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Review Article

CRITICAL CARE

Glucocorticoids with or without fludrocortisone in septic shock: a narrative review from a biochemical and molecular perspective

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Summary

Two randomised controlled trials have reported a reduction in mortality when adjunctive hydrocortisone is administered in combination with fludrocortisone compared with placebo in septic shock. A third trial did not support this finding when hydrocortisone administered in combination with fludrocortisone was compared with hydrocortisone alone. The underlying mechanisms for this mortality benefit remain poorly understood. We review the clinical implications and potential mechanisms derived from laboratory and clinical data underlying the beneficial role of adjunctive fludrocortisone with hydrocortisone supplementation in septic shock. Factors including distinct biological effects of glucocorticoids and mineralocorticoids, tissue-specific and mineralocorticoid receptor-independent effects of mineralocorticoids, and differences in downstream signalling pathways between mineralocorticoid and glucocorticoid binding at the mineralocorticoid receptor could contribute to this interaction. Furthermore, pharmacokinetic and pharmacodynamic disparities exist between aldosterone and its synthetic counterpart fludrocortisone, potentially influencing their effects. Pending publication of well-designed, randomised controlled trials, a molecular perspective offers valuable insights and guidance to help inform clinical strategies.

Keywords: adrenal insufficiency; corticosteroid insufficiency; critical illness; glucocorticoids; mineralocorticoids; stress response

Editor's key points

- A reduction in mortality has been reported in septic shock when adjunctive hydrocortisone is administered in combination with fludrocortisone. The mechanisms underpinning this mortality benefit remain unclear.
- The authors explored potential mechanisms derived from laboratory and clinical data, elucidating the distinct biological effects of glucocorticoids and mineralocorticoids, and the tissue-specific and receptor-independent effects.
- An understanding of the interplay between glucocorticoid and mineralocorticoid action in critical illness could help guide future clinical strategies.

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Adrenal insufficiency in critically ill patients is characterised by hypothalamic–pituitary–adrenal axis derangements and involves dysfunction in cortisol handling and the renin–angiotensin–aldosterone system (RAAS). Current guide lines suggest adding corticosteroid therapy for patients with septic shock requiring ongoing vasopressor support.[1,2] Although adjunctive hydrocortisone (i.e. cortisol) alone has not shown improvement in mortality in septic shock, two randomised controlled trials have demonstrated a mortality improvement when fludrocortisone is combined with hydrocortisone.[3–5] However, mechanisms underlying the observed comparative effectiveness of adjunctive fludrocortisone with hydrocortisone over hydrocortisone alone remain poorly understood.[6]

The Ger-Inf-05 trial examined the combination of fludrocortisone and hydrocortisone in septic shock.[3,4] A total of 300 patients were assigned to receive either the combination of a 50-mg i.v. bolus of hydrocortisone 6 hourly with 0.05 mg fludrocortisone daily administered through a nasogastric tube or placebo for 7 days.[3] Patients were classified as responders or non-responders based on a corticotropin test.[3] Combined low-dose hydrocortisone with fludrocortisone was observed to significantly reduce the risk of death in non-responders with septic shock (73 deaths [63%] in the placebo group, 60 deaths [53%] in the corticosteroid group [hazard ratio, 0.67; 95% confidence interval or CI, 0.47–0.95; P=0.02]). No significant mortality benefit was observed in responders (18 deaths [53%] in the placebo group and 22 deaths [61%] in the corticosteroid group, P=0.96). These results suggested that combining mineralocorticoid with glucocorticoid therapy improved mortality outcomes in septic shock.

Recent large, randomised, controlled trials examining the role of hydrocortisone in septic shock have shown divergent effects on mortality with fludrocortisone in combination with hydrocortisone treatment. *Corticosteroid Treatment and Intensive Insulin Therapy for Septic Shock in Adults* (COIITSS) was a randomised, 2×2 factorial trial of 509 patients with septic shock and multiple organ dysfunction assigned randomly to one of four groups: continuous i.v. insulin infusion with hydrocortisone alone, continuous i.v. insulin infusion with hydrocortisone and fludrocortisone, conventional insulin therapy with hydrocortisone alone, or conventional insulin therapy with i.v. hydrocortisone and fludrocortisone.[7] The primary objective was to assess the efficacy of intensive insulin therapy in patients with septic shock treated with hydrocortisone. The secondary objective was to evaluate the additive benefit of fludrocortisone administered for 7 days. Hydrocortisone was administered as a 50-mg i.v. bolus, followed by 50 mg i.v. 6 hourly for 7 days. A total of 245 patients received additional oral fludrocortisone at a dose of 0.05 mg daily. Overall, 105 of 245 patients treated with fludrocortisone died (42.9%) and 121 of 264 (45.8%) patients in the control group died (risk reduction, 0.94; 95% CI, 0.77–1.14; P=0.50). These findings provided evidence against intensive insulin therapy in patients receiving hydrocortisone for septic shock and indicated a lack of significant mortality benefit from the additional fludrocortisone. Notably, the COIITSS trial was underpowered as the mortality in the control group was underestimated and the sample size chosen to detect a large effect size (12.5% risk difference).[5] Furthermore, sample size calculation was based on the estimated effects of the insulin intervention, but not on the effects of the added fludrocortisone.[7] Thus, the trial was not powered to find a mortality difference with respect to the comparison of hydrocortisone with hydrocortisone plus fludrocortisone.

