



Review

2-Hydroxypropyl- β -cyclodextrin (HP- β -CD): A toxicology review

Sarah Gould *, Robert C. Scott

Safety Assessment, AstraZeneca UK Limited, Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG, United Kingdom

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Abstract

2-hydroxypropyl- β -cyclodextrin (HP- β -CD) is an alternative to α -, β - and γ -cyclodextrin, with improved water solubility and may be more toxicologically benign. This paper reviews the toxicity of HP- β -CD, using both literature information and novel data, and presents new information. In addition, it includes a brief review from studies of the metabolism and pharmacokinetics of HP- β -CD in both humans and animals.

This review concludes that HP- β -CD is well tolerated in the animal species tested (rats, mice and dogs), particularly when dosed orally, and shows only limited toxicity. In short duration studies, there were slight biochemical changes whereas studies of a longer duration, up to three months, produced additional minor haematological changes but no histopathological changes. When dosed intravenously, histopathological changes were seen in the lungs, liver and kidney but all findings were reversible and no effect levels were achieved. The carcinogenicity studies showed an increase in tumours in rats in the pancreas and intestines which are both considered to be rat-specific. There were also non-carcinogenic changes noted in the urinary tract, but these changes were also reversible and did not impair renal function. There were no effects on embryo-foetal development in either rats or rabbits.

HP- β -CD has been shown to be well tolerated in humans, with the main adverse event being diarrhoea and there have been no adverse events on kidney function, documented to date.

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Contents

1. Introduction	1452
2. Toxicology of HP- β -CD	1452
2.1. General toxicology	1452
2.1.1. Intraperitoneal administration	1452
2.1.2. Intravenous administration	1452
2.1.3. Acute intravenous studies	1452
2.1.4. Short-term studies	1453
2.1.5. Chronic oral administration (via diet)	1455
2.2. Genetic toxicity	1456
2.3. Carcinogenicity studies	1456
2.4. Developmental toxicity	1456

* Corresponding author. Tel.: +44 1625 512 648; fax: +44 1625 516 809.
E-mail address: sarah.gould@astrazeneca.com (S. Gould).

3. Human toxicity profile	1456
4. Animal pharmacokinetic and metabolic profile	1456
5. Human pharmacokinetic and metabolic profile	1457
6. Discussion	1457
References	1458

1. Introduction

Cyclodextrins (CDs) are useful formulation vehicles, which increase the amount of drug that can be solubilised in aqueous vehicles, thus increasing delivery of many useful medicinal agents to a biological system. Without a successful delivery system, many drugs could not be developed.

Cyclodextrins are cyclic amylose-derived oligomers composed of a varying number of α -1-4-linked glucose units. These glucose chains form a cone-like cavity into which compounds may enter and form a water-soluble complex and thus change the drug's physical-chemical properties. The number of units determines the size of the cone-like cavity and its corresponding name (Szejtli, 1998; Uekama et al., 1998). For example, the most common cyclodextrins used as formulation vehicles are α -, β - and γ -cyclodextrin, with the corresponding number of glucose units ($\alpha = 6$, $\beta = 7$, $\gamma = 8$). These cyclodextrin molecules, although similar in their unit make-up, possess slightly different absorption rates, possibly due to differences in degradation processes (Antlsperger and Schmid, 1996).

α -, β - and γ -cyclodextrins are all used successfully to incorporate drugs into aqueous vehicles (Antlsperger and Schmid, 1996) and their toxicity profile has been studied extensively (WHO, 1993; reviewed by Antlsperger and Schmid, 1996). The toxicity profile of CDs can differ depending on the route of administration. For example, β -cyclodextrin administered orally, induces limited toxicity (Olivier et al., 1991; Bellringer et al., 1995) and in both rats and dogs is considered non-toxic at a daily dose of less than 600 mg/kg bw or 3% and less in the diet (Fromming and Szejtli, 1996). However, if β -cyclodextrin is administered at higher doses in animals via a subcutaneous route, it will cause a decrease in body weight gain, a decrease in liver weight, and nephrotoxicity, with an increase in kidney weight, proximal tubular nephrosis and cellular vacuolation (Perrin et al., 1978; Fromming and Szejtli, 1996). Parenteral administration also induces similar changes to the kidney proximal tubules (Frank et al., 1976).

