

## Inadequate Timing Between Corticosteroid and Antibiotics Applications Increases Mortality Due to Sepsis

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This study tested the hypothesis that the use of corticosteroids prior to antibiotics can lower the mortality rate in severe infections by *S. aureus* or Gram-negative bacilli, using an animal model. This study was a prospective and controlled study, placed in a university laboratory. Seven hundred and sixty mice distributed into three groups (*Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae* infected). The interventions in each group were: I) infection control (intra-peritoneal); II) treatment solely with antibiotics (teicoplanin or amikacin); III) antibiotics administered prior to the corticosteroid (methylprednisolone); IV) antibiotics administered after the corticosteroid. Mortality in the *E. coli* group, subgroup I: 100%; subgroup II: 55% ( $p < 0.001$ ); subgroup III: 62.5% ( $p = 0.2488$ , compared to subgroup II); subgroup IV: 20% ( $p < 0.01$  compared to subgroups II and III). Mortality in the *K. pneumoniae* group: subgroup I: 100%; subgroup II: 72.5% ( $p < 0.01$ ); subgroup III: 80% ( $p = 0.215$  compared to subgroup II); subgroup IV: 45% ( $p < 0.01$  compared to subgroups II and III). Mortality in the *S. aureus* group: subgroup I: 82.5%; II: 42.5% ( $p < 0.001$ ); subgroup III: 77.5% ( $p = 0.2877$  compared to subgroup I); subgroup IV: 32.5% ( $p = 0.1792$  compared to subgroup II). The use of corticosteroids prior to antibiotics lowered the mortality rate caused by Gram-negative bacteria and did not affect the mortality caused by *S. aureus*. When used after starting treatment with antibiotics, the corticosteroid was not superior to the use of antibiotics alone in the case of the Gram-negative bacteria, and was not significantly different from non-treatment of the infection, in the case of *S. aureus*.

**Key Words:** Adrenal cortex hormones, antibiotics, sepsis, survival, mice, animals, laboratory, severe infection, peritonitis, shock, endotoxin.

The current line of thought regarding aggression to the host during severe infections is that the primary determinant is not the activity of the microorganisms themselves, but an uncontrolled response on the part of the host, culminating in a reaction of self-aggression and death. In fact, evidence continues to accumulate implicating numerous biochemical participants included in the collective "septic cascade"; which if not controlled ultimately lead to multiple organ failure [1-4]. The use of suitable anti-microbial medicines, even if they eliminate the microorganisms that triggered the septic process, often will not be enough to prevent the host's worsening and dying [5].

Several pro-inflammatory cytokines take part in this cascade, among them tumor necrosis factor-alpha (TNF-alpha) seems to be the most important and is the first to appear. TNF-alpha triggers activation of several other mediators, culminating in the metabolic, hemostatic and hemodynamic features of septic shock [6-10]. Although the lipopolysaccharide (LPS) produced by Gram-negative bacilli (GNB) is the most potent stimulus for the production of TNF-alpha, this cytokine is also produced during infections by Gram-positive bacteria (the main sensitizing factors seem to be the peptidoglycans and teichoic acid, which are components of the bacterial cell wall, as well as exotoxins), viruses, fungi and protozoans [11,12].

In the case of Gram-negative bacteria, the production and secretion of TNF-alpha is conditioned by the presentation of the bacterial lipopolysaccharide linked to a specific protein, the LPS binding protein [13,14], to receptor CD 14 of macrophages.

As long as the cell wall remains intact and the sensitizing portion of the LPS, called Lipid A, is hidden within it, the host will not produce an immunological reaction against the bacteria [15]. The toxic effects of Lipid A are only observed in situations in which the cell wall ruptures, i.e., during bacterial lysis [16], or during the processes of bacterial growth and multiplication, in which the LPS is also released into circulation [8]. The use of antibiotics can induce an exaggerated increase in the amount of LPS in the circulation during infections [17-19]; this allows us to make the assumption that rupture of the outer bacterial covering (induced by antibiotics) is associated with release of sensitizing factors, such as the LPS, triggering an unrestrained response on the part of the host [20]. Our objective was to determine if it was possible, in severe infections, to initiate corticosteroid therapy (inhibiting the macrophage response prior to the release of LPS) before starting antibiotics, and thereby inhibit the otherwise exaggerated response of the host.

