Intravenous endothelin-1 triggers pulmonary hypertension syndrome (ascites) in broilers

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ABSTRACT: Two experiments were conducted to evaluate the effect of endothelin-1(ET-1) on pulmonary arterial pressure (PAP) and pulmonary hypertension syndrome (PHS, ascites) morbidity in broilers. Two hundred and seventy of one-day-old Arbor Acre commercial broiler chickens were randomly allocated into two experiments. In Experiment 1, 40 broilers (28 days of age) were intravenously with five different dose groups of ET-1, and the PAP was measured from time 0 to 30 min. The results indicated that the PAP increased from time 0.5 to 5 min after the broilers were injected with ET-1 at concentrations of 24 ng/kg (Group T1), 120 ng/kg (Group T2), and 240 ng/kg (Group T3). When the broilers were injected with ET-1 at a concentration of 360 ng/kg (Group T4), the PAP decreased immediately from time 0 to 0.5 min but increased from time 0.5 to 10 min. The change from baseline (at time 0, before the injection) of PAP values from the four treatment groups were statistically compared with that from the control group (Group C). The statistical analysis has demonstrated that there is no significant difference of the changes from the baseline between Group T1 or Group T2 and control group (Group C). However, there are significant differences in the changes between Group T3 or Group T4 and control group (Group C). In Experiment 2, 230 broilers were divided into four groups: a control group (Group C, n = 50) and three treatment groups (Groups A, B, and D). The chickens in the two treatment groups (Groups A and B, n = 60 each) were intravenously injected with ET-1 at 240 ng/kg and 360 ng/kg, respectively. Those in Group D (n = 60) were exposed to cool temperatures (10°C to 14°C). The PHS morbidity, right/total ventricular weight (RV/TV) ratio, PCV, plasma nitric oxide (NO), and PAP were recorded and statistically compared. The results showed that PHS mortality did not occur in Group C, and trends toward increases in PHS mortality in Groups A, B and D were not significant. The RV/TV ratio and PAP were higher in groups A, B and D than in group C. The values for PCV and plasma nitric oxide (NO) followed the same tendencies as the RV/TV ratio. The results suggest that ET-1 at higher doses (240 ng/kg to 360 ng/kg) can initiate pulmonary hypertension and right ventricular hypertrophy, leading to PHS in broilers.

Keywords: endothelin-1; pulmonary hypertension syndrome; broilers; nitric oxide

Ascites is a common cause of mortality in fast-growing broiler chickens, especially during the cold months of the year (Sato et al., 2002). The term "ascites" refers to the fluid that leaks from the surface of the liver and accumulates in the abdominal cavity. Researches have indicated that the primary cause of ascites in broiler chickens is the pulmonary hypertension (PH) which will develop into pulmonary hypertension syndrome (PHS, ascites) (Julian, 1993; Odom, 1993). The frequent occurrence of PHS has been a detrimental to the poultry industry (Maxwell and Robertson, 1997, 1998). The surveys have shown that PHS accounts for over 25% of annual broiler losses in the United States (Odom,

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1993) at a cost to the industry about US $ 1 billion annually (Maxwell and Robertson, 1997). An incidence of 5% to 10% PHS was reported in China (Shi, 1993) compared to a global incidence of 4% to 5% (Maxwell and Robertson, 1997). The PHS was first recognized in fast-growing chickens reared at high altitudes of 3 500 meters above the sea level (Julian, 1993; Odom, 1993). The occurrence of PHS has been associated with the hyperphagia of fast-growing broilers (Dunnington and Siegel, 1996; Fedde et al., 1998), which in turn causes distension of the gastrointestinal tract and decreases ventilation and perfusion of the lungs (Fedde et al., 1998). The incidence of PHS is usually higher in broiler males than in broiler females and Leghorn males (Julian et al., 1986; Wideman et al., 2003). Birds that have developed PHS do not recover. Therefore, the consequences of the disease are premature death or condemnation at processing (Julian, 1988). The PHS will cause right-sided congestive heart failure in common and often accompanied with thickening (hypertrophy) of the right ventricular wall (RVW) (Julian, 1988; Wideman, 1988; Tan et al., 2005).

