Toxicological consequences of sea-dumped munitions

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introduction

Millions of tonnes of munitions that have been dumped after World War II pose an emerging new threat to the seas worldwide, since the metal vessels corrode and the toxic explosives trinitrotoluene (TNT) and metabolites leak into the environment. This does also apply to sunken war ship wrecks fully loaded with munitions [1]. While TNT is toxic and a potential carcinogen for humans, it is known from lab studies that exposure to TNT results in health effects to aquatic organisms, including impairment of growth, development and reproduction. While the mechanism of cellular toxicity of TNT is speculated to involve oxidative stress and reactive oxygen species (ROS), genetic biomarkers are urgently sought to better understand the mechanism and consequences of TNT intoxication in aquatic biota on a molecular level. This is especially important upon sublethal and chronic exposure to juvenile marine organisms living close to munition dumping sites or ship wrecks that are often used as nursery habitats.

Carbonyl reductase enzymes catalyze the Phase I detoxification of many carbonyl group bearing xenobiotics [2]. They also detoxify endogenous lipid peroxidation derived carbonyls (aldehydes and ketones) [3]. Thus, they protect against ROS generated oxidative and carbonyl stress and are involved in the (patho)physiology of frequent human diseases including neurodegenerative disorders (Fig.1) [4].

After a bioinformatics approach and molecular cloning of the carbonyl reductase gene, we could show in both laboratory and field studies that TNT induces a strong and concentration dependent gene induction in blue mussels [5].

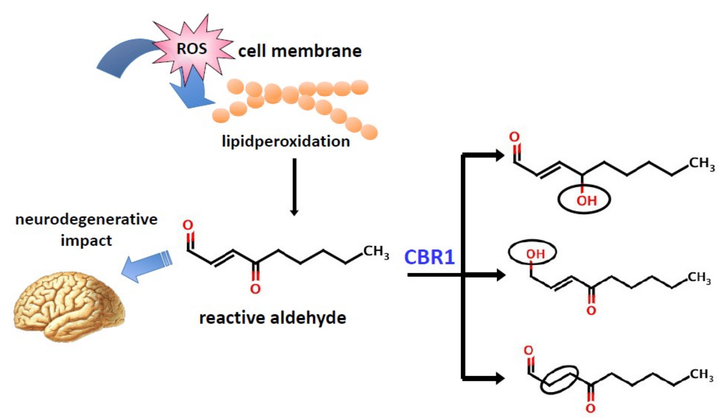


Figure 1: Human CBR1 mediated detoxification of reactive aldehydes derived from lipid peroxidation in cell membranes caused by reactive oxygen species (ROS).

material and methods

For identification and cloning of the *Mytilus* spp. carbonyl reductase gene and to obtain the optimum primer sequences for its amplification, a BLAST search for carbonyl reductase genes on *Mytilus* genomic data and various genomes of model organisms was performed (Fig.2). From the resulting "proposed" gene sequence primers were designed to obtain the coding sequence of the carbonyl reductase gene of *Mytilus* spp. used in the present study.

Lab study: Blue mussels (*Mytilus* spp.) were exposed to TNT (10 mg/l) for seven days. After treatment, mRNA was isolated from mussel tissues (gill, hepatopancreas, muscle, mantle) for RT-PCR analysis (Fig. 3 and 4). Gene specific primers were designed according to a bioinformatics analysis to infer the carbonyl reductase gene sequence and 18s RNA as the loading control, respectively, in *Mytilus* spp.

Field study: Two mussel bags with 15 mussels each were placed adjacent to corroding mines or close to blast craters with explosive materials in the vicinity in a depth of approximately 11 meters (Fig. 5). One of these bags was placed at the ground directly adjacent to a chunk of explosive material (7 U), the other at a height of 1 meter (7 O). The exposure period amounted to 58 days and the sampling was done by Kiel University research divers.

Gene expression experiments were performed by reverse transcription polymerase chain reaction (RT-PCR).

