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EFFECTS OF GLUCOCORTICOIDS IN VENTILATED PIGLETS WITH SEVERE PNEUMONIA.

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ABSTRACT

There is clinical evidence suggesting that glucocorticoids may be useful in severe pneumonia, but the pathogenic mechanisms explaining these beneficial effects are unknown.

The aim of the study was to determine the effects of adding glucocorticoids to antibiotic treatment in an experimental model of severe pneumonia.

Fifteen Lagerwhite-Landrace piglets were ventilated for 96h. After intubation, a solution containing 75 ml of *Pseudomona aeruginosa* (10^6 cfu/ml) was bronchoscopically inoculated. Twelve hours after inoculation, the animals were randomized into 3 group; 1) untreated, 2) treated with ciprofloxacin and 3) treated with ciprofloxacin plus methylprednisolone. Physiologic and laboratory parameters were monitored throughout the study. Proinflammatory cytokines were measured in serum and in bronchoalveolar lavage (BAL). Histopathology of the lungs and cultures from blood, BAL and lungs were performed.

At the end of the study, piglets receiving the antibiotic plus glucocorticoids showed: 1) a decrease in the concentration of IL-6 in BAL and 2) a decrease in the global bacterial burden both in BAL and lung tissue.

In conclusion, in this experimental model of pneumonia, the association of glucocorticoids with antibiotics attenuates local inflammatory response and decreases bacterial burden in the lung.

INTRODUCTION

The mortality rate in severe community or hospital acquired pneumonia requiring mechanical ventilation is very high (1;2). Moreover, despite advances in antimicrobial therapy and supportive measures, this rate has not changed over the last years (3-5), suggesting that other factors are also responsible for the poor outcome. The role of the host inflammatory response in the evolution of bacterial infections has been shown to be crucial with the release of cytokines and other inflammatory mediators from immune cells being key for the elimination of invading pathogens. However, an excessive release of these mediators can be harmful to the host and particularly to the lung. Different clinical studies have shown associations between the concentration of relevant inflammatory cytokines in bronchoalveolar lavage (BAL) fluid or serum and pneumonia severity (6;7). It has also been shown that persistent elevation of these cytokines has prognostic implications in severe pneumonia and in acute respiratory distress syndrome (ARDS) (7-10). Glucocorticoids (GC) decrease the local and systemic inflammatory response. These compounds inhibit the recruitment of leukocytes and monocyte-macrophages into affected areas (11), and the expression and activity of a great variety of cytokines relevant to inflammatory response in pneumonia, including IL-1 beta, IL-6 and TNF-alfa. (12)

Different controversial results have generated an intense debate regarding the benefits of prolonged GC treatment at low to moderate doses in patients with catecholamine-dependent septic shock (13-14) and acute lung injury and ARDS (15-17). Also, GC administration is indicated in severe *P.jiroveci* pneumonia (18). In addition, Confalonieri et al reported that in patients suffering from severe community-acquired pneumonia, continuous endovenous infusion of low dose hydrocortisone improved survival (19).

Multiple factors may influence the inflammatory response and the outcome of patients with severe pneumonia, particularly those receiving mechanical ventilation. In this sense, the

availability of an animal model of severe pneumonia may greatly enhance both our knowledge of the intimal mechanisms involving inflammatory response and the understanding of the efficacy of pharmacologic interventions. Moreover, the effects of GC on the modulation of inflammatory response in pneumonia and their potential microbiological and histopathological consequences may be better elucidated.

Marquette et al, have standardized an animal model of pneumonia in ventilated piglets (20). This model closely resembles human pneumonia and has proven to be very useful for evaluating different aspects related to the diagnosis and treatment of severe pneumonia (21-27). Using this animal model, we were able to reproduce severe pneumonia and to study the associated inflammatory response after inoculate high concentrations of *P.aeruginosa* (28), the most lethal causative microorganism both in CAP and ventilator-associated pneumonia (VAP) (2;8;29).

We hypothesized that the concomitant administration of GC with antibiotics might decrease the local and systemic inflammatory response with subsequent beneficial effects on the severity of pneumonia.

The aim of the present study was therefore, to investigate the potential benefits of GC in addition to antibiotic treatment in an experimental model of pneumonia induced by *P. aeruginosa* in ventilated piglets. We were particularly interested in knowing the effects of GC on inflammatory response (both local –lung and systemic –serum), and on clinical, microbiological and histopathological variables.

METHODS

Animal preparation.

Nineteen healthy domestic bred Largewhite-Landrace piglets, aged 3 months and weighting 20 ± 2 kg were anesthesized and orotracheally intubated. The femoral artery was cannulated for pressure monitoring and blood sampling, a catheter was inserted in the femoral vein for continuous infusion and a suprapubic urinary catheter was placed in the bladder through surgical midline minipelvitomy. The piglets were then placed in a prone position and were mechanically ventilated for 4 days. Ventilator parameters consisted of tidal volume (VT) of 10 ml/kg, a respiratory frequency of 15 breath/min, an inspiratory time of 33%, with an inspiratory fraction (FiO2) of 100% and a positive end-expiratory pressure of 0, as described previously (28).

Bronchial inoculation.

