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Original Research

Down-Regulation of the Mineralocorticoid Receptor (MR) and Up-Regulation of Hydroxysteroid 11-Beta Dehydrogenase Type 2 (HSD11B2) Isoenzyme in Critically Ill Patients

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Objective: The mineralocorticoid receptor (MR) has two ligands, aldosterone and cortisol. Hydroxysteroid 11-beta dehydrogenase (HSD11B) isoenzymes regulate which ligand will bind to MR. In this study we aimed to evaluate the expression of the MR and the HSD11B isozymes in peripheral polymorphonuclear cells (PMNs) in critical illness for a 13-day period.

Design: Prospective study

Setting: One multi-disciplinary intensive care unit (ICU)

Participants: Forty-two critically ill patients

Methods: Messenger RNA (mRNA) expression of *MR*, *HSD11B1*, and *HSD11B2*, aldosterone levels, and plasma renin activity (PRA) were measured in 42 patients on ICU admission and on days 4, 8, and 13. Twenty-five age and sex-matched healthy subjects were used as controls.

Results: Compared to healthy controls, *MR* expression in critically ill patients was lower during the entire study period. *HSD11B1* expression was also lower, while *HSD11B2* expression was higher. In patients, PRA, aldosterone, the aldosterone:renin ratio, and cortisol remained unaltered during the study period.

Conclusion: Our results suggest that, in our cohort of critically ill patients, local endogenous cortisol availability is diminished, pointing towards glucocorticoid resistance. Aldosterone probably occupies the MR, raising the possibility that PMNs might be useful to study to gain insights into MR functionality during pathological states.

Keywords: Aldosterone; Cortisol; Critical illness; Hydroxysteroid 11-beta dehydrogenase; Mineralocorticoid receptor; Neutrophils

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The mineralocorticoid receptor (MR) and the glucocorticoid receptor (GCR) are members of the steroid receptor superfamily. GCR is relatively ubiquitously expressed and exclusively binds glucocorticoids, which regulate genes controlling metabolism, stress, and immune response.[1] The MR shows a more restricted expression pattern; it is expressed at the greatest abundance in epithelial cells, where it is implicated in sodium reabsorption, water homeostasis, and potassium secretion.[2] The classical ligand for MR is aldosterone, the final step of the renin-angiotensin system.

Except aldosterone, cortisol also binds the MR with similar affinity. In the normal state, plasma glucocorticoid levels are more than 100 times higher than aldosterone levels, and most MRs are occupied by glucocorticoids. Hydroxysteroid 11-beta dehydrogenase (HSD11B) isoenzymes regulate whether cortisol or aldosterone will bind to MR.[3] The binding of cortisol or aldosterone to the MR results in different cellular responses.[4] HSD11B2 metabolises cortisol to inactive cortisone, which is unable to bind the MR, and thus, aldosterone activates the MR. HSD11B2 is largely restricted to the classical aldosterone (mineralocorticoid)-target tissues. HSD11B1 catalyses the regeneration of active glucocorticoids, is widely distributed, and amplifies glucocorticoid actions. Endogenous glucocorticoids play a critical role in controlling inflammatory responses. *In vitro*, co-localization of the two enzymes results in their reciprocal regulation to minimize simultaneous expression.[5]

We recently investigated the expression of *GCR* in polymorphonuclear cells (PMNs) of critically ill patients and showed that *GCR* was up-regulated.[6,7] *MR*s and *GCR*s are often co-expressed, and the expression of one receptor may cause compensatory changes in the other.[2,8,9] It remains currently unknown whether *MR* is also expressed in PMNs of intensive care unit (ICU) patients. The co-expression of the two receptors and the two isoenzymes is crucial for local cortisol availability.[2]

Given the above considerations, in the present study we measured *MR*, *HSD11B1* and *HSD11B2* expression in the same ICU patients, in whom *GCR* over-expression was previously demonstrated. The objectives were to assess the messenger RNA (mRNA) expression levels of *MR*, *HSD11B1,* and *HSD11B2* in ICU patients. Moreover, the relative expression of galectin-3 (*GAL3*) was additionally measured. Finally, the levels of the MR ligand, aldosterone, and its initial stimulator, plasma renin activity (PRA), were also determined.

Methods

Ethics Approval

The study was approved by the hospital's ethics committee (study approval number 48 - 3/03/2017). All procedures carried out were in compliance with the Helsinki Declaration. Informed written consent was obtained from all healthy subjects and patients' next-of-kin, prior to any study procedure.

Study Design

A prospective observational study of critically ill patients admitted in the multidisciplinary ICU of the hospital, over an 8-month period (April 2017 – November 2017).

Study Population

This prospective study included a cohort of 42 critically ill, mechanically ventilated patients suffering from medical, surgical, and trauma-related pathologies, the data of whom have been recorded in a different context.[6,7] The control group was comprised of 25 age- and sex-matched healthy blood donors, who were used for comparisons.