2-hydroxypropyl- β -cyclodextrin (HP- β -CD), a hydroxyalkyl derivative, is an alternative to α -, β - and γ -cyclodextrin, with improved water solubility properties (Uekama et al., 1998; Yoshida et al., 1988) and may be slightly more toxicologically benign.

This paper reviews the toxicity of HP- β -CD, using literature information together with novel in-house study data generated within AstraZeneca using HP- β -CD to assess the toxicology of a potential new drug with low water solubility. These new data add to the literature information regarding HP- β -CD and reduce the need for further assessments of this component in novel vehicles. A brief review of the metabolism and pharmacokinetics of HP- β -CD, with variants on the percentage administered and the route of administration is also included, as well as a limited review of human safety.

2. Toxicology of HP- β -CD

A number of toxicity studies have been conducted with HP- β -CD by either oral or intravenous administration in a variety of species including mice, rats, monkeys and dogs for up to a period of 12 months. In addition, carcinogenicity, genetic toxicology and developmental toxicity studies have also been done. AstraZeneca (AZ) completed a number of additional studies in rats and dogs up to a period of 1 month dosing. These studies are reviewed and summarized below (Table 1 summarizes data from AZ; Table 2 summarizes previously published data).

2.1. General toxicology

2.1.1. Intraperitoneal administration

In mice, up to 10,000 mg/kg bw HP- β -CD has been administered acutely by intraperitoneal (i.p.) injection and was neither lethal nor did it produce any toxicity (Fromming and Szejtli, 1996).

2.1.2. Intravenous administration

The intravenous administration of HP- β -CD has been studied in mice, monkeys, rats and dogs after single or repeated doses for up to 90 days.

2.1.3. Acute intravenous studies

In the *Cynomolgus* monkey, a single intravenous dose of 10,000 mg/kg of 50% w/v HP- β -CD was not lethal (Brewster et al., 1990). In mice, a single intravenous dose of up to 2000 mg/kg bw was also not lethal (Fromming and Szejtli, 1996).

Table 1
Summary of general toxicity studies with HP- β -CD (previously unpublished data from studies conducted by AstraZeneca)

Route	Study duration (days)	Species	Animal nos.	Dose (%) mg/kg/day	Data
i.v.	1	Rat	5	2250 (45%)	Premature deaths, adverse clinical signs: decreased activity, breathing irregularities
	1	Rat	5	1000 (20%)	No premature deaths, no adverse clinical signs
	7	Rat	10	225 (11.3%)	Histopathology: foamy macrophage infiltration, alveolitis haemorrhage, actaetasis, renal cortical tubular vacuolation, of proximal tubs, mild reduced splenic extramedullary haematopoiesis
	4	Rat	3	225 (11.3%)	Histopathology: foamy macrophage infiltration, alveolitis haemorrhage, actaetasis, renal cortical tubular vacuolation of proximal tubs, mild reduced splenic extramedullary haematopoiesis
	4	Rat	3	7200 (15%)	Histopathology: foamy macrophage infiltration, alveolitis haemorrhage, actaetasis, renal cortical tubular vacuolation of proximal tubules, mild reduced splenic extramedullary haematopoiesis
	7	Rat	3	2400 (5%)	Reduction in water consumption, reduced plasma cholesterol, increase in relative kidney weight, moderate renal cortical tubular vacuolation
Oral	7	Rat	10	4500 (45%)	Increases in ALT, AST, GLDH
	7	Rat	10	450 (45%)	NOEL
				2250 (45%)	Loose faeces, clinical pathology (increases in AST, ALT)
				4500 (45%)	Loose faeces, clinical pathology (increases in AST, ALT)
	28	Rat	20	450 (45%)	Increase ALT
				4500 (45%)	Increase in water consumption, Loose faeces, increases in lymphocytes, reduction in reticulocyte, HCT, increase in platelet count, increases in ALP, ALT, AST, reductions in creatinine, triglycerides, reduction in glucose concentration
MTD/14	Dog	6	540 (45%)	NOEL. No toxicological effects	
28	Dog	6	2250 (45%)	NOEL. No toxicological effects	

NOEL: no observed effect level; ALT: amino transferase; ALP: amino phosphatase; AST: aspartame transferase; MTD: maximum tolerated dose.

