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Effects of Endothelin A Receptor Antagonist BQ123 on Femoral Artery Pressure and Pulmonary Artery Pressure in Broiler Chickens

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ABSTRACT : Endothelin-1 (ET-1) is an important factor in regulation of cardiovascular tone in humans and mammals, but the biological function of ET-1 in the avian vascular system has not been determined. The purpose of this study was to characterize the role of endogenous ET-1 in the vascular system of poultry by investigating the effect of endothelin A receptor (ET_AR) antagonist BQ123 on the femoral artery pressure (FAP) and the pulmonary artery pressure (PAP) in broiler chickens. First, we found that plasma and lung homogenate ET-1 levels were both increased with age over the seven weeks life cycle of broiler chickens. Second, 60 min after intravenous injection, BQ123 (0.4 μg kg^{-1} and 2.0 μg kg^{-1}, respectively) induced a significant reduction in FAP and PAP ($p<0.05$). Third, chronic infusion of BQ123 (2.0 μg kg^{-1} each time, two times a day) into abdominal cavities led to significant decrease in systolic pressure of the femoral ($p<0.05$) and pulmonary arteries ($p<0.01$) in broiler chickens at 7 and 14 days after treatment. Taken together, the ET_AR antagonist BQ123 lead to a significant reduction of FAP and PAP, which suggests that endogenous ET-1 may be involved in the maintenance and regulation of systemic and pulmonary pressure in broiler chickens. (**Key Words**: BQ123, Endothelin-1, Femoral Artery Pressure, Pulmonary Artery Pressure, Broiler Chickens)

INTRODUCTION

Vascular endothelium plays important role in regulation of cardiovascular tone (Takahashi et al., 1998). It is not only the important barrier and semipermeable membrane, but also the important metabolizable and incretionary organ. The vascular endothelium controls vasomotor tone and microvascular flow and regulates trafficking of nutrients and several biologically active molecules (Langouche et al., 2005). Under normal conditions, the vascular endothelium secretes a variety of vasoactive substances including Endothelin (ET) (Mukai et al., 2006). ET, a 21-amino acid peptide having potential, strong and long-lasting vasoconstrictor activity, is important in the control of systemic blood pressure and/or local blood flow (Yanagisawa et al., 1988). ETs are a family of peptide hormones with three members, endothelin-1 (ET-1), endothelin-2 (ET-2) and endothelin-3 (ET-3) (Inoue et al., 1989), with ET-1 appearing to be the most important in vascular regulation (Levin, 1995). The effects of the ET peptides are mainly mediated through two known distinct specific ET receptors. They have been classified as type A and B receptors (ET_AR and ET_BR). The ET_AR has a greater affinity for ET-1 than for the other two ETs, whereas the ET_BR displays similar affinity for all three ETs (Sakurai et al., 1992). ET_AR is mainly located on vascular smooth muscle cells and mediates the vasoconstrictor and mitogenic effects of ET-1, and ET_BR is mainly located on vascular endothelium and mediates the vasodilator activity (Sakurai et al., 1992). Scientists provided the evidence of the high degree of similarity of the ET system in birds and mammals (Kempf et al., 1998). The ET_AR is conserved between birds and mammals, since the complete sequence of the cET_AR in bird displays a high identity with the sequence of the mammalian ET_AR (Kempf et al., 1998). At least two distinct types of ET receptors coexist on chick cardiac membranes: one of two has higher affinity for ET-1 and ET-2 than ET-3, and the other has preference toward ET-3 (Watanabe et al., 1989). The cDNA of the chick ET_AR has been cloned, sequenced and expressed and its affinity for ET antagonists is very similar to that shown by its mammalian counterparts (Kempf et al., 1998).

