DEVELOPMENT OF A RABBIT ANIMAL MODEL FOR THE ASSESSMENT OF THE ACUTE BYSSINOTIC REACTION FOLLOWING INHALATION OF COTTON DUST EXTRACT

S.K. Ainsworth,[1] M.P. Bishop,[1] P.A. Pilia,[1]
T.G. Mundie,[1] and W.B. Moorman[2]
[1]Medical University of South Carolina,
Department of Pathology, Charleston, SC 29425
[2]National Institute of Occupational
Safety and Health,
Division of Biomedical and Behavioral Science,
Cincinnati, OH 45226

Abstract

Rabbits were exposed acutely to cotton dust extracts (CDE) and histamine. Measurements of resistance (R) and compliance (C) show rabbits react to both CDE and histamine as evidenced by significant changes in resistance from baseline values. The development of this rabbit animal model with its ability to measure flow, volume and transpulmonary pressure is vital to our laboratory's overall assessment of causative agents, mediators and mechanisms in the acute byssinotic response.

Introduction

Several important studies have recently emphasized the need for the development of an animal model of byssinosis to better assess the causative agents, pathogenetic mechanisms, and the relationshop between the acute, reversible and chronic, irreversible effects. The World Health Organization's position paper in 1982 (1), and the 1982 report from the National Research Council of the National Academy of Sciences (2), along with reviews by Ainsworth and Pilia (3) and Butcher et al. (4), have discussed the etiopathogenic mechanisms, effects of cotton dust on humans and the importance of the animal model.

Other animal studies have previously revealed that: 1) a dose-dependent acute increase in airway resistance and decrease in respiratory volume occurs in cats and guinea pigs using an aqueous CDE (5); 2) dyspnea, fever or leukocytosis may occur in animals following inhalation of CDE (6); 3) inhalation of cotton dust at high concentrations produces changes in blood gas concentrations following several weeks of exposure (7); 4) CDE has been shown to cause recruitment of PMNs to the lungs (8-11).

The rabbit has proven to be very useful in our laboratory for characterizing inflammatory, immunologic and pharmacologic events in the lung following cotton dust extract (CDE) inhalation challenge. CDE inhalation assessed by bioassays of lung lavage produces chemotaxis of PMNs and macrophages, release of arachidonic acid metabolites and a selective increase in the permeability of alveoli to albumin. Interestingly, the peak chemotactic response parallels the release of thromboxane (TXB_2) and prostaglandin ($PGF_2\alpha$) at 4 hours (1). Studies in our laboratory have pioneered the usefulness of bronchopulmonary lavage for assessing cellular recruitment, bioactive mediator release, serum alveolar exudates and characterizing these substances as agents of pulmonary dysfunction causing altered mechanical properties and decreased ventilatory performance.

Bioassays have been used in our laboratory to ascertain the presence of histamine releasing agents, chemotaxins and smooth muscle constrictors in CDE and CBE. Such studies led us to propose that aracidonic acid metabolites ($PGF_2\alpha$, TXB_2 and leukotrienes), and 5HT, affect airway constriction in the acute byssinotic reaction (3,12-17). Macrophages release PGs, leukocytes&platelets release thromboxanes, and leukocytes release leukotrienes. Further in vivo rabbit studies were extremely important, and clearly demonstrated, the temporal relationship between the release of arachidonic acid metabolites and cellular recruitment to airways following acute CDE challenge (12).

This paper describes our preliminary efforts in developing the rabbit as a byssinotic animal model. In particular our advancements in technique and methodology to accurately assess pulmonary function changes (namely resistance and compliance) in rabbits following inhalation of standard cotton dust are presented and discussed.

Materials and Methods

Young New Zealand White rabbits (2.0-4.0 kg) anesthetized with ketamine (Vetalar; 0.25 ml/kg bw) and xylazine (Rompun; 0.02 ml/kg bw) are intubated with an endotracheal tube (North American Drager, Teleford, PA) of appropriate size: 2.0-2.5 kg, 3.5 mm I.D.; 2.5-4.0 kg 4.0 mm I.D.

