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Prodrug approach to improve absorption of prednisolone

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ABSTRACT

Amino acid and dipeptide prodrugs have been developed to examine their potential in enhancing aqueous solubility and permeability as well as to bypass P-glycoprotein (P-gp) mediated cellular efflux of prednisolone. Prodrugs have been synthesized and identified with LC/MS/MS and NMR. Prodrugs displayed significantly higher aqueous solubility relative to prednisolone. These compounds also exhibited higher stability under acidic conditions relative to basic medium. [14]-Erythromycin uptake remained unaltered in the presence of valine–valine–prednisolone (VVP) indicating lower affinity toward P-gp. Moreover, VVP generated significantly higher transepithelial permeability across MDCK-MDR1 cells compared to prednisolone. Importantly, [3H]-GlySar uptake diminished significantly in the presence of VVP indicating high affinity toward peptide transporters. Moreover, prednisolone was regenerated from VVP due to enzymatic hydrolysis in SIRC cell homogenate. Results obtained from these studies clearly suggest that peptide transporter targeted prodrugs is a viable strategy to improve aqueous solubility and overcome P-gp mediated cellular efflux of prednisolone.

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1. Introduction

Topical delivery of glucocorticoids is one of the most preferred and convenient method of administration to treat anterior ocular inflammatory conditions. Despite being highly patient compliant, topical administration faces numerous challenges such as rapid tear turnover, drainage to systemic circulation and non-specific absorption in other ocular tissues (Hariharan et al., 2009a). In addition, corneal epithelium expresses efflux proteins such as P-gp (Dey et al., 2003; Verstraelen and Reichl, 2013) and multidrug resistance associated proteins (MRPs) (Karla et al., 2007a; Karla et al., 2007b; Karla et al., 2009; Verstraelen and Reichl, 2014). These drug efflux pumps play an active role in exporting drug molecules from cornea to tear film resulting in poor drug accumulation in anterior ocular tissues (Dey et al., 2004; Hariharan et al., 2009a,b). Cumulative effects of these barriers cause very poor ocular absorption (<5%) of topically applied therapeutic agents.

Prednisolone, a corticosteroid is currently administered to treat ocular inflammation (Thomas and Melton, 1992). Prednisolone generates anti-inflammatory effects by binding to glucocorticoid receptors, thereby triggering signal transduction pathways (De Bosscher and Haegeman, 2009). In spite of high efficacy, ocular absorption of this steroid is very limited from topical administration. In addition to poor aqueous solubility, prednisolone is a known substrate of P-gp (Hariharan et al., 2009a; Karssen et al., 2002; Nakayama et al., 1999; Oka et al., 2002; Troutman and Thakker, 2003; Van der Heyden et al., 2012). Moreover, an optimum balance between hydrophilicity and lipophilicity is required to permeate cornea, which is lacking in prednisolone. Hence, an effective strategy to address challenges associated with topical prednisolone administration has been presented in this article.

Transporter targeted prodrug delivery approach has received considerable attention to improve corneal absorption of poorly permeable drugs (Anand et al., 2004; Gunda et al., 2006; Katragadda et al., 2006; Majumdar et al., 2009; Suresh et al., 2010; Vadlapudi et al., 2013; Vadlapudi et al., 2012; Vooturi et al., 2012). This approach involves chemical derivatization of drugs with membrane (influx) transporter targeting ligands. Among the basis of targeting ligands selection, the solubility of parent drug can also be improved significantly (Majumdar et al., 2005). Peptide transporters (PEPT1 and PEPT2) have been extensively utilized for prodrug derivatization due to their high substrate affinity and broad specificity. These transporters are primarily responsible for the transport of small peptides such as di- and tri-peptides. The structure, function, mechanisms and substrate specificity have been widely explored. Peptide transporters are highly expressed on cornea (Anand and Mitra, 2002; Kadam et al., 2013; Xiang et al., 2009). These transporters have been targeted to improve

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transcorneal absorption of poorly permeable drugs such as acyclovir (Anand et al., 2006; Anand and Mitra, 2002) and ganciclovir (Gunda et al., 2006; Majumdar et al., 2005) in our laboratory. Valine–valine–acyclovir and valine–valine–ganciclovir prodrugs demonstrated significantly higher aqueous solubility and transcorneal permeability compared to parent drugs i.e., acyclovir and ganciclovir (Anand et al., 2003; Majumdar et al., 2005). Moreover, valine based dipeptide prodrugs appear to substantially bypass P-gp mediated cellular efflux (Agarwal et al., 2008; Jain et al., 2005; Katragadda et al., 2006; Wang et al., 2012).

The primary objective of this study is to develop prodrugs of prednisolone to improve aqueous solubility, corneal permeability and circumvent P-gp mediated cellular efflux. Valine–valine–prednisolone (VVP) and valine–prednisolone (VP) have been synthesized. Interactions of VVP, VP and prednisolone with P-gp have been examined by uptake and transport studies across MDCK-MDR1 cells. This transfected cell line has been selected as it has been extensively employed to delineate P-gp interaction and transport of a wide range of compounds in our laboratory (Jain et al., 2004; Luo et al., 2011; Minocha et al., 2012; Patel et al., 2014a, b,c; Vadlapatla et al., 2011). The regeneration of prednisolone from VVP has been examined by conducting SIRC cell homogenate study.

