Chlorpyrifos- route of exposure and susceptibility for mammary line tumor development in Mice

*KHAWAJA RAEES AHMAD1, ASMATULLAH2, TAHIR ABBAS3, KAUSAR RAEES4 & SHAHZAD AHMAD MUFTI5

1 Department of Biological Sciences, university of Sargodha; Sargodha
2 Department of Zoology, University of the Punjab; Lahore
3 PhD Scholars, Department of Zoology, University of the Punjab; Lahore
4 Principal, Government college for women Farooq colony, Sargodha
5 Advisor Bio-sciences COMSATS, Islamabad

ABSTRACT

Susceptibility to chlorpyrifos (CPF) for mammary line tumor development, with respect to its route of exposure, was investigated in virgin female mice (Mus musculus). Sixty animals (3-4 months old) weighing between 30-35g were randomly divided in 3 groups (20 each). Females in group 1 and 2 received 60mg/kg CPF each orally and as subcutaneous injection in the dorsal cervical region respectively. The animals in group 3 were exposed to CPF fumes (25ml, 40EC) for 24 hours in a 10x12x10ft hermetically sealed room. After CPF exposure the Dams were kept for 3 months under the same housing conditions. No signs of overt toxicity were observed in any of these animals. Well recognizable mammary line tumors appeared in 04 animals within the 60 days after CPF treatment, in group 3. In group 1 subcutaneous dorso-cervical tumor (at the site of CPF injection) was developed only in one animal in the 2nd month after treatment. All other animals remained normal throughout this study. Both hemoglobin level and total leucocyte count declined in animals that developed tumors. Our findings suggest an obvious relationship between mammary line tumors and exposure to CPF fumes for 24 hours in mice.

Key Words: Mammary line cancer, Chlorpyrifos, Adenocarcinoma, Metaplastic Carcinoma, Mice

INTRODUCTION

With a continuous rise in its incidence frequency, breast cancer continues to be a major challenge for public health. It is the most common form of cancer among women in the West (Anni et al., 2007). According to a study conducted in New Zealand more than 50% of breast cancer is unexplained by known risk factors; however, evidences exist that environmental factors are involved in the rapidly increasing risk of breast cancer (Hodgen et al., 2002).

Mammary cancer in women and rodents begin to develop from the luminal epithelial cells lining the mammary ducts and alveoli (Nandi et al., 1995); that progressively pass from pre-malignant, malignant and metastasizing stages (Wellings et al., 1975). Mammary cancers in humans and mice are now classified according to the histopathology and their hormonal dependency (Mikaelian et al., 2004). Generally mammary cancers are hormone-independent at the time of detection in mice while in rats are hormone-dependent and in humans both types are found (Anni et al., 2007). However in breast cancer, ovarian and pituitary...
hormones are essentially involved in stimulating luminal mammary epithelial cell proliferation and the development of tumors (Nandi et al., 1995).

According to the U.S. Environmental Protection Agency, chlorpyrifos had an annual usage of 8–10 million pounds in the agricultural sector alone in 1999 (Donaldson et al., 2002). Approximately 800 registered products contain CPF; which are being used for a variety of purposes (Smegal, 2002). The carcinogenic risk factor of CPF exposure may be enhanced due to its potentials to cause DNA damage (Bagchi et al., 1995) and to modify endogenous antioxidant defense system through suppression of superoxide dismutase and glutathione peroxidase activity leading to an increase in oxidative stress (Bebe & Panemangalore, 2003). Also it induces endocrine disruptions causing potent anti-androgenic (Viswanath et al., 2010) and enhanced estrogenic effects (Kojima et al., 2004) thus increasing the possible risk for mammary line carcinogenesis (Mukherjee et al., 2006).

As experimental exposures, almost always, are given orally or as injectables, while the environmental exposure of insecticides most often occur in the form of fumes, thus we decided to find out any relationship between the route of exposure of CPF and the possible risk of carcinogenesis.