The tip (3 cm) of a latex balloon is securely tied around the open end opposite the luer end of a polypropylene catheter (Sherwood Mfg., St. Louis, MO.), the tip of which extends the length of the balloon. This unit is then placed in the lower 1/3 of the esophagus. (Placement of the balloon is crucial for proper phase alignment of the pressure and flow curves.) The luer end of the esophageal catheter is attached to a 3-way stopcock on the top of a Statham differential transducer (Statham Instruments, Inc. of Puerto Rico, Hato Rey, Puerto Rico). The side arm tap of the transducer dome is closed to the atmosphere; the additional transducer side tap is attached to the stainless steel connector of the pneumotachograph to incorporate static mouth pressure for transpulmonary pressure measurement. The esophageal balloon is inflated with 0.5 cc of air, and 0.6 cc of air is withdrawn to slightly deflate the balloon. This ensures minimal volume yet produces measureable pressure changes. The 3-way stopcock is closed to the syringe and opened to the transducer in the deflated state. Adjustments of the polygraph balance voltage is usually necessary to return the pressure curve to baseline.

To measure transpulmonary pressure, static pressure at the mouth is differentially compared to the changing pressures of the pleural space. The pleural pressure changes are approximated by the esophageal balloon. Static mouth pressure is sampled from the air flow in the endotracheal tube, but in order to conform to the concepts of minimal dead space and to produce a system containing no drastic changes in cross sectional diameter of the artificial airspace, a specially designed and constructed stainless steel connector (Pilia Products, Florence, SC) was built for the attachment of the endotracheal tube to the pneumotachograph.

The Statham differential transducer is connected to a Grass polygraph recorder (Grass Instrument Co., Quincy, MA) to measure transpulmonary pressure. The Fleisch pneumotachograph, heated with a variable resistor (reostat) to prevent moisture condensation, is connected to a Statham transducer to measure respiratory flow. The flow is integrated, thus giving tidal volume. All three curvilinear pens are adjusted to the center baseline. Since both resistance and compliance calculations depend only on relative changes of the three parameters, i.e. pressure, flow and volume, the baseline is not absolute and the balance voltage of each panel can be altered to shift the curves up or down. This will not alter the sensitivity, a parameter which must remain constant throughout. The paper speed is set at 100 mm/sec (maximum allowable speed). Calibrations are performed at the end of each run.

Bronchial challenges for each rabbit are made using a Bennett Model PR-2 Respiration Unit with nebulizer (Puritan Bennett, Norcross, GA). Both aerosol hose and manifold nebulizer unit are changed with each challenge substance. The rabbits are first challenged with 15 breaths of phosphate buffered saline (PBS;pH 7.3) at an inspiratory pressure of 18±2 cm H_2O. Pulmonary function is evaluated immediately. Animals are then challenged with either histamine (10 mg/ml) PBS or CDE (1 g non-lyophilized CDE/25 ml pyrogen free water or 0.01 g lypholized CDE /1 ml PBS lyophilized) 10-30 minutes following histamine challenge. Post-inhalation data is collected at varying intervals after histamine challenge and 10-30 minutes post CDE challenge at 5 minute intervals. The rabbits are inflated to 80% inspiratory vital capacity at 10-15 minute intervals post exposure to avoid atalectasis.

Calculations

From the simultaneous physiograph recordings the following parameters were measured: R = resistance,

C = compliance, $\dot{V}_i$ = flow at inspiration, $\dot{V}_e$ = flow at expiration, P = transpulmonary pressure, V = tidal volume. These measured parameters were used to calculate resistance and compliance according to the following previously established formulas:

$$R = \frac{\Delta P}{(\dot{V}_i + \dot{V}_e)} \quad \text{in} \quad \frac{\text{cm } H_2O}{\text{liter/sec}} \qquad (18)$$

$$C = \frac{\Delta V}{\Delta P} \quad \text{in} \quad \frac{\text{ml}}{\text{cm } H_2O}$$

Five determinations (breaths) per time interval were averaged for each separate event and for each animal to determine resistance and compliance.

Results

Resistance (R_L) and compliance (C_L) values and the percent change from baseline values were calculated as a function of post inhalation time for histamine and cotton dust extract (Table I).

