ELSEVIER

Biochemical Pharmacology

Biochemical Pharmacology 61 (2001) 61–66
Short communication

Characterization of multidrug transporters in a normal renal tubular cell line resistant to doxorubicin
Multidrug transporters in the LLC-PK$_1$ cell line and its resistant counterpart

Giuliana Decorti*, Anna Rosati, Luigi Candussio, Tullio Giraldi, Fiora Bartoli Klugmann

Department of Biomedical Sciences, University of Trieste, I-34127 Trieste, Italy

Received 19 October 1999; accepted 16 June 2000

Abstract

LLC-PK$_1$ is a proximal tubular cell line derived from normal pig kidney which has a structure and function similar to those of renal proximal tubular cells and which expresses baseline levels of P-glycoprotein. We isolated by drug selection a doxorubicin-resistant cell line (LLC-PK$_1$/ADR) that exhibited a multidrug-resistant phenotype; this cell line was characterized by reduced intracellular drug concentrations, an increased drug extrusion, and increased expression of a 170-kDa P-glycoprotein detected by Western blot analysis with monoclonal antibody C219. In addition, an increased expression of *MDR1* mRNA was seen by reverse transcriptase–polymerase chain reaction. These results suggest that it is possible to induce the overexpression of P-glycoprotein by chronic treatment with doxorubicin in a normal cell line that physiologically expresses low levels of this protein. This multi-resistant cell line could provide an interesting model for studying the role of P-glycoprotein and the consequence of its induction in a normal tissue. © 2000 Elsevier Science Inc. All rights reserved.

Keywords: Doxorubicin; LLC-PK$_1$ renal cell line; P-glycoprotein; Multidrug resistance

1. Introduction

Multidrug resistance P-glycoprotein is a 170-kDa transmembrane protein which is encoded by a small gene family comprising two members in humans (*MDR1* and *MDR2/3*). This protein is overexpressed in many drug-resistant tumors and acts as an ATP-dependent efflux pump to expel hydrophobic substances, including chemotherapeutics, from the cytoplasm [1]. P-glycoprotein is also present in various normal epithelial tissues, such as liver, intestine, kidney, and endothelia of the blood–brain barrier [2–4]. Although the physiologic role of P-glycoprotein is not clear at this time, possibilities include the protection of normal tissues from environmental and endogenous toxins, steroid secretion in the adrenal gland, secretion of bile salts in the bile canaliculi, and secretory functions in the kidney [5]. In addition to P-glycoprotein, several other proteins contribute to resistance by controlling drug transport and intracellular distribution. Among these, a 190-kDa MRP encoded by the *MRP* gene has been detected in several tumors and in most tissues of the human body [6]. It has previously been shown that expression of P-glycoprotein and of other proteins involved in multidrug resistance is regulated by chronic presence of substrates both *in vitro* [7,8] and *in vivo* [8,9]. Induction of these proteins could modify the pharmacokinetic profile and cause an alteration in treatment efficacy and in side effects of anticancer drugs.

The LLC-PK$_1$ cells are a proximal tubular renal cell line derived from pig kidney [10], which has a structure and function similar to those of renal proximal tubular cells [11]; these cells physiologically express low amounts of P-glycoprotein [12,13]. The aim of the study was therefore to evaluate if treatment with increasing concentrations of doxorubicin was able to induce the expression level of P-glycoprotein or of other proteins involved in multidrug resistance in this normal cell line. A multidrug-resistant line derived from normal proximal tubular cells should provide

* Corresponding author. Tel.: +39 40 676 7949; fax: +39 40 676 7838.

E-mail address: decorti@univ.trieste.it (G. Decorti).

Abbreviations: MDR, multidrug resistance; MRP, multidrug resistance-related protein; and RT–PCR, reverse transcriptase–polymerase chain reaction.

0006-2952/01/$ – see front matter © 2000 Elsevier Science Inc. All rights reserved.
PII: S0006-2952(00)00535-9

a suitable system in which to analyze the role of P-glycoprotein and the effects of its induction in normal tissues.

