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Canine Renal Failure Caused by Ochratoxin A and Citrinin in the Commercial Dog Food

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Abstract : Five dogs with renal failure were referred to the Veterinary Medical Teaching Hospital at Kangwon National University. These dogs had the common history of consumption of Pedigree dry dog food produced in Thailand plant for over 1 month. The dogs showed anorexia, emaciation, vomiting, and polydipsia/polyuria. And in one severely affected dog, bloody diarrhea and hypothermia were seen. The remarkable clinicopathological signs were high value of BUN and creatinine. In some dogs, GGT, phosphorus and lipase were increased. However, no significant changes of complete blood count were found. In urinalysis, hematuria, low specific gravity urine, proteinuria, and calcium oxalate-like crystals were observed. Two severely affected dogs were died. The remained dogs were recovered gradually after change of dog food and supportive therapy. Pathological findings were seen typically in kidneys. Renal atrophy, congestion of the glomerular capillary, and diffuse degeneration, necrosis, dystrophic calcification and regeneration in the tubular epithelium were seen. Yellowish brown fluorolucent laminated materials or particles were quite often found in the lumina of the necrotizing renal tubules of cortex and medulla. Proliferation of fibrous tissue in the interstitium was also seen. By the mycotoxin analysis of the Pedigree dry dog food, ochratoxin A (OTA) and citrinin were detected as much as the concentration of 372.8 ppb and 8.3 ppb, respectively. The final diagnosis of renal failure caused by OTA and citrinin toxicosis was made on the basis of history takings, clinical signs, clinicopathological and pathological findings, and analysis of mycotoxins.

Key words **:** Canine, renal failure, ochratoxin A, citrinin, Pedigree dog food.

Introduction

There are many nephrotoxins, such as the heavy metals, drugs, additives, fungi, plant toxins and organic compounds. The ochratoxin A (OTA) and citrinin are produced as the secondary metabolites of certain *Penicillium* and *Aspergillus* fungal species and are the common contaminant of human food stuffs and animal feeds (1,14,16). OTA has been found in barley, oats, rye, wheat, coffee beans, and other plant products (2). OTA in recent years has received considerable attention because not only can it seriously affect animal performance and well-being, but it may also have deleterious effects on humans (12). Of greatest concern in humans is its implicated role in an irreversible and fatal kidney disease referred to as Balkan endemic nephropathy (BEN) and its potent carcinogenic effects (12). The kidney is the main target organ for OTA toxicity and the mammalian kidney plays an important role as an elimination system (11). OTA was also proved to exhibit immunosuppressive and teratogenic properties (2,14,18).

The OTA and citrinin consumed by the dogs induced renal disease and apparently would have resulted in death. In experimental study with Beagle dogs, clinical signs of OTA and citrinin toxicosis included anorexia, emesis, retching, tenesmus, weight loss, polydipsia, polyuria, prostration and death (9). And the increases in urinary protein, ketone, glucose and granular casts were detected in the urine. In addition, urine specific gravity decreased to low value. The OTA is eliminated via urine and bile relatively slow, due to its high binding activity to serum albumin (5). The toxin present in the bound form and more than 60% of OTA bound to albumin in serum (4). The Joint FAO/WHO Expert Committee on Food Additives suggested that the tolerable weekly intake of OTA should be 112 ng/kg of body weight (BW) (6). This value, which is equal to 16 ng/kg BW per day, is considerably higher than that recommended by the researchers mentioned previously (12).

The major histopathological changes caused by citrinin mycotoxicosis were renal tubular degeneration and necrosis (10). In the dog, renal lesions of citrinin toxicosis included degeneration and necrosis of the tubular epithelium, primarily of the straight segments and distal convoluted tubules (3). When citrinin was given intraperitoneally to young dogs, evidence of renal damage included excessive BUN, glucosuria, proteinuria, low urinary specific gravity, increased lactic dehydrogenase, glutamic oxaloacetic transaminase and isocitric dehydrogenase and granular casts in the urinary sediment (9).

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In Korea, over one thousand of dogs were estimated to have suffered from the renal failure from the winter of 2003 after consumption of Pedigree dry dog feed produced in Thailand plant. The aims of this study are to diagnose the renal failure of dogs and to elucidate the relationship between the renal failure and the consumption of commercial dog food.

