Impact of Chlorpromazine Self-Association on Its Apparent Binding Constants With Cyclodextrins: Effect of SBE_7-β-CD on the Disposition of Chlorpromazine in the Rat

MICHELLE P. McINTOSH,[1] NATHANIA LEONG,[1] KASIRAM KATNENI,[2] JULIA MORIZZI,[2] DAVID M. SHACKLEFORD,[2] RICHARD J. PRANKERD[1]

[1]Drug Delivery, Disposition and Dynamics, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville Campus, 381 Royal Parade, Parkville, Victoria 3052, Australia

[2]Centre for Drug Candidate Optimisation, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville Campus, 381 Royal Parade, Parkville, Victoria 3052, Australia

Received 30 September 2009; revised 5 November 2009; accepted 20 November 2009

Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/jps.22064

ABSTRACT: Chlorpromazine is an antipsychotic agent with poor aqueous solubility. Complexation with SBE_7-β-CD can aid intravenous delivery through increasing the apparent solubility of chlorpromazine. However, chlorpromazine has also been known to self-associate. This self-association can influence its capacity to interact with other chemical species, such as cyclodextrins. This study aimed to characterise the self-association and cyclodextrin binding properties of chlorpromazine, and the effect on pharmacokinetic parameters in rats when dosed with a SBE_7-β-CD containing formulation. Pharmacokinetic studies of chlorpromazine in the presence and absence of SBE_7-β-CD were undertaken in rats. The binding constant of SBE_7-β-CD and chlorpromazine was studied relative to chlorpromazine concentration via fluorescence. The self-association of chlorpromazine was studied by fluorescence and UV–visible spectrophotometry. Urinary excretion of intact chlorpromazine increased in the presence of SBE_7-β-CD. The SBE_7-β-CD binding constant of chlorpromazine is highly concentration dependent and the variation can be attributed to the self-association of chlorpromazine. The apparent binding constant of chlorpromazine is highest at pharmacologically relevant concentrations, providing an explanation for the significant increase in renal chlorpromazine excretion observed in rats. © 2010 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 99:2999–3008, 2010

Keywords: physicochemical properties; cyclodextrins; physical; characterisation; micelle; formulation vehicle; inclusion compounds; preclinical pharmacokinetics; renal excretion

INTRODUCTION

SBE_7-β-CD has been used as an excipient in injectable formulations to increase the apparent solubility of poorly water-soluble drugs by the formation of drug–cyclodextrin (drug–CD) inclusion complexes. The intermolecular interaction between a drug and CD is often characterised by a binding constant, which is the equilibrium constant representing the affinity of a guest molecule for the CD host.

It has generally been accepted that drug–cyclodextrin complexes dissociate immediately and completely upon administration to the systemic circulation due to the effects of dilution in the blood.[1,2] Recently, a novel antimalarial compound, OZ209, has been reported to exhibit significantly different blood, plasma and urinary pharmacokinetic behaviour following intravenous administration to rats in an aqueous cyclodextrin vehicle, compared to administration of the same compound in a cyclodextrin-free solution.[3] The authors proposed that the altered pharmacokinetic profile was a consequence of an unusually high binding constant for OZ209 with sulfobutylether cyclodextrin (SBE_7-β-CD), which was determined to be $1.6 \times 10^6\,M^{-1}$.[4]

Chlorpromazine is an antipsychotic drug with low aqueous solubility[5] and as such may benefit from formulation with a CD such as SBE_7-β-CD. Given the impact of SBE_7-β-CD on pharmacokinetics of OZ209, pharmacokinetic studies of intravenous (IV) chlorpromazine were conducted in rats using a SBE_7-β-CD based and a CD-free formulation (i.e. the commercial Largactil® formulation) to determine if the presence

Abbreviations: CD; cyclodextrin; CMC; critical micelle concentration; SBE_7-β-CD; sulfobutylether 7-β-cyclodextrin.

