



**2-Hydroxypropyl- β -Cyclodextrin and Sulfobutylether- β -Cyclodextrin:
histopathology review of intravenous studies in the rat and dog.**

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TITLE

2-Hydroxypropyl-β-Cyclodextrin and Sulfobutylether-β-Cyclodextrin: histopathology review of intravenous studies in the rat and dog.

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RUNNING TITLE

Histopathology of two cyclodextrins

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KEYWORDS

Cyclodextrin; 2-Hydroxypropyl- β -cyclodextrin; Sulfobutylether- β -cyclodextrin; Histopathology; Rat;
Dog

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ABBREVIATIONS

CD	Cyclodextrin
2HPβCD	2-Hydroxypropyl-β-Cyclodextrin
SBEβCD	Sulfobutylether-β-cyclodextrin
d	day
w	week
LN	Lymph node
IS	Infusion site
N/A	Non applicable
Nb	Number

ABSTRACT

2-Hydroxypropyl- β -Cyclodextrin (2HP β CD) and Sulfobutylether- β -Cyclodextrin (SBE β CD) are 2 chemically modified cyclodextrins often used as vehicles to improve the solubility of a drug. The aim of this review is to provide histopathologic data that could be taken into account as background during safety evaluation. We analyzed a total of 25 intravenous rat and dog studies (308 and 108 animals respectively) performed over the past 4.5 years at Charles River Laboratories Montreal. The main target organs for both species were kidney, lung and liver with the main microscopic findings being tubular epithelial vacuolation in the kidney and foamy macrophages in the lung and liver. Other changes were observed in lymph nodes (foamy macrophages), urinary bladder (urothelial vacuolation), prostate (urothelial vacuolation), epididymis (tubular epithelial vacuolation), spleen (foamy macrophages) and at the infusion site (foamy macrophages). Only one change was specific for cyclodextrins: granular eosinophilic cytoplasmic inclusions in the tubular epithelium of the kidney. Changes were most numerous in continuous infusion studies as opposed to weekly infusions, regardless of the cyclodextrin dose. There were no major differences between rats and dogs. 2HP β CD caused more changes with a higher incidence and severity than SBE β CD. These studies confirm the safety of 2HP β CD and provide a basis regarding the histopathology of SBE β CD on which there are few publications. We also identified a potential higher sensitivity of Fisher 344 rats to cyclodextrins.

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INTRODUCTION

Cyclodextrins (CDs) are cyclic oligosaccharides produced from starch. They are composed of 6, 7 or 8 glucopyranose units for α -, β - and γ -cyclodextrins respectively, which are the natural CDs. Their 3 dimensional stable structure consists of a hollow cone with a hydrophobic cavity and hydrophilic exterior. The basis of their use as vehicles to increase the solubility of certain drugs is by enclosing the drugs within their cavity (Albers et al., 1995; Davis et al., 2004; Thompson, 1997). This can be very important since more than 40% of drug failures during development can be traced to poor biopharmaceutical properties, especially poor dissolution or poor permeability (Davis et al., 2004).

2-hydroxypropyl- β -cyclodextrin (2HP β CD) and sulfobutylether- β -cyclodextrin (SBE β CD) are chemically modified CDs. The aim of modifying the β -CDs was to increase their solubility and safety (Albers et al., 1995; Thompson, 1997). Indeed, β -CDs are highly nephrotoxic when given parenterally (Irie et al., 1997; Thompson, 1997), causing severe nephrosis and death in the rat (Frank et al., 1976), whereas 2HP β CD and SBE β CD are much safer.

This paper reviews the histopathology of 2HP β CD and SBE β CD in rats and dogs from studies performed at Charles River Laboratories Montreal over the past 4.5 years.

MATERIALS AND METHODS

Animals

Rats

Sprague Dawley rats (CrI:CD (SD) IGS BR) were used in every study except two. In one of these two studies, Wistar rats (CrI:WI[Han]) were used and in the other study it was Fischer 344 rats. They all came from Charles River (Raleigh, NC or St Constant, QC). Rats were 6 to 12 weeks old with body weight ranging between 100 and 400 g. They were housed individually in stainless steel wire mesh-bottomed cages equipped with an automatic watering valve, in air-conditioned rooms at 22 °C +/- 3 °C with a relative humidity of 50 % +/- 20 % and a 12-hour light/dark cycle. Rats were fed *ad libitum* except for one study where males received 22 g per day and females 16 g per day, with a standard certified pelleted commercial laboratory diet of PMI Certified Rodent Chow 5002 (PMI Nutrition International Inc.).

