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Suppression of P-glycoprotein expression by antipsychotics trifluoperazine in adriamycin-resistant L1210 mouse leukemia cells

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ABSTRACT

Multidrug resistance (MDR) to unrelated chemotherapeutic drugs can be mediated by overexpression of P-glycoprotein (P-gp), the *mdr* gene product. Trifluoperazine (TFP), a phenothiazine derivative antipsychotics, is known to reverse MDR of tumor cell lines by blocking P-gp efflux function. In the present study, we evaluated the effect of TFP on the expression of P-gp in multidrug-resistant L1210/Adr mouse leukemic cell lines, which are characterized by overexpression of P-gp. We found that TFP induced the downregulation of P-gp protein and *mdr1b* mRNA in a dose- and time-dependent manner in L1210/Adr cells. TFP reduction of *mdr1b* mRNA was paralleled by transcriptional suppression of the *mdr1b* promoter. Moreover, TFP restored the adriamycin-induced apoptosis in L1210/Adr cells. These results suggest that TFP may have utility as an adjuvant in the therapy of leukemia for the reversal of P-gp-dependent MDR as well as for the management of psychological symptoms in the cancer patients.

1. Introduction

Multidrug resistance (MDR) of tumor cells represents a development of clinical barrier to a wide range of structurally and functionally unrelated anti-cancer drugs (Gottesman and Pastan, 1993). Many mechanisms for the development of MDR have been described, including the up-regulation of membrane transporter, activation of pathway for detoxification of drugs, changes in DNA repair response and apoptotic responses to toxic drugs (Simon and Schindler, 1994). One of the most common and well-described mechanism in the development of MDR phenotype is the overexpression of P-glycoprotein (P-gp). P-gp is a transmembrane protein that acts as an ATP-driven efflux pump for a wide variety of structurally and functionally unrelated agents from cytoplasm, thereby reducing the intracellular drug content to a sublethal level (Fardel et al., 1996; Ambudkar et al., 2003).

Trifluoperazine (TFP) is a phenothiazine derivative which is widely used clinically to control psychotic disturbances, such as depression, agitation, anxiety, psychosis, and acute confusional state (Bruera and Nemann, 1998; Nordenberg et al., 1999). The therapeutic effects of TFP are attributed to the antagonism of dopamine D2 receptor and the inhibition of calmodulin (Nordenberg et al., 1999; Takano et al., 2003; Lahti et al., 1993). TFP, apart from their clinical use as a neuroleptics, has been shown to exert antiproliferative effects on many

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tumor cells (Osborn and Weber, 1980; Naftalovich et al., 1991; Bruera and Nemann, 1998; Nordenberg et al., 1999; Shin et al., 2004a). In addition, TFP has been shown to block the function of P-gp by modification of the membrane structure around the P-gp or by direct interaction with P-gp (Hendrich et al., 2001), and sensitizes MDR cells to chemotherapeutic agents (Ford et al., 1989; Ganapathi et al., 1991). It has been demonstrated that P-gp reversing agents, such as reserpine, verapamil and cyclosporine A, or bromocriptine, an ergot alkaloid known as a D2 dopaminergic receptor agonist, regulate P-gp gene expression (Sonneveld and Wiemer, 1997; Furuya et al., 1997).

The aim of the present study was to determine whether MDR reversing agent TFP could modulate P-gp gene expression. We found that TFP decreased levels of *mdr1b* mRNA and its product P-gp protein in adriamycin-resistant mouse leukemia L1210 model system.

2. Materials and methods

2.1. Materials

Trifluoperazine was purchased from RBI (Natick, MA). [α-^{32}P]dCTP and [^{14}C]chloramphenicol were from NEN Life Science Products, Inc. (Boston, MA). Acetyl coenzyme A was from Sigma (St. Louis, MO). Rabbit polyclonal P-Glycoprotein antibody was obtained from Oncogene (San Diego, CA) and anti-PARP, anti-NFκB, and anti-IκB antibodies were from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA).

2.2. Cell culture

L1210 mouse leukemia cell line was obtained from American Type Culture Collection (Rockville, MD). Multidrug resistant L1210 subline was obtained by long-term adaptation of parental L1210 cells to adriamycin (Kim et al., 1993). Additional details concerning cross-resistance responsible for MDR phenotype were described elsewhere (Kim et al., 1993; Kim and Kim, 1994; Kim, 1997). L1210 cells were grown in RPMI 1640 medium supplemented with 10% (v/v) heat-inactivated fetal bovine serum (Hyclone, Logan, UT). L1210/Adr cells were routinely maintained in a medium containing 0.2 μg/ml adriamycin.

