UNIVERSITE DE LAUSANNE – FACULTE DE BIOLOGIE ET DE MEDECINE

Service d'Anesthésiologie

Directeur : Professeur Donat R. SPAHN

EARLY BRONCHOCONSTRICTION IN ANESTHETIZED SHEEP : A SIMPLIFIED EXPERIMENTAL MODEL OF ASTHMA

THESE

préparée sous la direction du Dr Jean-Patrice GARDAZ, professeur titulaire

et présentée à la Faculté de Biologie et de Médecine de
l'Université de Lausanne pour l'obtention du grade de

DOCTEUR EN MEDECINE

par

LAURENT THIERRIN

Médecin diplômé de la Confédération Suisse

Originaire de Cheiry

Lausanne

2006

Remerciements

L'auteur tient à remercier chaleureusement le Dr J.-P. Gardaz pour son aide précieuse tout au long de l'élaboration de ce travail. De même, que soient remerciés le Dr P. Frascarolo ainsi que Mmes H. Capt et M. Augstburger pour leur assistance technique efficace.

Résumé

Cette étude décrit un modèle expérimental de bronchoconstriction précoce induite par aérosolisation d'un extrait *d'Ascaris suum* chez des moutons anesthésiés par de l'isoflurane et ventilés mécaniquement.

Dix moutons adultes ont été anesthésiés et ventilés mécaniquement puis ont été exposés à un stimulus bronchoconstrictif sous forme d'un aérosol d'extrait *d'Ascaris suum* durant 25 minutes. Tous les moutons ont été exposés deux fois à huit semaines d'intervalle à ce même stimulus. Les échanges gazeux ainsi que les paramètres respiratoires ont été mesurés régulièrement durant la période d'aérosolisation ainsi que durant les 60 minutes suivantes.

A la fin de la période d'aérosolisation, une augmentation significative ($p<0.05$) des pressions de crête (+114%) et de plateau (+148%), de la résistance expiratoire (+93%) et de la pression partielle artérielle de gaz carbonique PaCO2 (+25%) a été constatée, de même qu'une diminution significative ($p<0.05$) de la compliance respiratoire (-41%) et de la pression partielle artérielle d'oxygène PaO2 (-49%). Ces modifications sont restées stables durant toute la période d'observation.

Ce modèle expérimental animal de bronchoconstriction offre de nombreux avantages : la stabilité hémodynamique et le confort de l'animal sont améliorés et la réaction de stress est inhibée. Il permet de plus une distribution optimale de l'antigène respiratoire et finalement évite l'utilisation d'un pléthysmographe corporel.

Abstract

This study describes a simplified experimental model of early bronchoconstriction induced by aerosolization of *Ascaris suum* extract in isoflurane-anesthetized and mechanically ventilated sheep.

Ten adult sheep were anesthetized, mechanically ventilated and then challenged with an aerosol of *Ascaris suum* extract during 25 minutes. All of them were challenged twice at eight weeks intervals. During the bronchoconstrictive challenges and the following sixty minutes, gas exchange was measured and respiratory mechanics parameters computed from a lung mechanics calculator.

At the end of the challenge, a significant increase ($p<0.05$) was observed in peak (+114%) and plateau (+148%) pressures, expiratory resistance (+93%) and PaCO2 (+25%) along with a significant decrease ($p<0.05$) in respiratory compliance (-41%) and PaO2 (-49%). These changes remained stable throughout the 60 minutes study period.

This model offers several advantages : hemodynamic stability and animal welfare are improved and the stress response is blunted. It allows an optimal distribution of the antigen and finally avoids the need of a body plethysmograph.

1. Introduction

Due to a continuous increase in prevalence and morbidity, asthma will require considerable investigative effort in the future to better define its pathophysiology and to test new treatment modalities[1]. Only limited studies can be done in asthmatic humans for safety reasons. Appropriate animal models are therefore necessary to test hypotheses of underlying mechanism, to determine the significance of immunologic, pharmacologic, and cellular factors in human asthma.

