Drug Discovery Interface

Optimization of a Parallel Artificial Membrane Permeability Assay for the Fast and Simultaneous Prediction of Human Intestinal Absorption and Plasma Protein Binding of Drug Candidates: Application to Dibenz[b,f]Azepine-5-Carboxamide Derivatives

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ABSTRACT: Parallel artificial membrane permeability assay (PAMPA) has been successfully applied by pharmaceutical industries as high-throughput technique capable of screening compounds for passive oral absorption. Herein, the possible applicability of this assay to predict simultaneously biodistribution parameters such as plasma protein binding (PPB) and apparent volume of distribution (VD) was investigated for the first time. Apparent permeability (P_{app}) of 18 reference drugs was determined by nine PAMPA conditions and compared with the corresponding intestinal absorption fraction (Fa), PPB, and VD in humans. In all the models, P_{app} was not correlated with VD; however, it was correlated with Fa and PPB. In these cases, the best correlations ($r \geq 0.8953$) were found when using a membrane of 2% of L-α-phosphatidylcholine, at pH 6.5/7.4 for donor/acceptor compartments. Under these conditions, good correlation with traditional PAMPA ($r = 0.9633$) and Caco-2 models ($r = 0.9246$) were also achieved. This method correctly predicted compounds' permeability and was robust enough to distinguish compounds with high Fa ($P_{app} \geq 1.1 \times 10^{-6}$ cm/s) and PPB ($P_{app} \geq 1.0 \times 10^{-5}$ cm/s), with no false positives or negatives recorded. In addition, ultrafiltration method was used to determine the PPB of 10 tested derivatives of dibenz[b,f]azepine-5-carboxamide, which were also assessed through the new PAMPA model developed herein, and equal classifications were achieved. © 2011 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 101:530–540, 2012

Keywords: Biopharmaceutics Classification System; dibenz[b; f]azepine-5-carboxamide derivatives; high-throughput technology; intestinal absorption; parallel artificial membrane permeability assay; permeability; protein binding; transcellular transport; volume of distribution

INTRODUCTION

Drug discovery research aims to identify new chemical entities (NCEs) not only potent and selective but also with appropriate *in vivo* pharmacokinetic properties such as absorption, distribution, metabolism, and excretion in order to increase the success rate in clinical studies. Indeed, approximately 40% of active compounds fail during the drug development process due to their poor pharmacokinetic characteristics.[1–3]

One major hurdle for successful drugs is their permeability through biomembranes because this property may determine the extent of oral absorption,

Abbreviations used: BCS, Biopharmaceutics Classification System; CBZ, carbamazepine; CBZ-E, carbamazepine-10,11-epoxide; DDD, drug discovery and development; DMSO, dimethyl sulfoxide; ESL, eslicarbazepine acetate; Fa, intestinal absorption fraction; HTS, high-throughput screening; NCEs, new chemical entities; NSB, nonspecific binding; OXC, oxcarbazepine; PAMPA, parallel artificial membrane permeability assay; P_{app}, apparent permeability; PPB, plasma protein binding; *R*-Lic, *R*-licarbazepine; *S*-Lic, *S*-licarbazepine; *trans*-diol, 10,11-*trans*-dihydroxy-10,11-dihydro-carbamazepine; VD, volume of distribution; VD_{ss}, apparent volume of distribution at steady state.