Animals were inoculated with 75 mL of a suspension of 10^6 colony forming unities (cfu)/ml of pathogenic *P. aeruginosa* ATCC 27853, susceptible to ciprofloxacin [minimal inhibitory concentration (MIC) = 0.5 mcg/mL]. Fifteen ml of the infusion were evenly distributed in every lobe of each lung through the bronchoscope's channel.

Study Design.

To determine the effect of GC on different clinical, microbiological, histopathological variables and on inflammatory parameters, animals were randomized into 3 groups 12 hours after the inoculation of *P. aeruginosa.* The first group consisted of 5 animals receiving serum alone (control group). The second group included 5 animals receiving ciprofloxacin 200 mg/iv every 12 hours (CIP group) and the third group included 5 animals receiving ciprofloxacin 200 mg/iv every 12 hours plus glucocorticoids (methylprednisolone 0.5 mg/kg/iv every 12 hours)(CIP-GC

group). In keeping with our research protocol (28) piglets of each group were under mechanical ventilation for 84h. Piglets dying in the first 12 hours were excluded from the study.

Endpoints.

Clinical, hemodynamic, respiratory mechanics, biochemical data, inflammatory mediators in serum and BAL fluid (TNF-α, IL-1β, IL-6, IL-8 and CRP), type and degree of histopathologic pulmonary lesions and quantitative bacteriological studies were evaluated at different time-points in the three groups of animals studied. A summary of the study design is displayed in **figure 1**. Specific drug assignment was known by the investigators, however, biochemical, biological, microbiological and histopathological studies were performed blindly.

Samplings and procedures.

Heart rate, blood pressure, body temperature, mechanical ventilation parameters (airway pressures, static pulmonary compliance (30) and FiO_2, arterial blood gases (IL-1306; Instrumentation Laboraties, Milan, Italy), serum electrolytes (sodium, potassium) and lactate concentrations were monitored at 0, 2, 6, 12, 24, 36, 48, 60, 72, 84 and 96 hours. Blood biochemistry (glucose, creatinine, urea, bilirrubin, aspartat aminotransferase and alanine aminotransferase) and blood cells count were obtained at 0, 24, 48, 72 and 96 hours.

Bronchoalveolar lavage (BAL): five 20 mL aliquots of sterile saline solution (0.9% NaCl) were instilled through the bronchoscope's channel and subsequently aspirated by hand at hour 0 (immediately before *P. aeruginosa* inoculation) in the right middle lobe.

Inflammatory parameters.

C-Reactive Protein (CRP): CRP was quantified in serum and BAL fluid using a C-reactive protein kit (Biosystems S.A., Barcelona, Spain).

Cytokines in blood and BAL fluid: TNF-alfa, IL-1 beta, IL-6 and IL-8 levels were measured in serum and BAL supernatant using the ELISA method in specific porcine kits (R&D Systems Inc., Minneapolis, MN, USA).

BAL cytokine and CRP levels were determined at the time of intubation and at the end of the study. Serum cytokines and CRP were determined at the time of intubation and at 24, 48, 72 and 96 hours.

Sacrifice and post-mortem studies.

Sacrifice was performed at day 4 under general anesthesia by intravenous potassium chloride infusion. Collection of Lung Specimens: after death, animals remained mechanically ventilated up to the time of specimen collection. Lungs were aseptically exposed through a cervico-thoracic midline incision. BAL was performed in both, the macroscopically more preserved and more involved lung lobes. BAL specimens were processed for cytokine measurements and quantitative bacterial cultures. Thereafter, at least one lung tissue specimen (3 cm^3) was taken from the above mentioned lobes (the more preserved and the more involved) while the lungs were kept inflated. Specimens were cut into two parts for bacteriologic and pathologic studies.

Bacteriological studies.

Blood culture and quantitative BAL and lung tissue cultures were performed immediately postmortem with animals remaining under mechanical ventilation.

Bronchoalveolar lavage and lung tissue specimens were processed for quantitative bacterial cultures as described elsewhere (31) according to recommended laboratory methods (32).

Global bacterial burden: was assessed by calculating the median of the different bacterial counts samples taken from the more preserved lobe and the more involved lobe (both in BAL fluid and lung tissue).

Pathologic studies.

Lung tissue was processed according to standard methods. Pneumonia lesions were graded according to previously published criteria (33) in grades 0: no pneumonia; 1: purulent mucous plugging; 2: bronchiolitis; 3: pneumonia (consolidation coexisting with significant accumulation of polymorphonuclear leukocytes, fibrinous exudates and cellular debris into the alveolar space); 4: confluent pneumonia (extension along different secondary lobes); and 5: abscessed pneumonia (cellular necrosis coexisting with disruption of cellular architecture). Pneumonia was limited to the last 3 categories. Classification of each specimen was based upon the worst category observed.

Statistical Analysis.

All data are expressed as mean ± SD or SEM as appropriate. Qualitative or categorical variables were compared using the Chi-square test. Quantitative variables between the three groups were compared using oneway analysis of variance (ANOVA) test. Quantitative variables over the time were compared using Friedman non-parametric test. A p value <0.05 was considered statistically significant, all two-tailed.

Approval by the institutional committee.

All animals were treated in compliance with the guidelines of the Ethics Committee and Direction of Investigation of the Hospital de Clínicas “José de San Martín”, University Buenos Aires and the ***Guide for the Care and use of Laboratory Animals*** (NIH Publication No. 93-23, revised 1985).

