The Japanese Medaka, *Oryzias latipes*, as a New Model Organism for Studying Environmental Germ-cell Mutagenesis

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The effects of genotoxic substances on ecosystems should be assessed using various test systems with multiple genetic end points. The most widely used test system has been the specific-locus test developed by W.L. Russell, using the mouse. We are developing a new, nonmammalian test system using the Japanese medaka, *Oryzias latipes*. We have examined 625,926 embryos that correspond to 1,586,649 loci. In the medaka test system, four genetic end points are evaluated: dominant lethals, total mutations, viable mutations, and malformations. Because the medaka is an oviparous experimental animal, we were able to determine that approximately 90% of spontaneous as well as r-ray-induced total mutants died during development, irrespective of spermatogenesis stages at the time of exposure. Exposure of sperm and spermatids to ethylnitrosourea (ENU) also resulted in embryonic death of approximately 90% of total mutants. In sharp contrast, approximately 90% of total mutants recovered from ENU-exposed spermatogonia became viable mutants. These results indicate that the quantitative relationship between induction of specific-locus mutations and dominant lethals remains the same among spermatogenesis stages for r-rays, while it is biased excessively to the induction of specific-locus mutations in ENU-exposed spermatogonia. Thus, the assessment should integrate at least two factors, agent-specific and species-specific effects. — Environ Health Perspect 102(Suppl 12):33-35 (1994)

Key words: Japanese medaka *Oryzias latipes*, germ-cell mutagenesis, spermatogenic stage sensitivity, spontaneous rates, dominant lethals, specific-locus mutation, total mutation, r-rays, ethylnitrosourea

Introduction

The experimental basis for assessment of genetic risk in humans from exposure to environmental mutagens such as radiation and chemicals has been dependent on Russell’s mouse specific-locus test (SLT) system (1). The mouse data, therefore, have been the only data from which extrapolation to humans has been rationalized; and consequently, these data have often been regarded as being representative of the remaining nonhuman organisms. However, to address the issue of how best to assess the effects of genotoxic substances on ecosystems, it is important to remember biodiversity—the presence on earth of millions of species of animal and plants. About nine years ago, we began to develop a nonmammalian SLT system to study environmental germ-cell mutagenesis using a teleost, the Japanese medaka *Oryzias latipes* (2-4). During the initial phase of our study, r-rays from a 137Cs source were used to mutagenize wild-type males. As accumulation of data from irradiation experiments progressed, we also began to use a potent mutagenic alkylation agent, ethylnitrosourea (ENU). In this short report, some preliminary results on ENU-induced germ cell mutagenesis will be compared with our data obtained from irradiation experiments. The details of the ENU results will be published elsewhere (A Shimada and A Shima, unpublished data).

Materials and Methods

The experimental procedures for the medaka SLT have been previously described (2,3). Groups of 50 to 70 wild-type male medaka of the Sakura population were exposed to graded doses (0, 0.64, 1.90, 4.75, and 9.50 Gy; 0.95 Gy/min) of r-rays or various concentrations (0, 0.1, 0.5, and 1.0 mM) of ENU for 2 hr at 27°C. Each of the treated males was allowed to mate with a nontreated tester female which was homozygously recessive at three marker loci: b/b, h/h, and g/g/g. The fertilized eggs, collected daily after treatment of males, were carefully observed under a stereo microscope. During development of each embryo we scored dominant lethals (DL); total mutations (TM), which are specific-locus mutations that are phenotypically detected during early development and include mutations associated with dominant lethals; viable mutations (VM), which are viable specific-locus mutations in hatched embryos; and morphologic anomalies. Under specified conditions, the timing of spermatogenesis is well characterized in this species (5). The stage at the time of exposure is related to the number of days between treatment and embryo collection, as follows: 1 to 3 days, sperm; 4 to 9 days, spermatids; 10 to 15 days, spermatocytes; 16 to 29 days, differentiating spermatogonia; 30+ days, spermatogonia.

Results

Results obtained by July 8, 1993, using the medaka SLT system (Table 1) indicate that the medaka system is comparable to the mouse SLT system. For the purpose of simplicity, the spermatogenic stages included in Table 1 are limited to sperm, spermatids, and spermatogonia. In the control, 178,093 embryos were scored. The rate for spontaneous death during embryonic development was 5.8 x 10⁻²/gamete (90% confidence limits, 5.9 x 10⁻² and 5.7 x 10⁻²). These values are comparable to those from the mouse study (6). The spontaneous total mutation (TM) rate was 3.8 x 10⁻³/locus (90% confidence limits, 5.6 x 10⁻³ and 2.5 x 10⁻³). Because there exist no
Table 1. Numbers of scored animals included in this report.

