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ORIGINAL

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Gluco- and mineralocorticoid biological effects of a 7-day treatment with low doses of hydrocortisone and fludrocortisone in septic shock

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Abstract *Purpose:* The benefits of low-dose steroids in septic shock remain controversial. We investigated if these low doses were able to induce their expected hormonal effects by analyzing the biological modifications observed during the study, which first demonstrated the survival benefit of low-dose steroids. *Methods:* This was a multicenter, placebo-controlled, randomized, double-blind study in which 299 septic shock patients received a 7-day treatment with a combination of hydrocortisone (50 mg intravenously four times daily) and fludrocortisone (50 μg orally once daily) or matching placebos. Gluco- and mineralocorticoid biological effects observed during the 7 days of treatment were compared between groups. *Results:* Steroids significantly decreased eosinophil counts from day 2 to day 7. Steroids significantly increased plasma glucose from day 2 (compared with placebos: +0.8 mmol/l) to day 7 (+1.8 mmol/l) and cholesterol from day 3 (+0.54 mmol/l) to day 7 (+0.39 mmol/l). Steroids significantly increased plasma sodium from day 3 (+2 mmol/l) to day 7 (+5 mmol/l) and significantly decreased plasma potassium on day 7 (−0.2 mmol/l). Steroids significantly decreased urinary sodium/potassium ratio from day 2 (−47 %) to day 7 (−57 %) and sodium fractional excretion from day 3 (−25 %) to day 7 (−66 %). Steroids significantly increased urine output on day 4 and 5 and osmolar clearance from day 4 to day 7, and decreased free-water clearance from day 4 to day 7, this effect being significant on day 4 and 6. *Conclusions:* In septic shock, low-dose steroids induced both gluco- and mineralocorticoid biological effects and seemed to improve renal function. Most of these effects appeared after 2–3 days of treatment and lasted at least until the end of treatment.

Keywords Fludrocortisone · Glucocorticoid · Hydrocortisone · Mineralocorticoid · Randomized controlled trial · Septic shock

Introduction

Septic shock is frequently associated with relative adrenal insufficiency [1–3] or critical illness–related corticosteroid insufficiency [4]. This observation led to the concept of hormonal replacement in this indication and to a first trial (Ger-Inf-05 study) in which a combination of hydrocortisone (HC), 50 mg intravenously every 6 h, and fludrocortisone (FC), 50 μg orally once daily, was shown to improve the prognosis of patients with vasopressor-unresponsive septic shock, especially of those with relative adrenal insufficiency (nonresponders to a short corticotropin stimulation test) [5]. Widespread adoption of low-dose corticosteroids for treatment of severe sepsis and septic shock followed the publication of this trial [6], and the results obtained on tertiary endpoints were never published. However, similar data were not found when HC was given alone in less severe septic shock (CORTICUS study) [7] or when FC was given as an add-on drug in septic shock treated with HC (COIITSS study) [8]. These new results raised a controversy regarding the benefits and use of low-dose corticosteroids in severe sepsis and septic shock, leading to conflicting recommendations [9], even if, in the meantime, two meta-analyses confirmed the favorable effects of low-dose steroids in septic shock [10, 11]. Another controversy was raised regarding the interest in dual corticosteroid therapy combining HC and FC [12]. Indeed, there is doubt regarding the bioavailability of orally administered FC in patients with impaired hepatosplanchnic perfusion [13], and 200 mg per day of HC could provide enough mineralocorticoid supplementation, making the administration of FC irrelevant [4, 14]. Finally, it is now well established that critical illness is accompanied by tissue corticosteroid resistance [15], which could explain less biological effect of steroids. A possible way to progress in the understanding of these issues could be to assess the pharmacodynamics of these low doses of steroids.

In the Ger-Inf-05 study, we prospectively monitored many biological variables during the 7-day period of treatment (tertiary endpoints). Thus, we analyzed these data to determine if the doses used were sufficient to induce both gluco- and mineralocorticoid effects in septic shock patients. We also assessed if these effects differed in patients with and without relative adrenal insufficiency in order to evaluate if they could contribute to explain the clinical benefit observed in nonresponders.