**MATERIALS AND METHODS**

Sixty young (3 to 4 months old) virgin female (Swiss Webster) albino laboratory mice (Mus Musculus) ranging in weight between 30-35 grams were used in this study. They were divided into 3 groups of 20 animals each. Each animal in group 1 was delivered with an oral dose of 60mg/kg CPF by gavage. Females in group 2 were exposed to the same dose (60mg/kg) as intra-peritoneal injection in the dorso-cervical region. Group 3 Dams were kept in a 10x12x10ft hermetically sealed room for 24 hours where the walls of the room were freshly sprinkled with 25ml of 40EC Chlorpyrifos, with the help of a 5ml plastic syringe. All animals were kept in 18x18x12inch cages, under a standard protocol of 12hours light and dark periods for 3months after CPF exposure. The ambient temperature and humidity throughout this study were 25 +/- 2 0C and 30-40%. The animals were provided feed and drinking water ad-libitum. Dams were closely observed for the appearance of any sort of tumor formations daily throughout the post CPF exposure keep time.

The animals having developed tumors during post CPF exposure keep time were separated from the rest of their group members and kept in isolation in the separate cages. Each such animal was etherized to exteriorize tumor on the 20th day of its first appearance. Body weights of the intact animals were recorded. Tumors were exposed through an incision in the skin. In situ photographs were taken before and after exposure of the tumors in super-macro mode with 4MP (Kyocera M410) digital camera. After careful removal each tumor was weighed and fixed in Bouin’s solution for 48 hours for histological preparations. Body weight of the cancerous animals were recorded daily and represented in the form of a graph (Fig., 1). Hemoglobin level and total leucocytes count were obtained at the time of recovery (Table 1). Small pieces of tumor were processed for (8 micron thick) paraffinized sectioning and H & E staining. Photomicrograph of selected sections, on different magnifications, were obtained.
by a (Sony 7.2MP) digital camera mechanically fitted on (Labomid CXR2) trinocular microscope. The digital photographs were improved in CoralDRAW11 for color contrast adjustments, digital cropping and background changes.

RESULTS

Route of exposure and incidence of tumor: All members in the oral exposure group (group 1) remained healthy and no incidence of tumor formation was seen during the post exposure keep time (90 days). Among the animals in group 2, who received subcutaneous injections of CPF in dorsal cervical region, only one developed local tumor after 5 weeks (Fig 2.1Aa and Ab). Among the animals in group 3 (exposed to CPF fumes), four females out of 20 developed tumors (three animals in the 3rd week and one in the 5th weeks of exposure). Moreover, all tumors in ‘CPF fumes exposed group’ developed in the mammary line area (2.2Aa and Ab).

Histology of the Tumors

Histological preparations of the dorsal cervical tumor (group 2) reviled an apocrine metaplastic carcinoma of the sweat gland (Fig 2.1Ba and Bb), while the typical mammary line tumors appeared (group 3) to be the “mammary tubular adenocarcinoma showing ductal hyperplasia (Fig 2.1 Ba and Bb) with central necrosis (Comedo pattern)".

Recorded weights of the animal and tumors at the time of recovery are shown in the Table 1. Table 2 depicts the hemoglobin and TLC comparison of the tumoric animals with unexposed animal. Daily body weight response of the two selected tumoric animals are shown in Fig:1.

Fig., 1: Daily weight response of the animal with largest tumor of the mammary line area to that of the neck tumor.
Fig. 2.1Aa. A group1 female showing dorso-cervical

Fig. 2.1Ab. Dorso-cervical (exposed)

Fig. 2.1Ba. Histological section of the apocrine metaplastic carcinoma of the sweat glands (100X)

Fig. 2.1Bb. Metaplastic carcinoma of the sweat gland (400X)

Star: Malignant cell mass; Arrows: lobules filled with appocrine secretions

Fig. 2.2Aa. A group3 female showing mammary carcinoma Unexposed (Encircled)

Fig. 2.2Ab. A group3 female showing mammary carcinoma exposed (arrow)
**Fig. 2.2Ba.** Mammary tubular adenocarcinoma with ductal hyperplasia (100X)

