

Effects of L-arginine on Endothelium Derived Factors and Cyclic Nucleotides in Broilers under Low Ambient Temperature*

Bo Han**, Soonseek Yoon¹, Hongryul Han¹ and Xiaolong Wang²

College of Veterinary Medicine, China Agricultural University, Beijing 100094, P. R. China

ABSTRACT : A flock of AA breed chickens were reared in petershine brood-vat chamber and were provided with high energy pelleted feed. At 14 d of age, a total of 350 birds were randomly divided into 3 groups as follows: 100 birds were exposed to normal ambient temperature of 20°C for control group; 150 birds were exposed to lower ambient temperature of 11°C to induce ascites (treatment I); and another group of 100 birds were exposed to lower ambient temperature of 11°C and fed diet containing 1% L-arginine for ascitic prophylactic treatment (treatment II). Samples were collected from blood and abdominal fluid of chicken at 3, 4, 5, 6 and 7 wk of age subsequently, to analysis the contents of plasma endothelin (ET-1), angiotensin II (Ang II), cyclic adenosine monophosphate (cAMP), cyclic guanosine monophosphate (cGMP). The results indicated that the contents of cAMP, cGMP, and Ang II in treatment I and ascitic broilers were higher than the corresponding control group ($p < 0.01$, $p < 0.05$), ET-1 of preascitic broilers were control group ($p < 0.05$), while there was an insignificant difference with later ascitic broilers. The contents of cAMP and cGMP in treatment II were higher than the treatment I and control groups ($p < 0.01$, $p < 0.05$), whereas, the contents of Ang II were gradually decreased compared to the control group ($p < 0.05$), the contents of ET-1 were insignificantly different. On further analysis, the increased plasma Ang II at low ambient temperature condition in broilers made endothelium cell secretion of increased ET-1, cAMP, cGMP and decreased NO. Therefore, low temperature accelerated ascites syndrome in broilers. Supplemently L-arginine can decrease ET-1, and increase cAMP and cGMP. It is concluded that cAMP mediated in broilers pulmonary hypertension syndrome. (*Asian-Aust. J. Anim. Sci.* 2004, Vol 17, No. 11: 1570-1574)

Key Words : Ascites in Broilers, cAMP, cGMP, Ang II, ET-1

INTRODUCTION

Cyclic guanosine monophosphate (cGMP) has been reported to antagonize the positive effects of cyclic adenosine monophosphate (cAMP). There may also be either directly or indirectly inhibits calcium channels in myocytes (Gong et al., 1997). increases in cGMP reduce myocardial O₂ consumption, and causes vascular relaxation (Sperelakis et al., 1994). However, Weyrich et al. (1994) and Denninger et al. (1999) demonstrated that physiologic levels of nitric oxide, which increases the production of cGMP via cyclic guanylate stimulation, does not exert a major regulatory effect on cardiac function in unstimulated cardiac muscle or myocytes. cAMP induces iNOS expression and NO synthesis, as well as enhancing cytokine-induced NO synthesis in SMCs by stabilizing iNOS mRNA (Imai et al., 1994; Schini-Kerth et al., 1994). cAMP may have both intracellular and extracellular aims. Endothelin (ET) is a potent vasoconstrictor, which isolated from endothelium with important additional modulating

effects on a wide range of physiological processes. Current evidence indicates that circulating levels of ET, reflecting activation or inhibition of the ET axis, may, under certain conditions, have clinical diagnostic or prognostic importance and provide insight into the role of ET in the pathophysiology of a variety of cardiovascular and other disease processes. These include hypertension (Haak et al., 1992), congestive heart failure (Rodeheffer et al., 1992), and myocardial infarction etc. Angiotensin II can stimulate endothelin production, which also has been shown to stimulate oxidative stress (Rajagopalan et al., 1996). This may potentiate the vasoconstrictor effect of Ang II.

Generally, the pathogenesis of ascites in broilers raised at low altitude is thought to involve primary pulmonary hypertension resulting from hypoxaemia (Julian, 1993; Julian, 2004). In a simplified schematic outline the key features leading to the development of ascites are the following rapid growth; increased oxygen requirement; hypoxaemia; increased cardiac output; tissue hypoxia and hypoxia triggered pulmonary vasoconstriction, or a chain of metabolic events resulting in pulmonary hypertension. However, the actual haemobiochemistry of chicken pulmonary hypertension during the development of ascites have not been investigated. In the present study, experiments were carried out, therefore, to determine whether cAMP, cGMP, Ang II, and ET-1 functional or not during low ambient temperature condition and supplemented L-arginine in the diet for ascitic broiler

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** Corresponding Author: Bo Han. Tel: +86-10-62893801, Fax: +86-10-62891155, E-mail: hanbo@cau.edu.cn

¹ College of Veterinary Medicine, Seoul National University, Seoul 151-742, Korea.

