Short Communication

Prolongation of Pentoxifylline Aliphatic Side Chain Positively Affects the Reversal of P-Glycoprotein-Mediated Multidrug Resistance in L1210/VCR Line Cells

P. Dočolomanský[1], I. Fialová[2], M. Barančík[2], A. Rybár[3] and A. Breier[1]

[1] *Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Bratislava, Slovakia. E-mail: peter.docolomansky@savba.sk*
[2] *Institute for Heart Research, Slovak Academy of Sciences, Dúbravská cesta 9, P.O.Box 104, 840 05 Bratislava, Slovakia*
[3] *Institute of Chemistry, Slovak Academy of Sciences, Dúbravská cesta 9, 845 32 Bratislava, Slovakia*

Abstract. We reported previously that derivatives of pentoxifylline (PTX) reverse multidrug resistance (MDR) in P-glycoprotein (P-gp) positive L1210/VCR cells. Based on the results of a recent study using 25 N-alkylated methylxanthines with carbohydrate side-chains of various lengths, we formulated the following design criteria for a methylxanthine molecule to effectively reverse P-gp mediated MDR: i) a massive substituent at the N1 position is crucial for MDR reversal potency; ii) elongation of the substituents at the N3 and N7 positions (from methyl to propyl) increases the efficacy of a xanthine to reverse MDR; iii) elongation of the substituent at the C8 position (from H to propyl) decreases the efficacy of a xanthine to reverse MDR. Based on these criteria, we synthesized and tested for potency to reverse MDR a new PTX derivative, 1-(10-undecylenyl)-3-heptyl-7-methyl xanthine (PTX-UHM), with prolonged substituents at the N1 and N3 positions. The derivative was obtained by alkylation of 3-heptyl-7-methyl xanthine with 1-methylsulfonyloxy-10-undecylenyl. NMR and IR structural analyses proved the identity of the product. Cytotoxicity study showed that PTX-UHM is only slightly more toxic to L1210/VCR cells than PTX. We found that both PTX-UHM and PTX were able to reverse vincristine resistance of L1210/VCR cells, yet PTX-UHM was significantly more efficient in the reversal than PTX.

Key words: Alkyl xanthines — Pentoxifylline analogues — P-glycoprotein — L1210 cell line — Multidrug resistance

Correspondence to: Albert Breier, Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Vlárska 5, 833 34 Bratislava 37, Slovakia
E-mail: albert.breier@savba.sk

Xanthines are based on purine structures that give rise to a large scale of alkylated analogues. These compounds are mainly alkylated at positions N1, N3 (of pyrimidine), and N7 and C8 (of imidazole) with carbohydrate chains of various lengths. Alterations of side chains located at these positions were described to predetermine the effectiveness of xanthines in various regulatory activities, such as the effect on ADP-ribose-induced calcium release (Cavallaro et al. 1999), affinity to adenosine receptors (Klotz 2000; Strappaghetti et al. 2001), and ability to reverse multidrug resistance (MDR) in P-glycoprotein (P-gp) positive L1210/VCR* cells (Kupsáková et al. 2004).

P-gp is an integral membrane glycoprotein (170 kDa) located in the cell plasma membrane; the protein exports structurally diverse groups of substances out of cytoplasm (Kvačkajová-Kišucká et al. 2001). Several drugs, called chemosensitizers (calcium channel blockers, calmodulin inhibitors, cyclosporines, hormones, coronary vasodilators such as pentoxifylline (PTX), and others) are able to depress MDR through partial or full suppression of P-gp transport activity by several mechanisms (Breier et al. 2005). Based on the study using 25 structural analogues of PTX, we have formulated the following criteria that seem to be important for reversal of MDR of L1210/VCR cells (Kupsáková et al. 2004): i) a large substituent at the N1 position is the most important feature of a methylxanthine to be an effective MDR reversal agent; ii) alkylations of N1-5-oxohexyl xanthine on the N3 and N7 nitrogen atoms are beneficial to MDR reversal potency; iii) alkylation of N1-5-oxohexyl xanthine on the C8 carbon atom decreases the ability of the derivative to reverse MDR.