In the *Recombinant Human Activated Protein C and Low Dose of Hydrocortisone and Fludrocortisone in Adult Septic Shock* (APROCCHSS) trial, 614 patients were randomised to receive a combination of a hydrocortisone 50 mg i.v. bolus 6 hourly and fludrocortisone 0.05 mg daily through the nasogastric tube for 7 days, whereas 627 patients received hydrocortisone and fludrocortisone placebos.[4] The 90-day all-cause mortality at ICU discharge was observed to be lower in the hydrocortisone-plus-fludrocortisone group than among those who received placebo rather than hydrocortisone and fludrocortisone (35.4% *vs* 41.0%, P=0.04). The hydrocortisone-plus-fludrocortisone group, additionally, had more vasopressor-free and organ failure-free days.

Nonetheless, two randomised, placebo-controlled clinical trials revealed a survival advantage in septic shock through a combination of hydrocortisone and the mineralocorticoid fludrocortisone, compared with placebo. Another smaller trial (n=509 patients) contrasted hydrocortisone alone with hydrocortisone in conjunction with fludrocortisone. The findings did not exhibit a significant decrease in mortality with the addition of fludrocortisone but did show a 3% numerically lower mortality with combination therapy (relative risk, 0.94; 95% CI, 0.77–1.14; P=0.50). Thus, the unresolved potential value of combining fludrocortisone with hydrocortisone for septic shock treatment persists.

Apparently discrepant findings from these three large trials have led to divergent perspectives on the benefits of adjunctive hydrocortisone in combination with fludrocortisone in septic shock. However, distinctions and the limited power of these studies make direct comparisons difficult with respect to hydrocortisone and fludrocortisone.[1,6,8] Cortisol binds to both the glucocorticoid (GR) and the mineralocorticoid receptor (MR). One perspective suggests that as cortisol binds to the MR, the administration of high doses of hydrocortisone (≥200 mg daily) offers an adequate level of mineralocorticoid activity.[1,6] A dose of 240 mg of hydrocortisone confers a mineralocorticoid effect equivalent to 1.2 mg (1200 μg) daily of fludrocortisone and thus additional fludrocortisone therapy is not required.[9] An alternative perspective is that adjunctive hydrocortisone with fludrocortisone treatment is required in septic shock as although cortisol binds to the MR, the subsequent signalling pathways differ from those mediated by aldosterone (and by implication fludrocortisone).

Pending a well-designed, randomised, controlled trial on the role of fludrocortisone in combination with hydrocortisone in septic shock, a molecular perspective offers valuable guidance for clinical strategies.

Biological rationale for the combination of hydrocortisone with fludrocortisone in the management of septic shock

Sepsis, which is a life-threatening time-dependent organ dysfunction, arises from a dysregulated host response to pathogens.[10,11] This response manifests as an overwhelming systemic inflammatory response or immune dysfunction, and involves pattern recognition receptors sensing pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs).[10,11] The resultant injury occurs at the molecular, cellular, and organ levels and involves the breakdown of both the endothelial and epithelial barriers, and the microvasculature, compromising multiple circulatory beds.[12] Various organ systems, including the cardiovascular and pulmonary systems, renal system, the coagulation cascade, and the immune system become compromised and multiorgan

failure ensues.[10,12] In the blood–brain barrier, it causes perivascular oedema, oxidative stress, leukoencephalopathy, and neurotransmitter alterations.[12] The nervous system additionally plays an anti-inflammatory role, particularly in the early stages.[12] With regard to the immune system, this interaction leads to an excessive innate immune response.[10,12] The persistence of a severe and prolonged compensatory anti-inflammatory innate immune response results in a state of acquired immunodeficiency termed immunoparalysis.[13] Corticosteroids, recognised for their immunomodulatory properties, constitute an integral component of the host response to sepsis.[10,14]

Septic shock initiates a neuro-humoral response, which includes the activation of the RAAS.[15,16] The RAAS is a multi-organ system, which regulates homeostasis, inflammation, apoptosis, and fibrosis.[17] The classical RAAS pathway is considered a cardiovascular circulating hormonal system mediated by angiotensin II (AII), and the non-classical RAAS pathway, a local tissue-based system mediated by angiotensin-(1–7). These two systems can operate synergistically or independently.[18–20] In contrast to the classical RAAS and its circulating mediators, the non-classical RAAS comprises tissue axes, which regulate renal, cardiovascular, and nervous systems.[21,22]

Angiotensin-(1–7) is found in the heart, renal, and brain tissues, and in plasma.[19] Relative contributions of the classical (systemic) *vs* the non-classical (intra-renal) RAAS to blood pressure regulation remain debatable; however, the crosstalk between the RAAS and aldosterone production is well established.[21] As such, these two systems should not be seen in isolation.[23]

Both glucocorticoids and mineralocorticoids are integral components of the stress response.[10,14,24] Notably, steroidogenesis pathways also demonstrate overlap between mineralocorticoid and corticosteroid production and the synthesis of corticosterone, an aldosterone precursor, is exclusively triggered by corticotropin.[25] As such, adrenal corticosteroid insufficiency is likely to occur concurrently with mineralocorticoid dysfunction. Physiologically, daily adrenal production of cortisol oscillates between 5 and 11 mg m^{-2} day^{-1} with a mean of 7 mg m^{-2} day^{-1}, which equates to 20–30 mg daily of hydrocortisone in adults.[26,27] Production rates are highly variable between and within individuals.[26,27] Peak mean cortisol levels have been documented at 400 nmol L^{-1} with nadir levels of <50 nmol L^{-1}. In adrenal insufficiency, the traditional replacement dose from consensus guidelines is 15–25 mg of hydrocortisone daily.[28,29] In septic shock, the currently accepted dose for relative adrenal insufficiency is 100–200 mg daily.[1]

In contrast, aldosterone secretion is 200- to 500-fold lower at a rate of 50–200 μg daily, with plasma levels ranging 40–210 μg L^{-1}.[30,31] Isolated serum aldosterone measurement is of limited clinical value and is often interpreted alongside plasma renin activity.[32]

