LEAD ACETATE AND LEAD NITRATE INDUCED SEX-LINKED RECESSIVE LETHAL MUTATIONS IN MATURE SPERM OF Drosophila melanogaster

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ABSTRACT

The mutagenic effects of two lead salts were investigated: acetate and nitrate on mature sperm of Drosophila melanogaster. Oregon R adult males were injected intraperitoneally with different concentrations of the salts: 650, 750 and 850 ppm for lead acetate and 25, 50 and 75 ppm for lead nitrate. Treated males were mated with virgin BASC females and the F2 scored for the induction of sex-linked recessive lethal mutations. The higher doses of both salts were found to induce a significant number of these mutations at 5% level on the Kastenbaum-Bowman test (1970), lead acetate being less toxic and less mutagenic than lead nitrate.

RESUMEN

Se investigaron los efectos mutagénicos de dos sales de plomo: acetato y nitrato en espermatozoides maduros de Drosophila melanogaster. Machos adultos Oregon R fueron inyectados intraperitonealmente con diferentes concentraciones de las sales: 650, 750 y 850 ppm de acetato de plomo y 25, 50 y 75 ppm de nitrato de plomo. Los machos tratados fueron cruzados con hembras virgenes BASC y la F2 fue examinada para valorar la inducción de mutaciones letales recesivas ligadas al sexo. Ambas sales produjeron estas mutaciones en un nivel de 5% en la prueba de Kastenbaum-Bowman (1970), siendo el acetato de plomo menos tóxico y menos mutagénico que el nitrato de plomo.

INTRODUCTION

Drosophila melanogaster has been used for mutagenic studies ever since H. J. Müller developed the sex-linked recessive lethal test (SLRL) to detect the mutagenic effects of X rays (Muller 1927). He used the G1B chromosome which was later replaced by the Muller-5 or BASC chromosome. Using Drosophila, Auerbach and Robson (1946) were the first to discover the mutagenic effects of mustard gas. Since then a great number of chemicals have been tested for mutagenic activity in Drosophila (Lee et al. 1983).
Among the heavy metals, lead, has been shown to be widely distributed in the atmosphere, water, soils and foods (Beliles 1975). At the cellular level, lead inhibits the activity of enzymes that are dependent on the presence of free sulphydryl groups (SH). The clearest manifestation of these effects is the disturbance on the biosynthesis of heme, which in humans is accompanied by abnormalities in porphyrin metabolism (Valle and Ulmer 1972).

Lead acetate is used as a topical astringent and is found to be a renal carcinogen in rats (Bolyand et al. 1962, Van Esch et al. 1962, Roe et al. 1965, Mao and Molnar 1967, Choie and Richter 1974, Furst et al. 1976). In the Syrian hamster lead induces neoplastic changes in the bronchio-alveolar area (Kobayashi and Okamoto 1974, ICPEMC 1984). It also produces infertility in mice (Varma et al. 1974) and reduces the reproductive ability of rats (Stowe and Goyer 1971, Hackett et al. 1982, Hess and Sikov 1982).

Lead nitrate is employed in the manufacture of matches and explosives, as a mordant in dyeing of textiles, as well as a sensitizer in photography. It is embryotoxic to rats (McClain and Becker 1975) but not teratogenic (McClain and Sieker 1975). In Drosophila melanogaster lead induces enzymatic alterations in esterase and triose phosphate isomerase (Lower et al. 1976) and affects non disjunction (Ramel 1973).

Information about the mutagenic effects of lead salts in humans who are occupationally exposed to them and information obtained from in vitro studies are contradictory (Maki-Paakkanen et al. 1981).

We report the mutagenic effects of two lead salts, namely, acetate and nitrate on the induction of SLRL in mature sperm of Drosophila melanogaster.

MATERIALS AND METHODS

Lead acetate \([\text{(CH}_3\text{COO})_2\text{Pb} \cdot 3\text{H}_2\text{O}]\) (Baker) was employed at concentrations of 650, 750 and 850 ppm. Lead nitrate \([\text{Pb(NO}_3)_2]\) (Baker) was used at concentrations of 25, 50 and 75 ppm. The highest concentration of both salts is their LD$_{50}$. The salts were dissolved in distilled water.