Dogs

Beagle dogs (*Canis familiaris*) aged 5-16 months, body weight ranging between 5 and 11kg were used in these studies. They came from Marshall Farms (North Rose, NY) or Covance Research Products (Kalamazoo, MI). The dogs were housed individually in stainless steel cages equipped with a bar-type floor or a vinyl coated mesh floor and an automatic watering valve. Rooms were air-conditioned at 20 °C +/- 3 °C with a relative humidity of 50 % +/- 20 % and a 12-hour light/dark cycle. Dogs were fed an average of 400 g per day of a standard pelleted commercial dog food, 8727C Teklad Certified 25 % Lab Dog Diet (Harlan Teklad) or PMI certified dog chow 5007 (PMI nutrition international Inc.).

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Procedures

Infusion studies

In the infusion studies, animals had an indwelling catheter surgically implanted the day before the start of each study. Prior to surgery, the dogs were tranquilized with an intramuscular injection of acepromazine, butorphanol and glycopyrrolate. They were then anesthetized with an intravenous injection of thiopentone sodium 2.5 % prior to tracheal intubation and thereafter anesthesia was maintained using isoflurane and oxygen. Rats were anesthetized with an isoflurane/oxygen gas mixture. The surgical procedure was the same for rats and dogs. A small incision was made in the groin region and the femoral vein was isolated. A small phlebotomy was made in the vein and a medical-grade, silicone-based catheter was inserted with its tip placed in the vena cava at the approximate level of the kidneys. The catheter was secured in place at the vein insertion site and then brought subcutaneously to the exteriorization point at the nape of the neck. Both sites (femoral and exteriorization) were closed routinely and a topical antibiotic (polymyxin B, bacitracine, neomycin) was applied daily to the catheter exteriorization site and to the femoral site (until unnecessary). A jacket was then placed on the animal to hold the tether system. The catheter, pre-filled with 0.9% Sodium Chloride Injection, USP, was fed through the tether system and attached to a swivel secured to the outside of the cage. The upper portion of the swivel was connected to an infusion pump and all animals were continuously infused with 0.9 % Sodium Chloride Injection, USP, at a rate of 0.4 mL/hr for rats or 4mL/hr for dogs, until initiation of treatment.

Animals were humanely sacrificed the day after the last treatment or after a recovery period and a detailed necropsy was performed. For most studies, a full list of organs was collected, but in a few cases a reduced list of the major organs was collected. The tissues were fixed in 10 % buffered formalin.

These tissues were examined as part of the routine safety evaluation and findings entered directly into a computerized database PathData (Pathology Data Systems Ltd, Birsfelden/Basel, Switzerland).

Single dose intravenous injection studies

In these studies, animals received a single bolus injection by means of a syringe pump connected to a butterfly needle. The injection was made in the lateral tail vein of rats or in the cephalic or saphenous vein of dogs.

Animals were sacrificed humanely 1 to 14 days after initial treatment or after a recovery period. Tissues were handled as previously specified for infusion studies.

During the studies, the care and use of animals were conducted in accordance with the regulations of the USA National Research Council and the Canadian Council on Animal Care.

Evaluation of historical control data

All protocols from rat and dog intravenous injection or infusion studies on rats or dogs between April 2002 and October 2006 were evaluated for the use of a cyclodextrin as the vehicle. Thirteen rat studies and 12 dog studies were retrieved, with 9 and 5 respectively having a saline control group in addition to the vehicle control group. Only the data from vehicle and saline (when available) control animals were retabulated. Analysis was restricted to data from organs indicated in the pathology reports as being targets for cyclodextrins (i.e., kidney, liver, lung, infusion site, lymph node, urinary bladder, spleen, prostate and epididymis) and the microscopic findings retained were those present only in the vehicle control animals.

Treatment duration varied from 1 day to 13 weeks, of which a few (5 rat studies and 6 dog studies) had a 14 day recovery period and a 13 week dog study which had a 28 day recovery period. Two different cyclodextrins were used in the studies: 2HP β CD and SBE7 β CD. Infusions were administered

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continuously over several hours daily, once weekly or once every 3 weeks. Infusion rates ranged from 1 mL/hr to 20 mL/hr for rats or 1 mL/hr to 10 mL/hr for dogs.

None of the control animals receiving cyclodextrin died. Data were available from 308 rats (248 main study rats and 60 recovery) and 108 dogs (77 main study dogs and 31 recovery) with approximately equal numbers of males and females. Slight variations in the number of tissues examined stemmed from differences in a few study protocols.

