

Chromosome Aberrations Induction in Freshwater Mussel *Dreissena polymorpha* on 3-Methylcholanthrene Exposure

P. Woznicki*

Department of Ichthyology, University of Warmia and Mazury in Olsztyn, Oczapowskiego 5, 10-957 Olsztyn, Poland.

Received: October 25, 2004

Accepted: April 11, 2005

Abstract

The influence of 3-methylcholanthrene to zebra mussel (*Dreissena polymorpha*) larvae was studied. The artificial spawning of zebra mussels was used for obtaining larvae. Two different concentrations of 3-methylcholanthrene were used. The chromosome analysis showed a significant increase in chromosome aberrations (CA) at the higher concentration of the compound. The resistance of zebra mussel larvae to the lower concentration of 3-methylcholanthrene indicated that zebra mussel larvae are probably not sensitive enough for the study of genotoxicity of the compounds from the PAH group.

Keywords: zebra mussel, genotoxicity, PAH, chromosome aberrations

Introduction

In recent years much attention has been paid to the possibilities of detecting genotoxic substances in the water environment. It is very difficult to identify the impact of a genotoxic substance or another environmental stressor on higher levels of biological organization (population, ecosystem) [1]. The effect of genotoxic chemicals on the integrity of DNA is the first event in aquatic organisms following exposure [2]. Genotoxic substances can induce so-called adducts, single- and double-strand breaks and DNA crosslinks. As a result of changes in the sequence and organization of DNA, chromosome aberrations (Cabs) can be expressed after cell replication. Cabs can be measured as alteration in chromosomal structure or number in metaphase. Alternatively, micronuclei frequency can be evaluated in interphase of proliferant cells as an index of chromosomal damage. Despite increasing interest in the presence of genotoxic substances in the water environment, there are no meth-

ods which enable researchers to study genotoxicity under conditions similar to those in ecosystems. Much of the published surface water genotoxicity studies have employed the Salmonella/mutagenicity test. These studies demonstrated that river and coastal water worldwide are contaminated with different mixtures of genotoxic agents [1, 3]. Use of ecological test systems becomes a necessary condition for creating a scientific basis for the comparison of risk related to various genotoxic substances produced by man. Aquatic organisms such as teleosts and bivalves also have been used as sentinels in biomonitoring studies of surface waters. Larval stages of marine mussels and worms were successfully used for *in vivo* study of genotoxicity of different compounds [1, 4, 5]. As a freshwater organism, the zebra mussel (*Dreissena polymorpha*) fulfils the main criteria required from a sentinel species [6]. In addition, this is an invasive species of freshwater mussels that has become widely distributed throughout the world and appears to be an important organism from an ecological point of view. Many studies are available on the application of genotoxicity tests in zebra mussels [7-11]. The present work

*e-mail: pwozn@uwm.edu.pl

is the first case of using larval stages of the freshwater mussel for that purpose.

Materials and Methods

Zebra mussels (*Dreissena polymorpha*) 1.5-2.5 cm in length were collected from Wukśniki Lake near Olsztyn, Poland. In the laboratory, the animals were kept in a cooled and aerated aquarium at 10°C. Animals were artificially spawned using the 5-hydroxytryptamine stimulation as described by Ram et al. [12]. The 12h-old larvae of *Dreissena polymorpha* were taken for the experiment as described by Jha et al. [4] for *Mytilus edulis*. After 12h growth at 20°C the larvae suspension was divided into three equal samples 10 ml in volume. Two concentrations of 3-methylcholanthrene were chosen: 20 ppb and 200 ppb, because of the statistically significant increase of chromosome aberrations in *Mytilus edulis* treated by benzo [a] pyrene in concentrations from 1×10^{-8} M to 1×10^{-5} M (from 2.52 ppb to 2520 ppb) [1]. The solvent (0.005% DMSO solution) was used as a control sample. Pure water control was not used because of the lack of mutagenicity of the compound used as a solvent [1, 13]. The exposure period was 6 hours at 20°C. Before chromosome preparation, the samples were centrifuged (1 minute, 500rpm) and half of the volume of each sample was discarded (only supernatant). Then the 0.05% solution of colchicine in filtered water from Wukśniki Lake was added and the final concentration of the colchicine solution was 0.025%, as described for *Platynereis dumerilli* [14]. After 30 minutes of colchicine treatment the samples were centrifuged (5 minutes, 500rpm) and the hypotonic solution (distilled water/water from Wukśniki Lake at the ratio 1: 1) was added to each sample until the previous volume of 10 ml was obtained. The hypotonization period was 60 minutes. Following hypotonic treatment the larvae were fixed in methanol/acetic acid 3: 1 (Carnoy fixative) and the chromosome preparations were made as described by Jha et al. [15].