Clinically objective criteria were established enabling us to identify significant responders from nonresponders with each bronchial challenge. The critical clinical criteria for placement into the responder category was an increase in resistance of 125% of base value (saline). With this criteria uniformly applied to all rabbits, 9/14 rabbits were placed in the reactor category following cotton dust challenge and 12/14 were placed in the reactor category following histamine challenge. Two animals failed to react to both histamine and cotton dust extract.

Although three groups of animals were studied, i.e., using non-lyophilized cotton dust extract alone and with histamine and lyophilized cotton dust extract, no notable differences were observed between the two CDE preparations. These studies were compiled into one large group (R1-R14) for interpretive analysis.

Twelve of fourteen rabbits (less R5,14) can be classified as histamine reactors according to the clinically objective criteria of 125% increase from baseline values. Three of these twelve reactors (R6,12,13) demonstrate an immediate maximal response, all of which are significantly above the clinically objective criteria ranging from 182% to 282%. The remaining 9 animals demonstrated a 5-10 min delayed maximal response with 3/9 at 5 min and 6/9 at 10 min. These delayed maximal responses are more moderate on the average (with the exception of the 423% increase of R7) than the immediate maximal histamine responses.

Dividing the rabbits into groups of mild, moderate and severe responders to histamine using the criteria of mild (125-150%), moderate (150-175%) and severe (>175%), 4/12 (R3,4,9,10) are classified as mild responders, 2/12 (R1,11) as moderate responders and 6/12 (R2,6,7,8,12,13) are classified as severe responders. Thus, these initial studies with histamine bronchial challenge show that almost all rabbits respond to histamine, a known bronchoconstrictor, and this reactivity can be used as the standard pulmonary response on which to base unknown substances of bronchial challenge.

Ten of fourteen rabbits are defined by bronchial challenge data following CDE inhalation as clinically significant responders (R1-4,6-9,11,13), with all but two (R1,2) demonstrating an immediate response. R1 shows an extremely delayed and mild response at 30 min and R2 appears to have only a transient spike of reactivity at 15 min, whereas the remaining 8 rabbits continue to either increase (R11, 13) or decrease (R3,4,6,7,8,9) in responsiveness from a maximal immediate response . However, all but one (R9) of the rabbits that show a decrease in responsiveness from the immediate maximal response still demonstrate a clinically significant response over time. Using the divisions of mild, moderate and severe as previously established, we find that 3/9 (R1,2,9) are mild responders, 1/9 (R13) is a moderate responder and 6/9 (R3,4,6,7,8,11) are severe responders.

Comparing the CDE responders to histamine responders, the following are noted:

1) The two histamine non-responders are also CDE non-responders (R5,14).

2) Two CDE non-responders respond to histamine, one mildly (R10) and one severely (R12).

3) All CDE responders are also histamine responders.

Compliance and resistance are inversely proportional parameters, i.e. when resistance increases, compliance decreases. The compliance values are included in the table, but the focus of attention is on the variation of resistance values from baseline. This percent change is used in setting the criteria for reactivity.

Discussion

These studies demonstrate that pulmonary responses following inhalation of cotton dust extract can be successfully measured in rabbits by the techniques and equipment described. The rabbit, like our monkey animal model, shows significant pulmonary changes in resistance following bronchial challenge to CDE and histamine. We have assessed pulmonary function for histamine and CDE immediately following exposure and at 5 minute intervals for up to 30 minutes post-exposure. Six of 14 rabbits proved to be severe responders; one, moderate; three, mild; and four, nonresponders to CDE. These findings are similar to results of our bronchoprovocation studies with CDE in monkeys (19).

The substantial change in resistance from baseline (saline) with CDE challenge from baseline (saline) suggests CDE contains an acute pharmacologic bronchoconstricting agent or initiates immediate histamine release from lung tissues or cells. Our earlier work with the pig platelet histamine bioassay showed that numerous substances are contained in cotton dust and bract that cause non-antigenic histamine release (20). In addition, cotton dust has also shown to contain minute amounts of histamine(21), but it is thought the concentrations would have to be several orders of magnitude higher to affect the degree of FEV_1 drop seen in cotton mill workers.