Materials and methods

The Ger-Inf-05 study was a multicenter, placebo-controlled, randomized, double-blind trial in which patients received a combination of HC hemisuccinate (Roussel-Uclaf, Romainville, France), 50 mg intravenously every 6 h, and FC (Pharmacie Centrale des Hôpitaux, Paris, France), 50 μg orally once daily, or their respective placebos for 7 days. Before randomization, patients underwent a short corticotropin stimulation test with intravenous tetracosactrin (Synacthène Ciba, Rueil-Malmaison, France). They were graded as nonresponders for cortisol increase ≤9 μg/dl (250 nmol/l) and as responders otherwise.

The protocol of the study had been approved by an Institutional Review Board (Comité de Protection des Personnes dans la Recherche Biomédicale of St Germain en Laye, France). At the beginning of the study, written informed consent had to be obtained from the patients themselves or their relatives within 3 h of onset of shock [5]. However, this constraint made inclusions very difficult in research which could be considered to have major benefit for patients. Thus, the Institutional Review Board waived the need for informed consent 10 months after the beginning of the study [16].

Patients

Eligibility criteria have already been reported in detail [5]. Briefly, patients had to meet criteria for severe septic shock with (a) documented site (or at least strong suspicion) of infection, (b) temperature >38.3 °C or <35.6 °C, (c) heart rate >90 beats/min, (d) systolic arterial pressure <90 mmHg for at least 1 h despite adequate fluid replacement and >5 μg/kg/min of dopamine or current treatment with epinephrine or norepinephrine, (e) urinary output <0.5 ml/kg for at least 1 h or PaO_2/FiO_2 ratio <280 mmHg, (f) arterial lactate levels >2 mmol/l, and (g) need for mechanical ventilation. Patients had to be randomized within 8 h of onset of shock.

Variables

Blood and urine samples were drawn daily in the morning. The following hematological and biochemical variables were systematically recorded during the 7-day treatment period: hemoglobin, hematocrit, platelet count, leukocyte with neutrophil and eosinophil counts, plasma and urinary sodium, potassium, urea, creatinine, and glucose, plasma cholesterol, and urine output. Hematological and plasma and urinary biochemical variables were determined using standard methods in each participating center. The following variables were calculated: urinary sodium/potassium ratio; sodium fractional excretion (%) = (urine sodium × plasma creatinine)/(plasma sodium × urine creatinine); creatinine clearance (ml/min) = [(140 − age) × body weight/plasma creatinine] × 1.23 for male and × 1.04 for female, according to Cockcroft and Gault formula [17]; osmolar clearance

(ml/min) = [urinary osmolality × urine flow rate]/plasma osmolality; free-water clearance (ml/min) = urine flow rate − osmolar clearance. The renal Sequential Organ Failure Assessment (SOFA) score was also determined [18].

Statistical analysis

The statistical analysis was performed using SAS statistical software V9.2 (SAS Institute, Cary, NC). Variables are expressed as medians (interquartile ranges). All available data were included in the statistical analysis according to the intent-to-treat principle. Baseline variables were compared between the two groups of treatment and between responders and nonresponders by use of two-way (treatment, corticotropin response) nonparametric analysis of variance. The treatment effect compares patients who received steroids versus patients who received placebos. The corticotropin response effect compares responders versus nonresponders to the short corticotropin stimulation test. Evolution with time of biological variables was analyzed using three-way (treatment, corticotropin response, time) nonparametric repeated-measures analysis of variance (Friedman test). Three second-order interactions (treatment × corticotropin response, time × treatment, time × corticotropin response) and one third-order interaction (treatment × corticotropin response × time) were entered in the model. In case of significant treatment effect, time-by-time comparisons were performed by use of the least-squares means procedure. In time-by-time comparisons, reported effects are either differences between median values in placebo and steroids groups or percentages of variations between these median values with reference to placebo. All other reported percentages of variations between responders and nonresponders refer to median values. For all analyses, p value <0.05 was considered significant. All reported p values are two-sided.