2. Materials and methods

2.1. Establishment of doxorubicin-resistant clones

The pig kidney epithelial cell line LLC-PK_1, obtained from the American Type Culture Collection (ATCC-CRL-1392), was grown in medium 199 supplemented with 10% fetal bovine serum. The cells were selected for doxorubicin resistance by continuous stepwise increments of doxorubicin concentrations. Cells were seeded at a cell density of 5×10^4 cells/mL in 35-mm dishes in 2 mL of complete culture medium, and doxorubicin was then added for 48 hr. The initial concentration of doxorubicin to which cells were exposed was 0.1 μM, which was gradually increased to 20 μM. After incubation with doxorubicin, cells were washed and cultured in drug-free medium, and were again treated with increasing concentrations of doxorubicin when cell number increased at a similar rate as parental cells. A resistant subline tolerating 20 μM doxorubicin, LLC-PK_1/ADR, was collected and stored in liquid nitrogen. The drug resistance phenotype was stable for several months without further selection. Cells were used in passages 211 to 230 for LLC-PK_1 and 10 to 20 for LLC-PK_1/ADR lines.

Cells seeded as above were allowed to grow until confluency, and monolayers were observed under an inverted phase-contrast microscope for the morphological phenotype and formation of domes. Cells were similarly grown on 24-mm Transwell permeable supports (Corning Science Products), and the transepithelial electrical resistance was measured in triplicate at confluency using a Millicell-ERS (Millipore Corp.).

2.2. Growth inhibition assay

Cytotoxicity was determined by means of the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay of Mosmann [14] and the absorbance was measured on an Automated Microplate Reader EL311s (Bio-Tek Instruments) with a reference wavelength of 630 nm and a test wavelength of 540 nm. Each experiment was performed using 8 replicate wells for each drug concentration, and at least 3 separate experiments were carried out for each cell line. The IC_{50} was defined as the drug concentration required to reduce the optical density in each test to 50% of control. Resistance factor was defined as the ratio between the IC_{50} of the resistant subline and the IC_{50} of the parental control. Substances used for cell culture and the cytotoxicity test were from Sigma-Aldrich.

2.3. Doxorubicin and rhodamine 123 accumulation

LLC-PK_1 (4×10^4 cells/mL) and LLC-PK_1/ADR (20×10^4 cells/mL) cells were seeded in 35-mm dishes in 2 mL of complete medium; the uptake of doxorubicin and rhodamine 123 was measured on confluent cells on the 5th day after inoculation. Cell monolayers were incubated with test substances and treated as previously described [13]. To evaluate doxorubicin uptake, the final pellet was resuspended in 1 mL of 0.3 N HCl in 50% ethanol, mixed thoroughly in a vortex mixer, and centrifuged at 700 x*g*. Doxorubicin content in the supernatant fraction was determined fluorimetrically by the method of Bachur *et al*. [15]. To evaluate rhodamine 123 uptake, the cell pellet was suspended in 1 mL 1% Triton X-100, mixed thoroughly, incubated for 10 min at 37°, and the fluorescence was read on a spectrofluorimeter with excitation at 504 nm and emission at 540 nm.

2.4. Rhodamine 123 efflux

Efflux studies were performed by exposing cell monolayers to 3 μM rhodamine 123 at 37° for 2 (LLC-PK_1 cells) or 3 (LLC-PK_1/ADR cells) hr, respectively. Drug-containing medium was then removed, and monolayers were washed 3 times with ice-cold saline and incubated for different times in drug-free medium or in medium containing vinblastine (100 μM) or cyclosporin A (50 μM). Extraction of rhodamine 123 was performed following the procedure previously described. Protein concentration of samples was determined by the method of Lowry *et al*. [16]. Doxorubicin, vinblastine, colchicine, taxol, verapamil, and cyclosporin A were purchased from Sigma Chemical Co. All other chemicals were of analytical grade.

2.5. Western blot analysis

Cells were dissolved in sample buffer according to the method of Laemmli [17]. Approximately 30 μg of proteins [16] was separated on 8% SDS–PAGE, then transferred to nitrocellulose membrane. Western blot analysis was performed with C219 (CIS Bio International, 1:200, overnight, room temperature) [18] and MRPm6 (Chemicon International Inc., 1:250, 1 hr, room temperature) antibodies [19] in the same buffer used to block the membranes for 2 hr (Tris-buffered saline: 150 mM NaCl, 50 mM Tris–HCl, pH 7.5, containing 0.05% v/v Tween 20, added with 3% w/v BSA for C219 antibody and 1% non-fat milk for MRPm6 antibody). An anti-mouse secondary antibody conjugated with alkaline phosphatase (Sigma 1:30,000, 1 hr, room temperature) revealed the specific binding of antibodies. Markers were Kaleidoscope Prestained Standards (Bio-Rad Laboratories).