RESULTS

We studied a total of 15 animals after excluding 4 that died during the first 12 h before randomization.

Clinical, hemodynamic, and respiratory mechanics after inoculation of *P. aeruginosa* (before randomization):

As a result of the inoculation of *P. aeruginosa*, a series of clinical, hemodynamic, pulmonary mechanics and gas exchange alterations were observed in the animals studied (n=15). As shown in **figure 2**, early increases in body temperature and in heart rate were observed, being significant at hour 12. PaO_2/F_IO_2 values decreased significantly at hour 12 reflecting a marked impairment in pulmonary gas exchange. The Cst values also decreased after inoculation, although the differences did not reach statistical significance. No changes were observed in mean arterial pressure.

Outcome with specific treatments:

As stated above, 12 hours after the inoculation of *P. aeruginosa*, the animals were randomized into three groups. When we compared them, no significant changes were observed in the sequential physiologic measurements that were performed. However, regarding the laboratory parameters, we detected an increase in serum glucose levels (96 hours) and white blood cell count (84 and 96 hours) in the CIP+GC group (**table 1**). No differences in the rest of laboratory results were found (data not shown).

Respiratory Mechanics and Gas exchange:

Contrary to what occurred in the control and CIP groups, the animals in the CIP+GC group showed a progressive improvement in static compliance (**figure 3**). Gas exchange, reflected by the PaO_2/F_IO_2 ratio was impaired in both the control group and the CIP group. By contrast,

animals from the CIP+GC group demonstrated a discrete improvement in the PaO_2/FiO_2 ratio although the differences with the other two groups did not reach statistical significance (**figure 3**). pH, bicarbonate and lactate values remained within the normal range in all groups (data not shown)

Pulmonary and systemic inflammatory response:

At baseline, no differences were observed among the groups in the concentration of any of the different cytokines in BAL fluid **(figure 4**). At the end of the study, an increase was observed in the levels of the different cytokines evaluated with the highest concentration always seen in the control group and the levels of these cytokines in the CIP group were always lower than in the controls. Interestingly, in the CIP+GC group cytokine levels showed the lowest values with statistically significant differences in IL-6 concentrations **(figure 4).** Concentrations of CRP in BAL fluid followed the same pattern with low and homogeneous values at baseline and a marked increase at 96h in the control group, a moderate rise in the CIP group and the lowest increase in the CIP+GC group (**figure 4**).

Contrary to what was observed in BAL fluid, no consistent pattern was observed on the dynamics of the cytokines evaluated in serum (**figure 5**). Difference between groups were observed in serum IL-8 at hour 24 (p=0.04).

Microbiology.

Microbiological findings are shown in **table 2**.

In the control group, BAL and lung cultures demonstrated *P. aeruginosa* in all the samples evaluated. By contrast, in the treated groups (CIP and CIP + GC), *P. aeruginosa* was isolated in only 2 out of the 5 animals in BAL fluid. Cultures of lung tissue demonstrated the presence of *P. aeruginosa* in all but one piglet from each group. The global bacterial burden showed

significantly lower bacterial counts in both BAL and lung cultures in the CIP+GC group (p= 0.03 and p= 0.01 respectively) (**figure 6**).

Blood cultures were negative in all piglets except in one control animal with positive cultures for *P. aeruginosa.*

Histopathologic Findings:

According to the established criteria, 100% animals in the control group, 80% (4/5) in the CIP group and 40% (2/5) in the CIP+GC group showed evidence of pneumonia at the end of the study. Of the 30 lung samples examined, pneumonia was present in 80% (8/10) of the samples of the control group, in 60% (6/10) of the CIP group and in 30% (3/10) of the samples in the CIP+GC group (p=0.09).

Different grades of pneumonia severity were observed (**table 3)** Severe pneumonia was present in 60% of the pulmonary biopsies in the control group, 60% in the CIP group and 30% in the CIP+GC group (p=0.08).

No differences in other lesions, including pleural involvement, vascular abnormalities and alveolar damage, were found among the three groups of animals studied.

DISCUSSION

The results of the present study in an animal model of pneumonia due to *P. aeruginosa*, suggest that the addition of systemic GC to targeted antibiotic therapy diminish the lung associated inflammatory response and the bacterial lung burden.

Recent clinical evidence shows that low doses of hydrocortisone decrease mortality in severe CAP (19;34). Glucocorticoids might exert their beneficial effect by counteracting the excessive release of inflammatory mediators that occurs in severe pulmonary infections. In the present study, we show that in piglets treated with CIP plus GC, the concentration of IL-6 in the lung is attenuated. This attenuation is more pronounced than the one observed in animals receiving only targeted antibiotic. Levels of inflammatory mediators in BAL in the group of animals treated with CIP plus GC were comparable to those observed at baseline, before the inoculation of bacteria, suggesting an efficient anti-inflammatory role of GC particularly referring to IL-6. In this model we did not observe signs of severe sepsis, probably justifying the absence of an important systemic inflammatory response.