PHS can also be caused by inadequate gas exchange and vasoconstriction of pulmonary arterioles (Stenmark et al., 1987; Fedde et al., 1998). In addition, an increase in blood viscosity, caused by high altitude, rickets, respiratory disease, and reduced oxygen transfer can contribute to PHS (Fedde and Wideman, 1996). Recent investigations on the etiologies of the ascites in chickens have focused on three aspects: (1) PH, (2) miscellaneous cardiac pathology, and/or (3) cellular damage by reactive oxygen species (Currie, 1999). Right ventricular hypertrophy also occurs in response to PH (Julian, 1987). Indeed PH may be the most common cause of ascites (Julian, 1993), its pathogenesis originating from a high basal metabolic rate induced by many factors such as cold, activity, and hyperthyroidism.

The role of endothelin-1 (ET-1) is an active area of cardiovascular researc. Endothelin, a 21-amino-acid peptide, was initially identified as a potent vasoconstrictor substance isolated from the culture supernatant of porcine aortic endothelial cells (Yanagisawa et al., 1988). It causes extremely potent and long-lasting vasoconstriction in most mammalian species including humans, both *in vivo* and *in vitro* (Rubanyi and Polokoff, 1994). Pulmonary hypertension has been shown to be associated with increased production of ET-1 in various animal models as well as human patients (Jain et al., 2002). The plasma concentration of ET-1 was clearly correlated with the severity of disease (Yoshibayashi et al., 1991; Cacoub et al., 1993; Ishikawa et al., 1995). Nitric oxide (NO) and ET-1 are the major endothelium-dependent vasomediators. NO is a potent endogenous vasodilator produced by an enzyme – endothelial nitric oxide synthase (eNOS) in the lung and other tissues (Rubanyi and Polokoff, 1994); whereas ET-1 is a potent vasoconstrictive peptide that plays an important role in the regulation of pulmonary vascular tone (Miyazaki et al., 1989). In previous studies, what the role of endogenous ET-1 in PHS broilers had been evaluated. It was found that the plasma concentrations of ET-1 and NO clearly correlated with the severity of PHS in broilers. A positive correlation between ET-1 concentration and mean pulmonary arterial pressure (mPAP) was found (Zhou et al., 2004). However, the effect of exogenous ET-1 on PHS broilers has not been evaluated yet. In the present study, experiments were conducted to evaluate the effects of exogenous ET-1 on PAP and PHS morbidity in broilers.

MATERIAL AND METHODS

Experimental animals

Two hundred and seventy of one-day-old Arbor Acre commercial broiler chickens were obtained from a local hatchery (Wuhan Zhengda Breeding Co. Ltd., Wuhan, P.R. China) and randomly allocated into two experiments. Forty birds were allocated into Experiment 1. Two hundred thirty birds were assigned into Experiment 2 and divided into four treatment groups (Groups A, B, C and D; 50, 60, 60 and 60 birds, respectively). All birds were reared on fresh wood shavings. All birds in Experiment 1 and those in treatment groups A, B and C in Experiment 2 were brooded at 32°C and 30°C during weeks 1 and 2, and thereafter maintained at 24°C. The birds in Group D were kept at 33°C, 32.5°C and 32°C on Days 1, 3, and 5, respectively, and were lowered 1°C every other day until 28°C was reached on Day 14. From Day 15 to Day 42, the birds in Group D were exposed to low temperature (10–14°C) by closing the heating in order to induce ascites. Chinese standard corn-soybean meal-based broiler starter (CP, 21%; ME, 3 080 kcal/kg; calcium, 1%; phosphate, 0.45%; sodium, 0.3%) and grower (CP, 20%; ME, 3 110 kcal/kg;

calcium, 0.89%; phosphate, 0.42%; sodium, 0.3%) diets were provided *ad libitum.* The room humidity was maintained at 60–65%. Feed and water were provided for *ad libitum* consumption. All the birds were vaccinated at three weeks of age against infectious bronchitis and Newcastle diseases.