| Exposed stage of male gametes | Mutagens | Fertilized eggs | Dead embryos | Total mutations | Viable mutations |
|-------------------------------|----------|-----------------|--------------|----------------|-----------------|
| Sperm                         | γ-rays   |                 |              |                |                 |
| 0.64 Gy                       | 7,092    | 760             | 19/20,433    | 2/18,996       |
| 1.90 Gy                       | 3,976    | 687             | 33/11,202    | 2/29,840       |
| 4.75 Gy                       | 10,970   | 3,777           | 144/24,128   | 16/17,133      |
| 9.50 Gy                       | 8,612    | 5,013           | 203/12,838   | 8/6,099        |
| Reactor radiation             | 0.31 Gy  | 7,704           | 1,586,649    |                |
| 0.63 Gy                       | 8,243    | 1,237           | 33/23,553    | 5/21,018       |
| Ethylnitrosourea              | 0.5 mM   | 5,157           | 613          | 10/15,189      | 0/14,172        |
|                              | 1 mM     | 5,124           | 2,227        | 19/13,644      | 1/8,736         |
| Spermatids γ-rays             |          |                 |              |                |                 |
| 0.64 Gy                       | 13,620   | 1,076           | 17/39,624    | 1/37,632       |
| 1.90 Gy                       | 7,760    | 1,201           | 27/22,084    | 3/19,677       |
| 4.75 Gy                       | 17,069   | 4,014           | 118/36,223   | 10/30,084      |
| 9.50 Gy                       | 13,264   | 4,858           | 149/19,401   | 9/10,431       |
| Reactor radiation             | 0.31 Gy  | 14,808          | 844          | 19/43,597      | 1/41,892        |
| 0.63 Gy                       | 18,433   | 1,794           | 55/53,336    | 7/49,917       |
| Ethylnitrosourea              | 0.5 mM   | 11,499          | 2,135        | 16/33,488      | 1/28,082        |
|                              | 1 mM     | 11,396          | 6,349        | 29/24,675      | 2/15,111        |
| Spermatogonia γ-rays          |          |                 |              |                |                 |
| 0.64 Gy                       | 48,783   | 2,411           | 10/142,145   | 1/125,547      |
| 4.75 Gy                       | 71,152   | 5,431           | 67/151,547   | 8/145,239      |
| 9.50 Gy                       | 43,731   | 3,481           | 51/89,085    | 7/85,413       |
| Reactor radiation             | 0.31 Gy  | 17,726          | 741          | 2/43,443       | 1/41,955        |
| 0.63 Gy                       | 26,206   | 3,419           | 13/152,161   | 2/130,192      |
| Ethylnitrosourea              | 0.5 mM   | 15,543          | 1,199        | 8/45,292       | 4/43,032        |
|                              | 1 mM     | 33,165          | 1,986        | 33/96,687      | 30/93,537       |
| Control                       | 0 Gy     | 178,093         | 10,375       | 17/450,293     | 2/429,660       |
| TOTAL                         |          | 625,926         |              | 1,586,649      |                 |

*From Shima and Shimada (4); updated July 8, 1993. *Number of total mutants/(sum of effective numbers of marker loci of embryos). *Number of viable mutants/(number of hatched fry x number of marker loci per tester used).

Figure 1. Changes in dominant lethal rates as a function of time after treatment of wild-type males with γ-rays or ENU.

which corresponds to the spermatogonial stage. The increase in DL rates by ENU was essentially dose-dependent throughout 30 days after treatment, although the peak sensitivity was observed around 9 days, which corresponds to the exposure of male germ cells at the spermatid stage. These results indicate that the sensitivity, in terms of DL, of male germ cells changes remarkably with spermatogenic stages and also that the stage of peak sensitivity depends on the mutagen to which organism is exposed.

Dose-response relationships for DL induced by γ-rays (Figure 2a) or ENU (Figure 2b) also vary among sperm, spermatids, and spermatogonia. These results indicate that the approximate range of DL rates induced by γ-rays up to approximately 10 Gy is roughly comparable to that induced by ENU up to 1 mM. This apparent similarity does not necessarily indicate similarity in the underlying mechanisms. The common feature of γ-rays and ENU was that sperm and spermatids, postmeiotic male germ cells, are more sensitive than spermatogonia.

Figure 2. Induction of dominant lethals by γ-rays (a) or ENU (b) in sperm (Sm), spermatids (Sd), and spermatogonia (Sg).

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Although not presented as figures, the highest induced TM rates in our γ-ray results (sperm after 9.50 Gy) were approximately 1.6 \times 10^{-3} (203/12,838; from Table 1)/locus, while those in our ENU results (sperm exposed to 1 mM ENU) were approximately 1.4 \times 10^{-3} (19/13,644; from Table 1)/locus. In contrast to the above-mentioned similarities in the induced DL rates, which were roughly comparable between γ-ray dose of 9.50 Gy and ENU concentration of 1 mM, the TM rate induced by 1 mM ENU was about one order of magnitude lower than that induced by 9.50 Gy γ-rays. Such was also the case with VM rates.

Conclusions

In an attempt to understand both radiation- and chemical-induced germ-cell mutagenesis, relationships between specific-locus mutation rates and dominant lethal rates have been contrasted using both radiation and ENU. Figures 3a (γ-rays) and 3b (ENU) show relationships between specific-locus mutation rate (x-axis) and dominant lethal rate (y-axis) observed in postmeiots (sperm and spermatids, Pm) and spermatogonia (Sg). Data points obtained from γ-ray experiments, irrespective of spermatogenic stages at exposure, fell onto almost the same lines. In a sharp contrast to γ-ray results as well as results for Pm in Figure 3b, the induction of TMs and VMs in spermatogonia by ENU was essentially not accompanied by the induction of dominant lethals. These results suggest that rather uniform mechanisms would be operating in γ-ray mutagenesis in male postmeiots and spermatogonias of the medaka, while the overall mutagenic process by ENU would be quite different between the postmeiots and spermatogonia.

Thus, the assessment of the effects of genotoxic substances on ecosystems should integrate at least two factors; species-specific and agent-specific effects. The applicability of a particular test system to specified ecosystems should also be considered. Our medaka SLT system, as a late-starting study, benefits from the experience of the predecessor, Russell's mouse SLT. It appears that establishment of solid foundations of and continued progress in basic biology of the medaka now allow us to step into molecular mechanistic research [(8); Kubota et al., unpublished].

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