Animals treated with CIP plus GC not only had an attenuated local inflammatory response, remarkably they also had lower BAL and lung bacterial counts than the other two groups of animals, suggesting a more efficient bacterial eradication capacity when both compounds are associated. In fact, we observed decrements of 0.5 log cfu/ml and 0.8 log cfu/g in the mean BAL and lung tissue bacterial burden when comparing animals treated with CIP+GC to animals treated only with CIP. Although the small number of animals in these two groups precludes statistical comparisons, we believe that this decrement may be remarkable from a clinical view point. In fact, human studies with other types of respiratory infections have shown a parallel decrease in both bacterial burden and in lung inflammatory response (35)

The beneficial effect of the simultaneous administration of CIP and GC is also suggested by our findings in the histopathological analysis of lung samples. Animals treated with CIP plus GC showed pneumonia in only 30% of the lobes evaluated as compared with 60% in animals treated with CIP. Also, severity of pneumonia, defined as abscessed or confluent pneumonia, was present in 60% of the pulmonary biopsies in the group of animals treated with CIP plus GC as compared to 30% in animals treated with CIP. Again, and although differences were not statistically significant, probably due to the small number of animals studied, we believe that these results could have clinical significance.

Meduri et al (36) in an *in vitro* study have demonstrated that certain bacterial strains such as *P. aeruginosa* have receptors for the cytokines IL-1-beta and TNF-alfa and the exposure of bacteria to these cytokines enhance their growth and virulence. Glucocorticoids might restore the impaired capacity of phagocytic cells produced by an excessive inflammation. Exposing human monocytic cells (U937 cells) cells to progressively concentrations of LPS enhanced the intracellular survival and replication of different species of bacteria including *P. aeruginosa* (36). More importantly when exposed to graded concentrations of methylprednisolone, U937 cells previously stimulated with LPS were able to suppress bacterial replication efficiently in a concentration-dependent manner. Finally, mRNA levels of TNF-alfa, IL-1beta, and IL-6 in LPS-activated cells were reduced by treatment of such cells with methylprednisolone. (36).

These studies reinforce our findings in this animal model of severe pneumonia and suggest that the impaired ability of phagocytic cells to eradicate bacteria by an excessive inflammatory response may be counterbalanced by the administration of GC.

Some methodological considerations must be taken into account for the proper evaluation of the results. Animal models can not reflect all the physiopathological aspects of severe pneumonia pathogenesis, a dynamic process that involves a wide spectrum of pathogens and complex

interactions with host defenses favoring bacterial growth (37). Moreover, the exogenous administration of highly bacterial inoculums in a previously healthy animal does not necessarily reflect the complexities of the development of pneumonia.

Also, the potentially of harmful side effects due to GC treatment and the rebound effect that their tapering can cause in the evolution of the inflammatory process are matters of an intense debate (14, 16, 38-40). In this sense, failed or delayed recognition of infections in the presence of a blunted febrile response represents a serious threat. Also other adverse events such as hyperglycemia and neuromiopathy must be also taken into consideration when using GC as an adjuvant therapy. Strict infection surveillance programs and to find out the optimal dose of GC to be used can help in this issue. In the present study, we observed an increase in the white blood cell count and higher levels of glycemia in animals receiving GC. However, we did not observe other remarkable side effects at the steroid doses used.

Finally, we recognize that mechanical ventilation may indeed alter the inflammatory response. It is known that mechanical ventilation with high tidal volumes aggravates lung injury in patients with acute lung injury or ARDS. The effect of ventilation in patients without pre-existing lung injury is not so well characterized (41). Certainly, in our study we can not ruled out that mechanical ventilation had some influence in the observed inflammatory response. However, the potential influence of this factor may be partially offset by the fact that the three groups of animals compared were ventilated using the same settings.

In summary, in this experimental model of pneumonia in ventilated piglets, the addition of GC to targeted antibiotic treatment diminishes the local inflammatory response and decreases lung bacterial burden and might improve the histopathological severity of pneumonia. These effects could be potentially beneficial from the clinical point view. Further studies are necessary to elucidate not only which patients can potentially benefit from GC, but also what optimal doses

and duration of treatment are necessary to obtain an appropriate balance between the beneficial and harmful effects of inflammatory response.

FIGURE LEGENDS

Figure 1.- Scheme of determinations and different interventions in ventilated piglets.

Figure 2.- Mean sequential changes in temperature, heart rate, PaO_2/FiO2 ratio and Static Compliance during the first 12 hours after *P. aeruginosa* inoculation in all piglets studied (n=15). Data are expressed as mean + SD. p values are from Friedman paired test.

Figure 3.- PaO_2/FiO_2 ratio and Static Compliance measurements in the three groups of piglets studied. Results are expressed as percentage of change between hours 12 and 96. p values are obtained using ANOVA test.

Figure 4.- Values of bronchoalveolar lavage (BAL) concentrations of IL-1 beta, IL-6, IL-8, TNF-alfa and C-reactive protein (CRP) at baseline and at the end of the study (96 hours) in the three groups of piglets. Data are expressed as mean + SEM. At baseline, values are always homogeneus in the three groups ($p>0.1$). At hour 96, p values from ANOVA test are shown.

Figure 5.- Sequential determinations of IL-1 beta, IL-6, IL-8, TNF-alfa and C-reactive protein (CRP) in serum throughout the study are shown in the three groups of piglets. Data are expressed as mean + SEM. ANOVA test is used to compare the differences between the three groups every 24 hours. * represents $p<0.05$.

