Chronic Unilateral Occlusion of an Extrapulmonary Primary Bronchus Induces Pulmonary Hypertension Syndrome (Ascites) in Male and Female Broilers[1]

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ABSTRACT Previously, it was demonstrated that acute (4 min) and chronic (12 d) occlusion of an extrapulmonary primary bronchus triggers pulmonary hypertension but not pulmonary hypertension syndrome (PHS, ascites) in broilers. The present study was conducted to determine whether a more prolonged period of bronchus occlusion causes PHS similar to that induced by clamping one pulmonary artery. Male and female broiler chicks, 14 to 18 d old, were anesthetized, the thoracic inlet was opened, and a silver clip was positioned to fully obstruct the left extrapulmonary primary bronchus (BRONCHUS CLAMP group) or the left pulmonary artery (PA-CLAMP group). Sham-operated chicks were anesthetized and the thoracic inlet was opened; however, neither the pulmonary artery nor the bronchus was clamped (SHAM group). An electrocardiogram (ECG) was obtained whenever clinical ascites became apparent in individual broilers, or prior to the final necropsy for broilers surviving to the end (Day 36) of the experiment. The right:total ventricular weight ratio (RV:TV) was evaluated as an index of pulmonary arterial pressure. Early post-surgical mortality (up to 21 d of age) was higher in the PA-CLAMP group (27% for males and females combined) than in the BRONCHUS CLAMP (10%) and SHAM (2%) groups. Cumulative ascites mortality (Days 22 to 36) also was higher in the PA-CLAMP group (86% for males, 77% for females) than in the BRONCHUS CLAMP (69% for males, 41% for females) and SHAM (23% for males, 0% for females) groups. Ascitic birds in all treatment groups had higher RV:TV ratios and more negative ECG Lead II S-wave amplitudes than nonascitic birds, reflecting the right ventricular hypertrophy and generalized ventricular dilation typically associated with PHS. These results demonstrate that unilateral bronchus occlusion is an effective experimental model for triggering ascites at a lower incidence than that obtained by occluding one pulmonary artery. Following the onset of pulmonary hypertension, the pathophysiological progression leading to ascites appears to be similar for broilers with either unilateral bronchus or pulmonary artery occlusion.

(*Key words*: ascites, pulmonary hypertension, respiration, airway obstruction)

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INTRODUCTION

It previously was demonstrated that a primary increase in pulmonary vascular resistance (PVR), accomplished by permanently occluding one pulmonary artery, initiates a pathophysiological progression leading to pulmonary hypertension syndrome (PHS, ascites) in male broilers (Wideman and Kirby, 1995a, 1996). Occluding one pulmonary artery forces the right ventricle to increase pulmonary arterial pressure (PAP) to propel the entire cardiac output through the noncompliant vasculature of the unobstructed lung. In addition, unilateral pulmonary artery occlusion causes an immediate systemic arterial hypoxemia (undersaturation of the blood with oxygen), hypercapnia [elevated blood partial pressure of carbon dioxide (P_{CO2})], and acidosis (depressed blood pH), apparently because the increased blood flow rate past the gas exchange surfaces provides insufficient time for effective diffusive gas exchange (Wideman and Kirby, 1995b; Wideman *et al*., 1996a,b). Qualitatively similar responses, including pulmonary hypertension, hypoxemia, hypercapnia, and acidosis, were observed when a snare around an extrapulmonary primary bronchus was transiently tightened, although the increase in PAP during unilateral bronchus occlusion was lower than the increase in PAP observed in the same broilers during unilateral pulmonary artery occlusion (Wideman *et al*., 1996a). Permanently clamping one

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extrapulmonary primary bronchus for 12 d triggered an increase in the right:total ventricular weight (RV:TV) ratio, which is indicative of sustained pulmonary hypertension; however, the 7% incidence of ascites induced by this period of bronchus occlusion was not higher than in control or sham-operated broilers (0%), and was lower than the 52% incidence of ascites in broilers with unilateral pulmonary artery occlusion (Wideman *et al*., 1996a). The present study was designed to compare the incidence of ascites induced in male and female broilers during more prolonged unilateral occlusion of an extrapulmonary primary bronchus or pulmonary artery.