Management experimental treatments

In Experiment 1, at 28 days of age, the broilers were randomly divided into five treatment groups (Groups C, T1, T2, T3, and T4) with eight broilers each group. A modified right cardiac catheter was adopted to determine the PAP. Each bird was anesthetized to a surgical plane with an intramuscular injection of allobarbital (5,5-dial-barbituric acid; 15 mg/kg BW) before injecting ET-1(Human, 21 amino acids peptides; Summit SIGMA International Ltd., USA). Thereafter, the birds were fastened in dorsal recumbency on a surgical board thermostatically regulated to maintain a surface temperature of 30°C. An incision was made to open the neck, the subcutaneous connective tissues were separated and the vagosympathetic nerves parallel to the right jugular vein were separated from the right jugular vein. The jugular vein was ligated with threads and a small opening was cut. A 15 cm long polyethylene plastic catheter (0.7 mm, i.d.; 0.9 mm, o.d.), bent to a 60° to 90° angle at one end and filled with heparinized saline (1%: 1 ml of heparin (1 000 units/ml) with 1 liter 0.9% saline), was tied into the right jugular vein. The distal end of the polyethylene tubing was attached to a blood pressure transducer (YP100 blood transduser, Mei Yi Technology Ltd., Nanjing, China) and data acquired by a polygraph (MedLab-U/4CS polygraph, Mei Yi Technology Ltd., Nanjing, China). The transducer was placed at the level of the heart. The right heart catheter was located in the right pulmonary artery by monitoring the characteristic curves of the blood pressure recording to identify its location (Chapman and Wideman, 2001). The catheter reached the precaval vein at about 5 cm, the right atrium at about 5.5 cm, the right ventricle at about 6.5–7.0 cm, and the pulmonary artery at about 7.5–8.0 cm (Geng et al., 2004). The diastolic blood pressure (DBP) of the cardiac atrium and the blood pressure of the precava nearly reached to zero, right atrium systolic pressure exceed zero a little, right ventricular pressure (RVP) and PAP changed greatly but the latter was higher than the former. The left wing vein was cannulated with a polyethylene tubing for *i.v.* injections (1 ml/kg) with different concentrations of ET-1 dissolved at in PBS (PBS, phosphate buffered saline: kalium chloratum, 0.2 g; potassium dihydrogen phosphate, 0.2 g; sodium chloride, 8.0 g; hepta-hyd dibasic sodium phosphate, 2.16 g. They were dissolved into 1 liter distilled water, pH 7.4) (60, 120, 240, and 360 ng/ml, respectively). The baseline PAP data were recorded for 5 min before the ET-1 injection. ET-1 was then *i.v.* injected into the broilers of groups T1, T2, T3, and T_4. The broilers in Group C were injected with 1 ml/kg of PBS alone. The PAP data were collected at 0.5, 1, 5, 10, 15, 20 and 30 min after the injection.

In Experiment 2, at Day 15 of age, the broilers were daily intramuscularly injected with ET-1 at 240 ng/kg (Group A) or 360 ng/kg (Group B). Broilers in Group C were injected with 1 ml/kg of PBS alone at the same time until the experiment was completed. Throughout the experiment, mortality was recorded daily and the incidence of PHS was recorded weekly. Eight birds were randomly selected from each group (A, B, C, and D) weekly at 21 to 42 days of age. The PAP was obtained as described for Experiment 1. Blood was collected from the wing vein in heparinized vacu-containers after the determination of the PAP. The packed cell volume (PCV) was measured by microhematocrit centrifugation as described previously (Jain, 1986; Mirsalimi et al., 1992a, b). Plasma NO concentration was measured by nitrate reductase method (Zhou et al., 2004). Birds were then euthanized by cervical dislocation, the chest and abdomen were opened according to previously described methods (Julian et al., 1989; Monge and Leon-Velarde, 1991). The right ventricle (RV) was cut away from the left ventricle and septum, the RV and total ventricle (TV) weights were recorded. The right/total ventricular weight (RV/TV) ratio was calculated, as an indicator of prior exposure of the heart to elevated PAP. Birds having a RV/TV ratios ≥ 0.30 were classified as suffering from right ventricular failure and PH. Those with RV/TV ratios ≥ 0.27 but < 0.3 were considered as only PH whereas those with RV/TV ratios < 0.27 without abdominal or precardiac fluid were classified as non-PHS (Julian, 1987; Julian et al., 1989; Fedde and Wideman, 1996). Additionally, birds with an RV/TV ratio ≥ 0.27 or abdominal and precardiac fluid ≥ 10 ml were classified as PHS. Necropsies were performed to identify all PHS-related mortality occurring after Day 21

of age. If the birds died with ascites or they died with obvious pre-PHS symptoms, including right ventricular dilation, hydropericardium, and vascular congestion, the birds were included in the total PHS mortality.