During the last fifty years, induced allergic bronchoconstriction in animals has been extensively studied in three species[1-4], namely the Guinea pig[5], the Basenji-Greyhound dog[6] and the Rhesus monkey[7]. More recently, murine models have been developed to investigate the molecular and immunological mechanisms of bronchial asthma[8,9].

The pathophysiological features of the sheep asthma model are also well characterized[10-16]. Sheep frequently have a natural cutaneous sensitivity to *Ascaris suum* antigen, presumably resulting from exposure on the farm to pigs in whom roundworm infestation is endemic. Further active sensitization of the sheep can be achieved by repeated inhalational challenge with *Ascaris suum* antigen. Sensitized animals respond to this airway challenge with induced bronchoconstriction characterized by changes in respiratory mechanics, lung volumes and gas exchange. An increase in airflow resistance and a fall in dynamic lung compliance are observed, associated with pulmonary hyperinflation and a decrease in arterial oxygen tension, while carbon dioxide tension usually remains unchanged[1-4]. As seen in human asthma, allergic sheep often exhibit a dual respiratory response to inhaled *Ascaris suum* antigen : an immediate bronchoconstriction (early phase) followed 6 to 8 hours later in 30-50% of cases by a second more severe bronchial obstruction involving inflammatory mechanisms (late phase). Sheep have usually been studied in the conscious state by means of the body

plethysmograph technique[10-16]. Long et al. already described a mechanically ventilated and anesthetized sheep model of asthma which was however mainly used to study pulmonary and systemic hemodynamics[16]. In order to improve animal welfare and to assess more precisely the modifications of respiratory mechanics and gaz exchange, we designed an experimental model of early bronchoconstriction using isoflurane-anesthetized and mechanically ventilated sheep sensitized with *Ascaris suum* antigen.

2. Animals and methods

The study protocol was approved by the Local Committee on Animal Research and has been performed according to the Helsinki convention for the use and care of animals. Ten adult sheeps with a mean weight of 44 ± 9 kg (mean ± SD) were included in this study. All of them were challenged twice at eight weeks intervals.

After intramuscular premedication with xylazin (0.15 mg/kg), the sheep were anesthetized with intravenous thiopentone (10 mg/kg), their trachea intubated and connected to the ventilator in prone position. A continuous positive pressure ventilation with a tidal volume of 12-14 ml/kg, a PEEP of 5 cmH2O, an I:E ratio of 1:2 and an inspiratory pause of 10% of the respiratory cycle was used. Respiratory frequency was set to preserve normocarbia (PaCO2 4-5 kPa). Anesthesia was maintained with an inspired concentration of isoflurane 1% in a mixture of oxygen and air (FiO2 50%). To avoid distension of the rumen, a large-bore orogastric tube was inserted. Animals were perfused with 100-200 ml/h of ringer lactate solution and a peripheral arterial cannula was inserted. Electrocardiogram, invasive blood pressure and ear pulse oxymetry were continuously monitored.

For mechanical ventilation of the sheep and determination of their respiratory parameters, we used a Servoventilator 900 connected to a Lung Mechanics Calculator 940 (Siemens AG,

Munich, Germany)[17]. Inspired and expired tidal volumes, inspired and expired minute ventilation, respiratory frequency, as well as peak, plateau and end-expiratory pressures were directly measured. Computed inspiratory and expiratory resistances and respiratory compliance were continuously recorded.