MATERIALS AND METHODS

Male and female broiler chicks (n = 200 per sex) were wing-banded on the day of hatch (Day 1), and were reared on fresh wood shavings litter in four environmental chambers (8 m^2 floor space). They were brooded at 32 and 30 C during Weeks 1 and 2, respectively, and thereafter the temperature was maintained at 24 C. Throughout the experiment they were fed a corn-soybean meal-based broiler ration formulated to meet or exceed the minimum NRC (1984) standards for all ingredients, including 22.7% CP, 3,059 kcal ME/kg, 1.5% arginine, and 1.43% lysine. Feed and water were provided for *ad libitum* consumption.

Detailed descriptions of the surgical procedures have been provided previously (Wideman and Kirby, 1995a,b, 1996; Wideman *et al*., 1996a). Chicks between 14 and 18 d of age were anesthetized to a surgical plane with intramuscular injections of a 1:1 mixture of Ketamine HCl (Ketaset,[3] 100 mg/mL) and Xylazine (Rompun,[4] 100 mg/mL; injected volume of the mixture: 0.007 mL/100 g body mass). The thoracic inlet was opened, and a silver clip was positioned to fully occlude the left pulmonary artery (PA-CLAMP group) or the left extra-pulmonary primary bronchus (BRONCHUS CLAMP group). Sham-operated chicks were anesthetized and the thoracic inlet was opened; however, the pulmonary arteries and bronchi remained unoccluded (SHAM group). Surgical incisions were sealed with stainless steel wound clips and sprayed with a topical antibacterial powder, and the birds were placed under heat lamps until they recovered from the anesthesia. Following recovery from surgery, chicks were reared separately by sex but mixed by treatment group in the environmental chambers until they reached 36 d of age. An electrocardiogram (ECG) was recorded as described previously (Wideman and Kirby, 1996) whenever clinical ascites became evident in individual broilers, or on Day 36 for birds that survived to the end of the experiment. The ECG Lead II S-wave amplitude was measured as an index of right ventricular dilation and generalized ventricular hypertrophy (Wideman and Kirby, 1996). Broilers were killed by CO_2 inhalation and examined to verify clamp placement on the pulmonary artery or bronchus. The heart was removed, dissected, and weighed for calculation of the RV:TV ratio as an index of pulmonary hypertension (Burton *et al*., 1968; Cueva *et al*., 1974; Peacock *et al*., 1989). Broilers were diagnosed as having PHS (ascites) only when they exhibited abdominal fluid accumulation.

The incidences of early post-surgical mortality (Wideman and Kirby, 1995a) and ascites were analyzed by sex and treatment group using a G-test of independence with the Williams correction factor (Sokal and Rohlf, 1981). The SigmaStat® (Jandel Scientific, 1994) ANOVA procedure was used to analyze RV:TV ratios and ECG Lead II S-wave amplitudes by sex and treatment group, incorporating the Student-Newman-Keuls method to identify differences among means.