The crucial meaning of the substituent at the N1 position is consistent with the fact that compounds with bulkier side-chains in this position, such as 11-dodecylamino-10-hydroxy-undecyl (Ojima et al. 1998) or 5-imino-(2'-amino-1'-benzenehydroxy-sulphonyl)-hexyl (Kupsáková et al. 2002), were found to be more effective than PTX in reversal of MDR. Motivated by the above considerations, we undertook to prepare 1-(10-undecylenyl)-3-heptyl-7-methyl xanthine (PTX-UHM) and test its ability to depress vincristine resistance of L1210/VCR cells.

The PTX-UHM compound was synthesized by N1-alkylation of 3-heptyl-7-methyl xanthine (prepared previously, Kupsáková et al. 2004) with 1-methylsulfonyloxy-10-undecylenyl in dimethylsulfoxide (DMSO, Lachema Brno, Czech Republic). The reaction scheme is shown in Figure 1. The reaction was run for 24 h at 22 ℃ in a three-neck flask equipped with a stirrer, thermometer and drop funnel. 3-heptyl-7-methyl xanthine was dissolved to final concentration 11 mmol/l in dried DMSO and NaH (60 % suspension in DMSO) was dropped and mixed for 20 min. In the next step, fresh distilled 1-methylsulfonyloxy-10-undecylene was added and the reaction mixture was stirred for another 22 h. The reaction was carried out at 70–80 ℃ and controlled by thin-layer chromatography using 20 %

* The cell line obtained by adaptation of L1210 cells to vincristine (Poleková et al. 1992) that exerts massive overexpression of P-gp (Fiala et al. 2003; Sulová et al. 2005) associated with P-gp efflux activity (Orlický et al. 2005).