Corticosteroids, in contrast to other drugs proposed for the treatment of sepsis [21,22], may affect several phases of the septic process, including some very early stages in the cascade. Macrophage inhibition could theoretically block the entire septic cascade, if it is initiated before the macrophage is exposed to bacterial sensitizing factors. Other potentially beneficial effects of these drugs would be inhibition of the synthesis of TNF-alpha and interleukine-1 (IL-1), inhibition of phospholipase (and consequently inhibition of the synthesis of prostaglandins, leukotrienes and platelet

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activation factor), protection of the endothelium against free-oxygen radicals; inhibition of the function of polymorphonuclear leukocytes; and stabilization of the vascular response to vasopressors [23-25].

### Material and Methods

The care and handling of the animals included in this study were in accordance with the Ethical Principles of Animal Experimentation - International Council for Laboratory Animal Science-1990 USA, and was approved by the animal use review committees of all three institutions involved in this study (Universidade Federal do Paraná, Faculdade Evangélica de Medicina do Paraná and Instituto de Tecnologia do Paraná). All moribund animals were euthanized after the experiment.

Seven hundred and sixty male Swiss-albino mice, non consanguineous, lineage CF1 (Carawarth Farm 1), 45 days old, weighing between 18 and 22g, were obtained from the animal-rearing facility of the Institute of Technology of Parana. The mice were placed in groups of 10 in disinfected polypropylene boxes containing sterilized wood shavings and provided with water and food (Nuvital) *ad libitum*. The animals were kept in an acclimatized environment (18-22°C), with 12 hour light / dark cycles. The boxes were exchanged every 48 hours, providing fresh wood shavings and water [26]. The animals were divided into three groups: *Escherichia coli* (ATCC25922, sensitive to amikacin), *Klebsiella pneumoniae* (isolated from patients, sensitive to amikacin) and *Staphylococcus aureus* (ATCC 25923, not enterotoxigenic and sensitive to teicoplanin). All three groups were studied simultaneously, and each group was subdivided into seven subgroups according to treatment with antibiotics and/or corticosteroids, as well as the corresponding controls:

- **subgroup Control A:** 40 mice that received only antibiotics, intramuscularly (antibiotic control subgroup);
- **subgroup Control B:** a subgroup of 40 mice was considered common to all the other three groups, serving as a reference for the control of the diluent solution.
- **subgroup Control C:** 40 mice were treated only with corticosteroids, intraperitoneally (corticosteroid control subgroup);
- **subgroup I:** 40 mice were treated with the bacterial inoculum alone, intraperitoneally. (Infection control subgroup);
- **subgroup II:** 40 mice were treated with the bacterial inoculum; two hours later, the first dose of antibiotic was given;
- **subgroup III:** 40 mice were treated with the bacterial inoculum, two hours later the first dose of antibiotic was given, and four hours later they were treated with the corticosteroid;
- **subgroup IV:** 40 mice were injected with the bacterial inoculum, one hour later the corticosteroid was given, and 2.5 hours after the bacterial inoculum, they were treated with the first dose of antibiotic.

Total number of animals: 760 mice (instead of 840, because the diluent used in the *diluent control subgroup* -subgroup Control B- was the same for all three groups, thus only 40 mice were used (not 120) for this purpose).

### Preparation of the Bacterial Inocula

The strains were re-suspended in soy-casein agar, incubated for 48 hours at 35°C, and then utilized. After inoculation, the concentrations of the inoculum samples were checked by determining colony-forming units/mL in soy-casein agar [27]. The samples were diluted in decimal dilutions (1/10, 1/100, 1/1,000 and 1/10,000); 0.1 mL of each dilution was placed in a Petri dish and homogenized with a Drigalsky's loop. These were incubated at 35°C for 24 hours; the number of colonies was converted into colony-forming units /mL [28, 29].

### Inoculation of Mice

The animals were inoculated in the left inferior abdominal quadrant with 1 mL of the bacterial suspension; the same procedure was followed when the corticosteroid was inoculated, but then in a volume of 0.2 mL, carrying 30mg/kg of methylprednisolone [Solumedrol, Pharmacia & Upjohn, São Paulo-SP, Brazil] in a single dose. For the intramuscular administration of antibiotics, in the left posterior limb, 1 mL tuberculin-type syringes were used. An analysis after the inoculations confirmed the adequacy and the homogeneity of the inocula administered to each animal.

