INDUCTION OF NEPHROSIS BY β -CYCLODEXTRIN IN BEAGLES

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Abstract : The present study was conducted to examine nephrotoxicity of β -cyclodextrin (β -C) in dogs. Five female Beagle dogs were divided into 3 groups : Group 1 (2 dogs treated with 0.9 g/kg of β -C), Group 2 (2 dogs treated with 0.45 g/kg of β -C), and Group 3 (1 dog treated with saline). Durations of β -C injection were 7 and 10 consecutive days in Groups 1 and 2. Histopathological and histochemical examinations of the kidney were performed. Following 0.9 g/kg or long-term 0.45 g/kg treatment with β -C, the animals showed marked fatigue and elevated BUN and Cr. Histopathologically, β -C treatment resulted in vacuolation, necrosis, and regeneration in the proximal tubules. Activities of succinic dehydrogenase, β -glucuronidase, and glucose-6-phosphatase decreased histochemically after β -C treatment. Because signs of nephrotoxicity represented were noted in dogs after β -C treatment, as has been reported in rats, it seems likely that dogs also can serve as a model of nephrosis. If this model is to be applied to the study of renal carcinogenesis, the present study suggests the optimum dosing regimen for β -C to be 7-day 0.45 g/kg treatment. (J Toxicol Pathol 4 : 67~73, 1991)

Key words : β -cyclodextrin, Toxic nephrosis, Histochemistry, Dogs

Introduction

The establishment of an animal model of carcinoma similar to human carcinoma is important for analyzing various factors associated with the development and progression of cancer disease and obtaining basic data on its clinical diagnosis and treatment. We previously examined urinary bladder carcinogenesis in mice¹, rats^{2,3} and dogs^{4,5} and we established a dog model of bladder cancer which was the most appropriate for the evaluation of the process of carcinogenesis and the development of treatment methods.

Regarding renal carcinogenesis, Hiasa *et al.*

reported that β -cyclodextrin (β -C) induced development of nephrosis in rats⁶ and served to promote renal carcinogenesis in rats treated with N-ethyl-N-hydroxyethyl-nitrosamine (EHEN)⁷. However, if a model of renal carcinoma can be prepared in medium-sized animals such as dogs, it will allow to study into the course of renal cancer in a preclinical setting and will be available to clarify the natural history of renal cancer.

The present study was conducted to examine nephrotoxicity of β -C in dogs as the first step of preparing a dog model of renal cancer.

Materials and Methods

Animals

Five female Beagle dogs, weighing 7.2-8.4 kg (mean : 7.7 kg) (Chugai Pharmaceutical Co., Ltd. Toxicology Research Labs., Nagano, Japan) were used in this experiment.

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Chemicals

β -C (Nacalai Tesque Inc., Kyoto, Japan) was used dissolving in physiological saline at a concentration of 0.45 g/20 ml.

Experimental design

The animals were divided into three groups: Group 1 (2 dogs treated with 0.9 g/kg of β -C), Group 2 (2 dogs treated with 0.45 g/kg of β -C), and Group 3 (1 dog treated with 20 ml/kg of physiological saline). β -C or saline solution was subcutaneously administered to dogs using a 21 gauge needle. Durations of β -C injection were consecutive 7 days for one dog in each group and consecutive 10 days for the remainder. Group 3 served as control and duration of saline injection was consecutive 7 days.

On the day following the final injection, the animals were sacrificed. That is, Dogs 1 and 3 were sacrificed on Day 8, Dogs 2 and 4 on Day 11, and Dog 5 on Day 8 from the beginning of the experiment.

Histological examination

A part of the kidney was histopathologically examined with hematoxylin and eosin stain. The renal tissue was additionally examined histochemically using the following stains: succinic dehydrogenase (S.D.) stain (method of Nachlas *et al.*⁸); β -glucuronidase (β -G) stain (method of Hayashi *et al.*⁹); glucose-6-phosphatase (G6P) stain (method of Wachsteis and Meisel¹⁰); acid phosphatase (Ac.P) stain (method of Brustone¹¹); and alkaline phosphatase (Al.P) stain (method of Brustone¹¹). Furthermore, electron microscopical changes were also examined.

Biochemical examination

Prior to the study and on Days 3, 5, 8, and 11, blood was sampled from each dog for hematologic and biochemical tests.

Results

Physical and biochemical course of the experiment

In the 0.9 g/kg of β -C treated group (Group 1), both animals began to show marked fatigue and anorexia on Day 2, and one of them (Dog 2) died

on Day 3. This dog was immediately autopsied. The remaining dog (Dog 1) in this group was sacrificed on Day 3.

In the 0.45 g/kg dose group (Group 2), Dog 3, which was treated for 7 days, showed no abnormalities throughout the experiment, as also the untreated control (Dog 5). Dog 4 in the same dose group, which was treated for 10 days, began to

Fig. 1a. BUN levels of each dog during the experiment.

▨: normal range from our previous data (13-17 mg/dl).

Numbers in circles indicate dog number.

Fig. 1b. Creatinine levels of each dog during the experiment.

▨: normal range from previous our data (0.6-0.7 mg/dl).

Numbers in circles indicate dog number.

show marked fatigue and anorexia from Day 8, however, barely survived until the experimental termination and was sacrificed on Day 11. Changes in both of BUN and serum creatinine levels were shown in Figs. 1a and 1b.

Histopathological and histochemical changes of kidneys

Histopathological changes of the kidney were shown in Table 1. Marked cytoplasmic vacuolation, necrosis, and crystal formations in the proximal tubules were observed in the high dose of β -C group (Figs. 2a and 2b). In the low dose group, Dog 4, treated for 10 days, also showed marked necrosis, however, Dog 3 treated for 7 days showed only focal change of vacuolation.