5-hydroxytryptamine (5-HT) appears to be the major smooth muscle constrictor contained in cotton dust and bract (16). In vitro studies in our laboratory, using methods previously described, suggests 5-HT and arachidonic acid metabolites are important muscle contractors (13-16). Following inhalation of cotton dusts in rabbits, bronchopulmonary lavage was performed at various times for assessment of cell recruitment, 5-HT, PGE_1, $PGF_{2\alpha}$, and thromboxane release. Significant release of these mediators has been noted and their release from macrophages, polymorphonuclear leukocytes (PMNs) and platelets correlated with the maximum chemotactic response (mainly PMNs) in airways and alveoli (12).

The importance of pulmonary function testing is paramount in correlating mediator release with response. Additionally, both qualitative and quantitative pulmonary function responses for mechanical and ventilatory performance can be used to distinguish potential effects of different etiological agents, mediators, and mechanisms. Interestingly, different mediators released may be responsible for the different pulmonary function changes observed. Currently, studies are in progress using this animal model to assess potential causative agents, mediators and mechanisms responsible for the acute byssinotic response. The rabbit model will be used to pre-screen reactive vs. nonreactive substances. The monkey model with its exquisite sensitivity for quantitating human lung function parameters will serve as the final test for active agents and mediators.

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Dr. Mundie's present address is Walter Reed Army Institute of Research, Department of Respiratory Research, Washington, DC.

References

1. World Health Organization, Geneva 1983. Recommended health-based occupational exposure limits for selected vegetable dusts. Technical Report Series p.684.

2. National Research Council Committee on Byssinosis. 1982. Byssinosis: clinical and research issues. National Academy Press, Washington, DC.

3. Ainsworth, S.K., and P.A. Pilia. 1982. Etiologic agents and pathogenic mechanisms in the acute byssinotic reaction. In: Cotton Dust: Controlling an Occupational Health Hazard. Chapter 11, American Chemical Society, Ed. Joseph Montano, Washington, DC. pp. 163-186.

4. Butcher, B.T., O'Neil, C.E. and Jones, R.N. 1983. The Respiratory Effects of Cotton Dust. Symposium on Immune Factors in Pulmonary Disease. p.63-70.

5. Antweiler, A. 1974. The histamine releasing capacity of cotton dust extracts inhaled by guinea pigs. Annals NY Acad Sci. 221:136-140.

6. Pernis, B., E.C. Vighani, C. Cavagna, and M. Finulli. 1961. The role of bacterial endotoxins in occupational diseases caused by inhaling vegetable dusts. Br J Ind Med 18:120-129.

7. Kutz, S.A., M.S. Mentnech, N.C. Mull, S.A. Olenchock, and P.C. Major. 1980. Acute experimental responses to cardroom cotton dust. Arch Environ Health. 35:203-210.

8. Rylander, R., and A. Nordstrand. 1974. Pulmonary cell reaction after exposure to cotton dust extract. Br J Ind Med 31:220-223.

9. Mundie, T.G., Cordova-Salinas, Whitener, K.C. and Ainsworth, S.K. Changes in pulmonary lavage contents following acute inhalation of cotton dust extracts in rabbits. Presented at the 1983 Beltwide Cotton Production Research Conference. Proc. Seventh Cotton Dust Res. Conf., P.J. Wakelyn and R.R. Jacobs (eds.), National Cotton Council, Memphis, TN, pp. 101-103.

10. Kilburn, K.H., L.J. Williams, L.L. Tres, and W.N. McKenzie. 1973. Leukocyte recruitment through airway walls by condensed vegetable tannins and quercetin. Lab Invest. 28:55.

11. Walker, R.F., G. Edson, and J.D. Hatcher. 1975. Influence of cotton dust inhalation on free lung cells in rats and guinea pigs. Lab Invest. 33:28.

12. Mundie, T.G., C. Whitener and S.K. Ainsworth. Byssinosis: release of prostaglandins, thromboxane and 5-hydroxytryptamine in bronchopulmonary lavage fluid following inhalation of cotton dust extracts. In Press, Am J Path.

