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Effect of nonylphenol ethoxylates (NPEs) on barrier functions of epithelial cell membranes: Opening of tight junctions and competitive inhibition of P-gp-mediated efflux

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Abstract

The effect of nonylphenol ethoxylates (NPEs) on selected barrier functions of biological membranes, such as tight junction and P-gp efflux pump of epithelial membranes, against the transport of xenobiotics was examined. The Caco-2 cell line was used to evaluate the transport of mannitol and daunomycin across the cell monolayer as well as the cellular uptake of daunomycin. In the presence of NPEs, the transport of mannitol was increased, with NP-9 showing a maximal effect, and the transepithelial electrical resistance (TEER) was reduced. The onset of this effect of NP-9 was fairly rapid and reversible for a short term (e.g., 2 h) treatment, while irreversible for a long term (e.g., 72 h) treatment. In the presence of NP-9, the apical uptake of daunomycin was increased, suggesting competitive inhibition between NP-9 and daunomycin in the efflux via the P-gp system. However, a 72 h pretreatment of the cells with NP-9 (up to 1000 nM) did not affect the apparent cellular uptake of daunomycin, suggesting no significant effect of NPEs on the expression of P-gp. In conclusion, NPEs appear to rapidly open the tight junction of epithelial cell membranes and to competitively inhibit the efflux of P-gp substrates, thereby reducing the self-protection ability of the organism against xenobiotics or hazardous environmental compounds that are transported via the paracellular pathway (i.e., uptake) or the P-gp system (i.e., efflux).

Keywords: Nonylphenol ethoxylates; Barrier functions; Caco-2; Tight junction; P-glycoprotein

1. Introduction

Nonylphenol ethoxylates (NPEs, Fig. 1) are synthetic surfactants that are inexpensive enough to be commonly used in a variety of household products, and, as the result, are quite common in surface water

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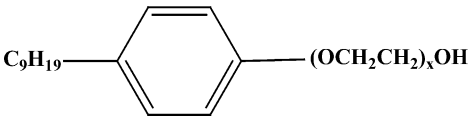

Fig. 1. Chemical structures of nonylphenol ethoxylates (NPEs) for NP-4, NP-7, NP-9, NP-10 and NP-35 which contain 4, 7, 9, 10 and 35 ethoxylates, respectively. The number following NP- indicates the average number of ethylene oxide units per molecule at position *x*.

and other aquatic environments that receive sewage discharges (Maguire, 1999). NPEs have been used as wetting agents and as intestinal permeability enhancers to improve oral drug delivery (Swenson et al., 1994a). They are rapidly absorbed orally and topically and are actively excreted into the urine of healthy subjects (Monteiro-Riviere et al., 2000, 2003; Charuk et al., 1998). It has been suggested that long term exposure to NPEs might have an impact on the reproductive systems in various animal species including humans (Kang et al., 2003), in that it exhibits a potent spermicidal effect in the testis (Kang et al., 2003). The inhibition of Ca^{2+} pumps on the testis endoplasmic reticulum has been suggested as a possible mechanism for this spermicidal effect (Monteiro-Riviere et al., 2003; Charuk et al., 1998; Minami et al., 2000). Recent reports suggest that NPEs may function as hormone disrupters (Monteiro-Riviere et al., 2003; Minami et al., 2000). Most interestingly, NPEs have been reported to be potential *Plasmodium falciparum* P-gp substrates and drug efflux inhibitors, and are under examination as reversal agents for malarial quinoline resistance (Crandall et al., 2000; Ciach et al., 2003).

Biological membranes serve as barriers against the permeation of xenobiotics into the systemic circulation or specific organs. Membrane junction complexes between the epithelial cells of the membrane, such as desmosomes, adherens junctions and tight junctions, appear to constitute the barrier function, with the tight junction making the major contribution (Erwin et al., 1999; Knipp et al., 1997). In general, surfactants are known to lower the barrier function of biological membranes through affecting the integrity (Eva et al., 1992) of the junction complexes. Thus, the effect of NPEs, a new category of surfactants, on the barrier function of tight junctions was studied. This is of special interest in that the tight junction contributes to the blood–testis barrier (Erwin et al., 1999; Holash et al., 1993; Janecki et al., 1992). In addition to the tight junction, the P-glycoprotein (P-gp), an ATP-dependent efflux pump, often also functions as a barrier against the permeation of xenobiotics across biological membranes (Monteiro-Riviere et al., 2003). NPEs are known to be substrates of P-gp (Charuk et al., 1998; Loo and Clarke, 1998). Thus, it seems reasonable to assume the existence of an interaction between NPEs and P-gp, which may influence the barrier function of the cell membranes. In this regard, the effect of NPEs on the function of P-gp was also investigated in the present study.

Information on this matter, as well as on the toxicity (Swenson et al., 1994a; Harrison et al., 1997), will aid in developing a better understanding, for example, of the effect of NPEs on the reproductive systems (Kang et al., 2003) and of the mechanism for malarial quinoline resistance reversal (Crandall et al., 2000; Ciach et al., 2003). The Caco-2 cell monolayer was utilized as a model biological membrane that contains tight junctions and the P-gp system.

2. Materials and methods

2.1. Materials

Unradiolabeled daunomycin was a kind gift from Dong-A Pharm. Co. (Kyounggi-do, Korea). [^{3}H]-Daunomycin (4.4 Ci/mmol) and [^{14}C]mannitol (50 mCi/mmol) were purchased from New England Nuclear Life Science Products (Boston, MA). Fetal bovine serum was purchased from Hyclone Laboratories (Logan, UT). Trypsin-EDTA was purchased from Gibco Laboratories (Gaithersburg, MD). NPEs which contain 4, 7, 9, 10 and 35 ethoxylates (i.e., NP-4, NP-7, NP-9, NP-10 and NP-35, respectively), Dulbecco's Modified Eagles's medium, nonessential amino acid solution, penicillin–streptomycin, Hank's balanced salt solution (HBSS), *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), 2-*N*-morpholinoethane sulfonic acids (MES), D-glucose and sodium bicarbonate were purchased from Sigma Chemical Co. (St. Louis, MO). All other reagents were of analytical grade.

2.2. Cell culture

Caco-2 cells (passage 45–55, American Type Culture Collection, Rockville, MD) were grown in the