

EFFECT OF SEVERAL NONYLPHENOL PRODUCTS ON *IN VITRO* VITELLOGENIN SYNTHESIS IN TILAPIA HEPATOCYTES

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Introduction

Nonylphenol (NP) is a degradation product of a widely-used nonionic surfactant group, alkylphenol polyethoxylates, and has estrogenic potential (Yadete et al., 1999). It has been reported that NP treatment induces appearance of a female-specific protein, vitellogenin (VTG) in the blood circulation of certain juvenile or male teleost fishes (Monteverdi and Di Giulio, 1999; Kinnberg et al., 2000). Although it is possible to purchase NP products from several companies for experimental uses, the purity and the mixtures of NP products are different from different companies and even among lots from the same company. Therefore, it is likely that the estrogenic potential of each NP is not constant. The aim of the present study was to compare estrogenic potential of NP from three companies using primary cultures of hepatocytes from tilapia (*Oreochromis mossambicus*). Medium VTG concentration was used as an indicator of estrogenic potential associated with NP.

Materials and Methods

Tilapia (200-300g) were collected using a casting net from rivers and maintained in concrete tanks (2 metric ton capacity) with filtered freshwater and aeration at ambient water temperature at the Sesoko Station, Tropical Biosphere Research Center, University of the Ryukyus, Okinawa, Japan.

Isolation and primary culture of tilapia hepatocytes were done in accordance with the method of Takemura and Kim (2001). Estradiol-17 β (E₂, Sigma, St. Louis, MO), three NP products [(A) Kanto Kagaku, Tokyo Japan; (B) Aldrich, Milwaukee, WI; (C) Wako Pure Chemicals, Tokyo, Japan] and tamoxifen (Sigma) were dissolved in ethanol and added to the culture media after 2 days of pre-culture. Medium VTG was measured by an enzyme-linked immunosorbent assay (Takemura and Kim, 2001).

Results and Discussion

At 3 days after onset of culture without hormone and NP treatments, the hepatocytes conjugated and formed chains, and started to joint together and formed a monolayer. Addition of NP from the companies A/B and C to the medium at 10⁻³ M caused death of cells and delay of cell adhesion, respectively. Death of hepatocytes was not observed in any media to which NP was added at 10⁻⁴ M. Adhesion of hepatocytes was slower in the media with NP from the companies A and B than the company C. It is considered that high concentration of NP is toxic against the hepatocytes.

Treatments of E₂ at 10⁻⁷ M and NP at 10⁻⁴ M from the companies A and B resulted in a significant increase of VTG synthesis, while NP from the company C did not induce VTG synthesis. Treatment of NP from the company B alone induced significant increase of VTG level in the medium of female hepatocytes. However, co-treatment of this NP and tamoxifen reduced VTG synthesis. Tamoxifen is known to be a nonsteroidal antiestrogen and binds strongly to ER. These results show that some NP have estrogenic effect in the primary culture of tilapia hepatocytes and act through ER. A similar effect of NP on VTG induction was reported in primary hepatocyte cultures of channel catfish, *Ictalurus punctatus*, (Monteverdi and Di Giulio, 1999) and rainbow trout, *Oncorhynchus mykiss* (Islinger et al., 2000).

Our results suggest differences in the induction level of VTG among the different NP products. According to a data sheet from the company C, it was ascertained that the NP from the company C had high purity and showed only a single peak by HPLC. Although information on purity of NPs from the companies A and B was not available at present, the estrogenic potential of NP products may perhaps be due to their impurity. Yadetie et al. (1999) induced expression of ER mRNA in the liver of juvenile Atlantic salmon, *Salmo salar*, with 85% pure NP product. In the present study, importance of impurity on estrogenicity could not be assessed, although it may be considered that the estrogenic potential of the NP product is synergistic by

admixtures. Our results suggest that the potential for differences in estrogenic activity may be applicable to other compounds and not just to NP. Consequently, comparison of experimental results from different laboratories may be risky, unless the products used are similar.

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Acknowledgements

This study was supported in part by Integrated Research Program for Effects of Endocrine Disruptors on Agriculture, Forestry and Fisheries and Their Action Mechanisms on Domestic Animals and Fishes.