Figure 6.- Global bacterial burden in the three groups of animals evaluated both in BAL (expressed as log cfu/ml) and in lung tissue (expressed as log cfu/gr). p values are obtained using ANOVA test.

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Table 1: Sequential measurements of physiologic and laboratory parameters during the study in the three groups of piglets. Data are expressed as mean ± SD.

		12 hours	24 hours	48 hours	72 hours	96 hours
Heart Rate, bpm	control	173 ± 36	142 ± 28	130 ± 18	136 ± 16	137 ± 22
	CIP	144 ± 53	136 ± 31	129 ± 45	132 ± 17	121 ± 14
	CIP+GC	141 ± 18	130 ± 10	116 ± 11	121 ± 06	131 ± 19
MAP, mmHg	control	105 ± 22	109 ± 11	116 ± 22	112 ± 26	110 ± 24
	CIP	118 ± 12	111 ± 14	114 ± 17	126 ± 30	120 ± 26
	CIP+GC	127 ± 16	105 ± 26	113 ± 21	118 ± 12	122 ± 14
Temperature, ºC	control	40,2 ± 1,2	40,0 ± 0,6	39,1 ± 1,0	39,1 ± 0.7	39,0 ± 1,4
	CIP	39,1 ± 1,7	38,6 ± 1,2	37,9 ± 1,7	37,9 ± 1,5	38,9 ± 2,3
	CIP+GC	40,3 ± 1,1	39,5 ± 1,2	38,9 ± 1,2	38,7 ± 1,4	37,8± 1,0
Glucose, mg/dL	control	115 ± 21	93 ± 27	97 ± 34	70 ± 34	64 ± 28*
	CIP	120 ± 55	122 ± 80	109 ± 40	124 ± 52	88 ± 32*
	CIP+GC	112 ± 41	104 ± 22	118 ± 18	140 ± 31	171 ± 52*
WBC, $x10^9/L$	control	16200 ± 8760	18720 ± 11450	15230 ± 4456	11610 ± 6120 *	13010 ± 3850 *
	CIP	17200 ± 10241	18920 ± 7391	21440 ± 12697	24400 ± 9693 *	20850 ± 9903 *
	CIP+GC	15600± 2899	27820 ± 8699	26800 ± 5338	28125 ± 5208 *	26900 ± 2787 *

MAP= Mean Arterial Pressure; WBC= White Blood Cell Count.
*p<0.05 between the three groups (ANOVA test)

Table 2: Bacteriologic results (BAL, lung tissue and blood cultures) performed at the end of the study.

Group	BAL culture (cfu/mL)		Lung culture (cfu/gr)		Blood
	More involved lobe	**More preserved lobe**	**More involved lobe**	**More preserved lobe**	
Control	*P. aeruginosa* (10^4)	*P. aeruginosa* (10^4)	*P. aeruginosa* (10^4)	*P. aeruginosa* (10^4)	Negative 2/2
Control	*P. aeruginosa* (10^4)	*P. aeruginosa* (10^4)	*P. aeruginosa* (10^4)	*P. aeruginosa* (10^4)	Negative 2/2
Control	*P. aeruginosa* (10^4) CN *Staphylococcus* (10^3)	*P. aeruginosa* (10^4) CN Staphylococcus (10^3)	*P. aeruginosa* (10^4)	*P. aeruginosa* (10^4)	Negative 2/2
Control	*P. aeruginosa* (10^4)	*P. aeruginosa* (10^4)	*P. aeruginosa* (10^4)	*P. aeruginosa* (10^4)	*P. aeruginosa* 2/2
Control	*P. aeruginosa* (10^5)	*P. aeruginosa* (10^4) CN *Staphylococcus* (10^2)	*P. aeruginosa* (10^4)	*P. aeruginosa* (10^4)	Negative 2/2
CIP	Negative	CN *Staphylococcus* (10^3)	*P. aeruginosa*(10^2)	CN *Staphylococcus* (10^3)	Negative 2/2
CIP	*P. aeruginosa* (10^3)	*P. aeruginosa* (10^2)	Negative	Negative	Negative 2/2
CIP	*P. aeruginosa* (10^4)	*P. aeruginosa* (10^2)	*P. aeruginosa* (10^4)	*P. aeruginosa* (10^4)	Negative 2/2
CIP	Negative	Negative	*P. aeruginosa* (10^2) CN *Staphylococcus* (10^3)	Negative	Negative 2/2
CIP	Negative	Negative	*P. aeruginosa* (10^4)	*P. aeruginosa* (10^4)	Negative 2/2
CIP+GC	*P. aeruginosa* (10^2)	*P. aeruginosa* (10^2)	*P. aeruginosa* (10^2)	*P. aeruginosa* (10^2)	Negative 2/2
CIP+GC	Negative	Negative	*P. aeruginosa* (10^2)	Negative	Negative 2/2
CIP+GC	Negative	Negative	*P. aeruginosa* (10^2)	Negative	Negative 2/2
CIP+GC	*P. aeruginosa* (10^2)	Negative	Negative	Negative	Negative 2/2
CIP+GC	*Negative*	*Negative*	*P. aeruginosa* (10^3)	*P. aeruginosa* (10^3)	Negative 2/2