Total RNA Extraction and Evaluation of Quality

Total RNA was isolated from PMN cells using Trifast (Peqlab) and following the manufacturer's instructions. Total RNA concentration and quality were determined spectrophotometrically at 260 nm and 280 nm. The final preparation of total RNA was free of DNA and proteins and had a 260/280 ratio of 1.8-2.1.

Reverse Transcription and Quantitative Real-Time PCR (RT-PCR)

Total RNA (1 μg) from each sample was reverse transcribed into single-stranded cDNA in a 20 μl reaction mixture, using the FastGene Scriptase II cDNA Kit (Nippon Genetics), following the manufacturer's instructions. Thereafter, a highly sensitive quantitative RT-PCR method was used for the quantification of *GAPDH* and target mRNAs using specific primers. The sequences of the primers used were: for *GAPDH* the forward primer, consisting of 19 nt, 5′-ATG-GGG-AAG-GTG-AAG-GTC-G-3′ and the reverse of 23 nt 5′-TAC-ATG-AGG-GCA-CGG-AAG-ATG-3′; *MR* forward primer5′-GAA-AGA-CGG-TGG-GGT-CAA-GTT-3′ (21 nt) and reverse primer 5′-ACC-GGA-AAC-ACA-GCT-TAC-GTT-3′ (21 nt); *HSD11B1* forward primer 5'-GCA-AAG-GGA-TCG-GAA-GAG-AGA-3' (21 nt) and reverse primer 5'-GCT-GAG-GCT-GCT-CCA-AGC-T-3' (19 nt); *HSD11B2* forward primer 5'-CCG-TAT-TGG-AGT-TGA-ACA-GCC-3' (21 nt) and reverse primer 5'-CAA-CTA-CTT-CAT-TGT-GGC-CTG-C-3' (22 nt) and *GAL3* forward primer consisting of 24 nt, 5'-ATG-CAA-ACA-GAA-TTG-CTT-TAG-ATT-3' and reverse primer consisting of 24 nt, 5'-AGT-TTG-CTG-ATT-TCA-TTG-AGT-TTT-3'.

Quantitative RT-PCR analysis was performed in 96-well plates on a CFX Connect thermocycler (Bio-Rad). The 20 μl reaction mixture contained 5 ng cDNA, 300 nM primers and 1X Kapa SYBR® Green PCR Master Mix. The thermal protocol conditions consisted of 3 min at 95 °C polymerase activation step, 40 cycles of denaturation at 95 °C for 10 s, primer annealing and extension at 60 °C for 20 s. All samples were amplified in triplicates and the average C_T values were calculated for their subsequent expression analysis. Following amplification, a dissociation curve was generated to distinguish the PCR products of interest from the non-specific ones or any primer-dimers, through their particular melting temperatures (Tm), recorded in the software. Using the comparative C_T method 2^{-DDC_T} [10] and the healthy donor samples as a calibrator, the relative quantification of the expression analysis of all blood samples from the critically ill

Table 1: Clinical characteristics and laboratory data of critically ill patients on ICU admission. Patients' outcome and demographics of the controls are also shown.

Characteristics	Patients	Controls	*P* value
Number of patients, N (%)	42	25	
Age (years), (mean ± SD)	56 ± 19	52 ± 7	0.37
Sex, N (%)			>0.99
Male	25 (59.5%)	15 (60.0%)	
Female	17 (40.5%)	10 (40.0%)	
Comorbidities, N (%)			
Hypertension	7 (16.7%)		
Diabetes	1 (2.4%)		
Coronary artery disease	2 (4.8%)		
COPD	1 (2.4%)		
Chronic renal failure	1 (2.4%)		
Diagnosis, N (%)		N/A	
Medical	11 (26.2%)		
Surgical/Trauma	31 (73.8%)		
Characteristics on ICU admission		N/A	
APACHE II score, (median, IQR)	16 (12-21)		
SOFA score, (median, IQR)	8 (7-10)		
Vitals signs		N/A	
Heart rate (bpm), (median, IQR)	78 (64-105)		
Mean Arterial pressure (mmHg), (mean ± SD)	81 ± 19		
Temperature (°C), (mean ± SD)	37 ± 1.3 °C		
Laboratory data		N/A	
Hemoglobin (mean ± SD)	10.15 ± 2.13		
White Blood Cell count (per µl), (mean ± SD)	13574 ± 6015		
Platelets (per µl), (median, IQR)	159000 (127000-229500)		
Creatinine (mg/dl), (median, IQR)	0.77 (0.62-0.94)		
Glucose (mg/dl), (median, IQR)	122 (99-166)		
Bilirubin (mg/dl), (median, IQR)	0.50 (0.34-0.85)		
Albumin (g/dl), (mean ± SD)	3.03 ± 0.67		
Lactate (mmol/l), (median, IQR)	1.2 (0.8-1.7)		
C-reactive protein (mg/dl), (median, IQR)	9.53 (4.68-17.34)		
Mechanical ventilation, N (%)	42 (100%)	N/A	
Septic status, N (%)		N/A	
Sepsis	7 (16.7%)		
Septic shock	5 (11.9%)		
No sepsis, no septic shock	30 (71.4%)		
Outcomes			
LoS in the ICU (days), (mean ± SD)	24 ± 18		
Duration of mechanical ventilation (days), (mean ± SD)	13 ± 10		
ICU mortality, N (%)	13 (30.9%)		
28-day mortality, N (%)	9 (21.4%)		