13. Mundie, T.G., M. Buck, M. Cordova-Salinas, R.E. Neuman, and S.K. Ainsworth. 1982. In vitro bioassays and cotton bract induced bronchoconstriction in human volunteers: a comparative study. Proc. Sixth Cotton Dust Res. Conf., P.J. Wakelyn (ed.), National Cotton Council, Memphis, TN, pp. 34-35.

14. Mundie, T.G., and S.K. Ainsworth. 1983. Platelet thromboxane release by cotton dust and bract extract. Beltwide Cotton Production Research Conference. Proc. Seventh Cotton Dust Res. Conf., P.J. Wakelyn and R.R.Jacobs (eds.), National Cotton Council, Memphis, TN, pp. 104-105.

15. Mundie, T.G., M. Cordova-Salinas, V.J. Bray and S.K. Ainsworth. 1983. Bioassays of smooth muscle contracting agents in cotton mill dust and bract extracts, arachidonic acid metabolites as possible mediators of the acute byssinotic reaction. Environ Res. 32:62-71.

16. Ainsworth, S.K., T.G. Mundie, P.A. Pilia, and R.E. Neuman. Byssinosis: 5-hydroxytryptamine, the significant smooth muscle contractor substance of cotton dust and cotton bract extracts. Submitted to Am Indus Hyg Ass J.

17. Mundie, T.G., J.D. Osguthorpe, C. Martin, B.T. Butcher, C.E. O'Neill, and S.K. Ainsworth. 1983. The effect of inhalation of cotton dust, and history of smoking, allergy, and byssinosis on immunoglobulin, complement, and thromboxane concentration in cotton mill workers. Proc. Seventh Cotton Dust Res. Conf., P.J. Wakelyn and R.R. Jacobs (eds.), National Cotton Council, Memphis, TN, pp. 26-28.

18. Frank, N.R., J. Mead, and B.G. Ferris, Jr. 1957. The mechanical behavior of the lungs in healthy elderly persons. J Clin Invest. 36:1680-1687.

19. Ainsworth, S.K., Moorman, W., Mundie, T.G., Pilia. P.A., Bishop, M.P. and Lewis, T. A primate model of byssinosis: pulmonary changes following inhalation of cotton dust extract. Proc. Eighth Cotton Dust Res. Conf., P.J. Wakelyn and R.R. Jacobs (eds.), National Cotton Council, Memphis, TN, pp. 87-91.

20. Ainsworth, S.K., Neuman, R.E., and Harley, R.A. 1979. Histamine release from platelets for assay of byssinogenic substances in cotton mill dust and related materials. Br J Industrial Med. 36:35-42.

21. Haworth, E. and MacDonald, A.D. 1957. On histamine in cotton dust and in the blood of cotton workers. Journal of Hygiene. 37:234-242.

Table I. PULMONARY FUNCTION: RESISTANCE (R) AND COMPLIANCE (C) FOLLOWING INHALATION CHALLENGE TO COTTON DUST EXTRACTS AND HISTAMINE

