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Effects of nonionic and ionic surfactants on survival, oxidative stress, and cholinesterase activity of planarian

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Abstract

Eight widely used surfactants (cetyltrimethylammonium bromide; CTAB, benzethonium chloride; Hyamine 1622, 4-nonylphenol; NP, octylphenol ethoxylate; Triton X-100, dodecylbenzene sulfonate; LAS, lauryl sulfate; SDS, pentadecafluorooctanoic acid; PFOA, and perfluorooctane sulfonate; PFOS) were selected to examine their acute toxicities and effects on oxidative stress and cholinesterase (ChE) activities in *Dugesia japonica*. The differences in acute toxicity among eight surfactants to planarians were at least in the range of three orders of magnitudes. The toxicity rank of surfactants according to estimated 48-h LC_{50} was SDS > NP > LAS > Hyamine 1622 > CTAB > Triton X-100 > PFOS > PFOA. The toxicity rank of surfactants according to 96-h LC_{50} was as follows: SDS > CTAB > NP > LAS > Hyamine 1622 > Triton X-100 > PFOS > PFOA. There were significant increases in catalase activities in planarians exposed to LAS at nominal concentrations of 0.5 or 1 mg l^{-1} and to PFOS at nominal concentrations of 5 or 10 mg l^{-1} after 48-h exposure. Inhibitions of ChE activities were found in planarians exposed to Hyamine 1622 at all concentrations tested, to PFOS at nominal concentration of 10 mg l^{-1}, to PFOA at nominal concentrations of 50 or 100 mg l^{-1} and to NP at nominal concentration of 0.5 mg l^{-1}. A significant increase in ChE activities was also observed in planarian exposed to Triton X-100 at nominal concentration of 5 mg l^{-1}. The implication of ChE inhibition by NP, PFOS and PFOA on neurological and behavioral effects in aquatic animals requires further investigation.

Keywords: *Dugesia japonica*; Lipid peroxidation; Cholinesterase activity; Antioxidant enzyme activity; Nonionic surfactants; Ionic surfactants

1. Introduction

Surfactants are widely used in everyday personal care and household products as well as in a variety of industrial applications. As a result, large amounts of surfactants are commonly discharged in large quantities to sewage treatment plants or directly to the aquatic environment in areas where there is no sewage treatment. In fact, many surfactants and their degradation products have been found worldwide in wastewater discharges, sewage treatment plant effluents, natural water and sediments (Ying, 2006). Because many surfactants are ubiquitous (Ying et al., 2002; Venhuis and Mehrvar, 2004), the potential toxic effects of these chemicals have attracted much research attention in the past several decades (Abel, 1974; Lewis and Suprenant, 1983; Lewis, 1991). However, previous investigations have concentrated mainly on anionic surfactants, and there is limited toxicological information on other types of surfactants or some new emerging surfactants such as 4-nonylphenol (NP), pentadecafluorooctanoic acid (PFOA), or perfluorooctane sulfonate (PFOS).

Many different mechanisms of toxicities exist for different types of surfactants and one single surfactant can produce its toxicity through more than one mechanism. In general, toxic effects of surfactants are observed via damage on gills and epidermis of aquatic vertebrate or disruption of cellular membrane of aquatic invertebrate (Abel, 1974). The toxicity of surfactants is primarily determined by the ability of the surfactants to adsorb and penetrate

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the cell membrane of aquatic organisms (Rosen et al., 2001). However, the molecular mechanisms of toxicities of surfactants are not well understood after surfactant adsorption on the membrane surface. What is known is that an interaction with lipid membranes appears to disrupt membrane integrity, thus causing toxic effects (Abel, 1974). The disruption of membrane integrity is possibly caused by interference with membrane permeability or membrane proteins. One possible mechanism of disruption of membrane integrity was oxidative stress which has detrimental effects on membrane integrity, leading to a loss of fluidity and increased ion permeability (Livingstone, 2003).