Data are expressed as percentages of total related variable (%), mean ± SD for normally distributed variables, or median (IQR) for skewed data. Definition of abbreviations: ICU= Intensive care unit; COPD= Chronic obstructive pulmonary disease; APACHE= Acute physiology and chronic health evaluation; SOFA= Sequential organ failure assessment; LoS= Length of stay.

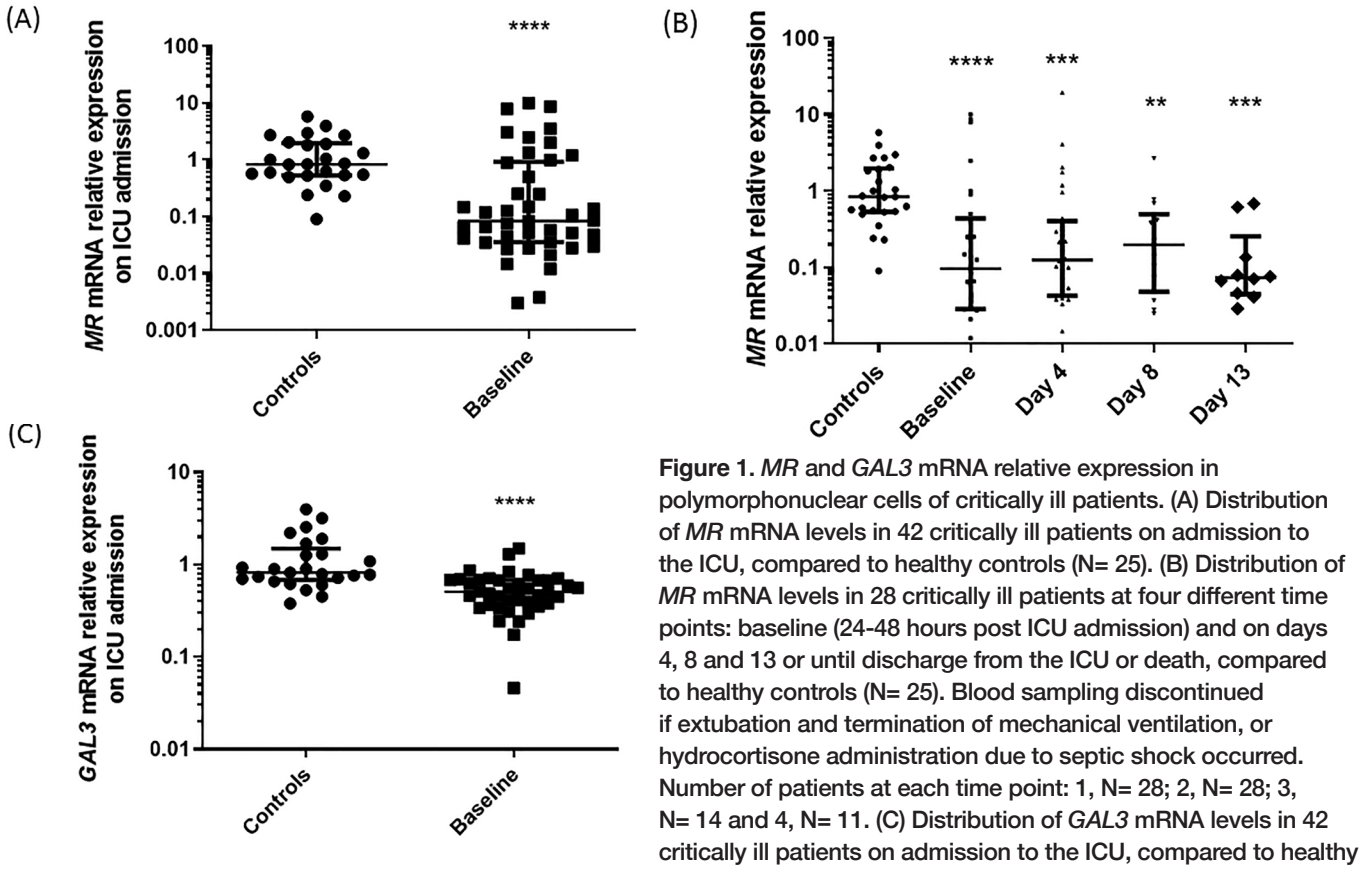

Figure 1. *MR* and *GAL3* mRNA relative expression in polymorphonuclear cells of critically ill patients. (A) Distribution of *MR* mRNA levels in 42 critically ill patients on admission to the ICU, compared to healthy controls (N= 25). (B) Distribution of *MR* mRNA levels in 28 critically ill patients at four different time points: baseline (24-48 hours post ICU admission) and on days 4, 8 and 13 or until discharge from the ICU or death, compared to healthy controls (N= 25). Blood sampling discontinued if extubation and termination of mechanical ventilation, or hydrocortisone administration due to septic shock occurred. Number of patients at each time point: 1, N= 28; 2, N= 28; 3, N= 14 and 4, N= 11. (C) Distribution of *GAL3* mRNA levels in 42 critically ill patients on admission to the ICU, compared to healthy controls (N= 25). Data are presented as scatter plots. Line in the middle, median value; lower and upper lines, 25th to 75th centiles. A, C: **** $P<0.0001$ from healthy controls using the non-parametric Mann-Whitney test for skewed data. B: ** $P<0.01$, *** $P< 0.001$, **** $P< 0.0001$ from controls using Kruskal-Wallis ANOVA followed by Dunn's multiple test.

patients was carried out. *GAPDH* expression was used for the normalization of the target gene expression levels between the different samples.

Human Plasma

A venous blood sample was drawn at four time points: within 24-48 hours after ICU admission (baseline), and on days 4, 8, and 13. Blood sampling discontinued earlier at extubation and termination of mechanical ventilation, hydrocortisone administration due to septic shock, or if death occurred. A single blood sample was also drawn from 25 healthy blood donors. Blood samples were collected in purple-topped Vacutainers tubes (BD), and plasma was prepared, according to the standard procedure.