Results

Baseline characteristics

All 299 septic shock patients analyzed in the primary analysis were included in this secondary, a priori planned, analysis. At baseline, the two groups (placebo and steroids) were balanced with respect to general characteristics, severity of illness, and type and site of infection [5]. Table 1 presents the characteristics of the patients at baseline according to treatment allocation (placebo and steroids) and to response to the corticotropin stimulation test (responders and nonresponders). There was no significant difference between the two treatment groups for any of the biological variables considered. Compared with responders, nonresponders had higher plasma creatinine, and lower plasma cholesterol and creatinine clearance.

Hematology

Steroids did not modify hemoglobin and hematocrit levels, and leukocyte and neutrophil counts but significantly decreased eosinophil counts from day 2 to day 7 and platelet counts (see Electronic Supplementary Material, ESM).

Glucocorticoid biochemical effects

Figure 1 shows the evolution with time of plasma glucose and cholesterol. There was a significant treatment effect on both variables ($p < 0.001$ for both), these effects being not different in responders and nonresponders (treatment × corticotropin response interaction, $p = 0.52$ for glucose and $p = 0.74$ for cholesterol). Steroids significantly increased plasma glucose between day 2 and day 7 (+0.8 mmol/l on day 2, +1.8 mmol/l on day 3, +1.3 mmol/l on day 4, +1.8 mmol/l on day 5, +2.3 mmol/l on day 6, and +1.8 mmol/l on day 7) and plasma cholesterol between day 3 and day 7 (+0.54 mmol/l on day 3, +0.71 mmol/l on day 4, +0.59 mmol/l on day 5, +0.42 mmol/l on day 6, and +0.39 mmol/l on day 7). There was also a significant corticotropin response effect for plasma cholesterol ($p = 0.001$) reflecting that nonresponders had significantly lower values of cholesterol (−12 %) compared with responders.

Mineralocorticoid biochemical effects

Steroids significantly increased plasma sodium from day 3 (+2 mmol/l) to day 7 (+5 mmol/l) and significantly decreased plasma potassium on day 7 (−0.2 mmol/l) (see ESM).

Figure 2 shows the evolution with time of urinary sodium/potassium ratio and sodium fractional excretion. There was a significant treatment effect on both variables ($p < 0.001$ for both), these effects being not different in responders and nonresponders (treatment × corticotropin response interaction, $p = 0.95$ for urinary sodium/potassium ratio and $p = 0.13$ for sodium fractional excretion). Steroids significantly decreased urinary sodium/potassium ratio between day 2 and day 7 (−47, −62, −61, −50, −42, and −57 %, respectively) and sodium fractional excretion between day 3 and day 7 (−25, −68, −37, −52, and −66 %, respectively). There was also a significant corticotropin response effect for both variables ($p < 0.001$ for both) reflecting that nonresponders had higher urinary sodium/potassium ratio and sodium fractional excretion values (+12 and +64 %, respectively) compared with responders.

