Chromosomal aberrations in germ cells of male mice immunised with attenuated viral vaccines (human)

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Summary. The cytogenetic effects of two attenuated viral vaccines (yellow fever vaccine and oral poliomyelitis vaccine) were assessed by means of the analysis of meiotic spermatocyte chromosomes in mice. In a dose of 0.5 ml, but not 0.1 ml, both vaccines induced a significant percentage of chromosomal aberrations after 7, 14 and 30 days. Euploidy was the major abnormality produced by yellow fever vaccine. The various abnormalities produced by oral polio vaccine were significant when pooled, but not when analysed individually. More abnormalities were produced by yellow fever vaccine than by oral polio vaccine.

Introduction

Since the discovery of viral-induced chromosomal aberrations by Hampar and Ellison,1 viral-induced cytogenetic effects have become well established,2-4 although information is limited. Some live viral vaccines, such as herpes simplex, smallpox and measles, have been shown to induce cytogenetic effects both in cell cultures and in the somatic cells of laboratory animals.5-7

Most studies of the cytogenetic effects produced by viruses and viral vaccines have been made in somatic cells. In the present study the cytogenetic effects of two live attenuated viral vaccines (yellow fever and oral poliomyelitis) were assessed by the analysis of meiotic spermatocyte chromosomes in mice. This test system is recommended8,9 for assessing the mutagenic potential of agents. These vaccines were selected for study to examine the possible genetic risk associated with their widespread use.

Materials and methods

Vaccines

Yellow fever vaccine was prepared by the Central Research Institute, Kasauli, India and obtained from the Institute of Preventive Medicine (Government of Andhra Pradesh), Hyderabad. This live attenuated vaccine was a suspension of chick embryo tissue infected with the pantropic 17D strain of yellow fever virus. Before administration the freeze-dried vaccine was re-constituted in cold sterile isotonic saline.

Oral poliomyelitis vaccine (Sabin; trivalent) was prepared by Haffkin's Institute, Bombay and obtained from Moti and Company, Pharmaceutical Distributors, Hyderabad. It was a live attenuated vaccine containing an aqueous suspension of type 1, 2 and 3 strains grown in cultures of monkey kidney tissue. For both vaccines the doses used were 0.5 ml (the human dose) and 0.1 ml.

Mice

Swiss Albino male mice, each weighing 25–30 g and aged 5–7 weeks, were obtained from the National Institute of Nutrition, Hyderabad.

Experimental design

The mice were randomly assigned to control and experimental groups. Yellow fever vaccine was administered intraperitoneally and polio vaccine orally, each in a single dose. Yellow fever vaccine control animals were inoculated with sterile isotonic saline intraperitoneally, and polio vaccine control animals received sterile distilled water by the oral route.

Six vaccinated and six control mice were killed by cervical dislocation 7, 14 and 30 days after inoculation. The testes were then removed and used to make preparations of metaphase chromosomes of the spermatocytes by an air-drying technique.10 We scored approximately 500 meiotic metaphases for numerical (euploids and aneuploids) and structural (univalencies and translocations) aberrations at each time interval from each set of animals.
Statistical analysis

The significance of the differences between the frequencies of chromosome abnormalities of control and treated groups were analysed by the chi-square (χ²) test.

Results

Table I shows that the ranges of percentages of normal bivalents and total aberrations in the four control groups of animals were 87.2-89.7% and 10.49-12.8%, respectively. These control values are in agreement with published data.

The total aberrations in mice inoculated with yellow fever vaccine varied from 13.8 to 22.3%. These percentages were significantly different (p < 0.01) from the control values 7, 14 and 30 days after inoculation with the 0.5-ml but not the 0.1-ml dose. Like yellow fever vaccine, oral polio vaccine in a dose of 0.5 ml, but not 0.1 ml, induced increases in total chromosomal aberrations 7 (p < 0.01), 14 (p < 0.05) and 30 (p < 0.05) days after inoculation.

The total anomalies in mice inoculated with oral polio vaccine varied from 11.43 to 17.8%. The total aberrations induced by yellow fever vaccine were higher than those induced by oral polio vaccine. The frequency of abnormal chromosomes did not differ among individual mice.

The numerical aberrations in control and vaccine-inoculated mice are presented in table II. The percentage of euploid cells (40 II or more) in control animals at three time intervals was in the range 4.35-5.8%. Animals inoculated with yellow fever vaccine exhibited a frequency of 7-12.5% for this anomaly. Euploidy, which was the major abnormality seen, was induced (p < 0.01) only by the 0.5-ml dose of yellow fever vaccine. Mice inoculated with oral polio vaccine showed 4.76-7.2% euploids. At both the doses and at all three time intervals, these percentages were not significantly different from the control values.

