*J Toxicol Pathol 2009; **22**: 289–292*

Case Report

Emphysematous Cystitis in a Chemically-Induced Diabetic Dog

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Abstract: Emphysematous cystitis is a rare disorder caused by bacterial infection and characterized by gas accumulation within the bladder wall with cyst formation. This report describes the histopathological characteristics of emphysematous cystitis found in a diabetic female beagle induced by streptozotocin and alloxan. Macroscopically, multiple cyst-like structures were observed on the cut surface of the urinary mucosa. During fixation, small specimens cut from the mucosa floated on the surface of the fixative solution. Histopathologically, multiple cysts were lined with a single layer of flattened cells found to be immunohistochemically positive for vimentin, partially positive for α-smooth muscle actin or macrophage scavenger receptor, class A, and thought to be myofibroblasts, fibroblasts or macrophages. Multinucleated giant cells were observed around the cysts, and gram-negative short bacilli were observed in the lumen of the urinary bladder. From these findings, this case was diagnosed as emphysematous cystitis. (J Toxicol Pathol 2009; **22**: 289–292)

Key words: cystitis, emphysema, diabetes mellitus, dogs, gases, cysts

Emphysematous cystitis is a rare disorder caused by bacterial infection and characterized by gas accumulation within the bladder wall accompanied by cyst formation[1,2]. The gas is thought to be produced by gas-producing bacteria as a result of fermentation of either glucose or albumin[3]. Cases of emphysematous cystitis have been reported in dogs, cats and humans. Most of the reported cases of emphysematous cystitis were associated with diabetes mellitus[4,5], but some cases were not. Clinical diagnosis of emphysematous cystitis in humans and domestic animals is usually based on evidence of gas accumulation within the bladder lumen and wall found with radiography and computerized tomography[2–6]. Patients and diseased animals recover well if administered antibiotics[5,6], and so few reports describe the histopathology in detail. In this report, we describe the histopathological characteristics of emphysematous cystitis found in a dog with streptozotocin and alloxan-induced diabetes.

A one-year-old female beagle was obtained from Chugai Research Institute for Medical Science (Shizuoka, Japan) and received a single intravenous injection containing a mixture of streptozotocin (STZ, 30 mg/kg) and alloxan (ALX, 50 mg/kg; Sigma Chemical, St Louis, MO, USA) to damage pancreatic β-cells. Clinically, the fasting plasma glucose level of this dog was in the range of 250–400 mg/dL during the 8 weeks after administration of STZ and ALX and in the range of 150–300 mg/dL from 28 weeks to 7 years following administration. Hematuria was occasionally noted in the animal during the period of half-year prior to sacrifice. The dog was sacrificed by exsanguination from the common carotid artery under pentobarbital anesthesia at 8 years old. All animal procedures were conducted in accordance with Chugai Pharmaceutical's "Guide for the Care and Use of Laboratory Animals", and all experimental protocols were approved by the Institutional Animal Care and Use Committee.

Macroscopically, the mucosa of the urinary bladder was irregular, and dark red areas were observed. On the cut surface, multiple cyst-like structures about 1 mm in diameter were observed in the elevated mucosa (Fig. 1a), and small specimens cut from the mucosa floated on the surface of the fixative solution. No cyst-like structures were observed in the other organs at necropsy.

For histopathological examination, the urinary bladder was fixed in 20% neutral-buffered formalin and embedded in paraffin. The sections were stained with hematoxylin and eosin (HE) and Gram stain. For immunohistochemical analysis, primary antibodies against vimentin (V9, monoclonal mouse anti-vimentin; Dako, Glostrup,

