Hyperuricemia of Chickens Caused by Inoculation with Nephropathogenic Infectious Bronchitis Virus

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Hyperuricemia was produced in chickens by artificial infection with nephropathogenic infectious bronchitis virus (IBV) and studied. Typical respiratory symptoms were found in all of the chickens after inoculation with the nephropathogenic AM-83 strain of IBV. Sixteen out of 30 chickens died from 4 to 14 days after challenge. The level of uric acid in plasma was very high at the day before death and the values in the majority of chickens was more than about 100 mg/dl. The chicken surviving against the IBV challenge had hyperuricemia for a longer time than the dead chickens. A few chickens had hyperuricemia for only one day with very low levels of uric acid.

Chickens in contact with the chickens challenged with AM-83 strain of IBV also died of hyperuricemia, but some of them were resistant to the challenge.

We demonstrated that the hyperuricemia in the chickens infected with IBV was divided into the 4 grades of uncertain, mild, moderate and severe.

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Key words : hyperuricemia, chicken, infectious bronchitis virus, gout, nephritis

Introduction

Nephritis and visceral gout are the most common findings during necropsy of chickens in the field. RANDALL *et al.* (1977) reported that mortality caused by kidney lesion was recorded as 20% in 2,615 specimens of chickens in laying flocks from the field.

Infectious bronchitis virus (IBV) is distributed worldwide and is a major cause of economic losses to the poultry farms. Infectious bronchitis was first noted as a respiratory disease of young chickens (SCHALK and HAWN, 1931), so, IBV has been considered a prime cause of respiratory disease in chickens. SINE WINTERFIELD and HITCHNER (1962) first reported outbreaks of nephrosis by IBV in the United States and CUMMINGS (1962, 1963) subsequently reported an IBV outbreaks causing severe kidney lesions in chickens in Australia, nephropathogenic strains of IBV were isolated from commercial chickens with visceral gout (BUTCHER *et al.*, 1988, 1990 ; KINDE *et al.*, 1991 ; SHIMAKURA and HIRAI, 1971). When the nephropathogenic strain was infected artificially to SPF chickens, gross renal lesions such as urate deposition and histologic renal changes were observed in the chickens (ALBASSAM *et al.*, 1988). However, we have little definitive knowledge on hyperuricemia of chickens after IBV infection.

The present study explains the details of hyperuricemia of chickens produced by artificial infection with a nephropathogenic strain of IBV.

Fig. 1. Incidence of respiratory signs after infection with nephropathogenic IBV and their mortality.
(●—●) respiratory signs, (▲—▲) mortality

Table 1. Change of uric acid in dead chickens by artificial challenge with nephropathogenic infectious bronchitis virus

Chicken No.	Days after challenge															
	-3	-1	1	3	4	5	6	7	8	9	10	11	12	13	14	
249	2.3	4.2	2.8	4.7	27.3	Dead										
234	5.2	4.4	3.3	4.3	3.9	7.1	53.4	Dead								
238	4.1	3.3	2.8	4.6	5.8	14.7	108.5	Dead								
240	5.9	6.0	5.2	6.1	6.7	28.1	143.8	Dead								
244	1.8	3.5	3.5	4.2	2.3	7.4	174.3	Dead								
251	5.3	6.8	6.5	6.2	10.9	17.5	47.9	Dead								
255	4.1	4.3	3.2	2.3	4.1	6.5	53.2	Dead								
257	5.4	5.8	3.3	4.3	5.7	4.6	35.3	Dead								
258	3.3	3.1	3.5	5.0	4.9	8.1	166.4	Dead								
239	3.3	5.0	4.1	5.6	5.3	8.6	30.3	165.2	Dead							
243	3.3	4.3	4.3	4.9	4.8	3.5	21.7	76.1	Dead							
246	2.8	4.5	2.8	3.5	2.9	3.8	4.5	12.3	151.0	Dead						
252	3.3	7.1	4.1	5.3	6.3	4.6	12.3	54.4	133.6	Dead						
232	2.5	4.5	3.9	3.8	5.4	3.9	4.4	6.1	12.4	219.7	Dead					
260	3.8	6.3	4.2	6.1	4.6	7.2	10.1	60.9	38.8	32.2	Dead					
248	2.9	4.6	3.4	5.4	3.4	3.4	9.0	4.8	2.6	6.6	27.5	35.7	60.5	186.9	Dead	

The concentration of uric acid was expressed as mg/dl.