2.3. Western blot analysis

Cells were lysed and protein samples (20 μg of each) were separated and transferred to nitrocellulose filters as described previously (Shin et al., 2004a). The blots were incubated with anti-P-glycoprotein antibody (Oncogene, La Jolla, CA) and developed with the enhanced chemiluminescence detection system (Amersham Pharmacia Biotech., Piscataway, NJ). For PARP cleavage assay, an anti-PARP antibody that detects both the native 113-kDa and the cleaved 89-kDa fragment was utilized (Santa Cruz Biotechnology, Inc., Santa Cruz, CA). The same blot was stripped and reprobed with anti-Erk1/2 or Erk2 antibody for use as an internal control. For detection of NFκB and IκB, L1210 cells were separated into cytosol and nuclear-enriched fractions as described previously (Shin et al., 2004b).

2.4. Northern blot analysis

Total RNA was prepared using STAT-60 reagent (TEL-TEST, Friendswood, TX) according to the manufacturer's protocol. Ten micrograms of total RNA was separated on 1.2% agarose gel, transferred to Hybond N$^+$ nylon membrane (Amersham Pharmacia Biotech., Piscataway, NJ), and hybridized with [α-^{32}P]dCTP-labeled mdr1 cDNA probe, an insert of pCHP1 (Riordan et al., 1985). The membranes were then washed with 2× SSC/0.1% SDS for 20 min at room temperature, 2× SSC/0.1% SDS at 42 °C for 30 min, and 0.5× SSC/0.1% SDS for 30 min at 65 °C. [α-^{32}P]dCTP-labeled *gapdh* cDNA was used as an internal control.

2.5. Generation of deletions of the rat mdr1b promoter constructs

Deletions of the rat mdr1b promoter fragments spanning from the −1237 to +42, −740 to +42, −370 to +42, and −205 to +42 were synthesized by PCR in a reaction containing the -1288 RMICAT plasmid, a rat *mdr1b*-CAT reporter (Zhou et al., 1996) which was a kind gift of Dr. M. Tien Kuo (University of Texas M.D. Anderson Cancer Institute, Houston, TX;), as a template with the following upstream primers: −1237; 5′-caGGTACCtacaaagggaagctgaagag-3′, −740; 5′-caGGTACCggactctgtctcaggttgac-3′, −370; 5′-caGGTACCgctgtcagaagggaactttta-3′, and −205; 5′-caGGTACCagagttacctgaacatgtagagac-3′. A *Kpn*I restriction site is indicated by uppercase letters. A single downstream primer (5′-cgTCTAGActcagcctcttacagcttca-3′) containing a *Bgl*II site, which is indicated by uppercase letters, was used in each PCR amplification. The PCR fragments were then digested with the restriction enzymes *Kpn*I and *Bgl*II. The fragments were extracted from the agarose gel and inserted into the *Kpn*I and *Bgl*II sites of the pGL3-basic luciferase-encoding reporter vector (Promega, Madison, WI, USA). The resultant constructs were verified by DNA sequencing and by restriction enzyme digests.

2.6. Transient transfection and reporter gene assay

For *mdr1b* promoter analysis, NIH3T3 cells were grown in DMEM supplemented with 10% heat-inactivated fetal calf serum. One day after seeding cells into 35-mm dishes (6×10^5 cells), the cells were co-transfected with 0.5 μg of 5′ deletion constructs of the *mdr1b* promoter and 50 ng of pRL/null plasmid, which expresses *Renilla* luciferase (Promega) using the Lipofectamine 2000 reagent (Life Technologies) according to the manufacturer's instructions. Twenty-four hours post-transfection, cells were treated with TFP. After 6–8 h, the cell lysates were assayed for luciferase activity as described in the manufacturer's protocol (Promega) using the TD 20/20 luminometer (Turner Designs, Sunnyvale, CA). *Renilla* luciferase activity was used to normalize the activity of the firefly luciferase.

2.7. Determination of DNA fragmentation

Cells (5×10^6) were lysed in a solution containing 5 mM Tris, pH 7.4, 20 mM EDTA, 0.5% Triton X-100. The lysate was extracted twice with phenol/chloroform and chloroform. Cytosolic frag-