RESULTS

Surgical procedures were considered successful when the broilers showed no signs of being over-anesthetized prior to surgery, and when pulmonary artery or bronchus clamps were positioned correctly without causing excessive bleeding. A total of seven surgical failures were excluded from the data sets of the three treatment groups combined. Three birds exhibited evidence of infection at the surgical incision site and were excluded from the data sets. The successful surgeries performed per group are shown in Table 1. More broilers were included in the PA-CLAMP and BRONCHUS CLAMP groups based on prior experience that higher total mortalities would occur in these groups. Broilers were considered to have succumbed to early post-surgical mortality when the surgery was successful but the bird died prior to Day 22. The incidence of early post-surgical mortality was higher for males in the PA-CLAMP group than in the BRONCHUS CLAMP or SHAM groups, and was higher for females in the PA-CLAMP group than for females in the SHAM group (Table 1). The male and female values within each treatment group did not differ ($P \geq 0.05$). The combined incidence of early post-surgical mortality was higher in the PA-CLAMP group than in the BRONCHUS CLAMP group, and higher in the BRONCHUS CLAMP group than in the SHAM group. The first death attributed to ascites occurred on Day 25. The incidence of ascites was higher in the PA-CLAMP group than in the BRONCHUS CLAMP group, and higher in the BRONCHUS CLAMP group than in the SHAM group regardless of the sex of the bird. Ascites occurred in only male broilers within the SHAM group (Table 1).

The RV:TV ratios for nonascitic broilers did not differ by sex or treatment group (Table 2). For ascitic broilers, the RV:TV ratios were higher for females and all broilers combined in the PA-CLAMP group than for females and all broilers combined in the BRONCHUS CLAMP group. Regardless of the sex of the bird or treatment group, ascitic broilers had higher RV:TV ratios than

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TABLE 1. Successful surgeries performed, early post-surgical mortality, and the incidence of ascites through Day 36 in sham-operated broilers (SHAM), broilers in which the left extrapulmonary primary bronchus was clamped (BRONCHUS CLAMP), and broilers in which the left pulmonary artery was clamped (PA-CLAMP)[1]

		Treatment group		
Category	Sex	SHAM	BRONCHUS CLAMP	PA-CLAMP
Number of successful[2] surgeries per group	Male (M)	45	58	77
	Female (F)	37	57	73
Post-surgical mortality[3] through Day 21	M[4]	2/45 (4%)[b]	6/58 (10%)[b]	25/77 (32%)[a]
	F	0/37 (0%)[b]	6/57 (11%)[ab]	15/73 (21%)[a]
	M + F	2/82 (2%)[c]	12/115 (10%)[b]	40/150 (27%)[a]
Incidence of ascites, Days 22 through 36	M[5]	10/43 (23%)[c]	36/52 (69%)[b]	42/52 (86%)[a]
	F	0/37 (0%)[c]	21/51 (41%)[b]	45/58 (77%)[a]
	M + F	10/80 (13%)[c]	57/103 (55%)[b]	90/110 (82%)[a]

[a–c]Means within a sex and across treatment groups with no common superscript differ significantly ($P \leq 0.05$).

[1]Data are number affected/total available (percentage).

[2]Procedure completed without over-anesthesia or excessive bleeding.

[3]Surgeries performed on Days 14 to 18; category includes all subsequent mortality through Day 21.

[4]No difference between males and females within this category.

[5]Males had a higher incidence of ascites than females in the Sham ($P \leq 0.001$) and Bronchus Clamp ($P \leq 0.005$) Groups.

nonascitic broilers (Table 2). The ECG Lead II S-wave amplitudes for nonascitic broilers were more negative in males and females of the BRONCHUS CLAMP and PA-CLAMP groups than in the SHAM group (Table 3). Regardless of the sex of the bird or treatment group, ascitic broilers had more negative Lead II S-wave amplitudes than nonascitic broilers. Ascitic males in the BRONCHUS CLAMP and PA-CLAMP groups had more negative Lead II S-wave amplitudes than ascitic females in either group or ascitic males in the SHAM group (Table 3).