² College of Veterinary Medicine, Nanjing Agricultural University, Nanjing 210095, P. R. China.

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Table 1. Percentage diet composition with calculated nutrient content

Pelleted diet	ME kcal/kg	CP (%)	Ca (%)	P (%)	Crude fiber (%)	Crude ash (%)	Arginine (%)	Lysine (%)
Starter diet	3,990	21.0	1.0	0.4	6.0	8.0	1.22	1.27
Grower diet	3,885	19.0	1.0	0.4	6.0	8.0	1.08	1.24

Table 2. The incidence of ascites in broiler chicken

Groups	Numbers	The sick No. of every week					Total ascites No.	Incidence (%)
		3 W	4 W	5 W	6 W	7 W		
Control group	100	0	0	0	2	1	3	3
Treatment I	150	0	0	2	3	9	14	9.33
Treatment II	100	0	0	0	2	1	3	3

chickens.

MATERIALS AND METHODS

Animals

Three hundred and fifty day-old broilers purchased from a commercial hatchery were housed in one petersime brood-vait cage of 30 birds each over two wk periods, and were brooded at 32 and 23°C during week 1 and 2, and were randomly divided into 3 groups from 3 wk of age. In each replication, 100 birds in the control group, the ambient temperature beginning on day 15 they were maintained at 20°C until the experiment terminated on day 49. The ambient temperature of both, 150 birds in the treatment group (treatment I), and 100 birds in the arginine group (treatment II), were decreased from 23 to 11°C in 3 wk of age until the ambient temperature terminated on day 49. All birds received a standard pelleted broiler starter crumble diet from 1 to 3 wk of age. A grower diet was fed from 4 to 7 wk of age. Treatment II chicks were fed the same diet but added to adjust the diet to 1% arginine prior to pelleting from 3 to 7 wk of age. The composition of the pelleted rations is presented in Table 1. Feed and water were provided for *ad libitum* consumption.

Vaccination

All birds were vaccinated using Newcastle (N₉₉) and infectious bursa disease (IBD) through eye and nasal organ at the day of 7 days and 21 days.

Samples collection schedule and biochemical measurements

Blood samples collected in heparin containing tubes, from the chicken heart at the end of 3 wk, 4 wk, 5 wk, 6 wk, and 7 wk. After centrifugation at 3,000 rpm for 10 minutes immediately, the supernatant was transferred to another tube, and stored at -20°C refrigerator for measurement of cGMP. cAMP, Ang II, ET-1, cGMP and cAMP were determined by radioimmunoassay, some plasma were homogenized in ethanol, the homogenate was centrifuged at 3,000 rpm. The supernatant was recovered. The combined supernatants were evaporated to dryness in a 60°C bath under water

stream, the final residue was dissolved in 1.0 ml of assay buffer (0.05 M sodium acetate, pH 5.8, containing sodium azide). These assays measure the competitive binding of ¹²⁵I-labeled cAMP and cGMP to cAMP and cGMP specific antibody. After construction of a standard curve, cAMP and cGMP levels were determined directly from the counts divided by the number of plasma per tube times. RIA kits come from Shanghai traditional medical university. Other plasma was assayed for ET-1 with a specific RIA similar to that described previously (Shaw et al., 1996). ET-1 RIA commercial kit comes from Institute of RIA, PLA General hospital, China. Another plasma for Ang II assay was obtained from blood collected in ice-chilled tubes containing protase inhibitors, each sample was immediately centrifuged at 4°C and stored at -20°C. Quantification for Ang II was achieved by a competitive protein binding radioimmunoassay (RIA) using an Ang II RIA kit, which comes from Beijing North Institute of Biological Technology.

Statistical analysis

Statistical analysis of the results was performed with the use of SAS software by analysis the variance (SAS Institute, 1994). A p value <0.05 was considered significant. All results are presented as mean±SEM.

RESULTS

Status of occurrence

Treatment I with low ambient temperature resulted in a significant increase of mortality from ascites (to 9.33%, Table 2). Treatment II with low ambient temperature and fed diet containing 1% L-arginine, however, caused a significant reduction in mortality from the syndrome (3%) compared with treatment I. The maximum mortality rates from the syndrome were seen during the seventh week of age, the control birds were without overt symptoms of ascites.