In AZ acute studies (see Table 1) in the rat (Alpk: AP_fSD; Wistar derived rat), a single intravenous dose of 2250 mg/kg of 45% w/v HP- β -CD was not tolerated: there were premature deaths and adverse clinical signs, including decreased activity, breathing irregularities and the animals were cold to touch (TLR3056). However, when the HP- β -CD was reduced to 1000 mg/kg of 20% w/v HP- β -CD, in a repeat study, there were no premature deaths and no adverse clinical signs. These acute studies included measurement of body weight and food consumption, in-life clinical observations and macroscopic examinations.

2.1.4. Short-term studies

AZ has completed a number of short-term studies (Table 1) in which several parameters were measured to determine overt toxicity including body weight and food consumption, in-life observations, haematology (haemoglobin, haematocrit, total red blood cell count, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, mean corpuscular volume, platelet count, total leukocyte count, lymphocytes, monocytes, neutrophils, basophils) and clinical chemistry parameters (glucose, urea, creatinine, total protein, albumin, total bilirubin, alkaline phosphatase, alanine transferase, aspartate transferase, sodium, potassium, cholesterol, glutamate dehydrogenase, triglycerides), bone marrow smear, organ weights (liver, kidney), microscopic exami-

nation of pelvic, thoracic and abdominal cavities and macroscopic examinations of the following tissues: adrenal glands, bone, brain, epididymides, eyes, heart, intestines, kidneys, liver, lung, pancreas, lymph nodes, pituitary gland, prostate, salivary gland, skeletal muscle, skin, spinal cord, spleen, stomach, testes, thymus, thyroid, tracheas, thyroid gland, tongue, urinary bladder, uterus, vagina, and any gross lesions noted in macroscopic examination.

2.1.4.1. Intravenous administration. Alpk:AP_fSD (Wistar-derived) rats were continuously infused for either 4–7 days with 225 mg/kg/day of 11.25% w/v HP- β -CD via the femoral vein. Histopathology changes included foamy macrophage infiltration of the lung, with some associated alveolitis haemorrhage, and atelectasis. In addition, renal cortical tubular vacuolation of the proximal convoluted tubules and mild reduced splenic extramedullary haematopoiesis were also noted. Similar histopathology changes were also recorded in rats dosed with 15% HP- β -CD and 2.5% dextrose for 4 days (Table 1).

In a 7-day rat study (Alpk:AP_fSD; Wistar-derived) 2400 mg/kg/day of 5% w/v HP- β -CD was administered by continuous intravenous infusion. In this study, there was a reduction in water consumption, reduced plasma cholesterol and minor changes in the kidney, which included an increase in relative kidney weight

Table 2
Data from toxicology studies conducted with HP- β -CD (external publications)

Route	Study duration	Species	Animal no's	Dose mg/kg/day	Data	Source	
i.v.	1	Monkey	4	10,000	No deaths	Brewster et al., 1990	
		Mouse	Unknown	2000	No deaths	Fromming and Szejtli, 1996	
	14/90	Rat	10	200	No toxicological effects	Brewster et al., 1990	
		Rat	Unknown	100	Swollen epithelial bladder cells, swollen and granular kidney tubular cells, increase in Liver Kupffer cells	Coussement et al., 1990	
	14/90 day	Rat	Unknown	400	Reduced body weight, food consumption, increase water consumption, decrease haematology parameters, and clinical chemistry, increase in spleen, adrenals kidneys, foamy cells in lungs, spleen hyperplasia, increased RES aggregates in the liver	Coussement et al., 1990	
					No significant toxicity		
	3 month	Monkey	4	200	NOEL	Brewster et al., 1990	
		Rat	Unknown	50 100	Minimal histological change in the bladder, kidney and liver	Coussement et al., 1990	
			Dog	Unknown	100	NOAEL	Coussement et al., 1990
					400	Slight increase in plasma liver enzymes and histopathology in lung, bladder and pelvis	
i.p.	Acute	Mouse	Unknown	1000	No deaths	Fromming and Szejtli, 1996	
Oral	1 year	Rat	100	500	NOEL	Van Cauteren et al., 1997	
				2000	Small reduction in body weight, minor haematology and clinical chemistry changes (including increased plasma liver enzymes) and histology changes urinary tract, liver, pancreas		
		Dog	Unknown	1000 2000	NOEL Loose faeces, urinary tract histopathology	Van Cauteren et al., 1997	

OEL : no observed effect level.