2. Materials and methods

2.1. Materials

Prednisolone and Boc-L-valine were purchased from Sigma–Aldrich (St. Louis, MO) and Bachem respectively. [3H]-GlySar (specific activity: 4 Ci/mmol) and [14C]-Erythromycin (specific activity: 51.3 mCi/mmol) were obtained from Moravek Biochemicals (Brea, CA, USA). MDCK cells, retrovirally transfected with the human MDR1 cDNA (MDCKII-MDR1) were generously donated by Drs. A. Schinkel and P. Borst (Netherlands Cancer Institute, Amsterdam, Netherlands). SIRC cell line was purchased from American Type Culture Collection (CCL-60; ATCC, Rockville, MD). Growth medium Dulbecco's modified Eagle's Medium (DMEM), Triple Express Trpsin®, non-essential amino acids, minimum essential medium (MEM), were procured from Life Technologies (Grand Island, NY). Fetal bovine serum (FBS) was obtained from Atlanta biological. Penicillin, Triton X-100, HEPES, D-glucose, streptomycin, sodium bicarbonate, cyclosporine A, GF 120,918, 4-(*N*,*N*-dimethylamino) pyridine (DMAP), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), triethylamine (TEA), trifluoroacetic acid (TFA), sodium chloride (NaCl), potassium chloride (KCl), sodium phosphate (Na_2HPO_4), potassium phosphate (KH_2PO_4), calcium chloride ($CaCl_2$), magnesium sulfate ($MgSO_4$), glucose and other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO). Culture flasks (75 and 25 cm^2), 12-well plates (3.8 cm^2 growth area/well) and Transwells® (Costar) were obtained from Fisher Scientific (Houston, TX, USA). All chemical agents procured were of special reagent grade and utilized without any further purification.

2.2. Methods

2.2.1. Synthesis of VVP

VVP was synthesized according to a procedure previous published from our laboratory (Fig. 1) (Agarwal et al., 2008). To synthesize VVP, VP was first synthesized by conjugating L-valine to prednisolone with an ester coupling agent such as EDC and DMAP. VP was then conjugated to L-valine to generate VVP using amide coupling agents, EDC and triethylamine (TEA).

In a round bottom flask, Boc-Val-OH (346 mg, 1.59 mmol) and EDC (304 mg, 1.59 mmol) was dissolved in anhydrous dimethyl formamide (DMF) and stirred at 0 °C for 45 min under nitrogen atmosphere (mixture 1). In a second round bottom flask, prednisolone (300 mg, 0.83 mmol) and DMAP (120 mg, 0.98 mmol) were dissolved in DMF at room temperature for 30 min to activate the terminal hydroxyl group of prednisolone (mixture 2). Mixture 2 was then added dropwise to the mixture 1 with the help of a syringe under constant stirring. The mixture was stirred for 24 h under nitrogen atmosphere. The reaction mixture was filtered and DMF was evaporated under reduced pressure to obtain crude product. The product (Boc-VP) was purified with silica based column chromatography by 5% methanol in dichloromethane (DCM) as eluent. Boc-VP was deprotected by the addition of 1:1 TFA/DCM at 0 °C over 50 min. VP was further purified by recrystallization in cold diethyl ether. The solvent was evaporated under reduced pressure to obtain a final dried product. The yield obtained was 86%.

VVP was generated by conjugating L-valine to VP with amide coupling agents such as EDC and TEA under similar conditions. TEA was added in the reaction medium to activate the amine group in VP. The product Boc-VVP was purified with column chromatography with 3% methanol in DCM as eluent. VVP was deprotected with 1:1 TFA/DCM at 0 °C for 50 min. The final product (VVP) was purified by recrystallization with cold diethyl ether. The yield obtained was 78%.

2.2.2. Identification of VP and VVP

VP and VVP were identified by LCMS and NMR analysis. LC/MS (*m*/*z*) for VP and VVP was +460.4 and +559.5, respectively. ^{1}H NMR analysis for VP (400 MHz, DMSO-d_6) δ: 0.80 (s, 3H), 0.90–0.95 (m, 1H), 0.91–1.02 (m, 9H) 1.18–1.65 (m, 14H) 1.68–2.40 (m, 4H), 4.18 (s, 1H), 5.23 (s, 2H), 5.90 (s, 1H), 6.17 (d, 1H), 7.25 (d, 1H), 8.05 (s, 2H) ^{13}C NMR analysis for VP (100 MHz, DMSO-d_6): 16.7, 17.5, 19.0, 20.1, 23.8, 27.6, 31.2, 31.8, 32.0, 32.5, 32.8, 33.2, 42.1, 47.2, 48.3, 49.8, 56.4, 67.5, 90.5, 121.5, 127.6, 152.4, 167.2, 169.0, 184.1, 203.7.

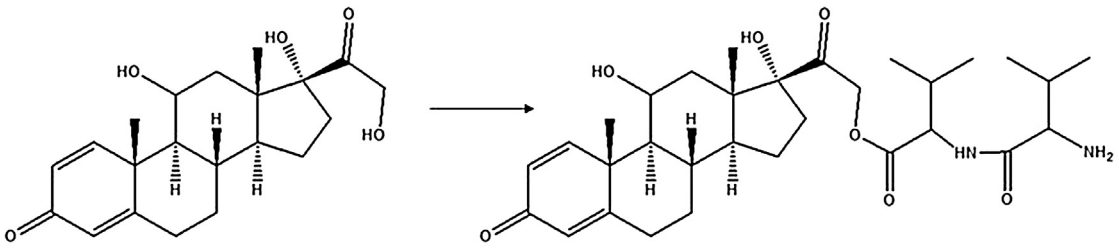

Fig. 1. Synthesis of VVP. To synthesize VVP, VP was first produced by conjugating valine to prednisolone. Another valine was then conjugated to VP to generate VVP. Reagents and conditions used for synthesis are explained in detail in Section 2.2.1.

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