Animal		SALINE	COTTON DUST EXTRACT					SALINE	HISTAMINE
		Baseline	Immediate	10 min	15 min	20 min	30 min	Baseline	Maximum Response
1	R	14.83±1.83	12.77±1.42	11.95±1.14	10.55±5.63	8.83±3.44	4.72±0.60	31.22±1.12	48.23±2.15
	%Δ		86%	81%	71%	60%	32%		(5) 155%
	C	4.51±0.27	5.11±0.72	5.27±0.25	5.48±0.34	5.27±0.61	5.99±0.52	3.30±0.10	2.83±0.07
	%Δ		113%	117%	122%	117%	133%		86%
2	R	13.30±1.97	14.38±1.76	6.80±2.74	18.62±1.70	14.75±2.30	12.64±1.63	14.40±7.61	41.65±10.04
	%Δ		108%	51%	140%	110%	95%		(5) 289%
	C	4.61±0.26	5.16±0.16	6.00±0.47	2.11±0.05	3.91±0.12	6.78±0.48	3.94±0.69	2.56±0.21
	%Δ		112%	130%	46%	85%	147%		65%
3	R	9.29±1.09	45.62±0.81	16.78±0.51	15.21±1.00	14.90±0.51	14.27±1.85	17.88±4.23	23.92±5.07
	%Δ		491%	181%	164%	160%	154%		(10) 134%
	C	8.49±0.25	3.65±0.11	5.54±0.33	6.83±1.22	6.04±0.27	6.44±0.38	3.69±0.15	4.06±0.11
	%Δ		43%	65%	80%	71%	76%		110%
4	R	5.40±2.72	14.49±1.25	12.77±2.36	14.15±0.32	10.25±1.43	5.45±1.55	24.94±2.92	37.14±6.33
	%Δ		268%	238%	262%	190%	101%		(10) 149%
	C	10.11±0.52	5.51±0.38	6.49±0.33	6.82±1.18	7.39±0.34	8.13±0.74	7.68±2.69	9.03±0.39
	%Δ		55%	64%	67%	73%	80%		118%
5	R	23.35±10.55	25.17±2.28	20.92±0.62			4.55±1.88	25.43±10.61	19.22±3.11
	%Δ		108%	90%			19%		(I) 76%
	C	6.48±0.32	8.30±1.70	6.44±0.35			15.50±5.07	3.64±0.16	3.40±0.11
	%Δ		128%	99%			239%		93%
6	R	10.63±2.07	28.68±2.50	27.87±3.93				10.63±2.07	29.98±3.14
	%Δ		270%	262%					(I) 282%
	C	4.30±1.08	2.63±0.12	2.80±0.14				4.30±1.08	2.81±0.43
	%Δ		61%	65%					65%
7	R	10.16±2.46	56.36±4.80	31.76±6.00				10.16±2.46	42.96±5.49
	%Δ		554%	313%					(10) 423%
	C	7.07±2.61	7.51±1.80	8.42±2.06				7.07±2.61	7.14±2.41
	%Δ		106%	119%					101%
8	R	20.63±3.44	56.43±7.82	45.96±8.35				20.63±3.44	38.70±4.45
	%Δ		274%	223%					(10) 188%
	C	3.17±0.14	2.38±0.13	2.27±0.22				3.17±0.14	1.78±0.06
	%Δ		75%	72%					56%
9	R	10.27±4.43	13.54±2.32	6.81±1.60				10.27±4.43	13.86±0.85
	%Δ		132%	66%					(10) 135%
	C	6.34±1.89	5.41±0.94	6.80±0.23				6.34±1.89	4.31±0.09
	%Δ		85%	107%					68%
10	R	14.53±4.17	15.22±3.69	15.47±5.06				14.53±4.17	19.91±3.44
	%Δ		105%	106%					(5) 137%
	C	4.56±0.75	4.10±0.37	3.67±0.06				4.56±0.75	3.63±0.50
	%Δ		90%	80%					74%
11	R	5.55±1.13	11.48±4.50	12.22±4.88				5.55±1.13	8.98±2.06
	%Δ		207%	220%					(10) 161%
	C	5.05±0.16	4.40±0.06	3.93±1.98				5.05±0.16	4.27±0.15
	%Δ		87%	78%					85%
12	R	14.13±2.00	17.44±2.83	14.66±2.50				14.13±2.00	25.70±3.14
	%Δ		123%	104%					(I) 182%
	C	7.77±0.55	7.22±0.21	6.64±0.42				7.77±0.55	4.87±1.24
	%Δ		93%	85%					63%
13	R	6.21±2.84	8.60±2.93	9.43±3.31				6.21±2.84	12.13±2.25
	%Δ		138%	152%					(I) 193%
	C	7.07±1.42	8.62±0.93	7.55±0.66				7.07±1.42	7.14±1.58
	%_		122%	107%					101%
14	R	24.68±1.94	21.30±1.58	20.78±2.45				24.68±1.94	23.97±2.74
	%Δ		86%	84%					(I) 97%
	C	3.74±0.08	3.48±0.47	3.31±0.34				3.74±0.08	3.23±0.44
	%Δ		93%	89%					86%

%Δ from baseline
( ) time
(I) immediate

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