(compared with body weight) and moderate renal cortical tubular vacuolation. In addition, mild foamy alveolar macrophages in the lung were also detected (Table 1).

In a 14-day subacute and 90-day subchronic intravenous toxicity studies, 200 mg/kg of 20% w/v HP- β -CD was administered to Sprague Dawley rats and *Cynomolgus* monkeys on alternate days (doses were given every second day). In these studies, there were no toxicologically adverse effects on the following parameters: body weight, body weight gain, food consumption, haematology, clinical chemistry, organ weights (cf brain or body weights) and macroscopic or microscopic histopathology (including kidney) (Brewster et al., 1990).

Two, three month intravenous dosing studies were conducted in rat and dog, and for both studies, HP- β -CD was administered at doses of 50, 100 or 400 mg/kg/day. In the rat, there were no adverse findings at 50 mg/kg/day. At 100 mg/kg/day, there were minimal histological changes in the urinary bladder (swollen epithelial cells), swollen and granular kidney tubular cells and an increase in Kupffer cells in the liver. At 400 mg/kg/day, there was a decrease in body weight

and food consumption, increase in water consumption, a decrease in haematocrit, haemoglobin and erythrocytes, and an increase in creatinine, bilirubin and aspartate and alanine aminotransferase plasma levels (AST and ALT, respectively). The weight of the spleen, adrenals and kidneys increased and histopathological changes included foamy cells in the lungs, increased spleen red pulp hyperplasia, increased rough endoplasmic RES aggregates in the liver and increased round cells in the Kieran space of the liver. Following a one month recovery period, most of the toxicological changes had reversed. However, there were still small elevations in AST and ALT levels and only a partial reversal of the urinary tract bladder and lung changes. In the dog, there were no adverse effects at 50 or 100 mg/kg/day. At 400 mg/kg/day, there were slight increases in ALT and AST and total bilirubin. Histological changes were seen in the lung (foamy cell) and there were swollen epithelial cells of the urinary bladder and renal pelvis. At the end of the recovery period, the toxicological changes had completely reversed except for an incomplete recovery of the swollen renal pelvis epithelium (Coussement et al., 1990).

2.1.4.2. Oral administration. AZ has completed subacute and subchronic toxicity studies in rats and dogs, which assessed the toxicity of HP- β -CD. Animals were weighed and food consumption monitored, standard haematology (red blood cell count, haemoglobin, haematocrit, mean cell volume, mean cell haemoglobin, mean cell haemoglobin concentration, total white blood cell count, differential white blood cell count, platelet count, prothrombin time and activated partial thromboplastin time), clinical chemistry (glucose, urea, creatinine, total protein, albumin, total bilirubin, alkaline phosphatase, alanine transferase, aspartate transferase, sodium, potassium, cholesterol, glutamate dehydrogenase, triglycerides) and urinalysis were completed both pre-study and during compound administration. At termination, organ weight measurements were taken (for 7-day studies only liver and kidney were taken) for 1-month studies organs weights included: adrenal glands, brain, epididymides, heart, kidney, liver (minus gall bladder), lungs, ovaries, pituitary, prostate gland, spleen, testes, thyroid glands (with parathyroid) and uterus (with cervix). Macroscopic and microscopic examinations were completed (see 2.1.4 for details for 7-day studies; for one-month studies the following tissues were taken: abnormal tissues, adrenal glands, aorta (thoracic) bone (femur) bone marrow (sternum), brain (whole), bronchus, cervix, epididymides, eyes, eyelids, gall bladder, heart (portions from 4 chambers and papillary muscle) intestine-duodenum, intestine-colon, intestine-rectum, kidneys, lacrimal glands, liver (left lateral and right median lobes), lungs, lymph node-bronchial, cervical, mesenteric, mammary gland (female), muscle (quadriceps), nerve (sciatic), oesophagus, ovaries, pancreas, pituitary gland, prostate gland, salivary gland-parotid, salivary gland-sublingual, salivary gland-submaxillary, skin (lateral thigh), spinal cord (lumbar and cervical) spleen, stomach, fundic area, stomach pyloric area, testes, thymus gland, thyroid and parathyroid, tongue, trachea, urinary bladder, uterus and vagina. For the dog studies, electrocardiogram and direct blood pressure measurements were also made at the start and end of the study period. Heart rate, P-R, QRS and Q-T intervals were derived from the ECG traces.

