Interactions of Pluronic Block Copolymers on P-gp Efflux Activity: Experience With HIV-1 Protease Inhibitors

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Received 7 December 2007; revised 29 January 2008; accepted 3 February 2008

Published online 4 April 2008 in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/jps.21372

ABSTRACT: The objective was to examine the influence of Pluronic block-copolymers on the interaction between the drug efflux transporter, P-glycoprotein and HIV-1 protease inhibitors (PIs). The ATPase assay determined the effect of various Pluronics on PI-stimulated P-gp ATPase activity. Cellular accumulation studies were conducted using MDCKII and LLC-PK1 cells transfected with human MDR1 to assess Pluronic modulation of PI efflux. Pluronic P85 inhibited both basal and nelfinavir-stimulated P-gp ATPase activity, while Pluronic F127 had no effect. In cell accumulation studies, Pluronic P85 restored the accumulation of nelfinavir in MDCKII-MDR1 cells while Pluronic F127 and F88 had no effect. Pluronic P85 increased saquinavir accumulation in wild-type and MDR1-transfected cells in both the MDCKII and LLC-PK1 cell models, suggesting inhibition of multiple transporters, including MRPs. In conclusion, this study provides evidence that a block-copolymer, Pluronic P85, effectively inhibits the interaction of P-gp with nelfinavir and saquinavir. These data indicate that effective inhibition of HIV-1 PI efflux by Pluronic P85 may influence the distribution of antiretroviral agents to sites protected by efflux mechanisms, such as the blood–brain barrier, and possibly increase the brain exposure of these drugs resulting in suppression of viral replication and reduction in the incidence of drug resistant mutants. J Pharm Sci 97:5421–5433, 2008

Keywords: efflux pumps; HIV-1 protease inhibitors; pluronics; polymers; ATPase P-glycoprotein; blood–brain barrier

INTRODUCTION

Protease inhibitors (PIs) are drugs used in the treatment of HIV-1 infection and are an integral part of active retroviral therapy (ART), which involves the use of a combination of different classes of antiretroviral drugs. PIs prevent the formation of mature HIV-1 particles by inhibition of the viral protease. By decreasing the morbidity and mortality related to HIV-1 infection and AIDS, PIs have become the mainstay of anti-HIV-1 ART therapy.

Given the frequent mutations the virus undergoes due to its retro-viral nature, development of resistance to PIs has been reported[1] and these mutants may persist even after PI therapy is stopped.[2] This phenomenon is more pronounced in tissues such as the brain, which has been implicated as a sanctuary site for HIV-1. Proliferation of the virus in brain tissue can lead to such conditions as the AIDS dementia complex and HIV-1 encephalopathy.[3] Many PIs have been shown to have much lower concentrations in CNS compared to plasma,[4,5] resulting in drug levels

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Journal of Pharmaceutical Sciences, Vol. 97, 5421–5433 (2008)

that provide an ideal environment for the evolution of more virulent forms of the virus.[6]

The low concentration of PIs in brain tissue has been shown to be a result of active efflux of these drugs out of the brain.[7–9] One of the main transport proteins responsible for this active efflux is P-glycoprotein. P-glycoprotein (P-gp, ABCB1) is a 170 kD trans-membrane protein belonging to the ATP binding cassette (ABC) super-family that is expressed in a number of tissues important for the ADME of drugs, such as the gut wall, liver canaliculi and proximal tubules in kidney.[10] In brain, P-gp is expressed at the luminal surface of the blood–brain barrier[11] and uses the energy generated by ATP hydrolysis to efflux drug substrates out of the brain against a concentration gradient, thereby decreasing brain levels. P-gp has been shown to efflux PIs both *in vitro* and *in vivo*,[7,12–15] in addition to a number of structurally diverse compounds. *In vitro* biochemical assays such as the ATPase assay have shown the ability of a number of PIs to stimulate P-gp ATPase activity.[16,17] Also, PIs have been shown to compete with substrates for the P-gp binding site.[9] *In vitro* cell accumulation studies have shown the ability of P-gp to limit cellular accumulation of PIs both in P-gp transfected cell models,[18] as well as primary cultures of brain capillary endothelial cells.[16] Directional flux studies across P-gp transfected cell monolayers have shown the preferential transport of PIs in the direction of P-gp mediated efflux.[8] There is some evidence regarding the ability of PIs to up-regulate P-gp expression.[19] *In vivo* studies in different animal models have shown that the brain-to-plasma ratio for PIs is much lower than unity following intravenous, as well as oral dosing.[8,14,15] Inhibition of P-gp using small molecule inhibitors has been shown to increase cellular accumulation and directional transport of PIs in P-gp over-expressing cells.[7,8] The brain-to-plasma ratio for PIs was significantly increased on treatment with P-gp inhibitors or in transgenic P-gp knockout mouse models.[18,20]

Small molecules have been traditionally employed as inhibitors for P-gp both *in vitro* and *in vivo*. Recent reports have provided evidence that surfactant and polymeric molecules, used primarily as excipients, have biologic activity against P-gp function.[21] Surfactants such as TPGS,[22–24] Cremophor EL,[25] Tween 80[26] and polymers such as Pluronics[27,28] and amphiphilic diblock copolymers[29] have been shown to inhibit P-gp function both *in vitro* and to enhance drug exposure *in vivo*. The effect of excipients on passive drug permeation across the plasma membrane has also been investigated.[30]

Pluronics consist of a combination of polyethylene oxide and polypropylene oxide chains arranged as a tri-block copolymer. Pluronic P85, an amphiphilic block copolymer with two chains of hydrophilic polyethylene oxide groups flanking a hydrophobic polypropylene oxide group, has been shown to inhibit P-gp efflux activity.[28]

The objective of the study was to evaluate the ability of Pluronic P85 to modulate the interaction of HIV-1 PIs with P-glycoprotein using the biochemical ATPase assay and *in vitro* cell accumulation studies.

MATERIALS AND METHODS

Chemicals

[^{3}H]-Vinblastine sulfate, [^{3}H]-saquinavir, and [^{14}C]-nelfinavir were obtained from Moravek Biochemicals (Brea, CA). Verapamil was obtained from the Sigma Chemical Co. (St. Louis, MO). Pluronics P85, F127, and F88 were a gift from BASF (Florham Park, NJ). Human P-gp expressing membranes were purchased from BD Biosciences (San Jose, CA). All other chemicals used were HPLC or reagent grade.

Cell Lines

Wild-type (WT) and MDR1-transfected epithelial Madin-Darby canine kidney (MDCKII) cells, as well as wild-type and MDR1-transfected porcine kidney epithelial cells (LLC-PK1 WT and LLC-PK1 MDR1) were obtained from Dr. Piet Borst (The Netherlands Cancer Institute, Amsterdam, The Netherlands). The MDCKII cells were maintained in Dulbecco's modified eagle medium (Mediatech, Inc., Herndon, VA) fortified with 10% heat deactivated fetal bovine serum (SeraCare Life Sciences, Inc., Oceanside, CA) and the LLC-PK1 cells were maintained in Medium 199 (Mediatech, Inc.) and fortified with 3% fetal bovine serum. Both the canine and porcine cell lines were treated with 100 U/mL of penicillin and 100 μg/mL of streptomycin (Sigma–Aldrich, St. Louis, MO) and incubated at 37°C under humidity and 5% CO_2 tension. The MDCKII-MDR1 cell growth media additionally contained 80 ng/mL of colchicine to maintain positive selection pressure