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Parallel artificial membrane permeability assay for blood–brain permeability determination of illicit drugs and synthetic analogues

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ABSTRACT

With the number of designer drugs on the streets rampantly on the rise, it's becoming more and more important to be able to rapidly characterize them in a biologically relevant way. Using a parallel artificial membrane permeability assay (PAMPA) to assess the blood brain barrier permeability has shown to be a high throughput way to compare new drugs with currently controlled substances via their effective permeability values. This combined with direct infusion electrospray ionization–mass spectrometry creates a rapid technique for characterization of new designer drugs. PAMPA has successfully determined the effective permeabilities of cocaine, methamphetamine, heroin, MDMA, and several tryptamine derivatives.

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1. Introduction

Analogues of controlled substances are a major source of disagreement in the legal community. The United States federal drug policy known as the Controlled Substances Act (1970) contains a drug analogue statute that criminalizes any analogue which has a structure or effect that is “substantially similar” to a scheduled drug. This vague terminology allows for much debate in a courtroom—what is “substantially similar”? In the justice system, conviction or acquittal in cases of analogues of scheduled drugs often comes down to the testimony of a scientist on similarity of structure or efficacy. A more scientifically relevant rubric could immensely improve the legal process by which novel designer drugs are relegated to scheduled drug status.

In order to meet this need, a biologically relevant characteristic of these compounds should be compared. Since psychoactive drugs must cross the blood–brain barrier in order to illicit the desired effect through interaction with various receptors, the permeability across this membrane is an ideal metric of comparison of potential drug analogues to currently scheduled substances.

Parallel artificial membrane permeability assay (PAMPA) is an in vitro process for membrane permeability assessment. It was initially developed and optimized to mimic the membrane of the gastrointestinal tract for use in the research of oral absorption of new pharmaceuticals [1–4]. This assay uses 2 well plates in a “sandwich”-like formation: a donor plate with porous membrane wells that are submerged in the wells of receiver plate. The porous membrane is coated with a lipid solution (initially lecithin in dodecane [1]) which forms a bilayer to mimic a biological membrane. The donor wells contain the drug in an aqueous buffer solution, and the receiver wells contain only buffer solution. Drugs that can permeate the membrane will passively diffuse into the receiver wells (Fig. 1). The solutions are generally kept at a pH of 7.4, though some studies have been conducted at other pHs [5].

By changing the lipid composition, different membranes can be simulated—PAMPA has since been modified to predict skin [6], retinal [7], and blood–brain barrier permeabilities [8–11]. Porcine polar brain lipids were used to more accurately mimic the BBB in these assays; this method has been determined to be an adequate high-throughput screening technique for BBB permeability when compared to MDCKII–MDR1 cell assays [12,13], Caco-2 cell assays [14,15], in situ brain perfusion assays [13], and in silico calculations [16,17].

Despite the method's apparent usefulness in filtering the viability of new pharmaceuticals, PAMPA–BBB has yet to be applied to controlled substances. The key advantage of this technique is its ability to rapidly assess the ability of drugs to pass the BBB. When coupled to electrospray ionization–mass spectrometry (ESI–MS) [18–21] rather than UV detection [1,8], even more advantages become apparent, such as increased sensitivity and capability of using a single assay for more than one drug. Using the following equation (Carrara et al. [22]) in order to

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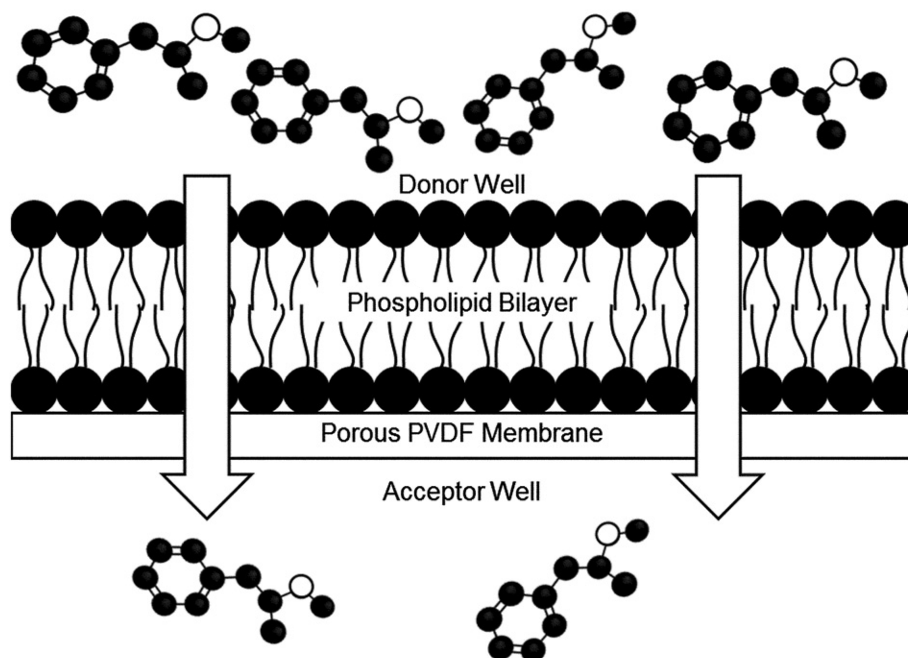

