Induction of Colon Tumors in C57BL/6J Mice Fed MelQx, IQ, or PhIP Followed by Dextran Sulfate Sodium Treatment

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Heterocyclic amines (HCAs) have been shown to induce tumors in several organs of rodents, but except for MeIQ and PhIP, other HCAs such as MelQx and IQ consistently failed to induce colon tumors in mice, whereas MeIQ, IQ, and PhIP exerted colon tumorigenicity in rats. Recently, we found that dietary MelQx induces genotoxicity in the colon as well as the liver of two different types of reporter gene transgenic mice at subcarcinogenic doses such as 300 ppm. However, in the present study, dietary MelQx did not significantly induce any tumors in C57BL/6J mice or gpt delta mice even when fed at 300 ppm for 78 weeks, suggesting that the treatment of MelQx alone was not sufficient to promote colon tumors. In order to clarify a possibility whether such HCAs can induce colon tumors, C57BL/6J mice were fed MelQx, IQ, or PhIP at a dose of 300 ppm for 12 weeks and, thereafter, twice received 1-week treatment with dextran sulfate sodium (DSS), 2 weeks apart. After 20 weeks, colon tumors including adenocarcinomas were found at incidences of 22%, 24%, and 45% in the groups receiving MelQx, IQ, and PhIP, respectively, which were significantly (p < 0.05 or 0.01) different from the DSS alone value (0%). Thus our results clearly indicate that, in addition to PhIP, MelQx and IQ can induce colon tumors in mice under an experimental condition promoting colon tumors.

Key Words: MelQx; heterocyclic amine; colon tumor; dextran sulfate sodium; mouse.

MelQx has never been shown to induce colon tumors in rodents (Kato et al., 1987; Ohgaki et al., 1988), whereas colon tumorigenicity was exerted by the other HCAs 2-amino-3,4-dimethylimidazo[4,5-f]quinoline (MeIQ) and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) in both rats and mice (Fujita et al., 1999; Ito et al., 1991; Kato et al., 1989; Tanaka et al., in press), and by 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) in rats but not in mice (Ohgaki et al., 1984; Takayama et al., 1984).

We have demonstrated that MelQx is genotoxic in the colon as well as the liver in lacI transgenic C57BL/6N (Itoh et al., 2000) or gpt delta (Masumura et al., 2003) C57BL/6J mice fed at a dose of 300 ppm. It was also documented that MelQx induces aberrant crypt foci (ACFs), an intermediate biomarker for colon tumorigenicity, in rats (Kasahara et al., 1997; Tanakamaru et al., 2001) and mice (Okonogi et al., 1997). Therefore, in the present study, in order to elucidate whether MelQx can induce colon tumors in such transgenic mice, a 78-week feeding study of MelQx was performed in gpt delta mice, and in order to neglect any possible involvement of the reporter genes to tumorigenicity, wild-type C57BL/6J mice were also fed MelQx for 78 weeks. Then, the colon tumorigenicity of dietary MelQx, IQ, or PhIP was investigated in C57BL/6J mice under a colon tumor-promotional condition due to dextran sulfate sodium (DSS), in order to confirm whether the positive genotoxicity of MelQx found in lacI transgenic or gpt delta mice is sufficient to initiate colon tumors.

MATERIALS AND METHODS

Chemicals and animals. DSS with a molecular weight of 40,000 was purchased from ICN Biochemicals, Inc. (Aurora, OH). MelQx, IQ, and PhIP were purchased from Toronto Research Chemicals (Ontario, Canada). Wild-type male C57BL/6J mice and C57BL/6J mice carrying about 80 tandem copies of the transgene lambda EG10 per haploid genome were obtained from Japan SLC (Shizuoka, Japan). They were housed in a room with a barrier system, and maintained under the following constant conditions: temperature of 24 ± 1 °C, relative humidity of 55 ± 5%, ventilation frequency of 18 times/h and a 12-h light–dark cycle with free access to Oriental CRF-1 basal diet (Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water. The protocols for this study were

