

Enhanced transport of anticancer agents and leukotriene C₄ by the human canalicular multispecific organic anion transporter (cMOAT/MRP2)

Takeshi Kawabe^{a,*}, Zhe-Sheng Chen^b, Morimasa Wada^a, Takeshi Uchiumi^a, Mayumi Ono^a, Shin-ichi Akiyama^b, Michihiko Kuwano^a

^aDepartment of Biochemistry, Kyushu University School of Medicine, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan

^bDepartment of Cancer Chemotherapy, Institute for Cancer Research, Faculty of Medicine, Kagoshima University, Sakuraga-oka 890-8520, Japan

Received 14 June 1999; received in revised form 10 July 1999

Abstract We established stable human canalicular multispecific organic anion transporter (cMOAT/MRP2) cDNA transfectants, CHO/cMOAT from non-polarized Chinese hamster ovary (CHO)-K1 and LLC/cMOAT from polarized pig kidney epithelial LLC-PK1. Human cMOAT was mainly localized in the plasma membrane of CHO/cMOAT and in the apical membrane of LLC/cMOAT. The ATP-dependent uptake of leukotriene C₄ (LTC₄) into CHO/cMOAT membrane vesicles was enhanced compared with empty vector transfectants. *K_m* values in CHO/cMOAT membrane vesicles were 0.24 μ M for LTC₄ and 175 μ M for ATP. Drug sensitivity to vincristine and cisplatin in human cMOAT cDNA transfectants decreased, but not to etoposide. Cellular accumulation of vincristine and cisplatin in human cMOAT cDNA transfectants decreased, but not of etoposide. The uptake of LTC₄ into CHO/cMOAT membrane vesicles was inhibited by exogenous administration of vincristine or cisplatin, but not that of etoposide. Moreover, this inhibition was more enhanced in the presence of glutathione. These consequences indicate that drug resistance to vincristine or cisplatin appears to be modulated by human cMOAT through transport of the agents, possibly in direct or indirect association with glutathione.

© 1999 Federation of European Biochemical Societies.

Key words: cMOAT; MRP family; ABC transporter; Multidrug resistance; Anticancer agent

1. Introduction

Both P-glycoprotein (Pgp) and multidrug resistance protein 1 (MRP1) belong to the ATP binding cassette (ABC) transporter superfamily, and mediate multidrug resistance through altered membrane transport of various anticancer agents in cancer cells [1–4]. Human canalicular multispecific organic anion transporter (cMOAT/MRP2) also belongs to the ABC transporter superfamily and shows a 49% amino acid identity to human MRP1 [5,6]. Rat [7] and rabbit [8] cMOAT transport a GSH conjugate, leukotriene C₄ (LTC₄), which is a high affinity endogenous substrate for MRP1 [9–12]. This structural and functional similarity between cMOAT and MRP1 suggests that overexpression of cMOAT may confer multidrug resistance. Human cMOAT is substantially overexpressed in several cell lines, and human cMOAT levels correlate with cisplatin resistance in a subset of resistant cell lines [5,6]. A

potent anticancer agent, cisplatin, which forms glutathione (GSH) conjugates, is predicted to be effluxed through the GS-X pump [13,14]. In our previous study, introduction of the human cMOAT antisense cDNA into human hepatic cancer HepG2 cells overexpressing human cMOAT results in increased sensitivity to cisplatin and vincristine, but not to etoposide [15]. In the human cMOAT antisense cDNA transfectants, cellular accumulation of cisplatin and vincristine increases, but cellular accumulation of etoposide does not change [15]. Transport of another anticancer agent, vinblastine, is also altered in polarized Madin-Darby canine kidney cells transfected with human cMOAT cDNA [16]. However, whether human cMOAT itself is involved in the membrane transport of any specific anticancer agent like MRP1 or Pgp remains unclear.

In this study, we established stable human cMOAT cDNA transfectants from non-polarized Chinese hamster ovary (CHO)-K1 and polarized LLC-PK1 cells, and examined if the drug sensitivity and cellular accumulation are altered in the transfectants. We further examined if human cMOAT transports LTC₄ which is a substrate for MRP1 [9–12] by using the membrane vesicles from human cMOAT cDNA transfectants, and if human cMOAT modulates the membrane transport of anticancer agents in association with GSH.

