growth and Survival. Daily maximums of about 23 and 21 g of BF diet (Group 2) and basic diet containing quercetin (Group 3) were eaten by each male and female rat, respectively. Each rat in unmedicated control Group 1 ate about 20 g of basic grain diet per day. Some dietary spillage occurred, so that this consumption estimate is probably excessive. The flavonol aglycones occur in BF as the glycosides astragalin, isoquercitrin, rutin, and tiliroside; no free aglycones have been detected in BF (12, 22). Following hydrolysis of BF, the aglycones, quercetin and kaempferol, were liberated and quantitated (24). The estimated mean total cumulative doses of these 2 flavonols, consumed by each male and female rat in Group 1 fed the basic grain diet, in Group 2 fed BF, and in Group 3 fed the quercetin-supplemented diet, are presented in Table 1.

During the first 20 weeks of the study, the mean weights of the male and female rats comprising the 3 groups were comparable (Chart 1). After this time and until the end of the study, male and female rats of Groups 2 and 3 gained weight at a comparable rate but grew significantly more slowly (p < 0.01) than did male and female rats of control Group 1.

Mean survivals ± S.D. of male and female rats of Groups 2 and 3 were not significantly different from those of control Group 1 (Table 1). All rats survived for at least 26 weeks. Rats of Group 2 that died prior to 43 weeks of study exhibited intestinal tumors with intussusception and obstruction. Some rats of Groups 1 and 3 died prematurely from pneumonia.

Incidence and Histological Classification of Intestinal and Bladder Neoplasms Produced in Rats Fed BF or Quercetin. The incidences of intestinal and bladder neoplasms present in rats fed BF (Group 2) or quercetin (Group 3) are presented in Table 1, and the histological classifications of these tumors are provided in Table 2. Intestinal and urinary bladder tumors were present only in rats fed BF or quercetin. Intestinal and bladder neoplasms were first detected at 34 weeks in rats fed BF and at 53 and 54 weeks, respectively, in rats fed quercetin (Table 2). Male and female rats appeared to be equally susceptible to BF and quercetin carcinogenicity. Rats (20 of 25) fed quercetin developed multiple tumors of the ileal segment of the small intestine. The intestinal tumors included 4 adenomas (Fig. 1), 7 fibroadenomas, and 9 adenocarcinomas (Figs. 2 and 3; Table 2). Male and female rats of Groups 2 and 3 were not significantly different from those of control Group 1 (Table 1). All rats survived for at least 26 weeks. Rats of Group 2 that died prior to 43 weeks of study exhibited intestinal tumors with intussusception and obstruction. Some rats of Groups 1 and 3 died prematurely from pneumonia.

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Treatment</th>
<th>Sex</th>
<th>Quercetin</th>
<th>Kaempferol</th>
<th>No. of rats that died or were killed</th>
<th>Intestinal tumors</th>
<th>Bladder tumors</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Basic diet</td>
<td>M</td>
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<td>&lt;0.03</td>
<td>9</td>
<td>5 ± 5</td>
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<td></td>
<td></td>
<td>F</td>
<td>&lt;0.03</td>
<td>&lt;0.03</td>
<td>10</td>
<td>9 ± 5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td></td>
<td></td>
<td>19</td>
<td>5 ± 5</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Bracken fern</td>
<td>M</td>
<td>5.8b</td>
<td>11.9b</td>
<td>8</td>
<td>7 ± 4</td>
<td>7 ± 4</td>
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<tr>
<td></td>
<td></td>
<td>F</td>
<td>5.2</td>
<td>10.8</td>
<td>11</td>
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<td>10 ± 4</td>
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<td></td>
<td>Total</td>
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<td></td>
<td>19</td>
<td>17 ± 4</td>
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<td>3</td>
<td>Quercetin</td>
<td>M</td>
<td>27.8</td>
<td>&lt;0.03</td>
<td>7</td>
<td>6 ± 4</td>
<td>6 ± 4</td>
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<tr>
<td></td>
<td></td>
<td>F</td>
<td>25.3</td>
<td>&lt;0.03</td>
<td>18</td>
<td>14 ± 4</td>
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<tr>
<td></td>
<td></td>
<td>Total</td>
<td></td>
<td></td>
<td>25</td>
<td>20 ± 4</td>
<td>20 ± 4</td>
</tr>
</tbody>
</table>

Mean ± S.D.

Present in BF as glycosides, i.e., astragalin, isoquercitrin, rutin, and tiliroside (12, 22).