### Monitoring

Each group was monitored for 10 days. During the first four days, mortality was assessed twice daily, then once daily thereafter.

An analysis made after the inoculations confirmed the adequacy and the homogeneity of the inocula administered to each animal; no significant differences were found compared to the samples collected prior to inoculation.

### Preparation and Administration of the Drugs

The antibiotics selected were teicoplanin [Targocid, Hoechst-Marion-Roussel, São Paulo-SP, Brazil] for *S. aureus* and amikacin [Novamin, Bristol-Myers-Squibb, São Paulo- SP, Brazil] for *E. coli* and *K. pneumoniae*. The sensitivity of the bacteria was previously confirmed *in vitro* for both antibiotics. The teicoplanin solution was administered in a load dose of 6 mg/kg and a maintenance dose of 6mg/kg/day, divided into doses of 3mg/kg in volumes of 0.1mL, given intramuscularly every 12 hours. Each antibiotic was administered during the first four days of the experiment, and then discontinued. The amikacin solution was administered at a dose of 15mg/kg/day, divided into doses of 7.5mg/kg, in volumes of 0.1mL, given intramuscularly every 12 hours. This antibiotic was used during the first four days of the experiment and then discontinued.

### Statistical Analysis

Analysis was made with the Z test to compare two proportions between independent samples. A p value < 0.05 indicated significance.

## Results

### Gram-Negative Bacilli

Survival Tables 1 and 2 summarize the outcomes with *E. coli* and *K. pneumoniae*, respectively. In all subgroups, the initial number of mice was 40. The control subgroups of antibiotic, corticosteroid and diluent yielded no mortality. During the first 12 hours of observation, the mortality in all subgroups was null.

After the third day there were no changes in the mortality rates in any subgroup. The final mortality in subgroup I (infection control subgroup) was 100%.

When the antibiotic was used alone (subgroup II; amikacin 15mg/kg/day for four days), the mortality rate decreased significantly ( $p < 0.00001$ , when compared to the infection control subgroup) and 18 mice survived (a mortality of 55%). The use of a corticosteroid prior to amikacin (subgroup IV) reduced the mortality even more: 32 of the initial 40 mice survived (mortality of 20%), giving a better result than amikacin alone ( $p < 0.001$ ). However, when the corticosteroid was administered after the antibiotic (subgroup III), the final result was inferior to its use prior to the antibiotic ( $p < 0.001$ ), and there was no significant difference compared to subgroup II (antibiotic alone,  $p = 0.2488$ ).

At the 24<sup>th</sup> hour of the experiment, subgroup II had a higher mortality rate when compared to the non-treated subgroup ( $p < 0.05$ ).

Mortality caused by *K. pneumoniae* (Table 2) was more pronounced than that induced by *E. coli* (comparison between both subgroups IV:  $p < 0.01$ ; comparison between both subgroups III:  $p < 0.05$ ; for subgroups II this difference in mortality caused by *E. coli* and *K. pneumoniae* was not quite significant:  $p = 0.051$ ). In the infection control subgroup, the final observed mortality was also 100%. When the infection was treated with the antimicrobial alone, the mortality decreased to 72.5% ( $p < 0.001$ ). An additional protective effect was seen when the corticosteroid was used prior to the antibiotic (subgroup IV); in this subgroup, the mortality (45%) was significantly lower than in subgroup II ( $p < 0.01$ ). On the other hand, when the corticosteroid was administered after the antibiotic (subgroup III), the final result was inferior compared to the administration of the corticosteroid prior to the antibiotic (subgroup IV,  $p < 0.01$ ), and there was no significant difference when compared to the results of the subgroup which received amikacin alone ( $p = 0.215$ ), which also resulted in a higher mortality within the first 24 hours (37.5%) when compared to the non-treated subgroup (5%,  $p < 0.001$ ).

**Table 1.** Survival in the *E. coli* group.

Time (days)	Number of mice surviving (initial n = 40 in all subgroups)														final											
	1	2	3	4	5	6	7	8	9	10	12	24	36	48		60	72	84	96	120	144	168	192	216	240	
ctrl. A	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40
ctrl. B	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40
ctrl. C	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40
I	40	35	28	16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
II	40	28	26	26	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18
III	40	30	21	21	17	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15
IV	40	36	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32

ctrl. A = antibiotic control; ctrl. B = diluent control; ctrl. C = corticosteroid control; subgroup I: infection control; subgroup II: mice treated only with antibiotics; subgroup III: mice treated with antibiotics prior to the corticosteroid; subgroup IV: mice treated with the corticosteroid prior to antibiotics.

