Difference in the Lethal Effects of Carcinogens/Mutagens among Three Cultured Goldfish (*Carassius auratus*) Cell Lines

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The sensitivities of three goldfish-cell lines to the lethal effects of four carcinogens/mutagens; 4-nitroquinoline-1-oxide (4NQO), 1-methyl-3-nitro-1-nitrosoguanidine (MNNG), mitomycin C (MMC) and bleomycin (BLM), were compared using colony-formation assay. RBCF-1 and CAF-MM1 cells were more sensitive to 4NQO, BLM and UV than were GEM 199 cells. CAF-MM1 and GEM 199 cells showed almost the same sensitivity to MNNG, while the RBCF-1 cells were more sensitive. RBCF-1 cells were also the most sensitive to MMC and BLM among the three. This suggests that there may be some defect in DNA repair system in RBCF-1 cell line.

INTRODUCTION

For the detection of carcinogens/mutagens in the aqueous ecosystem, fish has been used as a test animal. Cultured fish cells were also used to study the effect of chemicals. Recently, several goldfish cell lines with the high plating efficiency have been established, and the following characteristics of these fish cells have been reported on the lethal effects of radiation: a) all cell lines studied were found to be much more resistant to ionizing radiation than mammalian cells when D₀ values were compared, and both D₀ and D₉ values varied among these cell lines, b) ionizing radiation causes DNA-strand breaks in fish cells and most of them are rejoined immediately after irradiation, as in mammalian cells, c) the division delay after exposure to ionizing radiation was very short in fish cells, d) two cell lines derived from the fin tissue of goldfish were sensitive to UV irradiation than the other cell line derived from erythrophoroma of goldfish, e) all cell lines retain photoreactivability after UV irradiation, while no excision repair of pyrimidine dimers was demonstrated in any of these fish cell lines.

These results suggest that the DNA-repair mechanisms in fish cells may be different from those in mammalian cells and that the response of fish cells to DNA-damaging agents may also be different. In this report, sensitivities to carcinogens/mutagens of three fish cell lines with different sensitivities to radiation were studied.

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MATERIALS AND METHODS

Three established cell lines derived from goldfish (Carassius auratus) were used for the present study. RBCF-1 and CAF-MM1 were taken and cultured from the fin tissue of goldfish, and GEM 199 was grown from an erythrophoroma, a tumor originating from red pigment cells on the body surface of a goldfish. Cells were grown in HEPES-buffered (15 mM) medium 199 (GIBCO) supplemented with 10 per cent foetal bovine serum (FBS; Hyclone) and antibiotics at 26°C in air. The cells were inoculated into 60 mm plastic dishes and allowed to settle for 8–12 hours in medium 199 supplemented with 20 per cent FBS. Then the medium was discarded and the cells were washed once with the medium 199 without FBS and treated with each chemicals added to the medium 199 without FBS, for 60 min at 26°C. The cells were washed with the medium 199 twice after the chemical treatment, then fresh medium 199 supplemented with 20 per cent FBS was added. All the chemicals, 4-nitroquinoline-1-oxide (4NQO; Wako Pure Chemicals), 1-methyl-3-nitro-1-nitrosoguanidine (MNNG; Nakarai Chemicals), mitomycin C (MMC; Kyowa Hakko), and bleomycin (BLM; Nippon Kayaku), were dissolved in 70 per cent ethanol just prior to use (ethanol itself had no effect on the cellular survival under the present experimental concentration). After 10–14 days incubation at 26°C without medium change, the colonies were fixed and stained. Colonies containing 50 or more cells were counted as survivors. The surviving fraction for each point was normalized to those of control cultures processed in the same way at the same time.

RESULTS AND DISCUSSION

Table 1 summarizes the radiation sensitivities of the three cell lines along with the general growth characteristics of the cells compiled from the previous publications, the number of passages are same with those used in the present experiments. RBCF-1 and CAF-MM1 cells were more sensitive to UV irradiation than GEM 199 cells, while D0 values after γ-irradiation were not greatly variable.

Table 1. Comparison of radiosensitivities of goldfish cell lines in vitro.

