Chromosome Aberrations and Survival in Normal and Dechorionated Embryos of *Misgurnus anguillicaudatus* (Cobitidae, Pisces) Exposed to Mitomycin C

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**Summary** We studied chromosome aberrations and survival in normal and dechorionated embryos of *Misgurnus anguillicaudatus* (Cobitidae, Pisces) exposed to mitomycin C (MMC) at concentrations of 25, 50 and 100 μg/ml. The survival rates of dechorionated embryos exposed to MMC decreased dose-dependently but those of normal embryos exposed to 25 and 50 μg/ml of MMC were not significantly different from the controls. On the other hand, the frequencies of chromosome aberrant cells in both normal and dechorionated embryos, which were observed in the blastula stage, increased dose-dependently. The frequencies in dechorionated embryos were not significantly higher in all doses than those in normal embryos. These results suggest that in normal embryos a large amount of MMC passed through the chorion and induced chromosome aberrations. For detecting chromosome aberrations, dechorionated embryos are more sensitive but normal embryos may also be sufficiently sensitive. The use of both normal and dechorionated embryos may give more detailed and useful information related to the properties of genotoxic chemicals. A chromosome aberration test using fish embryos showing a clear dose-response would be useful for monitoring genotoxic contaminants in the water environment.

**Key words** Chromosome aberration, Survival, Fish embryo, Dechorionation, Mitomycin C, Cobitidae.

Since rodent chromosome aberration test has been widely used for detecting the genotoxicity of chemicals, the same test using fish should be useful for monitoring genotoxic contaminants in the water environment. In fish, however, only a few studies using the chromosome aberration test have been reported (Kligerman et al. 1975, Hooftman 1981, Hooftman and Vink 1981, Prein et al. 1978, Ueda et al. 1991, 1992). Fish chromosome aberration tests can be difficult to perform as fish chromosomes are generally small in size and large in number, and mitotic activity in adult fish may be too low for application. Mizell and Romig (1977) stated that vertebrate embryos are exquisitely sensitive to pollutants, carcinogens, viruses and other noxious agents. Ueda et al. (1991) reported that embryonic cells were adequate for chromosome aberration tests because embryos actively develop in water containing chemicals and good metaphase figures are easily obtained. However, it has been suggested that in fish embryos the chorion works as a protective barrier against the outer environment and therefore deleterious effects occur only at high concentration (Mizell and Romig 1997). Mizell and Romig (1997) and Villalobos et al. (2000) reported that the dechorionated zebrafish and medaka embryos were more sensitive to chemicals. However, there has been no report on the chromosome aberration test using dechorionated embryos.

To evaluate the chromosome aberration test using fish dechorionated embryos, we studied chromosome aberrations and survival in normal and dechorionated embryos of *Misgurnus anguillicaudatus*.

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M. anguillicaudatus (Cobitidae, Pisces) living in fresh water, exposed to mitomycin C (MMC), a well-known clastogen used as a positive control for the chromosome aberration test.

Materials and methods

Fish and artificial fertilization

M. anguillicaudatus, obtained from fish dealers in Saitama, Tottori, and Nara Prefectures (Japan), were bred in the laboratory at approximately 25°C. Both male and female specimens were intraperitoneally injected with gonadotrophin (Nippon Teikoku Zouki, Japan) at a dose of 10 IU/g (body weight), and eggs and sperm were artificially obtained 8 h after the injection. The eggs were inseminated in a petridish.

Dechorionation

To remove the chorion, the fertilized eggs were treated with 0.25% trypsin (1:250, Difco Laboratory, U.S.A.) solution dissolved in Dulbecco’s PBS(−) for about 2 min, 7 min after insemination. The dechorionated eggs were rinsed three times with 80% Holtfreter’s solution (Holtfreter’s solution contains NaCl 3.5 g, KCl 0.05 g, CaCl₂ 0.1 g in a liter solution).

Exposure to mitomycin C and observation of survival

The normal and dechorionated fertilized eggs were placed in the 80% Holtfreter’s solution containing 25, 50, and 100 µg/ml of mitomycin C (MMC) (Kyowa Hakko Kogyo, Japan) and kept at 25°C until hatching. The survival rates of 50 embryos were observed in each treatment. When the hatching rate of the control of normal embryos was less than 80%, the experiment was cancelled.

Chromosome preparation and observation of chromosome aberrations

Chromosome preparations were made using embryos in the blastula stage (6 h after fertilization). The embryos were directly treated with colcemid at a concentration of 0.2 µg/ml for 2 h. The embryonic cells were moved into microtubes (1.5 ml) and treated with 0.1% sodium citrate to obtain liberated cells. The cells were then hypotonized with 0.068 M KCl solution for 20 min and fixed with a mixed solution of acetic acid and methyl alcohol (1:3) three times. A drop of fixed cells was spread on a clean glass slide and air-dried. The slides were stained with 2% Giemsa and observed microscopically. Cells with chromosome structural aberrations, including gaps, were scored. The differences were statistically analyzed by the Fisher’s exact test.

