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Hypotension associated with systemic aggregated anaphylaxis is not attenuated by a selective endothelin-A receptor antagonist, BQ 610, in rabbits in vivo

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Abstract

Purpose. The present study was done to investigate the role of endothelin-1 (ET-1) in hypotension and bronchospasm provoked by anaphylaxis in rabbits in vivo.

Methods. Forty-five rabbits sensitized to horse serum were randomly allocated to five groups: Group 1 (n = 10) received 0.5 nmol·kg^{-1} of ET-1; Group 2 (n = 10) received 0.5 nmol·kg^{-1} of ET-1 and 200 nmol·kg^{-1} of a selective ET_A receptor antagonist, BQ 610, without anaphylaxis; Group 3 (n = 5) received 200 nmol·kg^{-1} of BQ 610 alone without anaphylaxis; Group 4 (n = 10) received normal saline alone before being antigen challenged to induce anaphylaxis; Group 5 (n = 10) received 200 nmol·kg^{-1} of BQ 610 before antigen challenge.

Results. Mean arterial pressure (MAP) values were significantly different between Groups 1 and 2. Heart rate (HR), central venous pressure (CVP), dynamic pulmonary compliance (C_{dyn}), and pulmonary airway resistance (R_L) did not differ significantly between Groups 1 and 2. MAP values were significantly decreased compared with baseline in both Groups 4 and 5; however, the values were not significantly different between two groups. CVP values were significantly different between Groups 4 and 5 only at the 15-min time point following antigen challenge. HR, R_L, and C_{dyn} values were not significantly different between Groups 4 and 5, nor were the survival rates.

Conclusion. BQ 610 does not improve hypotension or survival rates in systemic aggregated anaphylactic rabbits in vivo, implying that circulating ET-1 may not play an important role in anaphylaxis, although direct proof of production of circulating ET-1 or activation of ET_A receptors is lacking in this study.

Key words Anaphylaxis · Endothelin-1 · Hypotension · ET_A receptor antagonist · BQ 610

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Introduction

Endothelins (ETs) are a family of acidic 21-amino-acid constrictor peptides first isolated from vascular endothelial cells and are present in at least three distinct isoforms, ET-1, ET-2, and ET-3 [1,2]. ET-1 and other ET isopeptides are ubiquitous autacoids that are produced and released by many types of cells, both vascular and nonvascular, including vascular endothelial cells, smooth muscle cells, kidney and airway epithelial cells, mesangial cells, neurons, astrocytes, monocytes, and macrophages [3]. ET-1 is one of the most potent agonists of both vascular and airway smooth muscle to cause an increase in vascular and pulmonary resistance in several species in vivo [4]. In addition to vasoconstriction, ETs also have immunomodulating, endocrinological, and neurological effects that are exerted through at least two types of receptors, ET_A and ET_B [5]. Two different ET receptors, ET_A and ET_B, have been characterized, and many selective and nonselective receptor antagonists have been synthesized [6,7]. A selective ET_A receptor blockade by BQ 610 improves the hemodynamics in the intact circulation by causing a reduction in afterload and an increase in myocardial contractility [8]. It has become apparent that endothelial changes and increased production of vasodilating and vasoconstricting substances in the vessel wall, such as nitric oxide (NO) and ET, play an important role in the pathophysiologic changes of septic shock [9–13], adult respiratory distress syndrome [14], systemic inflammatory response syndrome [3], traumatic shock [15], and hemorrhagic shock [16].

In anaphylactic shock, many mediators such as NO, cytokines, platelet-activating factor, and so on, contribute to the pathophysiology of hemodynamics and pulmonary mechanics. With regard to a role of NO in the pathophysiology of anaphylactic shock, we reported that inhibition of NO production is detrimental to cardiac function and promotes bronchospasm, although

NO synthetase inhibitor improved vasodilation in anaphylaxis, suggesting that NO production may be beneficial to cardiac depression and bronchospasm in anaphylaxis in vivo [17]. The possibility of upregulation of circulating ETs and their implication in the pathophysiology of anaphylactic shock is conceivable. Filep et al. [18] report elevated plasma levels of immunoreactive ET during anaphylactic shock in guinea pigs. The purpose of the present study was to investigate the role of ET-1 in the hypotension and bronchospasm provoked by anaphylaxis in vivo. To determine whether inhibition of ET-1 would improve hypotension and bronchospasm in anaphylaxis, we administered BQ 610, the selective ET_A receptor antagonist, to fentanyl-anesthetized rabbits before the induction of anaphylaxis.

Materials and methods

Animal preparation

All experiments were performed in adherence to National Institutes of Health guidelines for the use of experimental animals. Approval of the Animal Use Committee of the Jichi Medical School was obtained before initiating the experiments.

