No Effects of Chlorophyllin on IQ (2-Amino-3-methylimidazo[4,5-f]quinoline)-genotoxicity and -DNA Adduct Formation in Drosophila

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Previously we demonstrated that chlorophyllin suppressed the genotoxicities of many carcinogens. However, the genotoxicity of IQ (2-amino-3-methylimidazo[4,5-f]quinoline), a carcinogenic heterocyclic amine, was not suppressed in Drosophila. On the contrary, it has been reported that chlorophyllin suppressed the genotoxicity of IQ in rodents, rainbow trout and Salmonella. We demonstrated that the chlorophyllin-induced suppression of MeIQx (2-amino-3,8-dimethylimidazo[4,5-f]quinoline)-genotoxicity was associated with a decrease in MeIQx-DNA adduct formation in Drosophila larval DNA. MeIQx represents another type of heterocyclic amine and is similar to IQ in structure. In this study we utilized 32P-postlabeling to examine whether chlorophyllin reduced IQ-DNA adduct formation in Drosophila DNA in the same way as MeIQx. The results revealed that the formation of IQ-DNA adducts was unaffected by treatment with chlorophyllin. This was consistent with the absence of any inhibitory effect on genotoxicity as observed in the Drosophila repair test. These results suggest that IQ-behavior in Drosophila is not affected by chlorophyllin, indicating that the process of IQ-DNA adduct formation followed by expression of genotoxicity in Drosophila may be different from that in other organisms.

Key words Drosophila melanogaster; IQ (2-amino-3-methylimidazo[4,5-f]quinoline); DNA adduct

Chlorophyllin is a chlorophyll-derived green pigment that is used as a food additive. Reports have appeared demonstrating its antimutagenic potency in bacteria, and transgenic mice. Its anticarcinogenic potency in animals and fish has been demonstrated recently for aflatoxin B1, and PhIP (2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine) and IQ (2-amino-3-methylimidazo[4,5-f]quinoline). However, chlorophyllin did not prevent IQ-induced genotoxicity in Drosophila larvae.

RESULTS AND DISCUSSION

The amount of IQ-DNA adducts formed in the DNA of treated larvae was measured by the 32P-postlabeling method. DNA was isolated from the brain using chloroform extraction. The extracted DNA was subjected to modified adduct-intensification analysis, the details of which have been described previously. Dashwood demonstrated the protective effect of chlorophyllin on the covalent binding of IQ to rat liver DNA and bacterial DNA in relation to the observed anticarcinogenicity and antimutagenicity. This discrepancy suggests that the genotoxic behavior of IQ in Drosophila is different from that in other organisms. In the present study we therefore examined whether IQ-DNA adduct formation in Drosophila was affected by chlorophyllin.

MATERIALS AND METHODS

Chemicals IQ (2-amino-3-methylimidazo[4,5-f]quinoline) was purchased from Wako Fine Chemicals (Tokyo). Sodium copper chlorophyllin was obtained from Nacalai Tesque (Kyoto).

Drosophila Strains and Treatment The Drosophila strain used and the protocols relating to treatment with heterocyclic amines and modulators have been described previously. The sc z w(y6) mei-9a mei-41D5/C(1)DX; yf strain consists of DNA repair-deficient males and DNA repair-proficient females. This strain was kindly provided by Dr. H. Ryo (Osaka University) and Dr. K. Fujikawa (Kinki University). The 3rd-instar larvae were fed a diet containing 154 ppm IQ (1 mg/6.5 g diet/bottle) for 6 h, with or without chlorophyllin (0—4.5%; 0, 100, 200 or 300 mg/6.5 g diet). A portion of these larvae was grown on a diet without mutagen and modulator. The male/female ratio is an indicator of somatic cell DNA lesions, with the test being an in vivo DNA repair test. DNA analysis required that the larvae be immediately frozen in liquid nitrogen until the DNA could be extracted.

32P-Postlabeling Analysis The amount of IQ-DNA adducts formed in the DNA of treated larvae was measured by the 32P-postlabeling method. DNA was isolated from the brain using chloroform extraction. The extracted DNA was subjected to modified adduct-intensification analysis, the details of which have been described previously. Dashwood demonstrated the protective effect of chlorophyllin on the covalent binding of IQ to rat liver DNA and bacterial DNA in relation to the observed anticarcinogenicity and antimutagenicity. This discrepancy suggests that the genotoxic behavior of IQ in Drosophila is different from that in other organisms. In the present study we therefore examined whether IQ-DNA adduct formation in Drosophila was affected by chlorophyllin.
that the amount of IQ-adducts did not change, even at the highest dose of chlorophyllin (Figs. 1C—E). Figure 2 shows the results of IQ and chlorophyllin feeding. The IQ-induced decrease in the sex ratio was not reversed by the addition of chlorophyllin. In contrast, the genotoxicity of MeIQx is suppressed by chlorophyllin,\(^1\) and DNA adduct formation also decreased following treatment with chlorophyllin,\(^2\) corresponding to complex formation. IQ is expected to form a complex with chlorophyllin since it possesses a planar structure similar to the structure of MeIQx. It has been shown that IQ also forms a complex with chlorophyllin with similar propensity as MeIQx.\(^3\) However, the genotoxic process of IQ was not disturbed by chlorophyllin in Drosophila. These results suggest that IQ may act in Drosophila in a different manner than in other organisms. Further studies are required to determine whether Drosophila is unique in its metabolism of IQ.

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**REFERENCES AND NOTES**

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**Table 1. Adduct Formation and DNA Damage**

<table>
<thead>
<tr>
<th>IQ (ppm)</th>
<th>Adducts (/10⁷ nucleotides)</th>
<th>DNA damage (male/female)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Data) Mean</td>
<td>(Data) Mean</td>
</tr>
<tr>
<td>0</td>
<td>0.07</td>
<td>(1.22, 1.54)</td>
</tr>
<tr>
<td>15</td>
<td>(5.4, 10.2)</td>
<td>(0.98, 0.21)</td>
</tr>
<tr>
<td>31</td>
<td>(12.6, 6.7)</td>
<td>(0.36, 0.17)</td>
</tr>
<tr>
<td>92</td>
<td>(22.9, 36.1)</td>
<td>(0.19, 0.08)</td>
</tr>
<tr>
<td>154</td>
<td>(53.0, 34.5)</td>
<td>(0.19, 0.12)</td>
</tr>
</tbody>
</table>

Data from two independent experiments.

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**Fig. 1. Autoradiograms of IQ-DNA-Adducts in the DNA of Drosophila Larvae Fed IQ with or without Chlorophyllin**

A, no IQ control; B, IQ 1 mg (per bottle) only; C, IQ + chlorophyllin 100 mg; D, IQ + chlorophyllin 200 mg; E, IQ + chlorophyllin 300 mg. Each one of these photos is a representative of 3 experiments. The origins for the TLC analyses are indicated by the arrows.

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**Fig. 2. Effects of Chlorophyllin on IQ-Adduct Formation and DNA Damage**

Drosophila larvae were fed IQ at 1 mg/6.5 g diet/bottle (154 ppm) for 6 h together with chlorophyllin (0–300 mg). The level of adducts/10⁷ nucleotides are shown by closed circles, and the sex ratios by open circles. Three independent experiments were performed. Statistical significance was evaluated using a Student’s t test.