EFFECT OF PENTACHLOROBIPHENYL ON ACCUMULATION AND TRANSEPITHELIAL TRANSPORT OF VINBLASTINE IN LLC-PK1 EXPRESSING HUMAN P-GLYCOPROTEIN

Shigemi Sasawatari[1], Hiroshi Fujise[1,2], Teruo Ikeda[2], and Kazumitsu Ueda[3]

[1]Laboratory of Pathobiochemistry, School of Veterinary Medicine, [2]High-Tech Research Center, Institute of Biosciences, and Azabu University, Sagamihara, Kanagawa, 229-8501
[3]Laboratory of Biochemistry, Division of Applied Life Science, Graduate School of Agriculture, Kyoto University, Kyoto 606-01, Japan

Introduction

3, 3', 4, 4', 5-Pentachlorobiphenyl (PeCB) is an isoform of coplanar polychloro-biphenyls (Co-PCBs). Co-PCBs are environmental pollutants which they may exert adverse effects even at very low concentrations[1, 2]. In particular, perinatal exposure to these chemicals reportedly disturbed the normal development of both fetus and neonate, and resulted in malformations and/or cancer[3, 4]. Soil, river, ocean and foods have been extensively polluted with these chemicals[5]. Thus, these chemicals might be highly accumulated in human and animals, especially in their lipid rich organs[5, 6].

In our experiment to search for p-glycoprotein which transport Co-PCBs, we found the effect of Co-PCBs on drug transport and accumulation mediated by p-glycoprotein. P-glycoprotein is a drug transport pump in cell membranes[7, 8]; it has the important role of extruding metabolites and toxic chemicals from epithelial cells. Here, we report the effect of PeCB on the accumulation and transepithelial transport of vinblastine in porcine kidney cell line, and its transformant cells expressing human p-glycoprotein..

Methods and Materials

Cells: Porcine kidney cells (LLC-PK1) and its transformant cells expressing human p-glycoprotein (LLC-COL) were employed. LLC-COL was prepared by transfection with human *MDR1* gene[9]. The cells were maintained in medium 199 supplemented with 10% fetal calf serum and 150μ M colchicine in 5% CO_2 at 37°C.

Cellular accumulation of vinblastine and its inhibition: For determination of cellular accumulation of the drugs, a coverslip and 24-well multi dish (Nalgen Nunc International) were used as reported elsewhere[10, 11]. The cells were seeded at 5 x 10^6/well in the medium, and incubated in 5% CO_2 at 37° C. After 6 days incubation, the medium was replaced with a fresh medium without colchicine, and they were incubated for 6 hr. Then, the medium was replaced with 750μ l fresh medium containing 11 nM [^{3}H]-vinblastine (5.16 kBq/ml) and with or without cyclosporinA or PeCB. After incubation for 1, 2, and 3 hr, the coverslip was removed, the cells were washed 3 times with PBS, lysed, and then the

radioactivity was measured in liquid scintillation counter. The accumulation of vinblastine in the cells was expressed as pmol/well.

Transepithelial transport and its inhibition: Transepithelial transports, basal-to-apical and apical-to-basal, were measured using a bottom-filtered well (Transwell, 3402, Coaster, Cambridge, MA) as reported elsewhere[8]. The cells were seeded on the bottom-filtered well the same as in the accumulation experiment. After 6 days incubation, the medium in either basal or apical side of the monolayers was replaced with 750μ l fresh medium containing 11 nM [^{3}H]-vinblastine (5.16 kBq/ml), 43.2μ g/ml [^{14}C]-inulin (4.0 kBq/ml) and with or without cyclosporin A or PeCB. An aliquot (25 μ l) of the trance side medium was taken up to 3 hr, and its radioactivity was measured by liquid scintillation counter. The transepithelial transport was indicated as percent of the whole radioactivity. The paracellular fluxes were monitored by measuring the appearance of inulin in the other side, and it was less than 5% in 3 hr as reported earlier.