Changes of plasma cAMP level in broiler chickens

The changes of plasma cAMP level in broiler chickens

are presented in Table 3. The contents of cAMP in treatment I, II were gradually higher during the experiment. However, the cAMP in control group was statistically lower than the above two groups ($p<0.05$, $p<0.01$). The cAMP contents in ascitic broilers were also significantly higher than the control group ($p<0.01$), whereas the cAMP contents in abdominal fluid were significantly lower than the control group ($p<0.01$).

Changes of plasma cGMP level in broiler chickens

As shown in Table 4, the changes of plasma cGMP contents in treatment I, II and ascitic broilers were significantly higher than the correspondent time control group ($p<0.05$, $p<0.01$).

Changes of plasma Ang II level in broiler chickens

The changes of plasma Ang II levels in broiler chickens were presented in Table 5. Ang II in control group and

treatment II were gradually decreased. While the Ang II in treatment I, ascitic broilers and abdominal fluid were higher than the control group ($p<0.01$).

Changes of plasma ET level in broiler chickens

As shown in Table 6, we summarize the changes in plasma ET level of broiler chickens. In the early time, ET-1 in treatment I, II were higher than the control group ($p<0.01$). In the later, ET-1 content were similar to control group. The ET-1 in ascitic broilers was also higher than the control group ($p<0.05$), but the ET-1 in abdominal fluid was insignificant difference ($p>0.05$).

DISCUSSION

The intracellular cAMP was produced by activated adenylate cyclase (AC) catalysing ATP. The second messenger cAMP, activated cAMP-dependent protein

Table 3. Changes of plasma cAMP level in broiler chickens (pM/ml)

Groups	3 W	4 W	5 W	6 W	7 W
Control	0.000±0.00	0.521±0.31 ^A	0.033±0.02 ^A	0.345±0.21 ^a	0.182±0.11 ^{aA}
Treatment I	0.048±0.11 ^a	0.724±0.35	0.117±0.05 ^B	0.40±0.03 ^a	0.85±0.06
Treatment II	0.063±0.11 ^a	1.163±0.79 ^B	0.381±0.11 ^C	0.462±0.28 ^b	0.482±0.13 ^b
Ascites in broilers			0.117±0.08 ^B	0.028±0.05	0.902±0.34 ^B
			n=2	n=3	n=9
Abdominal fluid					0.014±0.01 ^C
					n=9

Values are in case for N=7.

^{A-C} Means within column with no common superscripts indicate significant difference of ($p<0.01$).

^{a-c} Means within column with no common superscripts indicate significant difference of ($p<0.05$).

Table 4. Changes of plasma cGMP level in broiler chickens (pM/ml)

Groups	3 W	4 W	5 W	6 W	7 W
Control	0.082±0.087	0.542±0.21 ^a	0.057±0.021 ^A	0.463±0.24 ^{aA}	0.127±0.076 ^A
Treatment I	0.063±0.144	0.601±0.32 ^a	0.116±0.07 ^B	0.187±0.09	0.492±0.15 ^B
Treatment II	0.107±0.117	0.959±0.445 ^b	0.215±0.08 ^B	0.411±0.12 ^a	0.515±0.20 ^B
Ascites in broilers			0.021±0.029 ^C	0.051±0.171 ^B	0.577±0.674 ^B
			n=2	n=3	n=9
Abdominal fluid					0.121±0.07 ^A
					n=9

Values are in case for N=7.

^{A-C} Means within column with no common superscripts indicate significant difference of ($p<0.01$).

^{a-c} Means within column with no common superscripts indicate significant difference of ($p<0.05$).

Table 5. Changes of plasma Ang II level in broiler chickens (pg/ml)

Groups	3 W	4 W	5 W	6 W	7 W
Control	2,478.52±1,075 ^A	1,951.03±1,012 ^a	633.17±357 ^a	628.63±233 ^A	748.28±372 ^{aA}
Treatment I	3,025.57±1,054 ^B	1,022.53±858 ^b	893.25±332 ^b	867.31±547 ^A	900.36±462 ^b
Treatment II	2,451.30±1,539 ^A	1,195.21±1,026 ^b	627.91±270 ^a	1,238.04±327 ^B	840.72±314 ^a
Ascites in broilers			2,617.91±0	1,949.95±1,692 ^C	1,470.23±976 ^B
			n=1	n=3	n=9
Abdominal fluid					1,608.26±911 ^C
					n=9

Values are in case for N=8.

^{A-C} Means within column with no common superscripts indicate significant difference of ($p<0.01$).

^{a-c} Means within column with no common superscripts indicate significant difference of ($p<0.05$).