Aldosterone administration has been demonstrated to restore vascular endothelial alpha-1 receptor expression, improving sensitivity to catecholamines in septic shock models.[14,33] Fludrocortisone, a synthetic mineralocorticoid, may be used as replacement therapy for this purpose. The usual starting dose of fludrocortisone for adults with primary adrenal cortical insufficiency is 100 μg daily with a dose range of 100–500 μg.[34,35] Higher doses may be required in high salt-losing states, in paediatric patients because of relative renal resistance to mineralocorticoids, and during the last trimester of pregnancy because of the counter-regulatory effects of high progesterone levels.[29,36–38]

Mechanisms for the beneficial role of adjunctive use of fludrocortisone with hydrocortisone in septic shock

Reasons for the mortality improvement observed in the APROCCHSS and Ger-Inf-05 trials remain poorly understood.[6] Explanatory mechanisms include the mineralocorticoid activity of fludrocortisone, although high doses of cortisol purportedly possess such activity.[9,39] Furthermore, aldosterone has non-mineralocorticoid effects, which potentially play a role.[40] Although pharmacologically different, a similar implication can be extended to its synthetic counterpart, fludrocortisone.

The additional glucocorticoid effects of fludrocortisone should also be considered.[41,42] However, although fludrocortisone is a potent glucocorticoid,[41] its effects are largely mineralocorticoid at the low dosage used because of its significantly greater potency at the MR. Additionally, various genomic and non-genomic effects of fludrocortisone, and a yet unidentified mechanism resulting from the combination of hydrocortisone and fludrocortisone should also be considered.

Aldosterone has non-mineralocorticoid effects: classical mineralocorticoid receptor–mediated signalling *vs* alternate modes of signalling

Aldosterone has genomic and non-genomic actions (Fig. 1).[45] Genomic actions are the classic nuclear actions occurring over hours in epithelial and non-epithelial cells attributable to activation of the MR, a ligand-dependent transcription factor.[45] Aldosterone also displays rapid actions occurring over minutes. Termed non-genomic or rapid non-nuclear actions, these are independent of gene transcription and occur in classical MR targets and non-epithelial tissue and include actions such as the activation of sodium transport in target cells.[46,47] Rapid actions are clinically relevant in the acute setting as a result of their role in increasing peripheral vascular resistance and blood pressure.[48]

Notably, plasma aldosterone levels, not cortisol, rapidly change with posture as part of normal blood pressure postural control.[49,50] This suggests rapid cellular responses mediated by the acute increase in aldosterone and a role in blood pressure control in the acute setting.[48] Non-genomic actions were thought to be mediated through membrane-bound MR acting through second messenger pathways.[46] The G protein-coupled receptor, GPR30, or the G protein-coupled oestrogen receptor-1 (GPER-1), has been implicated as the receptor that mediates some of the rapid actions of aldosterone on vascular smooth muscle.[45,51–54] Experimentally, aldosterone has also been demonstrated to potentiate the constrictor effect of AII on vascular smooth muscle, which is mediated through the angiotensin receptor type 1 (AT1). Experimental evidence further suggests that GPR30 (GPER-1) has a high affinity for aldosterone but does not bind cortisol.[43]

Clinically, aldosterone (and by implication fludrocortisone) thus has MR-independent actions that are relevant in the acute setting with an effect on vascular smooth muscle and blood pressure control. The clinical rationale for fludrocortisone in combination with hydrocortisone therapy in critical illness should thereby not solely relate to aldosterone or fludrocortisone activation of the renal epithelial MR.[6,8]