For each study, the protocol was reviewed and details of animal age, weight, supplier, duration of study, flow rate, cyclodextrin type and concentration and other relevant details were recorded and are partly summarized in table 1.

RESULTS

In our studies, we identified several target organs: kidneys, liver, lung, lymph nodes, infusion site, urinary bladder, prostate, epididymis and spleen (table 2).

Kidney

The main microscopic change seen in the kidneys was vacuolation of epithelial cells from proximal convoluted tubules, which was observed in 45.5 % of dogs and 77.8 % of rats. It was described as focal, multifocal or diffuse, and bilateral, or rarely, unilateral. This finding consisted of small, mostly apical vacuoles in the epithelium of proximal tubules for the less severe changes. In the most severe changes, there was a unique large vacuole occupying most of the cell cytoplasm that displaced the nucleus to the periphery. This vacuolation was sometimes associated with the presence of eosinophilic cytoplasmic inclusions in the same cells (Figure 1). These inclusions consisted of finely granular eosinophilic material within the large vacuoles of epithelial cells. This finding was observed in 7.8 % of dogs and 4 % of rats. Tubular vacuolation was sometimes accompanied by eosinophilic hyaline droplets (Figure 2). Hyaline droplets are a common background finding in male rat kidneys and should not be confused with the eosinophilic cytoplasmic inclusions caused by CDs.

In some cases, there was degeneration and/or necrosis of proximal convoluted tubules, which was seen in 7.3 % of the rats. It was also observed in one dog study with 2HP β CD in 5/10 animals.

Finally, vacuolation of renal pelvic urothelium was observed only in dogs (27.3 %).

All these changes were observed with no significant sex difference. In one rat study, eosinophilic inclusions were only observed in females because there were no males in that study.

Liver

The main change seen in the liver of vehicle control animals was “reactive sinusoidal lining cells”. It was characterized by increased numbers of plump, but individual phagocytic cells lying within

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sinusoids (Figure 3). This finding was observed in 11.7 % of dogs and 8.5 % of rats with no significant sex difference. One study with Fisher 344 rats had unique findings of hepatocellular vacuolation in all males and in 3/5 females of the vehicle control group, whereas it was not seen in any of the 10 saline control animals. There was also single cell necrosis and infiltration of mononuclear cells, which tended to form microgranulomas in the more pronounced cases (Figure 4).

Lung

The only change observed in lung was histiocytosis characterized by multifocal or locally extensive aggregates of plump, active macrophages with finely vacuolated cytoplasm (Figure 5). It was present in 30.5 % of rats and 14.3 % of dogs and there was no sex difference.

Lymph nodes

Mandibular, mesenteric and grossly abnormal lymph nodes of various origins were examined in several studies and had histiocytosis that was characterized by accumulations of large, finely vacuolated macrophages within the sinuses of lymph nodes. This finding was present in few dogs (3.3 %), but it was observed in 18.2 % of rats, with an apparent predilection for mandibular lymph nodes. No sex difference was noted.

Infusion site

The hallmark change at the infusion site was the presence of large, finely vacuolated macrophages. However, the findings reported by pathologists were: “thrombosis with foamy macrophage accumulation” or “inflammation: vascular/perivascular, with foamy macrophages”. As thrombosis and inflammation are two common microscopic changes reported to be related to the infusion procedure, we grouped those two findings into one: “Thrombosis or inflammation with foamy macrophages accumulation”, and this was respectively seen in 12.7 % and 6.8 % of rats and dogs, with no sex difference.

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3 Necrotizing inflammation at the infusion site was seen in one rat study with 2HP β CD (6.1 % of all
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5 rats).

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8 *Urinary bladder, prostate and epididymis*
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10 Urothelial vacuolation in the urinary bladder and prostatic urethra and vacuolation of the tubular
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12 epithelium of the epididymis were observed in some animals.

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15 In rats, vacuolation of the urinary bladder urothelium was only observed in five females from one
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17 study (5.9 %), whereas it was seen in 28.6 % of all dogs. Vacuolation of the prostatic urethra and of
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19 the tubular epithelium of the epididymis (vacuolation of epithelial cells situated below the epididymal
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21 caput) was only observed in dogs from one study, in 5.9 % and 11.4 % respectively of all dogs.
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25 *Spleen*
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27 A vehicle-related change was seen in the normal macrophage aggregates present within the red pulp
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29 of the spleen. This change was characterized by a prominent vacuolation of these macrophages and
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31 was only observed in two (male) dogs from one study.
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35 *Recovery animals*
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37 All changes were at least partially reversible after a 14 or 28-day recovery period.