2.6. RT–PCR

Total RNA was extracted according to Chomczynski and Sacchi [20]. mRNA was purified using poly A^+ tract Isolation System IV (Promega). mRNA (100–500 ng) was reverse transcribed using Superscript II RNAse H- (GIBCO BRL), and

the cDNA were used in PCR reaction in a final volume of 10 μL. Synthetic oligonucleotide primers (Boehringer Mannheim) were used to investigate the presence of *MDR1*, *MDR2/3*, *MRP1*, and *MRP2/cMOAT* mRNA transcripts. *MDR1* sense, 5′-CCCATCATTGCAATAGCAGG-3′, antisense 5′-GTTCAAACTTCTGCTCCTGA-3′ (M14758 sequence); *MDR2/3* sense, 5′-TTATCTACGGTTGGCAGTTAACC-3′, antisense 5′-CGAAAACAACCGGCATAGGAA-3′ (M23234 sequence); *MRP1* sense1, 5′-CGGAAACCATCCACGACCCTAATCC-3′,antisense15′-TCCTCATTCGCATCCACCTTGG-3′ (L05628 sequence); *MRP1* sense2, 5′-TTCCCATTTCAACGAGACCTTGCTG-3′, antisense2, 5′-TCCGCCCCACGATGCCGACCT-3′ (L05628 sequence); *MRP2/cMOAT* sense, 5′TGGCTGAGATTGGAGAG-3′, antisense 5′-TTTGTCCTTTCACTAGTTC-3′ (X96395 sequence).

The amplification by *Taq* polymerase was performed using a PTC 100 DNA thermal cycler M. J. Research. After denaturing at 94° for 5 min, the reaction was conducted for 30 cycles (94° for 1 min, 60° for 1 min, 72° for 1 min) for *MDR2/3* and *MRP1*; there were 30 cycles for *MDR1* (94° for 30 sec, 55° for 1 min, 72° for 2 min, according to Noonan *et al*. [21]); for *MRP2/cMOAT* there were 35 cycles (94° for 1 min, 50° for 1 min, 72° for 1 min). For all primers the reaction ended with a final step at 72° for 10 min. PCR products were subjected to electrophoresis on 1.5% agarose gel and visualized by ethidium bromide staining. cDNA from pig liver was used as positive control. As an internal control primers amplifying a fragment of β-actin were used. The DNA marker was φx174DNA/Haelll (Promega Corp.).

3. Results and discussion

We have selected a drug-resistant renal proximal tubule cell line LLC-PK_1/ADR that exhibits the biochemical and pharmacological characteristics typical of the pleiotropic drug resistance phenotype. These cells were derived from the LLC-PK_1 cell line, established by Hull *et al*. [10] from male Hampshire pig renal tissue. This cell line maintains some functions of normal proximal tubular cells such as a Na^+-dependent transport system, enzymes located in the apical membrane [10,11], and an electric resistance of the monolayer that is typical of a leaky epithelium [22]. In addition, when grown in tissue culture dishes, these cells form domes as a result of transepithelial transport and accumulation of fluid between the cell monolayer and culture dish. Thus, this cell line is considered a relevant tool for the examination of the functions of proximal tubule [11].

Table 1
Results from cytotoxicity assays

	IC_{50} (μM)		RF[a]
	LLC-PK_1	LLC-PK_1/ADR	
Doxorubicin	0.35	2.5	7.14
Daunorubicin	0.2	1	5
Taxol	0.02	2	100
Colchicine	0.05	10	200

Three or more experiments were performed for each drug in each cell line.

[a]RF: resistance factor (IC_{50} resistant cells/IC_{50} sensitive cells).

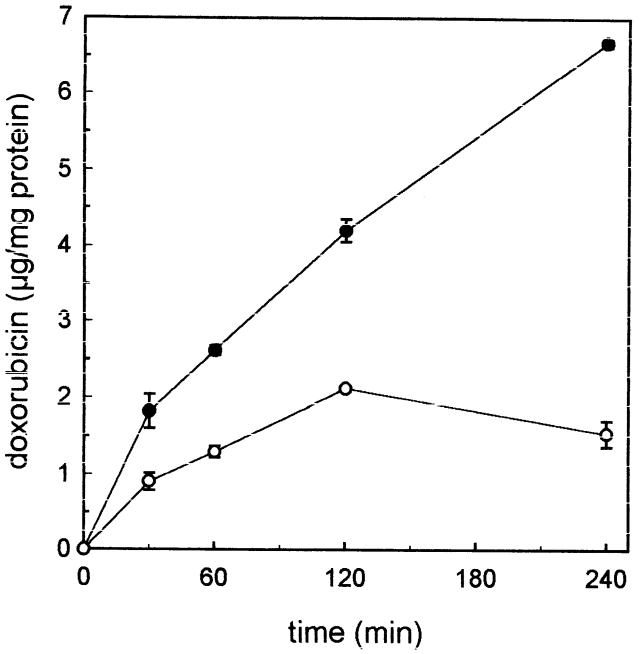

Fig. 1. Doxorubicin uptake in LLC-PK_1 (●) and LLC-PK_1/ADR (❍) cells. Cells were incubated with 50 μM doxorubicin in complete culture medium for up to 240 min. Each point represents the mean ± SE of data from three to six wells.