Renin-Angiotensin System Function

Morning aldosterone and plasma renin activity (PRA) were measured in 31 of the 42 patients. Blood was drawn between 08.00 and 08.30 h at four time points: within 24-48 hours after ICU admission (baseline), and on days 4, 8, and 13. Aldosterone was measured by radioimmunoassay (RIA) (Active Aldosterone RIA, Beckman Coulter) and was expressed in pg/ml (normal values: 49.3-175 pg/mL). PRA was measured by ELISA (PRA immunoassay, IBL). PRA was expressed in ng/(mL*h).

Statistical Analysis

Data are presented as individual values, mean ± standard deviation (SD) for normally distributed variables, or median with interquartile range (IQR) for variables with skewed distribution. Two-group comparisons were performed using the Student's *t*-test or the non-parametric Mann-Whitney test for skewed data, and associations between qualitative variables were examined by the chi-square test or the Fisher's exact when appropriate. One-way ANOVAs for repeated measures and Kruskal-Wallis ANOVA followed by Dunn's post hoc test for more than two group comparisons. All aforementioned analyses were performed using the GraphPad Prism 6 statistical program (GraphPad Software, Inc). All P values are two-sided; $P<0.05$ were considered significant.

Results

Study Population

The patient cohort consisted of 25 men and 17 women, with a mean age of 56 ± 19 years. Median admission APACHE II and SOFA scores were 16 and 8, respectively. The 28-day and ICU mortalities were 21% and 31%, respectively. The clinical characteristics and laboratory data of the critically ill patients on ICU admission, and the demographics of the controls are presented in Table 1.[7] None of our patients received steroids, spironolactone, or angiotensin converting enzyme inhibitors.