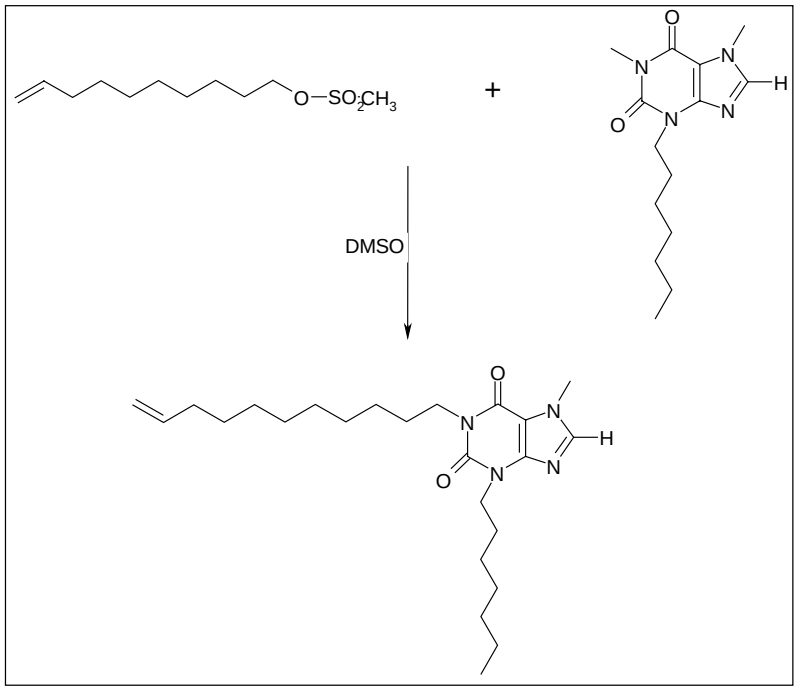


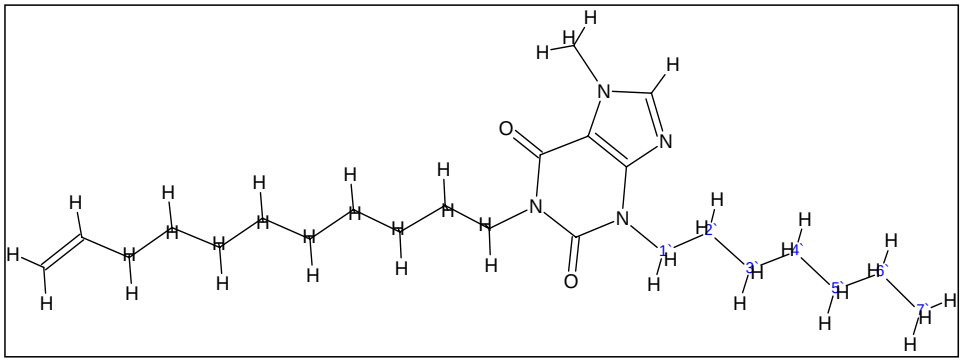
Figure 1. Reaction scheme of PTX-UHM preparation.

isohexane/dichlormethane as mobile phase. After separation of the product (yield 37 % of 3-heptyl-7-methyl xanthine) using column chromatography on Silicagel 200 (Sigma, USA), the structure of the final product was confirmed by IR spectrophotometry and ^{1}H-NMR spectroscopy (Table 1). IR spectrophotometry confirmed the presence of C=O binding (1580 cm^{-1}) in the purine structure.

L1210/VCR cells were cultivated in RPMI 1640 medium supplemented with L-glutamine (1 mg/ml), foetal bovine serum (4 %) and gentamycine (1 μl/ml; all from Gibco, Invitrogen Life Technologies, Scotland, UK), in atmosphere of 5 % CO_2, at 37 ℃ on 96 well plates (inoculation with 10^4 cells/200 μl). MDR reversal effect of xanthine derivatives was determined by cultivation of L1210/VCR cells in a medium containing vincristine (concentration range 0–6 mg/l) in absence or in presence of PTX or PTX-UHM (concentrations used: 10, 30, and 50 mg/l). Trypan Blue (Sigma, Germany) was used for staining of damaged cells after three day's cultivation and viable unstained cells were counted in a haemocytometer. Cytotoxicities of both alkylxanthines were determined as the decrease of cells' survival induced by presence of the respective alkylxantine in the cultivation medium. The MDR reversal effect was evaluated as the decrease in IC_{50} value for vincristine (computed as described elsewhere, Breier et al. 1994) induced by presence of the respective alkylxanthine.

Table 1. H^1-NMR (DMSO-d_6) values for 1-(10-undecylenyl)-3-heptyl-7-methyl xanthine (PTX-UHM)

Proton position	Signal	δ (ppm)
7'-CH_3	triplet	0.8
-CH_2-	singlet	1.22
-CH_2-	singlet	1.34
-CH_2-	multiplet	1.65
-CH_2-	multiplet	1.75
=CH-CH_2-CH_2-	quartet	2.02
N7-CH_3	singlet	3.99
N1-alkyl, N3-alkyl	multiplet	4.08
CH_2=	multiplet	4.95
=CH-	multiplet	5.81
H-8	singlet	7.53



We found that PTX-UHM is slightly more toxic to L1210/VCR cells than PTX (Figure 2A). This may be the result of improved xanthine hydrophobicity and consequently of the higher ability of the derivative to interact and/or pass through plasma membrane *via* passive diffusion. Both PTX-UHM and PTX are able to potentiate vincristine cytotoxicity (Figure 2B,C) and PTX-UHM was found to be much more effective than PTX (Figure 2D). At applied concentrations, both substances depressed the MDR of L1210/VCR cells only partially because IC_{50} for vincristine, characteristic for parental sensitive L1210 cells, is approximately 0.01 mg/l. Higher doses of xanthines that probably would reverse the MDR of these cells totally could not be used due to cytotoxicity of both xanthines at the respective doses. PTX-UHM was designed in accordance with the criteria based on P-gp reversal effectiveness of PTX derivatives (Kupsáková et al. 2004). The higher effectiveness of the PTX-UHM derivative compared to PTX confirms the plausibility of the above criteria. The voluminous 10-undecylenyl substituent at the N1 position of PTX-UHM has a non-polar character. It was found previously that