The ET system has been found to be involved in

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Table 1. Percentage of diet composition (% unless otherwise stated)

Ingredients	Starter period (day 1 to 15)	Grower period (day 16 to 49)
Crude protein	21.00	20.00
Crude fiber	6.00	6.00
Crude ash	8.00	8.00
Calcium	0.70-1.20	0.70-1.20
Total phosphonium	0.50-0.80	0.50-0.80
Common salt	0.30-0.60	0.30-0.60
Methionine+cystine	0.80	0.76
Arginine	1.22	1.08
Lysine	1.27	1.24
ME kcal/kg	3,990.48	3,885.67

multiple physiologic functions related to the nerve, renal, cardiovascular, respiratory, gastrointestinal and endocrine system (Gglie et al., 2004). Because of its vasoconstrictive and mitogenic properties, ET-1 affects cardiovascular, pulmonary and renal function, and may be involved in the development of several diseases such as atherosclerosis, myocardial infarction, renal disease and systemic and pulmonary hypertension in human (Ferri et al., 1995). The ascites syndrome (AS) in broiler chickens, also known as pulmonary hypertension syndrome (PHS), is characterized by pulmonary hypertension and right ventricular hypertrophy, which is pathophysiologically similar to pulmonary hypertension (PH) in humans and mammals. Recent study showed that ET-1 was associated in the development of PH in broilers (Ying et al., 2005).

Although the role of ET system has been well characterized in regulation of cardiovascular function in humans and mammals, the biological function of ET-1 in avian vascular system has not been identified. The purpose of this study is to characterize the role of endogenous ET-1 in poultry vascular system by investigating the effect of a highly selective ET_AR antagonist BQ123 on the femoral artery pressure (FAP) and the pulmonary artery pressure (PAP) in broiler chickens. This study could also provide the basic data for further investigating the relationship between PHS and ET system.

MATERIALS AND METHODS

Animals and drug preparation

One-day-old commercial AA male broiler chickens (Beijing Huadu Breeding Co. Ltd., Beijing, China) were maintained in environmental chambers at a normal temperature (23±1°C) and relative humidity (60±1%). During the brooding period, continuous lighting was used in the first 3 days. Then chickens were exposed to 23 h light and 1 h darkness from day 4 to 7 and 16 h light and 8 h darkness from day 8 to 49. Ambient temperature was set at 32±1°C on day 1 and then gradually decreased until 23±1°C on day 15. They were given free access to water and a commercial chick starter diet and grower diet (as shown in Table 1). Water and food were available at all times. Other standard experimental protocols included immunizing routinely and monitoring for overt signs of disease.

Dimethyl Sulfoxide (DMSO, Sigma Chemical Co., St Louis, MO, USA) was dissolved in normal saline. BQ123 (Peninsula Laboratories, Belmont, CA, USA) was dissolved in 0.3% DMSO. All other chemicals were analytical grade. Twice distilled water which had been de-ionized through a Millipore-Q system was used in all experiments.

Measurement of ET-1

We determined the levels of plasma and lung homogenate ET-1 in broiler chickens by radioimmunoassay (Ferri et al., 1995). Blood samples for the plasma ET-1 assay were collected into pre-chilled tubes containing EDTA-Na_2 (10%) and aprotinin (500 KIU/ml blood) and promptly centrifuged at 1,600×g at 4°C for 15 min. Supernatant was pipetted into polypropylene tubes and stored at -80°C until assayed. After measurement of the pressure, chickens were immediately killed by cervical dislocation. The lungs were dissected, snap-frozen in liquid nitrogen and stored at -80°C until processed for assays. Frozen lung tissue was homogenized at a ratio of 100 mg tissue/ml normal saline. The homogenate was centrifuged at 1,600×g at 4°C for 15 min to remove crude debris, and the supernatant was saved as samples for the assay. Commercial radioimmunoassay kit (Peninsula Laboratories, Belmont, CA, USA) was used to measure ET-1 concentration. Cross-reactivity of the system for endothelin-1 is 100%, but less than 7% for both endothelin-2 and endothelin-3 according to the manufacturer. Intra- and inter-assay coefficients of variation in our laboratory were 4< 10%. Recovery was 80%. Because of high degree of similarity of the ET system in birds and mammals, the same method can be used to measure the concentrations of plasma and lung homogenate ET-1 in broiler chickens and mammals.