2. Materials and methods

2.1. Drugs and chemicals

Cisplatin was donated by Bristol-Myers Squibb K.K. (Kanagawa, Japan). Vincristine was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Etoposide was obtained from Nippon Kayaku Co. (Tokyo, Japan). Lipofectamine and geneticin (G418) were purchased from Life Technologies, Inc. (Bethesda, MD, USA). [14,15,19,20-³H(N)]-LTC₄ was obtained from DuPont NEN (Boston, MA, USA).

2.2. Construction of human cMOAT expression vector

Previously described cDNA overlapping clones (L3, AL28-33, 4-1, 1-1, AK1-8) [5] were reconstructed into a full-length cDNA, which was inserted into the *NotI* site of the pCI-neo mammalian expression vector (Promega).

2.3. Cell culture and transfection with human cMOAT expression vector

CHO-K1 and LLC-PK1 cells were cultured in Eagle's MEM (Nissui, Tokyo) containing 10% newborn calf serum and Medium 199 (Nissui, Tokyo) containing 10% fetal bovine serum, respectively. Transfection of human cMOAT expression vector was performed using lipofectamine according to the manufacturer's protocol. The cells were incubated in a selection medium containing 800 μ g/ml G418 for 3–4 weeks. The stable transfectants expressing human cMOAT were selected using Western blot analysis among G418-resistant clones.

*Corresponding author. Fax: +81 (92) 642-6203.
E-mail: kawabe17@biochem1.med.kyushu-u.ac.jp

2.4. Production of human cMOAT antibody

Antibody against human cMOAT was elicited by synthetic peptide DYGLISSVEEIPEDAAS that corresponded to the unique amino acid sequence at the linker region of human cMOAT (amino acids 884–900). The amino acid sequence of the linker region was unique when we compared it with the amino acid sequences of the corresponding regions of MRP1 and MRP3-MRP6. The human cMOAT antibody was affinity purified with the glutathione S-transferase human cMOAT fusion proteins. This antibody did not cross-react with Pgp or MRP1 [15]. Double immunofluorescence using this antibody and anti-DPPIV antibody, and confocal laser scanning microscopy, showed localization of human cMOAT in the canalicular membrane domain of normal human hepatocytes but not in patients with Dubin-Johnson syndrome [17].

2.5. Western blot analysis and indirect immunofluorescence

An immunoblot analysis of human cMOAT was made as described by Koike et al. [15]. Aliquots of whole cell lysates (200 µg) were electrophoresed on 6% SDS-polyacrylamide gels and were analyzed by immunoblotting with human cMOAT antibody. Protein-antibody interaction was detected using the enhanced chemiluminescence technique.

Indirect immunofluorescence analysis was performed as described by Koike et al. [18].

2.6. Membrane vesicle preparation and [3 H]LTC₄ uptake in membrane vesicles

Membrane vesicles were prepared by the nitrogen cavitation method as described by Cornwell et al. [19] from CHO/CMV and CHO/cMOAT-1 cells.

LTC₄ uptake in membrane vesicles was measured by a rapid filtration technique essentially as described by Ishikawa et al. [13,20]. Briefly, the standard incubation medium contained membrane vesicles (50 µg of protein), 1.368 nM [3 H]LTC₄, 0.25 M sucrose, 10 mM Tris-HCl, pH 7.4, 10 mM MgCl₂, 4 mM ATP, 10 mM phosphocreatine and 100 µg/ml creatine phosphokinase in a final volume of 50 µl. The reaction was carried out at 37°C. The samples were passed through Millipore filters (GVWP, 0.22 µm pore size) under a light vacuum to measure the trapped [3 H]LTC₄ in the vesicles. The filters were washed and placed in scintillation fluid. The level of radioactivity was measured using a liquid scintillation counter. ATP-dependent accumulation was calculated from the difference in the radioactivity incorporated into the vesicles in the presence or absence of 4 mM ATP.

2.7. Chemosensitivity and cellular accumulation of vincristine, cisplatin and etoposide

A colony formation assay was made as described by Koike et al. [15]. The 50% lethal dose (IC₅₀) for each cell line was calculated from the dose-response curve. The relative resistance of human cMOAT cDNA transfectants was determined by dividing the IC₅₀ value for each transfectant by that of the empty vector transfectant. The accumulation of etoposide, vincristine and cisplatin was determined as described by Koike et al. [15].

