ABSTRACT

Charles University in Prague, Faculty of Pharmacy in Hradec Králové

Department of Pharmacology and Toxicology

Candidate: **MUDr. Libuše Smetanová**

Supervisor: **Prof. RNDr. Dr.h.c. Jaroslav Květina, DrSc., FCMA**

Title of Doctoral Thesis: **Transport mechanisms of xenobiotics (Caco-2 cell monolayer model) in relation to the Biopharmaceutics Classification System (BCS)**

Current technological levels enable to study transbarrier transports of xenobiotics (including gastrointestinal absorption) on *in vitro*, *in situ* and *in vivo* models. *In situ* and *in vivo* approaches enable to assess feedbacks at the organ or whole body level, in *in vitro* models extra barrier factors are eliminated and the models are aimed at the mechanisms of transport processes. *In vitro* models for studying of transport mechanisms through the intestinal wall are derived either from cell lines (e.g., Caco-2, MDCK, 2/4/A1 cells) or intestinal tissues (e.g., Ussing chamber). The Caco-2 cell monolayer model is one of the relatively most frequently used *in vitro* models.

Caco-2 cells are a cell line derived from colorectal adenocarcinoma. The main characteristic is their spontaneous differentiation with the creation of a monolayer of fully differentiated and polarized cells showing typical brush border and tight junctions under normal culture conditions. Usefulness of the Caco-2 model: a) transport studies - passive transcellular and paracellular transport, transport mediated by "uptake" and drug efflux mechanisms, b) inhibitory studies - the involvement of transporters in the drug transport and drug interactions, c) cumulative and uptake/efflux studies - intracellular accumulation of substances and substance uptake and efflux by intestinal cells, d) drug metabolism studies, and e) cytotoxicological studies.

The importance of Caco-2 model is not only in basic research but also in applied research (accepted by regulatory authorities as a standard method for the prediction of intestinal permeability and fraction absorbed in the new drug development). Factor "permeability" (= transbarrier transport) is then exploitable for completion of one of the parameters of the Biopharmaceutics Classification System (BCS), which sorts substances into four classes according to their solubility and permeability (Amidon et al. 1995). A substance is defined as “highly soluble” when the highest marketed dose strength is soluble in 250 ml of aqueous media over a pH range of 1 - 6.8 at 37°C and “highly permeable” when the extent of

absorption in humans is ≥ 85 % of an administered dose (EMEA-guideline 2010). Drug categorization according to the BCS: class I - highly soluble and permeable substance, class II - highly permeable with low solubility, class III - substances with low permeability and high solubility, and class IV - low solubility and permeability. Solubility parameter is determinable by physico-chemical techniques, however, parameter "permeability" requires biological experiments.

A relatively new approach in the approval process of new drug products is the BCS-based "biowaiver" approach, which means waiver of the bioequivalence study under certain well-defined conditions described in detail in regulatory directives. The BCS-based biowaiver approach is recommended for oral immediate-release products containing BCS class I drug with rapid dissolution equivalent to the reference product (EMEA-guideline 2010). EMEA- and WHO-guidelines extend the possibility of biowaiver to BCS class III drugs and WHO-guideline even to some substances from BCS class II.

The aim of the presented work was to use the *in vitro* Caco-2 cell method for studying of transcellular transport mechanisms of intentionally selected model drugs and for application of the results into the BCS. During the first phase, we introduced additional methods - cell viability assay, determination of the monolayer integrity using nontoxic phenol red, and cell accumulation. During the second phase, we used the validated Caco-2 cell monolayer model for studying of seven selected xenobiotics (ambroxol, 5-aminosalicylic acid (5-ASA), atenolol, caffeine, gliclazide, indapamide, and metformin) in respect to their permeability and mechanism of their transintestinal transport. The obtained results (parameter "permeability") of transport studies together with hydrophilic solubility of the selected compounds (data from literature) were used for the classification of the substances into one of four classes of BCS:

BCS class I

Ambroxol: determined high permeability and high solubility enable to classify ambroxol as class I of the BCS.

Caffeine: as highly soluble and highly permeable drug can be categorized into the class I of the BCS.

BCS class II

Gliclazide: with respect of low solubility and high permeability, gliclazide can be categorized as class II of the BCS.

Indapamide: with low solubility and high permeability can be categorized as class II of the BCS.

BCS class III

Atenolol: with high solubility and low permeability is indicated as BCS class III substance.

Metformin: with high solubility and low permeability can be ranked among class III compounds of the BCS.

BCS class IV

5-ASA: according to low permeability and low solubility, 5-ASA can be classified as class IV of the BCS.

The results of the transport studies of ambroxol and caffeine were published in scientific journals with impact factor, the results of 5-ASA transport studies have been accepted for publication in a scientific journal with impact factor.