Inhibition of 2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine-induced Lymphoma Formation by Oltipraz

Chinthalapally V. Rao, Abraham Rivenson, Edith Zang, Vernon Steele, Gary Kelloff, and Bandaru S. Reddy

Materials and Methods

Chemicals, Animals, and Diets. PhIP was purchased from Toronto Research Chemicals (Downview, Ontario, Canada). Oltipraz was kindly provided by Rhone-Poulenc (Paris, France) through the National Cancer Institute Chemoprevention Program. Weanling male F344 rats were purchased from Charles River Breeding Laboratories (Kingston, NY). All ingredients of the semipurified diet were obtained from Dyets, Inc. (Bethlehem, PA) and stored at 4°C prior to preparation of the diets. Male F344 rats received at weaning were quarantined for 10 days and had access to modified AIN-76A control diet (15). Following quarantine, all animals were randomly distributed by weight.
The stability of PhIP and oltipraz in the diet were analyzed by high-performance liquid chromatography, according to our procedures published previously (16). The results indicate that >98% of PhIP and >96% of oltipraz could be accounted for in feed samples stored in a cold room for 14 days. The stability of PhIP and oltipraz in the diet were analyzed by high-performance liquid chromatography, according to our procedures published previously (16). The results indicate that >98% of PhIP and >96% of oltipraz could be accounted for in feed samples stored in a cold room for 14 days.

Experimental Procedure. Beginning at 5 weeks of age, groups of animals were fed their respective experimental diets containing 400 ppm of PhIP alone or 400 ppm PhIP and approximately 200 or 400 ppm oltipraz. The dose selection of 400 ppm of PhIP was based on earlier published results (16). This level of PhIP in the control diet was found to be toxic. Due to retardation of body weight gain and toxicity of PhIP at 400 ppm, the level of PhIP in the diet was reduced to 200 ppm from the 10th week and then to 100 ppm from the 14th week until the termination at 52 weeks following the start of the experimental diets. Body weights were recorded every week until the 16th week and then every 2 weeks until the termination of the study. As scheduled, all rats in each group were sacrificed under CO2 euthanasia. All organs, including all of the lymphatic areas, were examined grossly under a dissection microscope for the presence of lymphomas. Tumors were fixed in 10% buffered formalin, embedded in paraffin blocks, and processed for histopathological evaluation.

Statistical Analysis. Body weights were compared among the animals fed control and experimental diets with Repeated Measures ANOVA. Animal survival rates were compared among various groups by ANOVA. Since several rats in the PhIP group died before the scheduled sacrifice at week 52, the incidence of lymphomas (total number of lymphoma-bearing rats with respect to the total number of rats at risk) between the rats fed the PhIP plus control diet and PhIP plus oltipraz was analyzed by Life-Table Analysis (adjusted for length of survival). The multiplicity of lymphomas, as the mean number of lymphomas/animal, was analyzed by the ANOVA and Tukey's test. Differences were considered statistically significant at P < 0.05.

Results

Effect of PhIP on Body Weight Gain. Fig. 1 and Table 1 summarize the body weights of rats and survival rates of rats fed the control diet and the experimental diets containing two dose levels of oltipraz with or without PhIP. Administration of PhIP in the diet produced a significant retardation of body weight gain when compared to diets without PhIP. However, no significant differences were observed in the body weight of animals fed the control and oltipraz diets without PhIP. It is noteworthy that the body weights of animals fed PhIP plus oltipraz were significantly higher (P < 0.0001) than the groups consuming the control diet with PhIP, indicating that the dietary oltipraz protects against the toxicity induced by PhIP (Fig. 1 and Table 1).

Survival Rates. Because of severe retardation of body weight gain and also reduced food intake due to PhIP-induced toxicity, several rats died or were sacrificed due to morbidity during the course of study. In addition, several animals were sacrificed during the course of the study due to severity of thymic lymphomas. The results summarized in Table 1 demonstrate that the administration of oltipraz protected against PhIP-induced toxicity (P < 0.02 to 0.005) in terms of survival in a dose-dependent manner. The number of rats surviving until termination of the experiment at 52 weeks was highest in the PhIP plus 400 ppm oltipraz group (89% survival), followed by PhIP plus 200 ppm oltipraz (72%), and PhIP alone (38%).