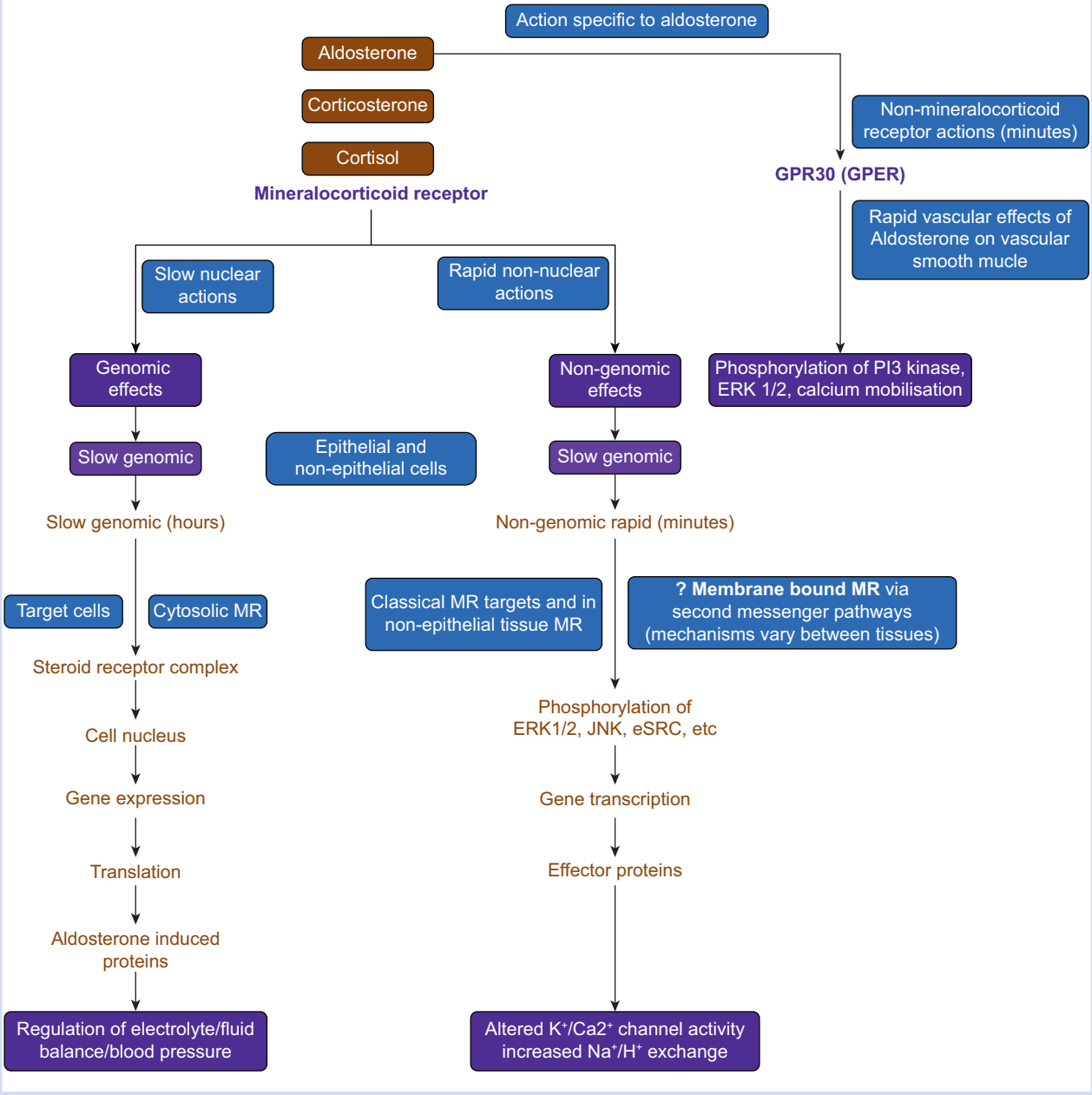


Fig 1. Genomic and non-genomic actions of aldosterone. Key features distinguishing genomic and non-genomic actions of aldosterone. Aldosterone action; genomic and non-genomic effects of aldosterone and cortisol. Classical slow genomic actions mediated through the mineralocorticoid receptor (MR) result in gene transcription and the production of effector proteins. Fast actions mediated through a surface receptor, which interact with the classical genomic actions, have recently been described to be mediated through the G protein-coupled receptor GPR30 (GPER), possibly through a membrane-bound MR, or both. Aldosterone action through GPR30 (GPER) is currently understood to be specific for aldosterone. BP, blood pressure; c-SRC, non-receptor tyrosine kinase c-SRC protein; ERK1/2, extracellular-signal regulated kinase 1/2; JNK, c-Jun NH2-terminal kinase; PI3-K, Phosphatidylinositol 3-kinase. Reused with permission from AME Publishing Company.[43,44]

The multifarious mineralocorticoid receptor

The MR and GR are members of the nuclear receptor super-family, which encompasses seven subfamilies (sub-group 0 to sub-group 6) and 48 members.[55] They act as ligand-activated transcription factors and consist of four structurally distinct domains namely: the amino terminal, a central DNA-binding domain, the hinge region, and the C-terminal ligand-binding domains.[56] Lipophilic ligands including steroids, retinoids, phospholipids, and as yet unidentified ligands (orphan members) regulate their activity.[55,57] Steroid receptors belong to sub-group 3, which includes the GR, MR, androgen receptor, progesterone receptor, and the oestrogen receptors (ERs): ERα and ERβ.[55] The MR and GR amino acid sequences exhibit

significant homology being 56% identical in the steroid-binding domain.[56] Distinctively, the MR has the longest amino terminal domain among steroid receptors, sharing less than 15% homology with the GR in this region.[56] Additionally, the MR has affinity for mineralocorticoids, endogenous glucocorticoids, and progesterone.[56,58–60]

In view of higher circulating levels of cortisol and equal affinity of the MR for cortisol and aldosterone, a system for receptor selectivity exists in some tissues. This is mediated at a pre-receptor level in mineralocorticoid target tissues by the 11β-hydroxysteroid dehydrogenase (11β-HSD) enzyme system.[58] Two 11β-HSD isoenzymes exist.[61,62] First, 11β-HSD type-1 (11β-HSD1) is a bi-directional enzyme *in vitro* and an activating enzyme *in vivo* by converting receptor-inactive cortisone to cortisol.[62] In contrast, 11β-HSD type-2 (11β-HSD2) is expressed in mineralocorticoid target tissues and is a deactivating enzyme through its conversion of cortisol to cortisone.[50–52,63] The role of 11β-HSD2 is to protect the MR from cortisol in mineralocorticoid target tissues, conferring specificity for aldosterone.[63,64] The observation that both cortisol and aldosterone have a high affinity for the MR indicates a lack of selectivity of the C-terminal ligand-binding domain for aldosterone (or cortisol), but not necessarily comparative activation of the receptor.[56,58–60] Notably, although the GR is activated by cortisol alone, not all glucocorticoids activate the MR. Synthetic glucocorticoids show minimal or no activity at the MR.[65–70]

The observation that different ligands have variable activity at the MR, and that not all glucocorticoids activate the MR, is supportive of the rationale that the choice of corticosteroid supplementation potentially influences clinical outcomes and that cortisol and aldosterone (and by inference their synthetic counterparts) do not necessarily have the same effect as ligands at the MR.