Table 1 Biological variables at baseline

Variables	Placebo, $n = 149$	Steroids, $n = 150$	p_1	Responders, $n = 70$	Nonresponders $n = 229$	p_2
Hemoglobin (g/dl)	9.9 [8.6–11.3]	9.6 [8.7–11.2]	0.66	10.3 [8.4–11.7]	9.7 [8.7–11.1]	0.39
Hematocrit (%)	30.0 [26.6–34.3]	29.5 [26.0–34.1]	0.62	30.3 [25.0–36.3]	29.6 [26.2–3.7]	0.40
Platelets (10^9/l)	153 [86–227]	130 [75–207]	0.16	163 [83–253]	134 [82–203]	0.15
Leukocytes (10^9/l)	12.2 [6.2–19.0]	11.6 [5.5–16.9]	0.91	13.7 [7.2–18.5]	11.5 [5.3–18.3]	0.15
Neutrophils (10^9/l)	11.2 [5.9–17.1]	9.9 [4.8–14.4]	0.42	11.4 [6.4–15.2]	10.3 [4.9–16.4]	0.42
Eosinophils (10^6/l)	0 [0–90]	0 [0–65]	0.22	0 [0–90]	0 [0–65]	0.59
Sodium (mmol/l)	137 [133–141]	137 [133–142]	0.59	138 [133–142]	137 [133–142]	1.00
Potassium (mmol/l)	4.0 [3.7–4.4]	3.9 [3.6–4.4]	0.99	4.0 [3.5–4.4]	4.0 [3.6–4.4]	0.44
Urea (mmol/l)	11.5 [7.5–20.2]	12.3 [9.0–19.4]	0.68	10.8 [6.5–16.8]	12.3 [8.4–20.3]	0.34
Creatinine (μmol/l)	153 [96–236]	164 [113–230]	0.53	132 [83–181]	165 [107–246]	**0.037**
Glucose (mmol/l)	8.2 [6.1–13.5]	7.8 [5.9–11.7]	0.24	8.3 [6.0–11.7]	7.9 [6.1–12.4]	0.58
Cholesterol (mmol/l)	1.87 [1.27–2.54]	1.67 [1.32–2.65]	0.24	2.14 [1.41–2.81]	1.69 [1.31–2.53]	**0.048**
Urinary sodium/potassium	1.2 [0.5–2.4]	1.0 [0.4–1.8]	0.06	0.9 [0.4–2.1]	1.2 [0.5–2.2]	0.46
Sodium fractional excretion (%)	0.75 [0.29–1.95]	0.80 [0.26–2.27]	0.57	0.41 [0.20–1.79]	0.85 [0.33–2.15]	0.22
Urine output (ml/min)	25 [0–70]	18 [0–50]	0.09	40 [10–70]	16 [0–50]	0.62
Creatinine clearance (ml/min)	41 [27–66]	40 [26–58]	0.31	51 [32–83]	38 [26–58]	**<0.001**
Osmolar clearance (ml/min)	0.98 [0.46–1.67]	0.82 [0.36–1.76]	0.24	0.82 [0.36–1.62]	0.94 [0.41–1.75]	0.47
Free-water clearance (ml/min)	0.00 [−0.16 to +0.13]	0.01 [−0.19 to +0.08]	0.52	−0.03 [−0.33 to +0.08]	0.01 [−0.12 to +0.15]	0.90

Data are medians [25th percentile to 75th percentile]

Data were available for at least 293 patients except for urine output ($n = 284$), neutrophils ($n = 219$), eosinophils ($n = 217$), cholesterol ($n = 193$), urinary sodium/potassium ($n = 188$), sodium fractional excretion ($n = 156$), and osmolar clearance and free water clearance ($n = 127$)

p_1 p value for "treatment" effect; p_2 p value for "corticotropin response" effect

Significant p values are in bold

Renal function

Steroids significantly increased urine output on day 4 and 5 but did not modify creatinine clearance and renal SOFA score (see ESM).

Figure 3 shows the evolution with time of osmolar clearance and free-water clearance, respectively. There was a significant treatment effect on both variables ($p = 0.003$ for osmolar clearance and $p = 0.009$ for free-water clearance), these effects being not different in responders and nonresponders (treatment × corticotropin response interaction, $p = 0.61$ for osmolar clearance and $p = 0.89$ for free-water clearance). Osmolar clearance increased, nonsignificantly on day 2 and day 3 (+0.17 ml/min for both days), and significantly from day 4 to day 7 (+0.56, +0.35, +0.73, and +0.23 ml/min, respectively). Free-water clearance decreased significantly on day 4 and day 6 (−0.27 and −0.35 ml/min, respectively). Although not significantly, it also decreased on day 5 and day 7 (−0.17 ml/min, $p = 0.15$, and −0.31 ml/min, $p = 0.06$, respectively).

Discussion

In this secondary analysis of the Ger-Inf-05 study, we showed that the combination of low doses of HC and FC induced both gluco- and mineralocorticoid effects. Glucocorticoid effects were illustrated by the increases of plasma glucose and cholesterol. Mineralocorticoid effects were illustrated by the increase of plasma sodium and the decreases of urinary sodium/potassium ratio and of sodium fractional excretion. All these effects were similar in responders and nonresponders. Steroids seemed also to improve renal function, especially in nonresponders, as shown by the increase of creatinine clearance and decrease of renal SOFA score observed in this subgroup. All these biological modifications appeared at the same time as the improvement of patients' outcomes (catecholamine requirement and survival) reported in the primary analysis [5].