Table 6. Changes of plasma ET level in broiler chickens (pg/ml)

Groups	3 W	4 W	5 W	6 W	7 W
Control	59.63±4.30 ^A	72.13±23.47 ^{aA}	93.89±10.43 ^d	95.04±18.42 ^a	95.31±12.33 ^a
Treatment I	83.81±22.57 ^B	94.15±21.53 ^B	106.36±25.66 ^b	90.38±12.40 ^d	91.37±23.54 ^a
Treatment II	150.64±9.60 ^C	90.14±17.15 ^b	101.22±17.91 ^b	98.12±26.71 ^a	88.59±22.95 ^a
Ascites in broilers					100.75±25.15 ^b n=8
Abdominal fluid					78.64±30.27 n=9

Values are in case for N=8.

^{A-C} Means within column with no common superscripts indicate significant difference of ($p < 0.01$).

^{a-c} Means within column with no common superscripts indicate significant difference of ($p < 0.05$).

kinase (APK), which made phosphorylation of target cell protein. cAMP can be decomposed finally through phosphodiesterase (PDE). Although the vascular effects of adenosine may, in part, be mediated via endothelial cell-derived NO, adenosine induces vascularization both in the presence and absence of the endothelium. However, the mechanisms for the endothelium-independent dilatory effects of adenosine are not fully understood. Recent studies show that several endogenous vasodilatory factors stimulate NO synthesis by SMCs. In this regard, cAMP, as well as agents that elevate cAMP levels, induce NO synthesis by SMCs and messenger cells via iNOS stimulation. Because treatment increases intracellular cAMP levels. In the present study, we found that the cAMP content persistently enhanced during the development of ascites. Meanwhile, the Ang II was also increased, anyway, cAMP and Ang II were attenuated gradually for control group and the diet with supplemented L-arginine preventive group. These results indicated that the chickens with cold ambient temperature stimulation increased oxygen requirement, hypoxaemia increased cardiac output, tissue hypoxia triggered much more Ang II and pulmonary vasoconstriction, or showed a chain of metabolic events resulting in high cAMP, pulmonary hypertension.

The cyclic AMP-adenosine pathway begins with the activation of adenylyl cyclase and has both intracellular and extracellular sites of adenosine production, within the cell, cytosolic PDE and cytosolic 5'-nucleotidase metabolize cyclic AMP to AMP and AMP to adenosine, respectively, and the adenosine formed reaches the extracellular space via facilitated transport. However, due to the competition of cytosolic 5'-nucleotidase and adenylyl kinase for AMP and the competition of transport mechanisms with adenosine kinase for adenosine, the intracellular formation of adenosine may be diminished. Therefore, the intracellular limb of the cyclic AMP-adenosine pathway may be quantitatively more important.

Moreover, our observations demonstrated that cGMP contents were significantly decreased for chickens in cold ambient temperature, while treatment II, in which chicken diet supplemented L-arginine, i.e., the substrate required by

NOS to liberty NO, the cGMP contents were relatively higher. A recent study has showed that L-arginine inhibits angiotensin II-induced hypertrophic reaction (Wang et al., 1996), and attenuated broilers pulmonary hypertension. L-arginine-NO-cGMP pathway might play an important role in the ascitic broilers syndrome. cAMP as a second messenger might play a certain role in the NO synthesis and formation of heart failure (Markovic et al., 2003; Han et al., 2004).

A previous report shown that the renin-angiotensin system plays a major role in hypertension. It has also been reported the mechanism of renin-angiotensin system-induced hypertension has generally been attributed to the vasoconstrictor effects of angiotensin II and the mineralocorticoid effects of aldosterone (Wang et al., 1996; Katayama et al., 2003). However, recent work has revealed an additional potential mechanism. Angiotensin II has been shown to stimulate O_2^- generation by increasing the activity of the enzyme NAD(P)H cytochrome p-450 oxidoreductase, more commonly termed NAD(P)H oxidase, in cultured rats vascular smooth muscle cells (Griendling et al., 1994; Tao et al., 2004) and in intact aortas of rats made hypertensive by angiotensin II infusion (Rajagopalan et al., 1996; Shah et al., 2000). This seems to be a fairly specific effect, as rats made hypertensive to a similar degree by infusion of nonadrenalin showed to increase in NAD(P)H oxidase activity.

In summary, L-arginine-NO-cGMP pathway might play an important role in the ascitic broilers syndrome. The cAMP as a second messenger might play a certain role in the NO synthesis. cAMP also increased Ang II contents, antagonist NO production and decreased vasodilation factors, enhanced vasoconstriction, and accelerated smooth muscle cell reproduction and platelet adhesion. The ensuing pathological changes resulting in right ventricle hypertrophy and its failure are thought to be the causes of ascites.

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