Measurement of FAP and PAP

In this study, FAP and PAP were used to represent the systemic and pulmonary vascular pressures, respectively. A modified method using a right cardiac catheter was adopted to determine the pulmonary artery systolic pressure (PASP) and the pulmonary artery diastolic pressure (PADP) just before killing (Guthrie et al., 1987). Chickens were restrained in a dorsal position on the operating-table and locally anesthetized with 5% procaine chloride in the middle of right neck and the inside of left thigh. The right jugular vein was isolated and a polyethylene plastic catheter (0.9 mm, external diameter) was passed into the pulmonary artery to monitor PASP and PADP continually. Meanwhile,

Table 2. Plasma and lung homogenate endothelin-1 (ET-1) levels in broiler chickens

	Days of age				
	15	22	29	36	43
Plasma (n = 10, pg ml^{-1})	40.82±1.90	46.07±8.21	52.92±5.36*	72.78±3.85**	93.70±11.19**
Lung (n = 10, pg g^{-1} wet weight)	503.84±27.40	567.20±52.55*	609.56±29.26**	676.58±28.44**	701.04±16.18**

The plasma and lung homogenate ET-1 levels were both increased with age over seven weeks' life circle of broiler chickens, and plasma ET-1 level was far lower than that of lung homogenate. All values were means±SD.

* $p<0.05$ compared with the values of day 15 within same row. ** $p<0.01$ compared with the values of day 15 within same row.

Table 3. Broiler plasma and lung homogenate endothelin-1 (ET-1) levels after chronic intraperitoneal administration of BQ123

	7 days after treatment		14 days after treatment	
	Plasma (pg ml^{-1})	Lung (pg g^{-1} wet weight)	Plasma (pg ml^{-1})	Lung (pg g^{-1} wet weight)
BQ123 (n = 10)	42.82±1.90	495.84±52.55	49.73±4.98	531.36±27.06
Control (n = 10)	46.07±8.21	567.20±29.26^{A}	52.92±6.57	609.56±87.10^{A}

Chronic intraperitoneal infusion of BQ123 (2.0 μg kg^{-1} each time, two times a day) induced significant decrease in ET-1 level of lung homogenate at 7 and 14 days after treatment.

^{A} $p<0.05$ compared with control group. All values were means±SD.

the left femoral artery was isolated and another catheter was placed into the left femoral artery for monitoring the femoral artery systolic pressure (FASP) and the femoral artery diastolic pressure (FADP). All catheters were flushed with sodium citrate in 0.9% sterile saline to avoid clotting. Mechanical responses were digitized, displayed, analyzed, stored and graphed by using Biopac System (BIOPAC Inc., Goleta, CA, USA). Anesthesia was supplemented with 5% procaine chloride as needed. The sensors were placed at the same level as the birds' heart.

Experimental protocols

To determine the effect of short-time ET_AR blockade on FAP and PAP in normal broiler chickens, 28-day-old birds were administered with 0.3% DMSO (n = 10, as control group), 0.4 μg kg^{-1} BQ123 (n = 10) and 2.0 μg kg^{-1} BQ123 (n = 10) respectively. Ten minutes after baseline hemodynamics measurement, different doses of BQ123 were infused into the wing vein. FASP, FADP, PASP and PADP were continuously recorded for 60 min after the infusion. To observe the changes of FAP and PAP after chronic administration of ET_AR antagonist, birds (n = 10) were abdominally injected with 2.0 μg kg^{-1} BQ123 (two times a day) at 16 to 30 days of age. The same volume of 0.3% DMSO was administrated into abdominal cavities in the control group (n = 10). At 23 and 30 days of age, hemodynamic indexes were measured. The doses of BQ123 were selected following preliminary studies based on the responses of several doses (range from 0.1 μg kg^{-1} to 2.0 μg kg^{-1}, data not shown).