Fig. 1. Membrane-permeable drugs will passively cross the membrane into the acceptor well. The rate at which they do this, permeability (cm/s), varies primarily based on lipophilicity and molecular weight.

determine the effective BBB permeability (P_{eff}) of currently scheduled drugs will facilitate the formulation of a scale by which to compare all new drug analogues that are confiscated by law enforcement.

$$P_{app} = -\left(\frac{V_r V_d}{(V_r + V_d)At}\right) ln\left(1 - \frac{Cr(t)}{C(t)}\right)$$

P_{app} is apparent permeability, V_d is the volume of the donor well, V_r is the volume of the receiver well, A is the filter area, and t is permeation time. $C_r(t)$ and $C(t)$ are the concentrations of the drug in the acceptor well and reference wells, respectively. This can be correlated to the mass spectral peak areas in the donor well and the reference well.

Direct infusion ESI–MS offers the advantages of faster analysis time and minimal sample volume over the traditionally used liquid chromatography—ESI–MS [18–21], but it is prone to suffer from matrix effects and ionization competition. Therefore, an internal standard with structural similarities to the analyte can be used to aid in quantitation [23]. By diluting the well contents with the internal standard after PAMPA, the concentrations can be represented as ratios of the peak area of the drug to the peak area of the internal standard.

In order to showcase the effectiveness of this method for both scheduled drugs and potential drug analogues, a rapid analogue synthesis was adapted from Singh et al. [24] to synthesize multiple tryptamine derivatives in one reaction by using multiple alkyl iodides in the reaction vessel with tryptamine. The synthesis of several potential analogues in a single reaction provides a few key benefits: decreased overall synthesis time (in comparison to synthesizing each one individually), simulation of drugs in evidence that may contain several substances of interest, and rapid population of an analogue database.

2. Materials and methods

2.1. Materials

Optima methanol, glacial acetic acid, 1-iodobutane, 1-iodohexane, and 1-iodooctane were obtained from Sigma Aldrich (Saint Louis, MO). HEPES buffer was ordered from Acros Organics (Geel, Belgium). Porcine polar brain lipids were from Avanti Polar Lipids, Inc. (Alabaster, AL). SDS and sodium bicarbonate were ordered from Fisher Scientific (Waltham, MA). All illicit drugs (heroin, MDMA, cocaine, methamphetamine) were provided by the Denton County Sheriff's Office and verified by a generic GC–MS method. Millipore Multiscreen 96-well assay plates (MAIPNTR10) and Multiscreen Transport Receiver Plates (MATRNPS50) were ordered from Fisher Scientific (Waltham, MA).

Analysis of all samples was conducted via direct sample infusion with a Chemyx Fusion 100 syringe pump (Stafford, TX) to the TurboSpray source of an API 3000 triple quadrupole mass spectrometer (AB Sciex, Framingham, MA). Nitrogen is used for the nebulizer and curtain gases. A spray voltage of 4500 V and a sample flow rate of 10 μL/min were used for all samples.

2.2. Synthesis of potential analogues

Tryptamine derivatives were synthesized by adding 50 mg tryptamine (0.31 mmol), 57 mg $NaHCO_3$ (0.68 mmol), 1 mL of water, 1.24 mg of SDS and 0.2 mmol each of the iodo-alkyls: 22.8 μL of 1-iodobutane, 29.5 μL of 1-iodohexane, and 36.1 μL of 1-iodooctane. This was heated at 80 °C for 1 h. After heating, the solution was allowed to cool and then the water layer was drawn off. The remaining brown, oily residue was dissolved in 1.2 mL of methanol. This was then diluted 1000-fold in 50/50 methanol/water, 1% acetic acid for analysis via positive mode ESI–MS as well as collision-induced dissociation (CID) for product structure determination. Collision energies were optimized for each compound separately.

To ensure fragmentation deconvolution, tryptamine was reacted with 1-iodobutane and 1-iodooctane in separate flasks. These syntheses were scaled down by half from the previous synthesis, using 25 mg of tryptamine in 0.62 mL water (with 0.6 mg SDS and 28.5 mg $NaHCO_3$) and 35.5 μL 1-iodobutane and 56.3 μL 1-iodooctane. This was then diluted 500-fold in 50/50 methanol/water, 1% acetic acid for analysis via positive mode ESI–MS and CID.

2.3. PAMPA

Each donor well in the top 96-well plate was coated with 4 μL of PBL solution. Solutions of 5 mg/mL cocaine, methamphetamine, ecstasy, and heroin were prepared in methanol and further diluted 200-fold in 10 mM HEPES buffer. The synthesis product solution was diluted 500-

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