RESEARCH ARTICLE

Suppository Formulations as a Potential Treatment for Nephropathic Cystinosis

BARBARA BUCHAN, GRAEME KAY, KERR H. MATTHEWS, DONALD CAIRNS

Institute for Health & Welfare Research, Robert Gordon University, Aberdeen, UK

Received 22 February 2012; revised 2 May 2012; accepted 8 June 2012

Published online in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jps.23246

ABSTRACT: Nephropathic cystinosis is a rare autosomal recessive disease characterised by raised lysosomal levels of cystine in the cells of all the organs. It is treated by the 6-h oral administration of the aminothiol, cysteamine, which has an offensive taste and smell. In an attempt to reduce this frequency and improve the treatment, cysteamine-containing polyethylene glycol suppositories were prepared and evaluated for dissolution and stability. The results demonstrated that cysteamine release was complete after 30 min, and that there was a uniform drug distribution within the formulations. Twelve-month stability tests highlighted a potential incompatibility among some excipients, although stability was demonstrated for the cysteamine suppositories up to 6 months. These suppositories may provide a useful alternative to the current oral therapy for cystinosis. © 2012 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci

Keywords: Cystinosis; Formulation; PEG; Physical Stability; Dissolution; Thermal Analysis; Pediatric

INTRODUCTION

Nephropathic cystinosis is a rare genetic disease characterised primarily by extremely high intracellular levels of the amino-acid cystine, electrolyte imbalance, proximal renal tubular dysfunction (Fanconi syndrome) and general failure to thrive.[1,2] The accumulation of cystine as crystals in most tissues leads to the progressive dysfunction of multiple organs.[3] The incidence of cystinosis is one in 100,000–200,000 live births, and affects approximately 2000 patients in the world, although there are believed to be many more cases undiagnosed.[2,4] Some infants will die because of dehydration and electrolyte imbalance from Fanconi syndrome without diagnosis.[5] In the US alone, there are 500–600 reported cases, with between 20 and 40 born each year.[5]

Cystinosis is categorised as a lysosomal storage disorder (LSD), which is a group of progressive disorders that share multi-organ failure as an endpoint.[3,5] As with all LSDs, no signs of abnormality are displayed at birth; the first symptoms of glomerular dysfunction begin to appear at around 6–12 months of age.[2] Without treatment most children will reach end-stage renal failure by age nine,[5] and grow at 50%–60% of the expected rate. By the age of eight, an untreated child with cystinosis will be of the height of a 4-year old,[5] and, if inadequately treated, may not reach 4 ft. in height.

The condition is caused by a defect in the *CTNS* gene. This is a gene which codes for a 367-amino-acid-lysosomal-transport protein called Cystinosin. In healthy cells, the amino-acid cystine is transported into the cytoplasm of the cell for processing. In cystinosis, however, it accumulates within the lysosomes to levels of 10–1000 times those seen in healthy cells, whereupon it crystallises from solution, causing cell death and organ failure.[6] This crystallisation is widespread throughout the majority of the tissues and organs in the body.[7] If left untreated, symptomatic deterioration leads to death by the second decade of life.[8]

The treatment for cystinosis involves the administration of the aminothiol cysteamine, which is used therapeutically in the bitartrate form as Cystagon™ (Mylan Laboratories, USA). With early and diligent therapy, cystinosis patients can prevent or delay most of the non-renal complications and extend their lives into a fourth or fifth decade, live independently and some women have borne children.[5,9] The renal damage is continuous; however, the decline is inevitable.[5]

Correspondence to: Graeme Kay (Telephone: +44-1224-262548; Fax: +44-1224-262555; E-mail: g.kay@rgu.ac.uk)

Journal of Pharmaceutical Sciences

Cysteamine causes a range of side effects and this is largely because of the high dose which is required as much of the drug is lost to first-pass metabolism.[9] High blood levels must be achieved, as a large proportion of the administered drug will bind to circulating proteins, and cannot be up taken by the cells.[10,11] Patients should aim to take their dose at regular 6-h intervals for the treatment to work effectively, as the plasma half-life of cysteamine is 1.88 h,[7] with blood levels peaking at 1 h, and rapidly declining thereafter.[5] This is a lifelong commitment and requires the patient to wake in the middle of the night.[9] The drug has a foul taste and smell akin to rotten eggs, which regularly induces vomiting after ingestion.[9] In approximately 10%–15% of patients, this can be severe enough to halt therapy.[5] Cysteamine and its metabolites are excreted in breath and sweat, and this is also an issue, especially when the child enters education. Cysteamine has the potential to cause potentially serious stomach irritations such as gastric acid hypersecretion, reverse peristalsis and bile reflux, and 97% of the patients report gastrointestinal symptoms,[12,13] which in many patients is severe enough to significantly limit the therapy.[14–16] Compliance can therefore be a major barrier to effective treatment, and lead to significant morbidity.