The rabbits were sensitized to horse serum (cat. No.1650-015. Life Technologies, Grand Island, NY, USA) with an initial 2-ml subcutaneous dose followed 2 days later by a 2-ml intravenous dose. A period of 14 days was then allowed to elapse before intravenous challenge with horse serum [17].

A total of 45 Japanese white rabbits of both sexes (body weight: 2.8–3.2 kg) were randomly selected and used in subsequent experiments. The rabbits were anesthetized with intravenous pentobarbital (25–50 mg) and were intubated with an endotracheal tube with an internal diameter of 4 mm. The rabbits were paralyzed with vecuronium (1 mg). Ventilation was maintained using a Harvard piston ventilator with 100% oxygen, and the tidal volume was adjusted to maintain an end-tidal CO_2 level between 30 and 40 mmHg. The rabbits received continuous infusion of fentanyl at a rate of $10\,\mu g \cdot kg^{-1} \cdot h^{-1}$ and vecuronium at a rate of $1\,mg \cdot h^{-1}$ after the initiation of controlled ventilation and before surgery to maintain adequate anesthesia. Nasal temperature was continuously monitored and variations in body temperature were prevented using a heating pad.

Heart rate (HR) was monitored with an electrocardiograph. Systolic blood pressure, diastolic blood pressure, and mean arterial pressure (MAP) were monitored with a high-fidelity transducer-tipped catheter (Miller Microtip catheter pressure transducer, 6F, SPC-360, Miller Instruments, Houston, TX, USA) placed in the ascending aorta via the left carotid artery. Two 20-G, 3.2-cm catheters (Terumo Tokyo, Japan) were placed in the left and right auricular veins: one for administration of lactated Ringer's solution at a rate of $10\,ml \cdot kg^{-1} \cdot h^{-1}$ throughout the experiment in all rabbits, and the other for the administration of reagents and antigen. A catheter was placed in the superior vena cava via the right jugular vein to monitor central venous pressure (CVP). All signals were monitored continuously using a multichannel polygraph (360, NEC San-ei, Tokyo, Japan). All parameters were recorded continuously throughout the experiments.

An esophageal balloon (Nihon Koden, Tokyo, Japan) was placed in the esophagus and positioned at the point where the minimum end-expiratory pressure was recorded. The balloon contained 0.2 ml of air. The transpulmonary pressure (Ptp) was measured with a differential pressure transducer (TP-603T, Nihon Koden) with one side connected to the esophageal catheter and the other side attached to an orifice in the side of the end of the endotracheal tube. Airflow was measured with a pneumotachograph head (Fleish type, TV-112, Nihon Koden) and a differential flow transducer integration of the flow signal. Pressure, flow, and volume signals were recorded with a thermal-tip recorder (Omnicorder 8M 15, NEC San-ei).

Dynamic pulmonary compliance (C_{dyn}) was calculated by dividing the tidal volume by the absolute difference in Ptp at zero flow. Pulmonary resistance (R_L) was calculated using the method described on earlier papers [17,19]. Relating the Ptp to specific points in the volume and flow-rate cycle can separate the elastic and flow-resistive components of Ptp. At points of equal lung volume during the respiratory cycle, the elastic forces must be approximately equal, and differences in Ptp reflect the resistance to airflow in the lungs and airway. R_L was calculated by dividing the difference in Ptp by the differences in flow between points of equal volume in the respiratory cycle. R_L and C_{dyn} were recorded continuously throughout the experiments and were reported as the mean values for three consecutive breaths.

Study design and experimental protocols

Three sets of experiments were performed to investigate the effects of ET-1 on cardiovascular changes and bronchospasm in anaphylactic shock (Table 1). In the first set of experiments (Groups 1 and 2), ET-1 was administered alone or in combination with a selective ET_A receptor antagonist, BQ 610, to evaluate the effect of BQ 610 on exogenous ET-1 without anaphylaxis. In the second set (Group 3), the effects of BQ 610 were evaluated in rabbits without anaphylaxis. In the third set (Groups 4 and 5), rabbits with induced systemic anaphylaxis were treated with or without BQ 610 to evaluate the role of endogenous ET-1 in the patho-

Table 1. Experimental design

Group	Exogenous endothelin-1[a]	BQ 610[b]	Anaphylaxis
1 (n = 10)	yes	no	no
2 (n = 10)	yes	yes	no
3 (n = 5)	no	yes	no
4 (n = 10)	no	no	yes
5 (n = 10)	no	yes	yes

[a] Rabbits intravenously received 10 ml of 0.9% NaCl solution (normal saline, NS) containing 0.5 nmol·kg^{-1} of endothelin-1 (Alexis, Laufelfingen, Switzerland) over 10 min
[b] Rabbits received 10 ml of NS containing 200 nmol·kg^{-1} of a selective endothelin-A receptor antagonist, BQ 610

physiological changes provoked by systemic aggregated anaphylaxis. In all sets of experiments, baseline measurements of experimental parameters were obtained after the stabilization of hemodynamics and pulmonary mechanics was confirmed.