Materials and methods

Clinical cases

From January to April of 2004, a total of 5 dogs with renal failure were referred to the Veterinary Medical Teaching Hospital at Kangwon National University. These dogs had the common history of consumption of Pedigree dry dog food produced in Thailand plant for over 1 month. Patients were in both sexes, broad range of age, and various breeds (Table 1).

Clinicopathological examination

The complete blood count (CBC), serum chemistry and urinalysis were conducted immediately after admission. CBC was conducted with the automatic analyzer (HEMACYTE™, Oxford Science, England). Serum total protein, albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), blood urea nitrogen (BUN), creatinine, creatine kinase, glucose, phosphorous, amylase, lipase, total bilirubin, gamma-glutamyl transferase (GGT) were analyzed by the chemistry analyzer (Vitros DT 60® System, Block Scientific Inc, USA). Urine samples collected by the urethral cannulation were subjected to urine strips with auto-analyzer (Combilyzer, Human, Germany). The urine sediment was also examined on microscope.

Pathological examination

One died dog (Case 1) was necropsied. After macroscopic examination, the tissue samples were removed, fixed in 10% neutral buffered formalin, embeded in paraffin, sectioned in 4 slices, and mounted on slides. After deparaffinizing, each specimen was stained with hematoxylin and eosin for an optical microscopic examination.

Determination of Ochratoxin A and citrinin

The OTA and citrinin in the Pedigree dry dog food (L11.3) determined at the laboratory (Scientec Lab Center Co, LTD, Korea) which was recognized under Korea Laboratory Accreditation Scheme by using the test kit (RIDASCREEN® Ochratoxin A, RIDASCREEN® Fastcitrinin, r-Biopharm GmbH, Germany). The protocol for the analysis of OTA and citrinin in feeds has been followed the recommendation by the Associate Referee of the Association of Official Analytical Communities. The detection limit of these kit were 0.1 ppb.

Results

Clinical signs

The dogs showed anorexia, emaciation, vomiting, and polydipsia/polyuria. And in one severely affected dog, bloody diarrhea and hypothermia were seen. Two out of 5 dogs were died and the remainder 3 dogs were recovered gradually after

Table 1. Signalment and history of 5 cases diagnosed with renal failure

Items	Case				
	1	2	3	4	5
Sex	F[A]	M[A]	F	M	F
Age (Month)	14	26	24	12	29
Breed	Cocker[B]	Shih Tzu	Shih Tzu	Shih Tzu	Peki[B]
Length of food consumption (Month)	1	2	9	1	9

[A]F; Female, M; Male.
[B]Cocker; Cocker spaniel, Peki; Pekingese.

Table 2. Clinical signs observed in 5 cases diagnosed with renal failure

Signs	Case				
	1	2	3	4	5
Anorexia	Yes	Yes	Yes	Yes	Yes
Emaciation	Yes	No	Yes	No	Yes
Vomiting	No	Yes	Yes	Yes	Yes
Polydipsia/Polyuria	No	Yes	No	Yes	No
Bloody diarrhea	Yes	No	No	No	No
Hypothermia	Yes	No	No	No	No
Prognosis	D[A]	D	R[A]	R	R

[A]D; died, R; recovered.

change of food to Hill's k/d and supportive therapy with antibiotics, metoclopramide, omeprazole, water soluble vitamins and fluid therapy with lactated Ringer's solution or 0.9% saline (Table 2).

Clinicopathological findings

No significant changes of CBC were found. Total protein were in low reference range in 2 cases (Table 3). Both BUN and creatinine concentrations were increased in all cases. Hyperphosphatemia (2 of 5) and modest increased lipase activity (3 of 3) were observed. GGT was markedly increased in 4 cases. In urinalysis, 2 cases (no. 2 and 3) formed isosthenuria and another 2 cases (no. 1 and 4) were showed dilute urine. These 4 cases were accompanied by hematuria. Mild proteinuria was observed in 1 case. Calcium oxalate-like crystals in the urine sediment were observed in 4 cases (Table 4).