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and multiplicity data were analyzed by the Student’s t-test or the Welch’s t-test. Significant increases were observed in Group 4 compared to Group 1 (p < 0.05 or 0.01) from the Group 1 value (0%). BrdU-labeling index on the nonlesional epithelia in Groups 1 and 4 showed statistically significant differences (p < 0.05 or 0.01) different from the Group 1 value (0%). The multiplicity of colon tumors per animal was 0.30, 0.34, and 0.62 in Groups 3–5, respectively, showing statistically significant differences (p < 0.05 or 0.01) from the Group 1 value (0%). BrdU-labeling index was 4.8% and 7.7% in Groups 1 and 4, showing a significant increase in Group 4 over Group 1 (Fig. 3).

**DISCUSSION**

The results in the present study showed that MeIQx, IQ, and PhIP induce colon tumors in mice within 20 weeks under a tumor-promotional condition, whereas 78-week feeding of MeIQx alone at the same dose (300 ppm) failed to induce colon tumors. Although MeIQ, IQ, and PhIP have exerted colon

**RESULTS**

**78-Week Feeding Bioassay of MeIQx in C57BL/6J and gpt delta Mice**

Only one animal given 30 ppm MeIQx and two nontreatment animals in gpt delta mice and one animal in wild-type C57BL/6J mice receiving 30 ppm MeIQx died at early stages of the experiment. As shown in Table 1, only a few tumors were sporadically found in the liver, lung, and hematopoietic systems of C57BL/6J mice or gpt delta mice. Thus no induction of intestinal tumors was noticed in any of the mice.

**Colon Tumorigenicity of MeIQx, IQ, and PhIP**

Only three in Group 3 and one each in Groups 4 and 5 died during the experiment, although they had no neoplastic lesions. Excluding these animals, ACFs involving four aberrant crypts or more were 0, 0.5, 2.9, 4.9, and 7.2 in Groups 1–5, respectively (Table 2). The multiplicities of ACFs in Groups 3–5 were significantly (p < 0.001) higher than that in Group 2 (Table 2). Colon tumors including adenomas and adenocarcinomas (Fig. 2) developed at incidences of 6 (22%), 7 (24%), and 13 (45%) in Groups 3–5, respectively, although no colon tumors developed in Groups 1 and 2 (Table 3). These incidences were statistically significantly (p < 0.05 or 0.01) different from the Group 1 value (0%). The multiplicity of colon tumors per animal was 0.30, 0.34, and 0.62 in Groups 3–5, respectively, showing statistically significant differences (p < 0.05 or 0.01) from the Group 1 value (0%). BrdU-labeling index was 4.8% and 7.7% in Groups 1 and 4, showing a significant increase in Group 4 over Group 1 (Fig. 3).
tumorigenicity in rats (Ito et al., 1991; Kato et al., 1989; Takayama et al., 1984). MeIQx failed to induce colon tumors in rats (Kato et al., 1988). MeIQ induces colon tumors in mice by itself (Fujita et al., 1999), and PhIP followed by DSS treatment also induces colon tumors in mice in line with our results in the present study (Tanaka et al., in press). However, both MeIQx and IQ induce only colonic ACFs but not colon tumors in mice (Kristiansen, 1996; Ohgaki et al., 1984; 1987; Okonogi et al., 1997). Thus, MeIQx has never induced any colonic tumors in rodents, in clear contrast to MeIQ (Fujita et al., 1989; 1991). In this context, our results first show that MeIQx and IQ can induce colon tumors in mice. Especially, absence of colon tumorigenicity of MeIQx has been interesting in terms of the epidemiological data showing close associations of MeIQx with risks of colon cancer (Marchand et al., 2002; Sinha, 2002).