CIP: Ciprofloxacin group; CIP + GC: Ciprofloxacin plus glucocorticoids group; CN: Coagulase-negative

Table 3: Histopathological findings

Group	Pneumonia score	
	More involved lobe	More preserved lobe
Control	Pneumonia	Bronchiolitis
Control	Confluent pneumonia	Confluent pneumonia
Control	Abscessed pneumonia	Confluent pneumonia
Control	Pneumonia	Purulent mucus plugging
Control	Abscessed pneumonia	Confluent pneumonia
CIP	Confluent pneumonia	Non specific abnormalities
CIP	Purulent mucus plugging	Non specific abnormalities
CIP	Confluent pneumonia	Bronchiolitis
CIP	Confluent pneumonia	Confluent pneumonia
CIP	Abscessed pneumonia	Confluent pneumonia
CIP+GC	Purulent mucus plugging	Bronchiolitis
CIP+GC	Purulent mucus plugging	Purulent mucus plugging
CIP+GC	Purulent mucus plugging	Non specific abnormalities
CIP+GC	Confluent pneumonia	Confluent pneumonia
CIP+GC	Confluent pneumonia	Purulent mucus plugging

CIP: Ciprofloxacin group; CIP + GC: Ciprofloxacin plus glucocorticoid group

Figure 1

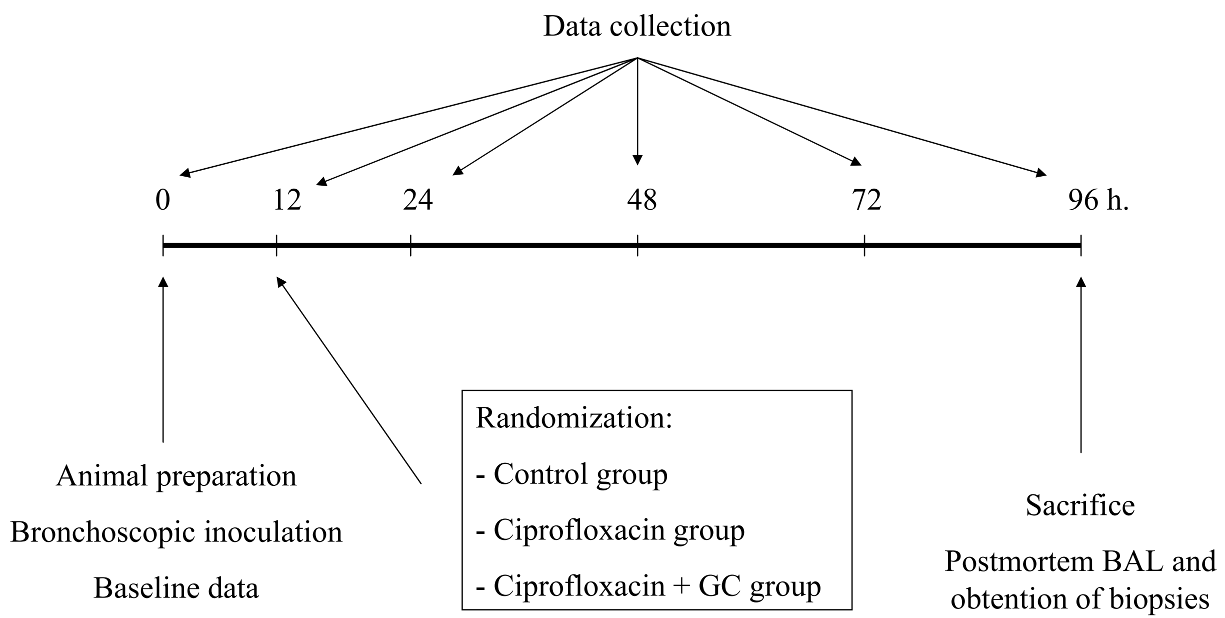
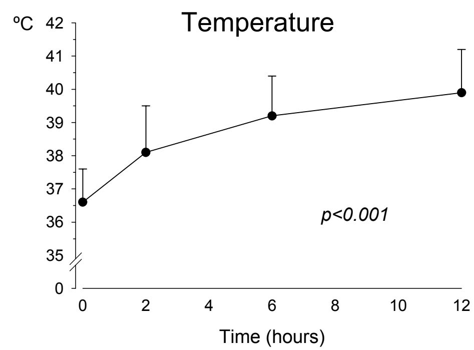
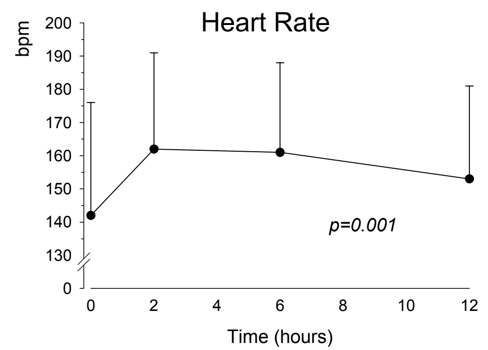
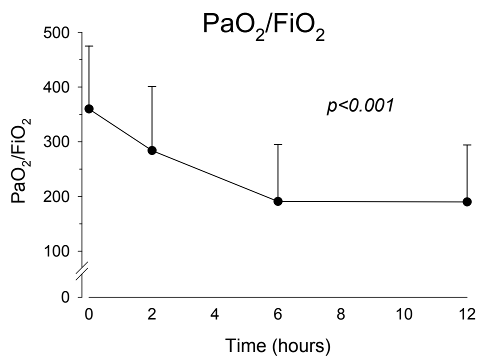