Statistical analysis

The PAP data from Experiment 1 were analyzed by the SAS software (SAS Institute, 1994) with main effect of change from baseline of PAP at 5% level of significance and 95% two-sided confidence intervals. No difference among the birds in the same treatment group in PAP values was presumed. The PAP data in the consequence time points from the same treatment group were considered as the pre-terminated birds' repeated measurements. Between-treatment group comparisons (Groups T1, T2, T3, T4 versus Group C) of change from baseline (before ET-1 injection) of PAP were performed using a mixed model for repeated measures with treatment group, time, individual birds, and individual birds-treatment group interaction as the fixed effects and individual birds as the random effect. Other data were subjected to one-way ANOVA (Snedecor and Cochran, 1989) (except that of the incidence of PHS; Table 1). A probability value of less than 0.05 was considered significant.

RESULTS

The mPAP in Experiment 1 are shown in Table 2 and the statistical analysis of the PAP data are shown in Table 3. Within 30 min of injecting PBS (Group C), there were no obvious changes in mPAP. The mPAP decreased to a minimum at 0.5 min and then

Table 1. The incidence of ET-1 induced and low temperature induced PHS in broilers from 3 to 6 weeks of age

Groups	Age (week)				Total PHS	Incidence (%)
	3	4	5	6		
C (n = 50)	0	0	1	1	2	2/50 (4%)
A (n = 60)	0	1	1	3	5	5/60 (8.33%)
B (n = 60)	0	2	2	4	8	8/60 (13.33%)
D (n = 60)	1	1	3	3	8	8/60 (13.33%)
Total (n = 230)	1	4	7	11	23	23/230 (10%)

Table 2. mPAP of broilers from time 0 to 30 min after the injection of different dose of ET-1 (0, 24, 120, 240, and 360 ng/kg BW) groups and dose

Time (min)	Groups and dose (n = 8)				
	C (0 ng/kg)	T1 (24 ng/kg)	T2 (120 ng/kg)	T3 (240 ng/kg)	T4 (360 ng/kg)
mPAP (kPa)					
0	2.86 ± 0.24	2.89 ± 0.16	2.81 ± 0.22	2.88 ± 0.12	2.84 ± 0.17
0.5	2.80 ± 0.17	2.63 ± 0.11	2.46 ± 0.12	2.48 ± 0.14	2.44 ± 0.14
1	2.77 ± 0.22	2.72 ± 0.13	2.64 ± 0.15	2.78 ± 0.13	2.83 ± 0.07
5	2.81 ± 0.20	3.03 ± 0.12	3.18 ± 0.10	3.33 ± 0.20	3.19 ± 0.1
10	2.85 ± 0.19	2.91 ± 0.27	3.06 ± 0.15	3.26 ± 0.15	3.46 ± 0.22
15	2.86 ± 0.16	2.85 ± 0.19	2.91 ± 0.13	3.15 ± 0.18	3.23 ± 0.15
20	2.87 ± 0.24	2.81 ± 0.19	2.91 ± 0.13	3.01 ± 0.17	3.14 ± 0.14
30	2.84 ± 0.18	2.85 ± 0.23	2.83 ± 0.13	2.92 ± 0.17	2.99 ± 0.16

Data are means ± SEM of eight replicates of mPAP per treatment

Table 3. Statistical analysis of change from baseline of PAP of broiler groups from 0.5 to 30 min after ET-1 treatment at different doses

Treatment group comparison	Least squares means difference estimate	Standard error of the means	*P*-value (95% confidence interval)*
C (*n* = 8) versus T1 ((*n* = 8)	0.0198	0.04619	0.6709 (–0.07408, 0.1137)
C (*n* = 8) versus T2 (*n* = 8)	–0.05346	0.04625	0.2557 (–0.0.1475, 0.04053)
C (*n* = 8) versus T3 (*n* = 8)	–0.1452	0.04618	0.0035 (–0.0.239, –0.05133)
C (*n* = 8) versus T4 (*n* = 8)	–0.2238	0.04617	< 0001 (–0.3177, –0.13)
T1 (*n* = 8) versus T2 (*n* = 8)	–0.07326	0.0464	0.1236 (–0.1676, 0.02103)
T1 (*n* = 8) versus T3 (*n* = 8)	–0.165	0.04616	0.0011 (–0.2588, –0.07118)
T1 (*n* = 8) versus T4 (*n* = 8)	–0.2436	0.04625	< 0001 (–0.3376, –0.1497)
T2 (*n* = 8) versus T3 (*n* = 8)	–0.09173	0.04637	0.0561 (–0.186, 0.002506)
T2 (*n* = 8) versus T4 (*n* = 8)	–0.1704	0.04619	0.0008 (–0.2643, –0.0765)
T3 (*n* = 8) versus T4 (*n* = 8)	–0.07865	0.04623	0.098 (–0.1726, 0.0153)