The study was performed in accordance with local ethical guidelines.

Statistical analysis

All data were presented as the mean±SD. Either the student's *t* test or one-way ANOVA with multiple comparison methods by SPSS for windows™ package (SPSS Inc., Chicago, IL, USA) was used for statistical analysis.

RESULTS

Changes of plasma and lung homogenate ET-1 levels

As shown in Table 2, plasma ET-1 (range from 40.82±1.90 pg ml^{-1} to 93.70±1.19 pg ml^{-1}) and lung homogenate ET-1 levels (range from 503.84±27.40 pg g^{-1} to 701.04±16.18 pg g^{-1} wet weight) were both increased with age over seven weeks' life circle of broiler chickens. The ET-1 concentrations of plasma were far lower than those of lung homogenate in broiler chickens. Sixty minutes after intravenous injection of BQ123 (0.4 μg kg^{-1} and 2.0 μg kg^{-1}) did not have the significant effect on plasma and lung homogenate ET-1 levels (data not shown). But as shown in Table 3, the chronic intraperitoneal injection of BQ123 (2.0 μg kg^{-1}) led to the significant reduction of lung homogenate ET-1 level at 7 and 14 days after treatment ($p<0.05$). The plasma ET-1 level didn't show the significant difference compared with the control group.

Effects of intravenous infusion of BQ123 on FAP and PAP

As shown in Figure 1 and 2, intravenous infusion of BQ123 (0.4 μg kg^{-1} and 2.0 μg kg^{-1}) caused the significant decreases in FASP, FADP, PASP and PADP ($p<0.05$). FASP and FADP reached the bottom values at 30 min after infusion of two doses of BQ123, and retuned to the baseline at 60 min after infusion. High dosage of BQ123 (2.0 μg kg^{-1}) caused more decrease in pressure than low dosage (0.4 μg kg^{-1}) at corresponding time point. The changes of PASP and PADP were similar to those of FASP and FADP, and differently, PASP and PADP reached the bottom values at 20 min after infusion.

