3
ATP-dependent glutathione conjugate transport in HL60 cells overexpressing the multidrug resistance associated protein (MRP)

G Jedlitschky,[1] I Leier,[1] U Buchholz,[1] K Barnouin,[1] M Center[2] and D Keppler[2]

[1]*Division of Tumor Biochemistry, Deutsches Krebsforschungszentrum, 69120 Heidelberg, Germany.*
[2]*Division of Biology, Kansas State University, Manhattan, KS, USA.*

ATP-dependent transport of the glutathione conjugate leukotriene C_4 (LTC_4) was studied in membrane vesicles from the human leukemia cell line HL60 and a subline isolated for resistance to adriamycin (HL60/ADR) which was shown to overexpress a 190 kDa glycoprotein encoded by the MRP gene. The function of this ATP-binding protein, which has a limited sequence similarity with P-glycoproteins, is so far unknown. The rate of ATP-dependent LTC_4 transport observed in membrane vesicles prepared from the HL60/ADR cells was more than 25-fold higher than in membrane vesicles from parent HL60 cells (25 pmol × mg protein^{-1} × min^{-1} versus < 1 pmol × mg protein^{-1} × min^{-1}). In photoaffinity labeling studies with these membranes a LTC_4-binding protein of about 190 kDa was detected only in the HL60/ADR membranes. The quinoline-based leukotriene receptor antagonist MK 571 completely inhibited the ATP-dependent LTC_4 transport by HL60/ADR membrane vesicles at a concentration of 5 μM. The [^{3}H]LTC_4 labeling of the 190 kDa glycoprotein was also competed for by this transport inhibitor. These data indicate that P-gp-independent, p190-mediated multidrug resistance is associated with an enhanced ATP-dependent transport of glutathione conjugates.

4
Characterization of the ATP-dependent export carrier for leukotriene C_4 and related conjugates in mastocytoma cells

I Leier, G Jedlitschky, U Buchholz and D Keppler

Division of Tumor Biochemistry, Deutsches Krebsforschungszentrum, 69120 Heidelberg, Germany.

Glutathione conjugation and subsequent ATP-dependent export during the biosynthesis of leukotriene C_4 (LTC_4) represent a pathway of metabolism and transport which is analogous to the inactivation and export of certain anti-cancer drugs. This transport system for LTC_4 and related conjugates was characterized using plasma membrane vesicles from murine mastocytoma cells. Selective inhibition of this LTC_4 export can be useful for characterization of the transport system and as a pharmacological approach to interfere with the export of conjugates, possibly related to certain non-P-glycoprotein mediated multidrug resistance phenomena. The effect was therefore studied of the following compounds on the ATP-dependent LTC_4 transport: the cysteinyl leukotriene analog MK 571 inhibited LTC_4 export with an IC_{50} value of 1 μM. Cyclosporin A interfered with this process with half maximal inhibition at 3 μM. The non-immunosuppressive cyclosporin analog PSC 833 was a much less effective inhibitor of LTC_4 transport (IC_{50} 21 μm). Using direct photoaffinity labeling with tritium-labeled LTC_4 as the photolabile ligand a predominant LTC_4-binding glycoprotein with a molecular mass of 190 kDa was identified. Specific labeling of a 190 kDa membrane glycoprotein by the glutathione conjugate LTC_4, which is competed for by a potent inhibitor of the ATP-dependent LTC_4 export carrier, pinpoints its involvement in the ATP-dependent transport of LTC_4 and related conjugates.

5
Neutralization of the biological effects of P-glycoprotein with the beta-amyloid polypeptide

Anwar A Hakim

Cellular and Molecular Biology, 180 Longwood Drive, PO Box 984, Kankakee, IL 60901, USA.

Multidrug resistance (MDR) results in humans from increased expression of MDR genes that encode the membrane P-glycoproteins (P-gp), an efflux pump which maintains intracellular concentrations of catabolites as well as chemotherapeutic agents at non-toxic levels. The P-gp contain two ATP-binding sites and binds to anti-neoplastic drugs causing their efflux from altered tumor cell membrane microfilament function (MF) and intracellular Ca^{2+} levels. By competitive inhibition, cytochalasin B and calcium channel blockers (CCB) inhibit the MF and P-gp blocking of the efflux from tumor cells. The beta-amyloid protein (AP), a polypeptide, is a major peptide and component of senile plaques which is composed of AP and neurofibrillary tangles (NFT) deposited by neural cells and fibroblasts of patients with Alzheimer disease (AD) in culture. AP-inhibits NF and modifies intracellular Ca^{2+}levels. The present study reports on the effect of AP and P-gp in MDR cells. The K562/ADM, X562/R7, 388, tamoxifen (T)-sensitive (TS5-21) and T-resistant (TR-5-23) and MCF-7 cell lines were maintained in RPMI 1640 supplemented with 10% FCS, 6 mM L-glutamine, 0.112% $NaHCO_3$ 20 mM HEPES, 250 mU/ml human insulin and gentamicin 20 μg/ml at 37°C in a humidified chamber containing 5% CO_2.[1–5] TR5-23 cells transfected with the AP gene followed by *in vitro* cultivation reverted to TS5-21, while when transfected with MDR1, CDNA, TS5-21 acquired resistance to T. During *in vitro* culture, MCF-7 human breast carcinoma ER+ cell line