3. Results

We established cell lines overexpressing human cMOAT by introducing the human cMOAT expression vector into non-polarized CHO-K1 and polarized LLC-PK1 cells. Western blot analysis with human cMOAT antibody showed the presence of human cMOAT of molecular weight 190–200 kDa in the human hepatic cancer HepG2 cells. Human cMOAT cDNA transfectants (CHO/cMOAT-1, 2 and LLC/cMOAT-1, 2 cells) expressed human cMOAT of molecular weight of 180–190 kDa, while cells transfected with empty vector alone (CHO/CMV and LLC/CMV cells) and parental cells (CHO-K1 and LLC-PK1 cells) did not express human cMOAT (Fig. 1).

The cellular distribution of human cMOAT was determined by indirect immunofluorescence with the human cMOAT antibody (Fig. 2). The photomicrographs of the horizontal

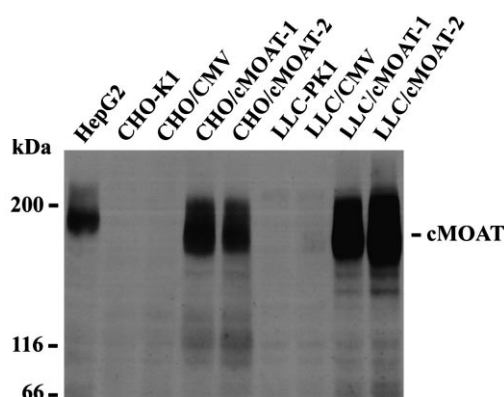


Fig. 1. Expression of human cMOAT in human cMOAT cDNA-transfected CHO-K1 and LLC-PK1 cells by Western blot analysis.

section of CHO/CMV (Fig. 2A), CHO/cMOAT-1 (Fig. 2B) and CHO/cMOAT-2 (Fig. 2C) cells show that the human cMOAT expression was observed in human cMOAT cDNA transfectants, and that human cMOAT was mainly localized in the plasma membrane. The photomicrographs of the top views of LLC/CMV (Fig. 2D), LLC/cMOAT-1 (Fig. 2E) and LLC/cMOAT-2 (Fig. 2F) cells show that the human cMOAT expression was also observed in human cMOAT cDNA transfectants. The photomicrograph of the vertical section of LLC/cMOAT-2 cell layer shows that the human cMOAT expression was mainly observed in the apical plasma membrane (Fig. 2G).

By using the membrane vesicles of human cMOAT cDNA transfectants, we examined if human cMOAT also transports LTC₄ like MRP1. Fig. 3A shows the time course of [3 H]LTC₄ uptake into membrane vesicles from CHO/CMV and CHO/cMOAT-1 cells in the absence or presence of ATP. We found a dramatic difference in LTC₄ uptake between CHO/CMV and CHO/cMOAT-1 vesicles when incubated in the presence of ATP. By contrast, any apparent LTC₄ uptake could not be observed between CHO/CMV and CHO/cMOAT-1 vesicles in the absence of ATP. The uptake was linear with incubation

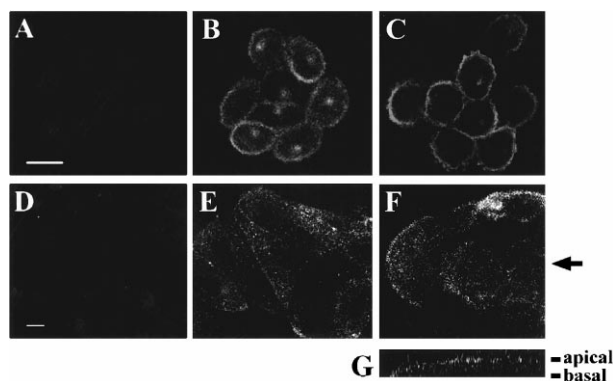


Fig. 2. Immunolocalization of human cMOAT in human cMOAT cDNA-transfected CHO-K1 and LLC-PK1 monolayers by confocal laser scanning microscopy. The panels of CHO/CMV (A), CHO/cMOAT-1 (B) and CHO/cMOAT-2 (C) show horizontal sections of the cell layers. The panels of LLC/CMV (D), LLC/cMOAT-1 (E), LLC/cMOAT-2 (F) show the top views of the monolayers. The vertical section (G) of the cell layer indicated by arrow in panel F shows appearance of human cMOAT in the apical plasma membrane. The positions of apical and basal plasma membrane are indicated on the right of panel G. Bar = 25 µm.