Table 2. Trace of uric acid in chickens surviving after

Chicken No.	Days after									
	-3	-1	1	3	4	5	6	7	8	9
235	3.9	4.3	4.8	4.8	5.1	4.1	6.5	8.7	7.7	8.1
237	2.9	3.1	2.6	4.8	3.8	4.1	6.0	4.6	3.3	10.3
247	3.3	3.1	3.5	4.9	5.4	4.8	5.6	5.9	7.3	6.9
231	5.8	6.0	5.7	5.8	5.8	7.5	9.6	15.4	16.0	12.7
233	2.5	3.3	3.1	4.1	3.3	2.3	3.7	3.4	1.5	5.7
241	3.8	5.1	4.9	5.3	4.3	5.7	11.3	11.9	4.8	9.2
242	2.6	3.6	4.7	5.1	3.8	5.2	6.9	6.8	10.1	42.5
245	2.3	3.5	3.1	5.4	13.1	22.3	22.9	50.4	22.5	24.9
253	2.9	6.2	5.1	4.8	4.0	6.5	6.1	7.6	26.1	22.6
254	3.5	3.8	4.1	2.9	6.1	4.5	9.2	5.6	7.3	6.8
259	3.5	5.8	3.5	5.9	3.4	9.0	14.2	6.7	8.7	8.9
232	3.3	4.3	5.4	5.4	4.0	4.4	6.1	7.1	5.0	7.3
250	2.5	5.1	2.8	5.7	8.9	9.0	8.5	7.4	7.7	9.2
256	2.6	4.6	4.1	3.8	3.8	4.6	4.8	4.1	4.0	3.1

The concentration of uric acid was expressed as mg /dl.

Materials and Methods

Virus

A nephropathogenic AM-83 strain of IBV was used. The strain was isolated from a broiler with lesion and gout in kidney at Amori Prefecture in Japan in 1983 (YACHIDA *et al.*, 1984). The virus was passaged 3 times in SPF chicken and the kidney was maintained as our stock collection. The virus titer was measured by amnioallantoid inoculation with five 10-day-old embryonated eggs each dilution.

Chicken

White Leghorn chickens, free from specific pathogens including IBV were used. They were maintained under isolated conditions in our laboratory by rotational crossbreeding without any vaccine (IRITANI and MIYAJIMA, 1979).

Inoculation of IBV

Preliminarily, the nephropathogenicity of a stock collection of AM-83 strain was tested in 4 week old SPF chickens by inoculation to the trachea. The chickens showed respiratory symptoms and diarrhea at 1 day postinoculation and then about half of the chickens died with visceral gout. In this study, IBV was inoculated at the rate of $10^{1.1}$ EID₅₀/chicken into the trachea of thirty 7-week-old chickens. Five other chickens were placed in the same room as the contact control with inoculated chickens. They were housed in pairs in wire bottomed battery cages. Feed and water were given ad libitum for the experiment. Feed was obtained from a commercial source for layer. Inoculated chickens were observed daily for clinical signs and were autopsied at death and at 22 days post inoculation.