Figure 2







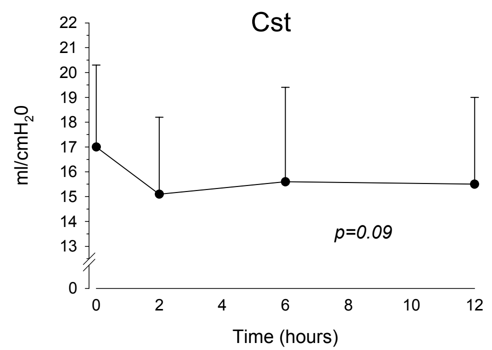


Figure 3

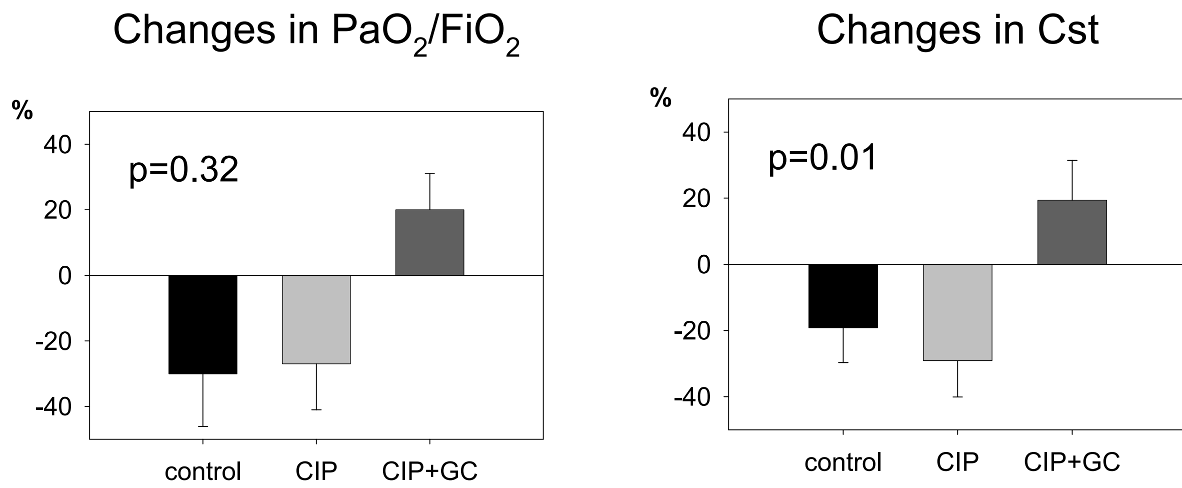


