TRANSEPITHELIAL TRANSPORT AND CELLULAR ACCUMULATION OF Co-PCBs IN PORCINE KIDNEY CELL LINE, LLC-PK1, AND ITS TRANSFORMANT CELLS TRANSFECTED WITH HUMAN MULTI-DRUG RESISTANT (MDR) GENE

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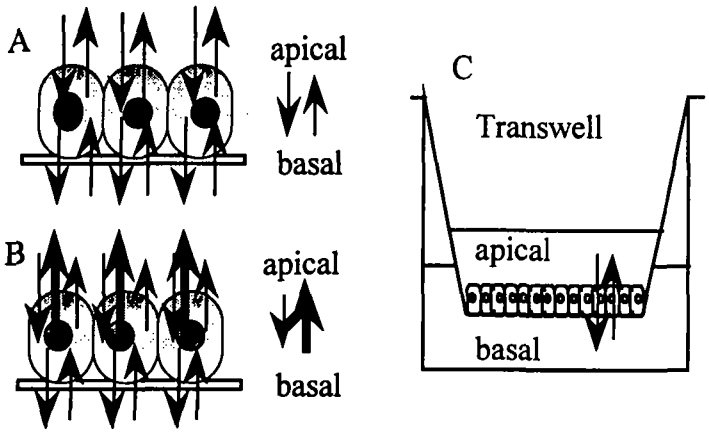
Introduction

Coplanar polychlorobiphenyl (Co-PCBs) are hardly metabolized by cytochrome P450 (CYP), and their lipophilic characteristics cause them to remain in the lipid tissues. In the animal body, these xenobiotics are firstly oxidized with CYP, then conjugated with glucuronic acid and the like. For the extrusion system, drug transport pumps exist in the intestinal tract, bile duct, urinary tubles and other organs[1]. The pumps are able to extrude not only water-soluble metabolites but also lipophilic chemicals. P-glycoprotein and MRP were among the pumps found[2,3]. P-glycoprotein is the protein responsible for multi-drug resistant (MDR), which is encoded MDR1 gene[4]. P-glycoprotein is capable of transporting lipophilic chemicals and a variety of structurally unrelated chemicals[5]. Thus, there is a possibility that Co-PCBs could be the substrate.

In the present report, transepithelial transport of Co-PCBs was measured in monolayer cells; so net basal-to-apical transport was measurable. For the monolayer cells, the porcine kidney epithelial cell line, LLC-PK1, and its transformant cells transfected with human MDR1 gene were used. As the representative of Co-PCBs, an isoform of the chemicals, 3, 3', 4, 4' tetrachlorobiphenyl (TeCB), was employed. Then, transepithelial transport and cellular accumulation of TeCB were examined and compared with that of an anticancer drug, vinblastine.

Methods and Materials

A wild type cell line derived from porcine kidney proximal tubules, LLC-PK1, and the transformant cells, LLC-GA5-COL300 (LLC-COL3), which expresses human P-glycoprotein on apical site because of transfection of human MDR1 cDNA, were used for transport and accumulation experiments[6]. LLC-PK1 was maintained in medium 199 supplemented with 10% fetal calf serum in an atmosphere of 5% CO_2 at 37°C, and LLC-COL3 were maintained in the same medium with 300 ng/ml colchicine.



Fig, 1. Scheme illustrating the transports of substrates at apical and basal membranes in LLC-PCK1 (A) and LLC-COL 3 (B), and the transports across monolayer cells in Transwells(C).

Transepithelial transports, basal-to-apical and apical-to-basal, were measured using a bottom-filtered well (Transwell, 3402, Coaster)[6] as shown in Fig. 1. LLC-PK1 and LLC-COL3 were seeded on the bottom-filtered well in the same medium as the maintenance culture. After 6 days incubation, the medium was replaced with a fresh one without colchicine, and they were incubated for 6 hr. Then, the medium in either the basal or apical side of the monolayers was replaced with 750 µl fresh medium containing 11 nM [^{3}H]-vinblastine (5.16 kBq/ml) or 11 nM (or 400 nM) [^{14}C]-TeCB (1.54 kBq/ml), with 6.89µg/ml [^{3}H]-inulin (3.7 kBq/ml) or 43.2µg/ml [^{14}C]-inulin (4.0 kBq/ml), but without colchicine. An aliquot (25 µl) of the trance side medium was taken at 1, 2 and 3 hr, and its radioactivity was measured by liquid scintillation counter. The transepithelial transport was indicated as a 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[7].

For determination of cellular accumulation of the chemicals, a coverslip and 24 well multi dish (Nalge Nunc International) were used. The cells were seeded on the coverslip in the well, and incubated in 5% CO_2 at 37°C. After 6 days, 750 µl incubation medium, which component was same as the transport experiment, was added into the well. After incubation for 1, 2, and 3 hr, the coverslip was removed, the cells were washed 3 times with PBS, then lysed, and the radioactivity was measured. The accumulation of the chemicals in the cells was expressed as pmol/well.