In a 7-day oral study in rats (Alpk:AP_fSD; Wistar-derived) 4500 mg/kg/day 45% w/v HP- β -CD caused changes in the plasma ALT, AST and glutamate dehydrogenase (GLDH) levels. The changes were particularly apparent in the females, but these changes were not accompanied by any histopathological changes (Table 1).

In another rat study, AZ administered 450, 2250 or 4500 mg/kg/day 45% w/v HP- β -CD to rats (Alpk:AP_fSD; Wistar-derived) for 7 days and 4500 mg/kg/day 45% w/v HP- β -CD for 14 days and showed that HP- β -CD was well tolerated at doses of up to 4500 mg/

kg/day. There were no toxicological findings at doses of 450 mg/kg/day and no treatment related effects seen on body weights, food or water consumption. For doses of 2250 mg/kg/day and above, loose faeces were seen from day 2. Clinical pathology showed marked increases in plasma GLDH activity at both 2250 and 4500 mg/kg/day and minor increases in plasma AST and ALT levels from day 4 in males dosed with 4500 mg/kg/day and day 8 in females dosed with 2250 mg/kg/day. There were no necropsy or histological changes in the liver, including at the electronic microscopy level or in the kidney (Table 1).

In a one-month rat study, Alpk:AP_fSD (Wistar-derived) rats were dosed orally with 450 and 4500 mg/kg/day 45% w/v HP- β -CD. Loose faeces were observed in animals dosed with 4500 mg/kg/day HP- β -CD from day 13. All male rats dosed with HP- β -CD showed an increase in water consumption and both sexes at 4500 mg/kg/day showed an increase in white blood cells (WBC) attributed to an increase in lymphocytes. Changes in red cell parameters were also detected, including a small reduction in reticulocytes and haematocrit, a slight increase in platelet count in males dosed with 4500 mg/kg/day and a decrease in haemoglobin (Hb) in males dosed with 450 mg/kg/day. Increases in plasma liver enzymes were detected in all animals dosed with HP- β -CD, including: increases in plasma ALP activity in males dosed with 4500 mg/kg/day; increases in plasma ALT activity in females dosed with 450 mg/kg/day and in both sexes dosed with 4500 mg/kg/day; an increase in AST activity in both sexes dosed with 4500 mg/kg/day and an increase in glutamate dehydrogenase (GLDH) activity in both sexes dosed with 4500 mg/kg/day. There were slight reductions in plasma creatinine and triglycerides concentrations in males dosed with 450 and 4500 mg/kg/day HP- β -CD and a reduction in plasma glucose concentration was detected in females dosed with 4500 mg/kg/day (Table 1).

In the Beagle dog, AZ conducted a maximum tolerated dose study for up to 14 days. The dose was with McIlvaines buffer containing a 540 mg/kg/day 45% w/v solution of HP- β -CD and there were no toxicological effects (Table 1). AZ also conducted a one-month oral toxicity study in Beagle dogs with 2250 mg/kg/day 45% w/v HP- β -CD and found no toxicological effects (Table 1).

2.1.5. Chronic oral administration (via diet)

Twelve-month oral toxicity studies in rat and dog were reported in the literature.

In the 12-month rat (Wistar) study, HP- β -CD was administered via the diet at doses 500, 2000 and 5000 mg/kg/day. At 500 mg/kg/day there were no toxicological effects. At 2000 mg/kg/day there was a small reduction in male body weight, increased serum chloride and liver plasma enzymes, reduced urinary pH and

volume in males, slight increase in pancreas weight and histopathology changes (swollen urinary tract epithelial cells, centrilobular swelling in the liver and focal hyperplasia in the pancreas). At 5000 mg/kg/day, toxicity was more pronounced with, in addition to the above changes, an increase in female body weight, and increase in food and water consumption, increase in white blood cells, decrease in thrombocytes, decrease in lipids, occult urinary blood in females, increased pancreas, kidney and lung weights, and increases in foamy cells in the lung (Van Cauteren, personal presentation, 1997).