Oregon R adult males were injected intraperitoneally (Felix and Rodriguez-Arnaiz 1968) with almost 0.2 \(\mu\)l of the salts solutions (Sega and Lee 1970) and after treatment the males were mated with BASC virgin females in a proportion of one male to two females, for three days. F$_1$ females fertilized by F$_1$ males were put into fresh medium, one per vial, and the F$_2$ scored for the induction of SLRL mutations by means of the absence of wild males. Only mature sperm were sampled (Vogel 1975). Controls were injected with 0.2 \(\mu\)l of 5% sucrose solution (Vogel and Sobels 1976). The Kastenbaum-Bowman test (1970) was employed in order to calculate the statistical significance of the results.

RESULTS AND DISCUSSION

Chemical agents which induce genetic damage constitute a risk for all populations, including human beings. The sex-linked recessive lethal test is capable of detecting chemicals which are capable of inducing heritable lethal mutations which may range from changes in only one base of the DNA, that is a point mutation, to small
deletions or chromosome aberrations. The SLRL test simultaneously examines 600 to 800 genes, which represents about 80% of the loci of the X-chromosome or roughly one fifth of the Drosophila genome (Lee et al. 1983).

Both lead salts induced SLRL (Table I), lead acetate was less toxic and less mutagenic than lead nitrate.

### TABLE I. INDUCTION OF SEX-LINKED RECESSIVE LETHALS BY LEAD ACETATE AND LEAD NITRATE ON MATURE SPERM OF Drosophila melanogaster

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>Number of tested chromosomes</th>
<th>Number of induced lethals</th>
<th>Percentage of lethals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1,178</td>
<td>7</td>
<td>0.59</td>
</tr>
<tr>
<td>Lead acetate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>650</td>
<td>1,285</td>
<td>11</td>
<td>0.86</td>
</tr>
<tr>
<td>750</td>
<td>1,061</td>
<td>10</td>
<td>0.94</td>
</tr>
<tr>
<td>850</td>
<td>1,327</td>
<td>28</td>
<td>2.11 *</td>
</tr>
<tr>
<td>Lead nitrate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>1,205</td>
<td>15</td>
<td>1.24</td>
</tr>
<tr>
<td>50</td>
<td>1,326</td>
<td>21</td>
<td>1.58 *</td>
</tr>
<tr>
<td>75</td>
<td>1,584</td>
<td>34</td>
<td>2.15 *</td>
</tr>
</tbody>
</table>

* Significant at 5% level on Kastenbaum-Bowman test (1970).

In the Tradescantia micronucleus test, the mutagenic effect of lead nitrate was found to be significant while lead acetate gave a borderline response (Ma et al. 1984).

In cattle, lead does not produce severe chromosome abnormalities (Leonard et al. 1974). In monkeys with a low calcium diet, lead acetate induces dicentrics, translocations and exchanges, while in groups receiving lead, irrespective of the diet, lead acetate produce gaps and fragments (Deknudt et al. 1977). In humans, reports about the induction of chromosomal aberrations in lead-exposed individuals are contradictory. Some have reported positive results (Garza-Chapa et al. 1977, Hogestead et al. 1979, Forni et al. 1980) while others have not found any increase in rates of chromosome aberrations (Schmid and Bauchinger 1973, O'Riordan and Evans 1974, Bijlsma and De France 1976, Horvat and Prpić-Majić 1977, Maki-Paakkannen et al. 1981. Qazi et al. (1980) observed a temporary increase in chromosome breakage in an infant exposed to lead while in utero. In some experiments, human lymphocytes exposed to lead salts in vitro, have been reported as unaffected (Deknudt and Deminatti 1978, Bauchinger and Gasiorek 1980), although Beek and Oboe (1974) obtained increased frequencies of chromosome aberrations.

In Drosophila we have found a statistical significant increase in the frequency of SLRL, mutations. However other experiments are needed to show the mode of action of these mutagens (Valencia et al. 1984).
REFERENCES