*These statistical inferences are from a mixed model for repeated measures with treatment group, treatment time, and the treatment-by-time interaction as fixed effects, individual bird as a random effect, and the baseline value as a covariate. Covariance matrix structure is chosen from Heterogeneous Compound Symmetry and Heterogeneous First-Order Autoregressive using Akaike's Information Criterion (AIC). The model with the smallest AIC value was chosen for this analysis. Group C = treatment with PBS alone; Group T1 = single dose of ET-1 at 60 ng/kg *i.v.* injection; Group T2 = single dose of ET-1 at 120 ng/kg *i.v.* injection; Group T3 = single dose of ET-1 at 240 ng/kg *i.v.* injection; Group T4 = single dose of ET-1 at 360 ng/kg *i.v.* injection

increased to a peak at 5 min when 60, 120, 240 ng/kg ET-1 was injected (Groups T1, T2, T3). In Group T4, the peak mPAP occurred at 10 min post-injection. The statistical data analysis using a mixed model as in Table 3 showed that there was no significant difference of PAP values between control group and the lower doses treatment groups (Groups T1 and T2, 60 and 120 ng/kg, *P*-values are 0.6709 and 0.2557, respectively). However, the treatments with higher doses (Groups T3 and T4 240 ng/kg and 360 ng/kg, *P*-values are 0.0035 and < 0.0001, respectively) were highly significantly different from the control group. There were no significant difference between Groups 1 and 2 or between Groups 3 and 4 but the differences between lower doses groups (Groups T1 and T2) and the higher dose groups (Groups T3 and T4) were statistically significant.

Mortality attributable to PHS is shown in Table 1. PHS mortality did not occur in Group C, and trends toward increases in PHS mortality in Groups A, B and D were not significant. Figure 1 shows blood pressures recorded as the right heart catheter was inserted into the right jugular vein and then advanced slowly to the right pulmonary artery. The

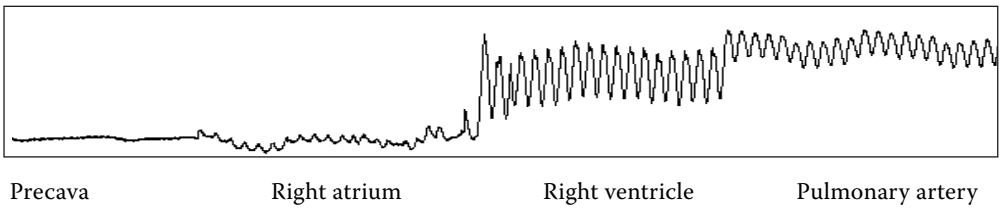

Figure 1. The changes of the pressuregraph of right ventricular pressure (RVP) and PAP after the right heart catheter was inserted into the right jugular vein and was pushed forward slowly to the right pulmonary artery. The diastolic blood pressure (DBP) of the cardiac atrium and the blood pressure of the precava nearly reach to zero, right atrium systolic pressure exceed zero a little, right ventricular pressure (RVP) and PAP changed greatly but the latter was higher than the former

Table 4. The changes of the PCV of broilers in different treatments at different ages[1]

Parameter and groups	Age (week)			
	3	4	5	6
PCV(%)				
C (n = 8)	27.85 ± 2.45^{b}	31.72 ± 3.47^{bA}	31.74 ± 2.16^{cA}	33.50 ± 2.10^{cA}
A (n = 8)	31.00 ± 3.12^{a}	35.08 ± 3.25^{bA}	34.94 ± 3.18^{cA}	37.22 ± 3.94^{cdA}
B (n = 8)	32.06 ± 2.14^{aC}	35.05 ± 3.31^{bBC}	37.35 ± 3.46^{bB}	41.91 ± 3.48^{bA}
D (n = 8)	30.56 ± 4.00^{abC}	35.09 ± 3.97^{bB}	37.66 ± 3.37^{bAB}	40.70 ± 4.57^{bdA}
PHS		45.13 ± 3.75^{a} (n = 3)	47.28 ± 5.30^{a} (n = 4)	51.05 ± 4.68^{a} (n = 6)

