Physicochemical Investigation of the Influence of Saccharide-Based Parenteral Formulation Excipients on L-*p*-Boronphenylalanine Solubilisation for Boron Neutron Capture Therapy

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ABSTRACT: This paper investigates the physicochemical properties of possible pharmaceutical alternatives to L-*p*-boronphenylalanine (BPA)–fructose intravenous formulation currently employed in boron neutron capture therapy. The physicochemical properties of BPA in the absence and presence of fructose, mannitol, trehalose and hydroxypropyl-β-cyclodextrin (HPCD) was investigated by determination of p*K*a values, solubility, precipitation and dissolution using a Sirius T3 instrument. Complex formation was also assessed using ^{10}B-Nuclear magnetic resonance (NMR). The results indicate that fructose and mannitol form a complex with BPA through a reversible interaction with the boronic acid group, determined by changes in the p*K*a of the boronic acid group, the ultraviolet and NMR spectra, and increase in kinetic solubility. Trehalose and HPCD did not undergo this reaction and, consequently, did not affect boronphenylalanaine physicochemical properties. Although mannitol is complexed with BPA in an identical manner to fructose, it is superior because it provides increased kinetic solubility. Replacement of fructose by mannitol in the current clinical BPA formulation is, therefore, feasible with advantages of increased dosing and removal of issues related to fructose intolerance and calorific load. Results also indicated that important pharmaceutical parameters are the complex's solubility and dissociation behaviours rather than, as originally assumed, the complex formation reaction. © 2011 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 101:223–232, 2012

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INTRODUCTION

Boron neutron capture therapy (BNCT) is a niche cancer radiotherapy treatment requiring administration and tumour-selective uptake of a boron-10-enriched compound followed by tumour irradiation with an external epithermal neutron beam. After scattering collisions in the patient, the neutrons become thermalised and BNCT is based upon nuclear reaction, which occurs when a boron-10 atom captures a thermalised neutron and subsequently undergoes a nuclear rearrangement to yield a high linear energy transfer (LET) alpha particle and a recoiling lithium-7 nuclei. The cytotoxic ionising effect of the LET species is limited to its path length in tissues around 5–9 μm or one cell diameter and, therefore, to cells containing the administered boron-10 compound. The therapeutic success of BNCT will depend upon the ability to deliver boron-containing drugs that selectively accumulate within the tumour coupled with tumour accessibility to neutrons. Current clinical application has, therefore, focused on the treatment of intracerebral, head and neck and skin tumours, which cannot be adequately treated, even with aggressive surgery, chemotherapy or conventional radiotherapy, and where the patient prognosis is poor.[1]

Boron-10-enriched L-*p*-boronphenylalanine (BPA) is the most commonly employed BNCT agent for human therapy[2,3] and is also a substrate for L-amino acid transporter, a specific cellular uptake

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mechanism for L-amino acids, which is upregulated in tumour cells including malignant glioma.[4,5] The initial synthesis of BPA was detailed in 1958[6] and it was applied to *in vivo* BNCT studies in the early 1960s,[7] but its aqueous solubility, at 1.6 g L^{-1}, limited its clinical application. In the late 1980s, Yoshino and co-workers[8,9] improved aqueous BPA solubility by utilising the complexation reaction which occurs between boronates and diols to provide a solubility of approximately 33 g L^{-1} in a 0.3 M fructose solution at pH 7.98. Since these initial publications, BPA has most commonly been clinically employed as an equimolar formulation with fructose,[10,11] administered by intravenous (i.v.) infusion at a dose of around 300 mg BPA kg^{-1} over 2 h. There is, therefore, a large body of clinical literature and knowledge based on the utilisation of this formulation.[1] However, the use of fructose in infusion fluids is no longer recommended due to hereditary fructose intolerance[12] which is an exclusion criterion in trials utilizing this BPA formulation.[13] In addition, utilisation of the fructose formulation leads to concomitant administration of approximately 13 gram of fructose per hour with associated calorie and metabolic sequelae. Finally, the BPA–fructose formulation is unstable, requiring relatively complex aseptic preparation no more than 48 h before administration.[14,15] These pharmaceutical limitations of the BPA–fructose formulation restrict its overall acceptability and utility and, in addition, limit the BPA dose which may be administered. This latter issue may limit BNCT efficacy, which is dependent on BPA accumulation in the tumour.[16]