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the biodistribution, and, consequently, the target tissues uptake. Nowadays, high-throughput screening (HTS) for compounds with reasonable membrane permeability has hence become a key goal at early stages of drug discovery and development (DDD) process.[4,5] One of the most interesting techniques that emerged as a HTS tool was the *in vitro* parallel artificial membrane permeability assay (PAMPA). This nonbiological model involves artificial lipid membranes that separate two compartments, the donor compartment containing buffer solution with the NCE and the acceptor compartment with only fresh buffer. Despite the absence of enzymes and transporters, PAMPA is able to measure the relative passive transcellular diffusion of compounds with reasonable accuracy, which is the most common pathway for drug intestinal absorption and biodistribution to target tissues.[6] Thereby, it is expected that PAMPA may predict not only the intestinal absorption fraction (Fa) of a set of NCEs but also their distribution characteristics. The first approach is actually well demonstrated in literature, providing significant correlations between the values of apparent permeability (P_{app}) experimentally obtained and human Fa and, therefore, it is applied at the beginning of DDD.[6–13] However, correlations between P_{app} values and main distribution pharmacokinetic parameters [apparent volume of distribution (VD) and plasma protein binding (PPB)] have not been investigated, in spite of the evident implications of distribution process in pharmacokinetics, pharmacodynamics, and toxicity of a drug. Empirically, it is expected that compounds with higher membrane permeation present higher values of VD. However, the ability of the drug to be distributed and/or accumulated through different compartments of the body is strongly dependent on several factors, particularly drug lipophilicity, charge state, affinity to tissues, and extension of binding to plasma proteins. Indeed, only the unbound drug is available to cross membrane barriers, be distributed to tissues, and exert the pharmacological and/or toxicological effects. A strong binding of drugs to plasma proteins (above 90%) brings a risk of highly varying free fraction of the drug in plasma, which can cause changes in its bioactivity and safety.[14–16] For these reasons, PPB is nowadays considered an important parameter in the optimization of drug properties in initial phases of DDD. As the VD, the PPB of a drug also depends on drug lipophilicity and ionization state.[14,17] Besides being used to predict the absorption of NCEs at the beginning of DDD, PAMPA is nowadays recognized as a good model of lipophilicity[18] and, taking into account the current state-of-art, the fact of lipophilicity and state of ionization are two important properties that govern PPB and VD, we were encouraged to investigate whether PAMPA could be used in initial phases of DDD because a HTS tool is able to provide a reproducible rank order of compounds based on their ability to be absorbed and distributed. According to the literature, a drug administered orally should present a Fa higher than 85% in order to guarantee reproducible drug plasma levels, whereas the PPB should be lower than 90% to minimize potential harm of drug–drug interactions. Drugs with VD lower than 0.7 L/kg are considered to be poorly distributed through the tissues, whereas those with VD higher than 10 L/kg exhibit an extensive level of drug–tissue partitioning.[17] Thus, the possible use of PAMPA to predict simultaneously the human Fa, PPB, and VD would be very useful for pharmaceutical industries in DDD programs. For example, in terms of drug design, where modulation of Fa of drug candidates is usually required, efforts are often focused on enhancing the lipophilicity but the opposite may be desirable when considering PPB. Thereby, the lipophilicity is a drug property with impact on Fa and PPB and a balance between these two parameters is required to make PAMPA a promissory tool likely to be used in this context. In addition, this would be attained rapidly and by using a small amount of NCE.

The present work aimed to assess the actual suitability and general applicability of PAMPA to simultaneously predict the absorption potential and biodistribution of drug candidates. Therefore, P_{app} values of 18 commercially available drugs with different structures and known human Fa, PPB, and VD were assessed using distinct experimental conditions of PAMPA (regarding lipid composition and pH of PAMPA compartments) in order to find the most discriminating one and to establish ranges that could distinguish those compounds with high or low Fa, PPB, and VD. The best PAMPA model herein developed was further applied to 10 chemically and structurally related dibenz[b,f]azepine-5-carboxamide derivatives, which were used as a set of test compounds to demonstrate the discriminating ability of the model to support drug discovery programs.

MATERIALS AND METHODS

Drugs and Reagents

Acetylsalicylic acid (A5376), atenolol (A7655), chloramphenicol (C0378), chlorothiazide (C4911), hydrochlorothiazide (H4759), ibuprofen (I4883), ketoprofen (075K1347), metoprolol (M5391), nadolol (N1892), naproxen (284785), norfloxacin (N9890), piroxicam (P5654), propranolol (P0884), ranitidine hydrochloride (R101), sulfasalazine (S0883), (S)-(−)-sulpiride (S777), theophylline (T1633), and (±)-verapamil hydrochloride (V4629) were purchased from Sigma–Aldrich (St. Louis, Missouri) and used