These observations are useful in the context of the controversy about (i) use of low doses of steroids in septic shock and (ii) the utility of FC in this indication. Septic shock is frequently associated with critical illness–related corticosteroid insufficiency, which could justify both gluco- and mineralocorticoid supplementation [2, 4, 5]. However, with respect to mineralocorticoid potency, 20 mg HC is considered to be equivalent to 50 μg FC [19, 20]. Therefore, a daily dose of 200 mg HC could be sufficient for mineralocorticoid replacement. In a recent study performed in healthy volunteers with experimentally induced hypoaldosteronism, we assessed the biological effects of single administrations of HC and FC, given alone or in combination, using the same single doses and routes of administrations as used in the present trial [21]. We found that 50 mg HC administered intravenously induced more marked and earlier

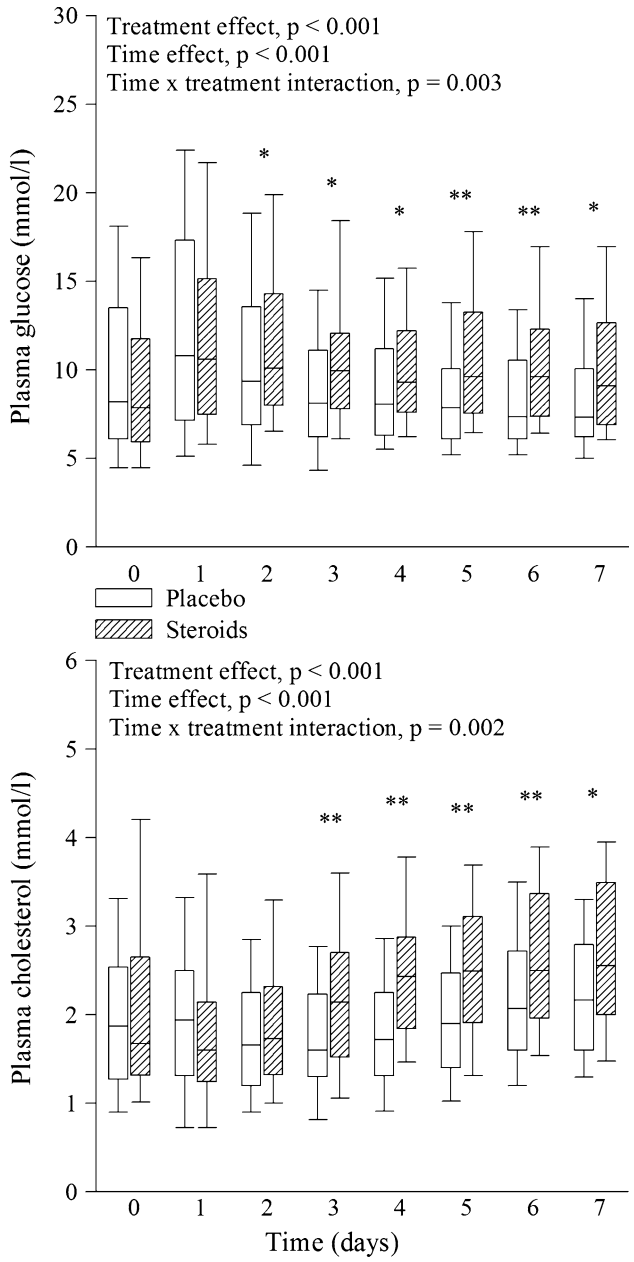

Fig. 1 Evolution with time of plasma glucose and cholesterol; $*p < 0.05$; $**p < 0.01$. Data are presented as *box plots*. The lower and higher boundaries of the *box* indicate the 25th and 75th percentile. The *line* within the *box* marks the median, and *error bars* below and above the *box* indicate the 10th and 90th percentiles