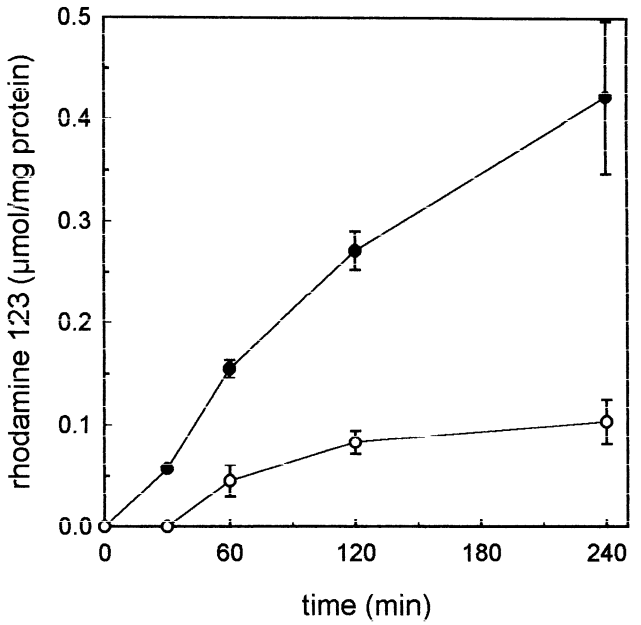

Fig. 2. Rhodamine 123 uptake in LLC-PK_1 (●) and LLC-PK_1/ADR (❍) cells. Cells were incubated with 3 μM rhodamine in complete culture medium for up to 240 min. Each point represents the mean ± SE of data from three to six wells.

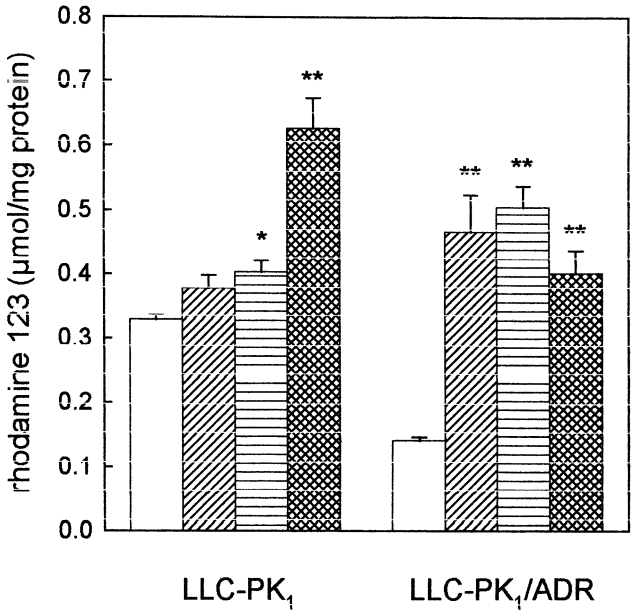

Fig. 3. Effect of 500 μM verapamil (▨), 100 μM vinblastine (▤), and 50 μM cyclosporin A (▩) on rhodamine 123 uptake in LLC-PK_1 and LLC-PK_1/ADR cells. The cells were incubated in medium containing rhodamine 123 (3 μM) without (□) or with the inhibitors for 240 min. Each bar represents the mean ± SE of data from three to six determinations from a typical experiment.

*: $P < 0.05$. **: $P < 0.01$; Student's *t*-test for independent data.

The morphological phenotype and transepithelial resistance (109 ± 10.5 for LLC-PK_1 and 102 ± 3.0 for LLC-PK_1/ADR) were maintained in the resistant cell line obtained in our laboratory; when cultured in drug-free medium for a maximum of 20 passages, no reversion to the drug-sensitive phenotype was observed. This cell line manifested doxorubicin resistance, with a resistance factor of 7.14. This level of resistance is lower compared to resistant tumor cell lines selected *in vitro*, but is probably more characteristic of *in vivo* drug resistance. Furthermore, there was cross-resistance between doxorubicin, daunorubicin, taxol, and colchicine, with a resistance profile that is that of classical multidrug resistance (Table 1). The intracellular uptake of doxorubicin (Fig. 1) and rhodamine 123 (Fig. 2) was drastically lower in the resistant subline, while treatment with some reverting agents reduced this drug accumulation defect (Fig. 3), suggesting that decreased drug accumulation contributed to P-glycoprotein-mediated multidrug resistance. Characterization of the efflux showed a reduction in the intracellular retention of rhodamine in LLC-PK_1/ADR cells which was almost completely reversed by cyclosporin A and vinblastine. A small but significant effect on retention was, however, also evident in the parental cell line, which is also consistent with a low P-glycoprotein expression in these cells (Fig. 4).

