Preclinical studies: biology

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Modulation by GTP/ATP ratio of the phosphorylation level of P-glycoprotein in plasma membrane vesicles from KB-V-1 multidrug resistant cells

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P-glycoprotein (P-gp), often encountered in pleiotropic resistance towards numerous chemotherapeutic agents, is known to function as an ATP-dependent efflux pump and to be phosphorylable *in vitro* by [γ^{32}P]ATP. Tryptic peptide mappings of *in vivo* labeled cells and of *in vitro* labeled plasma membrane vesicles suggest relatively few sites of phosphorylation, mainly on Ser residues. Various phosphorylation studies have attributed P-gp phosphorylation to several Ser/Thr protein kinases such as PKC, PKA and PKP. The implication of phosphoser residues in the linker region has been described. The functional significance of the phosphorylation/dephosphorylation process remains to be defined. It has previously been shown that GTP in place of ATP is also capable of supporting the transport of [^{3}H]vinblastine by P-gp in plasma membrane vesicles KB-VI cells. P-gp from the same transporting vesicles was also phosphorylated by [γ^{32}P]GTP *in vitro*, though to a lower extent than observed with [γ^{32}P]ATP. It has also been observed that the P-gp phosphorylation showed different behavior according to the [γ^{32}P]-nucleotide used for the labeling. With [γ^{32}P]ATP the expected decrease of labeling of P-gp was observed with increasing concentrations of cold ATP, while surprisingly the radiolabeling by [γ^{32}P]GTP was increased by increasing concentrations of cold GTP added. Moreover, increased concentrations of cold GTP strongly stimulated the phosphorylation by [γ^{32}P]ATP of the transporter, the level of phosphorylation depending on the GTP/ATP ratio. Similar modulation of the phosphorylation level of other plasma membrane proteins by the GTP/ATP ratio was also observed. Use of GTP analogs such as GMPPNP, GMPCPP and GMPPCP showed that the hydrolysis of GTP was required for the enhancement of P-gp labeling by [γ^{32}P]ATP. In this process the intervention of either a NDP-kinase or of casein kinase II was excluded. Both ATP or GTP modulated [γ^{32}P]ATP-labeling of P-gp required either Mg^{2+} or Mn^{2+}. Inability of specific inhibitors of PKA, PKC and cGMP-dependent protein kinase to affect the phosphorylation level of P-gp excluded the participation of these endogenous kinases under the conditions of the experiment. Comparison of the tryptic maps of P-gp phosphorylated peptides obtained after *in vitro* labeling either with ([γ^{32}P]ATP + cold ATP) or with ([γ^{32}P]ATP + cold GTP) showed a higher incorporation of label in one of the phosphopeptide spots, indicating that addition of GTP enables labeling of additional sites. These results suggest the superposing of a GTP regulation via GTP/ATP stoichiometry on the already complex P-gp phosphorylation process, probably implicating several protein kinases. This GTP mediated modulation of P-gp phosphorylation may occur as a response to the structural requirements of P-gp to achieve an appropriate phosphorylation level enabling recognition and efflux of unrelated chemical structures of the transported agents.

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Altered pharmacokinetics of vinblastine in mice with a homozygous disruption of the MDR1a P-glycoprotein gene

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The development of new and more effective reversal agents for circumvention of P-glycoprotein (P-gp) mediated multidrug resistance is in full swing, and various candidates are tested in (pre-)clinical investigations. The consequences of the use of such agents for the protective function of P-gp in normal tissues against cytotoxic drug induced toxicity is unclear. By standard knock-out technology mouse strains deficient for the MDR1a or MDR1b P-gp gene have recently been generated in the institute. By using a sensitive and selective high-performance liquid chromatographic procedure, the institute has extensively investigated the tissue distribution profile of vinblastine (VBL) in the MDR1a knock-out mice and their wild-type littermates. The absence of MDR1a P-gp had a clear effect on both the drug distribution and the retention of drug in several tissues; 4 h after the administration of a moderate dose (1 mg/kg) of VBL the drug levels in the intestine, heart, muscle and brain were respectively 2.9-, 3.4-, 6.7- and 22-fold higher, whereas the concurrent plasma level was 2-fold higher. Although 4 h after the administration of a relatively toxic dose of 6 mg/kg of VBL the differences were less pronounced, probably because of a saturation of P-gp activity, a 3- and 12-fold higher level was still found in the intestine and brain, respectively. Furthermore, profound differences in drug retention were seen in the brain and the heart which displayed respectively 23- and 14-fold higher VBL levels 24 h after drug administration. In conclusion, based on these findings, modulating agents may lead to increased and additional cytotoxic drug induced side effects. In particular, a combination with known cardiotoxic drugs like anthracyclines may lead to major clinical complications.