Latent Infection of Canine Herpes Virus in Laboratory Beagle Dogs

Katsuhiko Yoshizawa^{1,4}, Yuji Oishi¹, Kenjiro Tsubota¹, Kazuo Ieoka², Tsuneo Fujii², Osamu Ohe³, Hideto Senzaki⁴, and Airo Tsubura⁴

Department of ¹Toxicologic Pathology and ²Toxicology, Toxicology Research Laboratories, Fujisawa Pharmaceutical Co., Ltd., ³Contract Technical Division, Fujisawa Technical Service Co., Ltd., and ⁴Pathology II, Kansai Medical University

Abstract: The latent infection of canine herpes virus was investigated in laboratory beagles with no clinical abnormalities. In eight of twenty-three male dogs which were purchased from the same breeder at the age of six months, the pathogen was confirmed at the age of one year by immunological, histopathological or electron-microscopical examinations. The infected beagles seemed to be healthy in several clinical examinations including blood- and serum-biochemical analyses. The neutralizing titer of the canine herpes virus antibody test ranged from 64 to 128, compared to the undetectable titer in animals obtained from a different breeder and housed in the same conditions. Histopathological examinations revealed eosinophilic inclusion bodies in the cytoplasm of swollen or damaged epithelium of renal papillary ducts, papilla, and/or pelvis accompanied by chronic pelvic inflammation. The inclusion bodies showed positive reaction against anti-human herpes simplex virus antigen. Electronmicroscopically, these inclusion bodies consisted of numerous intracytoplasmic aggregates of virion. The virion particles were round to hexagonal, the envelope or capsid about 140 nm in diameter and arranged in a fine crystalline array. This evidence strongly suggests that the virions were excreted from the body by urination. Although these animals were housed in our laboratory for about half a year, the other beagles housed in separate cages in the same room were not infected by the virus. The results show the importance of preventative measures against latent canine herpes virus infection and maintenance of laboratory conditions to avoid this problem. (*J Toxicol Pathol* 2000; 13: 1~6)

Key words: Herpes virus, Cytoplasmic inclusion bodies, Kidney, Beagle dog, Immunohistochemistry

Introduction

Canine herpes virus (CHV) infection is a fatal systemic disease characterized by hemorrhagic and necrotic inflammation in the urinary system and other organs of neonatal puppies. In adult animals it rarely causes catarrhal tracheobronchitis with viral inclusions in the respiratory epithelia without clinical recognition¹⁻⁶. When this infection spreads in animal colonies of breeders or research laboratories, the pathogen must be cleaned out from the laboratories, because of the pernicious influence to the ongoing toxicological studies in the assessment of the toxicity.

The present note describes the latent infection of CHV in laboratory beagles without epidemic infection in the animals housed under the same conditions. Moreover, we clarified the characteristic epithelial inclusions in the renal tissues and mentioned the differences from several inclusions in the drug-induced or spontaneous conditions.

Materials and Methods

Twenty-three male beagle dogs vaccinated against several pathogens (Dohyvax[®]; mixed vaccin for canine distemper virus, adenovirus-2, parainfluenza virus, parvovirus, le-

ptospira canicola, and leptospira icterohaemorrhagiae, Kyouritsu Co., Tokyo, Japan) were purchased at six months of age from Fuji Animal Farm Co. (Shizuoka, Japan; Breeder A) and Ichiyangi Farm (Shizuoka, Japan; Breeder B). These animals had been checked for one month for medical inspection including parasite detection test and hematological and serum-biochemical analyses. The animals were housed individually in stainless steel cages in an environment of 23±2°C room temperature, 55±5% relative humidity, and a 12 hr light-dark cycle, and given daily 300 g of a commercial dog diet (TC-1, Maruha Pet Food, Tokyo, Japan) and water freely. Food consumption was recorded daily and bodyweight measured monthly. The dogs were housed in our laboratory to use for a pharmacotoxicity study for one to six months. During the study periods, the animals were routinely observed for clinical signs, weighed, and analyzed hematologically and serum-biochemically. Urinalysis was not done in any of the animals.

At the end of the study schedule, these beagles were sacrificed under anesthesia by exsanguination from the cervical aorta and received complete necropsy including measuring the weight of most of the organs. Specimens of all main organs were fixed in 10% buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (HE) for routine histopathological examination. Additional serial sections of some kidneys were stained by periodic acid-Schiff reaction (PAS), Grocott's stain, Warthin-Starry stain, Von Kossa's stain, and phosphotungstic acid hematoxylin (PTAH) stain. The other preparations were immunostained for rabbit anti-herpes simplex virus type 2 antigen (1:100 in dilution, DAKO, Glostrup, Denmark), using a labeled streptavidin-biotin staining kit (Dako) and antigen retrieval by microwave oven heating was essential⁷. This anti-herpes antibody reacts with all the major glycoproteins present in

Received: 6 September 1999, Accepted: 2 November 1999

Mailing address: Katsuhiko Yoshizawa, DVM, Msc., JCVF, and JSTP

Department of Toxicologic Pathology, Toxicology Research Laboratories, Fujisawa Pharmaceutical Co., Ltd., Kashima 2-1-6, Yodogawa, Osaka 532-8514, Japan

Phone number: +81-(0)6-6390-1167

Telefacsimile number: +81-(0)6-6304-5716

E-mail address: katsuhiko_yoshizawa@po.fujisawa.co.jp.