Rabbits in Group 1 (n = 10) received 10 ml of 0.9% NaCl solution (normal saline, NS) over 10 min followed by intravenous administration of 0.5 nmol·kg^{-1} of ET-1 (Alexis, Laufelfingen, Switzerland) over 10 min. Rabbits in Group 2 (n = 10) received 10 ml of NS containing 200 nmol·kg^{-1} of BQ 610 (homopiperidinyl-carbonyl-Leu-D-Trp [CHO]-D-Trp-OH, Alexis) over 10 min followed by intravenous administration of 0.5 nmol·kg^{-1} of ET-1 over 10 min. HR, MAP, and CVP values were recorded continuously for 45 min, and R_L and C_{dyn} values were recorded continuously for 40 min after the administration of ET-1.

Rabbits in Group 3 (n = 5) received 10 ml of NS containing 200 nmol·kg^{-1} of BQ 610 over 10 min followed by administration of 3 ml of NS over 30 s. All parameters were recorded continuously for 45 min after the administration of NS.

Rabbits in Group 4 (n = 10) received 10 ml of NS alone 10 min before antigen challenge as a control, and rabbits in Group 5 (n = 10) received 10 ml of NS containing 200 nmol·kg^{-1} of BQ 610 10 min before antigen challenge. After 10 min, 3 ml of horse serum was administered over 30 s into the systemic circulation to induce anaphylaxis. All parameters were recorded continuously for 45 min after antigen challenge because cardiovascular depression and bronchospasm return to the baseline 60 min after administration of the amount of antigen used to provoke anaphylactic shock in the present study.

In our earlier study using the same anaphylactic model, systemic hypoxemia does not occur after antigen challenge when animals are ventilated with 100% oxygen [17]. The survival rate was determined at 45 min after antigen challenge. Animals that were still alive at more than 45 min were killed by intravenous injection of pentobarbital and KCl.

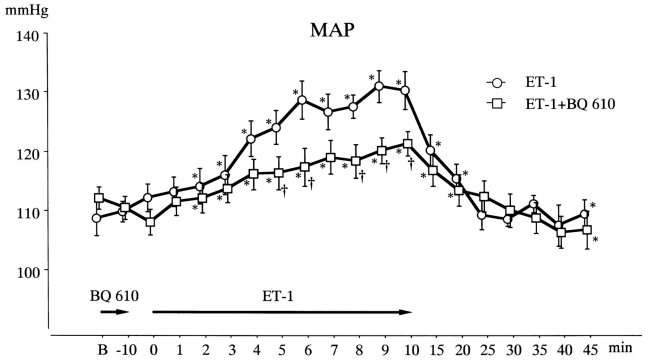

Fig. 1. Changes in mean arterial pressure (*MAP*) in rabbits receiving endothelin-1 (*ET-1*) alone (Group 1), and both ET-1 and BQ 610 (Group 2). *Open circle*, Group 1, n = 10; *open square*, Group 2, n = 10; *B*, baseline. *$P < 0.05$ *vs* baseline; †$P < 0.05$ Group 1 *vs* Group 2

Statistical analysis

All data are expressed as mean values ± SE. Statistical comparisons were performed using analysis of variance followed by the Fisher's protected least significant different test. Survival data were analyzed using the χ^2 test. A value of $P < 0.05$ was considered statistically significant.

Results

The baseline values of the parameters HR, MAP, CVP, R_L, and C_{dyn} did not differ significantly between Groups 1 and 2 (Figs. 1, 2, and 3). MAP values in both groups increased significantly during the period from 2 min to 20 min compared with baseline. Between the two groups, the values were significantly different during the period from 5 min to 6 min, and from 8 min to 10 min (Fig. 1). HR values in Groups 1 and 2 decreased significantly from baseline during the period from 0 min to 45 min. The values were not significantly different between the two groups (Fig. 2). CVP values in Group 1 decreased significantly from baseline during the period from 20 min to 30 min, and in Group 2, the values decreased significantly at 8 min and during the period from 20 min to 25 min compared with baseline. The values were not significantly different between the two groups (Fig. 2).

Compared with baseline values of R_L, Groups 1 and 2 showed significant differences at 30 min and 20 min, respectively, and there was a significant difference between the two groups at 30 min (Fig. 3). Values of C_{dyn} in Group 1 were significantly decreased from baseline from 6 min to 20 min, and in Group 2 the values were significantly decreased from 4 min to 40 min compared with baseline. The values were not significantly different between the two groups (Fig. 3).