$^{a–d}$means lacking a common superscript within a column are significantly different at $P < 0.05$

$^{A–C}$means lacking a common superscript within a row are significantly different at $P < 0.05$

[1]data are means ± SEM of 8 (Group A, B, C, and D) replicates per treatment or 3, 4, 5 (Group PHS) replicates from 4 to 6 weeks, respectively

PHS means the group PHS, and in this group the broilers were diagnosed as ascites broilers

Table 5. The changes of the right ventricle to total ventricle RV/TV ratios of broilers in different treatments at different ages[1]

Parameter and groups	Age (week)			
	3	4	5	6
RV/TV				
C (n = 8)	0.186 ± 0.031^{bB}	0.191 ± 0.029^{cAB}	0.219 ± 0.015^{bA}	0.204 ± 0.039^{cAB}
A (n = 8)	0.220 ± 0.031^{a}	0.229 ± 0.030^{b}	0.233 ± 0.019^{b}	0.245 ± 0.024^{b}
B (n = 8)	0.218 ± 0.046^{ab}	0.240 ± 0.045^{b}	0.245 ± 0.026^{b}	0.254 ± 0.034^{b}
D (n = 8)	0.203 ± 0.019^{ab}	0.221 ± 0.024^{bc}	0.214 ± 0.016^{b}	0.251 ± 0.030^{bA}
PHS		0.353 ± 0.026^{a} (n = 3)	0.336 ± 0.029^{a} (n = 4)	0.368 ± 0.031^{a} (n = 6)

$^{a–d}$means lacking a common superscript within a column are significantly different at $P < 0.05$

$^{A–B}$means lacking a common superscript within a row are significantly different at $P < 0.05$

[1]data are means ± SEM of 8 (Group A, B, C, and D) replicates per treatment or 3, 4, 5 (Group PHS) replicates from 4 to 6 weeks, respectively

PHS means the group PHS, and in this group the broilers were diagnosed as ascites broilers.

diastolic blood pressure (DBP) of the cardiac atrium and the blood pressure of the precava nearly reached zero, right atrial systolic pressure slightly exceeded zero, right ventricular pressure (RVP) and pulmonary arterial pressure (PAP) were substantially above zero, and PAP was higher than RVP. As shown in Table 4, when compared with control group C, the PVC was higher in Groups A and B during weeks 3, 5 and 6. The PVC in broilers that developed PHS was higher than in all other groups during weeks 4 to 6.

Intravenous injections of ET-1 (Groups A, B) as well as exposure to low temperature (Group D) increased the RV/TV ratios compared with that of the control group (Group C) at 3, 4, and 6 weeks of age ($P < 0.05$). The RV/TV ratios did not differ among Groups A, B, and D from 3 weeks of age to 6 weeks. However, the RV/TV ratios for PHS groups were higher than that of other groups. As shown in Table 6, the level of plasma NO did not differ among Groups A, B, C, and D at 3 and 4 weeks of age, there was no difference between Groups B

Table 6. The level of plasma NO for broilers in different treatments at different ages[1]

Parameter and groups	Age (week)			
	3	4	5	6
NO (nmol/ml)				
C (n = 8)	26.63 ± 2.17	28.78 ± 5.03	30.47 ± 2.66^{b}	35.82 ± 5.00^{bA}
A (n = 8)	27.88 ± 5.82^{C}	30.90 ± 3.19^{BC}	33.28 ± 2.98^{abAB}	37.01 ± 3.52^{bA}
B (n = 8)	29.99 ± 3.75^{C}	31.46 ± 3.60^{BC}	34.53 ± 3.52^{aB}	39.68 ± 3.97^{abA}
D (n = 8)	29.70 ± 2.76^{C}	30.13 ± 3.20^{C}	34.72 ± 2.73^{aB}	40.38 ± 4.50^{aA}
PHS		33.67 ± 2.80 (n = 3)	35.37 ± 2.07^{a} (n = 4)	42.37 ± 3.58^{aA} (n = 6)

$^{a–b}$means lacking a common superscript within a column are significantly different at $P < 0.05$

$^{A–C}$means lacking a common superscript within a row are significantly different at $P < 0.05$

[1]data are means ± SEM of 8 (Group A, B, C, and D) replicates per treatment or 3, 4, 5 (Group PHS) replicates from 4 to 6 weeks, respectively

