IN-VIVO CHROMOCLASTOGENIC EFFECTS OF AFLATOXIN B₁ IN THE HEPATOCYTES OF CHINESE HAMSTERS

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In-vivo effects of aflatoxin B₁ were studied in the chromosomes obtained from the regenerating hepatocytes of Chinese hamsters. Chromatid gaps were preferentially seen in the known heterochromatic areas of the chromosome pair one, X and Y. Asynchronous divisions of the centromere were seen in both the control and treated animals, however, the frequency was 8–10 times higher in the latter.

Introduction

Aflatoxin, a mycotoxin produced by Aspergillus flavus, has been shown to be carcinogenic (ASHLEY, et al., 1965; DICKENS & JONES, 1963 and KRAYBILL & SHIMKIN, 1964) and teratogenic (DI PAOLO, et al., 1967 and ELIS & DI PAOLO, 1967). In-vitro chromosome abnormalities have been produced by this compound in the seedling roots of Vicia faba (LILLY, 1965), roots of Allium cepa (REISS, 1971), in a cell line derived from the kidney of rat kangaroo (GREEN, et al., 1971), and human leukocyte chromosomes (DOLIMPIO, et al., 1968; PROMCHAINANT, et al., 1972 and WITHERS, 1966). Since the most dramatic and significant effect of aflatoxicoses is hepatomas (NEWBERNE & BUTLER, 1969), we decided to investigate the in-vivo effects of aflatoxin B₁ on the chromosomes obtained from regenerating hepatocytes (induced by partial hepatectomy) of the Chinese hamsters (Cricetulus griseus).

Material and methods

Crystalline aflatoxin B₁ (CalBiochem, San Diego, California) was used. As described previously (SRIVASTAVA, et al., 1973) a 30–50% liver was removed from the experimental animals by partial hepatectomy. At 24 hours after operation, the Chinese hamsters were injected with aflatoxin B₁, I/P, 1 mg/kg, in DMSO. Twenty-four hours after aflatoxin B₁ administration, colchicine, I/P, 4 mg/kg, was injected. A second dose of colchicine, 1 mg/kg, was also injected 4–5 hours, before sacrifice. The
animals were sacrificed by decapitation at 70-72 hours after partial hepatectomy. For controls, hepatectomized animals were injected with DMSO only. Chromosomes from hepatocytes were prepared by a previously described technique (Srivastava, et al., 1973). Histological sections were also prepared from the liver.

Results

Hepatic changes: The hepatic lesions in the aflatoxin B1 treated animals were not pronounced. A generalized fatty change and few focal areas of necrosis were most commonly seen. There was evidence of suppressed parenchymal regeneration. Biliary proliferation was not evident.

Cytogenetic changes: A total of 121 metaphases from 7 animals (2 males and 5 females) were examined and 82 of them showed aberrations (Table 1). The most common and frequent abnormality observed was chromatid gaps. These chromatid gaps were most frequently seen in the long arm of one of the X chromosomes, the long arm of the Y chromosome and in zones 3 and 12 of chromosome pair 1 (Hsu & Zenzes, 1964). A chromatid gap in the euchromatic area of chromosome pair 1 (Fig. 1) and pair 5 (Fig. 2) can also be seen. The chromatid gaps in metaphases were distributed as in Table 1. Beaded appearance of chromosomes was seen in 26 metaphases and asynchronous division of centromeres in 16 metaphases.

The next most frequent abnormality was asynchronous centromere division (i.e. the occurrence of divided and undivided centromeres within an individual fixed cell). This phenomenon was also seen in the control cultures, however, the frequency was 8–10 times higher in the aflatoxin B1 treated animals (Fig. 3).

In some metaphases a multiplicity of constrictions were seen throughout the entire length of chromosomes so that they assumed a “leaching-out” appearance or “beaded” pattern.

<table>
<thead>
<tr>
<th>Chromatid gaps</th>
<th>Beaded appearance</th>
<th>Asynchronous centromere division</th>
<th>Total</th>
<th>Normal metaphases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pair 1.</td>
<td>X</td>
<td>Y</td>
<td>7</td>
<td>27 6 26 16 82 39</td>
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TABLE 1
DISTRIBUTION OF ABNORMALITIES