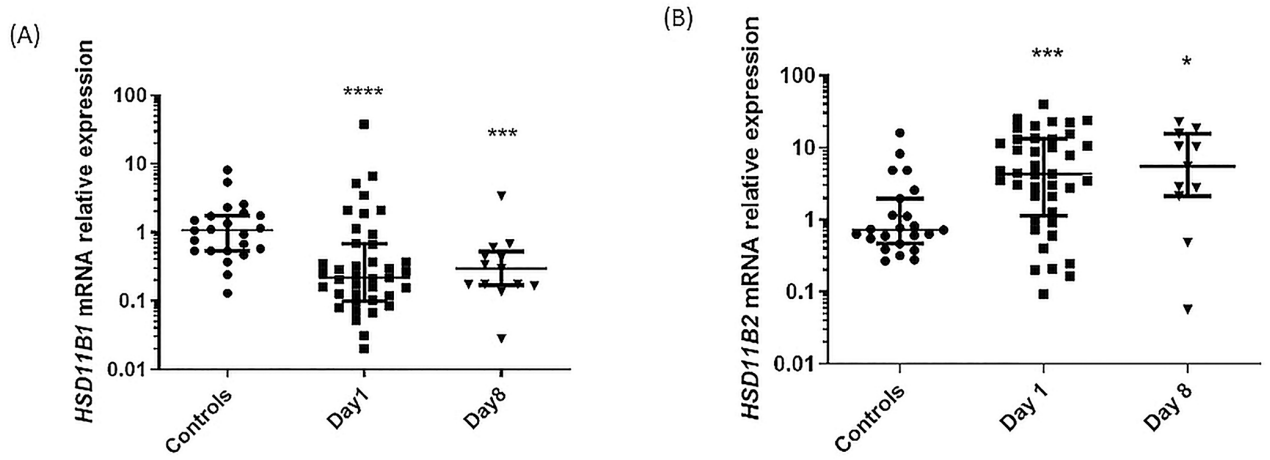

Figure 2. ***HSD11B1*** **and** ***HSD11B2*** **mRNA relative expression in polymorphonuclear cells of critically ill patients. (A) Distribution of** ***HSD11B1*** **mRNA levels in 42 critically ill patients on admission to the ICU (day 1) and day 8 (N= 14), compared to healthy controls (N= 25). (B) Distribution of** ***HSD11B2*** **relative mRNA levels in in 42 critically ill patients on admission to the ICU (day 1) and day 8 (N= 14), compared to healthy controls (N= 25). Blood sampling discontinued if extubation and termination of mechanical ventilation, or hydrocortisone administration due to septic shock occurred. Number of patients at each time point: Day 1, N= 42; Day 8, N= 14. Data are presented as box plots. Line in the middle, median value; lower and upper lines, 25th to 75th centiles; whiskers, range of values. A-B: * *P*<0.05, *** *P*< 0.001, **** *P*< 0.0001 from healthy controls using Kruskal-Wallis ANOVA followed by Dunn's multiple test.**

MR mRNA Expression in Critically Ill Patients

Compared to healthy controls, patients on admission to the ICU exhibited a 12-fold lower *MR* mRNA expression [0.08 (0.04-0.92); Figure 1A; *P*< 0.0001]. During ICU stay, *MR* expression levels remained reduced; more specifically, on day 4, patients exhibited 8-fold reduced levels [0.13 (0.04-0.41); Figure 1B; *P*< 0.001]; on day 8, 5-fold [0.20 (0.05-0.50); Figure 1B; *P*< 0.01]; and on day 13, 14-fold [0.07 (0.05-0.25); Figure 1B; *P*< 0.001]. The MR-downstream target gene *GAL3*, as expected, also exhibited reduced expression levels on ICU admission compared to healthy controls [0.51 (0.37-0.68); Figure 1C; *P*< 0.0001].

HSD11B1 and HSD11B2 mRNA Expression in Critically Ill Patients

Compared to healthy controls, critically ill patients on admission to the ICU exhibited a 5-fold lower *HSD11B1* mRNA expression [0.21 (IQR: 0.1-0.37); Figure 2A; *P*< 0.0001]. In contrast, patients exhibit a 4-fold increase in *HSD11B2* mRNA expression [4.34 (1.14-13.33); Figure 2B; *P*< 0.001]. During ICU stay, the expression levels of both enzyme types were maintained at the same levels. More specifically, on day 8, the expression of *HSD11B1* was 0.30 (0.17-0.53; Figure 2A; *P*< 0.001) and of *HSD11B2* 5.5 (2.15-15.38; Figure 2B; *P*< 0.05), compared to healthy controls. The relative mRNA ratio of *HSD11B2: HSD11B1* on days 1 and 8 was 25.39 (3.99-65.83) and 36.05 (1.64-63.16), respectively.

Renin-Angiotensin System Function in Critically Ill Patients

The change in aldosterone, PRA, the aldosterone:renin ratio, and cortisol from admission to day 13 is shown in Figure 3. Median aldosterone (Figure 3A), PRA (Figure 3B), aldosterone:renin ratio (Figure 3C), and cortisol (Figure 3D) remained unchanged during the study period.

Discussion

The main findings of the present study can be summarized as follows: (a) the MR is expressed in PMNs of critically ill patients, however, at a lower degree compared to normal controls; (b) the HSD11B isoenzymes are also expressed, however, *HSD11B2* expression is up-regulated, while *HSD11B1* is down-regulated. Taken together, these results indicate that MR is possibly occupied by aldosterone, and furthermore may indicate low cellular active cortisol availability, which might be associated with decreased sensitivity of PMNs to endogenous glucocorticoid action.