Metaphase spreads were examined using light microscope at a final magnification of x 1000. All complete metaphase spreads obtained were analysed (at least 100 per sample).

Chromosome aberrations (CA) were expressed as the percentage of aberrant cells and total aberrations per sample. Six to ten microscope slides with metaphases were analyzed per sample.

Statistical analysis of the differences between samples was performed by Kruskal-Wallis test at the level of significance $p < 0.05$ (*).

Results and Discussion

Three types of chromosomal aberrations were observed in *Dreissena polymorpha* larvae after 3-methyl-

cholanthrene treatment (Figure 1). Based on the analysis of at least 100 complete metaphase plates per treatment, the percentage of aberrant cells in the DMSO controls ranged from 0 to 9% per slide and the frequency of total aberrations ranged from 0 to 3 per slide. There was a distinct increase in both the percentage of aberrant cells and total aberrations for the larval stages of *Dreissena polymorpha* exposed to 3-methylcholanthrene. However, a statistically significant increase in these parameters was

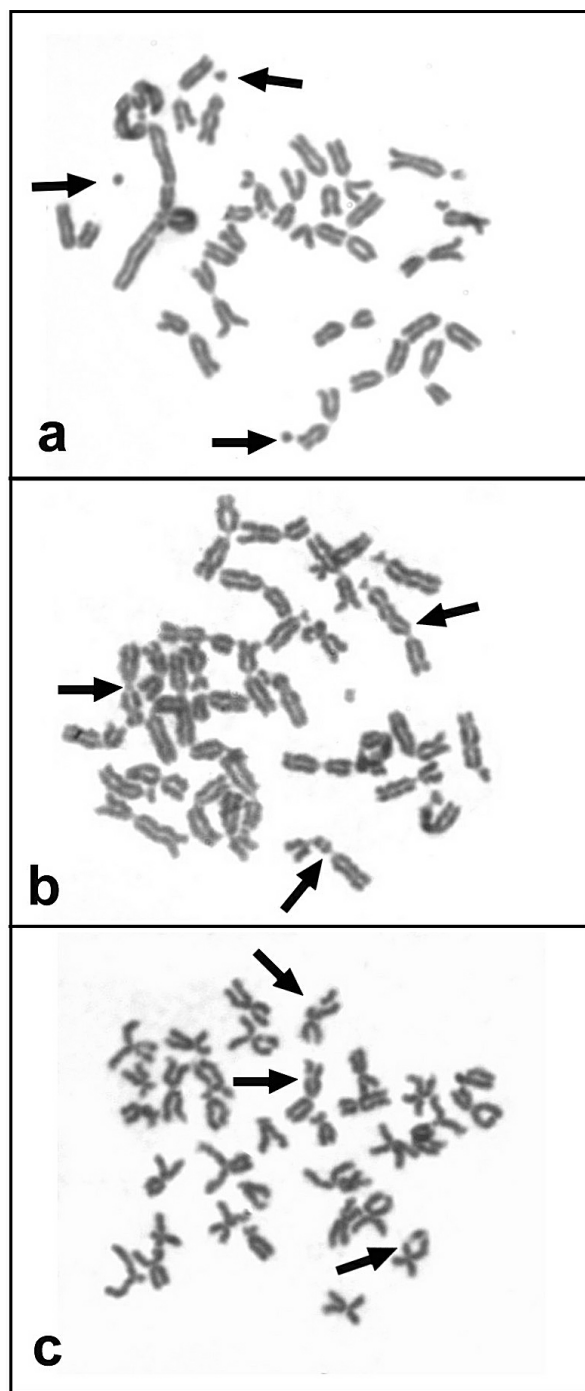


Fig. 1. Metaphase plates of *Dreissena polymorpha* with chromosome aberrations: a) chromosome fragments, b) dicentric chromosomes, c) chromatid breaks.

noticed only between the higher (200 ppb) concentration of that compound and the control group (Figs. 2, 3).

Zebra mussel (*Dreissena polymorpha*) is the only species of freshwater bivalves with free-living larval stages. The karyotype of *Dreissena polymorpha* consists of 32 chromosomes [16, 17]. It is quite suitable for analysis and scoring chromosome and chromatid aberrations. The embryo-larval stages of that species offers a chance to construct a model for detecting *in vivo* genotoxicity in freshwater habitats, analogous to models described by Jha et al. [1, 4, 14] and Hagger et al. [5] on marine invertebrates (*Mytilus edulis* and *Platynereis dumerilii*). The positive response of *Mytilus edulis* larvae exposed to benzo [a] pyrene occurred at the concentrations between 1×10^{-8} M and 1×10^{-5} M (2.52 ppb to 2520 ppb) [1]. In the zebra mussel, positive effects could be expected at concentrations higher than 20 ppb.