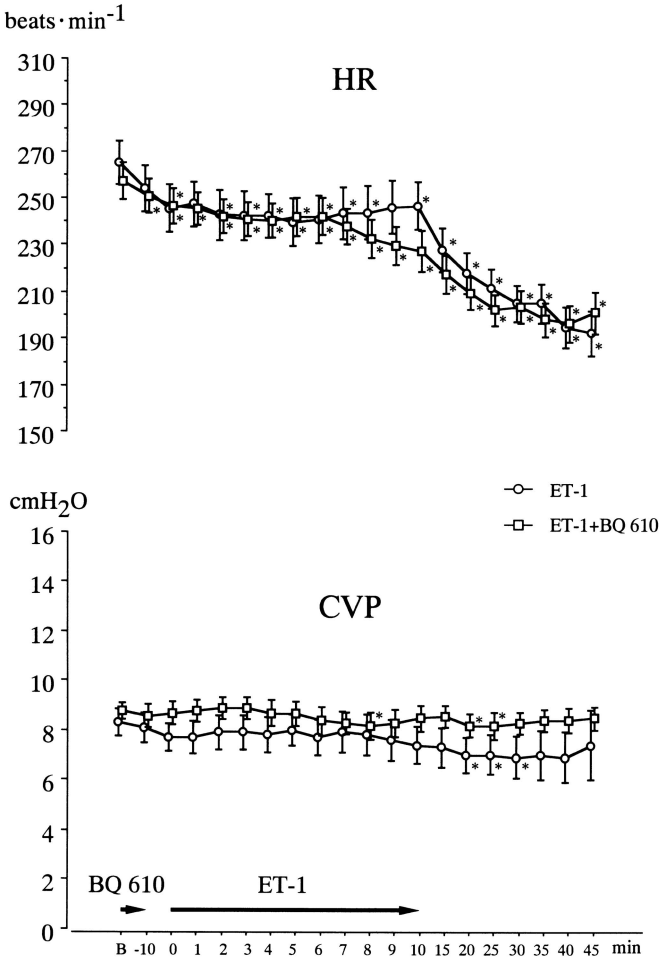

Fig. 2. Changes in heart rate (*HR*) and central venous pressure (*CVP*) in rabbits receiving ET-1 alone (Group 1), and both ET-1 and BQ 610 (Group 2). *Open circle*, Group 1, $n = 10$; *open square*, Group 2, $n = 10$; *B*, baseline. $*P < 0.05$ *vs* baseline

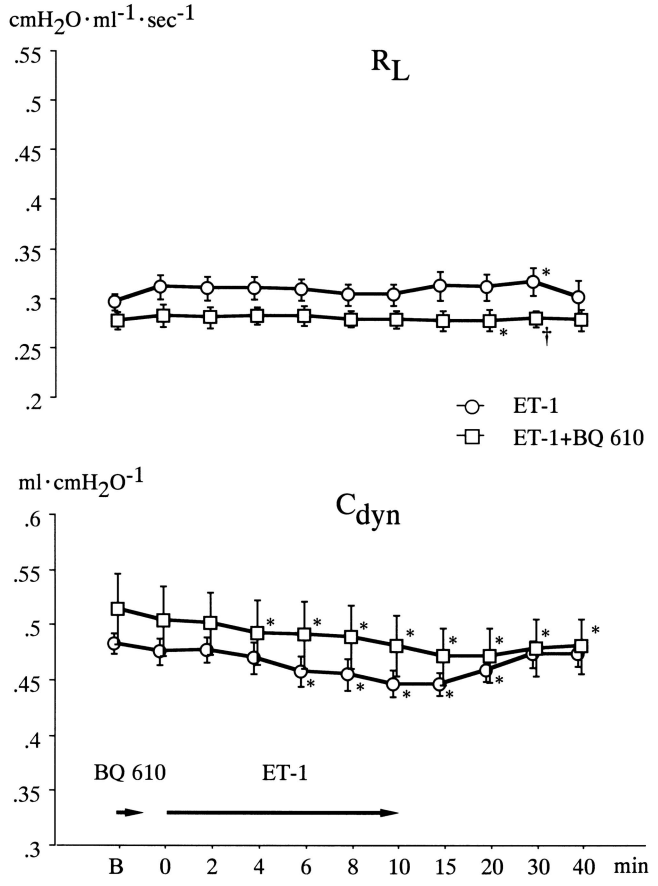

Fig. 3. Changes in pulmonary resistance (R_L) and pulmonary compliance (C_{dyn}) in rabbits receiving ET-1 alone (Group 1), and both ET-1 and BQ 610 (Group 2). *Open circle*, Group 1, $n = 10$; *open square*, Group 2, $n = 10$; *B*, baseline. $*P < 0.05$ *vs* baseline; $\dagger P < 0.05$ Group 1 *vs* Group 2

In Group 3, MAP, CVP, C_{dyn}, and R_L values were not significantly different from baseline throughout the experiment. HR values were significantly decreased at 9 min and during the period from 15 min to 45 min compared with baseline (Table 2).