Results and Discussion

Fig. 1 showed the accumulation of vinblastine, and the effects of cyclosporin A and PeCB on the accumulation in LLC-PK1 and LLC-COL. Intracellular accumulation of vinblastine was 0.21 and 0.018 mmol/well at 3 hr incubation in LLC-PK1 and LLC-COL, respectively. Thus, the accumulation of vinblastine was greatly decreased in LLC-COL compared to LLC-PK1 as reported previously[10, 11]. Increases of cellular accumulation of vinblastine were detected when cyclosporin A and PeCB were added in the medium in both cells. In Fig. 2, the relative effects of cyclosporin A and PeCB on the accumulation were compared. In LLC-COL, the accumulations were increased 8- and 3-fold by adding cyclosporin A and PeCB, respectively. Thus, PeCB might inhibit the extrusion of vinblastine by p-glycoprotein the same as in cyclosporin A. Not only in LLC-COL but also in LLC-PK1 the effects were detected, even the magnitude was different between the two cells. There was probably a endogenous drug extrusion system(s) in the wild type cells which was also inhibited by cyclosporin A and PeCB.

Fig. 3 showed the effect of cyclosporin A and PeCB on the transepithelial transport of vinblastine in LLC-COL. The net basal-to-apical transports of vinblastine were greatly increased in LLC-COL compared to LLC-PK1 as reported previously[10, 11]. By cyclosporin A and PeCB, the net basal-to-apical transports of vinblastine were increased by 40 and 54%, respectively. Thus, the transepithelial transport of vinblastine was inhibited by PeCB the same as for cyclosporin A.

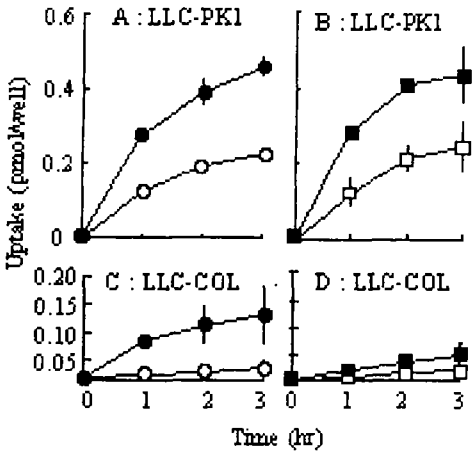

Fig. 1. Uptake of vinblastine as a function of time without (open symbols) or with cyclosporin A (A, C, filled circles) and PeCB (B, D, filled squares) in LLC-PK1 (A, B) and LLC-COL (C, D).

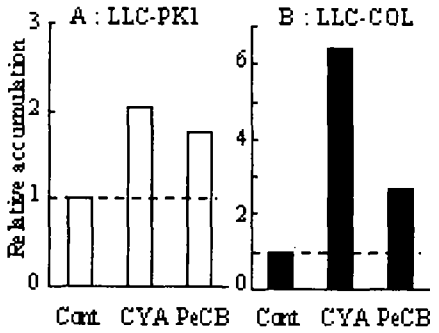

Fig. 2. Comparison of effects of cyclosporin A (CYA) and PeCB on the relative accumulation (with/ without chemicals) of vinblastine at 3 hr incubation in LLC-PK1 (A) and LLC-COL (B). Cont, control.

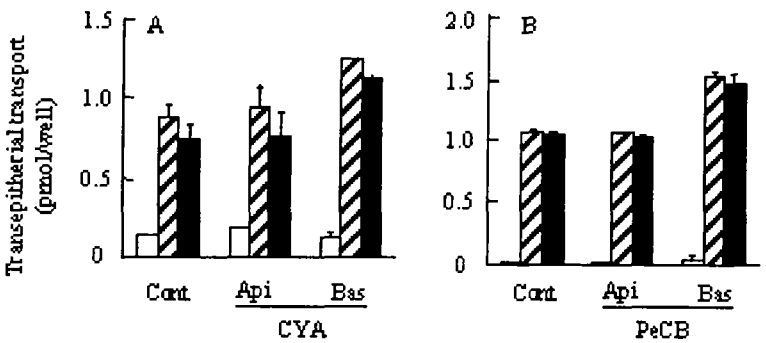

Fig. 3. Effect of cyclosporin A (A, CYA) and PeCB (B) on the transepithelial transport of vinblastine in LLC-COL. Cont, control; Api, chemicals in apical medium; Bas, chemicals in basal medium; open columns, apical-to-basal transport; hatched columns, basal-to-apical transport; filled columns, net basal-to-apical transport.

PeCB inhibited the accumulation and transepithelial transport of vinblastine the same as cyclosporin A. PeCB and the other Co-PCBs were highly lipophilic and accumulated in animal and human organs[5, 6]. Co-PCBs may inhibit the function of p-glycoprotein in many organs as shown in this experiment. The effects of Co-PCBs on p-glycoprotein have to be considered as the cause of the adverse effect of these chemicals.

Acknowledgments

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