The frequency of aneuploids (hypo- and hyperploidy) in the spermatocytes of control animals varied from 4.74 to 6.46%. In contrast, mice inoculated with yellow fever vaccine and oral polio vaccine showed frequencies of 4.49-6.35% and 5.71-8.8% respectively. With both the vaccines and at all three time intervals, these percentages were not significantly different from the control values.

The structural abnormalities found in the control and vaccinated animals are presented in table III. Univalent chromosomes were seen with a frequency of 0.1-1% in control animals, whereas the frequencies in mice inoculated with yellow fever vaccine and oral polio vaccine were 0.6-1.73% and 0.95-1.2% respectively. These percentages were not significantly different from the control values.

Table I. Normal bivalents and total chromosomal aberrations in the spermatocytes of control mice and of those inoculated with yellow fever vaccine (YFV) and oral polio vaccine (OPV)

<table>
<thead>
<tr>
<th>Dose (ml)</th>
<th>Treatment</th>
<th>Total metaphases</th>
<th>Normal bivalents (%)</th>
<th>Total aberrations (%)</th>
<th>Total metaphases</th>
<th>Normal bivalents (%)</th>
<th>Total aberrations (%)</th>
<th>Total metaphases</th>
<th>Normal bivalents (%)</th>
<th>Total aberrations (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>Saline (controls)</td>
<td>500</td>
<td>89-20</td>
<td>10-80</td>
<td>500</td>
<td>88-60</td>
<td>11-40</td>
<td>500</td>
<td>88-00</td>
<td>12-20</td>
</tr>
<tr>
<td>0.1</td>
<td>YFV</td>
<td>500</td>
<td>89-60</td>
<td>13-80</td>
<td>500</td>
<td>85-20</td>
<td>15-00</td>
<td>500</td>
<td>84-00</td>
<td>16-20</td>
</tr>
<tr>
<td>0.5</td>
<td>Saline (controls)</td>
<td>514</td>
<td>88-71</td>
<td>11-47</td>
<td>527</td>
<td>87-66</td>
<td>12-14</td>
<td>500</td>
<td>87-20</td>
<td>12-80</td>
</tr>
<tr>
<td>0.5</td>
<td>YFV</td>
<td>496</td>
<td>82-86</td>
<td>17-94†</td>
<td>489</td>
<td>82-00</td>
<td>18-40†</td>
<td>520</td>
<td>80-38</td>
<td>22-30†</td>
</tr>
<tr>
<td>0.1</td>
<td>Water (controls)</td>
<td>525</td>
<td>89-33</td>
<td>10-66</td>
<td>525</td>
<td>89-14</td>
<td>11-04</td>
<td>525</td>
<td>88-38</td>
<td>11-61</td>
</tr>
<tr>
<td>0.1</td>
<td>OPV</td>
<td>525</td>
<td>88-19</td>
<td>12-00</td>
<td>525</td>
<td>88-38</td>
<td>11-80</td>
<td>525</td>
<td>87-61</td>
<td>12-57</td>
</tr>
<tr>
<td>0.5</td>
<td>Water (controls)</td>
<td>505</td>
<td>89-70</td>
<td>10-49</td>
<td>500</td>
<td>89-20</td>
<td>10-80</td>
<td>480</td>
<td>87-90</td>
<td>12-29</td>
</tr>
<tr>
<td>0.5</td>
<td>OPV</td>
<td>525</td>
<td>84-50</td>
<td>16-19†</td>
<td>458</td>
<td>84-49</td>
<td>16-80*</td>
<td>500</td>
<td>84-00</td>
<td>17-80*</td>
</tr>
</tbody>
</table>

The sum of the total aberrations (%) and normal bivalents (%) is not always 100. This is because (1) the total aberrations included various types of abnormality, and (2) there was sometimes more than one type of aberration in the same metaphase stage entry. Normal bivalents percentage was calculated as follows:

\[
\text{Normal bivalents percentage} = \frac{\text{Number of normal bivalents}}{\text{Total number of metaphases screened}} \times 100.
\]

Values marked with * or † are significantly different from the control group (*p < 0.05; †p < 0.01).
Table II. Numerical chromosome aberrations in the spermatocytes of control mice and of those inoculated with yellow fever vaccine (YFV) and oral polio vaccine (OPV)