Groups 4 and 5 did not differ at baseline in the parameters HR, MAP, CVP, R_L, or C_{dyn} (Figs. 4 and 5). MAP values in both groups were significantly decreased from baseline between 2 min and 45 min, but there was no significant difference between the two groups (Fig. 4). HR values were not significantly decreased compared with baseline in Group 4. In Group 5, the values were significantly increased between 4 min and 10 min compared with baseline. The values were not significantly different between the two groups (Fig. 4). CVP values in Group 4 were significantly increased from baseline between 2 min and 25 min, and in Group 5 the values were significantly increased between 2 min and 6 min compared with baseline. Between the two groups, the values differed significantly only at 15 min (Fig. 4). R_L values in Group 4 were significantly increased from baseline between 2 min and 8 min. In Group 5, the values were significantly increased from baseline between 2 min and 10 min. The values were not significantly different between the two groups (Fig. 5). Values of C_{dyn} in both Groups 4 and 5 were significantly decreased from 2 min to 40 min compared with baseline. The values were not significantly different between the two groups (Fig. 5).

The survival rates did not differ significantly between the two groups. Five of ten rabbits in Group 4 and six of ten rabbits in Group 5 were alive 45 min after antigen challenge. All deaths were observed by 20 min after antigen challenge (Fig. 6).

Discussion

The results of the present study show that prior administration of BQ 610, a selective ET_A receptor antagonist,

Table 2. Changes in heart rate (HR), mean arterial pressure (MAP), central venous pressure (CVP), pulmonary resistance (R_L) and dynamic pulmonary compliance (C_{dyn}) in Group 3 rabbits ($n = 5$) receiving 200 nmol·kg^{-1} of BQ 610 over 10 min followed by administration of 3 ml of normal saline over 30 s

	5 min	6 min	7 min	8 min	9 min	10 min	15 min
HR (beats·min^{-1})	251 ± 11	252 ± 11	249 ± 12	247 ± 12	243 ± 14*	245 ± 13	243 ± 13*
MAP (mmHg)	99 ± 10	100 ± 9	97 ± 9	95 ± 9	95 ± 10	98 ± 10	95 ± 9
CVP (cmH_2O)	6.8 ± 0.4	6.8 ± 0.5	7.0 ± 0.4	6.8 ± 0.5	6.8 ± 0.5	6.6 ± 0.2	6.8 ± 0.6
R_L ($cmH_2O \cdot ml^{-1} \cdot s^{-1}$)		0.28 ± 0.2		0.28 ± 0.2		0.27 ± 0.02	0.28 ± 0.02
C_{dyn} ($ml \cdot cmH_2O^{-1}$)		0.45 ± 0.04		0.46 ± 0.04		0.45 ± 0.04	0.45 ± 0.04

	20 min	25 min	30 min	35 min	40 min	45 min
HR (beats·min^{-1})	237 ± 14*	235 ± 14*	235 ± 12*	234 ± 11*	231 ± 10*	223 ± 7*
MAP (mmHg)	96 ± 9	98 ± 10	97 ± 9	97 ± 9	96 ± 9	101 ± 5
CVP (cmH_2O)	6.8 ± 0.6	7.0 ± 0.5	6.8 ± 0.4	6.8 ± 0.4	7.0 ± 0.5	7.3 ± 0.6
R_L ($cmH_2O \cdot ml^{-1} \cdot s^{-1}$)	0.28 ± 0.02		0.28 ± 0.02		0.29 ± 0.02	
C_{dyn} ($ml \cdot cmH_2O^{-1}$)	0.45 ± 0.04		0.44 ± 0.04		0.44 ± 0.03	

* $P < 0.05$ compared with the baseline value

did not improve hypotension provoked by anaphylaxis. Although circulating ET-1 or ET_A expression was not measured in this rabbit anaphylactic model, plasma levels of immunoreactive ET during anaphylactic shock are elevated in guinea pigs [18]. The ET_A receptor is more important for producing the profound systemic hemodynamic changes induced by ET-1 in vivo, although both ET_A and ET_B receptors mediate vasoconstriction [20]. The ET_B receptor exists as at least two subtypes, the ET_{B1} receptor, located on the endothelium, and the ET_{B2} receptor, expressed on smooth muscle cells. The ET_A receptor, located on smooth muscle cells, together with the ET_{B2} receptor, mediates contraction, and ET_{B1} receptor activation causes relaxation through the release of NO and prostacyclin [5]. Therefore, a selective ET_A receptor antagonist, BQ 610, may prevent coronary vasoconstriction and consecutive myocardial ischemia, and may improve cardiac performance in anaphylactic shock. Hypotension due to vasodilation and fluid loss into the tissue space resulting from increased capillary permeability associated with anaphylaxis is observed. Mediators such as NO, cytokines, platelet-activating factor, and so on, contribute to this pathophysiology. Although ET-1 has a vasoconstriction effect and may compensate hypotension caused by vasodilators, ET-1 evokes losses in plasma volume and albumin escape via ET_A receptors. Although ET-1-mediated peripheral vasoconstriction supports MAP, increases in systemic arterial pressure to capillaries and/or local vasoconstriction may further aggravate losses in plasma volume and albumin escape [12]. Therefore, a selective ET_A receptor antagonist, BQ 610, was used in this study to evaluate the role of ET-1 in the pathophysiology of systemic anaphylaxis in vivo. In regard to hypotension associated with anaphylaxis, these results, along with the lack of improvement in survival rates in BQ 610-treated animals, imply that ET-1 might not play an important role in anaphylaxis, although direct proof of production of ET-1 or activation of ET_A receptors is lacking in this study.