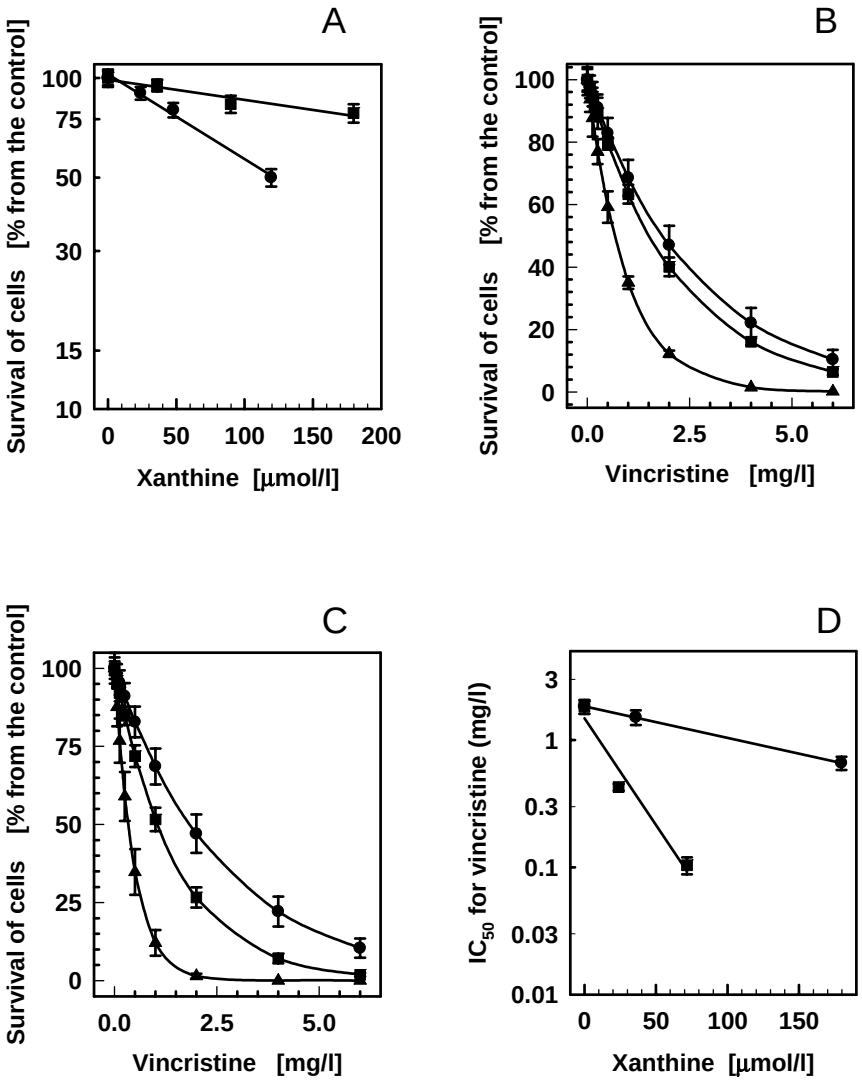


Figure 2. Reversal effects of PTX and PTX-UHM on P-gp-mediated MDR of L1210/VCR cells. **A.** Cytotoxicity of PTX (squares) and PTX-UHM (circles) on L1210/VCR cells. **B**. Influence of PTX on sensitivity of L1210/VCR cells to vincristine (PTX concentrations in μmol/l: 0 (circles), 36 (squares), 180 (triangles)). **C.** Influence of PTX-UHM on sensitivity of L1210/VCR cells to vincristine (PTX-UHM concentrations in μmol/l: 0 (circles), 24 (squares), 72 (triangles)). **D.** Relations between vincristine sensitivity of L1210/VCR cells characterized by IC_{50} value and concentration of PTX (circles) or PTX-UHM (squares). Data are reported as mean ± S.E.M. from six independent measurements.