Pathological findings

Case 1 was necropsied. In characteristic gross findings, hydrothorax, enlarged gall bladder and fibrinous peritonitis were observed. Hemorrhage was noted in stomach and small intestine. The kidney was showed congested, and its capsule was not easily detached. The yellowish spots were observed under the capsule of kidney. The cortex was atrophied and the ratio of cortex to medulla was about 0.4. The yellowish materials

Table 3. Serum chemistry in 5 cases diagnosed with renal failure

Serum chemistry	Reference range	Case				
		1	2	3	4	5
Total protein(g/dl)	5.5-7.5	4.9	6.3	7	6.1	5.3
Albumin(g/dl)	2.7-4.5	2.7	3.2	NA	3.4	3
ALT[A](U/L)	3-50	106	302	40	56	10
AST[A](U/L)	1-37	100	21	27	37	10
ALP[A](U/L)	20-155	86	NA	43	NA	85
GGT(U/L)	1-6	42	33	2	44	13
Glucose(mg/dl)	67-140	356	114	130	131	102
BUN[A](mg/dl)	7-28	288	183	130	148	39
Creatinine(mg/dl)	0.9-1.7	2.4	10.2	6.3	5.2	3.3
CK[A](mg/dl)	25-317	535	< 20	NA	NA	634
Calcium(mg/dl)	8-12	NA[B]	11.5	NA	NA	10.7
Phosphorous(mg/dl)	2.4-6.1	15	11.7	NA	NA	2.5
Amylase(U/L)	388-1250	893	541	720	529	NA
Lipase(U/L)	30-560	646	589	114	NA	NA
Total bilirubin(mg/dl)	0.1-0.3	NA	<0.1	NA	<0.1	NA

[A]ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; BUN, blood urea nitrogen; CK, creatine kinase.
[B]NA; Not assayed.

Table 4. Summary of urinalysis of 5 cases diagnosed with renal failure

Urinalysis profile	Case				
	1	2	3	4	5
Specific gravity	1.020	1.012	1.008	1.020	NA[A]
pH	6	5	6	6	NA
Protein	Neg[A]	+	Neg	Neg	NA
Glucose	Neg	Neg	Neg	Neg	NA
Urobilinogen	Neg	Neg	Neg	Neg	NA
Bilirubin	Neg	Neg	Neg	Neg	NA
Ketones	Neg	Neg	Neg	Neg	NA
Leucocytes	Neg	Neg	Neg	+++	NA
Blood	++	+++	++	+++	NA
Ascorbic acid	Neg	Neg	Neg	Neg	NA
Nitrite	NA[B]	NA	NA	Pos[C]	NA
Crystal in sediment	Pos[C]	Pos	Pos	Pos	NA

[A]Neg, Negative; [B]NA, Not assayed; [C]Pos, Positive.

or particles were observed on the medullary cut surface (Fig 1-A).

In histopathological findings, congestion of the glomerular capillary, and diffuse degeneration, necrosis, dystrophic calcification and regeneration in the tubular epithelium were seen (Fig 1-B). Yellowish brown fluorolucent laminated materials or particles were quite often found in the lumina of the necrotizing renal tubules of cortex and medulla (Fig 1-C and D). Proliferation of fibrous tissue in the interstitium, infiltration of inflammatory cell and desquamated tubular epithelium in the collection tubule were observed. Calcificating of mucosal cells of the stomach was observed. Congestion and atrophy of villi and infiltration of plasma cells in the interstitium of small intestine were also detected. Congestion in the lung and focal bacterial colony and infiltration of lymphoid cells in heart were observed. Splenic atrophy and diffuse blackish brown pigmentation and megakaryocyte were observed in spleen. In the liver, congestive dilatation of central zone with hemosiderosis and dissociation of hepatic cell cord were also detected.

Concentration of OTA and citrinin

OTA and citrinin were detected from the dog food. The concentrations of OTA and citrinin were 372.8 ppb and 8.3 ppb, respectively.