BQ 610 (100 nmol·kg^{-1}) pretreatment significantly attenuates 1 nmol·kg^{-1} of exogenous ET-1 induced hemodynamic changes in rats [21]. BQ 610 (100 μg·kg^{-1}) improves the hemodynamics in the intact circulation by causing a reduction in afterload and an increase in myocardial contractility in rats [8]. BQ 610 (100 μg·kg^{-1}) also attenuates 1 nmol·kg^{-1} exogenous ET-1-induced reduction of cardiac output and prevents exogenous ET-1-induced vasoconstriction in rats [22]. Schmitz-Spanke and Schipke [23] used 0.5 nmol·kg^{-1} (bolus) of ET-1 in healthy anaesthetized rabbits. Therefore, 0.5 nmol·kg^{-1} of ET-1 and 200 nmol·kg^{-1} (131.360 μg·kg^{-1}) of BQ 610 were used in the present study. With regard to BQ 610 administration 10 min before antigen challenge, the timing of the administration was according to the methods of Szalay et al. [24], in which BQ 610 is infused intravenously into systemic circulation over 10 min, followed which ET-1 in a 1-nmol·kg^{-1} dose is infused intravenously over 10 min and the circulatory effects are observed for another 70 min, and BQ 610 significantly attenuates the ET-1-induced increase in MAP and reduces the decrease in cardiac output.

In septic shock rats, Szalay et al. [24] demonstrate that endogenous ET-1 contributes significantly to the systemic hemodynamic alterations during hypodynamic circulatory response, and the inhibition of ET_A receptors improves global hemodynamic status in hypodynamic sepsis. The tissue expression of ET-1 mRNA is significantly increased 6 h after the in vivo injection of endotoxin [25]. The ET-1 level reaches its maximum 8 h after cecal ligation and perforation in rats; mean concentration of ET-1 increased from 1.8 pg·ml^{-1} at

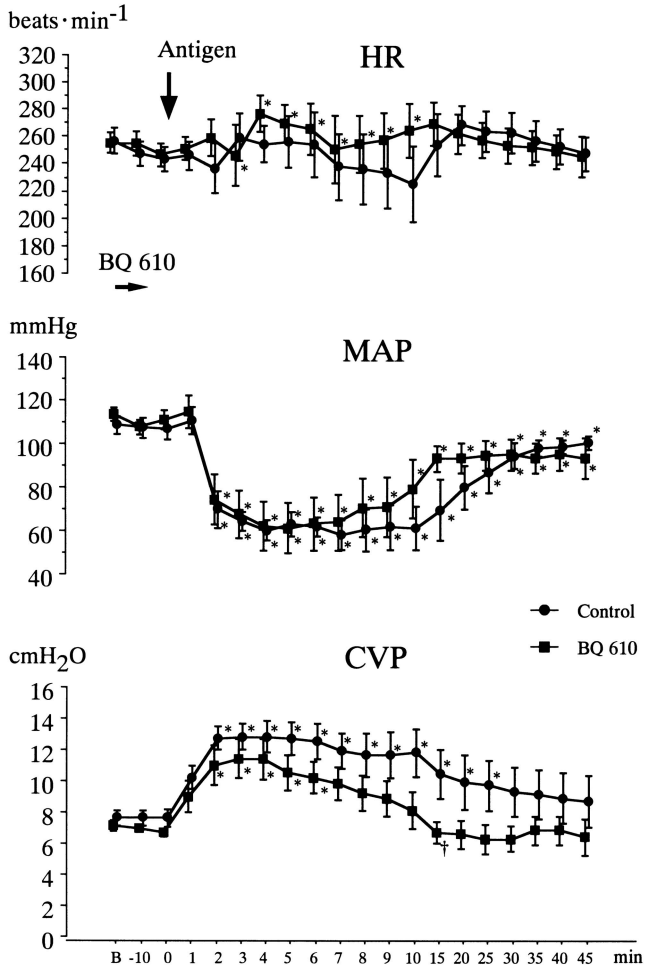

Fig. 4. Changes in heart rate (*HR*), mean arterial pressure (*MAP*), and central venous pressure (*CVP*) in control rabbits (Group 4) receiving 10ml of normal saline (*NS*) alone 10min before antigen challenge and in those receiving 10ml of NS containing 200nmol·kg^{-1} of BQ-610 10min before antigen challenge (Group 5). *Solid circle*, Group 4, $n = 10$, *solid square*, Group 5, $n = 10$; *B*, baseline. *$P < 0.05$ *vs* baseline; †$P < 0.05$ Group 1 *vs* Group 2