**Table 2.** Survival in the *K. pneumoniae* group.

Time (days)	Number of mice surviving in the different treatment groups (initial n = 40 in all subgroups)														final											
	1	2	3	4	5	6	7	8	9	10	12	24	36	48		60	72	84	96	120	144	168	192	216	240	
ctrl. A	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40
ctrl. B	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40
ctrl. C	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40
I	40	38	21	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
II	40	25	18	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11
III	40	30	23	21	21	10	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
IV	40	31	26	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22

ctrl. A = antibiotic control; ctrl. B = diluent control; ctrl. C = corticosteroid control; subgroup I: infection control; subgroup II: mice treated only with antibiotics; subgroup III: mice treated with antibiotics prior to the corticosteroid; subgroup IV: mice treated with the corticosteroid prior to antibiotics.

### Infection with *S. aureus*

Table 3 shows the results of the experiment with *S. aureus*. The final mortality of control subgroups of antibiotic, diluent and corticoid was zero. During the first 12 hours of observation, the mortality in all subgroups was zero.

Relevant differences in the results regarding Gram-negative bacteria were observed: at 24 hours, the mortality was higher in subgroup I than in subgroup II (62.5 and 30% respectively,  $p < 0.001$ ).

Although the final mortality in subgroup I (infection control) in the *S. aureus* group was lower than in the GNB groups, mortality in the first 24 hours for this same subgroup was considerably higher in the *S. aureus* experiment than in the *E. coli* or *K. pneumoniae* experiments ( $p < 0.0001$ ).

The difference in mortality between subgroups II and III (mortality rates of 42.5 and 32.5%, respectively) was not significant ( $p = 0.1792$ ); nevertheless, the mortality in these two subgroups was significantly lower than the mortality observed in the same subgroups in the experiments with Gram-negative bacilli.

In subgroup III, mortality was higher than in the subgroups that used only the antibiotic or the antibiotic after the corticosteroid ( $p < 0.0001$ ).

In subgroup I (infection control), mortality (82.5%) was significantly higher than in subgroups II and IV ( $p < 0.00001$ ). The incidence of mortality did not change after 48 hours.

### Discussion

We chose those three kinds of bacteria to include the most important mechanisms or models of sepsis in clinical practice, that is, Gram-negative bacilli (encapsulated and unencapsulated) and *S. aureus*.

The subdivision of the three groups (*S. aureus*, *E. coli* and *K. pneumoniae*) into seven subgroups allowed us to include all the possible controls. A subgroup treated only with corticosteroid was not included because this would only be used to treat endotoxic shock [31]. Given the effectiveness of corticosteroids for the treatment of endotoxic shock, we understood the importance of excluding the possibility of a large amount of pre-formed endotoxin in the inoculum (from

bacterial growth and multiplication) [32] in the bacterial solution. Without this precaution, any eventual beneficial effect of methylprednisolone in our study would only be due to its action against the effects of the endotoxin (consequently, a possible higher mortality in the subgroups not treated with this drug could be attributed to the effects of a large volume of injected endotoxin). The precaution of washing the bacteria was taken for this reason, and the bacterial suspension was injected into the mice immediately thereafter; this procedure minimizes the amount of endotoxin in the inoculum. The inoculation was also done immediately to prevent changes in the characteristics of the inoculum. To confirm that the inocula had not changed, colony forming counts per mL of the suspension were calculated in soy-casein agar.

The choice of antibiotics, amikacin for GNBs and teicoplanin for *S. aureus*, was made based on information available in the literature and on their use in humans. We administered the antibiotics to the animals using the same doses, routes and dosing intervals recommended for human treatments; these two drugs were selected because 1) the dosing intervals are longer (12/12h), which make the technicians' work easier; and 2) both can be administered intramuscularly (adequate and repeated IV administration of drugs in mice is quite difficult). The antimicrobials were used for four days and then discontinued; this procedure did not affect the evolution of disease in any group, as no further deaths occurred in the subgroups where antibiotics were used alone after the third day (in the *S. aureus* group, after the second day).

