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Design and evaluation of artificial receptors for the reversal of neuromuscular block

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ABSTRACT

Applying patient friendly and cost-efficient medications in healthcare will be a real challenge in the 21st century. Sugammadex is a selective, yet expensive agent used for the post-surgical reversal of neuromuscular block since 2008. A wide library of cyclodextrin-based follow-ups, having potentially similar affinity towards target aminosteroid type neuromuscular blocking agents has been established. Almost 20 compounds were assessed with respect to *in vitro* affinity against three commonly applied drugs. Based on the capillary electrophoretic screening, carboxymethylated and sulfobutylated gamma-cyclodextrin derivatives have the potential to be promising lead molecules for their affinity towards pipecuronium was identical or even superior to Sugammadex. Carboxymethylated gamma-cyclodextrin showed efficient and complete reversal of the pipecuronium induced neuromuscular block in an *ex vivo* rat diaphragm experiment.

1. Introduction

For the time being, Sugammadex (SGM)[1] is the only modern medical tool for the reversal of neuromuscular block (NMB) induced during surgery. However, this active pharmaceutical ingredient (API) has several drawbacks (costs, difficult synthesis and characterization) that leads to its limited application even if that would be beneficial for patients. In this work several potential follow-up molecules were strategically designed, prepared and evaluated that lack the disadvantageous properties of SGM, while they are potential lead compounds for a next generation of cyclodextrin-based artificial receptors.

During state-of-the-art surgery and anesthesia, neuromuscular block is frequently induced by aminosteroid type neuromuscular blocking agents (NMBAs) such as rocuronium, pipecuronium or vecuronium (Fig. 1). Each of these molecules contain a steroid structure/core and one or two/more amino functions.

After surgery, the neuromuscular block is traditionally reversed by acetylcholine esterase inhibitors, like neostigmine or edrophonium having numerous side-effects and moderate clinical efficiency Parlow et al., 1997; Srivastava and Hunter, 2009; Caldwell, 2009; Herbstreit et al., 2010). The first attempts to substitute such medications with macrocyclic chelators was reported by Cameron and Ming-Zhang, pioneers in this field, which finally led to the idea of using cyclodextrins (CDs) (Cameron et al., 2002). In the state-of-the-art anesthetic protocol, a highly selective complexing agent, SGM has been applied in clinical practice in the EU and more than 70 countries worldwide since 2008 and was also approved by the FDA in 2015. This single isomer γ-CD (GCD) derivative was tailor-made to encapsulate rocuronium with the highest possible affinity, creating a binding of almost covalent strength, thereby acting as an artificial receptor. Customization of the molecule includes complete conversion of all eight hydroxyl groups at C-6 position to carboxyethyl thioether groups, yielding an extended apparent cavity size and ability to induce electrostatic interactions, both favored for the target application. After the inclusion complex

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[1] List of abbreviations: API, active pharmaceutical ingredient; BCD, beta-cyclodextrin; BGE, background electrolyte; CD, cyclodextrin; CE, carboxyethyl; CM, carboxymethyl; CM-G-8, carboxymethyl maltooligomer; DMCMGCD, Octakis-(2,3-di-*O*-methyl-6-*O*-carboxymethyl)-γ-cyclodextrin; DMSO, dimethyl sulfoxide; DS, average degree of substitution; EOF, electroosmotic flow; GCD, gamma-cyclodextrin; MB, neuromuscular block; NMBA, neuromuscular blocking agent; pTSA, Toluene-4-sulfonic acid monohydrate; SB-G-8, sulfobutylether maltooligomer; SBE, sulfobutylether; SGM, Sugammadex; SPE, sulfopropylether; Succ, succinyl; TRIS, tris(hydroxymethyl)aminomethane

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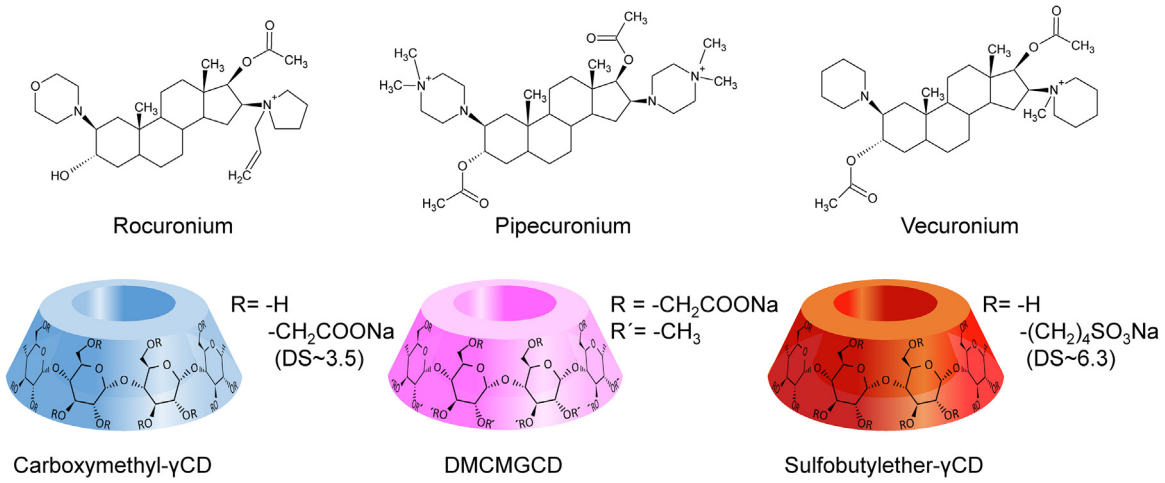