Table 1. Occurrence of CHV Infection in the Same Surroundings

Case No.	CHV antibody titer	Renal lesions					Breeder	Age (month)	CHV infection	
		Location of CHV inclusion bodies in epithelia		Pelvic changes		Others				
		Papillary duct	Covering epithelia in papilla	Pelvic epithelia	Lymphocyte infiltration					Epithelial hyperplasia
1	80	U	U	—	B (+)	U (+)	—	A	13	Infected
2	102	—	U	—	B (#)	—	Testicular atrophy	A	13	
3	128	—	—	—	U (+)	U (+)	Hepatic fibrosis	A	13	
4	80	—	—	—	B (#)	—	—	A	13	
5	64	—	—	—	—	—	Testicular atrophy	A	13	
6	NE	—	—	U	B (#)	U (+)	—	A	13	
7	NE	U	—	—	B (#)	U (+)	—	A	12	
8	NE	U	—	—	B (#)	—	—	A	12	
9	NE	—	—	—	U (+)	—	—	A	13	Infection suspected
10	NE	—	—	—	B (+)	—	SG lymphocyte infiltration	A	12	
11	NE	—	—	—	B (#)	U (+)	SG lymphocyte infiltration	A	12	
12	NE	—	—	—	B (+)	—	SG lymphocyte infiltration	A	12	
13	NE	—	—	—	B (+)	—	—	A	12	
14	<2	—	—	—	—	—	—	B	10	No evidence of infection
15	<2	—	—	—	—	—	—	B	9	
16	<2	—	—	—	—	—	—	B	8	
17	<2	—	—	—	—	—	—	B	13	
18	<2	—	—	—	—	—	—	B	11	
19	<2	—	—	—	—	—	—	B	11	
20	NE	—	—	—	—	—	—	A	13	
21	NE	—	—	—	—	—	SG lymphocyte infiltration	A	12	
22	NE	—	—	—	—	—	—	A	12	
23	NE	—	—	—	—	—	—	A	12	

NE : Not examined, U : Detected in unilateral, B : Detected in bilateral, + : moderate, # : severe, SB : Salivary gland

Fig. 1. Chronic pyelitis. Lymphocytes infiltrated severely with lymphoid follicular formation in the subpelvic area (arrowheads). Pelvic epithelia proliferated as papillary projection (arrows). Case No. 2, HE staining, $\times 40$.

both viral envelope and core protein of herpes simplex virus type 1 and 2, and does not react to cytomegalovirus. The previous study demonstrated reciprocal cross-neutralization between canine herpes virus and herpes simplex virus immunologically⁸. Additionally, some pieces of the renal papilla of case No. 1 were post-fixed in 2.5% glutaraldehyde and 1% osmium tetroxide solutions, then dehydrated in ethanol and embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate for electron-microscopic observation. For serological examination, serum samples of five beagles were examined at Marupi Lifetech Co., Ltd. (Osaka, Japan) by the immunoperoxidase method described in previous investigations⁹.

Results

There were no clinical abnormalities or controversial family histories on the breeder records of any animal, before arrival at our laboratory. Subsequent clinical and laboratory examinations including hematological and blood-chemical analyses did not reveal any abnormalities at all. According to the study, complete necropsy including measuring weights of most of the organs, showed no abnormalities in the examined beagles.

Results of histopathological examinations are given in Table 1. In twelve of twenty-three beagles, chronic pyelitis characterized by moderate to severe lymphocytic infiltration often accompanied by follicular formation was observed in the subpelvic area of the renal medulla (Fig. 1). The renal lesions were bilateral in ten and unilateral in two dogs (Table