DISCUSSION

The unilateral bronchus occlusion technique provides an effective experimental method for inducing ascites. Unilateral bronchus occlusion apparently induces a more moderate challenge than clamping one pulmonary artery, as reflected by the lower early post-surgical mortality and the lower incidence of ascites in the BRONCHUS CLAMP group than in the PA-CLAMP group. The incidence of ascites in the SHAM male broilers was higher in the present study than for sham-operated broilers in previous studies, in spite of the use of essentially identical anesthetic and surgical protocols (Wideman and Kirby, 1995a, 1996; Wideman *et al.*, 1996a). No unanesthetized, unoperated control broilers were included in this experiment due to the absence of differences between sham-operated and control groups in previous studies (Wideman and Kirby, 1995a, 1996; Wideman *et al.*, 1996a). The incidence of ascites in the SHAM males may reflect a degree of baseline respiratory distress elicited by anesthesia and opening the thoracic inlet and air sacs; nevertheless the incidence of ascites clearly was greatly amplified by unilateral occlusion of the bronchus or pulmonary artery.

The comparatively high incidence of early post-surgical mortality observed in PA-CLAMP groups in the

TABLE 2. Right ventricular weight to total ventricular weight ratio (RV:TV) in sham-operated broilers (SHAM), broilers in which the left extrapulmonary primary bronchus was clamped (BRONCHUS CLAMP), and broilers in which the left pulmonary artery was clamped (PA-CLAMP)

		Necropsy category	
Treatment group	Sex	Nonascitic RV:TV	Ascitic RV:TV
SHAM	Male (M)	0.305 ± 0.015[c]	0.428 ± 0.024[ab]
	Female (F)	0.281 ± 0.013[c]	. . .
	M + F	0.293 ± 0.010[c]	0.428 ± 0.024[ab]
BRONCHUS CLAMP	M	0.328 ± 0.015[c]	0.412 ± 0.011[ab]
	F	0.297 ± 0.020[c]	0.395 ± 0.010[b]
	M + F	0.310 ± 0.012[c]	0.406 ± 0.008[b]
PA-CLAMP	M	0.286 ± 0.021[c]	0.442 ± 0.011[a]
	F	0.337 ± 0.020[c]	0.430 ± 0.008[a]
	M + F	0.311 ± 0.017[c]	0.436 ± 0.007[a]

[a–c]Means ± SEM with no common superscript differ significantly ($P \leq 0.05$).

TABLE 3. Electrocardiogram Lead II S-wave amplitudes in sham-operated broilers (SHAM), broilers in which the left extrapulmonary primary bronchus was clamped (BRONCHUS CLAMP), and broilers in which the left pulmonary artery was clamped (PA-CLAMP)

Treatment group	Sex	Necropsy category: Nonascitic S-wave amplitude	Necropsy category: Ascitic S-wave amplitude
		(mV)	
SHAM	Male (M)	–0.059 ± 0.009[d]	–0.252 ± 0.050[b]
	Female (F)	–0.044 ± 0.008[d]	. . .
	M + F	–0.051 ± 0.006[d]	–0.252 ± 0.050[b]
BRONCHUS CLAMP	M	–0.157 ± 0.035[c]	–0.305 ± 0.023[a]
	F	–0.123 ± 0.011[c]	–0.256 ± 0.030[b]
	M + F	–0.134 ± 0.014[c]	–0.291 ± 0.019[a]
PA-CLAMP	M	–0.138 ± 0.042[c]	–0.367 ± 0.014[a]
	F	–0.144 ± 0.022[c]	–0.236 ± 0.014[b]
	M + F	–0.142 ± 0.019[c]	–0.301 ± 0.012[a]

[a–d]Means ± SEM with no common superscript differ significantly ($P \leq 0.05$).