Mineralocorticoid receptors are capable of divergent responses to different ligands

Glucocorticoid conformational mobility is another factor that confers mineralocorticoid action of glucocorticoids on the MR.[71–74] The molecular skeleton of steroids consists of four carbon atom rings defined as rings A, B, C, and D.[75] Steroid ring A conveys glucocorticoid action and is rigid in *pure* glucocorticoids and flexible in aldosterone. The structure of steroid ring C is required for mineralocorticoid action and is rigid in aldosterone (and relatively flexible in *pure* glucocorticoids). The exception is 11-deoxycorticosterone and its synthetic derivative, delta-11,12-deoxycorticosterone, which has a flexible C ring (and thus should be a pure glucocorticoid), but is actually a specific mineralocorticoid with marked glucocorticoid activity.[69,76] The lack of C-11 oxygenation of 11-deoxycorticosterone confers versatility.[76,77]

The MR exhibits ligand- and tissue-specific dichotomy. Cortisol's actions on the MR, although primarily agonist, can be antagonistic depending on the underlying pathophysiology.[78–81] The latter has been reported under certain experimental conditions in tissues in which 11β-HSD2 is not expressed (heart and specific regions in the central nervous system).[78–81] In conditions of tissue damage and in the presence of reactive oxygen species, cortisol acts as MR agonist.[80–82] Thus, functions of the MR are diverse, tightly regulated by ligand-specifying mechanisms, and are tissue-dependent.[61,83,84] Furthermore, MRs bound to aldosterone are demonstrably more resistant to proteolysis than MRs bound to cortisol, a result of ligand-induced conformational changes within the receptor protein.[85] Notably, the MR bound to fludrocortisone, aldosterone's synthetic counterpart, is highly resistant to degradation.[86–88] Thus, the MR is capable of divergent responses to different ligands.[39,89] Furthermore, receptor–ligand interactions differ according to ligand, and mineralocorticoid supplementation may help optimise MR activation.

Downstream signalling pathways between aldosterone and cortisol binding at the mineralocorticoid receptor differ

Although the MR binds multiple ligands, unique mineralocorticoid and glucocorticoid effects are evident. Their main ligands, aldosterone and cortisol, perform diverse roles and are governed by different regulatory mechanisms.[90–92] The effects of cortisol are influenced by various factors, including whether the signalling occurs through the GR, MR, or perhaps both.[93] Furthermore, therapeutic agents such as spironolactone, a competitive antagonist with weak agonist activity at the MR, are able to exert their protective effects not only through the exclusion of cortisol or aldosterone from the MR but potentially through the induction of protective factors.[94] Differences in differential downstream signalling pathways that have been observed when the MR is bound to aldosterone compared with cortisol are also likely to be cell- and tissue-specific.

Mineralocorticoid activity in the regulation of salt and water balance

Aldosterone release is the final step in the classical RAAS signalling system. In renal 11β-HSD2-expressing cells, cortisol is metabolised to bio-inactive cortisone. This pre-receptor exclusion of cortisol in 11β-HSD2-expressing tissues renders the MR aldosterone-selective, suggesting that cortisol and aldosterone may have similar or overlapping effects on the MR 11β-HSD2-expressing tissues.[95] This implicates the utilisation of similar signalling pathways between cortisol and aldosterone, specifically with respect to sodium and potassium regulation. The process of filtered sodium (Na^+) reabsorption in collecting duct cells begins with luminal Na^+ crossing the apical plasma membrane through epithelial sodium channels (ENaC) driven by its electrochemical gradient.[96] Subsequently, intracellular Na^+ is actively transported into the interstitial space by the basolateral Na^+/K^+-ATPase. Aldosterone-induced genes modulate ENaC subunit turnover, enhance ENaC synthesis, ENaC activity, and stimulate Na^+/K^+-ATPase activity.[71,96–98] The interaction of aldosterone with the MR leads to induction or repression of additional aldosterone-regulated genes, including serum and glucocorticoid-induced kinase (SGK1), which also play a key role in the regulation of Na^+ transport in distal kidney nephron epithelia.[99] SGK1 is the main regulator of ENaC.[100] Additionally, aldosterone has been found to have acute, non-mineralocorticoid effects on GPR30, which are not observed with cortisol.[51,53]

Mineralocorticoid activity in the brain

Neuroinflammation, along with ischaemic injury, neuronal cell death, and neuroanatomy changes, are major factors associated with cerebral dysfunction secondary to sepsis and septic shock.[101] Inflammation associated with sepsis and

septic shock has a direct impact on the structural integrity of the hippocampus and prefrontal cortex.[102,103] No effective therapeutic interventions exist; however, there is evidence to suggest that elevated levels of glucocorticoids may exacerbate neural inflammation, and evidence to the contrary.[102–105] The role of glucocorticoids, acting through both MR and GR, in modulating the immune response has been well studied.[10] Although, interest in the potential immune effects of mineralocorticoids has only recently emerged, the general consensus is that there is a role for both GR and MR in modulating the neuroinflammation associated with sepsis and septic shock.[10,102]

Compared with the GR, which is expressed ubiquitously in neurones and glial cells, the MR has a more restricted distribution, with the highest expression in limbic structures.[102,106] Aldosterone-selective neurones are limited to areas (periventricular, brain stem nucleus tractus solitarii) that regulate haemodynamic, fluid, and electrolyte homeostasis.[90]

Brain aldosterone levels are normally low because of the absence of aldosterone synthase and poor penetration across the blood–brain barrier caused by the presence of P-glycoprotein, an ATP-driven, drug efflux pump, which transports certain substrates across the cerebral vascular endothelium and into the circulation.[106–108] In contrast, cortisol (or corticosterone in rodents) circulates at significantly higher levels (100–1000 fold), acting as the main ligand for brain MR and GR.[109] Although MR signalling in the brain has been demonstrated to induce a pro-inflammatory response, the results of MR activation in the brain can vary depending on the ligand.[10,110]

Numerous studies show the diverse impacts of cortisol and aldosterone on brain MR.[111,112] Despite this variability, experimental data indicate that both hormones, when interacting with the brain cortisol-responsive MR, can influence cellular activity in similar ways through both genomic and non-genomic pathways.[113] Notably, these neurones also contain 11β-HSD1, which regenerates cortisol. However, it is essential to consider that poor penetration of the blood–brain barrier by aldosterone may be a limiting factor in its effects on brain MR activation.