Lymphoma Incidence. Administration of PhIP in the diet induced lymphomas primarily in the thoracic area (thymoma) and to a lesser extent in other organ sites. The lymphomas developed in PhIP-treated rats started in the thymic area. Large thymic lymphomas were observed as early as 16 weeks following the administration of the PhIP diet. The thymic lymphomas were large, greatly expanding the mediastinum, occupying almost the entire thoracic cavity and resulting in collapse of the lungs (Fig. 2). Because of these symptoms, most of the rats exhibited heavy breathing and reduced food intake that lead to the death or early sacrifice of these animals. Histologic evaluation indicated that large sheets of small- and medium-sized lymphoid cells arranged in uniform and compact patterns. Also, clusters of larger histoid or lymphoblastic cells were scattered throughout the tumor. Most of the rats with large thymomas also had highly enlarged spleen and enlarged lymph nodes in various areas of the lymphatic system. They also displayed the same histological characteristic features described for the mediastinal tumor, erasing the normal organ structure and infiltrating the neighboring tissues. The incidence of PhIP-induced lymphomas, including thymomas and enlarged spleens, are summarized in Table 2. Administration of 0.01–0.04% PhIP in the diet produced lymphomas in 75% of animals. Most of these lymphomas were of thymic origin. Since some of the rats with thymic lymphoma died during the course of the study, the tumor incidence data were analyzed by Life-Table Analysis. There is no evidence of these pathological abnormalities in rats fed the control diet or experimental diets containing oltipraz without PhIP.

Table 1 Effect of dietary oltipraz on PhIP-induced body weight gain and survival rate

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>No. of animals</th>
<th>% of body weights compared groupwise by repeated measures (ANOVA)</th>
<th>% of rats that survived until termination</th>
<th>%s for survival by pairwise comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control diet</td>
<td>36</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PhIP + 200 ppm oltipraz</td>
<td>36</td>
<td>0.0001*</td>
<td>72</td>
<td>0.02*</td>
</tr>
<tr>
<td>PhIP + 400 ppm oltipraz</td>
<td>36</td>
<td>0.0001*</td>
<td>89</td>
<td>0.005*</td>
</tr>
<tr>
<td>Control diet</td>
<td>12</td>
<td></td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>400 ppm oltipraz</td>
<td>12</td>
<td></td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

* Values are significantly different from the PhIP diet group (Fig 1.).

Values are not significant between control diet and oltipraz diet without PhIP. NS, not significant.
findings are that the animals fed 400 ppm oltipraz suppressed the incidence of PhIP-induced thymomas by 90–100%, and none of the rats fed oltipraz showed PhIP-induced spleen enlargement.

Intestinal Tumor Incidence. In the present study, administration of PhIP induced the intestinal tumor incidence in about 28% animals. Most of these tumors were localized in the small intestine (20% incidence), and very few tumors were localized in the colon (8% incidence). Because of low PhIP-induced intestinal tumor incidence, the administration of oltipraz in the diet had no significant effect on tumor incidence (data not shown).

Discussion

The major findings of this study were that the administration of PhIP in the diet induced lymphomas and that the induction of PhIP-induced lymphoma formation was inhibited by dietary oltipraz. These findings are very significant because the present study has identified not only an animal model to study the lymphomas induced by a food-borne carcinogen but also demonstrated inhibition of these lymphomas by oltipraz, a synthetic analogue of naturally occurring di-thiolethione. Research conducted over the past few years provided evidence that among heterocyclic amines, PhIP occurs in high concentrations in broiled meat (7, 8). At present, there are few epidemiological studies to suggest that consumption of meat would increase the risk of lymphomas (5–8). In experimental animals, administration of heterocyclic amines induce tumors in various organ sites (7). The present investigation provided a very useful animal model to study lymphoma formation with the naturally occurring carcinogen, PhIP.