Correspondence to: Michelle P. McIntosh (Telephone: +61-3-9903-9531; Fax: +61-3-9903-9583;
E-mail: michelle.mcintosh@pharm.monash.edu.au)

Journal of Pharmaceutical Sciences, Vol. 99, 2999–3008 (2010)

of SBE_7-β-CD impacts on the pharmacokinetics of chlorpromazine. The binding constant for chlorpromazine with SBE_7-β-CD was also determined using fluorescence spectroscopy, although measurement of the binding constant was complicated by the fact that chlorpromazine exhibits self-association behaviour.[6–11] Several publications, using a range of techniques including isothermal titration calorimetry,[10] octanol–water partitioning,[8] surface tension measurements,[7] stearic acid spin label solubility measurements,[9] fluorescence[6] and UV–visible spectrophotometry[11] have reported CMCs for chlorpromazine covering a wide range (Tab. 1). It has been proposed that self-association of phenothiazines, such as chlorpromazine, is a sequential process of adding monomeric units as concentration increases, rather than classical micellisation according to the pseudo-phase separation model, where near monodisperse micelles are spontaneously formed above a critical concentration.[7,12] The self-association of chlorpromazine is a significant factor in physicochemical and biological studies, as it may limit the availability of monomers for biological activity or interaction with other species,[13] such as CDs.

MATERIALS AND METHODS

Materials

Chlorpromazine hydrochloride and potassium fluoride were procured from Sigma-Aldrich (St. Louis, MO), Largactil® (Aventis Pharma Pty Ltd, NSW, Australia) was purchased from a local pharmacy (ampoules containing chlorpromazine hydrochloride solution equivalent to 50 mg base in a 2 mL volume, i.e. 25 mg/mL). SBE_7-β-CD (Captisol®) was a gift from Cydex Pharmaceuticals, Inc. (Lenexa, KS). Isoflurane (Forthane®) was obtained from Abbott Australasia (Kurnell, Australia), Complete® tablets were obtained from Roche Diagnostics (Indianapolis, IN) and pentobarbitone was obtained from Virbac Australia Pty Ltd (Peakhurst, NSW, Australia). Heparin Sodium Injection BP was from Mayne Pharma Ltd (Mulgrave, VIC, Australia) and ethylenediaminetetraacetic acid disodium salt (EDTA) was from Merck Pty Ltd (Kilsyth, VIC, Australia). Sodium dihydrogen orthophosphate (AnalaR, BDH Chemicals Australia Pty Ltd, VIC, Australia); disodium hydrogen phosphate, urea, sodium chloride, dipotassium hydrogen phosphate were obtained from Ajax Chemicals (NSW, Australia) and used as received. Sodium hydroxide (AnalaR) and potassium dihydrogen phosphate (AnalaR) were obtained from Merck (VIC, Australia) and used as received. Phenylalanine (Sigma Chemical Company, St. Louis, MO) and tryptophan (Sigma Chemical Company) were used as received. Water was obtained from a Milli-Q water purification system (Millipore Corporation, Bedford, MA) and organic solvents used for LC-MS analysis were HPLC grade. All other reagents were analytical grade.

Determination of Chlorpromazine *In Vitro* Binding Affinity

Preparation of Solutions

Phosphate Buffer, pH 6.0. Appropriate amounts of disodium hydrogen and sodium dihydrogen phosphates (50 mM total phosphate) and sodium chloride (30 mM) were dissolved in Milli-Q water and pH adjusted to 6.0 with sodium hydroxide before making up the volume to 500 mL.

Urine Substitute Buffer. Appropriate ratios of disodium hydrogen and sodium dihydrogen phosphates (25 mM total phosphate), sodium chloride (25 mM), urea (750 mM), tryptophan (0.01 mM) and phenylalanine (0.015 mM) were dissolved in Milli-Q water and the pH adjusted to 6.0 before making up the volume to 500 mL.

SBE_7-β-CD Solutions. A stock solution of 0.9 mM (2 mg/mL) of SBE_7-β-CD was prepared in pH 6.0 phosphate buffer. The stock solution was subsequently diluted as required for the various chlorpromazine concentrations.

pH Measurements. pH measurement was performed using an Orion 520A+ pH Meter (Thermo Electron Corporation, MA), with a Corning semi-micro combination electrode, calibrated daily using a pH 7.00 standard solution (Merck, VIC). The Nernst

Table 1. Previously Reported CMC Values for Chlorpromazine

Method	Temperature (°C)	CMC (mM)	Other Observations
Photon correlation spectroscopy[7]	25	4.5	CMC decreases as electrolyte concentrations increase
Surface tension[7]	25	2.0	
Isothermal titration calorimetry[10]	25	3.2 (pH 6.5)	CMC increases with temperature
Fluorescence[6]	37	0.05 (pH 7.0)	
Stearic acid spin label solubility measurements[9]	22	0.2 (pH 7.4)	
		2 (pH 5.6)	
UV–Vis spectrophotometry[11]	25	5.6	Association of chlorpromazine below CMC
Gel filtration chromatography[11]	25	5.3	No pH specified