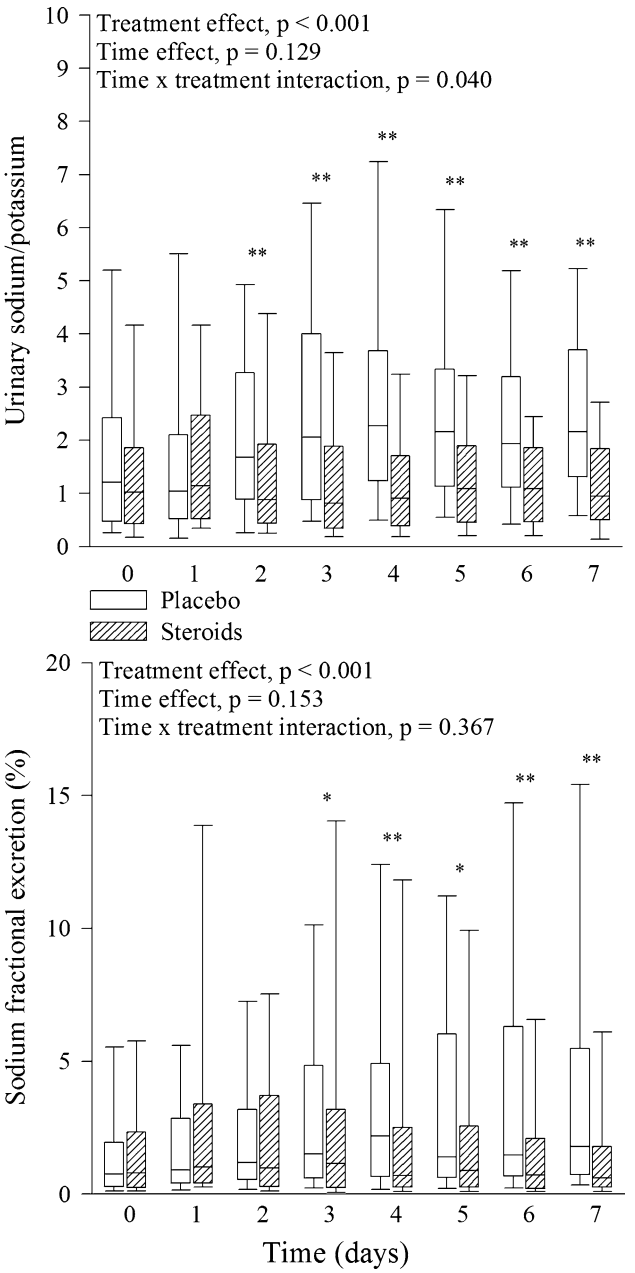

Fig. 2 Evolution with time of urinary sodium/potassium and sodium excretion fraction; $*p < 0.05$; $**p < 0.01$. Data are presented as *box plots*. The lower and higher boundaries of the *box* indicate the 25th and 75th percentile. The *line* within the *box* marks the median, and *error bars* below and above the *box* indicate the 10th and 90th percentiles

mineralocorticoid effects than 50 µg FC administered orally, and that the effects of HC and FC were additive. However, these biological effects appeared between 4 and 8 h following treatment administration, i.e., far earlier than the effects observed in the present study. These different kinetics of effects between patients and healthy volunteers could result from decreased glucocorticoid access to tissues, decreased glucocorticoid receptor number/affinity [22], and altered cortisol metabolism due to increased 11β-hydroxysteroid dehydrogenase activity [23]. This suggests that FC could have contributed to the mineralocorticoid effects observed in our patients. However, in another study which assessed the effect of low-dose HC alone in septic shock [24], mineralocorticoid

