Synthesis and Characterization of Antiepileptic Nanomedicine for Transbuccal Delivery

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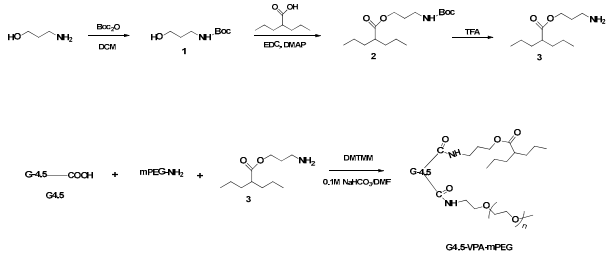
Statement of Purpose: Epilepsy therapeutic treatment is aided with a variety of formulations such as sustained release tablets, sprinkle tablets, delayed release capsules and syrup solutions, which primarily rely on oral administration. Using nanoparticles to deliver central nervous system (CNS) drugs can help enhance brain uptake. However, intravenous injection is often applied for nanoparticle-based therapeutics and likely cause poor patient compliance among patients suffering from such chronic disease as epilepsy. The purpose of this work is to develop antiepileptic nanomedicine and nanofiber formulation and to explore the buccal mucosa as a non-invasive adsorption site for delivery of nanomedicine. Antiepileptic drug valproic acid (VPA) as a model drug and highly branched nanoscale polyamidoamine (PAMAM) dendrimer as the underlying carrier were used to construct nanomedicine. The synthesized VPA nanomedicine was then loaded to electrospun nanofiber scaffold to make a mucoadhesive formulation and tested for transport across the buccal mucosa.

Methods:



Scheme 1. Synthesis of VPA nanomedicine.

*Synthesis of tert-butyl 3-hydroxypropylcarbamate (**1**).* To a solution of 3-aminopropan-1-ol (2 g, 27 mmol) and Et_3N (3.5 g, 35 mmol) in 15 ml of dry dichloromethane at 0°C a solution of Boc_2O (7.5 g, 34 mol) in dichloromethane was added. The reaction mixture was stirred at 0°C for 1h and then at room temperature for 24h. *Synthesis of 3-((tert-butoxycarbonyl)amino)propyl 2-propylpentanoate (**2**).* To a solution of **1** (3 g, 17 mmol), valproic acid (3.7 g, 26 mmol), and DMAP (2 g, 16 mmol) in 25 ml DCM at 0 °C EDC (4.77g, 25mmol) was added. The reaction mixture was stirred for 1 h at 0°C and allowed to warm slowly to room temperature. *Synthesis of 3-aminopropyl 2-propylpentanoate (**3**).* A mixture of **2** (1 g, 3.3 mmol) and 5 ml of TFA in 15 ml of DCM was stirred at 0°C for 1 h and at room temperature for 1 h. *Synthesis of G4.5-VPA-mPEG (**4**).* To a solution of PAMAM dendrimer G4.5 (EDA core, 20mg, 0,85µmol) and mPEG-NH_2 (110mg, 55µmol) DMTMM (17mg, 63µmol) was added. After reaction mixture was stirred for 7h at room temperature, a solution of **3** (12mg, 60µmol) in 500µl of DMF was added followed by addition of DMTMM (17mg, 63µmol). The reaction mixture was stirred for 12 h. The solvents were removed by rotary evaporation. The remained residues were redissolved in water, filtered, and dialyzed against water for 12 h. To remove unreacted **3** freeze-dried product was precipitated in ether, filtered and dried under vacuum.

Nanofiber formulation. Gelatin nanofibers are obtained via electrospinning and cross-linked with PEG diacrylate ethanol solution containing VAP nanomedicine (Donald A, Acta Biomaterialia, in revision).

Ex vivo buccal mucosa transport. The setup and test are conducted following our previous work (Yuan Q. ACS Chem. Neurosci. 2011;2:676-683)

Results: PAMAM dendrimer has been shown to be able to enhance therapeutic transport across the buccal musosa by our early work. Furthermore, dendrimers are highly branched, enabling a high drug payload of VPA. Methoxypolyethylene glycol (mPEG) was attached to the dendrimer due to its non-immunogenicity, biocompatibility and its ability to increase circulation time and tissue distribution. So far, we have successfully synthesized all intermediates and the final product according to ^{1}H NMR characterization. ^{1}H NMR (Figure 1) confirms the attachment of Valproic acid and mPEG to PAMAM dendrimer G4.5 (Figure 1).

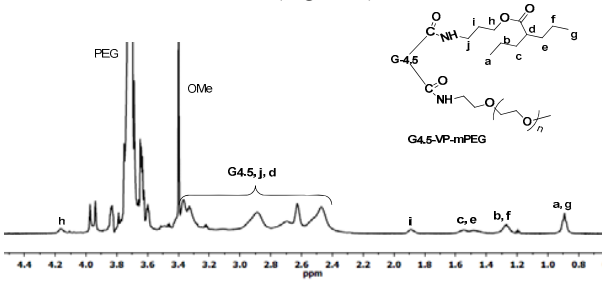


Figure 1. ^{1}H NMR spectrum of G4.5-VPA-mPEG.

Conclusions: We successfully conjugated valproic acid to Generation 4.0 dendrimer as confirmed by ^{1}H NMR. Ongoing studies include measurements of permeability and drug release studies during transbuccal transport.

Acknowledgements: This research was supported, in part, by NIH R21NS063200 and NSF CAREER Award CBET0954957. D.A. is a recipient of SREB-State Doctoral Fellowship.