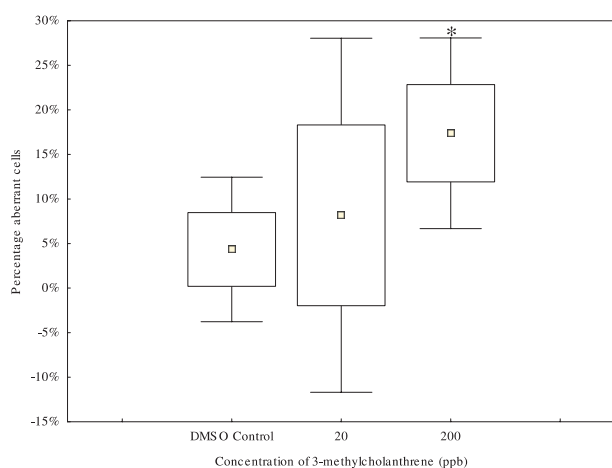


Fig. 2. Percentage of aberrant cells in larvae of *Dreissena polymorpha* treated with different concentrations of 3-methylcholanthrene.

* Significant difference ($p < 0.05$).

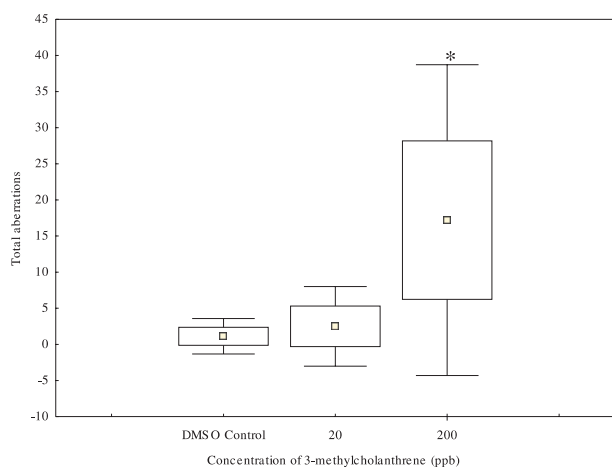


Fig. 3. Total aberrations in larvae of *Dreissena polymorpha* treated with different concentrations of 3-methylcholanthrene.

* Significant difference ($p < 0.05$).

The benzo [a] pyrene treatment (75 ppb and 750 ppb) of adult *Dreissena polymorpha* (and also *Anodonta anatina*) specimens showed no statistically significant differences (analyzed by comet assay – Woznicki, unpublished). In contrast, the Chinese mussel (*Anodonta woodiana*) showed significant increase in DNA damage even at 75 ppb of benzo [a] pyrene exposure [13]. The significant response of zebra mussel larvae to 3-methylcholanthrene suggested that the embryo-larval stages of this freshwater species possessed an active cytochrome P₄₅₀ system, as described in larval stages of marine invertebrates [1, 15]. The lack of response to the lower concentration of the compound suggested that the sensitivity of *Dreissena polymorpha* larvae is lower than in the species studied by Jha et al. [1, 15]. Aquatic organisms possess an MXR mechanism which removes from the cells and organisms both endogenous chemicals and xenobiotics, including some man-made chemicals [18]. MXR in aquatic organisms represents a general biological first-line defence mechanism for protection against environmental toxins [18]. This mechanism is responsible for species-specific differences in susceptibility to environmental genotoxic agents, including PAH. The present paper shows that the larval stages of *Dreissena polymorpha* can respond to high concentrations of 3-methylcholanthrene, but they are not susceptible enough to be model organisms in environmental studies of PAH genotoxicity.

Acknowledgements

This study was supported by project No. 3 P04G 033 24 financed by KBN Warsaw, Poland.