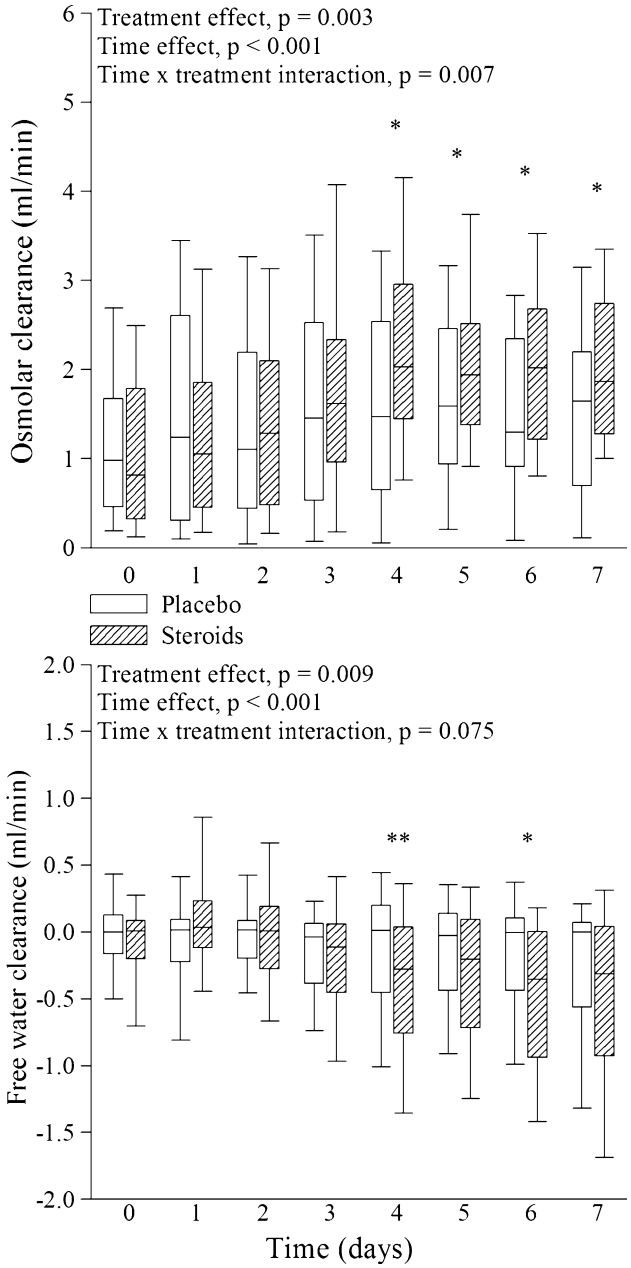

Fig. 3 Evolution with time of osmolar clearance and free-water clearance; *$p < 0.05$; **$p < 0.01$. Data are presented as *box plots*. The lower and higher boundaries of the *box* indicate the 25th and 75th percentile. The *line* within the *box* marks the median, and *error bars* below and above the *box* indicate the 10th and 90th percentiles

effects were also observed (at day 3, there was a significant increase in plasma sodium in the HC group compared with the placebo group). These similar kinetics of effects, in similar patients, suggest that the addition of FC did not accelerate (nor contribute to) mineralocorticoid effects in our patients. Thus, additional studies have to be performed before reaching a definitive conclusion on this issue.

Concerning glucocorticoid effects, as could be expected, we observed an early and sustained increase of blood glucose in the steroid group [8, 21]. It is noteworthy that blood glucose, which can be considered as a pharmacodynamic biomarker of the glucocorticoid effect, can easily be managed using conventional insulin therapy [8]. In our study, started before the publication of the first trial demonstrating the importance of glucose control in critically ill patients [25], the protocol did not recommend insulin therapy, and blood glucose levels were therefore markedly higher than in more recent trials using steroids in septic shock [8]. We also observed an early and sustained increase of plasma cholesterol in the steroid group. This effect can be related to the anti-inflammatory properties of glucocorticoids, since low plasma cholesterol concentrations correlate with the degree of inflammation and are related to poor prognosis in severe sepsis [26]. Moreover, there was a decrease of eosinophil count in the steroid group without any significant effect on neutrophil count. This effect on eosinophil count can also be related to the anti-inflammatory properties of glucocorticoids, which inhibit the interleukins implicated in eosinophil growth and differentiation [mainly interleukin (IL)-3, IL-5, and granulocyte macrophage colony-stimulating factor (GM-CSF)] and promote eosinophil apoptosis [27]. In a previous subgroup analysis of our trial performed in patients with acute respiratory distress syndrome (ARDS), we showed that steroids significantly decreased plasma IL-6 levels with a rapid onset (starting on day 2) and long duration (lasting until day 7) [28]. The size of the effect on eosinophils could argue for a marked anti-inflammatory effect of low-dose steroids, as compared with effects induced on other biomarkers. Overall (all times being combined), steroid-treated patients had an 88 % decrease of eosinophils as compared with placebo. This effect was higher than those on glucose (+15 %), cholesterol (+18 %), urinary sodium/potassium ratio (−48 %) or sodium fractional excretion (−43 %). The number of circulating eosinophils is also correlated to adrenal function, and an increase in circulating eosinophils in critically ill patients is associated with clinical signs of relative adrenal insufficiency [29]. Therefore, the decrease of eosinophil count observed in our study in the steroid group may reflect both the anti-inflammatory effect of glucocorticoids and the improvement of patients' status.