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39 Renal tubular vacuolation was still present in 41.7 % of all rats and in 16.1 % of all dogs. Renal
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41 degeneration and/or necrosis was seen in one rat and renal eosinophilic cytoplasmic inclusions in one
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43 dog.
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46 Only two dogs had reactive sinusoidal lining cells in the liver and three rats had hepatocellular
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48 vacuolation.
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51 Histiocytosis or foamy macrophage accumulations were observed in the lung of one rat, at the
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53 infusion site of one dog and in the lymph nodes of two rats and two dogs.
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Urothelial vacuolation was still present in the kidney of three dogs, in the urinary bladder of four dogs and the prostate of two dogs. There was also tubular vacuolation of the epididymis in these last two dogs.

Comparison of the cyclodextrins

The incidence and severity of changes were higher in animals receiving 2HP β CD in comparison to the animals that received SBE β CD (table 3).

Rats treated with 2HP β CD showed either renal tubular degeneration/necrosis (75 %) or renal tubular vacuolation (21.4 %) but not both. Among the 56 rats given 2HP β CD, 54 (96.4 %) had renal tubular vacuolation or degeneration and/or necrosis, whereas renal tubular vacuolation was observed in 78.6 % of the rats treated with SBE β CD (renal tubular degeneration and vacuolation were present simultaneously in 6 rats treated with SBE β CD).

Renal tubular epithelial eosinophilic cytoplasmic inclusions were also present in 10 female rats in a 2HP β CD study. In the dog studies, renal tubular degeneration and/or necrosis or renal tubular eosinophilic cytoplasmic inclusions were observed in 2HP β CD-treated dogs. Renal tubular vacuolation and renal urothelial vacuolation were present with an incidence of 63.6 % and 81.8 %, respectively, among 2HP β CD-treated dogs, whereas these incidences were 42.4 % and 18.2 %, respectively, among SBE β CD-treated dogs.

A similar trend of 2HP β CD causing more changes than SBE β CD was seen in the other organs (table 3).

DISCUSSION

All studies examined provide histopathologic data that confirms the safety of 2HP β CD. Moreover, they constitute a basis regarding the safety of SBE β CD, on which there are few publications, despite the fact that it is being used more frequently as a vehicle.

The tubular vacuolation observed in the kidney has been well explained by Frank et al. (1976). It is the result of a series of alterations in vacuolar organelles of the proximal tubule. These changes begin as an increase in size of apical vacuoles that is followed by the appearance of giant lysosomes. A transient increase in size of apical vacuoles is also observed as an adaptative response to the excretion of osmotic agents such as glucose, mannitol and dextran at extremely high concentrations. Unlike such osmotic agents, β -CDs cause additional cellular changes that are irreversible and ultimately toxic to cells. These compounds tend to recrystallize in the proximal tubules of the kidney. Repeated administration of large doses of CDs results in numerous giant lysosomes distorted by the enclosed microcrystals. These could correspond to the granular eosinophilic cytoplasmic inclusions sometimes observed in rats or dogs in our studies. And these changes observed in our 2HP β CD and SBE β CD studies were reversible, which confirms that these compounds are safer than β -CDs by the intravenous route.

In the liver, lung, lymph node and spleen, the presence of large foamy macrophages demonstrates an uptake of CDs by these cells. This is a non-specific change that is often observed with the administration of various compounds.

Urothelial vacuolation in the urinary bladder has sometimes been observed in other studies after intravenous administration of 2HP β CD to rats and dogs (Coussement et al., 1990; Van Cauteren et al., 1997). It is reported to be a result of osmotic “necrosis” (Gould et al., 2005), which is thought to be a

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transient, adaptive change following intravenous administration of plasma expanders, hypertonic sugar solutions and dextrans, as is also the case in the proximal tubules of the kidney. Coussement et al. (1990) also reported vacuolation in the renal pelvis urothelium in dogs as we did have found in our studies. Additionally, we observed such vacuolation in the prostatic urothelium in one dog study. These changes most likely have the same pathogenetic mechanism as in the urinary bladder.

Finally, splenic changes have periodically been reported with intravenous administration of 2HPβCD and consisted of reduced extramedullary hematopoiesis (Gould et al., 2005) or spleen hyperplasia (Coussement et al., 1990). These changes were not found in our studies.