Discussion

Nephrotoxic effects due to OTA and citrinin have been described in the dog (3,8-10,17). In experimental study with dogs, clinical signs of OTA and citrinin toxicosis included anorexia, emesis, retching, tenesmus, weight loss, polydipsia, polyuria, prostration and death (9). In this study, the dogs showed anorexia, emaciation, vomiting, polydipsia/polyuria and death. And in one severely affected dog, bloody diarrhea and hypothermia were seen. These clinical signs are considered to be of mycotoxicosis of OTA and citrinin. The pathogenesis of polydipsia and polyuria was not specifically studied. It has been suggested, however, widespread gradual destruction of nephrons can cause polyuria by causing diminished circulating antidiuretic hormone or by changing mechanism concerned with producing a hypertonic interstitial fluid in the renal medulla (13,15). The bloody diarrhea in one dog of this study was likely induced by gastrointestinal hemorrhage and by the parasym-

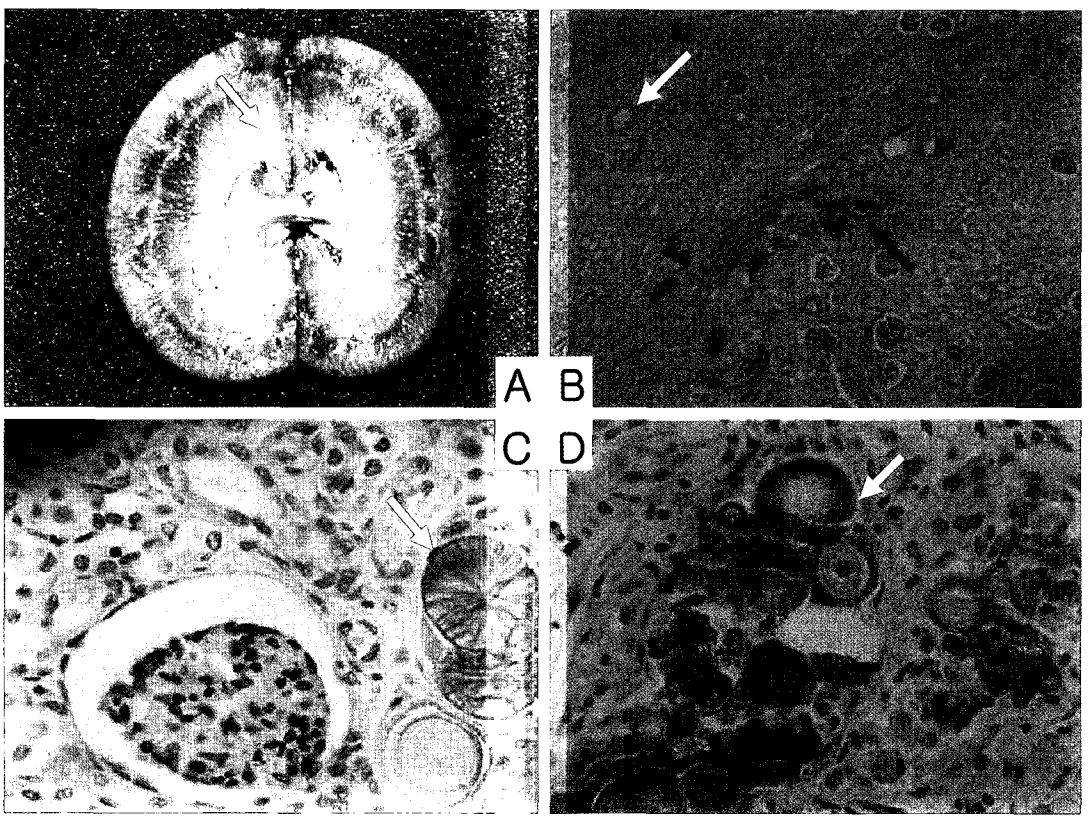

Fig 1. Macroscopic (A) and microscopic pathological findings (B-D) of the kidney from the dog with renal failure. A, Cortex of the kidney was atrophied, as represented by the decreased ratio of cortex to medulla, 0.4 (black arrow). Yellowish particles were observed on medullary surface (white arrow). B, The kidney was characterized by tubular degeneration and necrosis with dystrophic calcification, followed by regeneration (white arrow). Also, note the yellowish brown fluorolucent laminated materials or particles within the tubular lumina (black arrow). C, Magnification of B. Note the yellowish brown fluorolucent laminated materials or particles within the tubular lumina (white arrow). D, The yellowish laminated materials were also existed in medulla (arrow).

pathomimetic activity of citrinin (7,9). Death must be in part be attributed to vascular shock subsequent to intestinal abnormality and renal failure.