**Fig. 2.2Bb.** Tubular adenocarcinoma comedo pattern (enlarged single duct: 400X).

**Arrows:** Adenocarcinoma comedo pattern: central tissue necrosis surrounded by malignant cells

**Table 1:** Showing Weight of the female at the time of recovery of the tumor

<table>
<thead>
<tr>
<th>ANALYSIS</th>
<th>Unexposed animal</th>
<th>Mammary Line Tumor</th>
<th>Subcutaneous (Neck) Tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin</td>
<td>12g/dl</td>
<td>8.1g/dl</td>
<td>7.8g/dl</td>
</tr>
<tr>
<td>Total Leucocytes</td>
<td>9200/mm³</td>
<td>3200/mm³</td>
<td>2800/mm³</td>
</tr>
</tbody>
</table>

**Table 2:** Blood analysis (hemoglobin ant total leucocyte count) of the dams with subcutaneous and mammary tumor to that of an untreated animals

<table>
<thead>
<tr>
<th>Type of tumor</th>
<th>Weight of the Dams (g)</th>
<th>Weight of Tumor (g)</th>
<th>Percent fractional weight of tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mammary line (Fig. 2.2Aa and Ab)</td>
<td>34.163</td>
<td>6.930</td>
<td>20.28%</td>
</tr>
<tr>
<td>Mammary line</td>
<td>28.286</td>
<td>4.342</td>
<td>15.35%</td>
</tr>
<tr>
<td>Mammary line</td>
<td>25.197</td>
<td>1.378</td>
<td>5.47%</td>
</tr>
<tr>
<td>Mammary line</td>
<td>29.455</td>
<td>5.412</td>
<td>18.37%</td>
</tr>
<tr>
<td>Neck (Fig. 2.1Aa and Ab)</td>
<td>24.892</td>
<td>4.679</td>
<td>18.79%</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Among organophosphorus group of insecticides Dichlorvos and Malathion are identified as endocrine disruptors and carcinogenic whereas Chlorpyrifos is also a definite endocrine disruptors (DeBruin & Josephy, 2002; Brody & Rudel, 2003) and recently has been clamed to cause cancer. Bebe & Panemangalore, (2003) found that exposure to Endosulfan and CPF can modify the activity of the endogenous antioxidants like superoxide dismutase. Chlorpyrifos intoxication has been found to cause a significant decrease in the reduced glutathione, catalase and glutathione- S-transferase activities (Goel et
Modification of endogenous antioxidants activities may lead to the development of oxidative stress in specific tissues (Zama et al., 2007). Whereas continued oxidative stress usually causes inflammation, that in turn may lead to chronic diseases including cancer (Reuter et al. 2010).

Completion of mammary gland development takes place following puberty. This process is mainly dependent on the estrogen and progesterone (Mueller et al., 2002). Hypothalamic neurons that regulate the reproductive axis through gonadotropin-releasing hormone (GnRH) are targets of environmental endocrine disrupting chemicals.

In this connection Gore (2002) found that treatment of CPF for 24 hours to the immortalized hypothalamic GT1-7 cells led to a significant alteration in the transcription of GnRH gene and GnRH mRNA levels. Keeping in view the potentials of CPF to alter GnRH biosynthesis in the hypothalamic immortalized cells in vitro, we suggest a similar endocrine disruption activity of GnRH neurons in vivo (particularly when the animals are exposed with the fumes of CPF) that must be involved (at least partially) in the development of mammary line tumors in the preset study.

Factor such as oxidative stress and estrogenic stimulation of CPF exposure may contribute towards mammary line oncogenesis. Results obtained in the present study, however, give an indication that neuro-endocrine disruptions may have a key role in mammary oncogenesis. Consistent inhalation of CPF fumes seems to disrupt the hypothalamic-pituitary-gonadal axis leading to the mammary line tumor development. Further in depth studies (like critical hormone bioassays) are required to obtain a clear picture about the mammo-oncogenesis under CPF-fumes exposure. Conclusively our results suggest a strong relationship between mammary oncogenesis and prolonged exposure to the aerial fumes of CPF.

REFERENCES