Fig. 1. Chemical structures of the applied aminosteroid type NMBAs and 3D structures of some CD derivatives applied in the study.

formation, the muscle relaxants, unable to bind the acetylcholine receptor, are excreted selectively along with the CD host (Bom et al., 2002; Welliver, 2006; Miller, 2007). The product, containing SGM, marketed under the trade name Bridion® (Merck) is in clinical practice to reverse rocuronium, pipecuronium, pancuronium and vecuronium induced neuromuscular block with significantly reduced or eliminated adverse effects and faster recovery time compared to traditional medications (Naguib, 2007; Decoopman et al., 2007; Pühringer et al., 2008; Bom et al., 2009; Lemmens et al., 2010; Duvaldestin et al., 2010; Blobner et al., 2010; Ledowski et al., 2014; Tassonyi et al., 2015).

However, Bridion® therapy has certain drawbacks, like high costs (Chambers et al., 2010), troublesome synthesis and work-up with moderate yield (Zhang et al., 2000; Ponnaiah et al., 2013, 2011; Gorin et al., 1996; Adam et al., 2002), numerous potential impurities (resulting in challenging purification as they are structurally related GCD derivatives with comparable physicochemical characteristics, molecular weights, pharmacological and toxicological profile to those of the active substance) (European Medicines Agency, 2008; Pharmaceutical and Medical Devices Agency, 2009) and heavily patented intellectual property environment (the substance is protected until 2020, afterwards, several process patents on intermediates and synthetic processes will survive) (Zhang et al., 2000; Ponnaiah et al., 2013, 2011; Wiebe and Diakur, 2005; Hattori, 2008; Hecht et al., 2008; Anon, 2015a,b). Moreover, SGM may cause severe hypersensitivity reactions and hemorrhagic side effects (Blobner et al., 2010; Sacan et al., 2007). Its non-selective interaction with aminosteroid type NMBAs raised issues with parallel applied medications (e.g. contraceptives) (Rex et al., 2010; Aniskevich and Brull, 2012), and SGM is not efficient against other families of NMBAs (e.g. benzylisoquinolines) that account for 1/3 of the NMBA market (Miller and Bom, 2001).

The idea of designing SGM follow-ups was first raised for Calabadion, an acyclic member of the cucurbituril family. Calabadion is an experimental agent that binds both to aminosteroid and benzylisoquinoline type NMBAs (the *in vitro* rocuronium binding affinity was reported to be comparable to SGM, the molar potency to reverse vecuronium and rocuronium effects was higher compared with that of SGM), however, its *in vivo* disposition and efficacy are unknown (Ma et al., 2012; Hoffmann et al., 2013; Zhang and Isaacs, 2014; Haerter et al., 2015; Ganapati et al., 2016).

The purpose of this study was to design potential and suitable CD-based candidates with simple, cost-effective and environment-friendly syntheses to act as SGM follow-ups that efficiently bind pipecuronium, rocuronium or vecuronium and are selective antagonists accordingly. To understand the key elements of SGM's success at a molecular level, a three-way strategy was set to explore three types of potential candidates:

- opened-ring CD derivatives (following the example of acyclic Calabadion),
- single isomer CD derivatives other than SGM and
- statistically substituted CD isomeric mixtures differing in cavity size and type, number and position of substituents.

The prepared host library was evaluated with respect to *in vitro* binding affinity against pipecuronium, rocuronium and vecuronium and the feasibility of the concept was proven on an *ex vivo* animal model.

2. Material and methods

2.1. Materials

Randomly substituted cyclodextrin derivatives with low DS like carboxymethyl-β-CD DS-3.5, carboxyethyl-β-CD DS-2.8, sulfobutylether-β-CD DS-4.2, DS-6.5 (DEXOLVE™), and DS-10.4, carboxymethyl-γ-CD DS-4.2, carboxyethyl-γ-CD DS-3.0, succinyl-γ-CD DS-3.8, sulfoprotylether-γ-CD DS-2.6, sulfobutylether-γ-CD DS-4.3 are all in sodium salt form and are commercially available fine chemical products of CycloLab Ltd. (Budapest, Hungary, Fig. 1). Gamma cyclodextrin was a product of Wacker Chemie AG (Munich, Germany). Sodium chloroacetate (98%), sodium hydroxide (≥98%), 1,4-butane sultone (≥99%) were product of Sigma Aldrich (St Louis, Missouri). Palladium/charcoal activated (10% Pd) was a product of Merck (Darmstadt, Germany). Strong ion-exchange resins were from Purolite (Llantrisant, UK), PFA400OH and C100C. Charcoal was from Cabot-Norit (Boston, Massachusetts), Norit C extra USB. The membrane dialysis was a Spectra/Por® Dialysis tubing with a cut-off of 100–500 Da. Sintered glass filters were Duran® sintered disc filter funnels of porosity 4. The membrane filters were from Labex Ltd. (Budapest, Hungary), Filter-Bio® CA membrane 0.45 μm pore size.

Toluene-4-sulfonic acid monohydrate (pTSA), tris(hydroxymethyl)aminomethane (TRIS), monosodium phosphate monohydrate, sodium hydroxyde and dimethyl sulfoxide (DMSO) used for