Results and Discussion

The transepithethial transport of TeCB and vinblastine as a function of time were shown in Fig. 2. The transport of vinblastine in LLC-PK1 was measured for both direction, 6.2%/3 hr for basal-to-apical and 2.8%/3 hr for apical-to-basal, and the net basal-to-apical transport was 3.4%/ 3 hr (Fig. 2 A). In LLC-COL3, the transport of basal-to-apical was increased and that of apical-to-basal was

decreased, thus increasing the net basal-to-apical transport to 7.5%/3 hr (Fig. 2 B). The net basal-to-apical transport through the monolayer of LLC-COL3, which expressed with human P-glycoprotein, was 2-fold greater than in LLC-PK1 as reported earlier[8].

The transport of apical-to-basal could be considered as a diffusion transport, so the diffusion transport of TeCB was detectable and the values was about one-half of vinblastine. In TeCB, however, the transepithelial transport of TeCB in LLC-PK1 and LLC-COL3 were not different in either direction (Fig. 2 C, D). Thus, the net basal-to-apical transport was not detected in both cells.

The cellular accumulation of the chemicals in the coverslip as a function of time was shown in Fig. 3. The accumulation of vinblastine was very low, 0.1%/well/3 hr, in LLC-COL3, though that in

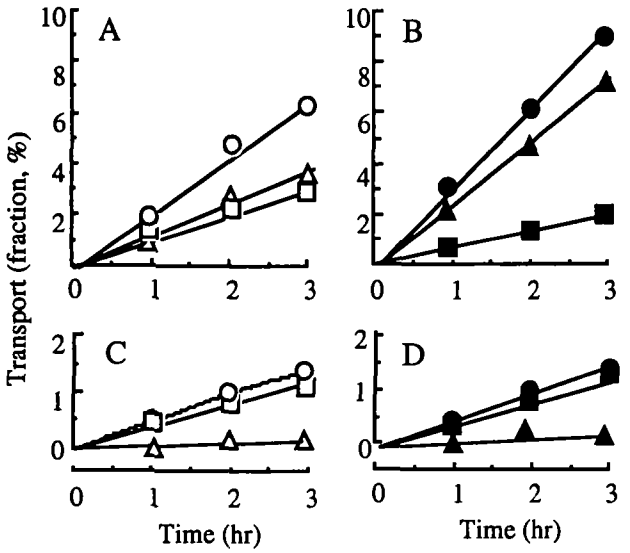


Fig. 2. Transport of vinblastine (A ,B) and TeCB (C , D) across monolayer cells in LLC-PK1 (A, C, open symbols) and LLC-COL 3 (B ,D, filled symbols). Circles, basal-to-apical; squares, apical-to-basal; triangles, net basal-to-apical. Values are means of triplicate determination.

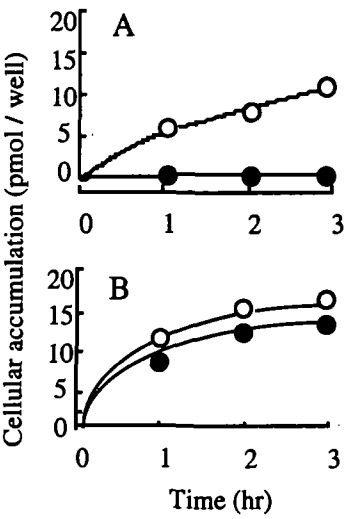


Fig. 3. Cellular accumulation of vinblastine (A) and TeCB (B) in LLC-PK1 (open circles) and LLC-COL 3 (filld circles). Values are means of triplicate determination.

LLC-PK1 was high, 2.0%/well/3 hr; 0.6 pmol/well/3 hr in the former cells and 10.8 pmol/well/3 hr in the latter cells. Therefore, LLC-COL3 could exclude 17-fold more vinblastine to the apical side than in LLC-PK1. The accumulation of TeCB clearly detected both in LLC-PK1 and LLC-COL3, was 5.7 and 4.8 %/well/3 hr, respectively; 16.5 pmol/well/3 hr in the former cells and 14.7 pmol/well/3 hr in the latter. Thus, there is no difference in the accumulation between the two cells. The correlation between the net basal-to-apical transport and the low accumulation in vinblastine is comparable to earlier report[9]; the higher the extrusion activity, the lower the intracellular accumulation.

TeCB is a very lipophilic chemical, so it may easily enter the cells or their membranes. Thus, the cellular accumulation for TeCB might be higher than for vinblastine. TeCB was not transported by wild type human P-glycoprotein. However, there is a possibility that some mutant P-glycoprotein may transport TeCB. An amino acid substitution in human P-glycoprotein altered the affinity to the substrates and the viability to drugs[10,11]. A mutant or isoform of P-glycoprotein may therefore exert the ability for transport of a chemical which is not the substrate of a wild type human P-glycoprotein. There is a possibility that TeCB could be a transport substrate for some mutant or isoform of P-glycoprotein.

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