Kidney P-glycoprotein has been localized in the apical brush border membrane of the proximal tubuli [2], where it is expected to participate in the excretion of xenobiotics, as well as in proximal tubular cells in culture [13,23,24]. The results of the present study revealed an increase in *MDR1* mRNA levels in LLC-PK_1/ADR cells compared with parental cells (Fig. 5). These findings were correlated with levels of P-glycoprotein detected by the monoclonal antibody C219 (Fig. 6). LLC-PK_1/ADR cells exhibited a significant induction of P-glycoprotein as compared to the parent line, which expressed low but detectable amounts of the protein. The positive control cells, P388/ADR, also exhibited considerable induction. The expression level of *MDR2/3* mRNA was extremely low and unchanged in the two cell lines (data not shown). This finding is in agreement with the current knowledge that *MDR2/3* in humans is not involved in the phenomenon of drug resistance, its main function being the transport of phospolipids into the bile [25].

Besides P-glycoprotein, another pump, the multidrug re-

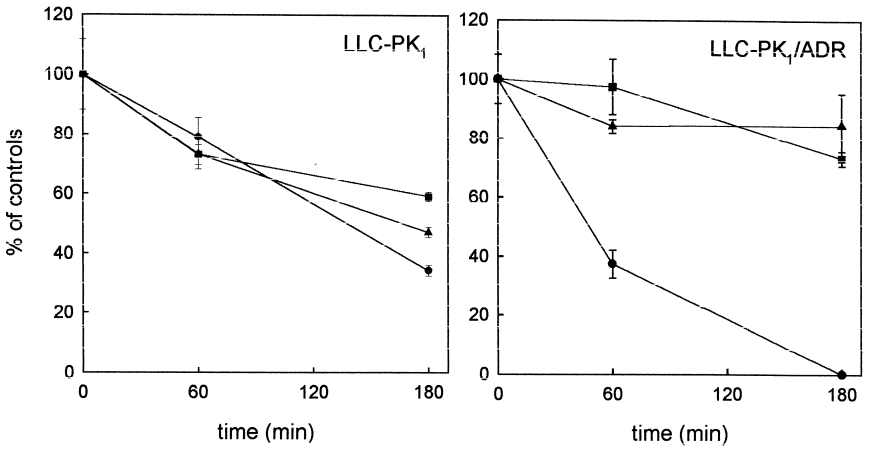

Fig. 4. Kinetics of rhodamine 123 release from LLC-PK_1 and LLC-PK_1/ADR cells incubated in complete medium containing rhodamine. After 2 (LLC-PK_1) or 3 (LLC-PK_1/ADR) hr of incubation, the cells were washed with ice-cold PBS to remove free rhodamine (time 0, total uptake) and incubated at 37° in drug-free medium (●) or in medium containing cyclosporin A 50 μM (■) or vinblastine 100 μM (▲). Rhodamine remaining in the cells was measured at 60 and 180 min. Each point represents the mean ± SE of data from three to six determinations from a typical experiment.