<table>
<thead>
<tr>
<th></th>
<th>CAF-MM1</th>
<th>RBCF-1</th>
<th>GEM 199</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Origin</strong></td>
<td>Normal Fin</td>
<td>Normal Fin</td>
<td>Erythrophoroma</td>
</tr>
<tr>
<td><strong>P.E. (%)</strong></td>
<td>15–20</td>
<td>15–20</td>
<td>60–80</td>
</tr>
<tr>
<td><strong>Doubling Time (hour)</strong></td>
<td>29</td>
<td>20</td>
<td>21</td>
</tr>
<tr>
<td><strong>γ-ray</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D0 (Gy)</td>
<td>3.25</td>
<td>2.75</td>
<td>3.25</td>
</tr>
<tr>
<td>Dq (Gy)</td>
<td>9.75</td>
<td>4.50</td>
<td>16.25</td>
</tr>
<tr>
<td><strong>UV</strong></td>
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</tr>
<tr>
<td>D0 (J/m²)</td>
<td>1.7</td>
<td>0.9</td>
<td>3.2</td>
</tr>
<tr>
<td>Dq (J/m²)</td>
<td>2.6</td>
<td>2.2</td>
<td>11.4</td>
</tr>
</tbody>
</table>
Fig. 1. Dose-survival curves of three goldfish cell lines (●; GEM 199, ○; CAF-MM1 and ×; RBCF-1 cells) after 4NQO, MNNG, MMC and BLM treatment for 60 minutes. Averages of triplicate experiments were used for each point with S.E.

MNNG, MMC and BLM for 60 minutes at 26°C. CAF-MM1 and RBCF-1 cells were more sensitive to 4NQO killing than GEM 199 cells. The colony forming ability of the RBCF-1 and CAF-MM1 cells was more sensitive to 4NQO as well as to UV than that of the GEM 199 cells. The responses to killing and the mutagenic effects of 4NQO in mammalian cells and bacteria
have been shown to be similar to those to UV when cell strains with and without DNA repair capacities were compared\(^2\). Since the excision repair of the pyrimidine dimers induced by UV irradiation has not been detected in cultured fish cells\(^4, 9\), the different UV and 4NQO sensitivities may not be attributed to the difference in the DNA repair capacities. The presence of enhancement of UV killing by caffeine in fish cells\(^5, 10\) suggests that repair system other than excision repair may be involved.

RBCF-1 cells were also more sensitive to MNNG than GEM 199 and CAF-MM1 cells, which showed almost the same sensitivity. The sensitivity to MNNG in human cells has been reported to correlate with that of ionizing radiation, but not with those of UV or 4NQO\(^13\), and similar relative sensitivities to MNNG and γ-rays were demonstrated in these fish cells (Fig. 1 and Table 1), although the difference in γ-ray sensitivity was small.

The increasing sensitivities to MMC were in the following order: GEM 199, CAF-MM1 and RBCF-1. Using the repair-deficient Chinese master mutant cells, Thompson et al., suggested that the MMC sensitivity reflected the repair capacity for UV or ionizing radiation of the cell\(^14\). At least in comparison with UV, such correlation was demonstrated in these fish cell lines.

GEM 199 cells were also more resistant to BLM than CAF-MM1 and RBCF-1 cells which showed almost the same sensitivity. The biphasic survival curve reported for the mammalian cells\(^15, 16\) was not obvious in the fish cells. BLM releases thymine bases and produces DNA-strand breaks, and most of its effects on cellular survival in human cells have been reported to be similar to those of ionizing radiation\(^17\). The responses of fish cells were quite different from that of mammals and were similar to the response to UV and 4NQO.

The relative sensitivities to 4 chemicals among 3 fish cell lines were not in complete agreement with the previous reports of the relative sensitivities in mammalian cells or bacteria. This could be due to the DNA repair mechanisms in fish cells which appear to be different from mammalian cells or bacteria. RBCF-1 cells are most sensitive to both radiation and 4 chemicals used in present experiments among three cell lines, which suggesting that there may be some defect in DNA repair system in these cells.

REFERENCES

LETHAL EFFECTS OF MUTAGENS TO FISH CELLS