In the 12 month dog study, HP- β -CD was administered by oral gavage at dose levels of 500, 1000 and 2000 mg/kg/day. The no effect dose level was 1000 mg/kg/day. The higher doses showed softened faeces and urinary tract histological changes. (Van Cauteren, personal presentation, 1997).

2.2. Genetic toxicity

The available literature reports were limited in detail. In an Ames assay (up to 1000 μ g/plate) and an in vivo micronucleus test (up to 5000 mg/kg/day; species unknown) there was no evidence that HP- β -CD was genotoxic (Coussement et al., 1990). HP- β -CD has also been reported to be negative in an unscheduled DNA synthesis test (UDS) assay (for DNA damage), a mouse lymphoma assay (for gene mutation) and in a human lymphocyte test (for chromosomal aberration) (Van Cauteren, personal presentation, 1997).

2.3. Carcinogenicity studies

There are reports in the literature of the findings from an 18-month mouse (Swiss strain) and a 2-year rat (Wistar Strain) carcinogenicity study, which both dosed HP- β -CD in the diet, at dose levels of 500, 2000 and 5000 mg/kg body weight/day.

In the mouse study, there was no effect on survival and no increase in total tumour incidence of individual tumour type and thus; the study concluded there was no evidence of primary carcinogenic potential in the mouse (Van Cauteren, personal presentation, 1997).

In the 2-year rat study, there was no effect on survival or increase in total tumour incidence at doses of up to 5000 mg/kg body weight/day. However, there were some changes including: increases in polypoid tumours of the large intestine at the high dose (incidence 0/100 controls, 4/50 males, 2/50 females), tumours of the exocrine pancreas at all dose levels and changes in the urinary tract (swelling and vacuolation of renal cortical tubules, urothelium of pelvis and urinary bladder, enlargement of secondary lysosomes filled with heterogeneous inclusions). Changes in the pancreas were initially seen at 12 months, with exocrine pancreatic hyperplasia, which

developed to exocrine pancreatic neoplasia by 24 months. Changes in the urinary tract were also seen by light microscope as swelling and vacuolation in cells of the renal cortical cells, urothelium of pelvis and urinary bladder. When examined by electron microscopy, there was evidence of enlarged secondary lysosomes filled with heterogeneous inclusions (Van Cauteren, personal presentation, 1997).

2.4. Developmental toxicity

Developmental toxicity studies, in rats and rabbits using either oral or intravenous administration are reported in the literature.

In an intravenous embryo-foetal development study in rats (dosing day 6–16 of pregnancy), 400 mg/kg/day caused slight maternal toxicity, but there were no adverse effects observed in the offspring. In a similar study in rabbits (dosing day 6–18 of pregnancy), there were no adverse effects at doses of up to 400 mg/kg/day (Coussement et al., 1990).

In an oral teratogenic and embryotoxicity study in rats (dosing day 6–16 of pregnancy), maternal toxicity, embryotoxicity and teratogenicity were not present at doses of up to 400 mg/kg/day. In an oral study in rabbits (dosing day 6–18 of pregnancy), slight maternal toxicity and embryotoxicity was present at 1000 mg/kg (Coussement et al., 1990).

3. Human toxicity profile

A number of clinical studies are reported in the literature and have shown that HP- β -CD was well tolerated and safe in the majority of patients receiving HP- β -CD at daily oral doses of 4–8 g for at least 2 weeks (Irie and Uekama, 1997). Higher oral daily doses of 16–24 g when given for 14 days to volunteers, resulted in increased incidences of soft stools and diarrhoea. Therefore, based on these clinical data, HP- β -CD was considered to be non-toxic (at least for 14 days) if the daily dose is <16 g.

In an intravenous dosing study (Seiller et al., 1990) single doses up to 3 g were found to have no measurable effect on kidney function and were well-tolerated by all volunteers. Following a 1 week intravenous study at a single dose level of 1 g, no adverse effects were reported (Janssen Technical Bulletin, 1992).

4. Animal pharmacokinetic and metabolic profile

The pharmacokinetics and metabolism of HP- β -CD have been examined in rats and dogs following single and repeat intravenous (Monbaliu et al., 1990) and oral administration (Monbaliu et al., 1990; Gerloczy et al., 1990).