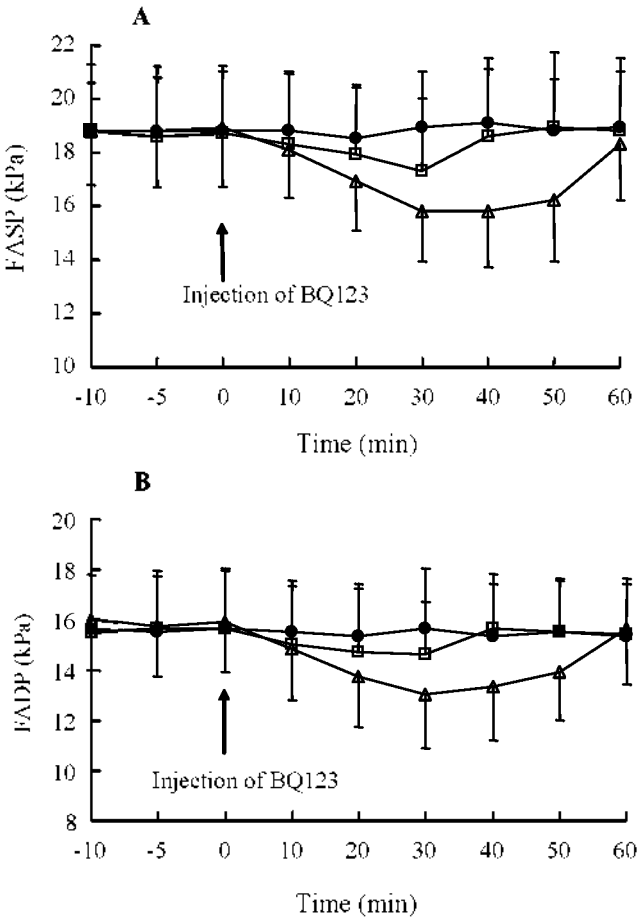

Figure 1. Effects of intravenous infusion of BQ123 on FASP (Figure 1A) and FADP (Figure 1B). FASP and FADP continued to decrease after intravenous infusion of BQ123 at 0.4 μg kg^{-1} (□, n = 10) or 2.0 μg kg^{-1} (Δ, n = 10). BQ123 at 2.0 μg kg^{-1} caused more decrease in pressure than at 0.4 μg kg^{-1} at the same time point. Chickens in control group (●, n = 10) with infusion of 0.3% DMSO. All values were means±SD. FASP, femoral artery systolic pressure; FADP, femoral artery diastolic pressure.

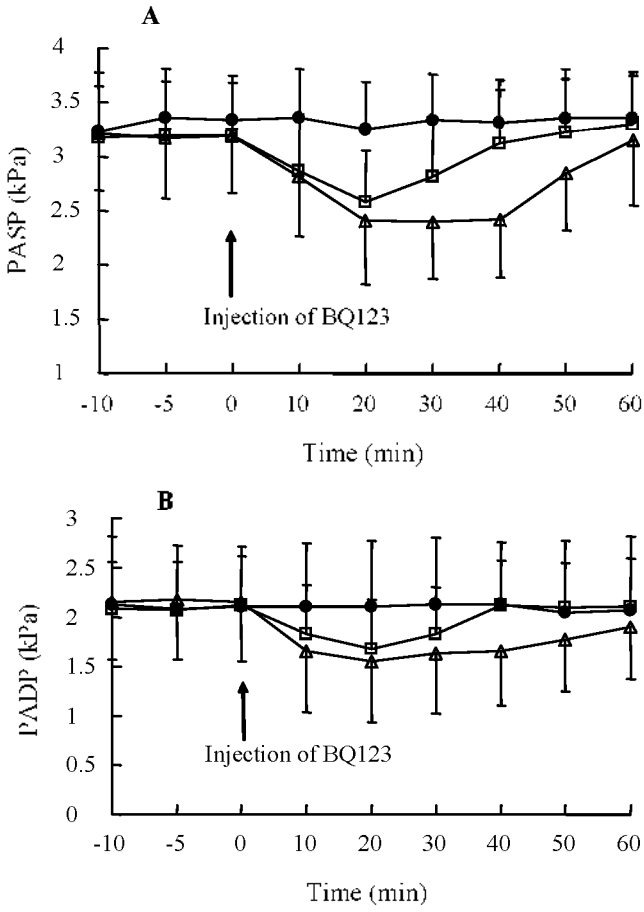

Figure 2. Effects of intravenous infusion of BQ123 on PASP (Figure 2A) and PADP (Figure 2B). FASP and FADP significantly decrease after intravenous infusion of BQ123 at 0.4 μg kg^{-1} (□, n = 10) or 2.0 μg kg^{-1} (Δ, n = 10). BQ123 at 2.0 μg kg^{-1} caused more decrease in pressure than BQ123 at 0.4 μg kg^{-1} at the same time point. Chickens in control group (●, n = 10) with infusion of 0.3% DMSO. All values were means±SD. PASP, pulmonary artery systolic pressure; PADP, pulmonary artery diastolic pressure.

Effects of chronic administration of BQ123 on FAP and PAP

As shown in Figure 3, at 7 and 14 days after chronic intraperitoneal injection of 2.0 μg kg^{-1} BQ123, FASP ($p<0.05$) and PASP ($p<0.01$) decreased significantly, and no obvious differences between treatment and control groups were found in FADP and PADP.