Indirect evidence of downstream signalling differences is seen in murine models, where corticosterone antagonises aldosterone-mediated hypertension at the brain MR.[114] The brain shows widespread 11β-HSD-1 expression, whereas 11β-HSD-2 is limited to neurones in the fasciculus solitarius.[61,70,106] Aldosterone interactions with these neurones regulate salt appetite.[112] The subsequent blood pressure response to dietary sodium is inhibited by spironolactone.[115] The presence of 11β-HSD-1 and lack of 11β-HSD-2 imply significant roles for both cortisol and aldosterone in the brain, beyond salt regulation. In these cells, salt regulation is not the primary function, providing indirect evidence of the requirement for both cortisol and aldosterone in those tissues, although the specific role of aldosterone is these tissues is not well defined.

Our hypothesis is that in aldosterone-selective tissues, downstream signalling mechanisms are similar between the two ligands, hence the pre-receptor exclusion of cortisol to prevent MR activation by cortisol, which is ubiquitous. In non-aldosterone selective tissues, downstream signalling mechanisms activated by the MR differ between ligands, and pre-receptor modulation of access is therefore not required. These variable downstream actions resulting from aldosterone and cortisol binding at the MR may extend to their synthetic counterparts.

Mineralocorticoid activity in the heart

Differences in aldosterone and corticosterone signalling have been described in murine cardiomyocytes.[116,117] Both GR and MR are present in the heart. In a murine model, the activation of MR and GR resulted in both common and distinct responses, suggesting similarities and differences in signalling. In a study using isolated neonate cardiomyocyte cells *in vitro*, both aldosterone and corticosterone induced acceleration of spontaneous cardiomyocyte contractions with a chronotropic response to corticosteroids mediated by both MR and GR.[116,117]

Increased chronotropy was dependent on low threshold T-type calcium channel expression, which was disrupted by GR antagonism, but not by MR antagonism. Aldosterone's chronotropic action was further found to be additive to forskolin's effect on intracellular cyclic adenosine monophosphate (cAMP) levels, an additive response selectively abolished by GR inhibition.[118] Moreover, GR antagonism prevented cardiomyocyte hypertrophy induced by aldosterone, whereas MR antagonism (spironolactone) had limited effect. This suggests that, in murine cardiomyocytes, complete electrical remodelling and the maximal chronotropic response to corticosteroids require both GR and MR activation, with the GR alone involved in sensitising cells to aldosterone's chronotropic regulation through the cAMP pathway and in the hypertrophic response.[117]

Further evidence suggestive of signalling differences between cortisol and aldosterone in cardiomyocytes include well-established recognition of rapid non-genomic effects unique to aldosterone, not mimicked by cortisol.[119] Acute non-genomic effects of aldosterone in cardiac and other tissues mimicked by classical mineralocorticoid agonists, fludrocortisone, and deoxycorticosterone, but not by cortisol, have been demonstrated.[119] These effects are not blocked by spironolactone.[119] In a murine cardiomyocyte model, aldosterone specifically increased [^{3}H]leucine incorporation into protein, which was not observed with MR occupancy by corticosterone.[120]

The observation that reduced GR signalling and increased MR signalling are associated with an increased risk of heart disease, whereas alterations that favour increased GR signalling and diminished MR signalling exhibit cardioprotective effects, provides further indirect evidence of differences in signalling between glucocorticoids and mineralocorticoids.[121] GR and MR have complementary roles in regulating the hypothalamic–pituitary axis. The balance of glucocorticoid signalling through GR and MR has been demonstrated to play a role in disease.[90,92,121] Increased GR signalling and decreased MR signalling is considered cardioprotective, with unopposed MR signalling, irrespective of ligand (cortisol or aldosterone), associated with accelerated cardiovascular disease in experimental models. These responses can also be viewed as being anti-inflammatory or pro-inflammatory, respectively.[94,122]

Molecular interactions of the MR that differ by ligand have been described, providing compelling evidence of distinct MR conformation when bound to aldosterone *vs* cortisol.[91,109,123–125]

Collectively, these findings support the view that downstream signalling pathways vary depending on whether the MR is bound to aldosterone or cortisol and emphasise the

clinical implications of unique signalling pathways for cortisol, aldosterone, and their synthetic analogues. Although these represent potential mechanisms and, in a sense, proof-of-concept, a link to specific responses or outputs remains to be fully elucidated in humans.

Aldosterone *versus* fludrocortisone mechanism of action: is there a difference?

Synthetic hormones structurally vary from physiological steroid hormones, leading to differing effects.[126–128] Although they may mimic selected biological actions of endogenous hormones, they may not exert identical effects at a molecular level.[90,126,127,129–133]

Pharmacokinetic differences between synthetic and physiological steroid hormones are evident in their binding to corticosteroid-binding globulin (CBG). The majority of cortisol (>90%) is bound to CBG, whereas synthetic glucocorticoids (prednisone, prednisolone, dexamethasone, fludrocortisone) have lower CBG affinity, leading to increased free fractions.[67,134] This difference in bioavailability supports additive *glucocorticoid* activity as another mechanism explaining the mortality improvement in patients administered hydrocortisone in combination with fludrocortisone in septic shock. In contrast to fludrocortisone, aldosterone plays a minimal role in glycogen deposition, glycogen phosphorylation, glycogenolysis, insulin resistance, or sensitivity.[76] Thus, additive fludrocortisone may exacerbate hyperglycaemia compared with hydrocortisone alone.