Recent epidemiologic studies have suggested that high red meat diets probably increase the risk of colorectal cancer (Butler et al., 2003; Norat and Riboli, 2001; Sandhu et al., 2001). Red meat, especially when cooked well done, may be a source of exposure to chemical carcinogens such as HCAs, polycyclic aromatic hydrocarbons, and other pyrolysis products (Butler et al., 2003; Sugimura, 1985). A number of HCAs have been identified in cooked meat and fish at levels that vary according to cooking methods, temperature and duration, and type of meat (Layton et al., 1995; Skogg et al., 1998), and PhIP and MeIQx are the most significant HCAs in terms of human exposure and carcinogenic potency (Layton et al., 1995). A dose-dependent association was found between the HCA intake estimates and male rectal cancer, and this association was the strongest for MeIQx (Marchand et al., 2002).

These HCAs have different organ specificity, although they have similar chemical structures and common metabolic pathways. MeIQ, unlike other HCAs including MeIQ, IQ, and PhIP, is activated to genotoxic and carcinogenic intermediates through N-oxidations catalyzed by P450 1A2 in the liver (Rich et al., 1992; Shimada et al., 1989; Turesky et al., 1998) and further activated by N-acetyltransferases or sulfotransferases (Chou et al., 1995; Minchin et al., 1992; Turesky et al., 1991). N2-(deoxyguanosin-8-yl)-MeIQx is a major DNA adduct detected in the liver and the other tissues of animals treated with MeIQx (Ochiai et al., 1991; Schut and Snyderwine, 1999; Snyderwine et al., 1993). Although the level of MeIQx in cooked foods is the second highest following PhIP in this group of compounds, the level of human exposure is estimated to be 107- to 108-fold lower than the doses for the rodent bioassays (Kobayashi et al., 2002; Layton et al., 1995; Ushiyama et al., 1991). It is important that the level of MeIQx-DNA adducts is greater in the human colon than in the colon of the rat or mouse strains, while the colon bioavailability of MeIQx and the types of DNA adducts appear similar between human and rodents (Mauthe et al., 1999).

In comparison of three HCAs tested in the present study, the colon tumor-inducibility was the greatest with PhIP, followed by IQ and MeIQx, although the latter two were almost comparable. It has been reported that in vitro potencies of bacterial genotoxicity and micronucleus induction are in the order MeIQx > IQ > PhIP (Pfau et al., 1999). It has also been shown that in vivo potencies of genotoxicity in the colonic mucosa of mice by alkaline single cell gel electrophoresis (Comet) assay are almost comparable among the three HCAs (Sasaki et al., 1998). Although the reasons for discrepancy between these previous data and the results in the present study remain unknown, one of the possibilities may be the differences in the tumor-promoting activity of the HCAs.

DSS is known to cause colitis in experimental animals, and DSS-induced colitis has been used as a model for ulcerative colitis in human (Okayasu et al., 1990). Although DSS itself is not genotoxic (Mori et al., 1984), it has been shown that DSS enhances colon tumorigenesis and even induces colon tumors in rodents after long-term feeding, suggesting that DSS is a nongenotoxic colon carcinogen or a colon tumor-promoter (Hirono et al., 1981; 1983). The data for BrdU-labeling index

### Table 2

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of mice</th>
<th>Incidence (%)</th>
<th>1AC</th>
<th>2AC</th>
<th>3AC</th>
<th>&gt;4AC</th>
<th>Total ACFs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. MeIQx alone</td>
<td>15</td>
<td>14 (93)</td>
<td>10.1 ± 3.7b</td>
<td>1.9 ± 1.4</td>
<td>0.1 ± 0.4</td>
<td>0</td>
<td>12.1 ± 4.6</td>
</tr>
<tr>
<td>2. DSS alone</td>
<td>15</td>
<td>15 (100)</td>
<td>14.0 ± 3.2c</td>
<td>6.4 ± 2.0c</td>
<td>2.7 ± 1.8c</td>
<td>0.5 ± 0.9c</td>
<td>23.6 ± 6.3c</td>
</tr>
<tr>
<td>3. MeIQx/DSS</td>
<td>27</td>
<td>27 (100)</td>
<td>42.5 ± 8.0c</td>
<td>16.8 ± 6.1c</td>
<td>7.1 ± 3.9c</td>
<td>2.9 ± 3.3c</td>
<td>69.3 ± 20.0c</td>
</tr>
<tr>
<td>4. IQ/DSS</td>
<td>29</td>
<td>29 (100)</td>
<td>47.0 ± 9.7c</td>
<td>19.8 ± 4.5c</td>
<td>10.7 ± 4.6c</td>
<td>4.9 ± 3.3c</td>
<td>82.4 ± 20.1c</td>
</tr>
<tr>
<td>5. PhIP/DSS</td>
<td>29</td>
<td>29 (100)</td>
<td>47.4 ± 10.9c</td>
<td>21.1 ± 8.2c</td>
<td>12.5 ± 5.6c</td>
<td>7.2 ± 4.6c</td>
<td>88.2 ± 28.1c</td>
</tr>
</tbody>
</table>

