Hydroxy Propyl-Beta-Cyclodextrin

Summary for IND / IRB Submissions

1) Chemistry and Manufacturing

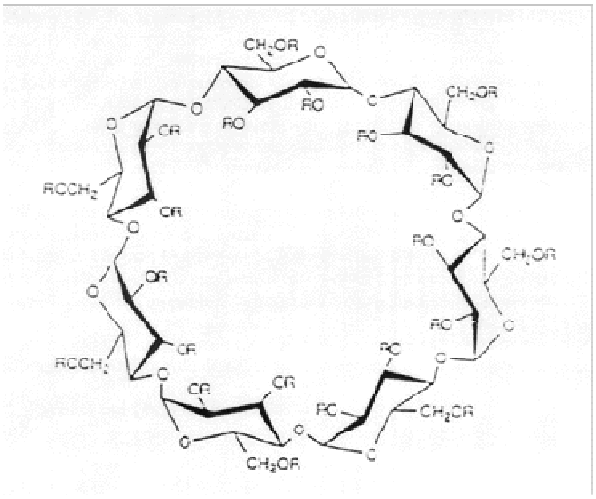
a) Chemistry

Cyclodextrin molecules are relatively large (molecular weight ranging from almost 1000 to > 2000 Da) with a large number of hydrogen donors and acceptors, and are consequently poorly absorbed through biological membranes.

Cyclodextrins are non-reducing cyclic glucose oligosaccharides resulting from the cyclomaltodextrin glucanotransferase (E.C. 2.4.1.19; CGTase) catalyzed degradation of starch. Their structures have been reviewed. There are three common cyclodextrins with 6, 7 or 8 D-glucopyranonsyl residues (α-, β-, and γ-cyclodextrin respectively) linked by α-1,4 glycosidic bonds. The glucose residues have the $^4C_1$ (chair) conformation. All three cyclodextrins have similar structures (that is, bond lengths and orientations) apart from the structural necessities of accommodating a different number of glucose residues.

Hydroxypropyl Beta Cyclodextrin (HPCD) (cas#94035-02-6) is a partially substituted poly(hydroxpropyl) ether of beta cyclodextrin (BCD). The empirical formula is: (C42 H70-n O35) . (C3 H7 O)n It contains not less than 10.0 percent and not more than 45.0 percent hydroxypropoxy(-OCH2CHOHCH3) groups. the structure is shown below where R represents either hydrogen or a hydroxypropoxy group.

R= CH2CH(OH)CH3 or H



The solubility of HPBCD is quite high, exceeding 600 mg/ml. Viscosity is not an issue in concentrations below 55%.

b) Source

Trappsol® brand of endotoxin controlled HPBCD will be obtained from Cyclodextrin Technologies Development, Inc. 27317 NW 78th Ave, High Springs, FL 32643 (386)-454-0887. The product is referenced in Drug Masterfile 10772.

c) Sterile Parenteral Manufacturing

HPBCD for parenteral administration will be compounded by the hospital pharmacy where the drug is administered. The product will consist of HPBCD in sterile water filled under aseptic conditions and subjected to sterility and pyrogenicity testing prior to use.

2) Pharmacology & Toxicology

a) Excipients use

An excellent review of cyclodextrins in drug delivery can be found at:

http://www.hi.is/~thorstlo/general.pdf

Examples of marketed products containing **2-Hydroxypropyl-β-cyclodextrin**

Cisapride	Rectal	Propulsid Europe
Hydrocortisone	Buccal	Dexocort Europe
Indomethacin	Eye drops	Indocid Europe
Itraconazole	Oral, IV	Sporanox Europe, USA
Mitomycin	IV	Mitozytrex USA

b) In Vitro Pharmacology

The physiological importance of cholesterol in the cell plasma membrane has attracted increased attention in recent years. Consequently, the use of methods of controlled manipulation of membrane cholesterol content has also increased sharply, especially as a method of studying putative cholesterol-enriched cell membrane domains (rafts). The most common means of modifying the cholesterol content of cell membranes is the incubation of cells or model membranes with cyclodextrins, a family of compounds, which, due to the presence of relatively hydrophobic cavity, can be used to extract cholesterol from cell membranes. However, the mechanism of this activity of cyclodextrins is not completely established.

Abulrob et.al. (J Neurochem. 2005 Mar;92(6):1477-86) demonstrated the neuroprotective activity of some CD derivatives against oxygen-glucose deprivation (OGD), N-methyl-D-aspartic acid (NMDA) and glutamate in cortical neuronal cultures. Although all CDs complexed with NMDA or glutamate, only beta-, methylated beta- and sulfated beta-CDs displayed neuroprotective activity and lowered cellular cholesterol.