Moreover, fludrocortisone is a synthetic steroid with potent mineralocorticoid and substantial glucocorticoid activity (Fig. 2a) which was developed from the addition of a fluorine molecule at the 9-alpha-position to hydrocortisone (Fig. 2b and c).[76,87,136–139] It is a structural analogue of hydrocortisone with the same A ring, but is functionally similar to aldosterone.[140] Fluorination results in inert bonds, a prolonged half-life, improved bioavailability, and increased affinity for GR.[86–88] Fludrocortisone has similar effects to hydrocortisone, but has marked effects on electrolyte balance and carbohydrate metabolism with 10-fold higher glucocorticoid activity than hydrocortisone.[86]

Although human comparative pharmacokinetic and pharmacodynamic data for aldosterone and fludrocortisone dosing are limited, during the characterisation of the novel MR modulator, AZD9977, in a murine model, distinct mineralocorticoid effects of fludrocortisone compared with aldosterone were observed.[76,133] These effects were subsequently extrapolated to humans using preclinical and clinical data modelling. The mechanism of this effect remains unexp lained.

Comparison of aldosterone and fludrocortisone revealed distinct effects.[128] A 1-mg dose of intramuscular aldosterone exhibited an electrolyte regulating effect equivalent to a daily oral dose of 250 μg of fludrocortisone.[128] However, fludrocortisone exerted a more prolonged effect. A 1-mg dose of intramuscular aldosterone showed a greater effect on eosinopenia than 1-mg dose of oral fludrocortisone, indicating that fludrocortisone's effect on immune cell function requires much higher doses than aldosterone.[128,141] Notably, differences in the administration routes of aldosterone and fludrocortisone may have contributed to the observed effects. Fludrocortisone also exhibited pituitary adrenocorticotropic

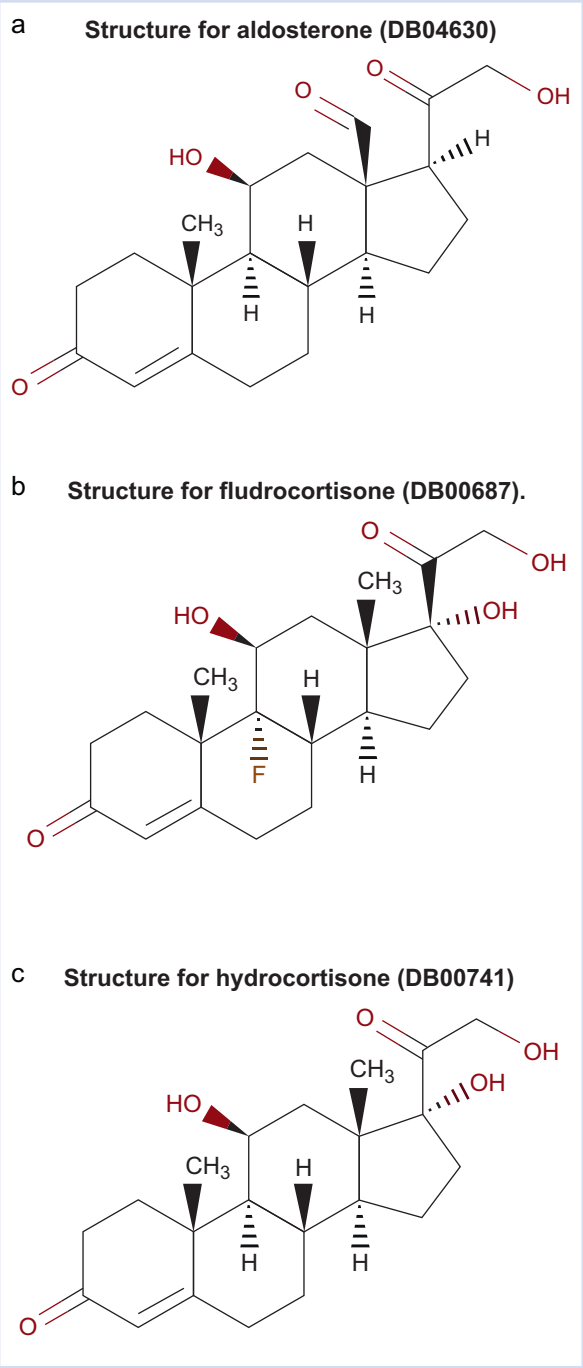


Fig 2. Molecular structure of (a) aldosterone (DB04630), (b) fludrocortisone (DB00687), and (c) hydrocortisone (DB00741).[135] Molecular structures are depicted in a standard format. Distinguishing features between depicted steroids: fludrocortisone has a fluorine atom on C9 replacing hydrogen and aldosterone has an aldehyde group on C18. Bond line notation: double lines indicate a double bond, single arrows indicate a single bond, dashed lines indicate that the bond extends behind the surface plane, bold wedged lines indicate bonds protruding out form the surface plane. DB, DrugBank Accession number.

hormone (ACTH) inhibition, consistent with glucocorticoid activity, an effect not observed with aldosterone, providing further mechanisms whereby fludrocortisone may compound the hyperglycaemia and immune changes observed with hydrocortisone administration.[3,4,128,142]

Thus, although both aldosterone and fludrocortisone are classified as mineralocorticoids, their pharmacological actions differ and may translate into distinct clinical effects. However, although both fludrocortisone and aldosterone have demonstrable non-genomic effects in murine models, evidence for this mechanism in humans as an explanation for the mortality improvement observed with adjunctive fludrocortisone with hydrocortisone in septic shock is minimal.[143]

Role of adrenal age-related changes and mineralocorticoid supplementation

The traditional concept of compartmentalised enzymatic reactions within distinct zones of the adrenal cortex, known as functional zonation, has been challenged.[144] Adrenal cortex zones, namely the zona glomerulosa, zona fasciculata, and zona reticularis, were thought to have distinct roles in synthesising aldosterone, glucocorticoids, and androgens, respectively.[145,146] The distribution of enzymes and receptors does not, however, suggest autonomous steroid production by each adrenal zone. This is particularly relevant for the glomerulosa, which seemingly lacks the complete suite of the necessary enzymes for aldosterone biosynthesis.[147,148] Hence, the adrenal gland, seen as an integrated structure, diminishes the likelihood of diseases such as septic shock being restricted to a particular zone.[147]