After a stabilization period of 30 minutes in prone position, a set of control measurements including respiratory mechanics, arterial blood gas and hemodynamics were performed. Then the endotracheal administration of the *Ascaris suum* extract aerosol lasting 25 minutes was started. A volume of 2.5 ml of *Ascaris suum* concentrated extract (Greer Laboratories Inc., Lenoir, NC, USA) was diluted with physiologic sodium chloride to obtain an aerosol solution of 5 ml with a concentration of 18000 protein nitrogen units (PNU) per ml. The aerosol was generated using a Pneumatic Drug Nebulizer (Drägerwerk, Lübeck, Germany) which was connected to the endotracheal tube and synchronized with the beginning of the inspiratory cycle of the ventilator. This system has been demonstrated to provide an aerosol with 80% of the dry *Ascaris suum* extract delivered in the range between 0.1 and 1 μm droplets diameter, the mean value being 0,5 μm[18]. To avoid contaminations, a filter was placed between the nebulizer system and the Y-piece of the circuit.

During this period and the following 60 minutes, all parameters previously described were continuously monitored and recorded every 20 minutes. One hour after the end of the sensitization, anesthesia was stopped and the sheep were awakened. A bronchodilator aerosol of salbutamol (Ventolin®) 5 mg was systematically administered before extubation.

All data were expressed as means ± standard deviation (SD). A two-ways ANOVA for repeated measurements on one way (time) was performed to assess differences between the two sessions. A Dunnett's test was performed to detect significant differences between post-

challenge measurements and baseline values. Differences were considered significant at *P* value < 0.05. Repeatability was tested according to the method of Bland & Altman[19].

3. Results

All animals tolerated the procedure well during the 2 sessions and demonstrated typical responses of early bronchoconstriction after *Ascaris suum* airway challenge.

During the first session, we observed a significant increase ($p<0.05$) in peak pressure (31 ± 11 vs 14 ± 3 cmH2O), plateau pressure (26 ± 10 vs 10 ± 3 cmH2O), expiratory resistances (26 ± 6 vs 13 ± 2 cmH2O/L/s) and PaCO2 (5.5 ± 1.1 vs 4.5 ± 0.3 kPa) along with a significant decrease ($p<0.05$) in respiratory compliance (26 ± 10 vs 45 ± 10 ml/cmH2O) and PaO2 (16 ± 9 vs 32 ± 5 kPa) at the end of the *Ascaris suum* aerosol (T0 + 25') in comparison with baseline values (Table 1 and Fig 1). All these changes were maximal at the end of the challenge. During the following sixty minutes period, they remained stable and different ($p<0.05$) from control values.

During the second session, the response to *Ascaris suum* challenge was similar to the first session but less pronounced. Peak pressure (31 ± 12 vs 15 ± 4 cmH2O), plateau pressure (26 ± 12 vs 11 ± 3 cmH2O), expiratory resistances (24 ± 6 vs 13 ± 3 cmH2O/L/s), respiratory compliance (29 ± 12 vs 48 ± 11 ml/cmH2O), PaO2 (17 ± 7 vs 33 ± 9 kPa) and PaCO2 (5.7 ± 2.1 vs 4.5 ± 0.5 kPa) changes were indeed significant at the end of the challenge and also remained stable during the next sixty minutes period (Table 2 and Fig 2).

For the two sessions, cardiovascular parameters were not altered by the *Ascaris suum* challenge. Repeatability coefficients were 13 cmH2O for peak pressure, 12 cmH2O for plateau pressure, 15 cmH2O/L/s for expiratory resistance, 17 ml/cmH2O for respiratory

compliance, 15 kPa for PaO2 and 2.2 kPa for PaCO2. The two-ways ANOVA demonstrated no change for peak and plateau pressure, PaO2 and PaCO2, whereas expiratory resistances and respiratory compliance were significantly different between the two sessions ($p<0.05$).

4. Discussion

This experimental model of early bronchoconstriction offers significant advantages in comparison with the traditional animal models of asthma. First, the animal is not stressed during the bronchoconstrictive challenge. Secondly, since the resistance is measured via a calibrated electronic flowmeter and a pressure transducer incorporated within the ventilator, this model does not require a body plethysmograph and thereby simplifies the procedure. This animal model offers the possibility to challenge at least twice the same animals after a recovery period. Despite a less pronounced bronchoconstrictive response during the second session in comparison with the first one, that response is still appropriate because changes remain significantly different from control values and stable throughout the study period. It should be mentioned however that some animals were initially insensitive to *Ascaris suum* antigen and required several challenges before obtaining an early bronchoconstrictive response.