In the infusion studies, the presence of changes was determined by several factors such as the frequency of administration, the type of CD and the dose. Of these factors, the frequency of administration seems to be the major factor. When the infusion was continuous, there always were changes. This was also true for daily infusions of 1 to 6hr/d in our studies. In the case of weekly or less frequent infusions, changes were less frequent, regardless of the dose. Thus, rats and dogs that received 2100 mg/kg/d and 1935 mg/kg/d of SBEβCD, respectively, once a week over 8 weeks did not have any changes. On the other hand, rats and dogs that received 750 mg/kg/d and 900 mg/kg/d of SBEβCD, respectively, 6 hours a day for 14 days had kidney changes. As long as the frequency of administration did not overwhelm the recovery rate of the animal, no changes were observed. The concentration of CD and rate of administration per hour determine the daily-administered dose, but do not directly influence the presence of changes.

The dose was a key factor in single-dose studies. We only reviewed three rat studies and one dog study with single injections. Nevertheless, these studies showed a No Observed Effect Level for single intravenous administration of SBEβCD at 550 mg/kg/d in the rat and 330 mg/kg/d in the dog.

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6 Although Gould et al. (2005) reported that dogs seemed slightly less sensitive than rats with orally
7 administered CDs, it is difficult here to make a similar statement for intravenous studies, because the
8 protocols for rat and dog studies were often different. Nevertheless, we observed that the main target
9 organs were the same in both species: kidney, lung and liver. The urothelium seemed to be more
10 frequently targeted in dogs than in rats. Finally, both species had a similar recovery capacity after CD
11 administration.
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22 Two different CDs were used in our studies: 2HP β CD, which has been widely studied and SBE β CD
23 which is the most recently commercialized. Numerous publications have been made about the safety of
24 2HP β CD, but very little regarding the safety of SBE β CD. However, in these few studies, SBE β CD is
25 described as safer than 2HP β CD (Thompson, 1997) and this was confirmed in our studies.
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32 There were changes in every study using 2HP β CD, even with a dose as low as 100 mg/kg/d (1 hr/d,
33 13 weeks) in dogs. Not only were the changes previously described present in every study we
34 reviewed but they were also observed in a great majority of the animals (often every vehicle control
35 animal) and were more severe than with SBE β CD. Indeed, 2HP β CD caused necrotic changes in the
36 kidney and, in one study, at the infusion site.
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44 However, one of our studies was an exception from among these observations: a single-dose study
45 with SBE β CD in Fisher 344 rats. These rats had kidney vacuolation with single cell necrosis and
46 hepatic changes that were not seen in the other SBE β CD studies (i.e. hepatocellular vacuolation, single
47 cell necrosis and mononuclear cell infiltrations). The fact that the CD was administered at a high dose
48 (6000 mg/kg) in only a few minutes could potentially explain those changes. However, another
49 possible explanation is that Fisher rats might be more sensitive to CDs. Three studies were relatively
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recently reported (Olivier et al., 1991; Toyoda et al., 1995; Bellringer et al., 1995) that used the parent β -CD more toxic than the chemically modified β -CDs. In each of these studies, the β -CD was administered orally (the safest route for CDs) at dose levels of 0, 1.25, 2.5, 5 and 10% of the daily diet. Olivier et al. (1991) used OFA rats which were derived from Sprague-Dawley rats, dosed for 90 consecutive days and did not find any changes. In contrast, Toyoda et al. (1995) using Fisher 344 rats, which were also dosed for 90 consecutive days, observed a dose-dependent increase in the severity of inflammatory cell infiltration in the liver and focal hepatocellular necrosis was present in both sexes of the 10 % group and males of the 5 % group. Similar but in general less severe changes were reported by Bellringer et al. (1995) to occur in Sprague-Dawley rats when the β -CD was administered for the much longer time period of 52 weeks. Their rats had liver changes of single cell necrosis, prominent sinusoidal lining cells, centrilobular hepatocyte enlargement, portal inflammatory cell infiltration and an increase in foci of hepatocytes with rarefied cytoplasm in males of the 5 % group and females of the 2.5 and 5 % groups. The results of these three studies, together with our results, support the probability that Fisher 344 rats are more sensitive to CDs.

The aim of this work was to give a detailed review of the histopathologic changes induced by chemically modified β -CDs administered by the intravenous route in rats and dogs. These changes, although mostly not specific for cyclodextrins (except for the eosinophilic cytoplasmic inclusions in the kidney), should be known and taken into account as vehicle-related changes for such studies because despite their minimal toxicological significance, they can complicate the interpretation of lesion during routine toxicological evaluations.

