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DRUG DISCOVERY TODAY TECHNOLOGIES
Transporter assays

In vitro methods in drug transporter interaction assessment

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Drug transporter proteins recruit to pharmacological barrier tissues and profoundly affect the ADME properties of a large number of drugs. *In vitro* assays optimized for drug transporters have grown into routine tools in the determination of molecular level interactions as well as prediction of barrier penetration and system level pharmacokinetics. Regulatory position mandates increasing interest in the application of these assays during drug development.

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Introduction

Drug transporters are multispecific transmembrane proteins that facilitate the membrane passage of a large number of drugs [1]. Drug transporters have a distinct expression pattern in the human body lining pharmacological barrier tissues, most importantly the small intestinal epithelium [2], the endothelial cells in the blood–brain barrier [3], the epithelium of the proximal tubule cells in the kidney [4], and hepatocytes in the liver [5] (Fig. 1).

The potential impact of drug transporters on general pharmacokinetics of drugs is well established [6,7], and regulatory bodies recently began to request transporter interaction data, based on physicochemical properties, expected comedication and available *in vivo* data [8]. In the recent years, the Food and Drug Administration as well as the European Medicines Agency issued guidelines (FDA Guidance for Industry: Drug Interaction Studies – Study Design, Data Analysis, Implications for Dosing, and Labeling Recommendations: http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM292362.pdf; EMA Guideline on the Investigation of Drug Interactions: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2012/07/WC500129606.pdf) that explicitly list transporters considered to be the most relevant, as well as outline preferred methods for characterization. The cornerstone of these guidelines is a set of cut-off values that trigger *in vivo* or clinical transporter mediated drug-drug interaction studies (Fig. 2).

Driven mainly by the needs of the industry, *in vitro* methods that assess transporter interactions have matured into routine tools in the past two decades and are widely applied to predict *in vivo* and clinical phenomena as well as to characterize interactions on a molecular level. In this paper the four predominantly used *in vitro* methods are discussed: vesicular transport, ATPase, cellular uptake and monolayer assay.

Assays with membrane preparations

The substrate binding sites of efflux transporters face the cytoplasm and/or cytoplasmic leaflet, and are freely accessible to high passive permeability compounds only [9]. Thus in a more physiological setup, such as an assay applying cells, the difference between nominal and effective concentration at the binding sites can be substantial, and is not straightforward to determine.

One method to expose binding sites is preparation of plasma membrane fractions from transporter-expressing

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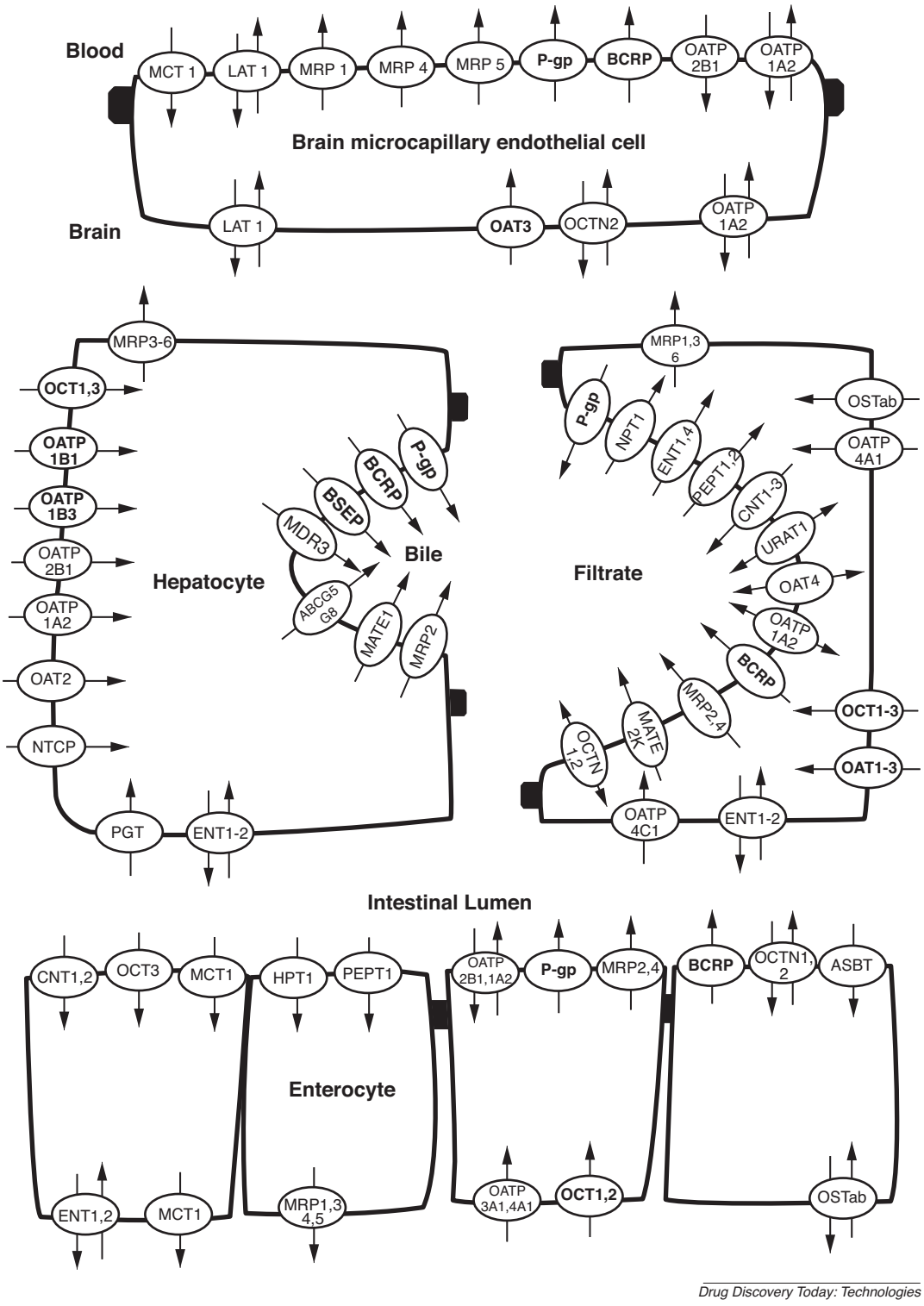

Figure 1. Expression of transporters implicated in drug transport on the four major pharmacological barriers (top to bottom, right to left: blood–brain barrier, hepatocyte, proximal tubule cell, enterocyte). Presence of transporters shown in the figure was confirmed through functional studies and mRNA detection. Directions of transport are shown with arrows. Transporters highlighted in regulatory documents are typed in bold. Generic name of the transporters is shown.

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