Our model differs from the one described by Long et al.[16] regarding the anesthetic technique. To obtain good hemodynamic stability during and after the bronchoconstrictive challenge, we have choosen isoflurane for the maintenance of anesthesia. Pentobarbital administered by repetitive intravenous bolus cannot provide stability of heart rate and arterial pressure[16,20]. In contrast, our modified model allows to test specifically respiratory effects of new bronchodilatory compounds without interference with hemodynamic parameters.

From an ethical point of view, the use of general anesthesia offers advantages. The stress response induced by the conscious tracheal intubation and the bronchoconstrictive challenge with consecutive dyspnea, hypercapnia and hypoxemia is blunted[21]. Moreover, instrumentation of the animals (tracheal intubation, vascular accesses, aerosol administration, e.g.) is greatly facilitated. Finally, the use of controlled ventilation and of a pneumatic nebulizer permits a synchronized administration of the aerosol with the inspiratory cycle of the ventilator and a homogeneous distribution of the aerosol along the entire bronchial tree[18].

Ideally, the anesthetic should not influence the airway reactivity. Although barbiturates have no significant effect on airway reactivity, we did not choose them for maintenance of anesthesia due to their rapid accumulation when administrated by continuous intravenous infusion[22]. The choice of the anesthesia technique was therefore restricted to either intravenous administration of propofol or inhalational agents. All these drugs are known to have direct and neurally-mediated bronchodilating properties[23-25]. However, at steady state this effect is constant and obviously allows to study bronchoconstriction due to *Ascaris suum* exposure. In addition, the observed bronchoconstriction was at least as severe as in awake animals[10,12], indicating again that the isoflurane-induced bronchodilation was relatively mild and thus not basically interfering with the antigen-induced bronchoconstriction.

In conclusion, our simplified experimental model of antigen-induced early bronchoconstriction in sheep under general anesthesia with controlled ventilation offers several advantages. In comparison with other models, animal welfare is improved, the stress response is blunted and the hemodynamic stability is maintained. Our model also allows an optimal distribution of the antigen by synchronized pneumatic nebulization and finally avoids the need of a body plethysmograph.

References

1. Szelenyi I. Animal models of bronchial asthma. Inflamm Res 2000; 49: 639-54.
2. Bice DE, Seagrave J, Green FH. Animal models of asthma: potential usefulness for studying health effects of inhaled particles. Inhal Toxicol 2000; 12: 829-62.
3. Patterson R, Kelly JF. Animal models of the asthmatic state. Annu Rev Med 1974; 25: 53-68.
4. Wanner A, Abraham WM. Experimental models of asthma. Lung 1982; 160: 231-43.
5. Broder I, Rogers S, Chamberlain DW, Milne EN. Model of allergic bronchoconstriction in the guinea pig. I. Characteristics of the system. Clin Immunol Immunopathol 1978; 9: 1-15.
6. Hirshman CA, Malley A, Downes H. Basenji-Greyhound dog model of asthma: reactivity to Ascaris suum, citric acid, and methacholine. J Appl Physiol 1980; 49: 953-7.
7. Pare PD, Michound MC, Hogg JC. Lung mechanics following antigen challenge of Ascaris suum-sensitive rhesus monkeys. J Appl Physiol 1976; 41: 668-76.
8. Dohi M, Tsukamoto S, Nagahori T, Shinagawa K, Saitoh K, Tanaka Y, Kobayashi S, Tanaka R, To Y, Yamamoto K. Noninvasive system for evaluating the allergen-specific airway response in a murine model of asthma. Lab Invest 1999; 79: 1559-71.
9. Neuhaus-Steinmetz U, Glaab T, Daser A, Braun A, Lommatzsch M, Herz U, Kips J, Alarie Y, Renz H. Sequential development of airway hyperresponsiveness and acute airway obstruction in a mouse model of allergic inflammation. Int Arch Allergy Immunol 2000; 121: 57-67.
10. Wanner A, Mezey RJ, Reinhart ME, Eyre P. Antigen-induced bronchospasm in conscious sheep. J Appl Physiol 1979; 47: 917-22.