5-oxohexyl and 5-hydroxyhexyl at this position give final derivatives with higher effectiveness than n-hexyl (Kupsáková et al. 2004). Therefore, it still remains to be resolved whether the effectiveness of PTX-UHM could be further improved by replacement of 10-undecylenyl at the N1 position with a more polar hydroxy or oxo 10-undecylenyl side-chain.

Acknowledgements. This study was supported by grants from the Slovak Grant Agency for Science VEGA (2/4154/4, APVT 51-013802, and APVT 51-027-404.

References

Breier A., Barančík M., Štefanková Z., Uhrík B., Tribulová N. (1994): Effect of pentoxifylline on P-glycoprotein-mediated vincristine resistance of L1210 mouse leukemic cell line. Neoplasma **41**, 297—303

Breier A., Barančík M., Sulová Z., Uhrík B. (2005): P-glycoprotein – implications of metabolism of neoplastic cells and cancer therapy. Curr. Cancer Drug Targets **5**, 457—468

Cavallaro R. A., Filocamo L., Galuppi A., Galione A., Brufani M., Genazzani A. A. (1999): Potentiation of cADPR-induced Ca^{2+}-release by methylxanthine analogues. J. Med. Chem. **42**, 2527—2534

Fiala R., Sulová Z., El-Saggan A. H., Uhrík B., Liptaj T., Dovinová I., Hanušovská E., Drobná Z., Barančík M., Breier A. (2003): P-glycoprotein-mediated multidrug resistance phenotype of L1210/VCR cells is associated with decreases of oligo- and/or polysaccharide contents. Biochim. Biophys. Acta **1639**, 213—224

Klotz K. N. (2000): Adenosine receptors and their ligands. Naunyn Schmiederbergs Arch. Pharmacol. **362**, 382—391

Kupsáková I., Dočolomanský P., Rybár A., Barančík M., Breier A. (2002): Carbonyl group of aliphatic side chain of pentoxifylline does not play role for P-glycoprotein antagonizing effect of pentoxifylline. Gen. Physiol. Biophys. **21**, 471—478

Kupsáková I., Rybár A., Dočolomanský P., Drobná Z., Stein U., Walther W., Barančík M., Breier A. (2004): Reversal of P-glycoprotein mediated vincristine resistance of L1210/VCR cells by analogues of pentoxifylline. A QSAR Study. Eur. J. Pharm. Sci. **21**, 283—293

Kvačkajová-Kišucká J., Barančík M., Breier A. (2001): Drug transporters and their role in multidrug resistance of neoplastic cells. Gen. Physiol. Biophys. **20**, 215—237

Ojima I., Bounaud P. Y., Oderda C. F. (1998): Recent strategies for the treatment of multidrug resistance in cancer cells. Expert Opin. Ther. Pat. **8**, 1587—1598

Orlický J., Sulová Z., Dovinová I., Fiala R., Zahradníková A. Jr., Breier A. (2004): Functional fluo-3/AM assay on P-glycoprotein transport activity in L1210/VCR cells by confocal microscopy. Gen. Physiol. Biophys. **23**, 357—366

Poleková L., Barančík M., Mrázová T., Pirker R., Wallner J., Sulová Z., Breier A. (1992): Adaptation of mouse leukemia cells L1210 to vincristine. Evidence for expression of P-glycoprotein. Neoplasma **39,** 73—77

Strappaghetti G., Corsano S., Barbaro R., Giannaccini G., Betli L. (2001): Structure-activity relationship in a series of 8-substitued xanthines as A1-adenosine receptor antagonists. Bioorg. Med. Chem. **9**, 575—583

Sulová Z., Orlický J., Fiala R., Dovinová I., Uhrík B., Šereš M., Gibalová L., Breier A. (2005): Expression of P-glycoprotein in L1210 cells is linked with rise in sensitivity to Ca^{2+}. Biochem. Biophys. Res. Commun. **335**, 777—784

Final version accepted: September 30, 2005