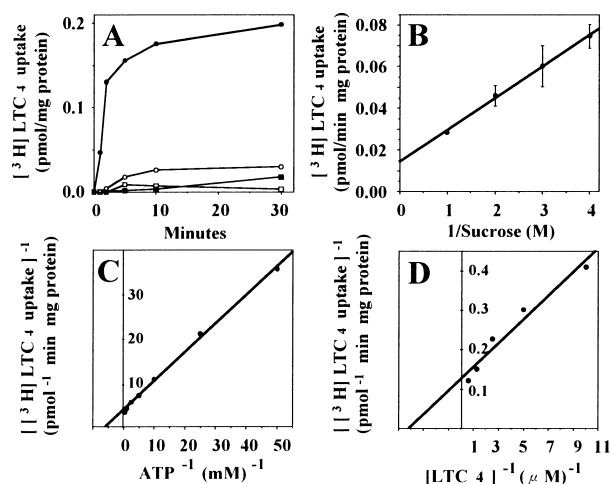


Fig. 3. Transport studies of $[^3\text{H}]\text{LTC}_4$ by membrane vesicles. A: Time course of $[^3\text{H}]\text{LTC}_4$ accumulation in membrane vesicles from CHO/CMV and CHO/cMOAT-1 cells in the presence or absence of 4 mM ATP. CHO/CMV–ATP ($\square$), CHO/CMV+ATP ($\blacksquare$), CHO/cMOAT-1–ATP ($\circ$) and CHO/cMOAT-1+ATP ($\bullet$). B: Effect of osmolarity on the ATP-dependent $[^3\text{H}]\text{LTC}_4$ accumulation in membrane vesicles from CHO/cMOAT-1 cells ($\bullet$). The $[^3\text{H}]\text{LTC}_4$ accumulation was measured at increasing concentrations of sucrose (0.25–1 M) in the presence or absence of 4 mM ATP for 2 min. C: Effect of ATP concentration on the rate of $[^3\text{H}]\text{LTC}_4$ uptake in membrane vesicles from CHO/cMOAT-1 cells ($\bullet$). The $[^3\text{H}]\text{LTC}_4$ uptake was measured at various concentrations of ATP for 2 min. D: Effect of LTC_4 concentration on the rate of $[^3\text{H}]\text{LTC}_4$ uptake in membrane vesicles. Membrane vesicles (25 μg of protein) from CHO/cMOAT-1 cells ($\bullet$) were incubated at various concentrations of LTC_4 in the presence or absence of 4 mM ATP for 2 min. A, C, D: Each point represents the average of two separate experiments; bars, S.E.

time for 2 min (Fig. 3A). To confirm that $[^3\text{H}]\text{LTC}_4$ uptake reflects the transport of substrate into the vesicle lumen rather than the surface or intramembrane binding, we analyzed the osmotic sensitivity of $[^3\text{H}]\text{LTC}_4$ uptake. High osmotic pressure reduces the intravesicular space by shrinking the membrane vesicles, resulting in reduced uptake capacity. Osmotic sensitivity was examined by analyzing ATP-dependent $[^3\text{H}]\text{LTC}_4$ uptake by CHO/cMOAT-1 vesicles incubated in various concentrations of sucrose. The amounts of $[^3\text{H}]\text{LTC}_4$ accumulated in CHO/cMOAT-1 vesicles decreased proportionally to the increasing osmolarity of the extravesicular medium, indicating that a significant amount of $[^3\text{H}]\text{LTC}_4$ was actually transported into the intravesicular lumen of the CHO/cMOAT-1 vesicles (Fig. 3B). We further examined the ATP concentration dependence of $[^3\text{H}]\text{LTC}_4$ uptake. Lineweaver-Burk analysis indicated that $[^3\text{H}]\text{LTC}_4$ uptake was saturable with respect to ATP concentration. Kinetic analysis revealed that the K_m value for ATP in CHO/cMOAT-1 vesicles was 175 μM (Fig. 3C). The LTC_4 concentration dependence of $[^3\text{H}]\text{LTC}_4$ uptake was analyzed. Lineweaver-Burk analysis indicated that $[^3\text{H}]\text{LTC}_4$ uptake was saturable with respect to LTC_4 concentration. Kinetic analysis revealed that the K_m value for LTC_4 in CHO/cMOAT-1 vesicles was 0.24 μM (Fig. 3D).