DISCUSSION

Under normal physiological conditions, ET-1 is not a circulating hormone, and rather acts as autocrine/paracrine factor at multiple sites. The lung represents a primary target for ET-1 effects and is a special site for ET-1 metabolic pathways (Gglie et al., 2004). Since it is necessary to determine the levels of plasma and lung homogenate ET-1 for studying the function of endogenous ET-1, we measured the ET-1 levels in broiler chickens, and obtained the following findings. First, plasma and lung homogenate ET-1 levels were both increased with age over seven weeks' life circle of broiler chickens, consistent with the previous report of Bo et al. (2004) who also measured the ET-1 concentrations in broiler chickens. In addition, earlier study (Battistelli et al., 1996) also demonstrated that plasma ET concentrations were also increased with age in humans. Second, the ET-1 level of lung homogenate was far higher than that of plasma, which suggested that the lung might also be an important target for ET-1 effects in broiler chickens. Third, the plasma ET-1 levels (above 41 pg ml^{-1}) of broiler chickens were much higher than those (all below 10 pg ml^{-1}) of human (Apostolopoulou et al., 2003; Gglie et al., 2004) and other mammals (rabbit, Gratton et al., 1997 and canine, Willtte et al., 1997; rat, Ronald et al., 2000). The role of high plasma ET-1 levels in broiler chickens

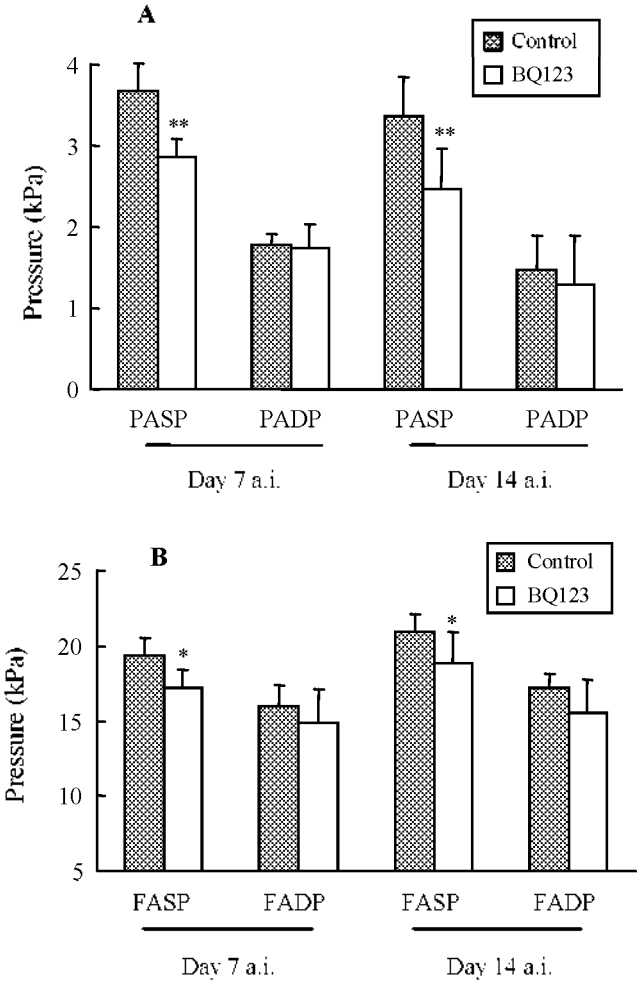

Figure 3. Effects of chronic administration of BQ123 on pulmonary artery pressure (Figure 3A) and femoral artery pressure (Figure 3B). At 7 and 14 days after chronic intraperitoneal injection of 2.0 μg kg^{-1} BQ123, FASP and PASP in broilers (n = 10) significantly decreased. * $p<0.05$ and ** $p<0.01$, compared with control group (n = 10, infusion of 0.3% DMSO). All values were means±SD. FASP, femoral artery systolic pressure; FADP, femoral artery diastolic pressure; PASP, pulmonary artery systolic pressure; PADP, pulmonary artery diastolic pressure; a.i., after injection.

remains to be further investigated.