Most surfactants have an ionic or polar head group connected to a hydrophobic tail with a straight or branched hydrocarbon chain. Hydrocarbon metabolisms of surfactants by aquatic animals might be produced highly reactive oxygen species and to cause oxidative stress in organisms. Information of surfactant-induced oxidative stress in aquatic organisms is still very limited and effects of different classes of surfactants *in vivo* need further investigations. In addition, some studies have recently indicated that some surfactants may inhibit cholinesterase (ChE) activity in aquatic animals (Guilhermino et al., 1998, 2000a; Garcia et al., 2000). Indeed, the chemical properties of surfactants can alter enzyme activities by binding or disrupting enzyme structure (Cserháti et al., 2002). However, most ChE inhibitions were observed *in vitro* experiments (Guilhermino et al., 1998; Garcia et al., 2000) but very few *in vivo* experiments (Guilhermino et al., 2000a). Because of their common occurrence in aquatic environment, it will be of important to examine effects of different surfactant types on ChE inhibition in aquatic animals.

Freshwater free-living planarians are distributed worldwide in unpolluted streams and an important component of the aquatic ecosystem. Traditionally, planarians have been a favored animal model in developmental biology (Newmark and Alvarado, 2002) and neuroscience research (Pagan et al., 2006). Furthermore, they have been suggested as test organisms for various types of short-term toxicity bioassays because they are sensitive to different classes of environmental pollutants (Horvat et al., 2005; Pra et al., 2005). In addition, they can be easily collected in large numbers, require only low culture and test medium volumes, and can be kept inexpensively in laboratory for toxicological testing. These characteristics make planarian a suitable organism for studying the effects of environmental pollutants in aquatic environment.

The objective of this study was to evaluate aquatic toxicity of surfactants using a freshwater planarian, *Dugesia japonica*, as an animal assay. Eight commonly used surfactants were selected. Their adverse toxicities on aquatic invertebrates were determined by examining the effects of these surfactants on survival, oxidative stress and ChE activity in *D. japonica*. These different surfactants were selected in view of their known widespread human exposure and environmental occurrence (Venhuis and Mehrvar, 2004; Lehmler, 2005). Cetyltrimethylammonium bromide (CTAB) and benzethonium chloride (Hyamine 1622) are used primarily in cosmetics and shampoos for its antimicrobial and cationic surfactant properties. Octyl phenol ethoxylate (Triton X-100) and NP are nonionic surfactants widely used in industrial and household products. Of four anionic surfactants chosen, sodium dodecylbenzene sulfonate (LAS) and lauryl sulfate (SDS) are commonly used as active ingredients in household and personal care products as well as in specialized applications, while PFOS and PFOA are two fluorinated surfactants with growing environmental concerns and are widely applied to fabrics, carpets and paper (Renner, 2005).

2. Materials and methods

2.1. Chemicals

NP was purchased from Riedel-de Haën (Sigma–Aldrich, USA), with a chemical purity of 94%. PFOS (>98%) was obtained from Fluka. Hyamine 1622, Triton X-100, LAS (80%), PFOA (>98%), SDS (99%) and CTAB (99%) were obtained from Sigma–Aldrich. The properties of surfactants and the range of nominal testing concentrations for each surfactant are listed in Table 1. In this study, all stock solutions and test beakers for surfactants tested were used glass containers, except for PFOS and PFOA treatments which used polyprolene containers for stock solutions and test vessels because these two chemicals have potential to be adsorbed onto glass surface. In addition, all biochemical materials for enzyme assays were purchased from Sigma–Aldrich. HPLC grade acetone was purchased from Mallinckrodt. NP was dissolved in acetone for preparing the test stock solution. All other stock solutions for testing chemicals were prepared in dechlorinated tap water.

2.2. Test organisms

D. japonica was collected from Nan-shi stream located in Wu-lai, Taipei County, Taiwan in 2004. Since then, the planarians have been maintained in dechlorinated tap water at our laboratory. Animals were fed with raw chicken liver once a week. Culture medium was renewed weekly after feeding.

2.3. Acute toxicity test

The planarians (body length $= 0.9 \pm 0.1$ cm) were exposed to different surfactants with at least five different concentrations or dechlorinated tap water as a control group. For each concentration, five animals were kept in 50 ml of test solution in a beaker and each treatment was replicated five times during the experiment. All acute toxicity experiments were conducted in a temperature incubator at 25 ± 1 °C with a 12 L:12 D illumination. The animals were not fed and inspected every 24 h for mortality during