We examined if the drug sensitivity to vincristine, cisplatin and etoposide is altered in human *cMOAT* cDNA transfectants. Dose-response curves were assayed by colony formation

assay in the presence of various doses of each drug. CHO/cMOAT-1, 2 cells showed about 2-fold higher resistance to vincristine and to cisplatin, respectively (Fig. 4A and B) than CHO/CMV cells. LLC/cMOAT-1, 2 cells showed about 7-fold higher resistance to vincristine (Fig. 4D) and about 2-fold higher resistance to cisplatin (Fig. 4E) than LLC/CMV cells. However, no change appeared in the drug sensitivity to etoposide between empty vector transfectants and human *cMOAT* cDNA transfectants (Fig. 4C and F).

We examined if human *cMOAT* changes cellular accumulation of vincristine, cisplatin and etoposide. Cellular accumulation of $[^3\text{H}]\text{vincristine}$ in both human *cMOAT* cDNA-transfected CHO-K1 (Fig. 5A) and LLC-PK1 (Fig. 5B) cells decreased greatly compared with empty vector transfectants. Cellular accumulation of cisplatin in both human *cMOAT* cDNA transfectants also decreased significantly compared with empty vector transfectants. The decreased cellular levels of $[^3\text{H}]\text{vincristine}$ and cisplatin in both human *cMOAT* cDNA transfectants were about 50% and 80%, respectively, compared with those in empty vector transfectants. However, no

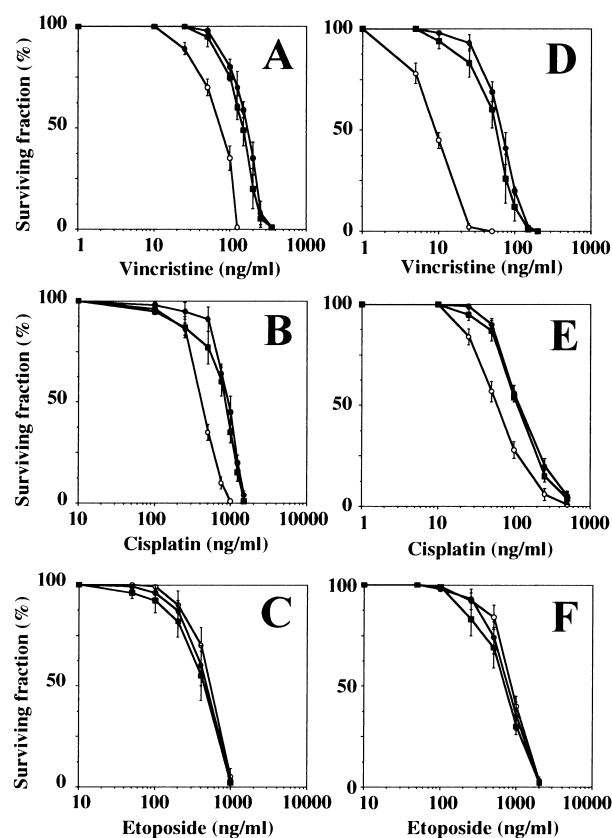


Fig. 4. Comparison of dose-response curves to vincristine (1–350 ng/ml), cisplatin (1–1500 ng/ml) and etoposide (10–2000 ng/ml) in human *cMOAT* cDNA transfectants and empty vector transfectants in CHO-K1 (A, B, C) and LLC-PK1 (D, E, F) cells. IC_{50} values (ng/ml) for vincristine were 78 ± 7 (CHO/CMV) and 9 ± 1 (LLC/CMV); those for cisplatin were 425 ± 5 (CHO/CMV) and 62 ± 4 (LLC/CMV); and those for etoposide were 580 ± 8 (CHO/CMV) and 880 ± 10 (LLC/CMV). IC_{50} values were almost the same between empty vector transfectants and parental cells (data not shown). Vincristine (A, D); cisplatin (B, E); and etoposide (C, F). CHO/CMV and LLC/CMV ($\circ$), CHO/cMOAT-1 and LLC/cMOAT-1 ($\bullet$), CHO/cMOAT-2 and LLC/cMOAT-2 ($\blacksquare$). 100% corresponds to the number of colonies in the absence of any drug. Each point indicates the average of three separate experiments; bars, S.D.

change appeared in the cellular accumulation of [3 H]etoposide between empty vector transfectants and human *cMOAT* cDNA transfectants (Fig. 5A and B).