The F344 rat lymphoma model developed in the present study has several advantages over the CDF, mouse model because a low dose of PhIP induced a high incidence of lymphomas (>75%) with much shorter time (<30 weeks), whereas in CDF, mice, the incidence of lymphomas was very low (<25%), even after 70 weeks of continuous feeding of 0.04% PhIP (9). The results of the present study are also somewhat comparable with the thymic lymphomas induced by N-propyl-N-nitrosourea and related synthetic chemical carcinogens in rats (17). Although the development of lymphomas in mice with retroviruses has been extensively studied (18), the relevance of retroviruses and synthetic carcinogens such as N-propyl-N-nitrosourea on lymphoma formation is not fully explored. On the other hand, PhIP-induced lymphomas in rats provide a realistic model to study the lymphoma genesis without viral implications or less rationalistic chemical carcinogens. Since most of the heterocyclic amines, including PhIP, is formed during the normal cooking of meat and thus humans may be exposed continuously to these genotoxic agents in their diet, the etiological role of this class of compounds in human cancer (lymphoma) development has been of high concern. At present, we are exploring the exact origin and specific type of lymphomas (T- or B-cell) induced by the administration of PhIP or related heterocyclic amines. The precise mechanism by which PhIP induces lymphomas is unclear; but it is possible that PhIP may alter the lymphatic cellular DNA or modifications in other macromolecules. In this connection, it is interesting to note that Morgenthaler and Holzhauser (19) demonstrated the induction of several mutations by PhIP in human lymphoblastoid cells. Several studies have also provided evidence of PhIP or heterocyclic amine(s)-DNA adducts in various target organs (8).

Another important finding of the present study is that the incidence of PhIP-induced lymphomas is significantly suppressed by oltipraz (20). In addition, the administration of oltipraz significantly protected the animals against the PhIP-induced toxicity. To our knowledge, this is the first study to demonstrate the inhibitory properties of dietary oltipraz against lymphoma formation in laboratory animals. These results extend the possibility of potential chemopreventive activity of this agent in human clinical trials. The precise mechanism by which oltipraz exerts its inhibitory effects on PhIP-induced toxicity and
lymphoma development has not been established. It is possible that modulation of several detoxification pathways and several cellular events by oltipraz might be responsible for its chemopreventive activity against the PHIP-induced lymphomas and toxicity. Previous studies from our laboratory and elsewhere showed that oltipraz protects against the toxicity of various chemicals, including aflatoxin B1, and inhibits tumor formation in several organs by a wide variety of carcinogens (12, 13). The relative rates of metabolic activation and/or detoxification of carcinogens can be critical determinants in tumor initiation and other events in subsequent tumor formation. Like most chemical carcinogens, PHIP undergoes enzymatic biotransformation in vivo (14). This process occurs mostly in the liver involving oxidation followed by esterification or conjugation. Oxidation results in the formation of N-hydroxy and/or ring-hydroxy derivatives of PHIP (7, 14). Esterfication of N-hydroxy PHIP by acetyltransferase or sulfotransferase generates highly reactive carcinogens: heterocyclic amines in cooked food. In: D. V. Parke, C. Ioannides and R. Walker (eds.), Food, Nutrition and Chemical Toxicity, pp. 259—276. London: Smith-Gordon, 1993.

In conclusion, the present study demonstrated that PHIP, a potential human carcinogen, induces lymphomas, thus providing a realistic model to analyze development of lymphoma without viral implications. In addition, dietary administration of oltipraz significantly protected against the PHIP-induced lymphoma and toxicity in male F344 rats.

Acknowledgments

We thank Laura Nast for the preparation of the manuscript and Dr. Karam El-Bayoumy for reviewing the manuscript. We are also thankful to Barbara Simi, Jeff Rigotty, and Beverly Gambrell for expert technical assistance.

References

Inhibition of 2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine-induced Lymphoma Formation by Oltipraz


Cancer Res 1996;56:3395-3398.

Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/56/15/3395

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.