Steroids increased osmolar clearance and decreased free-water clearance together with an increase of urine output. Osmolar clearance might have increased as a result of glycosuria, because many patients who received steroids had blood glucose levels over 10 mmol/l. In addition, steroids probably increased catabolism, which augmented urinary urea excretion and therefore urine osmolality. The decrease of free-water clearance could be the consequence of an increase of plasma vasopressin

induced by steroids. Such an increase has been described after HC in a post hoc analysis of the VASST trial [30]. It is noteworthy that the increase of urine output is not in contradiction with the hypothesis of an increase of plasma vasopressin induced by steroids. In fact, it could be attributed to the natriuretic action of vasopressin when high plasma concentrations are achieved [31]. It could also result from the improvement of patients' hemodynamic status. However, given that free-water clearance and urine output were improved with steroids, we can also suggest that these effects reflect an improvement of renal function. The fact that, in the VASST trial, patients treated with both vasopressin and HC had an improvement of renal dysfunction also argues in favor of this hypothesis [30]. Acute renal failure is a common complication of septic shock. In this situation, improvement of renal function is characterized by an increase of urine flow rate with increases of creatinine and osmolar clearances and decreases of free-water clearance and of sodium fractional excretion [32]. Such improvements have already been observed in a canine endotoxic shock model with an inducible nitric oxide synthase (iNOS) inhibitor and were suggested to result from blockade of excessive NO production leading to enhanced water absorption at the collecting duct [33]. Improvement of renal function with steroids has also been described in patients with Addison's disease [34]. Interestingly, in our study, creatinine clearance and renal SOFA score improved with steroids, but only in the subgroup of nonresponders, i.e., the patients who benefited most from steroids [5]. Taken together, our data suggest that steroids improved water balance and electrolytes, resulting in an improvement of renal function that was more marked in nonresponders. This issue remains to be further evaluated, since creatinine clearance was not significantly different between steroid and placebo groups at any time point and the improvement of creatinine clearance in nonresponders with steroids was mild. However, the method we used to estimate creatinine clearance (namely the Cockcroft and Gault formula) has recently been shown to underestimate renal function in critically ill patients [35]. Finally, we cannot discard the hypothesis of insufficient statistical power to explain the absence of effect in the subgroup of responders. Therefore, it can be speculated that the benefit observed in renal function could have been underestimated in our study.

The biological effects of low-dose steroids were quite similar in responders and in nonresponders on the variables related to both gluco- (glucose, cholesterol, eosinophils) and mineralocorticoid (plasma sodium and potassium, urinary sodium/potassium ratio, and sodium fractional excretion) effects. These observations suggest that there was no relationship between these effects and disease severity. Thus, these biological effects can better be viewed as biomarkers of steroid administration rather than as biomarkers of the mechanisms underlying improved clinical outcome.

In conclusion, we showed that low doses of HC and FC induced both gluco- and mineralocorticoid effects and seemed to display favorable effects on renal function, especially in nonresponders. These pharmacological effects, which appeared after 2–3 days of treatment and lasted until the end of treatment, could be viewed as biomarkers of steroid effects in septic shock.

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Appendix: Ger-Inf-05 Study

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Independent Diagnosis Validation Committee: Jean Carlet (Paris), Didier Dreyfuss (Colombes), Philippe Veyssier (Compiègne).

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