11. Abraham WM, Delehunt JC, Yerger L, Marchette B. Characterization of a late phase pulmonary response after antigen challenge in allergic sheep. Am Rev Respir Dis 1983; 128: 839-44.

12. Clark JM, Abraham WM, Fishman CE, Forteza R, Ahmed A, Cortes A, Warne RL, Moore WR, Tanaka RD. Tryptase inhibitors block allergen-induced airway and inflammatory responses in allergic sheep. Am J Respir Crit Care Med 1995; 152: 2076-83.

13. Ahmed T, D'Brot J, Abraham WM, Lucio J, Mendelssohn R, Robinson MJ, Shakir S, Sanpedro B: Heterogeneity of allergic airway responses in sheep : differences in signal transduction. Am J Respir Crit Care Med 1996; 154: 843-9.

14. Huang SW, Kao KJ, Abraham WM. Plasma thrombospondin levels in sheep with allergic asthma. Chest 1996; 109: 1614-7.

15. Baile EM, Pare PD, Ernest D, Dodek PM. Distribution of blood flow and neutrophil kinetics in bronchial vasculature of sheep. J Appl Physiol 1997; 82: 1466-71.

16. Long WM, Yerger LD, Martinez H, Codias E, Sprung CL, Abraham WM, Wanner A. Modification of bronchial blood flow during allergic airway responses. J Appl Physiol 1988; 65: 272-82.

17. Jonson B, Nordstrom L, Olsson SG, Akerback D. Monitoring of ventilation and lung mechanics during automatic ventilation. A new device. Bull Physiopathol Respir (Nancy) 1975; 11: 729-43.

18. Persons DD, Hess GD, Muller WJ, Scherer PW. Airway deposition of hygroscopic heterodispersed aerosols: results of a computer calculation. J Appl Physiol 1987; 63: 1195-204.

19. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1986; 1: 307-10.

20. Gilmore JP. Pentobarbital Sodium Anesthesia in the Dog. Am J Physiol 1965; 209: 404-8.

21. Deegan R, He HB, Wood AJ, Wood M. Effects of anesthesia on norepinephrine kinetics. Comparison of propofol and halothane anesthesia in dogs. Anesthesiology 1991; 75: 481-8.

22. White PF. Clinical uses of intravenous anesthetic and analgesic infusions. Anesth Analg 1989; 68: 161-71.

23. Brown RH, Greenberg RS, Wagner EM. Efficacy of propofol to prevent bronchoconstriction: effects of preservative. Anesthesiology 2001; 94: 851-5.

24. Hirshman CA, Edelstein G, Peetz S, Wayne R, Downes H. Mechanism of action of inhalational anesthesia on airways. Anesthesiology 1982; 56: 107-11.

25. Katoh T, Ikeda K. A comparison of sevoflurane with halothane, enflurane, and isoflurane on bronchoconstriction caused by histamine. Can J Anaesth 1994; 41: 1214-9.