PHS means the group PHS, and in this group the broilers were diagnosed as ascites broilers

Table 7. Changes of mPAP of broilers in different treatments at different ages[1]

Parameter and groups	Age (week)			
	3	4	5	6
mPAP (kPa)				
C (n = 8)	2.42 ± 0.23^{bC}	2.70 ± 0.25^{cB}	3.06 ± 0.27^{cA}	3.28 ± 0.30^{cA}
A (n = 8)	2.45 ± 0.20^{abD}	2.75 ± 0.20^{cC}	2.99 ± 0.30^{cB}	3.49 ± 0.19^{cA}
B (n = 8)	2.66 ± 0.19^{aD}	3.09 ± 0.29^{abC}	3.47 ± 0.28^{bB}	3.93 ± 0.30^{bA}
D (n = 8)	2.52 ± 0.22^{abD}	2.99 ± 0.18^{bC}	3.47 ± 0.30^{bB}	3.86 ± 0.34^{bA}
PHS		3.32 ± 0.25^{aC} (n = 3)	3.84 ± 0.19^{aB} (n = 5)	4.30 ± 0.45^{aA} (n = 6)

$^{a–c}$means lacking a common superscript within a column are significantly different at $P < 0.05$

$^{A–D}$means lacking a common superscript within a row are significantly different at $P < 0.05$

[1]data are means ± SEM of 8 (Group A, B, C, and D) replicates per treatment or 3, 4, 5 (Group PHS) replicates from 4 to 6 weeks, respectively

PHS means the group PHS, and in this group the broilers were diagnosed as ascites broilers

and D, Groups A and C from 3 to 6 weeks of age. The level of plasma NO in Group B and D was higher than that of Group A and it was higher in PHS groups than that of other groups. As the results shown in Table 7, the peak mPAP response to Group C and 240 ng/kg ET-1 (Group A) could not be surpassed in amplitude by daily injections ET-1 up to 360 ng/kg BW (Group B). There were no differences between Group C and A, Group B and D, but the mPAP of Group C. The mPAP of the PHS group were highest.

DISCUSSION

The pharmacological actions of exogenously applied ET have been extensively investigated. ET-1 causes extremely potent and long-lasting vasoconstriction in most mammalian species including humans, both *in vivo* and i*n vitro* (Rubanyi and Polokoff, 1994). The *in vivo* hemodynamic responses to intravenously injected ET-1 are complex, depending upon the vascular bed, and include both direct vasoconstrictor and indirect effects (Lerman

et al., 1991). ET-1 exerts vasoconstrictor and vasodilator actions through stimulation of ETA receptors in vascular smooth muscle and ETB receptors in endothelial cells, respectively (Gardiner et al., 1994; Teerlink et al., 1994). In the present study, it was clearly demonstrated that injection ET-1 caused an initial reduction in PAP from 0 to 30 s post-injection, followed by a significant increase in PAP. When the broilers were injected with ET-1 at doses of 24, 120, 240 ng/kg BW, the mPAP reached the highest level at the time of 5 min but it returned to pre-injection levels by 10, 15 and 30 min. When broilers were injected with ET-1 at a dose of 360 ng/kg BW, mPAP reached a peak at 10 min and mPAP did not return to baseline until 30 min post-injection. Thus, higher concentrations of ET-1 elicit more sustained increases in mPAP. ET-1 can produce either dilation or constriction of blood vessels depending on concentration. Prostanoids appear to mediate vasodilation induced by the lowest concentration of ET-1 yet contribute to constriction induced by higher concentrations of ET-1 (Armstead et al., 1989). The results in the present study indicated that ET-1 effects on mPAP in broilers that are similar to those seen in mammals.

In this study, cool temperatures were used in the present study to induce PHS in broilers of Group D, as a comparison with the efficacy of ET-1 in Group A and B. Cool temperatures significantly amplified the incidence of PHS under commercial and experimental conditions (Julian et al., 1989, 1992; Scheele et al., 1992; Julian, 1993; Wang and Hacker, 1993). Compared with the control group which birds were bred under a common condition, the total mortalities of Group A (5/60, 8.33%), Group B (8/60, 13.33%) and Group D (8/60, 13.33%) were high. It indicated that the higher concentration of ET-1 was injected into the broilers has the tendency that the higher mortality of PHS in broilers was obtained, and ectogenic ET-1 was directly increased the mortality of PHS in broilers when the broilers were supplied high level of ET-1 (≥ 240 ng/kg BW).