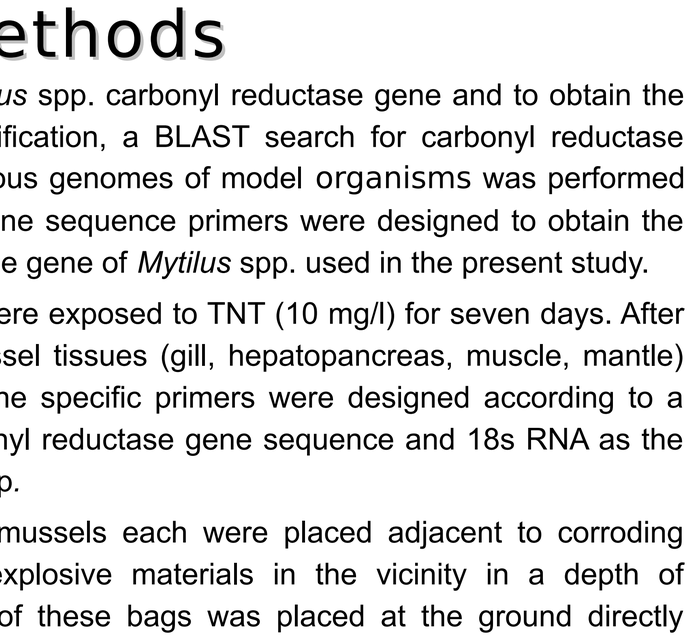


Figure 2: Carbonyl reductase genes in model organisms: *Drosophila*, *Daphnia* and humans. Two isoforms of carbonyl reductases exist in most mammalian genomes like the human genome. The fruit fly *Drosophila* only has a gene for *sniffer*, often referred to as functional homolog of CBR1. *Daphnia* express a gene for *sniffer* along with four copies of the carbonyl reductase gene. It should be noted that "CR1", "CR2", "CR3", and "CR4" are generic names, i.e., "CR2" is not a homolog of the nonmammalian CR2 and "CR4" is not a homolog of the human CBR4 (SDR45C1). (Adapted from [6]; with kind permission from FEBS Journal). Here, carbonyl reductase has been identified in the blue mussel *Mytilus* spp.

experimental results

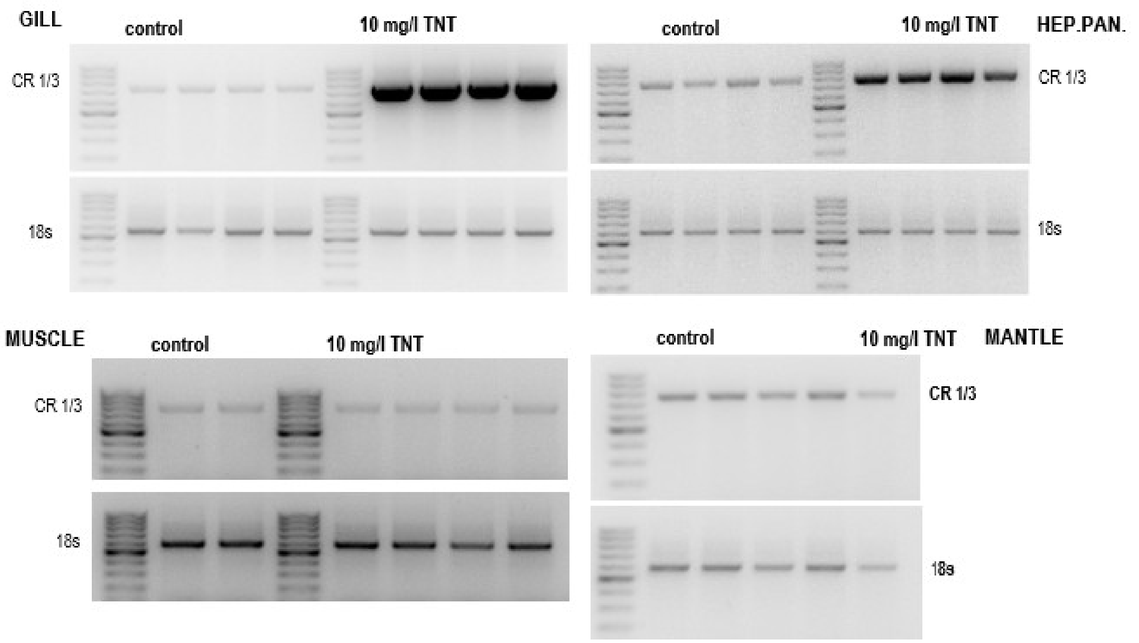


Figure 3: RT-PCR results of mussel tissues after TNT exposure (selection)