Furthermore, with progressing age (from about 30 yr), adrenal topographical changes occur, resulting in zona fasciculata enlargement, reduction in zona reticularis and glomerulosa cell masses, and the development of aldosterone-synthase expressing cell clusters at the border of the zona glomerulosa with the zona fasciculata.[147,149,150] Such histological changes suggest reduced functional differentiation between zones. Of clinical relevance is that the reduction in the aldosterone-producing cell mass and observed decrease in aldosterone release with advancing age suggests that adjunctive mineralocorticoid supplementation in septic shock may be more effective in the older population.[149–151] This aligns with the demographic profile of participants in the APROCCHSS trial, where the average age was 66 yr.[4] Thus, adjunctive hydrocortisone and fludrocortisone therapy may hold greater promise for improving outcomes in septic shock in older patients.

Impact of bioavailability of orally administered fludrocortisone

Fludrocortisone differs from other corticosteroids in its 100% bioavailability and long half-life (18–36 h) and duration of action (1–2 days). It is dosed orally and is stable as a solid.[140] However, pharmacokinetic data on the bioavailability of oral fludrocortisone in critical illness is limited. In one-third of patients with septic shock, a single oral dose of 50 μg led to undetectable plasma levels in one study.[152] The usual replacement dose of fludrocortisone in primary adrenal insufficiency is 100–200 μg, a dose significantly higher than the 50 μg administered in studies conducted in septic shock. More recently, an average of 100 μg fludrocortisone has been used in septic shock.[5] Additionally, the different mineralocorticoid effects of synthetic fludrocortisone compared with endogenous aldosterone suggested by preclinical data highlights that translation of the mineralocorticoid effects of fludrocortisone to those of aldosterone requires further exploration.[133,153]

Clinical applications and suggestions for clinical practice

Which population is likely to benefit?

The decline of the aldosterone-producing cell mass with age and the consequent reduction in aldosterone release suggests that adjunctive mineralocorticoid supplementation in septic shock may be more efficacious in the older population. Based on the demographic profile of participants in the APROCCHSS trial, consideration of adjunctive mineralocorticoid therapy in septic shock may be more beneficial in the population group above 60 yr of age.

Dosage and mode of fludrocortisone administration

Current recommendations suggest adjunctive hydrocortisone at a non-tapered dose of 200 mg daily for 7 days if used in septic shock. If adjunctive fludrocortisone therapy is to be commenced in combination with hydrocortisone, we suggest a dose of 50 μg daily for 7 days without tapering. Notably, a recent international survey of clinician preferences for corticosteroid prescription in septic shock suggests a low preference for fludrocortisone.[154] Well-powered RCTs to investigate patient selection, optimal dosage, and treatment regimen are required before any firm recommendations can be made regarding fludrocortisone dosing.

Adverse effects of hydrocortisone and fludrocortisone use

Risks associated with adjunctive corticosteroid therapy in severe sepsis and septic shock include gastroduodenal bleeding, superinfection, neuromuscular weakness, hyperglycaemia, and hypernatraemia.[142] Findings of a systematic review on corticosteroids in the treatment of severe sepsis and septic shock in adults suggested no significant differences in rates of gastroduodenal bleeding, superinfections, or neuromuscular weakness between treated and control patients. However, there were higher risks of hyperglycaemia (nine trials; 51.6% in the treatment group [363 out of 703] *vs* 46% in the control group [308 out of 670]; $P<0.001$; $I^2=0\%$) and hypernatraemia (three trials; 31.4% in the treatment group [127 out of 404] *vs* 19.2% in the control group [77 out of 401]; $P<0.001$; $I^2=0\%$) observed in the treated group.[142] In the APROCCHSS trial, the use of hydrocortisone in combination with fludrocortisone significantly increased the risk of hyperglycaemia (relative risk, 1.07; 95% CI, 1.03–1.12; $P=0.002$). There were no significant differences in the risks of gastroduodenal bleeding (relative risk, 0.88; 95% CI, 0.58–1.34; $P=0.56$) or superinfection (relative risk, 1.09; 95% CI, 0.92–1.30; $P=0.30$) when comparing hydrocortisone plus fludrocortisone with placebo. The relatively minimal adverse implications, along with the substantial potential benefit, would seem to favour adjunctive hydrocortisone and fludrocortisone administration in septic shock.

Conclusions

Human and laboratory data provide insights into potential mechanisms explaining the beneficial role of adjunctive

hydrocortisone with fludrocortisone in septic shock. Cortisol and aldosterone actions at the MR differ and by implication their synthetic counterparts, however, this has not yet translated into compelling clinical evidence of effect. Aldosterone exhibits mineralocorticoid and clinically relevant non-mineralocorticoid effects; thus, when considering potential beneficial mechanisms, the focus should not solely be on the activity of aldosterone or fludrocortisone at the MR. Signalling pathways activated by aldosterone on the MR are likely distinct from those of fludrocortisone. Consequently, specific effects of adjunctive fludrocortisone in combination with hydrocortisone in septic shock are still not well defined.

The precise targets of mineralocorticoid therapy within the context of sepsis require further elucidation. Additionally, the effect of 'pro-inflammatory' mineralocorticoid actions in relation to immunoparalysis in septic shock and on vascular reactivity requires further elucidation.Additionally, there is clearly a need for further investigation into the pharmacokinetics of orally administered fludrocortisone in critical illness.

Authors' contributions

Researched the scientific literature: GDN
Read and approved the final manuscript: all authors

Declaration of interest

The authors declare that they have no competing interests.

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