After a single 200 mg/kg intravenous dose in rats and dogs, ^{14}C -HP- β -CD was eliminated rapidly (more than 90% in 4 h), almost completely as the intact compound and mostly by renal excretion. The excretion in faeces and expired air was minimal. The plasma elimination half-life was 0.4 h in rats and 0.8 h in dogs. After oral administration of HP- β -CD in both rats and dogs, 86% was excreted via the faeces in both species, where as less than 5% was excreted in the urine. In rats, about 10% of the administered dose was found in the expired air. The absolute bioavailability was calculated from this study and estimated at 3.3% in the dog and less in the rat. In both rats and dogs following intravenous administration, tissue distribution was limited: in rats the highest concentration was found in the kidney and lung and in dogs, the highest concentrations were in the kidney and the liver. The concentration of HP- β -CD was higher in the renal cortex than the medulla. A 3-month intravenous toxicity study was conducted in both rat and dog at doses of 50, 100 or 400 mg/kg/day HP- β -CD and showed that plasma concentrations increased linearly with dose in both species (Monbaliu et al., 1990).

A separate oral study in rats was also conducted by Gerloczy et al. (1990) and showed that up to 6% of a single oral dose was absorbed from the gastrointestinal tract within 5 min. Faecal excretion was found to be the main route of elimination. Of the dose administered, approximately 3% was eliminated by the kidney in the urine and 70% in the faeces within 72 h. This finding concurs with Monbaliu et al. (1990) data following oral administration. Analysis of exhaled air showed that 3% of the total dose was metabolised. Tissue distribution analysis showed that the largest amounts of HP- β -CD were detected in the liver (3–5% of the dose) and kidneys (0.2–0.35%) (Gerloczy et al., 1990), suggesting that there may be a slight difference in distribution depending upon the route of administration; Monbaliu et al. (1990) had shown the kidney as the major organ containing HP- β -CD following intravenous administration.

The steady state volume of distribution (V_{dss}) for HP- β -CD in rats (Monbaliu et al., 1990), dogs (Monbaliu et al., 1990) and humans (Messens et al., 1991) corresponds well with the extracellular fluid volume of each species suggesting that there are no deep compartments or storage in pools. The total plasma clearances (CL_T) in all species tested are similar to that of inulin and clearance through the kidneys is independent of dose administered and nearly equivalent to the glomerular filtration rate thus, elimination is dependent on renal function (Frijlink et al., 1991).

5. Human pharmacokinetic and metabolic profile

The pharmacokinetics of HP- β -CD have been studied in healthy volunteers after single intravenous and oral

dosing (Szathmary et al., 1990). Following intravenous dosing at 0.5, 1.0, 1.5, 2.0, 2.5 or 3.0 g, plasma levels of unchanged HP- β -CD 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 V_{dss} were shown to be independent of dose and urine levels suggested that elimination was almost totally via the kidneys with no sign of tubular reabsorption. It was postulated that any remaining drug might be eliminated by other pathways, most likely by metabolism. Following oral administration, HP- β -CD could not be detected in either the plasma after 1 h or urine indicating that there was no absorption from the gastrointestinal tract and that oral bioavailability in humans was low (Szathmary et al., 1990).

6. Discussion

The available literature shows that the toxicity of HP- β -CD in animals has been extensively studied. HP- β -CD is well tolerated in most species, particularly if dosed orally and shows limited toxicity, depending upon dose and route of administration. HP- β -CD is also well tolerated in humans, with the main adverse effect being diarrhoea with no effects, documented to date, on kidney function.

In the animal studies, when administered orally, HP- β -CD, at either high single doses or a prolonged period of administration, induces minor biochemical changes. For example, after 7 days administration in the rat, 450 mg/kg/day was found to be a no effect dose level and 450 and 4500 mg/kg of 45% HP- β -CD when administered for one month produced only minor haematology changes and increases in plasma liver enzyme levels. There was no evidence of any histopathological changes, even at the highest oral dose, demonstrating that HP- β -CD was well tolerated and toxicologically benign. Comparison of effects in different species shows that the dog may be slightly less sensitive to the effects of HP- β -CD than the rat. For example, in the one month dog study, an oral a dose of 2250 mg/kg/day 45% HP- β -CD was a no effect dose, whereas in the rat, only 7 days administration at this dose caused minor increases in AST and ALT. Chronic oral dosing in the rat produced limited toxicity, with only findings at the highest doses of either 5000 mg/kg/day or 2000 mg/kg/day; however, in this chronic study the HP- β -CD was administered via diet, which may have reduced the bioavailability and maximum systemic exposure compared with oral gavage dosing.