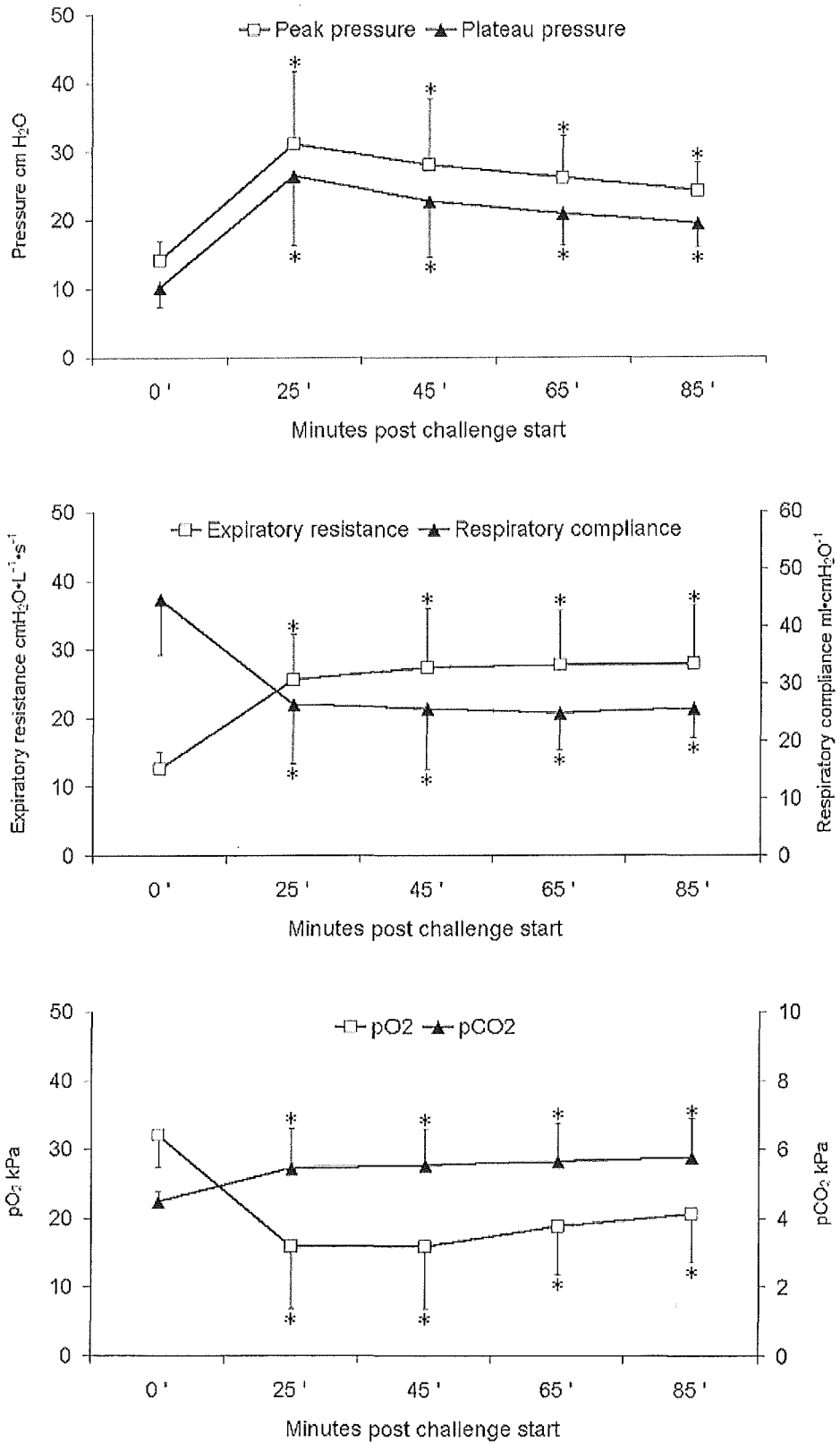

Figure 1. Time course of peak and plateau pressures, expiratory resistance, respiratory compliance, PaO2 and PaCO2 after *Ascaris suum* 1st session airway challenge. *Significantly different from baseline values.