In this study, concentration of serum BUN and creatinine of severely affected dogs were consistently increased above the reference range of normal dogs. It seems that the increase of these enzymes were caused by impairment of removal function of metabolized materials through kidneys. The increase in serum hepatic enzymes was considered to be failure of excretion of these enzymes by kidneys and mild hepatic damage by OTA and citrinin (7).

When OTA and citrinin were given to young Beagle dogs, evidence of renal damage included excessive BUN, glucosuria, proteinuria, low urinary specific gravity, increased lactic dehydrogenase, glutamic oxaloacetic transaminase and isocitric dehydrogenase and granular casts in the urinary sediment (9). Proteinuria is common after renal tubular necrosis with release of proteins from cells and failure of tubular resorption. However, in this study hematuria, mild proteinuria, dilute urine and crystaluria were observed in some of cases.

Dogs given experimentally combined doses of OTA and citrinin had degeneration and necrosis in proximal and distal tubules, and in thin segments and the collecting ducts. There were desquamated cells and granular casts in the lumina (10). Dogs given OTA had necrosis of lymphoid tissues in the spleen, tonsil, thymus peripheral lymph nodes and lymph nodules of the ileum, colon and rectum. There was ulceration of the mucosa of the intestine in dogs given large combined doses of OTA and citrinin (10). The most of important pathological findings observed in dogs given experimentally citrinin and OTA were also observed in natural clinical cases in this study.

Pedigree company had recalled products after their inspectors found molds in 2 kinds of ingredients for dry dog food in its Thailand plant. It is likely that clinical signs seen in patients were the result of ingestion of one or more mycotoxins that have been identified in raw materials at the Thai plant and in returned finished products. And Pedigree said that OTA found in corn at the Thai site. Moreover, citrinin had been identified in one sample of dry dog food made at the Thai plant.

Both OTA and citrinin are known as nephrotoxins and are considered likely contributing causes to this syndrome. Therefore, we analysed OTA and citrinin in the dog food at the laboratory. In analysis result, OTA and citirinin concentrations in Pedigree dry dog food were 372.8 ppb and 8.3 ppb, respectively. The Joint FAO/WHO Expert Committee on Food Additives was recommended provisional tolerable weekly intake of 112 ng/ of body weight (6). Consequently, it is considered that OTA in Pedigree dry dog food was enough amount to cause mycotoxicosis in dogs.

In this study, one died dog had very similar pathological findings in BEN of human and mycotoxicosis by ochratoxin and citrinin of dogs. In addition, the dogs showed common history, clinical signs, and clinicopatholoical findings. In accordance with history takings, clinicopathological and pathological findings, these cases were tentatively diagnosed as renal toxicosis caused by OTA and citrinin. After determination of OTA and citrinin in dog food samples, these cases were finally diagnosed as the renal failure caused by OTA and citrinin in Pedigree dry dog food.