AHI (ascites heart index) that is, the ratio of right ventricle weight to total ventricle weight, was suggested to be a sensitive indicator of prior exposure of the heart to increased PAP (Burton et al., 1968). Broilers with an AHI < 0.27 without fluid in the abdomen were regarded as normal, whereas those with an AHI ≥ 0.30 with fluid accumulation were regarded as having pulmonary hypertension (Cawthon et al., 2001). In this study (Table 5), the AHI of the injection ET-1 groups (Group A and Group B) and cool temperature group (Group D) significantly increased compared with the control group (Group C, $P < 0.05$). It indicated that ET-1 not only promoted the mPAP of chicken broilers directly but also induced right ventricular hypertrophy of boilers. In chronic heart failure rats (permanent coronary artery ligation) with severe PH, continuous infusion of BQ123 (ETA receptor antagonist) significantly reduced both right ventricular systolic pressure and central venous pressure. The endothelin receptor antagonists thus far investigated are remarkably efficacious in most animal models of PH (Sakai et al., 1996). In the recent study, ET-1 appeared to cause progressive aggravation of the failing myocardium.

During the development of ascites syndrome, birds exhibited classic hematological changes. Decreased erythrocyte deformability combined with an increased PCV should increase the blood viscosity and thus increase the resistance to blood flow (Chien et al., 1967). A higher PCV indicates higher blood viscosity, which was said to be one of the causes of ascites (Julian, 1993). Broilers may be particularly susceptible to increase in blood viscosity because their non-inflatable lungs are restricted by the size of the thoracic cavity, and their blood capillaries are small and relatively non-compliant. Low capillary expandibility and reduced erythrocyte deformability may be important in the development of cardiac overload and failure. The results of Table 4 demonstrated that high level of ET-1 possibly caused increases in PCV and reductions in erythrocyte deformability, which together increased the resistance to pulmonary blood flow through an inadequate pulmonary vasculature and caused pulmonary hypertension. Therefore, the PAP was increased (Table 7) when the broilers were supplied high level of ET-1 daily.

Pulmonary hypertension (PH) has been shown to be associated with increased production of ET-1 in various animal models as well as human patients. The plasma concentration of ET-1 clearly correlated with the severity of disease (Yoshibayashi et al., 1991; Cacoub et al., 1993; Ishikawa et al., 1995). Infusion of BQ123 reduced the progression of the disease and right ventricular hypertrophy, and prevented the characteristic thickening of the pulmonary arterial media (Miyauchi et al., 1993). In addition to ET-1, vascular endothelium has been shown to produce a factor which can induce relaxation of vascular smooth muscle cells (Furchgott and

Zadawzki, 1980). This endothelium-derived relaxing factor, which was discovered to be NO, is synthesized in endothelial cells from L-arginine by NO synthase (Moncada et al., 1991). It is known that NO produced by endothelial NOS serves as a key modulator of flow-dependent pulmonary vasodilation, and it is likely that NO generated by iNOS also contributes to the pulmonary vasodilator response (Wideman et al., 2004). It appears likely that the pulmonary vascular endothelium releases NO that in turn reduces the pulmonary vascular resistance or attenuates myocardial contractility in broiler chickens (Wang et al., 2002; Weidong et al., 2002). In broilers with PHS, the elevated plasma level of ET-1 and NO dramatically increased following the severity of disease and plasma ET-1 levels increased progressively, preceding the development of PH (Zhou et al., 2004). In this study, both plasma ET-1 and plasma NO were increased. These findings suggested that not only local production of ET-1 but also exogenous ET-1 might contribute to the pulmonary vascular abnormalities associated with PH in broilers. Although NO can induce relaxation of vascular smooth muscle cells and can relax blood vessel, it can't counteract the extremely potent and long-lasting vasoconstriction effects which ET-1 or other vaso-excitor material mediated.

In summary, this study has established the facilitative effect of ET-1 on PHS in broilers. Ectogenous ET-1 advanced the progression of this disease and right ventricular hypertrophy, promoted the characteristic hypertension of the pulmonary artery, induced high susceptibility and mortalities of PHS in broilers. In addition to ET-1, NO as a vascular relaxation factors also took part in the development of PHS in broilers and took an important role in it.

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