Monnaert et.a. (JPET 310:7445-751, 2004) using an in vitro model of the blood-brain-barrier examined various cyclodextrin derivatives for their toxicity and permeability. The authors concluded that some cyclodextrins cross the BBB slightly in normal conditions.

c) In Vivo Pharmacology

After a single 200mg/KG intravenous dose and rats and dogs 14C-HPBCD was illuminated rapidly (more than 90% in 4 hours), almost completely as the intact compound and mostly by renal excretion. The plasma elimination half-life was 0.4 hours and rats and 0.8 hours and dogs. In both rats and dogs following intravenous administration, tissue distribution was limited: in rats are highest concentrations were in the kidney and lung and in dogs the highest concentration was in the kidney and liver. The total plasma clearance in all species tested are similar to that of insulin and clearance through the kidneys and is independent of dose administered and nearly equivalent to the glomerular filtration rate. Thus the elimination is dependent on renal function.

d) Use in NPC models

Niemann Pick type C is a rare autosomal recessive lysosomal storage disease and is related to mutation of the NPC1 protein responsible for cholesterol trafficking between the late endosomal and lysosomal compartments to the cytosol of all cells. As a result, clinical manifestations are organ dysfunction and ultimately neurological decline

secondary to neurodegeneration. A homozygous mutant mouse (NPC-/-) has been identified that manifests many of the same defects seen in humans. This model has been used to elucidate the various functioning of the NPC1 gene and to screen for compounds with potential therapeutic potential.

Liu et.al. (J. Lipid Res, 2008,vol 49, pp663-669) demonstrated that a single 4000 mg/kg s.c. injection of HPBCD at 7 days of age prolonged the life of NPC-/- mutant mice. The effect size in that study, however, was a modest 15%. More recently, Walkley's group at Albert Einstein report (Abstract, Lysosomal Disease Network Annual Meeting, February 18-20, 2009) that NPC-/- mice treated daily from day 7 with 4000 mg/kg HPBCD demonstrated a 3 week delay in onset of clinical symptoms and survived more than double that of untreated NPC-/- mice.

Camargo et.al. (Life Sci. 2001, 70(2):131-42) found a similar small effect of HPBCD. They concluded the slight effects of the HPBCDs on neurological symptoms may be partially due to their apparent non-permeation of the blood-brain barrier (BBB). Intrathecal delivery of HPBCD by an Alzet osmotic minipump did not improve its efficacy in ameliorating neurological symptoms.

Mellon allopreganolone studies with HPPBCD as dilluent time course.

e) Animal toxicology

For an excellent review see: Gould and Scott "2-Hydroxypropyl-beta-cyclodextrin: A toxicology review (Food and Chemical Toxicology 43 (2005) 1451-1459.

i) Oral

Acute toxicity testing has not determined an LD(50) for HPBCD. 5000mg/kg have been administered orally to rats and no mortality was observed. (1)

C14 labeled HPBCD has been administered orally to determine the metabolic fate of the HPBCD Most of the label was excreted in the feces. 3-6% of the label was absorbed and some label appeared in the blood about five minutes after administration indicating some absorption from the stomach. About 3% of the label appeared in the urine and another 3.25% in the exhaled CO2. The HPBCD preparation did contain some propylene glycol that was also labeled. The amount of label absorbed corresponded to the amount of proplyene glycol present.

Teratogenicty and embryotoxicity studies have been done in rats and rabbits at doses up to 5000 mg/Kg per day in rats and 1000mg/Kg per day in rabbits. No maternal

toxicity, embryotoxicity or teratogenicity was found in rats. In rabbits, no teratatogenicity was observed, but slight maternal and embryotoxicity was observed at 1000 mg/Kg.

Chronic studies were done using both mice and rats. The study with mice was terminated after 104 weeks. The mice received 500mg/Kg HPBCD. No adverse histopathology was noted that could be attributed to HPBCD and the mice given HPBCD had a lower incidence of tumors than the controls. A two-year study was also done with rats with a dose of 500 mg/Kg per day. No effects were observed that were attributed to HPBCD.

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ii) Parenteral

In an acute study with cynomologus monkeys, a dose of 10, 000 mg/Kg was not lethal

HPBCD is quickly cleared after parenteral administration. After a single intravenous administration of (14)C labeled HPBCD, a half-life in the plasma of 0.4 hours and 0.8 hours was found for rats and dogs respectively. A plasma clearance of 512 and 188 ml/kg/h was calculated for rats and dogs.

The subchronic toxicity studies with rats, no adverse effects were found in rats treated intravenously with 50 mg/Kg HPBCD. At 100 mg/Kg HPBCD. At 100 mg/Kg some minimal histological changes were found in the epithelial cells of the urinary bladder, kidney tubular cells and in the liver. At 400 mg/Kg, there was a decreased body weight and food consumption, increased water consumption, decreased hematocrit, hemoglobin and erythrocyte levels, increased creatinine, total bilirubin and aspartate and alanine aminotransferase levels. Some organ weights also increase. Most of these changes were reversible after one month except for slightly elevated aspartate and alanine aminotransferase levels and histological changes in the lung and urinary tract that were only partially reversible.