present (Table 1) and previous studies (Wideman and Kirby, 1995a) presumably reflects the inability of susceptible individuals to survive the acute challenge of having the entire cardiac output forced at an elevated PAP through the vasculature of only one lung (Wideman *et al*., 1996a,b). Most broilers in the PA-CLAMP group that died during the early post-surgical period exhibited an obvious cyanosis immediately after the pulmonary artery was clamped, suggesting that these individuals developed a ventilation-perfusion mismatch and succumbed to extreme blood gas disturbances (Wideman and Kirby, 1995b; Wideman *et al*., 1996a,b). As a caveat to this interpretation, in 6- to 7-wk-old broilers, the systemic hypoxemia induced by transiently occluding one bronchus was more severe than that induced by transiently occluding one pulmonary artery (Wideman *et al*., 1996a). Accordingly, the difference in early post-surgical mortality between the BRONCHUS CLAMP and PA-CLAMP groups may be more closely associated with the ensuing increase in PAP, which in 6- to 7-wk-old broilers was relatively modest during transient bronchus occlusion but very dramatic during acute occlusion of the pulmonary artery (Wideman *et al*., 1996a). If increases in PAP are proportionately transmitted beyond the primary pulmonary resistance vessels, then the gradual onset of pulmonary edema could account for the intergroup differences in early post-surgical mortality. Similarly, RV:TV ratios are highly correlated with PAP under chronic conditions known to induce PHS (Burton *et al*., 1968; Cueva *et al*., 1974; Peacock *et al*., 1989), and the RV:TV ratios of the combined male and female ascitic broilers were higher in the PA-CLAMP group than in the BRONCHUS CLAMP group. Overall, these observations suggest the incidences of early post-surgical mortality and ascites induced by the BRONCHUS CLAMP and PA-CLAMP models were influenced mainly by the magnitude of the pulmonary hypertensive response rather than by the degree of systemic hypoxemia or susceptibility to a ventilation-perfusion mismatch.

After pulmonary hypertension had been initiated by occluding one bronchus or pulmonary artery, the resulting ECG Lead II S-wave amplitudes, RV:TV ratios, and gross necropsy observations indicate that broilers in both the BRONCHUS CLAMP and PA-CLAMP groups proceeded through a similar pathophysiological progression to terminal ascites. However, differences presumably exist in the mechanisms by which the bronchus occlusion and pulmonary artery occlusion models trigger pulmonary hypertension. Physiological evaluations of broilers during acute unilateral pulmonary artery occlusion indicate the right ventricle attempts to overcome the direct elevation in PVR by increasing PAP to propel the cardiac output through the low compliance vasculature of the unobstructed lung (Wideman *et al*., 1996a,b). These PAP responses are consistent with those observed previously during acute unilateral pulmonary artery occlusion in adult ducks and White Leghorn chickens (Burton *et al*., 1968; Powell *et al*., 1985). In contrast, the bronchus clamp model directs all of the air flow to the unobstructed lung, whereas pulmonary arterial blood continues to perfuse both lungs. Consequently the blood exiting the poorly ventilated lung remains essentially venous in its composition, and presumably it is the resulting systemic respiratory acidosis, arterial hypoxemia, or regional air capillary hypoxia that triggers pulmonary vasoconstriction and an overall increase in PVR (Burton *et al*., 1968; Besch and Kadono, 1978; Peacock *et al*., 1989; Owen *et al*., 1995; Wideman *et al*., 1996a). It is this secondary increase in PVR, dependent on the vasoconstrictive responsiveness of the pulmonary vasculature to acidosis, hypoxia, or hypoxemia, that apparently challenges the right ventricle to develop an increased PAP in the bronchus clamp model (Wideman *et al*., 1996a).

These different techniques for initiating pulmonary hypertension suggest the pulmonary artery clamp and bronchus clamp models may prove useful for dissociating the multiple physiological factors suspected of contributing to the overall susceptibility of broilers to ascites. The pulmonary artery clamp and bronchus clamp models also can be used for selecting broilers that are highly resistant to ascites (Rhoads *et al*., 1995). For

large-scale genetic selection programs, considerably less surgical expertise is required to expose and clamp one bronchus than to clear the connective tissues surrounding a pulmonary artery and correctly position a clamp on this thin-walled vessel. However, the pulmonary artery clamp model rigorously eliminates the high percentage of male and female broilers that are unable to accommodate having the entire cardiac output forced to flow through only one lung.

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