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3 Finally, we focused here on only one route of administration but there are others to be studied, such
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5 as inhalation, which is being used with increased frequency because of its simplicity. It would be
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7 interesting to know if cyclodextrins induce any changes via this administration route.
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TABLES

Table 1 - Summary of intravenous studies with 2HPβCD and SBEβCD conducted at Charles River Laboratories Montreal.

	Number of animals	Duration	Dose rate (mL/kg/hr)	Vehicle	CD dose (mg/kg/day)	Target organs
RAT STUDIES	24	Continuous 14 d	1.5 (SBEβCD and 2HPβCD) 3 (2HPβCD)	4 or 8 % SBEβCD 8 % 2HPβCD	SBEβCD 4 % 1440 SBEβCD 8 % 2880 2HPβCD 8 % 2880 (1.5 mL/kg/hr) or 5760 (3 mL/kg/hr)	SBEβCD: kidney 2HPβCD: kidney, IS
	10	Continuous 14 d	1	5 % 2HPβCD	1200	Kidney, lung, IS
	20	Continuous 14 d	2	5.5 % SBEβCD	2640	Kidney, LN, liver, urinary bladder
	50	Continuous 3 or 14 d	2	5.5 % SBEβCD	2640	Kidney, lung, LN
	4	Continuous 7 d	1	5 % 2HPβCD	1200	Kidney, lung, LN
	20	Continuous 5d	2	11 % SBEβCD	5280	Kidney
	20	6 hr/d 14 d	2.5	5 % SBEβCD	750	Kidney
	20	4 hr/d 14 d	5	20 % 2HPβCD	4000	Kidney, lung, liver, LN. IS
	60	30 min weekly, 3 w	20	3 or 30 % SBEβCD	3 %: 300 30 %: 3000	3 %: - 30 %: Kidney
	30	Once weekly 9 hr, 8 w	6 mL/kg 30 min then 1.5 mL/kg/hr	13.3 % SBEβCD	2100	-
	20	Single dose	20 mL/kg	30 % SBEβCD	6000	Kidney, liver
	10	Single dose	5 mL/kg	40 % 2HPβCD	2000	Kidney
	20	Single dose	5 mL/kg	11 % SBEβCD	550	-
DOG STUDIES	20	Continuous 3 or 14 d	1	11 % SBEβCD	2640	Kidney, urinary bladder
	2	Continuous 4 d	2	5 % 2HPβCD	2400	Kidney, lung
	8	Continuous 24 hr	1.5	11 % SBEβCD	3960	Kidney, liver
	12	1 hr/d 13 w	10	10 % 2HPβCD	100	Kidney, lung, LN, liver, urinary bladder, IS, other
	6	6 hr/d 14 d	3	5 % SBEβCD	900	Kidney
	2	4 hr/d 7 d	2	15 % SBEβCD	1200	Kidney
	12	9 hr	7 mL/kg 30 min then 1.29 mL/kg/hr	13.3 % SBEβCD	1935	Kidney
	4	9 hr	7 mL/kg 30 min then 1 mL/kg/hr	13.3 % SBEβCD	1600	-
	12	Once weekly 9 hr, 8 w	7 mL/kg 30 min then 1.29 mL/kg/hr	13.3 % SBEβCD	1935	-
	12	Once weekly 9 hr, 7 w	3.25 mL/kg 30min then 1 mL/kg/hr	13.3 % SBEβCD	1350	-
	10	2 cycles of 9 hr every 21 d	3.25 mL/kg 30min then 1 mL/kg/hr	13.3 % SBEβCD	1350	-
	8	Single dose	3 mL/kg	11 % SBEβCD	330	-

Table 2 - Percentages of changes in target organs in males and females rats and dogs from intravenous studies with 2HP β CD and SBE β CD.