A previous study from our group described the expression of *GCRA* in ICU patients.[7] We showed that *GCRA* expression displays a biphasic pattern; acutely, patients have significantly higher levels of *GCRA*, while during the sub-acute phase, patients' *GCRA* mRNA expression declined. The possibility that PMNs of critically ill patients also express MR remains unknown. In the present study, we measured *MR* expression in the same group of ICU patients in whom *GCRA* was found to be up-regulated. Patients' PMNs expressed the *MR;* however, it was consistently down-regulated for the entire study period compared to healthy-controls. As a ligand-activated transcription factor, MR controls the expression of its target genes. The expression of

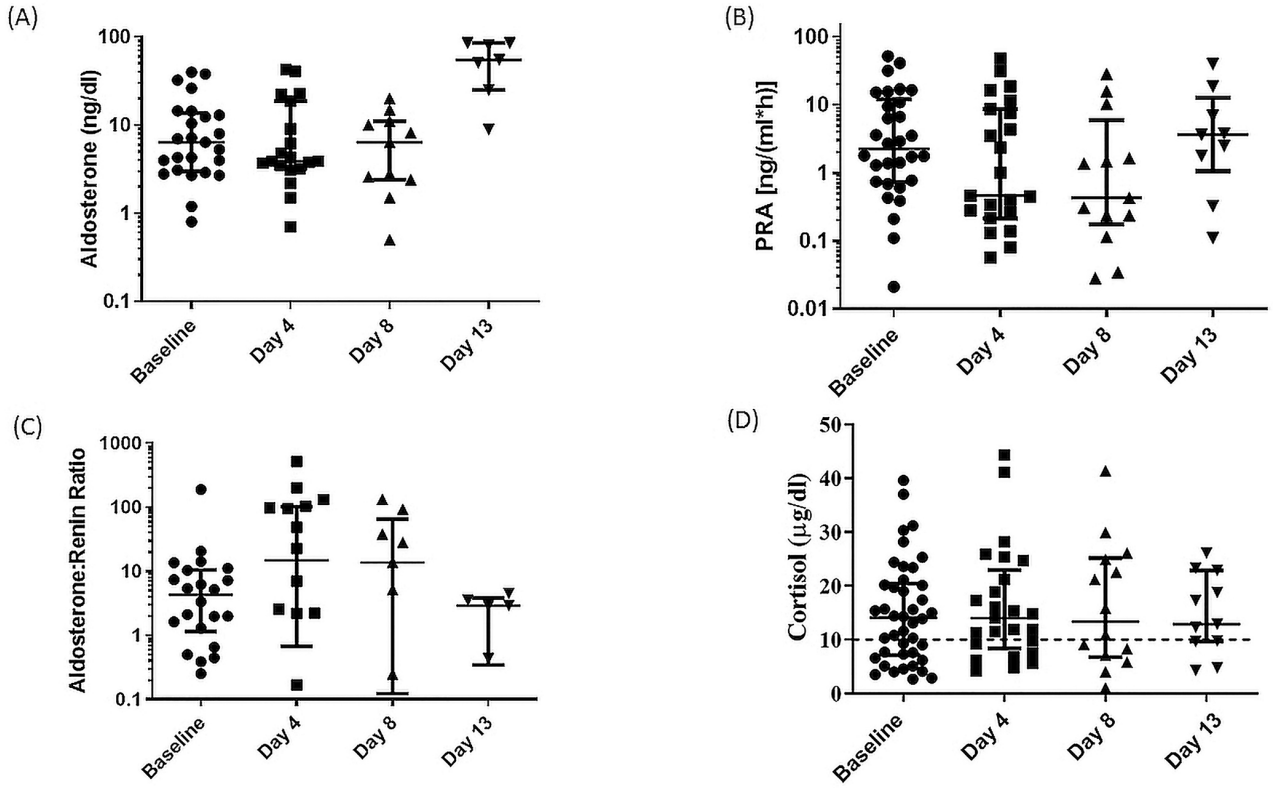

Figure 3. Aldosterone (A), plasma renin activity (PRA) (B), aldosterone:renin ration (ARR) (C), and cortisol (D) levels in critically ill patients on ICU admission and during the 13-day ICU stay. Aldosterone (A) and PRA (B) were measured in critically ill patients at four different time points: baseline (24-48 hours post ICU admission) and on days 4, 8 and 13 or until discharge from the ICU or death. Blood sampling discontinued if extubation and termination of mechanical ventilation, or hydrocortisone administration due to septic shock occurred. Number of patients at each time point: 1, N= 31; 2, N= 24; 3, N= 14 and 4, N= 9. Data are presented as scatter plots. Scatter plots: line in the middle, median value; lower and upper lines, 25th to 75th centiles.

GAL3, which is required for aldosterone/MR-mediated mechanisms, was also down-regulated. Taken together, the results of our previous and present study suggest the two receptors are co-expressed in PMNs; however, *CGRA* is up-regulated, while *MR* is down-regulated. GCR-α is responsible, at least in part, for the dampened MR expression. Theoretically, favoring GCR-α signalling over MR signalling in PMNs could be interpreted as a mechanism facilitating the well-known beneficial anti-inflammatory effects of endogenous corticosteroids in PMNs. Steroids contribute to the increase of PMNs in the circulation through demargination of neutrophils from the endovascular lining, delayed migration from the circulation into the tissues and improved survival of circulating PMNs by suppressing apoptosis, and release of immature neutrophils from the bone marrow into the circulation.[11] In circumstances of uncontrolled inflammation, PMNs can become detrimental by causing tissue injury and organ damage in critical illness.[12] In critical illness, the renin–angiotensin–aldosterone system plays a key role in maintaining hemodynamic stability, vascular tone, and electrolyte homeostasis. Various disturbances in the renin–angiotensin–aldosterone system have been documented, including primarily high renin or PRA with low aldosterone.[13] In the present study, we found that PRA and aldosterone levels remained within normal limits during the course of critical illness.