References

- JHA A. N., CHEUNG V. V., FUOLKES M. E., HILL S. J., DEPLEDGE M. H. Detection of genotoxins in the marine environment: adoption and evaluation of an integrated approach using the embryo-larval stages of the marine mussel, *Mytilus edulis*. *Mutat. Res.* **464**, 213, **2000**.
- SHUGART L. Biological monitoring: testing for genotoxicity. In: McCarthy JF, Shugart LR, ed., *Biomarkers of environmental contamination*. Lewis: Chelsea, pp. 217-227, **1990**.
- OHE T., WATANABE t., WAKABAYASHI D. K. Mutagens in surface waters: a review, *Mutat. Res.* **567**, 109, **2004**.
- JHA A. N., HAGGER J. A., HILL S. J. Tributyltin induces cytogenetic damage in the early life stages of the marine mussel, *Mytilus edulis*. *Environ. Mol. Mutag.* **35**, 343, **2000**.
- HAGGER J. A., FISHER A. S., HILL S. J., DEPLEDGE M. H., JHA A. N. Genotoxic, cytotoxic and ontogenetic effects of tri-n-butyltin on the marine worm, *Platynereis dumerilii* (Polychaeta: Nereidae). *Aquat. Toxicol.* **57**, 243, **2002**.
- MERSCH J., BEAUVAIS M. N. The micronucleus assay in zebra mussel, *Dreissena polymorpha* to *in situ* monitor genotoxicity in freshwater environments. *Mutat. Res.* **393**, 141, **1997**.

7. DE LAFONTAINE Y., GAGNE F., BLAISE C., COSTAN G., GAGNON P., CHAN H. M. Biomarkers in zebra mussels (*Dreissena polymorpha*) for the assessment and monitoring of water quality of the St. Lawrence River (Canada). *Aquat. Toxicol.* **50**, 51, **2000**.
8. KLOBUCAR G. I. V., PAVLICA M., ERBEN R., PAPES D. Application of the micronucleus and Comet assays to mussel *Dreissena polymorpha* haemocytes for genotoxicity monitoring of freshwater environments. *Aquat. Toxicol.* **64**, 15, **2003**.
9. PAVLICA M., KLOBUCAR G. I. V., MOJAS N., ERBEN R., PAPES D. Detection of DNA damage in haemocytes of zebra mussel using comet assay. *Mutat. Res.* **490**, 209, **2001**.
10. PAVLICA M., KLOBUCAR G. I. V., VETMA R., ERBEN R., PAPES D. Detection of micronuclei in hemocytes of zebra mussel and great ramshorn snail exposed to pentachlorophenol. *Mutat. Res.* **465**, 145, **2000**.
11. BOLOGNESI C., BUSCHINI A., CARBONI P., FURLINI M., MARTINO A., MONTEVERDE M., POLI P., ROSSI C. Comet and micronucleus assay in zebra mussel cells for genotoxicity assessment of surface drinking water treated with three different disinfectant. *Sci. Total Environ.* **333**, 127, **2004**.
12. RAM J. L., CRAWFORD G. W., WALKER J. U., MOJARES J. J., PATEL N., FONG P. P., KYOZUKA K. Spawning in the zebra mussel (*Dreissena polymorpha*): activation by internal or external application of serotonin. *J. Exp. Zool.* **265**, 587, **1993**.
13. WOŹNICKI P., LEWANDOWSKA R., BRZUZAN P., ZIOMEK E., BARDEGA R. The level of DNA damage and the frequency of micronuclei in haemolymph of freshwater mussels *Anodonta woodiana* exposed to benzo [a] pyrene. *Acta Toxicol.* **12** (1), 41, **2004**.
14. JHA A. N., HUTCHINSON T. H., MACKAY J. M., ELLIOTT B. M., DIXONS D. R. Evaluation of the genotoxicity of municipal sewage effluent using the marine worm *Platynereis dumerilii* (Polychaeta: Nereidae). *Mutat. Res.* **391**, 179, **1997**.
15. JHA A. N., HUTCHINSON T. H., MACKAY J. M., ELLIOTT B. M., DIXON D. R. Development of an in vivo genotoxicity assay using the marine worm *Platynereis dumerilii* (Polychaeta: Nereidae). *Mutat. Res.* **356**, 141, **1996**.
16. WOŹNICKI P., BORONĀ A. Banding chromosome patterns of zebra mussel *Dreissena polymorpha* (Pallas) from the heated Konin lakes system. (Poland). *Caryologia* **56** (4), 427, **2003**.
17. BORONĀ A., WOŹNICKI P., SKUZA L., ZIELIŃSKI R. Cytogenetic characterization of the zebra mussel *Dreissena polymorpha* (Pallas) from Miedwie Lake, Poland. *Folia Biol. (Kraków)*, vol. **52** (1-2), 33, **2004**.
18. KURELEC B. A new type of hazardous chemical: the chemosensitizers of multixenobiotic resistance. *Environ. Health Perspect.* **105**, Suppl. 4, **1997**.