		RAT				DOG			
		Male	%	Female	%	Male	%	Female	%
Kidney	Nb examined	117		131		38		39	
	Vacuolation, tubular	89	76	104	79.4	17	44.7	18	46.1
	Degeneration/necrosis, tubular	10	8.5	8	6.1	2	5.3	3	7.7
	Eosinophilic cytoplasmic inclusions	0	0	10	7.6	2	5.3	4	10.3
	Vacuolation, urothelium	0	0	0	0	10	26.3	11	28.2
Lung	Nb examined	75		89		38		39	
	Histiocytosis	27	36	30	33.7	6	15.8	5	12.8
Liver	Nb examined	117		131		38		39	
	Reactive sinusoidal lining cells	8	6.8	13	9.9	4	10.5	5	12.8
	Cytoplasmic rarefaction	0	0	0	0	2	5.3	1	2.6
	Vacuolation, hepatocellular	5	4.3	3	2.3	0	0	0	0
	Single cell necrosis	3	2.6	0	0	0	0	0	0
	Mononuclear cell infiltration	5	4.3	0	0	0	0	0	0
Infusion site	Nb examined	112		126		37		37	
	Thrombosis or inflammation with foamy macrophage accumulation	10	8.9	19	15.1	2	5.4	3	8.1
	Inflammation vascular/perivascular necrotizing	7	6.3	7	5.6	0	0	0	0
Urinary bladder	Nb examined	75		85		37		39	
	Vacuolation, urothelium	0	0	5	5.9	9	24.3	13	33.3
Lymph node	Nb examined	161		179		93		90	
	Histiocytosis	32	19.9	30	16.8	3	3.2	3	3.3
Prostate	Nb examined	75		N/A		34		N/A	
	Vacuolation, urothelium	0	0	N/A	N/A	2	5.9	N/A	N/A
Epididymis	Nb examined	75		N/A		35		N/A	
	Vacuolation, tubular epithelium	0	0	N/A	N/A	4	11.4	N/A	N/A
Spleen	Nb examined	85		95		37		38	
	Prominent vacuolated macrophages	0	0	0	0	2	5.4	0	0

Table 3 - Percentages of changes caused by 2HPβCD vs SBEβCD

	RAT				DOG			
	2HPβCD	%	SBEβCD	%	2HPβCD	%	SBEβCD	%
Kidney Nb examined	56		192		11		66	
Vacuolation, tubular	42	75	151	78.6	7	63.6	28	42.4
Degeneration/necrosis, tubular	12	21.4	6	3.1	5	45.5	0	0
Eosinophilic cytoplasmic inclusions	10	17.9	0	0	6	54.5	0	0
Vacuolation, urothelium	0	0	0	0	9	81.8	12	18.2
Lung Nb examined	34		130		11		66	
Histiocytosis	31	91.2	26	20	10	90.9	1	1.5
Liver Nb examined	56		192		11		66	
Reactive sinusoidal lining cells	17	30.4	4	2.1	8	72.7	1	1.5
Cytoplasmic rarefaction	0	0	0	0	0	0	3	4.5
Vacuolation, hepatocellular	0	0	8	4.2	0	0	0	0
Single cell necrosis	0	0	3	1.7	0	0	0	0
Mononuclear cell infiltration	0	0	5	2.6	0	0	0	0
Infusion site Nb examined	56		182		11		63	
Thrombosis or inflammation with foamy macrophage accumulation	25	44.6	4	2.2	5	45.5	0	0
Inflammation vascular/perivascular necrotizing	13	23.2	1	0.5	0	0	0	0
Urinary bladder Nb examined	30		130		11		65	
Vacuolation, urothelium	0	0	5	3.8	9	81.8	13	20
Lymph node Nb examined	67		273		26		157	
Histiocytosis	32	47.8	30	11	4	15.4	2	1.3
Prostate Nb examined	10		65		4		30	
Vacuolation, urothelium	0	0	0	0	2	50	0	0
Epididymis Nb examined	10		65		4		31	
Vacuolation, tubular epithelium	0	0	0	0	4	100	0	0
Spleen Nb examined	40		140		9		66	
Prominent vacuolated macrophages	0	0	0	0	2	22.2	0	0

FIGURES

Figure 1. – Granular eosinophilic cytoplasmic inclusions and vacuolation in the tubular epithelium of the kidney. Sprague-Dawley rat. H&E. original magnification of 400x.

Figure 2. – Vacuolation in the tubular epithelium of the kidney. Note the presence of eosinophilic droplets in the bottom left that should not be confused with granular eosinophilic inclusions caused by cyclodextrins. F344 rat. H&E. original magnification of 400x.

Figure 3. – Reactive sinusoidal lining cells in the liver. Sprague-Dawley rat. H&E. original magnification of 400x.

Figure 4. – Single cell necrosis, cellular vacuolation and mononuclear cell infiltrations in the liver. F344 rat. H&E. original magnification of 200x.

Figure 5. – Alveolar histiocytosis with plump foamy macrophages. Sprague-Dawley rat. H&E. original magnification of 400x.

Figure 6. – Vascular inflammation at the infusion site with presence of numerous plump foamy macrophages (vascular lumen at the bottom left). Sprague-Dawley rat. H&E. original magnification of 400x.