In immune cells, HSD11B1 is primarily expressed in macrophages and lymphocytes, especially during inflammation.[14-16] On the other hand, HSD11B2 protects the MR from illicit occupancy by cortisol by inactivating cortisol within cells. We found a lower expression of *HSD11B1* and a higher expression of *HSD11B2*. These indicate that in the PMNs of our critically ill patients, MR is possibly occupied by aldosterone. The role of HSD11B2 expression in PMNs remains currently unclear. Some reports have shown a pro-inflammatory effect in PMNs,[17,18] while one report showed anti-inflammatory effects in neutrophils.[19]

In human T-lymphoblastic leukaemia cells, both *HSD11B2* expression and reciprocal regulation of *HSD11B1* and *HSD11B2* have been shown to be associated with glucocorticoid resistance.[20,21] Data for tissue resistance to glucocorticoid activity are limited in critical illness. Indirect evidence suggesting altered

tissue HSD11B activity comes from studies that found increased plasma cortisol:cortisone ratio in critically ill septic and trauma patients.[22,23] A recent study showed that in septic shock patients, sensitivity to glucocorticoids does not appear to be mediated by changes in the expression of the HSD11B2 isozyme.[24] In our study, both at the acute (on admission) and sub-acute phase (day 8), patients exhibited low *HSD11B1* and high *HSD11B2* expression levels, pointing possibly towards decreased sensitivity of PMNs to endogenous glucocorticoid action. Current guidelines suggest the use of hydrocortisone supplementation in patients with septic shock refractory to vasoactive agents, however, not all patients respond appropriately, and this could be partly due to glucocorticoid resistance.[25]

A limitation of our study was the relatively small number of patients, but similar to other investigations. Larger number of patients are needed to validate the results of this study. Furthermore, since monocytes are cells of interest in the immunomodulatory effects of glucocorticoids, future research should look at the expression of the steroid receptors and HSD11B isozymes in isolated monocytes. Nevertheless, PMNs, which are easily accessible cells, seem to be a target for aldosterone given the co-expression of *MR* and *HSD11B*s, raising the possibility that these cells could be useful in exploring the MR/aldosterone effects in various clinical entities, including critical illness.

Conclusion

In the last years it has become evident that glucocorticoid activity in humans is affected by a number of factors, including the HSD11B enzymes. HSD11B1 amplifies glucocorticoid effects, while HSD11B2 prevents inappropriate exposure of the MR to glucocorticoids. We have previously shown that PMNs of ICU patients express the GCR.[6] The present investigation demonstrated that these cells also express the MR, although to a lesser extent. Moreover, PMNs express both HSD11B1 and HSD11B2, which undergo a reciprocal change in expression, resulting in the conversion of endogenous cortisol to inactive cortisone. These results possibly point towards low sensitivity of peripheral PMNs to endogenous glucocorticoids in critically ill patients. Whether this is part of an adaptive response to inflammation or contributes to glucocorticoid resistance remains to be established.