In subchronic toxicity studies, no adverse effects were found in rats treated intravenously with 50mg/Kg or in dogs receiving 100 mg/KG HPBCD. At 400 mg/Kg in dogs there were slight increases in serum alanine and aspartate aminotransferase and total bilirubin. Histological changes werefound in the lung and epithelial cells of the urinary bladder and renal pelvis. All of the changes were reversed within a month after treatment except for incomplete re- versibility of the swollen renal plevis epithelium.

Teratogenicity and embryotoxicity studies have been done in rats and rabbits at doses up to 400mg/Kg per day. Slight maternal toxicity was observed in rats at 400mg/Kg but there were no primary adverse effects in the offspring. No adverse effects were observed in the rabbits.

iii) Dermal

Dermal irritation studies were done with albino rabbits. No erythema, edema or other dermal effects were observed and the material was considered to be a nonirritant to the skin. A dermal sensitization study was done using guinea pigs. No irritation was detected upon the initial intradermal injection of HPBCD. Upon challenge of applying HPBCD to the surface of the skin, low incidences of very faint erythema were found in 30% of the animals at 48 hours and 10% of the animals at 72 hours.

iv) Mutagenicity

HPBCD is nonmutagenic. Mutagenicity was tested using S. typhimurium and E. coli WP2 strains both with and without microsomal activation and found to be non-mutagenic. Testing with mammalian cell culture, with and without microsomal activation, also found HPBCD to be non-mutagenic.

f) Human pharmacology & toxicology

A number of clinical studies are reported literature and have shown that HPBCD was well-tolerated in safe in the majority of patients receiving HPBCD at daily oral doses of the 4-8 g for at least two weeks (Irie, T and Uekama,K. (1977) J. Pharm. Sci 86, 147-162.). Higher moral daily doses of 16 to 24 g and use for 14 days to volunteers resulted in increased incidence is of sauce tools in diarrhea. Therefore based on these clinical data, HPBCD was considered to be non toxic release for 14 days if a daily dose is less than 16 g.

In an intravenous dose and study single doses up to 3 g were found and no measurable effect and kidney function and well tolerated by all volunteers (Seiller et.al (1990) in: Duchene, D. (Ed). Minutes of the 5th International Symposium on cyclodextrins, Editions de Sante. Paris, pp 518-540). Following one week intravenous study and a single dose level 1 g no adverse effects were reported (Janssen Technical Bulletin,(1992). Encapsin HPB hydroxypropyl-beta-cyclodextrin. A real solution for real drug delivery problems. Janssen Biotech. N.V. pp 1-7)

The pharmacokinetics of HPBCD have been studied and healthy volunteers after single intravenous and oral dosing. Following intravenous dose in at 0.5, 1.0, 1.5, 2.0, 2.5 or 3 g, plasma levels of the unchanged HPBCD declined rapidly and showed a bi-phasic decline. There were no differences between males and females and dose proportionality was demonstrated. Pharmacokinetic parameters such as half-life clearance and the Vdss were shown to be independent of dose and urine levels suggesting that elimination was almost totally via the kidneys and no sign of tubular

reabsorption. Following oral administration HPBCD could not be detected in either the plasma after 1 hour or urine indicating that there was no absorption from the GI tract and that oral bioavailability in humans was low (Szathmary, S.C et.al. (1990) in: Duchene, D. (Ed). Minutes of the 5th International Symposium on cyclodextrins, Editions de Sante. Paris, pp 535-540).

3) Pediatric therapeutic use

Carpenter et. Al. (J. Pediatrics, 1987, pp507-512) reported treating a 3 year old hypervitaminosis A patient with infusions of HPBCD in an effort to solubilize retinoids and enhance their urinary excretion. The patient received a continuous intravenous infusion at 470 mg/kg/24hrs for a total of 30 g over 4 day period in the form of 5% aquous solution in water.. Other than generalized irritability and leg pain associated with vitamin A toxicity, no adverse events were reported, although cholesterol levels decreased by 20-30% during the cyclodextrin infusions.

HPBCD has been used in children as young as 7 month as a solubilizer for the parenteral administration of anti-fungal agents. Abdel-Rahman et. al. (Antimicrobial Agents and Chemotherapy, 2007, pp2668-2673, vol 51 #8) safely administered itraconazole 2.5 mg/kg in 0.1 –g/kg HPBCD as a 1 hr intravenous infusion. The authors measured HPBCD pharmacokinetics and observed that levels fell below quantifiable limits by 12 hrs. The volume of distribution approximated the extracellular fluid space. The total plasma clearance of HPBCD approximated estimates of the glomerular filtration rate. The population estimated value of clearance (CL) was 5.27 liters/hr for a 30-kg child.

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