Trace of uric acid levels in plasma

Blood was collected daily at 9 o'clock in the morning from before 3 days until after

challenge with nephropathogenic infectious bronchitis virus

challenge									Kidney lesion
10	11	12	13	14	15	18	20	22	
7.8	6.6	3.5	4.7	5.0	5.1	5.3	6.1	6.1	+
4.3	3.9	4.4	3.4	4.3	4.2	3.3	4.3	4.3	—
5.7	5.5	6.4	5.3	4.1	6.1	3.3	6.6	4.6	—
10.9	7.9	7.3	5.5	7.9	10.5	5.1	11.3	4.9	+
12.0	8.6	8.0	5.8	4.2	4.6	4.8	4.1	4.1	—
16.5	10.9	7.6	5.3	5.5	6.6	9.8	12.4	8.2	+
19.0	9.7	6.6	5.1	4.9	5.9	5.7	6.3	5.8	+
14.1	12.2	11.4	7.1	6.1	5.6	4.4	4.6	4.1	—
17.1	13.6	10.7	8.7	8.6	7.3	6.4	9.3	7.2	+
5.8	5.6	5.4	7.4	5.1	3.8	8.9	6.4	5.2	+
8.6	8.9	7.4	7.3	8.6	7.5	6.6	6.5	7.1	+
6.9	10.5	28.0	10.8	17.3	9.0	12.9	14.4	12.9	—
12.1	11.1	9.1	12.1	8.4	40.3	9.7	11.1	11.0	+
3.9	4.7	4.7	8.0	3.5	2.8	3.8	3.8	3.8	+

Table 3. Hyperuricemia in contact chickens with artificial infected chickens with nephropathogenic infectious bronchitis virus

Chicken No.	Days after contact									Urate deposition	Kidney lesion
	10	11	12	13	14	16	18	20	22		
261	3.0	6.1	4.3	11.6	174.0	Dead				+	—
262	6.0	6.2	6.8	42.0	10.7	6.0	7.4	4.8	6.3	—	+
263	4.4	4.8	4.5	4.6	4.5	4.0	3.6	4.2	3.2	—	+
264	3.8	15.6	Dead							+	—
265	4.3	5.2	3.6	3.9	3.6	10.1	5.3	Dead		+	+

The concentration of uric acid was expressed as mg/dL.

22 days postinoculation with the nephropathogenic AM-83 strain of IBV. Plasma was obtained from the heparinized blood by centrifugation and then cooled. Uric acid was measured by the uricase-peroxidase method (KABASAKALIAN *et al.*, 1973).

Results

Some of the chickens inoculated with nephropathogenic AM-83 strain, had respiratory symptoms and a typical diarrhea at the day of postinoculation, and then cumulative incidence of the respiratory symptoms was elevated with rapidity. It reached 100% at 4 days postinfection. The high incidence lasted for 4 days and then declined. By contrast, the chicken died 5 days postinoculation, there after, the cumulative mortality increased and finally became about 50%. In these dead chicken visceral gout in heart and kidney was found at necropsy.

Table 1 shows the uric acid levels in the plasma of dead chickens before and after infection with AM-83 strain of IBV. The uric acid in the chickens was very low for 3 days before and after inoculation. There was no difference in the concentration of

uric acid during these 3 days. However, hyperuricemia was found in the chickens after 4 days postinfection. The levels were 27.3 mg/dl at the lowest and 186.9 mg/dl at the highest. Moreover, before death, a uric acid level of more than 7.2 mg/dl lasted in 13 of 16 chickens for not more than 5 days and not less than 2 days. In a large majority of the dead chicken, the highest concentration of uric acid was found at the day before death. As a rare case, in chicken No.260 the highest uric acid of 60.9 mg/dl was detected at 3 days before death, but it was not low at the day before death.

In the chickens surviving the challenge with AM-83 strain of IBV some found kidney lesions were found at necropsy, but a visceral urate deposition was not observed in any of the chicken. Hyperuricemia with more than 7.0 mg/dl was also found in the surviving chickens. About half of the chickens had hyperuricemia for more than 4 days. Chicken No. 250, had hyperuricemia for the longest duration of 19 days. The hyperuricemia in chicken No. 254 was transient and a few chickens such as chicken No. 237 only showed hyperuricemia for 1 day. A high uric acid of more than 26 mg/dl was also measured in the plasma of 5 surviving chickens after resistance to infection. These chickens did not die in spite of the same high levels of uric acid as in dead chickens shown in chick No. 249 and 243.