<table>
<thead>
<tr>
<th>Results of examinations made at (days after inoculation)</th>
<th>7</th>
<th>14</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose (ml) Treatment</td>
<td>Euploids (%)</td>
<td>Aneuploids (%)</td>
<td>Euploids (%)</td>
</tr>
<tr>
<td>---------------------</td>
<td>--------------</td>
<td>----------------</td>
<td>--------------</td>
</tr>
<tr>
<td>0.1 Saline (controls)</td>
<td>5.00</td>
<td>4.80</td>
<td>5.20</td>
</tr>
<tr>
<td>0.1 YFV</td>
<td>7.00</td>
<td>5.40</td>
<td>7.60</td>
</tr>
<tr>
<td>0.5 Saline (controls)</td>
<td>5.25</td>
<td>4.86</td>
<td>5.50</td>
</tr>
<tr>
<td>0.5 YFV</td>
<td>4.38</td>
<td>5.33</td>
<td>4.57</td>
</tr>
<tr>
<td>0.1 Water (controls)</td>
<td>4.76</td>
<td>5.90</td>
<td>4.95</td>
</tr>
<tr>
<td>0.1 OPV</td>
<td>4.35</td>
<td>5.54</td>
<td>4.60</td>
</tr>
<tr>
<td>0.5 Water (controls)</td>
<td>6.66</td>
<td>8.19</td>
<td>6.55</td>
</tr>
</tbody>
</table>

The percentage of each anomaly was determined as
\[
\text{Number of each type of anomaly} \times 100. \\
\text{Total number of metaphases screened}
\]

The total numbers of metaphases screened are given in table I.
Euploidy includes 40 II, 60 II, 80 11, and 100 11.
Aneuploidy includes hypodiploidy (16 11, 17 11, 18 11, and 19 11) and hyperdiploidy (21 11, 22 11, 23 11, and 24 11).
* Significantly different from the control group (p < 0.01).

Table III. Structural chromosome aberrations in the spermatocytes of control mice and of those inoculated with yellow fever vaccine (YFV) and oral polio vaccine (OPV)

<table>
<thead>
<tr>
<th>Results of examinations made at (days after inoculation)</th>
<th>7</th>
<th>14</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose (ml) Treatment</td>
<td>Univalencies (%)</td>
<td>Translocations (%)</td>
<td>Univalencies (%)</td>
</tr>
<tr>
<td>---------------------</td>
<td>----------------</td>
<td>-------------------</td>
<td>----------------</td>
</tr>
<tr>
<td>0.1 Saline (controls)</td>
<td>0.80</td>
<td>0.20</td>
<td>0.80</td>
</tr>
<tr>
<td>0.1 YFV</td>
<td>1.00</td>
<td>0.40</td>
<td>1.00</td>
</tr>
<tr>
<td>0.5 Saline (controls)</td>
<td>0.77</td>
<td>0.58</td>
<td>0.94</td>
</tr>
<tr>
<td>0.5 YFV</td>
<td>0.60</td>
<td>1.20</td>
<td>1.02</td>
</tr>
<tr>
<td>0.1 Water (controls)</td>
<td>0.76</td>
<td>0.19</td>
<td>0.57</td>
</tr>
<tr>
<td>0.1 OPV</td>
<td>0.95</td>
<td>0.38</td>
<td>0.95</td>
</tr>
<tr>
<td>0.5 Water (controls)</td>
<td>0.59</td>
<td>0.00</td>
<td>0.40</td>
</tr>
<tr>
<td>0.5 OPV</td>
<td>0.95</td>
<td>0.38</td>
<td>1.09</td>
</tr>
</tbody>
</table>

Univalency includes autosomal and sex chromosomes.
Translocation includes rings and chains.
The percentage of each anomaly was calculated as described in table II.
The total numbers of metaphases screened are given in table I.

uncommon in the spermatocytes of control mice (0.19-1.0%). The frequencies in mice inoculated with yellow fever vaccine and oral polio vaccine were also very low (0.4-1.73% and 0.19-0.6% respectively) and did not differ significantly from the control values.

Discussion

Various investigations with somatic cells have shown that vaccines induce chromosome damage, but information on the effects of vaccine on germ cells is limited to a few studies. Illinskikh
demonstrated the induction of chromosomal abnormalities by measles and tickborne encephalitis vaccines in the germ cells of mice. Yellow fever vaccine was shown to induce chromosome breaks in leucocytes and poliomyelitis type 2 vaccine induced a high percentage of diffuse and numerical chromosome abnormalities in the bone marrow cells of mice. Poliomyelitis type 1 virus also induced chromosome damage in cell cultures. Specific antigens of poliomyelitis virus persisted for long periods in mouse bone-marrow cells after vaccination. It has also been shown that live replicating and live non-replicating influenza viruses were equally capable of inducing a high percentage of chromosome abnormalities in the germ cells of mice.

These observations are comparable with those presented in this paper. Chromosomal abnormalities were produced at a greater frequency by yellow fever vaccine than by oral polio vaccine (table I). This may be attributed to the ability of the yellow fever virus to replicate in mice. The lower frequency of abnormalities produced by oral polio vaccine may be attributed to the inability of polio viruses to replicate in mice and to the loss of some vaccine as a result of oral administration.

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REFERENCES