Functional significance of genotoxicity in fish germ cells

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1. Introduction

Aquatic ecosystems are commonly considered as the ultimate receptacle for anthropogenic compounds. Recent works show that about one third of them could have genotoxic properties. If some great progress have been made in understanding the implications of genotoxin exposure to human health through water use, a huge gap remains in our understanding of the impacts on aquatic species. Indeed, DNA damage has been used since quite a long time as an exposure biomarker in aquatic ecotoxicology, but the functional significance of this damage is still poorly understood, in particular in fish.

Genotoxins are prone to affect offspring directly or indirectly through the reproductive process. All this could influence the recruitment rate and hence the population dynamics [1]. However, assessment of the ecological risks associated with environmental genotoxic exposure is usually based on individual responses. Thus, there is a need for a better understanding of the long term and population level implications of genotoxic insults in fish.

While low levels of DNA damage in somatic cells and oocytes can be efficiently repaired, mature sperm cells, i.e. spermatozoa, are susceptible to accumulate damage due to their lack of repair capacity. The present work aims to track the transfer of toxic effects across generations by studying the link between the level of DNA damage in fish sperm after parental exposure to the genotoxic compound methyl methane sulfonate (MMS) and the rate of development abnormalities measured in the offspring.

Three different fish species were chosen based either on their ecological importance or on their reproduction behavior, respectively brown trout (*Salmo trutta*), Arctic charr (*Salvelinus alpinus*) and threespine stickleback (*Gasterosteus aculeatus*).

2. Materials and methods

A first experiment was carried out on 2 years old male brown trout and 3 years old male Arctic charr reared at the INRA hatchery (Thonon les Bains, France) in outdoor tanks continuously supplied with 36m deep water from Lake Geneva. Animals were fed daily *ad libitum* with dry pellets. Different modes of exposure to MMS (through water, food or intraperitoneal injection) were tested in preliminary experiments, and finally intraperitoneal injection was found to be the most effective (data not shown). Thus, male fish (n=31 trouts, n=28 chars) were injected intraperitoneally with MMS previously dissolved in coconut oil. Exposed fish received 50 mg MMS / kg fresh body weight, control receiving vehicle alone (trout n=16, charr n=18). After 3 weeks of exposure DNA damage was measured in mature spermatozoa through the comet assay. Aliquotes from one egg pool obtained from nonexposed females were fertilized by each sperm of both control and exposed males. The rate of abnormalities development was then measured in the resulting offspring at various embryonic stages (neural plate, eyed stage) and larval stages (hatching, yolk resorption, swimming stage), as well as the survival during the first month post yolk resorption.

A second experiment following the same protocol is currently in progress in stickleback and will be presented.

3. Main results

3.1. Sperm DNA damage

After 3 weeks of MMS exposure, a significant increase in sperm DNA damage was measured using the Comet assay reaching in average a 30% tail DNA value in both species (10 fold increase in trout and 20 fold

increase in charr compared to the control, respectively). However fertilization rate was not affected by MMS treatment of male fish since it ranged in average 95% in trout and 84% in charr, whatever the treatment.

3.2. Embryo and larval development

Embryo abnormality rate clearly increased in offspring stemming from MMS exposed male fish but remains at these development stages to a low value (for example, 0.6% in treated trout versus 0.2 % in control). Male exposure to MMS led also to a large array of abnormalities when measured at later stages i.e. after hatching and in particular during yolk resorption (Figure 1). Such an effect was significant only in trout and reached 2% of abnormal larvae.

Figure 1. Different types of larvae abnormalities. A and E: yolk oedema in trout and charr respectively; B and C: trout spine deformation; D: charr jaw deformation; F: charr Siamese larvae

3.3. Skeleton development and larvae survival

If we look more accurately at the effects, bony staining reveals very frequent spine and cephalic deformation in swimming larvae stemming from DNA damaged sperm (Figure 2). It has to be stressed that those abnormalities reached around a 20 % value what is much higher than those observed at earlier stages in embryos. So it was observed that consequences of original sperm DNA damage appear to increase along with the progeny development. Trout larvae survival monitored one more month after the end of yolk resorption was 5 fold lower in larvae from MMS treated group compared to the control one.

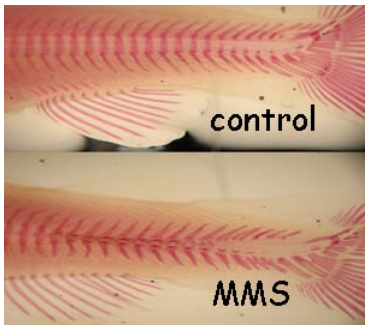

Figure 2. Spine deformation frequently observed in fish larvae

4. Discussion and perspectives

This work demonstrates the interest of studying the impact of genotoxic compounds on spermatozoa, a cell type that exhibits a high genotoxic response, possibly due to low DNA repair and to low biotransformation capacities contrary to oocytes. Consequently, spermatozoa are susceptible to accumulate DNA damage under chronic and low-dose exposure to environmental genotoxins. These results highlight the possible transfer of genetic damage from adult to offspring in freshwater fish as recently demonstrated in marine invertebrates [2]. Other experiments are currently carried out in stickleback to confirm such an effect.

The increase in abnormality rate along the offspring development time underlines the interest to further study more integrated responses such as fish growth, survival and F1 reproductive success. Moreover, it would be interesting to evaluate both the oocyte capacity to repair spermatozoa DNA damage, and the contribution of a potential oocyte DNA damage to the reproduction impairment.

This work is a first step towards the understanding of the functional significance of fish germ cell primary genotoxic damage, revealed as a reproductive impairment.

5. References

[1]. Newman M.C., Clements W.H. 2008. Ecotoxicology: a comprehensive treatment, CRC Press, 252 p.

[2]. Lewis C., Galloway T. 2009. R eproductive consequences of paternal genotoxin exposure in marine invertebrates. Environ. Sci. Technol. 43: 928-933.