1). Moreover, the lesion was accompanied by pelvic epithelial hyperplasia such as papillary projection in five dogs (Fig. 1). In five of twenty-three dogs, the cytoplasm of the papillary ducts and/or covering epithelia of the papilla were filled with eosinophilic inclusion bodies (Fig. 2a). In this field, several affected cells were broken, compressed, and detached into the lumen. Furthermore, in one animal, the hyperplastic pelvic epithelia contained a few inclusions in the cytoplasm (Fig. 2b). These inclusions were negative for PAS, Grocott's, Warthin-Starry, Von Kossa's, and PTAH stainings. The inclusion bodies showed positive reaction against herpes simplex virus antigen, whereas the normal epithelial cells were completely negative (Fig. 2c). Electron-microscopically, these inclusion bodies consisted of numerous intracytoplasmic aggregation of virus particles which were regularly arranged in a crystalline array (Fig. 3a). Each virion was of about 140 nm in diameter, round to hexagonal in shape, and possessing envelope or capsid surrounding a dense nucleoid (Fig. 3b). These viral inclusion bodies were not observed in any other organs of the beagles examined. Moreover, there were no lesions related to viral infections in the renal parenchyma or vascular system in any dogs. Testicular atrophy, hepatic fibrosis, or chronic inflammation of the salivary gland was detected in some beagles (Table 1), but these lesions were apparently not related CHV infection.

With or without detecting renal viral inclusions, the neutralizing titer for CHV antibody ranged serologically from 64 to 128 in five beagles (Table 1). On the other hand, six of the animals derived from another farm (Breeder B) and housed in the same surroundings, showed no abnormalities in

Fig. 2. Cytoplasmic inclusion bodies of canine herpes virus. (a) Many eosinophilic inclusions are present in the swollen epithelial cells of renal papillary ducts (arrowheads). Some infected epithelia are detached into the lumen. Intratubular mineral deposition in the same manner as normal beagles (arrow). Case No. 2, HE staining, $\times 400$. (b) Cytoplasmic inclusion bodies in the hyperplastic pelvic epithelia. Case No. 1, HE staining, $\times 600$. (c) Positive staining of cytoplasmic inclusion bodies against anti-herpes antigen in immunohistochemistry. Case No. 2, Anti-human herpes simplex, $\times 400$.

serology or histopathology.

Discussion

Canine herpes virus (CHV) causes a systemic disease of neonatal puppies that is characterized by hemorrhagic necrotic foci and in a wide variety of organs, especially the lung and renal cortex^{3,6,10}. Newborns, especially those within two week of age, are susceptible to the virus infection but older puppies are not so susceptible. In adult dogs, the pathogen does not usually induce histopathological changes; it rarely causes catarrhal tracheobronchitis with formation of viral inclusions in the respiratory epithelia without clinical recognition²⁻⁵.

In this study, viral inclusion bodies and particles were present in the epithelial cells of the renal papilla, papillary duct and pelvis, both histopathologically and electronmicroscopically. From the immunohistochemical and electronmicroscopical findings, the virion was considered to be CHV. As the cells bearing viral inclusions were often detached into the lumen, this available evidence was strongly suggestive of viral urinary excretion. Although these animals were housed in our laboratory for about half a year, the other beagles housed in separate stainless cages in the same room were not infected by CHV. The cages were washed daily with a neutralized cleanser, Neoperex[®] (Kao, Co. Ltd.,

Tokyo, Japan), and routine maintenance of cleanness in the housing room contributed to preventing epidemic infections in addition to the housing environment.

The affected beagles might have been infected at the breeder A, because other beagles housed in the same conditions but from breeder B were negative for CHV antibody test. Moreover, the lesions including the characteristic renal inclusion related to the infection were not detected in these dogs. In addition, as we have purchased all animals from the other farm since the present occurrence, no beagles have been affected by CHV up to the present. CHV has been associated with abortion, stillbirth, and infertility and viral recrudescence can be induced in recovered animals by corticosteroids¹⁰. In the farm from which the animals were obtained, the breeder did not record such abnormalities or drug-therapy.

The systemic disease is characterized by hemorrhagic necrosis in a wide variety of organs in neonatal puppies and also renal and retinal dysplasia in puppies are caused by neonatal infection by canine herpes virus^{1,11}. These lesions were not detected in the animals. Therefore, the animals would have been infected in the growing period after weaning and before arrival at our laboratory.

Interstitial nephritis and pyelitis are associated with *leptospira interrogans* infection in beagles¹². In the animals, other pathogens including spirochaeta and mycotic path-

Fig. 3. Electronmicrographs of virus particles in the epithelial cells of papillary ducts. Case No. 1. (a) Full crystalline array of numerous viral particle in cytoplasm. bar : 2 μ m. (b) Each virus has an envelope or capsid surrounding dense nucleoids. bar : 300 nm.

ogens were not detected by PAS, Grocott, or Warthin-Starry stainings. Though the herpes virus infection is characterized by multifocal necrosis with intranuclear inclusion bodies in puppies as one of several pathological lesions, older animals do not always undergo such specific changes³. Likewise, the animals revealed only chronic inflammation with cytoplasmic inclusions in the kidneys, but possessed no specific figures. Therefore, the animals might be related to the persistent CHV infection for longer period of time. Moreover, it was suspected that five dogs (Case Nos. 9-13) without renal inclusion bodies were infected by CHV, because of chronic perivascular changes likely in CHV-infected beagles.