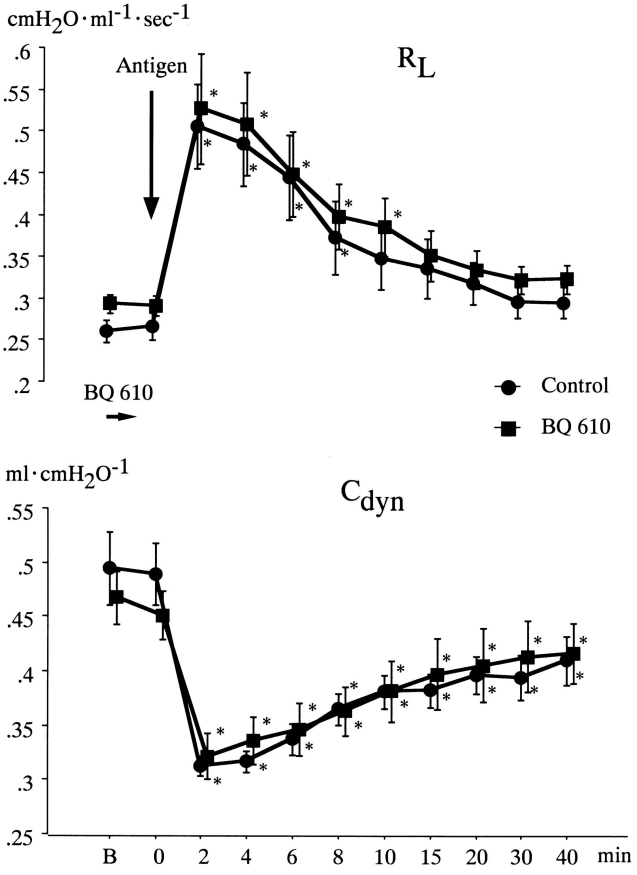

Fig. 5. Changes in pulmonary resistance (R_L) and pulmonary compliance (C_{dyn}) in control rabbits receiving 10ml of NS alone 10min before antigen challenge (Group 4) and in those receiving 10ml of NS containing 200nmol·kg^{-1} of BQ 610 10min before antigen challenge (Group 5). *Solid circle*, Group 4, $n = 10$; *solid square*, Group 5, $n = 10$; *B*, baseline. *$P < 0.05$ *vs* baseline; †$P < 0.05$ Group 1 *vs* Group 2

baseline to a peak of 30.3pg·ml^{-1}, an increase of 1580% [26]. ET-1 mRNA is significantly increased in the lungs 2h after trauma in rats [15]. A much longer period of time is required for the stimulation of gene expression in septic shock and traumatic shock. In anaphylactic shock, the plasma immunoreactive ET level increased on average by 76% 5min after antigen challenge [18]. Because changes in hemodynamics in the early phase of systemic anaphylaxis occur so rapidly after antigen challenge, production of ET may not be high enough to affect hemodynamics or it may not occur at all. Therefore, in this study, ET-1 may not be involved in the pathophysiology of systemic anaphylaxis, at least in the early phase. Filep et al. [18] show that the plasma immunoreactive ET level is significantly elevated in response

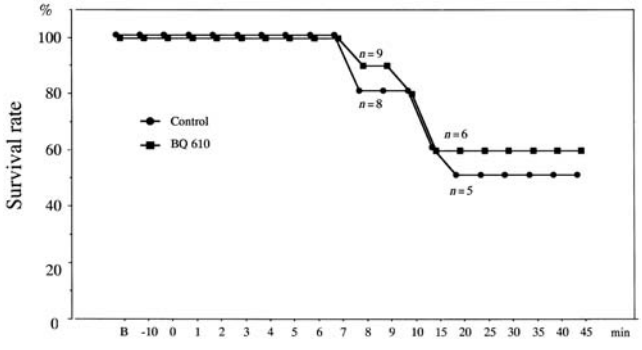

Fig. 6. Survival rates in control rabbits receiving 10ml of NS alone 10min before antigen challenge (Group 4) and in those receiving 10ml of NS containing 200nmol·kg^{-1} of BQ 610 10min before antigen challenge (Group 5). *Solid circle*, Group 4, $n = 10$; *solid square*, Group 5, $n = 10$

to antigen challenge in either actively or passively sensitized guinea pigs; however, they conclude that the elevated plasma level of immunoreactive ET during anaphylactic shock is independent of hypotension, hypovolemia, and respiratory insufficiency. This observation is in accordance with our results in regard to changes in hemodynamics in systemic anaphylaxis if ET-1 increased after antigen challenge in the animal studied in the present study.