Figure 4

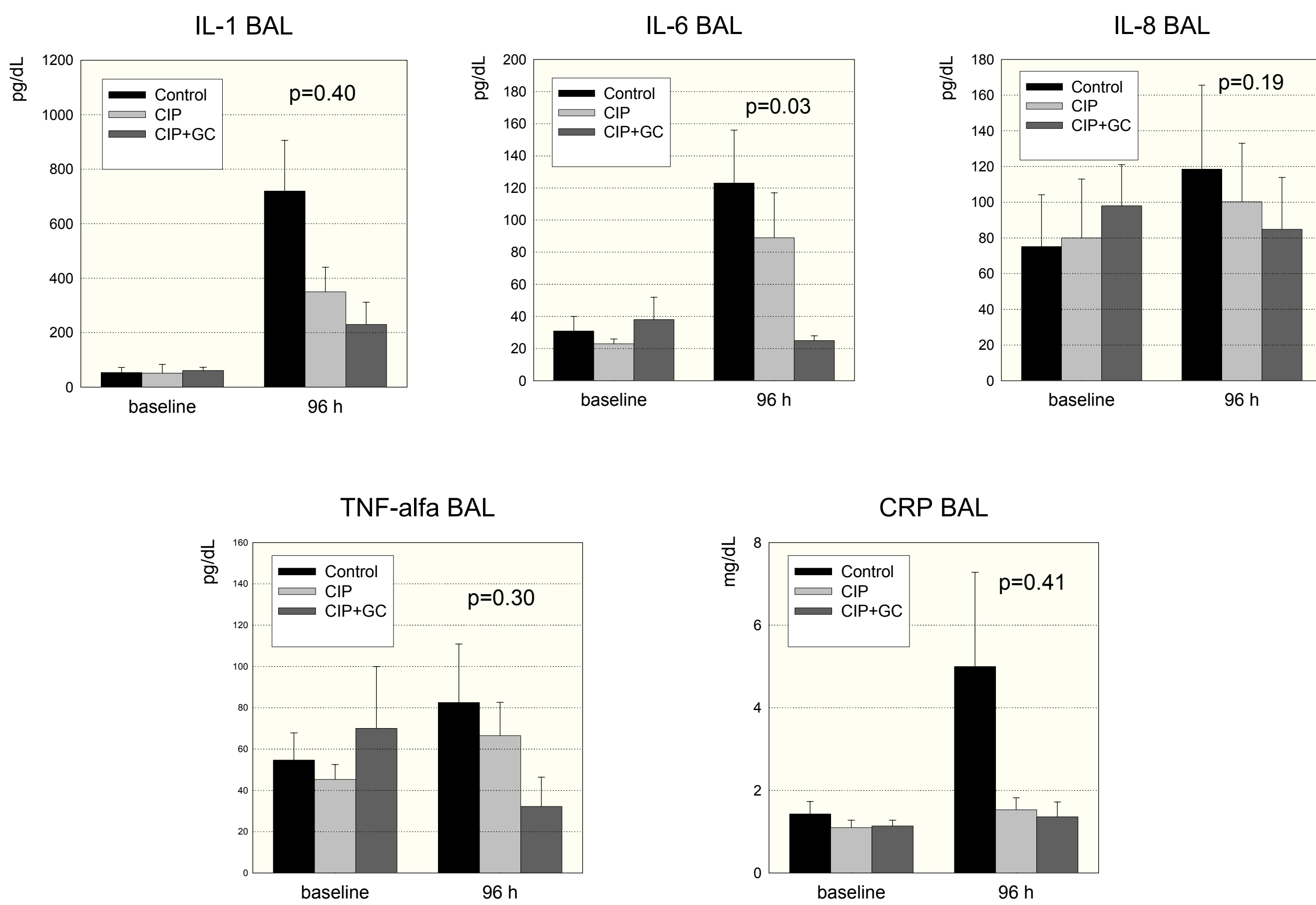


Figure 5

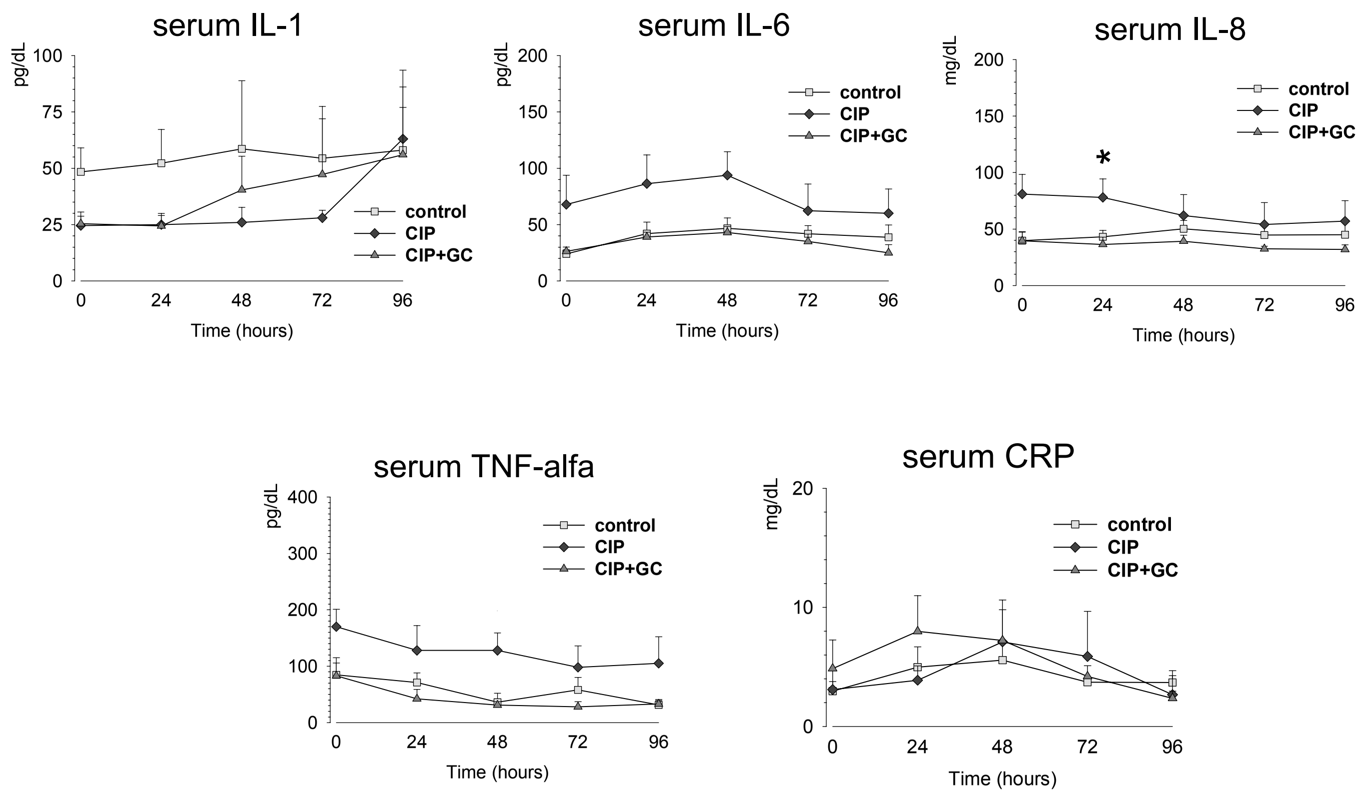


Figure 6