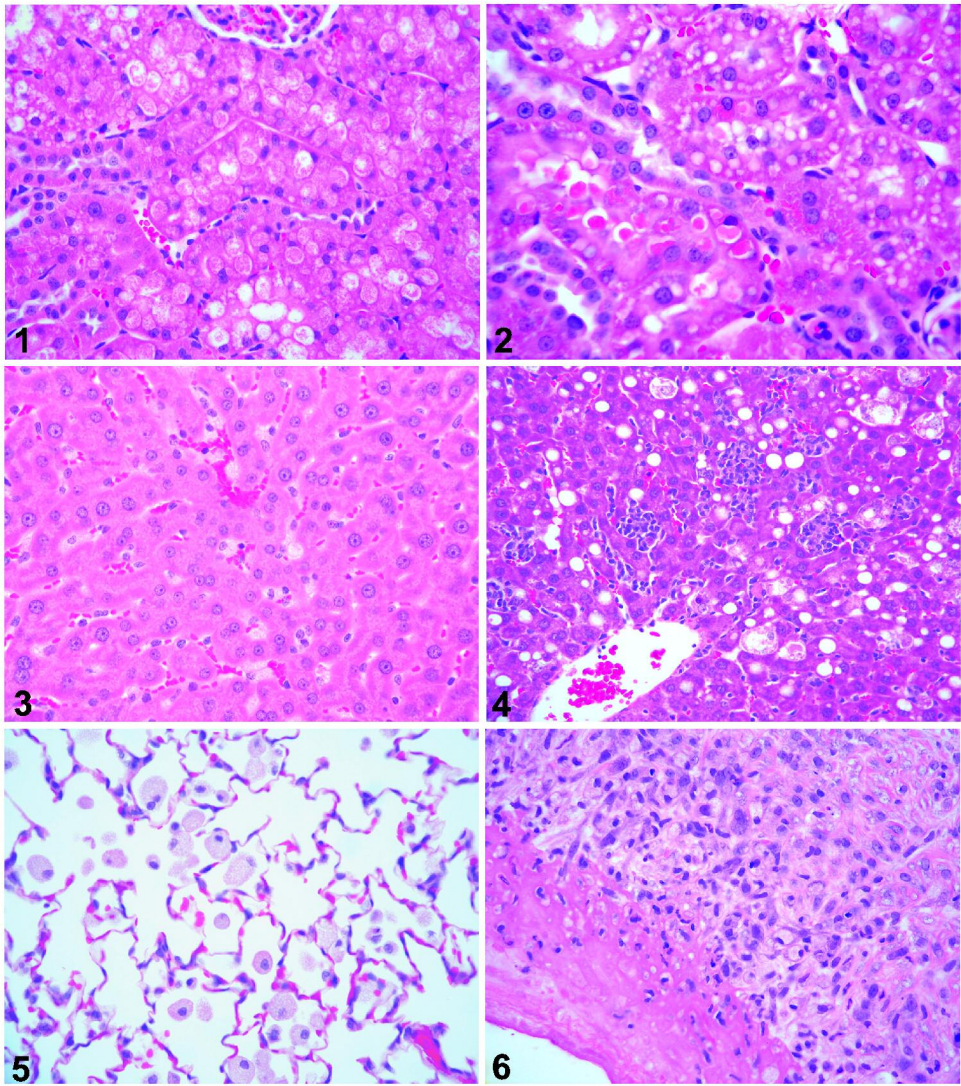


Figure 1. □ Granular eosinophilic cytoplasmic inclusions and vacuolation in the tubular epithelium of the kidney. Sprague-Dawley rat. H&E. original magnification of 400x. **Figure 2.** □ Vacuolation in the tubular epithelium of the kidney. Note the presence of eosinophilic droplets in the bottom left that should not be confused with granular eosinophilic inclusions caused by cyclodextrins. F344 rat. H&E. original magnification of 400x. **Figure 3.** □ Reactive sinusoidal lining cells in the liver. Sprague-Dawley rat. H&E. original magnification of 400x. **Figure 4.** □ Single cell necrosis, cellular vacuolation and mononuclear cell infiltrations in the liver. F344 rat. H&E. original magnification of 200x. **Figure 5.** □ Alveolar histiocytosis with plump foamy macrophages. Sprague-Dawley rat. H&E. original magnification of 400x. **Figure 6.** □ Vascular inflammation at the infusion site with presence of numerous plump foamy macrophages (vascular lumen at the bottom left). Sprague-Dawley rat. H&E. original magnification of 400x.