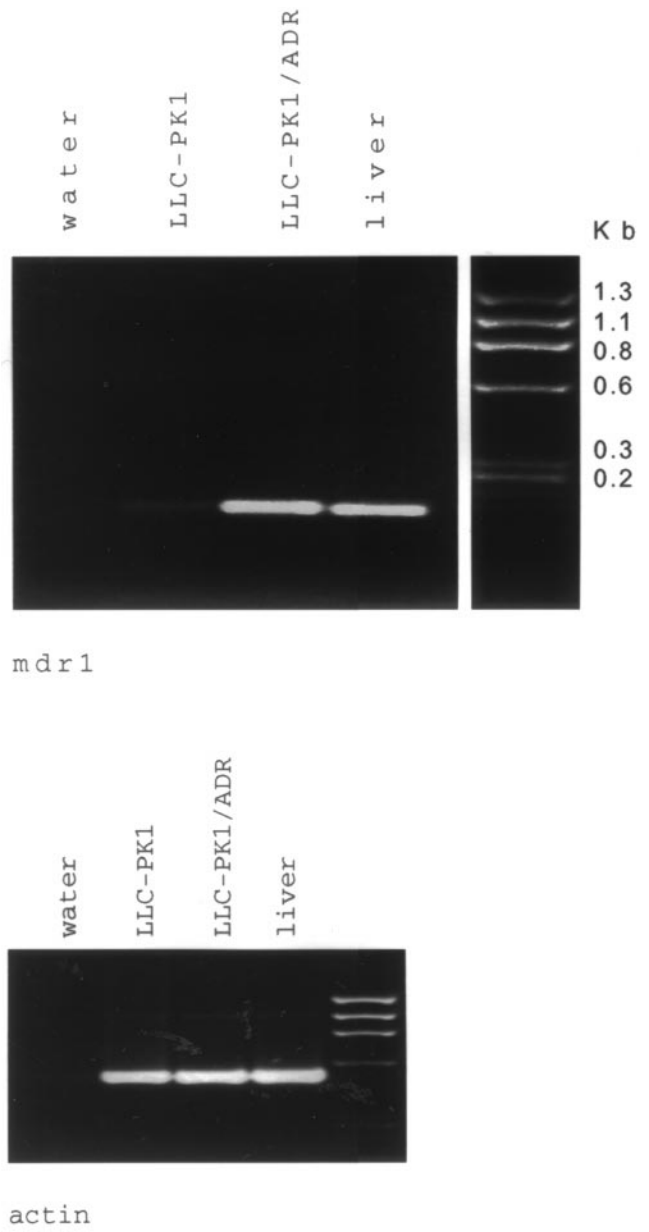

Fig. 5. RT-PCR analysis of *MDR*1 expression in LLC-PK$_1$ and LLC-PK$_1$/ADR cells and in pig liver. RNA (100–500 ng) was reverse transcribed, and the resulting cDNA was tested in PCR reaction using specific primers as described in Materials and Methods. The amplification of a β-actin fragment was used as an internal control. The size of the amplified product was determined from the mobility of the DNA size markers.

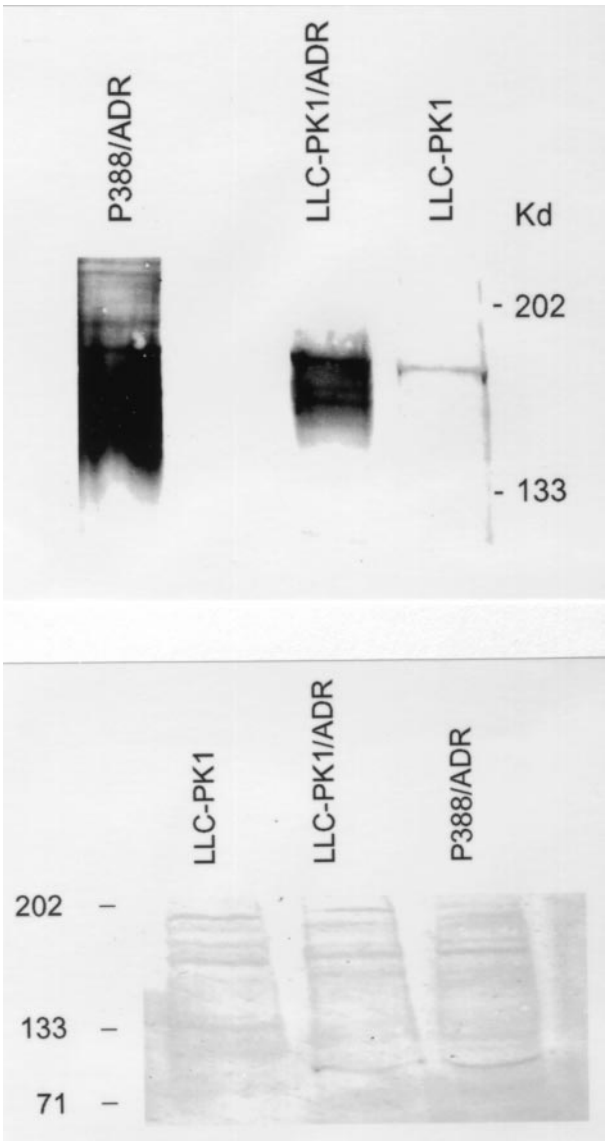

Fig. 6. Western blot analysis of proteins from P388/ADR, LLC-PK$_1$, and LLC-PK$_1$/ADR cells. Cell lysates (30 μg) were probed with monoclonal antibody C219 (upper panel) or MRPm6 (lower panel). Molecular size standards are indicated in kDa.