Table 1

Treatment schedules, sex, estimated mean total cumulative flavonol dosages, mean survivals, and incidences of intestinal and bladder neoplasms produced in albino rats fed BF or quercetin.
Table 2

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Treatment</th>
<th>Tissue</th>
<th>Incidence at following times of neoplasms detection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>wk</td>
</tr>
<tr>
<td>2</td>
<td>Bracken fern</td>
<td>Intestine</td>
<td>Adenoma</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Adenocarcinoma</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Adenocarcinoma and fibrosarcoma</td>
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<tr>
<td></td>
<td></td>
<td>Urinary bladder</td>
<td>Fibrosarcoma</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Papilloma</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Transitional cell carcinoma</td>
</tr>
<tr>
<td>3</td>
<td>Quercetin</td>
<td>Intestine</td>
<td>Adenoma</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fibroadenoma</td>
</tr>
<tr>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Urinary bladder</td>
<td>Transitional cell carcinoma</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Transitional cell carcinoma with fibromyxoma</td>
</tr>
</tbody>
</table>

* Male rats; undesignated, female rats.

Chart 1. Changes in body weights of male (●) and female (○) rats fed a basic grain diet (— — — —) or a basic diet supplemented with 33% BF (-----), or 0.1% quercetin (———) with respect to time. Points, mean weights of surviving rats. Bars, S.D.

2) Most adenocarcinomas penetrated through all layers of the intestinal wall including the serosa. In 3 rats fed quercetin, mesenteric lymphatic metastases were present. Bladder tumors induced with quercetin in 5 rats were all grossly detectable and consisted of 3 papillary and 2 sessile transitional cell carcinomas (Fig. 4), with one tumor exhibiting fibromyxoma (Table 2). Histological features of these intestinal and urinary bladder tumors were indistinguishable from those of BF-induced tumors in this and other studies (14–16). However, there was a significant (p < 0.01) difference between the incidences of bladder tumors present in rats of Group 2 and in those of Group 3. Those rats fed BF developed more bladder tumors (74%) than did those fed quercetin (20%). No significant incidences of other neoplastic lesions were observed in the untreated control Group 1 rats or in the other organs examined.

DISCUSSION

The data presented here clearly demonstrate that quercetin is carcinogenic for the intestinal and bladder epithelium of the rat. Prior studies suggesting that quercetin or other flavonoid compounds fed to rats or mice were devoid of oncogenic activity either did not describe complete histopathological examination of susceptible target tissues (7) or did not define the source and purity of the quercetin studied (19). The neoplasms produced in quercetin-fed rats were grossly and histologically identical to those produced in BF-fed rats (6, 12, 14–16). The ingested quantity of quercetin, found in BF as the glycosides isoquercitrin and rutin (12, 22), was about 5 times that ingested by BF-fed rats (Table 1). In addition, BF-fed rats received exposure to the flavonol kaempferol, occurring in BF as the glycosides astragalin and tiliroside (12, 22), at levels about twice those of quercetin. These data suggest that kaempferol, a structural analog of quercetin and a known mutagen for S. typhimurium TA 98 and TA 100 (5, 9, 10, 19, 20), may also participate in BF oncogenesis in rats. We recently have isolated from BF, but not yet completely characterized, a third mutagenically active compound, not seemingly structurally related to quercetin or kaempferol. It is possible that this substance may also participate in the carcinogenesis exhibited by BF. We demonstrated previously (16) that thiamine (Vitamin B₁) administration to rats fed BF significantly augmented the incidence of urinary bladder tumors produced. In this study, the low incidence of these neoplasms in rats fed quercetin (Table 1, Group 3) may be related to the absence of thiamine supplementation to these rats. The carcinogenicity displayed by quercetin suggests that it is a principal effector of BF oncogenesis and that tannin, previously isolated from BF (21), does not play a significant role in the production of rat intestinal or vesical neoplasms (15).

The mechanisms of carcinogenicity of quercetin are not known. Quercetin has demonstrated significant effects on DNA synthesis, lactate production, and cyclic adenosine 3':5'-monophosphate levels in neoplastic cells (17). Comparative structure-activity mutagenicity studies of quercetin and related aglycones and glycosides have been reported previously (4, 5, 9, 19, 20). The 3-flavonol glycosides studied (5), quercetin, rutin, and robinin, were not directly mutagenic but could be activated by a variety of mixed glucosidases. It is possible that intestinal bacteria contain similar enzymes that may liberate...
the aglycones. Previous studies (12) on the inhibition of BF-induced intestinal and bladder neoplasms are consistent with this interpretation. Essential structural features for mutagenic activity displayed by flavonols and related analogs have been described previously (5, 9, 20). It is not known if these requirements (5, 9, 20) are operative in oncogenesis.

ACKNOWLEDGMENTS

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REFERENCES

Fig. 1. Adenoma (adenomatous polyp) of ileum of rat fed quercetin. H & E, × 70.
Fig. 2. Adenocarcinoma of ileum of rat fed quercetin. Note many mitotic figures. H & E, × 100.
Fig. 3. Adenocarcinoma of ileum of rat fed quercetin. Note extension of carcinoma through the muscularis propria into the submucosa. H & E, × 80.
Fig. 4. Transitional cell carcinoma, infiltrating into the wall of the urinary bladder, from a rat fed quercetin. H & E, × 110.
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