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C-547, a 6-methyluracil derivative with long-lasting binding and rebinding on acetylcholinesterase: Pharmacokinetic and pharmacodynamic studies

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ABSTRACT

C-547, a potent slow-binding inhibitor of acetylcholinesterase (AChE) was intravenously administered to rat (0.05 mg/kg). Pharmacokinetic profiles were determined in blood and different organs: *extensor digitorum longus* muscle, heart, liver, lungs and kidneys as a function of time. Pharmacokinetics (PK) was studied using non-compartmental and compartmental analyses. A 3-compartment model describes PK in blood. Most of injected C-547 binds to albumin in the bloodstream. The steady-state volume of distribution (3800 ml/kg) is 15 times larger than the distribution volume, indicating a good tissue distribution. C-547 is slowly eliminated ($k_{el} = 0.17\ h^{-1}$; $T_{1/2} = 4$ h) from the bloodstream.

Effect of C-547 on animal model of *myasthenia gravis* persists for more than 72 h, even though the drug is not analytically detectable in the blood. A PK/PD model was built to account for such a pharmacodynamical (PD) effect. Long-lasting effect results from micro-PD mechanisms: the slow-binding nature of inhibition, high affinity for AChE and long residence time on target at neuromuscular junction (NMJ). In addition, NMJ spatial constraints i.e. high concentration of AChE in a small volume, and slow diffusion rate of free C-547 out of NMJ, make possible effective rebinding of ligand. Thus, compared to other cholinesterase inhibitors used for palliative treatment of *myasthenia gravis*, C-547 is the most selective drug, displays a slow pharmacokinetics, and has the longest duration of action. This makes C-547 a promising drug leader for treatment of *myasthenia gravis*, and a template for development of other drugs against neurological diseases and for neuroprotection.

1. Introduction

Translational drug research implies a tight coupling between molecular mechanisms, modeling, pharmacokinetics and pharmacodynamics. This approach has been implemented for the discovery of new drugs for the treatment of *myasthenia gravis* (MG). In this respect, C-547, an alkylammonium derivative of 6-methyluracil (Scheme 1) — potent inhibitor of acetylcholinesterase (AChE) — was considered as a promising drug (Kharlamova et al., 2016; Petrov et al., 2011). Unlike its non-charged analogue (C-35) that is of potential interest for the treatment of Alzheimer disease (Semenov et al., 2015), C-547 does not cross the blood-brain barrier

Abbreviations: ACh, acetylcholine; AChE, acetylcholinesterase; ATC, acetylthiocholine; BBB, blood brain barrier; BChE, butyrylcholinesterase; BK, binding kinetics; BSA, bovine serum albumin; C-547, 1,3-bis[5(diethyl-o-nitrobenzylammonium)pentyl]-6-methyluracil dibromide; ChE, cholinesterase; CMAP, compound muscle action potential; DTNB, dithiobisnitrobenzoic acid; EAMG, experimental autoimmune *myasthenia gravis*; EDL, Extensor digitorum longus muscle; *iso*-OMPA, tetraisopropyl pyrophosphoramide; MG, *myasthenia gravis*; MRT, mean residence time; NMJ, neuromuscular junction; PK/PD, pharmacokinetics/pharmacodynamics; RBC, red blood cell.

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