sistance protein, is involved in multidrug resistance. MRP is a 190-kDa member of the family of ATP-binding cassette (ABC) membrane transporters that, similar to P-glycoprotein, mediates resistance to a range of structurally and functionally unrelated drugs [6]. Our results show that *MRP1* is expressed in both cell lines, but no overexpression could be observed in the resistant subline. Indeed, Western blot analysis with MRPm6 monoclonal antibody revealed a weak band of about 190 kDa in the two cell lines, but not in the P388/ADR cells (Fig. 6); data were confirmed by RT-PCR, which showed that the expression of *MRP1* as well as of *MRP2/cMOAT* genes was significant, but unchanged in the two lines (data not shown). The role of *MRP1* in the kidney is still unclear, although recent evidence suggests that it could act as an excretory pump as does P-glycoprotein [26]. While MRP2, the hepatocyte canalicular isoform of the multidrug resistance protein, also called cMOAT [27], has been identified in the brush border of proximal convoluted tubule cells [28], MRP was found in the glomeruli, ascending limb cells, and basolateral membranes of the distal and collecting tubule cells of the kidney, but not in proximal tubular cells [29].

Clinical and *in vitro* studies [7–9,30] provide evidence for the regulated increase in functional expression of P-glycoprotein and related proteins in normal tissues as a consequence of chronic presence of the substrates. For kid-

ney, where expression will influence excretory capacity, this will substantially alter pharmacokinetic profiles and may be relevant to the treatment efficacy and side effects of drugs. This phenomenon seems to be particularly important since many P-glycoprotein substrates are widely used in clinical practice, both in antineoplastic multicombination therapy and for indications unrelated to oncology. Therefore, a multidrug-resistant cell line derived from the normal proximal tubule could represent a useful system for studying the effects of P-glycoprotein induction in normal tissues.

Acknowledgments

This research was supported by grants from the Ministero Università e Ricerca Scientifica e Tecnologica 60% (University of Trieste) and the Programmi di Ricerca Scientifica di Rilevante Interesse Nazionale (Farmaco-tossicologia di antracicline antitumorali: meccanismi e basi molecolari).