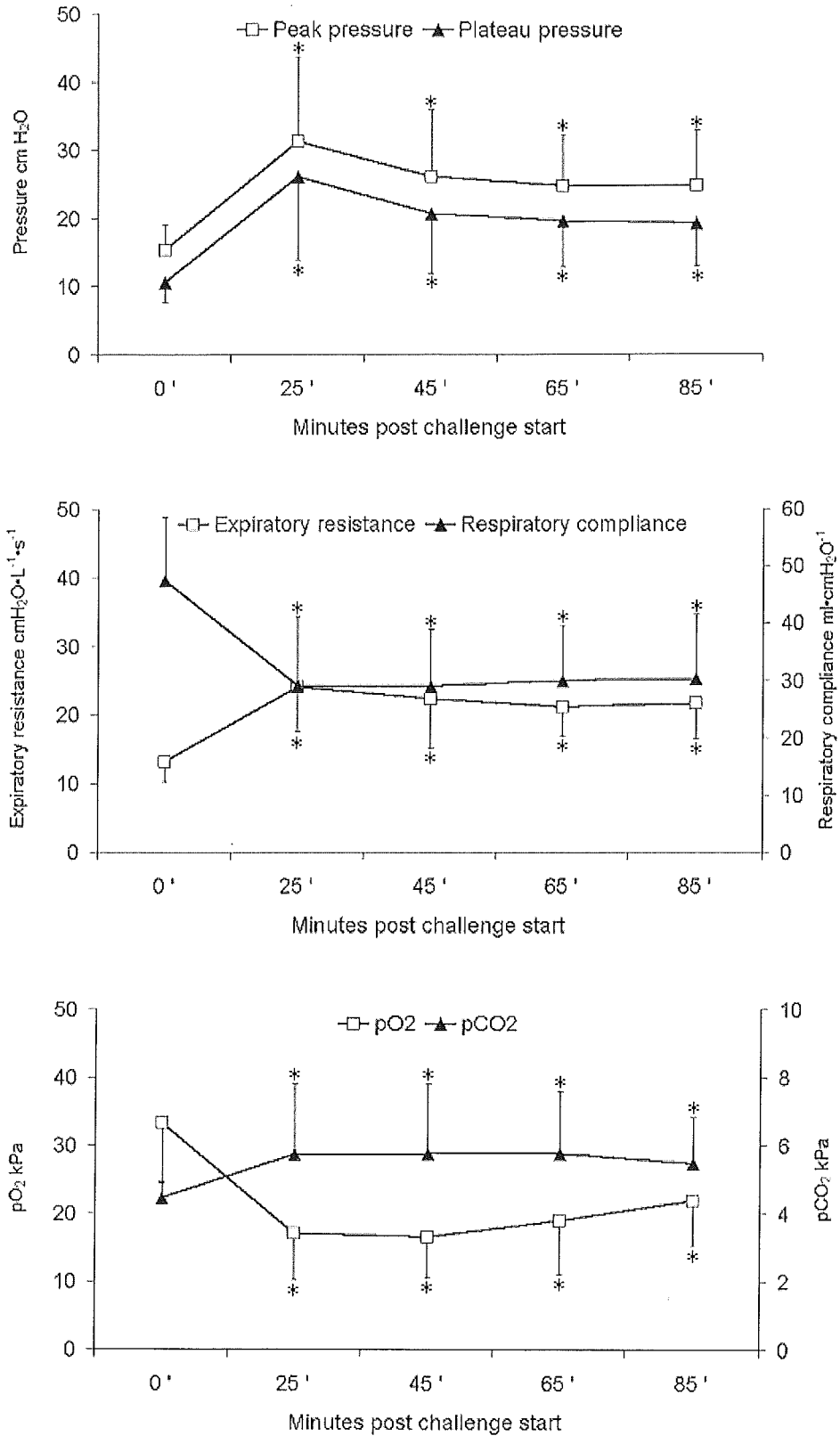

Figure 2. Time course of peak and plateau pressures, expiratory resistance, respiratory compliance, PaO2 and PaCO2 after *Ascaris suum* 2nd session airway challenge. *Significantly different from baseline values.