Histochemically, high dose group showed reduction in S.D., β -G, and G6P activities (Table 2). Similar changes were also marked after 10-day 0.45 g/kg treatment (Dog 4) and were observed only slightly after 7-day 0.45 g/kg treatment (Dog 3).

Electron microscopical changes of kidneys

Electron microscopical changes in kidneys of all β -C treated dogs showed crystals within irregularly shaped lysosomes, cytoplasm which contained numerous vacuoles of irregular size demarcated with a single membrane. Lysosomes were attached to some vacuoles and brush borders remained. In addition, an irregular distribution of slightly smaller mitochondria and hyperplasia of basal membrane were observed, although invagination of the basal membrane was not evident. (Figs. 3a and 3b)

Discussion

The purpose of the present study was to determine whether or not subcutaneous treatment with β -C would induce nephrosis in dogs, as has been reported in rats. If affirmative, this model would be applicable to experimental study of renal carcinogenesis and allow determination of the optimum dose of β -C to serve as a promoter. Because many reports have been published regarding experimental assessment of nephrotoxicity of β -C and analogous substances in rats, it is

Table 1. Histopathological Changes of the Kidney in Each Dog

Dog No.	Dose	Days	Histopathological findings			
			vacuolation	necrosis	crystal	regeneration
①	0.9 g/kg	2	⦿	+	⦿	—
②	0.9 g/kg	2	⦿	+	⦿	—
③	0.45 g/kg	7	⦿*	—	±	—
④	0.45 g/kg	10	—	⦿	+	⦿
⑤	saline	7	—	—	—	—

⦿ : marked, + : moderate, ± : mild, — : negative * : focal changes

Table 2. Histochemical Changes of the Kidney in Each Dog

Dog No.	Dose	Days	Histochemical findings				
			S.D.	β -G	G6P	Ac.P	Al.P
①	0.9 g/kg	2	+	+	+	⦿	⦿
②	0.9 g/kg	2	+	+	—	+	+
③	0.45 g/kg	7	⦿*	⦿	⦿*	⦿	⦿
④	0.45 g/kg	10	+	+	—	+	⦿
⑤	saline	7	⦿	⦿	⦿	⦿	⦿

S.D.: succinic dehydrogenase, β -G: β -glucuronidase, G6P: glucose-6-phosphatase, Ac.P: acid phosphatase, Al.P: alkaline phosphatase

⦿ : marked, + : moderate, ± : mild, — : negative * : focal changes

Figs. 2a and 2b. Histological findings of the kidney of Dog 2.

Marked cytoplasmic vacuolation, necrosis, and crystal formations in the proximal tubules were observed. Hematoxylin and eosin stain, $\times 160$.

Figs. 3a and 3b. Electron microscopical findings of the kidney of Dog 2.

Electron microscopical findings showed β -C crystals within irregularly shaped lysosomes (3a). Lysosomes were attached to some vacuoles and brush borders (3b). Bar=0.1 μ m.

significant to compare the results from this study in dogs with those from such previous studies.

Histopathologically, Hiasa⁶ and Frank *et al.*¹² reported vacuolation and crystal formation in the proximal tubules of rats. Similar findings were obtained also from Beagle dogs in the present study. Based on the concept of osmotic nephrosis proposed by Allen *et al.*¹³ Kimura reported vacuolar swelling of the proximal tubules in the rat kidney after low-molecular-weight dextran treatment¹⁴. Although acute renal failure does not occur in osmotic nephrosis, it occurred in cases with nephrosis induced by low-molecular-weight dextran. Kimura¹⁴ attributed it to vacuolation and inferred that lysosomes were associated with vacuolation. Also in the present study, treatment with 0.9 g/kg or long-term treatment with 0.45 g/kg of β -C resulted in the elevation of BUN and creatinine levels, leading to death or marked fatigue indicative of acute renal failure.

Dogs in the present experiment showed reduced S.D., β -G, and G6P activities in the epithelial cells of the proximal tubules histochemically, similar to the finding in rats⁶. Frank *et al.*¹² documented by electron microscopy that increased numbers of apical vacuoles and changes in the epithelial cell structure lead to a reduction in the activity of various enzymes. As reported by Kimura¹⁴, enzyme activities are enhanced in the early stage of vacuolation and decrease after intense vacuolation. Electron microscopic study of the nephrosis induced by β -C or its analogs in rats has been reported by several investigators. Frank *et al.*¹² reported that nephrosis occurred after an increase in the presence of apical vacuoles and the appearance of giant lysosomes (with acicular microcrystals deposited in the lysosome matrix), and that an advance in nephrosis probably leads to the appearance of giant vacuoles, resulting in enzyme activity reduction and cell death. Kimura¹⁴ reported that lysosomes, instead of mitochondria or basal membrane, are directly associated with vacuolation. In the present study, uptake of β -C crystals into lysosomes and attachment of crystal-deposited lysosomes to vacuoles were observed, while mitochondria and basal membrane appeared normal. These results from previous^{6,12} and present studies indicate that

lysosomes play a role in vacuolation.

In summary, β -C treatment induced nephrosis in dogs, similar to that seen in rats. What should follow next is the application of this model to the study of renal carcinogenesis. If β -C could be used as a promoter in EHEN-induced renal carcinogenesis in dogs, the present study suggests the optimum dosing regimen for β -C to be 0.45 g/kg for 7 consecutive days, because 0.9 g/kg treatment and long-term 0.45 g/kg treatment caused death or fatigue due to acute renal failure.

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