Methylprednisolone was chosen among all corticosteroids because there was data concerning optimal dosages in mice [32]. An intraperitoneal route was chosen, instead of intramuscular, because it provides better and quicker absorption.

It could be argued that the large surface of the peritoneum facilitates rapid mobilization of bacteria into the circulation, making this model closer to one of endotoxic shock. But if this was true, then the survival rates would have been similar for the subgroups that received the corticosteroid prior to the antibiotic (subgroups IV) and those that received it after the

**Table 3.** Survival in the group *S. aureus*.

Number of mice surviving in the different treatment groups (initial n = 40 in all subgroups)																					
Time (days)	1		2		3		4		5		6		7		8		9		10		final
Time (hours)	12	24	36	48	60	72	84	96	120	144	168	192	216	240							
ctrl. A	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40
ctrl. B	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40
ctrl. C	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40
I	40	15	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
II	40	28	23	23	23	23	23	23	23	23	23	23	23	23	23	23	23	23	23	23	23
III	40	12	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
IV	40	32	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27

ctrl. A = antibiotic control; ctrl. B = diluent control; ctrl. C = corticosteroid control; subgroup I: infection control; subgroup II: mice treated only with antibiotics; subgroup III: mice treated with antibiotics prior to the corticosteroid; subgroup IV: mice treated with the corticosteroid prior to antibiotics.

antibiotic (subgroups III), because corticosteroids alone can abort endotoxic shock. Consequently, even if a human sepsis model is not fully mimicked by experimental models, we believe that our study design was adequate to test the benefits of corticosteroids given early and prior to antibiotics.

Analysis was simplified by the large differences found. Though the mortality rates were higher with *K. pneumoniae* when compared with *E. coli* (we attributed this fact to the pathogenic characteristics of *K. pneumoniae*, due mainly to the protective capsule), we found similar trends in the results found with these two species; for this reason we discuss them together, separately from the results obtained with *S. aureus*.

The severity of infection with GNB was clear from the high mortality (100%) of the infection control subgroups (subgroups I). The final mortality in the subgroups treated with corticosteroid prior to the antibiotic (subgroups IV) was considerably lower when compared to the subgroups treated only with antibiotics (subgroups II) ( $p = 0.0009$  for the *E. coli* group and  $0.0073$  for the *K. pneumoniae* group). However, when the administration sequence was inverted and antibiotics started prior to corticosteroid treatment (subgroups III), these effects were not apparent and mortality was not significantly different from that found in subgroups where only amikacin was administered.

Mortality due to GNB during the first 24 hours was lower in subgroup I than in subgroup II ( $p < 0.05$  in the *E. coli* group and  $p < 0.001$  in the *K. pneumoniae* group). Although a superficial analysis might conclude that this finding is paradoxical, it actually reinforces the previously-discussed postulates: this mortality, initially higher in the subgroup treated with antibiotics can be understood as secondary to the sudden release of large amounts of LPS, due to bacterial lysis, which was induced by the antibiotic. In the subgroup treated with methylprednisolone prior to amikacin, the steroid probably inhibited the macrophages, so that subsequent reactions were blocked, resulting in lower mortality rates in this subgroup throughout the study (Tables 1 and 2). In the *K. pneumoniae*-infection group, whenever the corticosteroid was administered after the antibiotic (subgroup III), mortality increased enormously after the withdrawal of the antibiotics, suggesting that the use of corticosteroids after beginning antibiotics can be counterproductive; possibly it would be helpful if antibiotics were continued for a longer period.

Results regarding *S. aureus*. The reaction in the *S. aureus* group was quite different from that with GNB. Though the differences of mortality between subgroups II or IV and subgroup I were significant ( $p < 0.00001$ ), the small advantage observed in the survival of subgroup IV over subgroup II (27 survivors against 23) was not quite significant ( $p = 0.0603$ ).

Contrary to what was found with the GNB, the *S. aureus* group did not reproduce the initial difference in mortality (during the first 24 hours) favorable to subgroup I (infection control) in comparison with subgroup II (treated only with antibiotics): the difference was quite significant in favor of

this latter subgroup ( $p = 0.0023$ , Table 3). Nevertheless, in the subgroup treated with antibiotics prior to the corticosteroid (subgroup III), the mortality in 24 hours was higher than in all the other infected and treated subgroups ( $p < 0.001$ ) and not significantly different from subgroup I ( $p = 0.2388$ ). Possibly, in the case of *S. aureus*, the corticosteroid given after the antibiotic only exerts its immune-depressant effects, thus accelerating the death of the animals and overcoming the effects of the antibiotic, leading to a kind of response that is equivalent to non-treatment of infection.