Table 1. Physiological parameters before and after *Ascaris suum* 1st session airway challenge

	Base line (T0)	End challenge (T0 + 25 min)	20 min (T0 + 45 min)	40 min (T0 + 65 min)	60 min (T0 + 85 min)
Peak pressure, cmH2O	14 ± 3	**31 ± 11**	**28 ± 10**	**26 ± 6**	**24± 4**
Plateau pressure, cmH2O	10 ± 3	**26 ± 10**	**23 ± 8**	**21 ± 5**	**19 ± 3**
Expiratory resistance, cmH2O/L/s	13 ± 2	**26 ± 6**	**27 ± 9**	**28 ± 8**	**28 ± 8**
Respiratory compliance, ml/cmH2O	45 ± 10	**26± 10**	**26 ± 10**	**25 ± 6**	**26 ± 5**
PaO2, kPa	32 ± 5	**16 ± 9**	**16 ± 9**	**19 ± 7**	**21 ± 7**
PaCO2, kPa	4.5 ± 0.3	**5.5 ± 1.1**	**5.5 ± 1.1**	**5.6 ± 1.1**	**5.7 ± 1.1**
pH	7.51 ± 0.06	7.47 ± 0.10	7.47 ± 0.09	7.45 ± 0.08	7.46 ± 0.08
Inspiratory tidal volume, ml	618 ± 82	627 ± 80	627 ± 80	628 ± 80	628 ± 78
Heart rate, beats/min	85 ± 11	84 ± 14	84 ± 15	86 ± 15	86 ± 15
Mean arterial pressure, mmHg	80 ± 9	84 ± 24	83 ± 20	81 ± 21	82 ± 22
Temperature, °C	38.5 ± 0.9	38.8 ± 0.8	38.8 ± 0.8	38.9 ± 0.9	38.9 ± 1.0

Values are means +/- standard deviations (SD). Bold-faced values are significantly different from baseline values ($p<0.05$).

Table 2. Physiological parameters before and after *Ascaris suum* 2nd session airway challenge

	Base line (T0)	End challenge (T0 + 25 min)	20 min (T0 + 45 min)	40 min (T0 + 65 min)	60 min (T0 + 85 min)
Peak pressure, cmH2O	15 ± 4	**31 ± 12**	**26 ± 10**	**25 ± 8**	**25 ± 8**
Plateau pressure, cmH2O	11 ± 3	**26 ± 12**	**21 ± 9**	**20 ± 7**	**19 ± 6**
Expiratory resistance, cmH2O/L/s	13 ± 3	**24 ± 6**	**22 ± 7**	**21 ± 4**	**22 ± 5**
Respiratory compliance, ml/cmH2O	48 ± 11	**29 ± 12**	**29 ± 10**	**30 ± 10**	**30 ± 11**
PaO2, kPa	33 ± 9	**17 ± 7**	**17 ± 6**	**19 ± 8**	**22 ± 7**
PaCO2, kPa	4.5 ± 0.5	**5.7 ± 2.1**	**5.8 ± 2.1**	**5.8 ± 1.8**	**5.4 ± 1.4**
pH	7.52 ± 0.06	7.47 ± 0.13	7.47 ± 0.13	7.47 ± 0.13	7.48 ± 0.12
Inspiratory tidal volume, ml	677 ± 63	669 ± 73	679 ± 63	677 ± 62	677 ± 61
Heart rate, beats/min	83 ± 11	90 ± 21	90 ± 19	87 ± 18	85 ± 11
Mean arterial pressure, mmHg	84 ± 16	95 ± 24	92 ± 23	93 ± 22	92 ± 21
Temperature, °C	38.6 ± 0.5	38.8 ± 0.6	38.8 ± 0.6	38.9 ± 0.6	38.9 ± 0.6

Values are means +/- standard deviations (SD). Bold-faced values are significantly different from baseline values ($p<0.05$).