Results

The chromosome number of M. anguillicaudatus was 2n=50 (Fig. 1), and the karyotype consisted of 5 pairs of metacentric, 2 pairs of submetacentric, and 18 pairs of acrocentric chromosomes as reported by Ojima and Takai (1979) (Fig. 2).

In embryos exposed to MMC, various types of chromosome structural aberrations resulted from gaps, breaks, and exchanges were observed (Figs. 3, 4). The frequencies of cells with chromosome structural aberrations in-
duced in normal and dechorionated embryos exposed to MMC showed a similar dose-response. Significant increases from the control data were shown in all embryos treated with MMC (p<0.01 or p<0.05: the latter was only in normal embryos treated with 25 μg/ml). The ratios (D/N) of the frequencies in dechorionated embryos (D) to those in normal embryos (N) were 1.44 in 25 μg/ml of MMC, 1.39 in 50 μg/ml of MMC and 1.17 in 100 μg/ml of MMC (Table 1), but the differences were not significant.

The survival rates of normal and dechorionated embryos at 6 h (blastula stage) and 21 h (hatched time) after fertilization, are shown in Table 1. The survival rates of normal embryos exposed to 25 and 50 μg/ml of MMC were not significantly different from the controls at 6 h and 21 h, but those exposed to 100 μg/ml of MMC significantly decreased to 70% at 6 h and to 0% at 21 h (p<0.01). The survival rates of dechorionated embryos exposed to 25, 50 and 100 μg/ml of MMC decreased dose-dependently, the decreases in 50 and 100 μg/ml of MMC at 21 h being significant (p<0.01).

Discussion

A clear threshold level of MMC exposure was observed with respect to normal embryo sur-
vival, but none was observed in the induction of chromosome aberrations. The dechorionated embryos showed no threshold level of MMC exposure with respect to both survival and induction of chromosome aberrations. The frequencies of chromosome aberrant cells in dechorionated embryos were not significantly higher in all doses than those in normal embryos. In the survival test, the chorion appeared to be working effectively as a protective barrier against the outer environment. However, the chromosome aberration test suggests that a large amount of MMC passed through the chorion and induced chromosome aberrations. For detecting chromosome aberrations, dechorionated embryos would be more sensitive but normal embryos may be also sufficiently sensitive so far as MMC is concerned. These results suggest that the chromosome aberration test using normal embryos carried out under natural conditions is adequate for monitoring genotoxic contaminants in the water environment and that using dechorionated embryos is effective for more sensitive detection of genotoxic chemicals.

Effects of genotoxic chemicals to normal embryos should vary by their trans-chorionic permeability, which may be affected by lipid solubility (Helmstetter and Alden III 1995), molecular size, and/or other factors. The chromosome aberration test using both normal and dechorionated embryos may give more detailed and valuable information related to the properties of genotoxic chemicals. The lower survival rates of the controls of dechorionated embryos probably resulted from the damage caused by the trypsin treatment for dechorionation and sensitivity to dechorionated condi-

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Fig. 4. Representative chromosome structural aberrations in embryos of *Misgurnus anguillicaudatus* exposed to mitomycin C, observed in the blastula stage. Bar scale $=5 \mu m$.

<table>
<thead>
<tr>
<th>MMC (µg/ml)</th>
<th>Chromosome aberrant cells (%) in the blastula stage (6 h)</th>
<th>Survival (%) after fertilization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal embryo</td>
<td>0</td>
<td>0 (0/99)</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>6.0 (4/67)*</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>8.9 (17/196)**</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>18.8 (31/165)**</td>
</tr>
<tr>
<td>Dechorionated embryo</td>
<td>0</td>
<td>0 (0/90)</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>8.6 (9/105)**</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>12.3 (15/122)**</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>22.0 (31/141)**</td>
</tr>
</tbody>
</table>

* and ** indicate significant differences from controls (0 µg/ml) at p<0.05 and p<0.01, respectively.
tions. Therefore, care is necessary in the handling of the dechorionated embryos.

Mizell and Romig (1997) emphasized that zebrafish and medaka are useful for toxicity test. However, we propose that using the embryos of M. anguillicaudatus is superior due to the larger number of embryos obtained from one ovulation (about 5000) and the shorter hatching time.

The present study demonstrated that the chromosome aberration test using fish embryos showing a clear dose-response would be useful for detecting genotoxic chemicals in water. But the present study was limited to MMC exposure. Further investigation using other genotoxic chemicals is necessary for the establishment of this testing method.

References