References

1. Kadmiel M, Cidlowski JA. Glucocorticoid receptor signaling in health and disease. Trends Pharmacol Sci. 2013;34(9):518-530. doi:10.1016/j.tips.2013.07.003
2. Gomez-Sanchez E, Gomez-Sanchez CE. The multifaceted mineralocorticoid receptor. Compr Physiol. 2014;4(3):965-994. doi:10.1002/cphy.c130044
3. Funder JW. Glucocorticoid and mineralocorticoid receptors: biology and clinical relevance. Annu Rev Med. 1997;48:231-240. doi:10.1146/annurev.med.48.1.231
4. Nethathe GD, Cohen J, Lipman J, Anderson R, Feldman C. Mineralocorticoid Dysfunction during Critical Illness: A Review of the Evidence. Anesthesiology. 2020;133(2):439-457. doi:10.1097/ALN.0000000000003365
5. Chapman KE, Coutinho AE, Zhang Z, Kipari T, Savill JS, Seckl JR. Changing glucocorticoid action: 11β-hydroxysteroid dehydrogenase type 1 in acute and chronic inflammation. J Steroid Biochem Mol Biol. 2013;137:82-92. doi:10.1016/j.jsbmb.2013.02.002
6. Vassiliou AG, Floros G, Jahaj E, et al. Decreased glucocorticoid receptor expression during critical illness. Eur J Clin Invest. 2019;49(4):e13073. doi:10.1111/eci.13073
7. Vassiliou AG, Stamogiannos G, Jahaj E, et al. Longitudinal evaluation of glucocorticoid receptor alpha/beta expression and signalling, adrenocortical function and cytokines in critically ill steroid-free patients. Mol Cell Endocrinol. 2020;501:110656. doi:10.1016/j.mce.2019.110656
8. Bigas J, Sevilla LM, Carceller E, Boix J, Pérez P. Epidermal glucocorticoid and mineralocorticoid receptors act cooperatively to regulate epidermal development and counteract skin inflammation. Cell Death Dis. 2018;9(6):588. Published 2018 May 22. doi:10.1038/s41419-018-0673-z
9. Oakley RH, Cruz-Topete D, He B, et al. Cardiomyocyte glucocorticoid and mineralocorticoid receptors directly and antagonistically regulate heart disease in mice. Sci Signal. 2019;12(577):eaau9685. Published 2019 Apr 16. doi:10.1126/scisignal.aau9685
10. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001;25(4):402-408. doi:10.1006/meth.2001.1262
11. Ronchetti S, Ricci E, Migliorati G, Gentili M, Riccardi C. How Glucocorticoids Affect the Neutrophil Life. Int J Mol Sci. 2018;19(12):4090. Published 2018 Dec 17. doi:10.3390/ijms19124090
12. McDonald B. Neutrophils in critical illness. Cell Tissue Res. 2018;371(3):607-615. doi:10.1007/s00441-017-2752-3
13. Bitker L, Burrell LM. Classic and Nonclassic Renin-Angiotensin Systems in the Critically Ill. Crit Care Clin. 2019;35(2):213-227. doi:10.1016/j.ccc.2018.11.002
14. Bene NC, Alcaide P, Wortis HH, Jaffe IZ. Mineralocorticoid receptors in immune cells: emerging role in cardiovascular disease. Steroids. 2014;91:38-45. doi:10.1016/j.steroids.2014.04.005
15. Cole TJ, Young MJ. 30 Years of the Mineralocorticoid Receptor: Mineralocorticoid receptor null mice: informing cell-type-specific roles. J Endocrinol. 2017;234(1):T83-T92. doi:10.1530/JOE-17-0155

16. Zhang Z, Coutinho AE, Man TY, et al. Macrophage 11β-HSD-1 deficiency promotes inflammatory angiogenesis. J Endocrinol. 2017;234(3):291-299. doi:10.1530/JOE-17-0223
17. Gilet A, Zou F, Boumenir M, et al. Aldosterone up-regulates MMP-9 and MMP-9/NGAL expression in human neutrophils through p38, ERK1/2 and PI3K pathways. Exp Cell Res. 2015;331(1):152-163. doi:10.1016/j.yexcr.2014.11.004
18. Walczak C, Gaignier F, Gilet A, Zou F, Thornton SN, Ropars A. Aldosterone increases VEGF-A production in human neutrophils through PI3K, ERK1/2 and p38 pathways. Biochim Biophys Acta. 2011;1813(12):2125-2132. doi:10.1016/j.bbamcr.2011.07.010
19. Bergmann A, Eulenberg C, Wellner M, Rolle S, Luft F, Kettritz R. Aldosterone abrogates nuclear factor kappaB-mediated tumor necrosis factor alpha production in human neutrophils via the mineralocorticoid receptor. Hypertension. 2010;55(2):370-379. doi:10.1161/HYPERTENSIONAHA.109.141309
20. Sai S, Esteves C, Kelly V, et al. Reciprocal Regulation of HSD11B1 and HSD11B2 Predicts Glucocorticoid Sensitivity in Childhood Acute Lymphoblastic Leukemia. J Pediatr. 2020;220:249-253. doi:10.1016/j.jpeds.2019.12.054
21. Sai S, Nakagawa Y, Yamaguchi R, et al. Expression of 11beta-hydroxysteroid dehydrogenase 2 contributes to glucocorticoid resistance in lymphoblastic leukemia cells. Leuk Res. 2011;35(12):1644-1648. doi:10.1016/j.leukres.2011.07.002
22. Cohen J, Smith ML, Deans RV, et al. Serial changes in plasma total cortisol, plasma free cortisol, and tissue cortisol activity in patients with septic shock: an observational study. Shock. 2012;37(1):28-33. doi:10.1097/SHK.0b013e318239b809
23. Venkatesh B, Cohen J, Hickman I, et al. Evidence of altered cortisol metabolism in critically ill patients: a prospective study. Intensive Care Med. 2007;33(10):1746-1753. doi:10.1007/s00134-007-0727-7
24. Cohen J, Pretorius CJ, Ungerer JP, et al. Glucocorticoid Sensitivity Is Highly Variable in Critically Ill Patients With Septic Shock and Is Associated With Disease Severity. Crit Care Med. 2016;44(6):1034-1041. doi:10.1097/CCM.0000000000001633
25. Singer M, Deutschman CS, Seymour CW, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA. 2016;315(8):801-810. doi:10.1001/jama.2016.0287

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