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Measurement of polymorphic P-glycoprotein activity in cell cultures: a review.

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Introduction: The P-glycoprotein (P-gp) is an efflux pump widely expressed in the organism that exports xenobiotic compounds out of the tissue where it is expressed. It plays a central role in the Blood-Brain Barrier permeability, being responsible of Central Nervous System side-effects or ineffectiveness of many drugs. Several single-nucleotide polymorphisms (SNPs) in ABCB1 (the gene encoding for P-gp) have been identified. The most relevant ones, C3435T, C2677T/A and C1236T have been associated with variable pharmacokinetic parameters in healthy volunteers that received single oral doses of antidepressants and antipsychotics. There is no consensus regarding the in vivo effect they have. Here, we compile relevant information in order to simplify the understanding of materials, methods and cell lines classically used to assess polymorphic P-gp activity in in vitro cell culture models.

Methods: A comprehensive research of the studies performed in this regard has been accomplished. More than 389 articles have been reviewed, corresponding to the topics “ABCB1 polymorphisms”, “assessment of P-gp function” and “P-gp expression in cell cultures” published in PubMed Search Engine.

Results: Twenty-four articles have been summarised and classified. Site-directed mutagenesis has been acknowledged as a convenient approach to obtain cell lines expressing mutant P-gp. Ten different techniques have been identified as key in the assessment of P-gp function: Transfection; Western Blot; Flow Cytometry; Transcriptional Analysis; TEER measurements; Calcein-AM, Rhodamine-123 or Radioactivity based accumulation and transport assays; Transwell® inserts; MTT viability assays.

Conclusion: Site-directed mutagenesis performed in plasmids that contain wild-type ABCB1, followed by transfection of the plasmid into cells (HeLa, Caco-2 cells) may lead to cell lines expressing P-gp with the SNPs of interest (C3435T, C2677T/A and C1236T). Assessment of P-gp function may be accomplished by Calcein-AM or Rhodamine-123 accumulation assays. The actual effect of these SNPs in P-gp on antidepressants or antipsychotics efflux through membranes could be assessed by Transwell® insert transport assays.

Keywords: ABCB1, P-glycoprotein, P-gp, cell cultures, Transwell, Transfection, site-directed mutagenesis, antidepressants, antipsychotics.
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