Loe et al. [13] have reported that exogenous addition of vincristine and vinblastine which are substrates for MRP1 inhibit the MRP1 mediated ATP-dependent transport of LTC₄, and that the inhibition is significantly enhanced by physiological concentration of GSH. We then examined if human *cMOAT* modulates the membrane transport of anticancer agents in association with GSH. We examined the effect of exogenous addition of vincristine, cisplatin and etoposide on the human *cMOAT* mediated ATP-dependent LTC₄ uptake in the absence (Fig. 6A) or presence (Fig. 6B) of 3 mM GSH. The administration of vincristine and cisplatin at 100 μ M significantly blocked the ATP-dependent uptake of LTC₄ in the absence or presence of GSH. The administration of vincristine and cisplatin at 10 μ M also significantly ($P < 0.05$) blocked the ATP-dependent uptake of LTC₄ in the presence of GSH (Fig. 6B). In contrast, there appeared no such inhibition of the ATP-dependent uptake of LTC₄ by exogenous addition of etoposide at 10 and 100 μ M even in the presence of GSH.

4. Discussion

In this study we isolated transfected cell lines expressing human *cMOAT* predominantly on the plasma membrane. The size of the transfected human *cMOAT* protein is smaller than the endogenous human *cMOAT* protein in HepG2 cells. This difference may correspond to a different extent of glycosylation because of a species difference.

Our study revealed that human *cMOAT* had a high affinity K_m value of 0.24 μ M for LTC₄. Human MRP1 also transports LTC₄ and had K_m values of 0.035 μ M to 0.105 μ M for LTC₄ [9,11,12,21]. The K_m values for LTC₄ of human *cMOAT* appear to be about 2-fold to 7-fold higher than those of human MRP1. Rat *cMOAT* shows K_m values of 0.25–0.32 μ M for LTC₄ [22,23], which appear to be comparable to that of human *cMOAT*. Thus, the transport of LTC₄ was also modulated by human *cMOAT* like as MRP1.

This study indicated that human *cMOAT* cDNA transfectants showed about 2-fold to 7-fold higher resistance to vin-

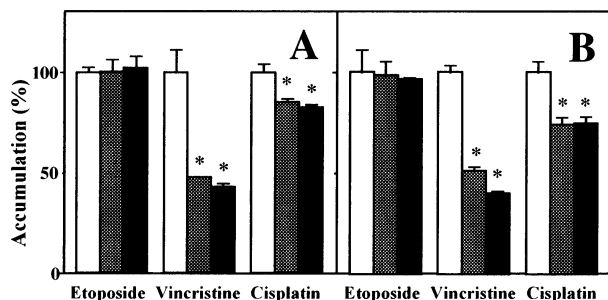


Fig. 5. Comparison of cellular accumulation of [3 H]etoposide, [3 H]vincristine, and cisplatin in CHO/cMOAT transfectants (A) and LLC/cMOAT transfectants (B). A: CHO/CMV ($\square$), CHO/cMOAT-1 (gray box) and CHO/cMOAT-2 ($\blacksquare$) cells. B: LLC/CMV ($\square$), LLC/cMOAT-1 (gray box) and LLC/cMOAT-2 ($\blacksquare$) cells. All results were normalized to cellular protein. 100% corresponds to the accumulation of each drug in each empty vector transfectant. Each value represents the average of three separate experiments; bars, S.D. *, $P < 0.01$ compared with CHO/CMV cells or LLC/CMV cells.

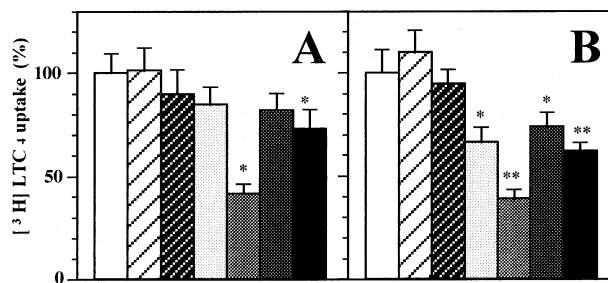


Fig. 6. Effect of vincristine, cisplatin and etoposide on ATP-dependent [3 H]LTC₄ uptake in the absence (A) or presence (B) of 3 mM GSH. Membrane vesicles from CHO/cMOAT-1 cells were incubated with three different anticancer agents at 10 or 100 μ M for 2 min. Control ($\square$); etoposide (10 μ M) (light dashed box); etoposide (100 μ M) (dark dashed box); vincristine (10 μ M) (light gray box); vincristine (100 μ M); cisplatin (10 μ M) (dark gray box); cisplatin (100 μ M) ($\blacksquare$). 100% corresponds to ATP-dependent [3 H]LTC₄ uptake in each control. Bars represent means ($\pm$ S.D.) of triplicate determinations. *, $P < 0.05$ compared with control. **, $P < 0.01$ compared with control.