Received: 1 June 2009, Accepted: 18 August 2009
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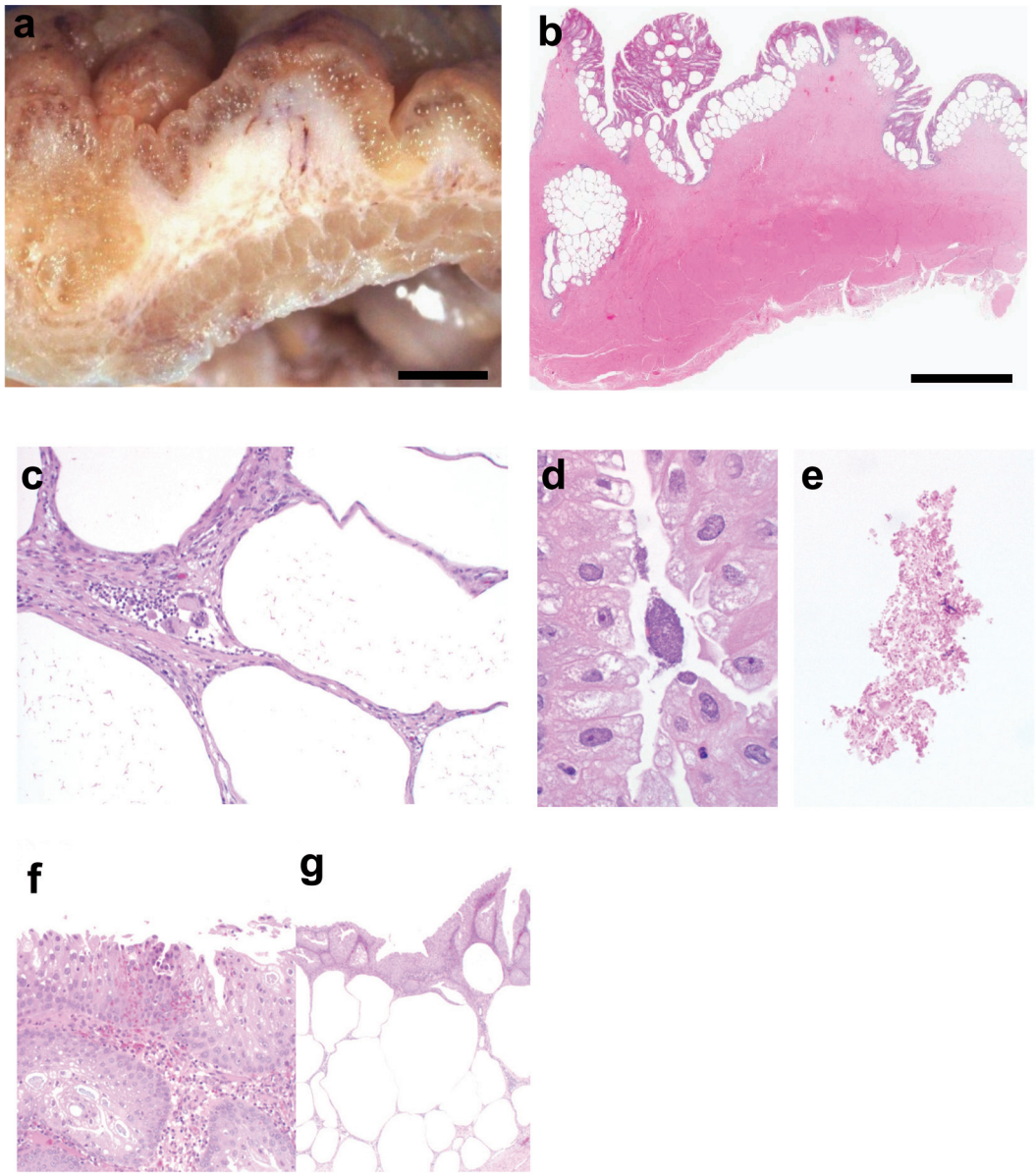

Fig. 1. Macroscopic (a) and microscopic (b–g) findings of the urinary bladder. (a) The cut surface of the urinary bladder. Multiple cyst-like structures about 1 mm in diameter were observed in the elevated mucosa. Bar: 5 mm. (b) Multiple cyst-like structures varying in size were observed in the area from the lamina propria to the muscle layer. HE. Bar: 5 mm. (c) Cysts were lined by a single layer of flattened cells. Multinucleated giant cells and neutrophils were observed around the cysts. HE, ×100. (d) Small colonies of short bacilli were observed in the lumen of the urinary bladder. HE, ×400. (e) Short bacilli were negative for gram staining. Gram stain, ×400. (f) Degeneration and desquamation of the mucosal epithelium, hemorrhage and inflammatory cell infiltration of neutrophils, lymphocytes and macrophages were observed in the mucosal epithelium and lamina propria. HE, ×100. (g) Papillary and nodular hyperplasia of the transitional epithelium was observed. HE, ×20.

Denmark); α-smooth muscle actin (α-SMA, 1A4, monoclonal mouse anti-human smooth muscle actin; Dako); macrophage scavenger receptor, class A (MSR-A, SRA-E5, monoclonal mouse anti-human macrophage scavenger receptor; Trans Genic Inc., Kumamoto, Japan); cytokeratin (AE1/AE3, monoclonal mouse anti-human cytokeratin; Dako); cytokeratin, high molecular weight (34βE12, monoclonal mouse anti-human cytokeratin; Dako); and CD31 (JC70A, monoclonal mouse anti-human CD31; Dako) were used, and immunohistochemical staining was performed according to the polymer-immuno complex method using an ENVISION+ kit (Dako) or the labeled streptavidin-biotin (LSAB) method using an LSAB 2 kit (Dako). The immunoreaction was visualized by a peroxidase-diaminobenzidine reaction. Finally, the sections were counterstained with hematoxylin.

Histopathologically, multiple cyst-like structures varying in size were observed in the area from the lamina propria to the muscle layer (Fig. 1b). Most of the cysts were lined by a single layer of flattened cells, but they were partially lined by the transitional epithelium continuous from the epithelium of the urinary bladder or were not lined by any cells (Fig. 1c). Multinucleated giant cells and neutrophils were observed around the cysts (Fig. 1c). Gram-negative short bacilli were observed sporadically in the lumen of the urinary bladder (Fig. 1d, e). In the mucosa, degeneration, desquamation, erosion and papillary/nodular hyperplasia of the transitional epithelium were observed (Fig. 1f, g). Hemorrhage and inflammatory cell infiltration of neutrophils, lymphocytes and macrophages were observed in the mucosal epithelium and lamina propria (Fig. 1f). Immunohistochemistry revealed that the flattened cells covering the cysts were positive for vimentin, partially positive for α-SMA or MSR-A and negative for cytokeratins and CD31 (Fig. 2).

