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Formulation of hydrophobic therapeutics with self-assembling peptide and amino acid: A new platform for intravenous drug delivery

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ABSTRACT

Clinical application of hydrophobic therapeutics is restricted by lack of an efficient vehicle which permits their solubility in aqueous environments. We have previously developed a novel formulation strategy to deliver a hydrophobic Src inhibitor, PP2, involving combinations of one self-assembling peptide (SAP) and one of 4 selected amino acids (AAs). The present study aims to develop a generalized drug delivery platform for intravenous application of hydrophobic drugs by combining self-assembling peptide, amino acid and low concentration of co-solvent. A multi-step screening pipeline is established which includes assessment of drug solubility and physicochemical characteristics, as well as functional efficacy and safety *in vitro* and *in vivo*. Using PP2 as an exemplary hydrophobic compound, 480 different combinations of 6 SAPs, 20 naturally existing AAs at 2 concentrations, and 2 co-solvents were evaluated. Among the combinations, 60 formulae dissolved PP2; 10 of which significantly reduced thrombin-induced IL-8 production, a sign of inflammatory response, in normal human lung epithelial BEAS2B cells. These formulations did not show cytotoxicity alone, but 2 reduced cell viability with presence of thrombin. We then performed a double-blinded test in a rat model of pulmonary ischemia-reperfusion. PP2 formulated with EAK16-I peptide plus methionine and 2% ethanol were administrated intravenously, significantly reducing severity of lung injury. The SAP-AA formulation strategy was also successfully applied to other hydrophobic compounds, suggesting this strategy could be applicable to other hydrophobics for a variety of clinical applications.

1. Introduction

Research in nano-sized biological materials has provided the field of medicine with novel, innovative methods of tissue engineering and drug delivery [1]. A unique class of biomaterials in particular, called self-assembling peptides (SAPs), has paved its way into applications as 3D scaffolds for tissue engineering [2–4]. These peptides have exemplified great biocompatibility and safety *in vitro* and *in vivo* [5,2]. Contemporary studies have directed attention toward utilizing self-assembling peptides for drug delivery: their ability to be customized through rational design is appealing in maximization of compatibility with diverse materials, as well as regulating their assembly into stable secondary structures [6]. Furthermore, self-assembling peptides have the potential to overcome a major limitation pertaining to the delivery of hydrophobic therapeutics which cannot dissolve in aqueous, hydrophilic environments such as blood [7,8]. The amphipathic properties of self-assembling peptides allow them to serve as a qualified medium between hydrophobic compounds and aqueous environment, in order to improve bioavailability and absorption which depend highly upon drug solubility [9]. This also diminishes the use of organic solvents that are not clinically applicable due to toxicity [10]. Self-assembling peptides have proved their efficacy as delivery vehicles in several *in vitro* studies, and potential usage *in vivo* has been validated through intra-peritoneal route [11]. However, due to the low osmolarity of the preparations, they cannot be used for intravenous injections [12].

Src family protein tyrosine kinases (PTKs) have been reported to regulate acute inflammatory responses in multiple diseases including acute lung injury (ALI), brain injury, stroke, and myocardial infarction. These complications can be attenuated by introduction of Src PTK inhibitors such as PP2 [13–15]. We previously demonstrated that PP2 prevented ischemia-reperfusion-induced ALI in rats, when dissolved in high concentration of organic solvent dimethyl sulfoxide (DMSO) [15]; however, DMSO is not suitable for clinical settings due to the documented toxicity

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[14,16]. In light of a clinically-applicable vehicle for PP2, we developed a novel strategy combining a self-assembling peptide (EAK16-II) with one of four selected amino acids for the delivery of PP2. This formulation reduced the amount of DMSO as a co-solvent down to 1%, and increased the osmolarity to a clinically-acceptable range - therefore improving biocompatibility and safety. The functional efficacy of the PP2 formulation was verified through successful inhibition of Src PTK activity in cultured cells, and reduction of lipopolysaccharide-induced ALI in mice [14].