cristine and about 2-fold higher resistance to cisplatin, but a similar sensitivity to etoposide compared with empty vector transfectants. The growth rate is the same between human *cMOAT* cDNA transfectants and empty vector transfectants (data not shown) and is not likely contributing to the change of resistance observed. In this study we also observed that cellular accumulation of vincristine and cisplatin in human *cMOAT* cDNA transfectants decreased, but not of etoposide. Moreover, the *cMOAT* mediated ATP-dependent LTC₄ uptake by membrane vesicles was inhibited by exogenous addition of vincristine and cisplatin, but not that of etoposide. Although human *cMOAT* is reported not to be expressed on plasma membrane in non-polarized cells transfected with human *cMOAT* cDNA [16], our data clearly show that human *cMOAT* is expressed on plasma membrane in non-polarized CHO-K1 as well as polarized LLC-PK1 cells transfected with human *cMOAT* cDNA. Forced expression of human *cMOAT* on plasma membrane is expected to enhance export of drugs through plasma membrane, resulting in reduction of cellular level of drugs. These consequences suggest that vincristine and cisplatin show some affinity to human *cMOAT*, and that human *cMOAT* confers drug resistance to vincristine and cisplatin by the export of them. Thus, human *cMOAT* might be involved in the membrane transport of some anticancer agents and confer drug resistance similar to MRP1, but with some differences. Drug sensitivity to vincristine and etoposide is markedly altered, but not for cisplatin in *MRP1* cDNA-transfected cells or *mnp*(-/-) cell line [3,4,24]. The transport of etoposide might be rather specifically mediated through MRP1 than through human *cMOAT*. During the preparation of this report, Cui et al. have reported that forced expression of human *cMOAT* confers drug resistance to cisplatin and vincristine [25], consistent with our present study. However, their human *cMOAT* cDNA transfectants are also resistant to etoposide [25]. It remains unclear why drug sensitivity to etoposide is altered in their human *cMOAT* cDNA transfectants, unlike ours. Further study should be required to examine if any difference in experimental assay system, cell lines employed, and also cellular distribution of human *cMOAT* is responsible for the discrepancy.

The human cMOAT mediated LTC₄ uptake by membrane vesicles was more greatly inhibited by each vincristine or cisplatin in the presence of GSH than in the absence of GSH. Loe et al. [12] have reported that GSH significantly enhances the ability of vincristine and vinblastine to inhibit MRP1 mediated ATP-dependent transport of LTC₄, suggesting that either efflux of certain drugs requires activation of MRP1 by GSH or a co-transport mechanism with GSH is involved in the MRP1-dependent drug transport [26]. Such co-transport mechanism might be also partly operated in the human cMOAT-dependent transport of vincristine or cisplatin with GSH.

In conclusion, human cMOAT, a family of MRP, shows a high affinity to LTC₄ like MRP1. Drug resistance to vincristine or cisplatin appears to be modulated by human cMOAT through transport of the agents, possibly in direct or indirect association with GSH.

Acknowledgements: This study was supported by grants from Second-Term Comprehensive Ten-Year Strategy for Cancer Control from the Ministry of Health and Welfare, Japan, and also CREST (Core Research for Evolutional Science and Technology) of Japan Science and Technology Corporation (JST).