The mortality rate in the infection control subgroup during the first 24 hours was much higher in the *S. aureus* group than in the equivalent subgroups of the other bacteria. The final rate of mortality in this subgroup was not 100%, as occurred with the GNB, but it was high enough (82.5%) to demonstrate the severity of the infection.

These differences reinforce the hypothesis that the pathogenic mechanisms of *S. aureus* are considerably different from those of GNB; the response of the host apparently follows different pathways in such a way that it is not affected by the corticosteroids. Similar findings, using antibodies against TNF- $\alpha$  instead of corticosteroids, and infection by other Gram-positive bacteria (*Streptococcus pyogenes*), were reported by Wayte [33].

These differences between GNB and *S. aureus* infection reinforce the hypothesis that the evolution of sepsis at the biomolecular level is quite different for Gram-positive and Gram-negative bacteria. Possibly, in the case of *S. aureus*, there are one or more parallel chain-reactions, besides that of TNF- $\alpha$ , which are not responsive to the blockages exerted by corticosteroids.

Although corticosteroids have been successfully used in experimental studies in the past [32,34,35], their use in human sepsis has not always proved beneficial. Unfortunately, studies in human beings are not easy to interpret, given the lack of a precise definition for the term sepsis and the impossibility of determining the exact moment infection begins. Consequently, the recommended "early" use of the corticosteroids may not have been early enough to guarantee efficacy. Schumer [36] concluded that they could be effective; however, this was refuted by subsequent studies [37-39]. Even if all these authors were concerned about the early use of corticosteroids, none of them addressed the relative timing between corticosteroids and antibiotics, which we think is critical and therefore controlled in our experiment. Nevertheless, controversy concerning human sepsis continues [40-43]; even if in more recent years the work by Annane et al. seems to have changed current opinion to favor the use of corticosteroids in human sepsis [44 - 46], they and others [47] suggest using these drugs only in low doses, as replacement therapy in an adrenal failure setting or for peripheral resistance to endogenous glucocorticoids. In previous experimental studies, the early application of corticosteroids may have allowed these drugs to begin

action before the antibiotic had caused the rupture of a large number of bacteria. High doses corticosteroids would have inhibited the macrophages before these cells had contact with large amounts of lipopolysaccharide, thus preventing triggering of the septic cascade.

Our opinion is that corticosteroids should be used early and prior to the use of antibiotics, in order to achieve the best results.

### Study Limitations

- 1) We did not check the minimum inhibitory concentrations (MIC) of the antibiotics used for each microorganism; even though the good survival rates for infected mice treated only with antibiotics suggests an adequate MIC, it would have been useful to check this item. However, an antibiogram by Kirby-Bauer's method was provided.
- 2) Unfortunately endotoxins were not measured in our study, so that our conclusions regarding endotoxin release rest on the logic of the vast literature concerning this issue.
- 3) We did not proceed to a *post-mortem* study.
- 4) Finally, TNF-alpha and other mediators were not measured; such measurements might have reinforced our conclusions, but we did not have sufficient funds for such analyses.

### Conclusions

- 1) The use of methylprednisolone early and prior to antibiotics reduced the mortality in this model of severe experimental infection by *E. coli* or *K. pneumoniae*.
- 2) The response of *S. aureus* to application of methylprednisolone prior to the antibiotic did not coincide with that observed in the Gram-negative bacilli experiments, failing to promote a significant reduction in mortality;
- 3) The use of methylprednisolone after the antibiotic had already been started was not better than the use of the antibiotic alone against infection with Gram-negative bacilli, and it increased the mortality rate in the case of *S. aureus* infection.

This is a primary observation in an experimental model; clearly, these conclusions should not be translated to humans. However, a potential clinical implication of this work would be to use corticosteroids in patients not yet managed with antibiotics who arrive in the emergency room with a disease that provokes high mortality rates, such as, for example, acute severe community-acquired pneumonia. More preliminary studies must be done with animal models before attempting this in humans.

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