The value of uric acid in chicken in contact with infected chicken at challenge room was 3.8 and 4.3 mg/dl on the average before for 3 days and after 9 days contacts, respectively. There were no chickens with a value of more than 7.0 mg/dl during these periods. However, 3 chickens died of hyperuricemia from 11 days after the contact. Deposites of urea and lesions in the kidney were observed at necropsy. In 2 other chickens, hyperuricemia was found in one but not in the other. However, both chickens had kidney lesions without urate deposition at necropsy.

Discussion

Visceral urate deposition is generally due to a failure of urinary excretion. WINTERFIELD and HITCHNER (1962) first reported that gross kidney lesions, swelling paleness and degenerative changes were seen by artificial inoculation of IBV into chickens. ALBASSAM *et al.* (1988) reported that nephropathogenic strains of IBV produced acute renal changes consisting of tubular damage and interstitial inflammatory cell infiltration and edema. Recently, outbreaks of very virulent nephropathogenic IBV were found in commercial farms (BUTCHER *et al.*, 1990 ; COWEN *et al.*, 1987 ; KINDE *et al.*, 1991).

By contrast, these reports did not describe the uric acid in plasma. Studies on uric acid in contacted or inoculated chickens by IBV have only been conducted by NEWTON and SIMMONS (1963). In the present study, we enhanced the change of uric acid caused by IBV infection. It is an interesting and reasonable fact that the levels of uric acid in dead chickens by IBV challenge were very high at the day before death and the values in the major chickens were more than about 20 times before challenge. Chicken No. 249 died even though the value of uric acid was lower than that of other dead chickens. The time of sampling until death was longer than other dead chickens. Hyperuricemia occurred by IBV challenge and lasted for several days in the dead and surviving

chickens. Therefore, it may be useful as a screening model to develop a drug for human hyperuricemia. The values of uric acid in both chickens were much higher than that reported by NEWTON and SIMMONS (1963), it must be caused by a difference in the pathogenicity to chickens among the IBV strains used. It may be also supported by the fact that dead chickens were also found among those in contact with the chicken artificially inoculated with IBV. The chickens surviving the IBV challenge had kidney lesions at necropsy but not some of them. A favorable turn may occur in the chickens with mild hyperuricemia because the absence of a kidney lesion.

From present study, we divided the hyperuricemia caused by IBV challenge into the 4 grades of uncertain, mild, moderate and severe. The duration of hyperuricemia and value of uric acid were short and low in the uncertain group of chickens. Further studies are necessary. However, we believe that the biochemical data obtained in the present study may be useful for clinical diagnosis at the field.

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腎炎型鶏伝染性気管支炎ウイルス接種により 引き起こされる鶏の高尿酸血症

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腎炎型鶏伝染性気管支炎ウイルス (IBV) AM-83 株を、人為的に7週齢 SPF 鶏に接種し、高尿酸血症を引き起こし、その血清尿酸値を追跡した。AM-83 株接種鶏は、接種1日目より呼吸器症状、下痢などの臨床症状が認められ、発症率は4日目で100%となり、7日目以降から減少した。一方、死亡鶏は5日目より出現し、累積死亡率は約50%に達し、全死亡例に実質臓器の尿酸沈着を認めた。死亡鶏において、血清尿酸値は大部分が死亡前日が最も高く、その値は100 mg/dl 以上を示した。尿酸血症の持続は死亡鶏よりも生存鶏の方が長い傾向に

あった。生存鶏における持続性は連続的または、間歇的であり一過性のものは少なかった。IBV 感染鶏と同室に飼育された同居鶏にも同様の高尿酸血症と死亡鶏を認めた。今回の成績から、IBV によって惹起される高尿酸血症を軽度 (一過性)、軽度 (持続性)、中度 (持続性) および重度 (死亡) の4群に区別した。

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