The rectal route of administration can largely avoid the phenomenon of first-pass effect. This results from one of the three rectal veins (upper vein) draining into the hepatic system, whereas the middle and lower veins bypass this and drain directly into the systemic circulation. If the suppository is positioned correctly, the drug should not be subjected to the first-pass effect. This potentially allows a smaller dose to be administered, thereby reducing or eliminating some of the unpleasant side effects. They may also be beneficial for treating conditions in infancy, when capsules are difficult to administer, or when the oral route is compromised. Rectal formulations are useful tools, particularly in a case such as this where the taste and side effects render the task of swallowing a tablet very unpleasant and foreboding.

Aims

Formulation science may provide a way to improve the current medication, significantly improving the lives of sufferers and those who care for them. By eliminating the taste and frequency of administration through alternative dosage forms, ease of administration of cysteamine could be improved. The aim of this work was to develop alternative formulations of cysteamine which could reduce or eliminate some of the side effects experienced using the current oral capsule, thereby improving the quality of life for those affected. An improvement in the ease of administration of cysteamine to infants and young children was a central objective, therefore suppositories were investigated. By avoiding the first-pass metabolism of cysteamine, a lower dose should be achievable, whereas the taste and upper-gastric side effects should be eliminated. A study conducted by van't Hoff et al. was previously undertaken where a cysteamine-loaded suppository gel, for use in cystinosis, was evaluated. However, this rectal formulation was eliminated before cysteamine absorption was completed.[17]

MATERIALS AND METHODS

Materials

Cysteamine hydrochloride and polyethylene glycol (PEG) grades 400, 600, 1000, 1500, 3000, 4000, 6000, 8000 and 14,000 were obtained from Sigma (St. Louis, Missouri). Witepsol W35 was obtained from Gattefosse (St-Priest, France). Gelucire 39/01 was purchased from Sasol GmbH (Witten, Germany). Poloxamer F68 was bought from BASF SE (Ludwigshafen, Germany). Ellman's Reagent, 5,5′-dithiobis(2-nitrobenzoate) (DTNB) was purchased from Molekula (Gillingham, UK). Tris buffer and Tween 80 were bought from Fisher (Loughborough, UK).

Synthesis of N,N-(Bis-L-Phenylalanyl)Cystamine Bistrifluoroacetate (Phenylalanine Conjugate)

Cysteamine does not possess a chromophore and therefore is ultraviolet (UV) transparent; thus, monitoring its release from formulations is very difficult. Initially, a phenylalanine conjugate was developed to tag the molecule, allowing quantitative determination of release of the active from the dosage form via UV spectroscopy (Fig. 1). Cystamine, the oxidised disulphide of cysteamine, was used in the synthesis. The phenylalanine conjugate was subsequently replaced with cysteamine hydrochloride with DTNB detection (see the section *Dissolution Studies*).

To a stirring solution of cystamine dihydrochloride (1 g, 0.00444 mol) in anhydrous dichloromethane (DCM) (20 cm^3) at room temperature, 1,8-diazabicycloundec-7-ene (1.33 mL, 0.0089 mol) was added. The reaction mixture was then stirred at room

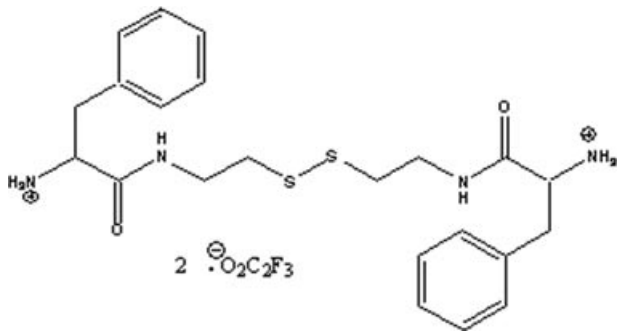

Figure 1. N,N-(bis-L-phenylalanyl)cystamine bistrifluoroacetate, (Phenylalanine conjugate).

 DOI 10.1002/jps