With the urinary bladder, emphysematous cystitis, glandular cystitis and cystic cystitis are known to have cyst-forming lesions in the bladder wall[9–11]. The criteria for diagnosis of glandular cystitis and cystic cystitis is the presence of an urothelium or columnar epithelium outlying the cysts that secretes mucin into the lumina[9–11]. On the other hand, emphysematous cystitis and other emphysematous disorders such as intestinal emphysema in pigs or pneumatosis cystoides intestinalis in humans are characterized by gas-filled cysts lined with fibrous tissue, macrophages and endothelial cells suggesting lymphatic vessels[1,5,7,9,12,13]. In the present case, the cystic structures were lined with flattened myofibroblasts, fibroblasts or macrophages and did not have an urothelium or columnar epithelium. In addition, multinucleated giant cells were observed around the cysts, which were thought to be a characteristic of emphysematous disorders and possibly a foreign reaction to the gas[14,15]. Moreover, since the small specimens cut from the mucosa of the urinary bladder floated in the fixative solution, we speculated that the cyst-like structures were filled with gas in the present case. From these findings, this case was diagnosed as emphysematous cystitis.

In dogs and humans, gram-negative bacteria such as *Escherichia coli*, *Klebsiella spp.*, *Pseudomonas spp.*, *Proteus spp.* and *Enterobacter spp.* are known to be causal gas-forming bacteria in urinary tract infections, and the gas in the lumen and within the wall of the urinary bladder are considered to be produced by these bacteria from fermentation of glucose in the urine[2–6]. The glucosuria, lowered resistance to infection and dysuria associated with diabetic neuropathy in diabetic animals and patients are thought to contribute to bacterial infection in the urinary bladder[3–5]. In the present case, bacterial infection in the urinary bladder was indicated by the gram-negative short bacilli in the lumen, which were thought to be gas-forming bacteria. Glucosuria was suggested by the persistent hyperglycemia due to the STZ/ALX-induced diabetes. In STZ/ALX-induced diabetes model dogs, diabetic neuropathy in the vagal and tibial nerve has been observed in all model animals, including the present case[16]. Therefore, it is possible that diabetic neuropathy may affect urination and bring about bacterial infection of the urinary bladder. In addition, chronic inflammation of the urinary bladder was indicated by the continuation of hematuria for half-year and the hyperplasia of the transitional epithelium. Taken together, these considerations led to a diagnosis of emphysematous cystitis in the chemically-induced diabetic model dog.

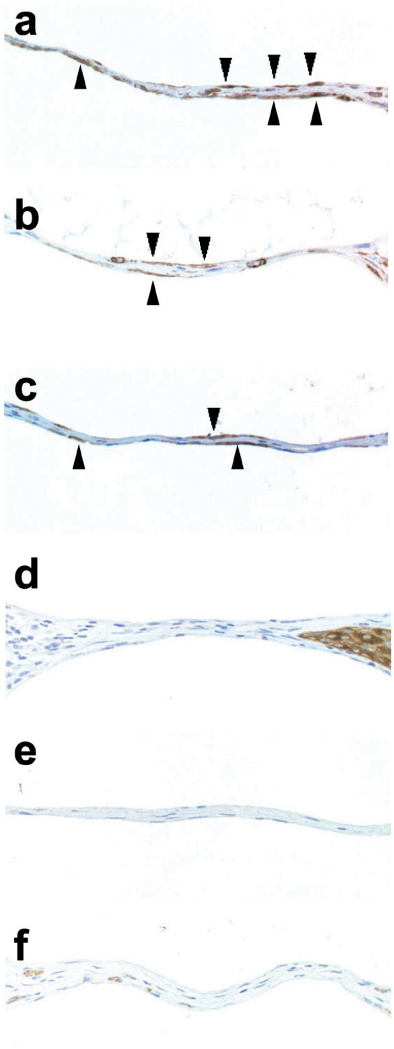

Fig. 2. Immunohistochemical staining of the flattened cells covering the cysts. The flattened cells were positive for vimentin (a), partially positive for α-SMA (b) and MSR-A (c) and negative for cytokeratin (AE1/AE3) (d), high molecular weight cytokeratin (e) and CD31 (f). Arrow heads indicate positive cells. ×200.

Emphysematous cystitis is a rare disorder found in dogs, cats and humans, and therefore little is known about its histopathological characteristics. This report offers valuable, detailed information on the histopathological

characteristics of emphysematous cystitis found in a chemically-induced diabetic dog.

Acknowledgments: We would like to thank Dr. Tomomi Soshin, Dr. Etsuko Fujii, Ms.Yoshimi Ohtani, Ms. Asuka Ikeda, Ms. Yayoi Takai and Mr. Toshihiko Shiga for their scientific and skillful technical assistance.

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