*Aberrant crypt.
*Mean ± SD.
*Significantly different from Group 1 or 2 at *p* < 0.01.
*Significantly different from Group 1 at *p* < 0.05.
*Significantly different from Group 1 at *p* < 0.01.
in our preliminary study clearly indicate that DSS could be a strong colon tumor-promoter, although other factors such as inflammatory cytokines remain unknown. In the present study, under the condition that the DSS alone treatment did not induce any colon tumors, the HCAs MeIQx, IQ, and PhIP induced colon tumors. The fact that MeIQx induces colon tumors is very important, because cross associations between intake of MeIQx and risk of colon cancer have been reported in epidemiological studies (Marchand et al., 2002; Sinha, 2002). In this context, colon tumor-promotional conditions such as ulcerative colitis could be important to achieve the colon tumorigenicity of MeIQx.

Recently, we reported that MeIQx induces significant genotoxicity in the colon as well as the liver of gpt delta mice when given for 12 weeks (Masumura et al., 2003), but in the present study MeIQx failed to induce any tumors in both gpt delta and wild C57BL/6J mice even after feeding for 78 weeks. Although the transgenic reporter genes are known not to be transcribed (Ono et al., 1995), wild mice were also tested for carcinogenicity in order to confirm that these genes would not influence genotoxicity or carcinogenicity. The results in the present study suggested that these animals carrying the transgene lambda EG10 should be biologically normal. In conclusion, our results clearly indicate that MeIQx, as well as IQ, can induce colon tumors in mice after sufficient tumor-promotion. This fact is in good agreement with epidemiological data and suggests that tumor-promoting factors are extremely important in colon carcinogenesis as well as tumor-initiating factors including HCAs. Further studies are needed to elucidate or neglect the possibilities that specific chemicals may promote spontaneously initiated cells. Not every tumor-promoter may promote all initiated cells the same way (Lee, 2000), or transcriptional alterations may be involved in the targeted genes (Thilly, 2003; Trosko, 1997).

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**TABLE 3**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Incidence (%)</th>
<th>Multiplicity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AD</td>
<td>ADC</td>
</tr>
<tr>
<td>1. MeIQx alone</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2. DSS alone</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3. MeIQx/DSS</td>
<td>5 (19)</td>
<td>2 (7)</td>
</tr>
<tr>
<td>4. IQ/DSS</td>
<td>5 (17)</td>
<td>4 (14)</td>
</tr>
<tr>
<td>5. PhIP/DSS</td>
<td>5 (17)</td>
<td>10 (34)</td>
</tr>
</tbody>
</table>

*a*Significantly different from Group 1 or 2 at *p* < 0.05.  
*b*Significantly different from Group 1 or 2 at *p* < 0.01.

**FIG. 2.** Photomicrographs showing colon tumors. (A) Adenocarcinoma found in a mouse given MeIQx + DSS. (B) Adenoma found in a mouse given IQ + DSS. H-E stain, original magnification ×180.

**FIG. 3.** BrdU-labeling index in nonlesional colonic epithelia of C57BL/6J mice given MeIQx and/or DSS. Data represent mean ± SD. *p < 0.01 versus MeIQx alone group.

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REFERENCES