A proposed Cancer Research UK sponsored trial of BNCT in malignant glioma required a BPA formulation that could be prepared to good manufacturing practice standards to meet European Union Clinical Trials Directive requirements. In addition, the ability to administer higher doses than possible with the current fructose formulation was required. In this paper, we investigate the physicochemical properties of the existing fructose formulation in comparison with alternative formulations utilising parenterally acceptable sugar-based excipients, mannitol, hydroxypropyl-β-cyclodextrin (HPCD) and trehalose and the influence of these on the physicochemical properties and solubility of BPA. The possible substitution of fructose with mannitol bears several pharmaceutical and clinical advantages because mannitol removes issues related to hereditary fructose intolerance, calorific load and may improve stability and permit higher BPA doses to be infused. In addition, for glioma therapy, mannitol also has the ability to disrupt the blood–brain barrier, potentially raising the intratumoural concentration of BPA, a feature which is known to improve the therapeutic outcome.[16] Finally, a large body of published clinical data is available on BPA–fructose formulation[1]; demonstration of pharmaceutical equivalence will assist comparison of these data with that of any new proposed formulation.

MATERIALS AND METHODS

Materials

L-*p*-boronphenylalanine (^{10}B) was synthesized by Syntagon AB, (Box 2073, Tallvagen 2, S-151 02, Sweden) and used as received; chromatographic purity ≥98% peak area for BPA, enantiomeric purity ≥99% (w/w), water content <1% (w/w) and ^{10}B isotopic purity >98%. Mannitol and fructose [pharmacopoeial grade (European Pharmacopoeia)] were obtained from Fluka (Sigma–Aldrich, Poole, Dorset, UK); D(+)-trehalose dihydrate 99% was from Acros, Loughborough, Leicestershire, UK, via Fisher Scientific (Loughborough, UK); and HPCD (Cavitron 82004) was from Food and Pharma Specialities division of Cargill (Cedar Rapids, Iowa). Water was either double distilled or obtained using an Elga (Marlow, Buckinghamshire, UK) UHQ2 system; HCl and KOH (CO_2-free ampoule) standard volumetric solutions (0.5 M) were obtained from Fisher Scientific. Nuclear magnetic resonance (NMR) reagents and KCl were obtained from Sigma–Aldrich. Argon gas was obtained from Air Products, Crewe, Cheshire, UK.

Methods

Investigation of BPA Complex Formation

Potentiometric pKa and Spectrophotometric Experiments. All measurements were performed using a Sirius T3 apparatus (Sirius Analytical Instruments Ltd, East Sussex, UK) at a temperature of 25 ± 1°C fitted with an Ag/AgCl double-junction reference electrode, an ultra-mini immersion probe attached to an MMS UV–VIS Carl Zeiss Microimaging spectrophotometer (Welwyn Garden City, Hertfordshire, UK) and a stirrer, controlled by a Dell computer running Sirius software. Potentiometric p*K*a titrations were carried out in ion-strength-adjusted water (0.15 M KCl), titrating with 0.5 M KOH and 0.5 M HCl, respectively, under an Argon atmosphere. Triplicate titrations were carried out in the pH range of pH 1.8 (starting pH) to pH 11 with an initial concentration of BPA of approximately 3.6 mM (0.75 g L^{-1}) in a volume of 1.5 mL. These studies were conducted with BPA alone or in the presence of mannitol, fructose and HPCD. Spectrophotometric data were also collected during titrations to determine changes in BPA ultraviolet (UV) absorbance properties with pH; reference spectra were collected at the start of the titration and data recorded as relative absorbance.

^{10}B Nuclear Magnetic Resonance. A 15% reference solution of boron trifluoride ethyl etherate (BF_3OEt_2)

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