In guinea pig anaphylaxis, plasma levels of immunoreactive ET reach a maximum at 5 min and are inversely correlated with arterial blood PO_2 [18]. This implies that hypoxemia provoked by antigen challenge in the circulation may account for the release of ETs, although it is uncertain whether or not this observation may solely be attributed to hypoxemia because severe hypoxia (10%–12% O_2) in rats is necessary to detect a significant increase in plasma immunoreactive ET [27,28]. Because our animals did not have systemic hypoxemia after antigen challenge when the animals were ventilated with 100% oxygen using the anaphylactic shock rabbit model [17], it is conceivable that ET-1 may not release after the initiation of anaphylaxis without hypoxemia in this study.

In BQ 610-treated rabbits with systemic anaphylaxis, CVP decreased significantly only at 15 min after antigen challenge. In this model, right heart failure induced by severe bronchoconstriction resulted in an increase in CVP. Therefore, the reduction in the increase in CVP after the initiation of anaphylaxis may presumably reflect improvement in the right heart failure provoked by bronchoconstriction resulting from systemic anaphylaxis. Changes in CVP reflected the degree of cardiac depression in the rabbit model used in the present study because the major hemodynamic effects in this model are reduced cardiac output and blood pressure due to impaired left ventricular filling pressure, with no significant change in peripheral vascular resistance [29]. Although the effects of ET-1 on ventricular function have been controversial, ET is reported to be a potent vasoconstrictor of the resistance coronary vessels, producing a redistribution of transmural blood flow and a decrease in myocardial contractility secondary to ischemia in the intact heart of anesthetized dogs [30]. In the present study, BQ 610 significantly attenuated increases in CVP only at 15 min after antigen challenge; however, the inhibitor did not attenuate decreases in MAP provoked by systemic anaphylaxis. It was possible that the amount of BQ 610 used in the present study was not enough to block the effect of circulating ETs after antigen challenge. Cardiac depression induced by systemic anaphylaxis might be attributable to ET-1-mediated effects if it can be shown that ETs are released at high enough levels in the early phase. Further investigations should be performed to evaluate the role in cardiac function associated with systemic anaphylaxis and to confirm the presence and production of ETs.

Bronchial hyperresponsiveness induced by ET-1 is mediated by ET_A receptor activation in rabbits [31]. ET_A receptor antagonists might be most useful in blocking the respiratory effects of anaphylaxis. In the present study, ET-1 decreased C_{dyn} and BQ 610 did not inhibit the effects of exogenous ET-1 in intact rabbits without anaphylaxis. The amount of BQ 610 administered intravenously was not enough to alleviate pulmonary changes associated with the administration of exogenous ET-1. D'Agostino et al. [31] administered aerosolized ET-1 and ET_A receptor antagonists to the lung via an endotracheal tube and showed bronchial hyperresponsiveness mediated by ET_A receptor activation. Therefore, the present data were not evaluated in regard to the role of ET-1 in pulmonary changes provoked by systemic anaphylaxis. It would be necessary to increase the amount of BQ 610 to confirm the effects of ET antagonists on pulmonary changes in systemic anaphylaxis. Increased epithelial airway ET-1 levels contribute to the anaphylactic reaction of guinea pig airways in vitro [32]. Conversely, Redington et al. [33] show that allergen exposure in asthma does not result in immediate release of immunoreactive ET. The presence and production of ETs by pulmonary cells, the wide distribution of specific ET receptors in airway and pulmonary vasculature, and their actions on pulmonary circulation and airways imply a role for these peptides in the regulation of pulmonary function under physiological and pathological conditions [34]. ETs play an important role in pulmonary pathophysiology by regulating pulmonary vascular and airway tone, activation of inflammatory cells, and cellular growth and/or differentiation [34]. Further studies on the production of ETs and the activation profile of the ET receptor subtypes in anaphylaxis are needed to clarify the pulmonary pathophysiology of anaphylaxis.

Severe bronchospasm provoked by antigen challenge in the anaphylaxis model used in this study was also observed in preliminary studies. Therefore, we used a paralytic agent to minimize the influence of chest wall compliance, permitting accurate measurements to be obtained for R_L and C_{dyn}, and ensuring adequate oxygenation during artificial ventilation throughout the experiment. Continuous infusion of fentanyl at a rate of $10\,\mu g \cdot kg^{-1} \cdot h^{-1}$ is enough anesthetic in animals because of no changes in MAP and HR during the operation before the antigen challenge.

In conclusion, BQ 610 does not improve hypotension provoked by systemic aggregated anaphylaxis in rabbits in vivo, implying that the associated hypotension is not attributable to circulating ET-1.

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