Administration of HP- β -CD via intravenous infusion induced some minor clinical observations, as well as biochemical and histopathology changes. The target organs were the lungs, where there was an increase in

macrophage infiltration and also the liver and kidney (in some studies histopathology findings were noted in both organs). In studies, which looked for a dose response a no effect dose level was achieved and where reversibility was studied, all findings were reversible.

Comparison of the toxicity profile from oral and intravenous administration studies show a similar target organ profile. When comparing the shorter duration studies, the intravenous studies induced histopathological changes in the target organs: lungs, spleen, liver, urinary tract and kidney, whereas only biochemical and clinical observations were detected on the short-term oral studies. In order to induce histopathological changes via the oral route, the literature indicates that longer term dosing of up to a year is required, at higher doses.

The kidney is one of the target organs, which is comparable to the toxicity profile of other cyclodextrins (Antlisperger and Schmid, 1996). HP- β -CD forms stable complexes with cholesterol in blood, and due to an abnormal absence of free cholesterol in the urine, the complex dissociates in the kidney, and results in the accumulation of cholesterol and cholesterol esters in the urinary bladder and renal pelvis, the latter being responsible for the induction of kidney changes (Frijlink et al., 1991).

The carcinogenic studies showed no increased tumours in mice. However, in the rat, there was evidence of changes in the urinary tract at all doses and an increase in tumours: in the pancreas at all doses, in the intestine at high doses. Colonic mucosal hyperplasia has been found in rats after oral administration of raw starches, caramels, sugar alcohols and lactose and this hyperplasia is considered to be the result of increased osmotic activity of the intestinal contents. It is therefore possible that HP- β -CD could cause a similar physiological adaptation and increase the osmotic forces in the large intestine. Changes in the pancreas were initially seen at 12 months as exocrine pancreatic hyperplasia, which developed to exocrine pancreatic neoplasia by 24 months. Mechanistic studies have shown this to be a consequence of HP- β -CD complexing bile acids and leads to an increase in cholecystokinin (CCK), a peptide that can act as a mitogen and in the rat, producing increased cellular proliferation of acinar cells of the exocrine pancreas (Van Cauteren, personal presentation, 1997). In mice, non-human primates and man, CCK has no mitogenic effect and therefore these pancreatic tumours are considered a rat-specific effect with low clinical relevance (Van Cauteren, personal presentation, 1997). The changes in the urinary tract have been reported to be a result of osmotic 'necrosis', i.e. swelling and vacuolation of cells in the proximal cortical cells, which is believed to be a transient, adaptive change, and is seen following the intravenous administration of plasma expanders, hypertonic sugar solutions and dex-

trans, in both animals and patients. This change does not produce impaired renal function and is reversible (Van Cauteren, personal presentation, 1997).

The findings from these toxicology studies indicate that HP- β -CD is toxicologically safe, with clear no effect dose levels and reversible histopathological and biochemical changes. The main target organs were the kidney, liver, lungs and spleen. The changes in the liver and kidney, such as renal cortical tubular vacuolation are consistent with those seen in studies with the other α -, β - and γ -cyclodextrins (Olivier et al., 1991; Bellringer et al., 1995; Antlisperger and Schmid, 1996).

The pharmacokinetic analysis shows that bioavailability of HP- β -CD given orally is low in rat, dog and human. Distribution analysis shows that the target organs are the kidney and the liver, although via intravenous administration, the lung and the kidney are the major organs, and this is reflected in the target organ profile of the toxicology studies. Dependent upon the route of administration, the main elimination route is different: for both rat and dog, HP- β -CD following oral administration is mainly excreted via the faeces, whereas via intravenous administration, HP- β -CD is excreted via the kidneys. For humans, excretion is mainly via the kidneys.

It is anticipated that the data from the AZ studies, essentially conducted to understand the utility of a 45%w/v HP- β -CD aqueous dosing vehicle, in early drug safety assessment studies will be useful to other preclinical scientists. We have found this vehicle useful with poorly aqueous drugs; a problem frequently encountered in our experience. These data may reduce or eliminate the need for this vehicle to be repeatedly assessed as a useful tool in early (particularly relatively short term) toxicology studies.

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