Biosensor Analysis of the Interaction between Drug Compounds and Liposomes of Different Properties; a Two-Dimensional Characterization Tool for Estimation of Membrane Absorption

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ABSTRACT: The interactions between 78 drug compounds and immobilised liposomes were investigated using an assay based on surface plasmon resonance technology. The drugs were screened at a single concentration and allowed to interact simultaneously with two different types of liposomes. When the drug–liposome responses are plotted against one another they generally fall into three distinct bands: low response—low percent fraction absorbed in humans (Fa), medium response—medium Fa, and high response–high Fa. For drugs with medium to high Fa values, basic compounds could be resolved from acidic and neutral compounds to a large extent. This technique has the potential to be utilized as a screening tool for binning novel compounds into low, medium, or high Fa based on a simple experimental measurement. The assay was applied to 11 kinase inhibitors, 9 thrombin inhibitors, and 11 carbonic anhydrase inhibitors highlighting a subset that may have incomplete intestinal absorption (low to medium Fa). Assay conditions were optimized making the assay suitable for routine analysis and for compound characterization early in drug discovery where solubility may be an issue.
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INTRODUCTION

It has become widely recognized that early estimates of the pharmacokinetic properties of drug candidates is important in the drug discovery process. The rate at which screening data is obtained from combinatorial synthesis, using different molecular scaffolds, and from high throughput screens, is constantly increasing. This is paralleled by an increasing interest in rapid *in vitro* assays as well as improved *in silico* approaches[1] for prediction of absorption, distribution, metabolism, and excretion (ADME) properties. For orally administered drugs one key factor is the gastrointestinal absorption level. Several simplified medium throughput *in vitro* assays are available and have been reviewed.[2,3]

The currently most widely accepted *in vitro* model system is the use of cultured cells such as Caco-2, MDCK, and HT-29.[4–6] These cell monolayer assays mimic different transport routes through a membrane as they have tight intercellular junctions, microvilli, and express both enzymes and transport proteins. Advantages and disadvantages of cell monolayer assays are continuously being discussed,[2,3] with some of the main

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disadvantages being low throughput, overexpression of P-glycoproteins, high interlaboratory variability, and poor correlation with absorption *in vivo* for compounds showing low to medium permeability rate.

To further simplify the studies of drug membrane interactions it is also common to use octanol buffer distribution coefficient (log*D*) or artificial membranes such as immobilized artificial membrane (IAM) columns,[7] liposome partitioning,[8] or parallel artificial membrane permeation assay[9] (PAMPA) instead of cells. These *in vitro* assays are often used with the main objective of reducing the number of compounds for the more laborious and expensive assays.

Biosensor technology has been shown to be of value within ADME for screening of drug plasma protein binding[10,11] and for estimation of drug membrane absorption.[12,13] In the latter report a correlation between published fraction absorbed values (Fa %) and drug absorption to liposomes captured on a lipophilic sensor chip was demonstrated. Further, the effect of liposome composition, temperature, and dimethylsulfoxide content on drug/liposome absorption was investigated. The reproducibility and stability of the liposome surfaces was shown to be high. The main advantages of biosensor technology (here a surface plasmon resonance, SPR, sensor) is that not only the binding level but also interaction kinetics are monitored directly without the use of labels, using general detection based on changes in refractive index at a sensor surface. Sample consumption is low, and both the running and data evaluation of the assay are highly automated.[14,15]

In this article we present a method, based on the new concept of allowing the compound to simultaneously interact with two different types of liposomes, for classification of compounds into high, intermediate, or low level of passive membrane absorption and for characterization of compound charge.

MATERIALS AND METHODS

Instrumentation and Assay Temperature

Biacore® S51 from Biacore AB, Uppsala, Sweden, was used (Fig. 1), with on-line degassing of buffers by Degasys Ultimate DU2010, Uniflows, Co., Ltd, Tokyo, Japan. Interaction analysis was performed at 37°C.

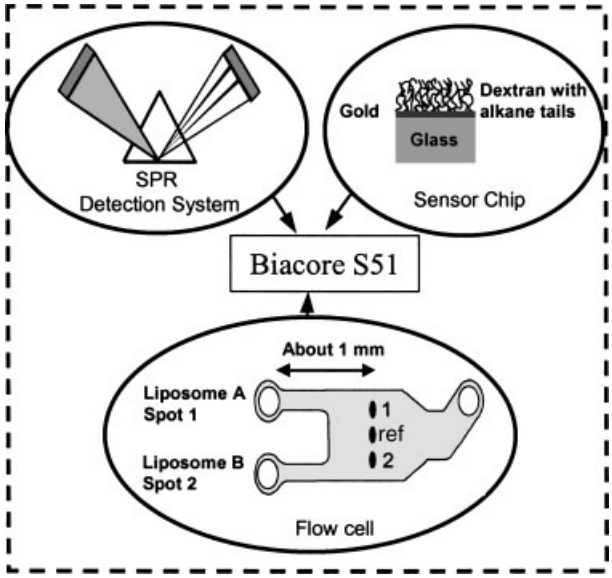

Figure 1. The detection system, sensor surface, and flow cell of Biacore S51. A sensor chip surface is placed in contact with the flow cell and the detection unit. Buffer flows continuously through the flow cell and over the three detection spots. Liposomes are immobilized to spots 1 and 2. Samples are injected in parallel over the three spots, using the autoinjector, and refractive index changes are detected by the detection unit.

Buffers and Regeneration Solution

Buffer A: 1 litre × 10 concentrated PBS stock buffer; 43.5 g $NaH_2PO_4 \cdot H_2O$, 32.9 g $Na_2HPO_4 \cdot 2H_2O$, 2.0 g KCl, 56.7 g NaCl, pH 5.8. Buffer B: 103 parts of buffer A diluted with 897 parts of water. Buffer C: 97 parts of buffer B mixed with 3 parts dimethylsulphoxide (DMSO) obtaining the running buffer (50 mM PBS with 3% DMSO, pH 6.5). Using these buffers, buffer constituents such as salts as well as DMSO concentration could be carefully matched in samples, running buffer, and solutions for correction of DMSO bulk differences, which is crucial for reproducible data. Isopropanol:50 mM NaOH at a ratio of 40:60 (v/v) was used as regeneration solution.

Preparation and Immobilization of Liposomes

1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), cholesterol and Synthetic phospholipid blend, PE:PS:PC at 5:3:2 (w/w) consisting of 1,2-Dioleoyl-*sn*-glycero-3-phosphoethanolamine, 1,2-Dioleoyl-*sn*-glycero-3-(phospho-L-serine), 1,2-Dioleoyl-*sn*-glycero-3-phosphocholine (Avanti Blend) were from Avanti Polar Lipids, Alabama, USA. Aliquots of lipids, solubilised in chloroform, were