References

- [1] Gottesman, M.M. and Pastan, I. (1993) *Annu. Rev. Biochem.* 62, 385–427.
- [2] Germann, U.A. (1996) *Eur. J. Cancer* 324, 927–944.
- [3] Cole, S.P.C., Sparks, K.E., Fraser, K., Loe, D.W., Grant, C.E., Wilson, G.M. and Deeley, R.G. (1994) *Cancer Res.* 54, 5902–5910.
- [4] Loe, D.W., Deeley, R.G. and Cole, S.P.C. (1996) *Eur. J. Cancer* 32A, 945–957.
- [5] Taniguchi, K., Wada, M., Kohno, K., Nakamura, T., Kawabe, T., Kawakami, M., Kagotani, K., Okumura, K., Akiyama, S. and Kuwano, M. (1996) *Cancer Res.* 56, 4124–4129.
- [6] Kool, M., de Hass, M., Scheffer, G.L., Scheper, R.J., van Eijk, M.J.T., Juijn, J.A., Bass, F. and Borst, P. (1997) *Cancer Res.* 57, 3537–3547.
- [7] Ito, K., Suzuki, H., Hirohashi, T., Kume, K., Shimizu, T. and Sugiyama, Y. (1998) *J. Biol. Chem.* 273, 1684–1688.
- [8] van Aubel, R.A., van Kuijk, M.A., Koenderink, J.B., Deen, P.M., van Os, C.H. and Russel, F.G. (1998) *Mol. Pharmacol.* 53, 1062–1067.
- [9] Leier, I., Jedlitschky, G., Buchholz, U., Cole, S.P.C., Deeley, R.G. and Keppler, D. (1994) *J. Biol. Chem.* 269, 27807–27810.
- [10] Müller, M., Meijer, C., Zaman, G.J.R., Borst, P., Scheper, R.J., Mulder, N.H., de Vries, E.G.E. and Jansen, P.L.M. (1994) *Proc. Natl. Acad. Sci. USA* 91, 13033–13037.
- [11] Jedlitschky, G., Leier, I., Buchholz, U., Barnouin, K., Kurz, G. and Keppler, D. (1996) *Cancer Res.* 56, 988–994.
- [12] Loe, D.W., Almquist, K.C., Deeley, R.G. and Cole, S.P.C. (1996) *J. Biol. Chem.* 271, 9675–9682.
- [13] Ishikawa, T. and Ali-Osman, F. (1993) *J. Biol. Chem.* 268, 20116–20125.
- [14] Ishikawa, T., Wright, C.D. and Ishizuka, H. (1994) *J. Biol. Chem.* 269, 29085–29093.
- [15] Koike, K., Kawabe, T., Tanaka, T., Toh, S., Uchiumi, T., Wada, M., Akiyama, S., Ono, M. and Kuwano, M. (1997) *Cancer Res.* 57, 5475–5479.
- [16] Evers, R., Kool, M., van Deemter, L., Janssen, H., Calafat, J., Oomen, L.C.J.M., Paulusma, C.C., Oude Elferink, R.P.J., Bass, F., Schinkel, A.H. and Borst, P. (1998) *J. Clin. Invest.* 101, 1310–1319.
- [17] Toh, S., Wada, M., Uchiumi, T., Inokuchi, A., Makino, Y., Horie, H., Adachi, Y., Sakisaka, S. and Kuwano, M. (1999) *Am. J. Hum. Genet.* 64, 739–746.
- [18] Koike, K., Uchiumi, T., Ohga, T., Toh, S., Wada, M., Kohno, K. and Kuwano, M. (1997) *FEBS Lett.* 417, 390–394.
- [19] Cornwell, M.M., Gottesman, M.M. and Pastan, I.H. (1986) *J. Biol. Chem.* 261, 7921–7928.
- [20] Ishikawa, T. (1989) *J. Biol. Chem.* 264, 17347–17348.
- [21] Paul, S., Breuninger, L.M., Tew, K.D., Shen, H. and Kruh, G.D. (1996) *Proc. Natl. Acad. Sci. USA* 93, 6929–6934.
- [22] Ishikawa, T., Müller, M., Klünemann, C., Schaub, T. and Keppler, D. (1990) *J. Biol. Chem.* 265, 19279–19286.
- [23] Böme, M., Büchler, M., Müller, M. and Keppler, D. (1993) *FEBS Lett.* 333, 193–196.
- [24] Rappa, G., Lorico, A., Flavell, R.A. and Sartorelli, A.C. (1997) *Cancer Res.* 57, 5232–5237.
- [25] Cui, Y., König, J., Buchholz, U., Spring, H., Leier, I. and Keppler, D. (1999) *Mol. Pharmacol.* 55, 929–937.
- [26] Loe, D.W., Deeley, R.G. and Cole, S.P.C. (1998) *Cancer Res.* 58, 5130–5136.