References

1. Abramson D, Usleber E, Martlbauer E. An indirect enzyme immunoassay for the mycotoxin citrinin. Appl Environ Microbiol 2001; 61: 2007-2009.
2. Bennett JW, Klich M. Mycotoxins. Clin Microbiol Rev 2003; 16: 497-516.
3. Carlton WW, Sansing G, Szczech GM, Tuite J. Citrinin mycotoxicosis in beagle dogs. Food Cosmet Toxicol 1974; 12: 479-490.
4. Chang FC, Chu FS. The fate of ochratoxin A in rats. Food Cosmet Toxicol 1977; 15: 199-204.
5. Fuchs R, Hult K. Ochratoxin A in blood and its pharmacokinetic properties. Food Chem Toxicol 1992; 30: 201-204.
6. JECFA. Toxicological evaluation of certain food additives and contaminant in food. WHO food additive series 1996; 35: 363-376.
7. Jeong WI, Do SH, Jeong DH, Chung JY, Yang HJ, Yuan DW, Hong IH, Park JK, Goo MJ, Jeong KS. Canine renal failure syndrome in three dogs. J Vet Sci 2006; 7: 299-301.
8. Kitchen DN, Carton WW, Hinsman EJ. Ochratoxin A and citrinin induced nephrosis in beagle dogs. III. Terminal renal ultrastructural alterations. Vet Pathol 1977; 14: 392-406.
9. Kitchen DN, Carton WW, Tuite J. Ochratoxin A and citrinin induced nephrosis in beagle dogs. I. Clinical and clinicopathological features. Vet Pathol 1977; 14: 154-172.
10. Kitchen DN, Carton WW, Tuite J. Ochratoxin A and citrinin induced nephrosis in Beagle dogs. II. Pathology. Vet Pathol 1977; 14: 261-272.
11. Luhe A, Hildebrand H, Bach U, Dingermann T, Ahr HJ. A new approach to study ochratoxin A (OTA)-induced nephrotoxicity: expression profiling in vitro employing cDNA microarrays. Toxicol Sci 2003; 73: 315-328.
12. Marquardt RR, Frohlich AA. A review of recent advances in understanding ochratoxicosis. J Anim Sci 1992; 70: 3968-3988.
13. Meyer T, Scholey J, Brenner B. Nephron adaptation to renal injury. In: The kidney, 4th ed. Philadelphia: WB Saunders. 1991: 1871-1908.
14. O'Brien E, Heussner AH, Dietrich DR. Species-, sex-, and cell type-specific effects of ochratoxin A and B. Toxicol Sci 2001; 63: 256-264.
15. Polzin DJ, Osborne CA, Jacob F, Ross S. Chronic renal failure. In: Textbook of veterinary medicine, 5th ed. Philadelphia: WB Saunders. 2000: 1634-1662.
16. Rahimtula AD, Bereziat JC, Bussacchini-Griot V, Bartsch H. Lipid prroxidation as a possible cause of ochratoxin A toxicity. Biochem Pharmacol 1988; 37: 4469-4477.
17. Szczech GM, Carlton WW, Tuite J. Ochratoxicosis in Beagle dogs. I. Clinical and clinicopathological features.Vet Pathol 1973; 10: 135-154.
18. Zepnik H, Pahler A, Schauer U, Dekant W. Ochratoxin A-induced tumor formation: is there a role of reactive ochratoxin A metabolites. Toxicol Sci 2001; 59: 59-67.

시판 사료에 오염된 Ochratoxin A와 Citrinin에 의한 개의 신부전

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요 약 : 태국에서 생산된 Pedigree사의 개 사료를 1개월 이상 섭취한 5마리의 개가 식욕절폐, 쇠약, 구토, 다음다뇨 증상을 보였으며 증상이 심한 개는 혈액성 설사와 저체온증을 보였다. 특징적인 임상병리학적 소견으로 BUN과 creatinine이 높았으며 일부의 개에서는 GGT, 무기인과 lipase가 높았으나 CBC는 특이적인 임상병리학적 소견이 없었다. 요분석에서는 혈뇨, 저비중뇨, 단백뇨와 calcium oxalate와 유사한 결정이 관찰되었다. 증상이 심한 두 마리는 폐사하였으며 나머지의 개는 사료의 변경과 보조요법에 의하여 점차 회복되었다. 전형적인 병리학적 소견은 신장에서 관찰되어 신장의 위축, 일부 사구체의 모세혈관 충혈, 세뇨관의 미만성 변성과 이영양성 석회화, 세뇨관의 괴사와 재생, 세뇨관 내강 내에 황갈색 판상 형광성 물질 또는 파편이 관찰되었다. 섭취한 사료와 동일한 개봉하지 않은 사료 내의 ochratoxin A와 citrinin의 농도를 분석한 결과 각각 372.8ppb와 8.3ppb가 검출되었다. 병력, 임상증상, 임상병리소견, 병리학적 소견 및 사료 내의 곰팡이독소의 분석을 통하여 ochratoxin A와 citrinin의 중독에 의한 신부전으로 최종진단을 내렸다.

주요어 : 개, 신부전, ochratoxin A, citrinin, Pedigree 개 사료.