References

[1] Preiss R. P-glycoprotein and related transporters. Int J Clin Pharmacol Ther 1998;36:3–8.
[2] Thiebaut F, Tsuruo T, Hamada H, Gottesman MM, Pastan I, Willingham MC. Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues. Proc Natl Acad Sci USA 1987;84:7735–8.
[3] Cordon-Cardo C, O'Brien JP, Boccia J, Casals D, Bertino JR, Melamed MR. Expression of the multidrug resistance gene product (P-glycoprotein) in human normal and tumor tissues. J Histochem Cytochem 1990;38:1277–87.
[4] Cordon-Cardo C, O'Brien JP, Casals D, Rittman-Grauer L, Biedler JL, Melamed MR, Bertino JR. Multidrug-resistance gene (P-glycoprotein) is expressed by endothelial cells at blood–brain barrier sites. Proc Natl Acad Sci USA 1989;86:695–8.
[5] Gottesman MM, Pastan I. Biochemistry of multidrug resistance mediated by the multidrug transporter. Annu Rev Biochem 1993;62:385–427.
[6] Cole SP, Deeley RG. Multidrug resistance mediated by the ATP-binding cassette transporter protein MRP. Bioessays 1998;20:931–40.
[7] Hauser IA, Koziolek M, Hopfer U, Thevenod F. Therapeutic concentrations of cyclosporine A, but not FK506, increase P-glycoprotein expression in endothelial and renal tubule cells. Kidney Int 1998;54:1139–49.
[8] Garcia del Moral R, O'Valle F, Andujar M, Aguilar M, Lucena MA, Lopez-Hidalgo J, Ramirez C, Medina-Cano MT, Aguilar D, Gomez-Morales M. Relationship between P-glycoprotein expression and cyclosporin A in kidney: An immunohistological and cell culture study. Am J Pathol 1995;146:398–408.
[9] Jette L, Beaulieu E, Leclerc JM, Beliveau R. Cyclosporin A treatment induces overexpression of P-glycoprotein in the kidney and other tissues. Am J Physiol 1996;39:F756–65.
[10] Hull RN, Cherry WR, Weaver GW. The origin and characteristics of a pig kidney cell strain, LLC-PK1. In Vitro 1976;12:670–7.
[11] Handler JS, Perkins FM, Johnson JP. Studies of renal cell function using cell culture techniques. Am J Physiol 1980;238:F1–9.
[12] Crivellato E, Candussio L, Rosati AM, Decorti G, Bartoli Klugmann F, Mallardi F. Kinetics of doxorubicin handling in LLC-PK_1 kidney epithelial cell line is mediated by both vesicle formation and P-glycoprotein drug transport. Histochem J 1999;31:635–43.
[13] Decorti G, Peloso I, Favarin D, Klugmann FB, Candussio L, Crivellato E, Mallardi F, Baldini L. Handling of doxorubicin by the LLC-PK1 kidney epithelial cell line. J Pharmacol Exp Ther 1998;286:525–30.
[14] Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J Immunol Methods 1983;65:55–63.
[15] Bachur NR, Moore AL, Bernstein JG, Liu A. Tissue distribution and disposition of daunomycin (NSC-82151) in mice: Fluorometric and isotopic methods. Cancer Chemother Rep 1970;54:89–94.
[16] Lowry OH, Rosenbrough NJ, Fark AL, Randell LJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265–75.
[17] Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970;227:680–5.
[18] Georges E, Sharom FJ, Ling V. Multidrug resistance and chemosensitization: Therapeutic implications for cancer chemotherapy. Adv Pharmacol 1990;21:185–220.
[19] Flens MJ, Izquierdo MA, Scheffer GL, Fritz JM, Meijer CJ, Scheper RJ, Zaman GJ. Immunochemical detection of the multidrug resistance-associated protein MRP in human multidrug-resistant tumor cells by monoclonal antibodies. Cancer Res 1994;54:4557–63.
[20] Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction. Anal Biochem 1987;162:156–9.
[21] Noonan KE, Beck C, Holzmayer TA, Chin JE, Wunder JS, Andrulis IL, Gazdar AF, Willman CL, Griffith B, Von Hoff DD, Roninson IB. Quantitative analysis of *MDR1* (multidrug resistance) gene expression in human tumors by polymerase chain reaction. Proc Natl Acad Sci USA 1990;87:7160–4.
[22] Rabito CA. Occluding junctions in a renal cell line (LLC-PK_1) with characteristics of proximal tubular cells. Am J Physiol 1986;250:F734–43.
[23] Ernest S, Bello-Reuss E. Expression and function of P-glycoprotein in a mouse kidney cell line. Am J Physiol 1995;38:C323–33.
[24] Lacave R, Ouar Z, Paulais M, Bens M, Ricci S, Cluzeaud F, Vandewalle A. Lysosomotropic agents increase vinblastine efflux from mouse MDR proximal kidney cells exhibiting vectorial drug transport. J Cell Physiol 1999;178:247–57.
[25] Smit JJ, Schinkel AH, Oude Elferink RP, Groen AK, Wagenaar E, van Deemter L, Mol CA, Ottenhoff R, van der Lugt NM, van Roon MA, et al. Homozygous disruption of the murine mdr2 P-glycoprotein gene leads to a complete absence of phospholipid from bile and to liver disease. Cell 1993;75:451–62.
[26] Kim WJ, Kakehi Y, Hirai H, Arao S, Hiai H, Fukumoto M, Yoshida O. Multidrug resistance-associated protein-mediated multidrug resistance-associated modulated by cyclosporin A in a human bladder cancer cell line. Jpn J Cancer Res 1995;86:969–77.
[27] Evers R, Kool M, van Deemter L, Janssen H, Calafat J, Oomen LC, Paulusma CC, Elferink RP, Baas F, Schinkel AH, Borst P. Drug export activity of the human canalicular multispecific organic anion transporter in polarized kidney MDCK cells expressing cMOAT (MRP2) cDNA. J Clin Invest 1998;101:1310–9.
[28] Schaub TP, Kartenbeck J, Konig J, Vogel O, Witzgall R, Kriz W, Keppler D. Expression of the conjugate export pump encoded by the *mrp2* gene in the apical membrane of kidney proximal tubules. J Am Soc Nephrol 1997;8:1213–21.
[29] Peng KC, Cluzeaud F, Bens M, Van Huyen JP, Wioland MA, Lacave R, Vandewalle A. Tissue and cell distribution of the multidrug resistance-associated protein (MRP) in mouse intestine and kidney. J Histochem Cytochem 1999;47:757–67.
[30] Gomez-Morales M, Bustos M, Montes A, Andujar M, Medina-Cano MT, Ramirez C, O'Valle F, Aguilar D, Aneiros J, Garcia del Moral R. Influence of intrarenal deposits of ciclosporin A on acute renal transplant rejection. Nephron 1995;70:402–9.