In addition, testicular atrophy, hepatic fibrosis, or chronic inflammation of the salivary gland was detected in the examined animals including CHV-affected dogs. As these lesions are often seen in normal laboratory beagles without viral inclusions, they were not related to CHV infection.

Several inclusions, whether drug-induced or spontaneous, are recognized in the renal tubules in the assessment of routine toxicity studies^{13,14}. Nonspecific lysosomal and lipochrome inclusions are often located in the cytoplasm of epithelial cells in the kidney¹⁴. These inclusions are less eosinophilic and granular than the viral inclusions¹⁴. In addition, several xenobiotics produce eosinophilic inclusions (hyaline droplets)^{14,15}. The droplets are often located in proximal tubules and positive for PAS reaction. From the point of morphological figures including special stainings, it would be easy to differ spontaneous or drug-induced inclusions from the present viral inclusions, if we have apprehen-

sions for them. Moreover, in the assessment of routine toxicity studies, it is important to understand the present latent infection, so as to prevent the spread of pathogen in laboratory canine colonies.

Acknowledgment : We thank Ms F. Tanaka, Ms T. Akamatsu, and Ms Y. Morioka for their skillful technical assistance at Osaka Saiseikai Nakatsu Hospital, Kansai Medical University, and Fujisawa Pharmaceutical Co. Ltd., respectively.

References

1. Albert DM, Lahav M, Carmichael LE, and Percy DH. Canine herpes induced retinal dysplasia and associated ocular anomalies. *Invest Ophthalmol* 1976; **15**: 267-278.
2. Cornwell HJC and Wright NG. Neonatal canine herpesvirus infection: A review of present knowledge. *Vet Rec* 1969; **84**: 2-6.
3. Jones TC, Hunt RD, and King NW. Herpesviridae. In: *Veterinary pathology*, 6th ed. TC Jones, RD Hunt, and NW King (eds), Baltimore: Williams & Wilkins, 216-241, 1997.
4. Schulze C and Baumgartner W. Nested polymerase chain reaction and insitu hybridization for diagnosis of canine herpesvirus infection in puppies. *Vet Pathol* 1998; **35**: 209-217.
5. Yamamura T, Minato Y, Kojima A, and Okaniwa A. Electron microscopy of renal arterial lesion in a pup infected with canine herpes virus. *J Vet Med Sci* 1992; **54**: 779-780.
6. Hashimoto A, Hirai K, Miyoshi A, Shimakura S, Yagami K, Kato N, Kunihiro K, Fujiura A, Kitazawa K, Okada K, and Fujimoto Y. Naturally occurring canine herpesvirus infection

- in Japan. *Jpn J Vet Sci* 1978; **40**: 157-169.
7. Oyaizu T, Takahashi H, Senzaki H, Oishi Y, Tsubura A, and Morii S. Antigen retrieval based on microwave-exposure in immunostains of myosarcoma tissues. *Acta Histochem Cytochem* 1993; **26**: 429-433.
 8. Manning A, Buchan A, Skinner GRB, Durham J, and Thompson H. The immunological relationship between canine herpesvirus and four other herpesviruses. *J Gen Virol* 1988; **69**: 1601-1608.
 9. Soma T, Ishii H, and Yonehara Y. Serological surveys of canine herpesvirus infection by immunoperoxidase method. *J Jpn Vet Med Assoc* 1998; **51**: 438-441.
 10. Jubb KVF, Kennedy PC, and Palmer N. Herpesvirus infections of the fetus and newborn. In: *Pathology of domestic animals*, Vol. 3, KVF Jubb, PC Kennedy, and N Palmer (eds), Florida: Academic Press, 361-365, 1997.
 11. Maxie MG. Renal dysplasia. In: *Pathology of domestic animals*, Vol. 2, KVF Jubb, PC Kennedy, and N Palmer (eds), Florida: Academic Press, 351-353, 1997.
 12. Scanziani E, Crippa L, Giusti AM, Luini M, Pacciarini ML, Tagliabue S, and Cavalletti E. *Leptospira interrogans* serovar sejroe infection in a group of laboratory dogs. *Lab Anim* 1995; **29**: 300-306.
 13. Racusen LC and Solez K. Nephrotoxic tubular and interstitial lesions: morphology and classification. *Toxicol Pathol* 1986; **14**: 45-57.
 14. Casey HW, Ayers KM, and Robinson FR. Nonviral cellular inclusions in the urinary tract. In: *Pathology of laboratory animals*, Vol. 1, K Benirschke, FM Garner, and TC Jones (eds), New York: Springer-Verlag, 155-157, 1978.
 15. Alden CL and Frith CH. Mechanisms of toxicity. In: *Handbook of toxicologic pathology*, WM Haschek and CG Rousseaux (eds), California: Academic Press, 327-354, 1991.