High-Throughput Screening Using Caco-2 Cell and PAMPA Systems

Cheng Li, Sam Wainhaus, Annette S. Uss, and Kuo-Chi Cheng

Abstract This chapter will focus on the two most commonly used high-throughput screening methods detecting cellular/bio-membrane permeability in the pharmaceutical industry: Caco-2 cells and the parallel artificial membrane permeability assay (PAMPA). Both assays have advantages and disadvantages, and it is essential to understand these limitations. Since it is well recognized that human intestinal absorption cannot be precisely predicted by a single screening assay, it is important to utilize various in vitro and in vivo preclinical studies during lead optimization in drug discovery.

Keywords: High-throughput screening; Caco-2 monolayer; PAMPA; In vitro absorption models

Abbreviations

BCRP Breast cancer resistance protein
NCEs New chemical entities
PAMPA Parallel artificial membrane permeability assay
P-gp P-glycoprotein
TEER Transepithelial electrical resistance

18.1. Introduction

Lead optimization of new chemical entities (NCEs) based on pharmacokinetic behavior plays a major role in modern drug discovery. Despite advancement of drug delivery methods, the oral route remains the most frequent route of administration for approved new drugs. Therefore, during lead optimization it is essential to identify NCEs with sufficient oral absorption predicted using a variety of in vitro and in vivo assays. It is well recognized that in order for a NCE to achieve reasonable oral absorption, it will need to have adequate aqueous solubility, as well as intestinal permeability [1]. Recent advancements in chemistry, such as parallel and combinatorial synthesis, have resulted in a multifold increase in the number of compounds that are available for evaluation in new drug discovery. Furthermore, a variety of improved structural chemistry
tools, such as X-ray crystallography, structural modeling and ligand/substrate docking algorithms, and improved molecular biology tools, such as high-throughput binding targets and cell-based activity assays, provides new drug discovery scientists with an unprecedented level of structure-based designs to further guide the synthesis of new chemotypes as potential drug leads. Along with the advancement of chemistry and biology, new automated screening tools have become commercially available that can carry out complex, programmable, and adaptable robotic operations to test hundreds of thousands of compounds in a speedy and precise manner. As a result, these new forces have worked together to increase our ability to create NCEs that exhibit targeted pharmacological activity. Hence, the task of screening compounds for their biopharmaceutical properties, such as solubility, permeability, and metabolic stability, has become a major challenge in drug discovery. This change, in turn, has compelled the invention and implementation of high-throughput screening methods that predict in vivo oral absorption.

Drug absorption through the gastrointestinal (GI) tract following oral administration is a rather complex and dynamic process. Passive diffusion can occur through the cell membranes of enterocytes (transcellular) or the tight junctions between the enterocytes (paracellular) [2–4]. Influx and efflux through various drug transporters also play roles in dictating drug absorption. Since many processes are occurring simultaneously, it is often impossible for a single in vitro model to simulate the entire in vivo process. However, two in vitro screening models, Caco-2 cell and parallel artificial membrane permeability assay (PAMPA), have been developed over the last decade and are currently used by most major pharmaceutical companies in their drug discovery efforts. In the following pages, we will describe these two assays, their pros and cons, and how to use them for lead optimization in the discovery process.

18.2. Caco-2 Cell Monolayer System

There are several cell monolayer models that are frequently used for the evaluation of drug permeability and absorption potential (Table 18.1). For a more detailed discussion, please refer to Chap. 8. Caco-2 cells (adenocarcinoma cells derived from colon) are the most extensively characterized and frequently used of the available cell lines [5–9]. A unique feature of Caco-2 cells is that they undergo spontaneous enterocyte differentiation in cell culture. Unlike intestinal enterocytes, Caco-2 cells are immortalized and replicate rapidly into confluent monolayers. When the cells reach confluency during culture on a semi-porous membrane, they start to polarize and form tight junctions, creating an ideal system for permeability and transport studies. During the past decade, use of

<table>
<thead>
<tr>
<th>Cells</th>
<th>Species/tissue origin</th>
<th>Cell type</th>
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<tbody>
<tr>
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<td>Human/colon</td>
<td>Epithelial</td>
</tr>
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