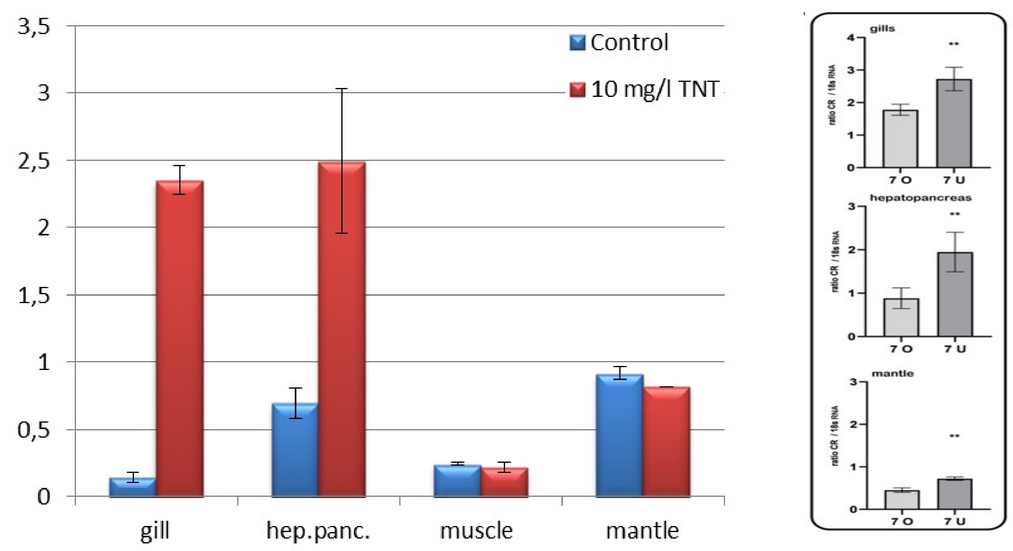
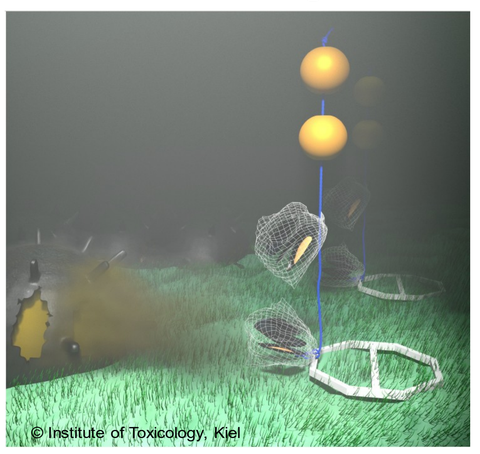


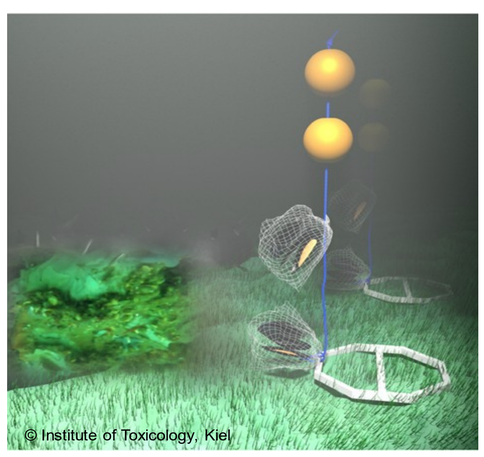
Figure 4: Mean values (± SD) of the semiquantitative analysis of blue mussels exposed to TNT and a control group (n = 1-4) in lab studies (left) and field studies in the Baltic Sea (right).

Close to corroding mines



- Mussels close to corroding mines can (still) be eaten.
- However, they show adverse health effects (oxidative stress).

At free-lying chunks of MC



- Consuming mussels from free-lying chunks of MCs bears a carcinogenic risk for the consumer.

Figure 5: Toxicological risk assessment for humans and mussels at corroding mines (left) or free-lying chunks of munitions compounds (MCs) in blast-in-place (BiP) craters [7-9]

conclusion

- After a bioinformatics approach and molecular cloning of the carbonyl reductase gene, it was shown in both laboratory and field studies that TNT induces a strong and concentration dependent gene induction in mussels.
- The results also clearly demonstrate that organs with high blood supply (gill) or involved in digestion (hepatopancreas) show a much higher expression of carbonyl reductase than organs like muscles or mantle.
- Carbonyl reductase may thus serve as a biomarker and early warning system for TNT exposure in marine systems.
- A toxicological risk assessment indicated that mussels close to corroding mines can still be eaten, however they show symptoms of oxidative stress (induction of the carbonyl reductase gene).
- Consuming mussels from a free-lying chunk of TNT bears a carcinogenic risk for the human consumer.

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