The objective of the present study was to further develop the self-assembling peptide–amino acid strategy into a potential platform for intravenous hydrophobic drug delivery. Using PP2 as an exemplary hydrophobic compound, we established a multi-step screening pipeline (Fig. 1A). The primary step focused on the solubility of the drug: interchanging 6 self-assembling peptides (Fig. 1B), 20 naturally existing amino acids at 2 different concentrations, and ethanol as an alternate, safer co-solvent to DMSO. Selected formulations were then tested for

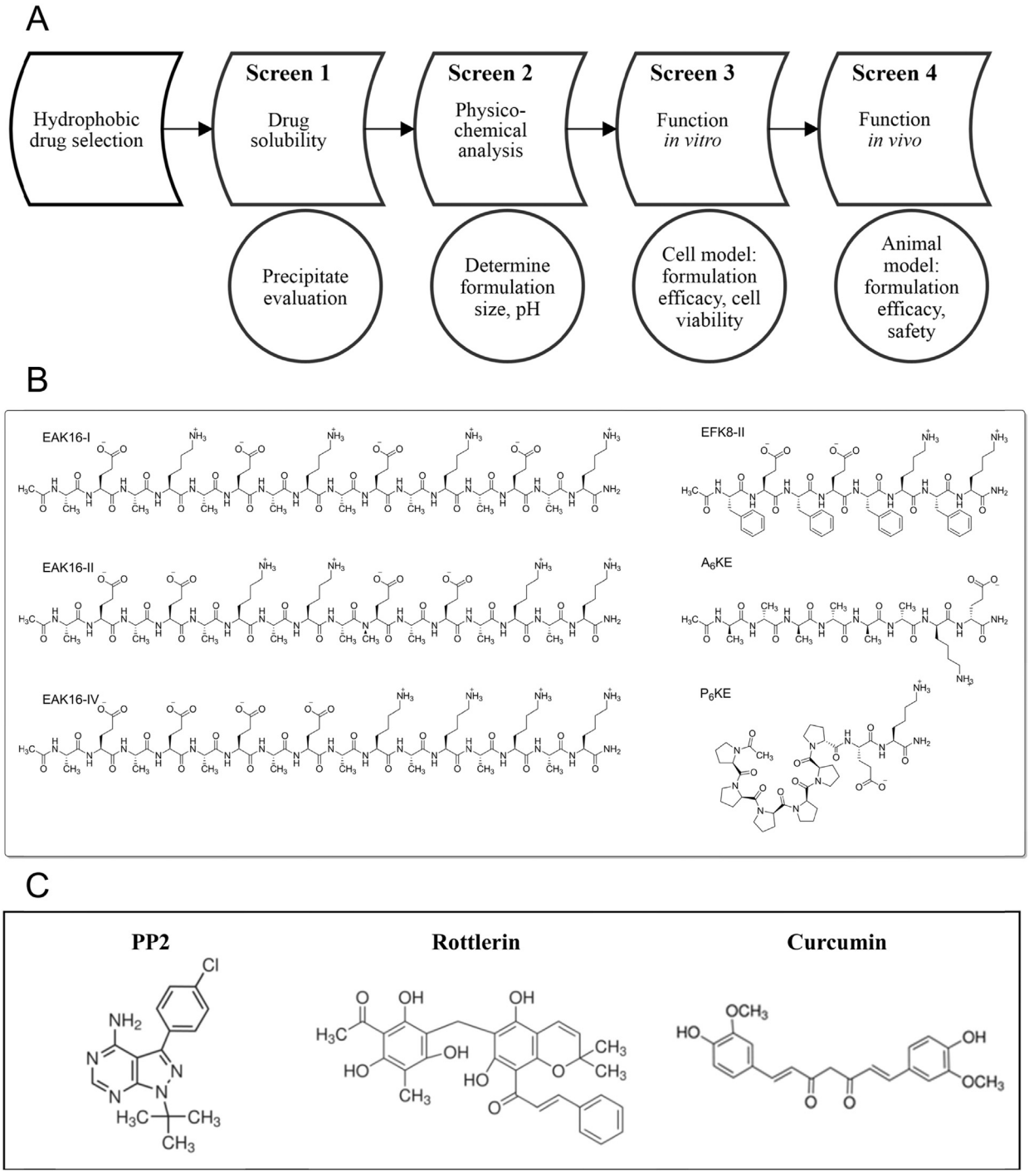

Fig. 1. Experimental protocol for selection of formulae. A) A pipeline for drug formulation and selection. A set of screens have been established in order to systematically optimize the self-assembling peptide–amino acid combination for a given hydrophobic compound. Screening includes assessment of drug solubility, physicochemical characterization, and functional efficacy/safety *in vitro* and subsequently *in vivo*. B) Chemical structures of six self-assembling peptides. Left: long-chain peptides (16 amino acids), all part of EAK family. Right: short-chain peptides (8 amino acids). EFK8-II is also part of the EAK family, whereas A6KE and P6KE are surfactant-like. C) Relevant hydrophobic compounds with differing chemical structures tested in the present study. The solubility information for these compounds, according to suppliers, is as follows: PP2 – soluble in DMSO (25 mg/mL); Rottlerin – soluble in DMSO (30 mg/mL) or Ethanol (1 mg/mL); Curcumin – soluble in DMSO (>11 mg/mL), ethanol (10 mg/mL).