Many studies have shown that endogenous ET-1 contributes to the maintenance of basal vascular tone and blood pressure in human and mammal (Haynes et al., 1994; Haynes et al., 1996). The vasoconstrictor effect of ET-1 is induced by ET_AR (Yanagisawa and Masaki, 1989). BQ123 (-D-Asp-L-Pro-D-Val-L-Leu-D-Trp-) was found to selectively inhibit the binding of ET-1 to the ET_AR and to functionally antagonize ET-1-induced vasoconstriction. It is a useful tool to assess the physiological and pathophysiological roles of ET-1 and ET_AR. In previous studies, BQ123 was mainly used to study the role of ET-1 in regulating cardiovascular tone in mammals. In this paper we used two experimental protocols to indirectly characterize the role of endogenous ET-1 in avian vascular system. In first experiment, the intravenous infusion of BQ123 was conducted to observe the transient changes of FAP and PAP by directly antagonizing the pressor effect of endogenous ET-1. Our data showed that intravenous infusion of BQ123 didn't cause significant changes in plasma and lung homogenate ET-1 levels, but led to the significant decreases of FASP, FADP, PASP and PADP in broiler chickens. Furthermore, high dosage of BQ123 (at 2.0 μg kg^{-1}) induced the more reduction in systemic and pulmonary pressure compared with low dosage (at 0.4 μg kg^{-1}), and longer time was needed to return to baseline in high dosage group. So BQ123 reduced blood pressure in a dosage-sensitive manner. Some related studies on human and mammal got the similar results. Bigaud and Pelron (1992) reported a decrease in femoral arterial blood pressure in the anaesthetized rats following intravenous administration of BQ123. BQ123 also antagonized ET-1-induced contraction of the canine pulmonary artery (Willtte et al., 1997).

In second experiment, the chronic intraperitoneal infusion of BQ123 was used to observe the effect of BQ123 on FAP and PAP in broiler chickens with long-term adaptation to antagonism. We found that chronic intraperitoneal administration of BQ123 led to the significant decrease of PASP and FASP, and the change of PAP was more distinct than that of FAP. Although, at two weeks after treatment, the concentrations of lung homogenate ET-1 significantly decreased in broiler chickens, we could see, as shown in Table 2 and Figure 3, that the degree of pressure reduction was much greater than that of ET-1 level decrease. Those results indicated that the reduction of FAP and PAP might not be completely induced by the decreased ET-1 levels, and was probably in part secondary to the antagonism of BQ123.

It is recognized that vascular endothelial dysfunction contributes to the development and perpetration of PH by disturbing the balances between vasodilating and vasoconstrictive forces, and between proliferative and antiproliferative forces. ET-1, binding to the ET_AR, has been shown to be involved in the pathogenesis of PH. In mammals, ET-1 receptor antagonists not only reduced PH, but also resulted in reversal of vascular remodeling and right ventricular hypertrophy (Langleben et al., 1999). Ascites syndrome is a condition in which the abdominal cavity is filled with serous fluid, leading to death or potential condemnation (Bo et al., 2005). It is characterized by chronically elevated pulmonary pressure, right ventricular hypertrophy and failure and central venous congestion, which eventually results in the accumulation of fluid in the abdominal cavity (Kai et al., 2006). The ascites has been a worldwide source of concern to the poultry

industry for several decades (Ipek et al., 2006). Therefore our study can provide the basic data for further investigating the relationship between the ET system and the ascites in broiler chickens which is pathophysiologically similar to PH in humans and mammals.

In conclusion, the ET_AR antagonist BQ123 leads to the significant reduction of FAP and PAP in broiler chickens, which suggests that endogenous ET-1 may be involved in the maintenance and regulation of systemic and pulmonary pressure in broiler chickens.

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