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Graphical Abstract

Glucocorticoid replacement therapies: past, present and future
Su-Yi Liew, Scott A Akker, Leonardo Guasti, James FH Pittaway

Highlights

- Adrenal insufficiency was first described by the English physician Thomas Addison in 1855.
- Hundred percent of mortality was observed until the discovery and synthesis of adrenocortical hormones in the 1930s.
- Since 1970, treatment options have changed little, but associated morbidity is better defined.
- Novel preparations of glucocorticoid replacement aim to more closely mimic innate physiology.
- Adrenocortical cells, regenerated from differentiated cells, provide hope for future therapy.
Glucocorticoid replacement therapies: past, present and future

Su-Yi Liew, Scott A. Akker, Leonardo Guasti and James F. H. Pittaway

Abstract
Since the original description of adrenal insufficiency by Thomas Addison in 1855, there has been an exponential growth in the understanding of adrenal gland biology and its role in the hypothalamic–pituitary–adrenal axis. Despite this, the mainstay of therapeutic glucocorticoid replacement for most clinicians has remained unchanged for nearly 50 years. More recently, there has been better recognition of the morbidity and mortality associated with current approaches and the challenges to tackle in reducing this and improving clinical outcomes. In this review, we have summarised the history of glucocorticoid replacement therapy from its nascent in the 1930s, through common practice and culminating in more recent glucocorticoid replacement strategies plus the potential of stem cell therapy in the future.

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Glucocorticoids (GCs) are produced from the middle layer of the adrenal cortex, the zona fasciculata, under the control of the hypothalamic–pituitary axis. The GCs, cortisol and corticosterone, exert their effect through the GC receptor and, among many physiological effects, influence carbohydrate metabolism and mediate the mammalian stress response.

Adrenal insufficiency
Adrenal insufficiency is the term given when steroid hormone production of the adrenal cortex does not meet the physiological demand from the body. This predominantly refers to GC action, but in the case of primary adrenal insufficiency (PAI), it also affects mineralocorticoid and adrenal androgen reserve.

PAI has many potential causes, but most cases arise as a consequence of autoimmune-mediated atrophy, tuberculous destruction (uncommon in developed countries but a serious problem worldwide) or surgical removal (e.g. as a definitive treatment for Cushings syndrome or because of bilateral phaeochromocytoma) or owing to inherited enzymatic defects in adrenal hormone synthesis (e.g. congenital adrenal hyperplasia due to 21-hydroxylase deficiency) [4]. In the last case, there is an added disease burden over and above the requirement for daily adrenal replacement therapy as abnormal growth and sexual development are often also part of the clinical picture.

Introduction
Structure and function of the adrenal cortex
The human adrenal cortex is divided into three histologically and functionally distinct layers. The zona reticularis is the innermost layer of the adrenal cortex and produces a series of sex hormones that are collectively referred to as adrenal androgens. Regulation of adrenal androgens is partly mediated through the action of adrenocorticotropic hormone (ACTH), released from the pituitary gland [2]. These hormones have little innate androgenic effect but provide a source of precursors from which more potent androgens and estrogens are formed [3].

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Secondary adrenal insufficiency is caused by a failure in function of the hypothalamic-pituitary-adrenal (HPA) axis, resulting in insufficient ACTH stimulus to produce physiological GC levels. This is commonly caused by disorders of the pituitary gland, namely, tumours, and as a result of their treatment with surgery or radiotherapy [5]. Long-term treatment with exogenous GCs for inflammatory conditions can also lead to suppression of the HPA axis and may precipitate adrenal insufficiency when tapered or withdrawn. More recently, with the wider use of immunomodulatory therapies in oncology, monoclonal antibodies have been shown to interrupt the HPA axis at all levels, leading to adrenal insufficiency [6].

Adrenal insufficiency is a serious medical condition which, if untreated, has a high morbidity and mortality [7,8]. This is most pronounced in PAI that is characterised by profound extracellular fluid volume depletion with hypotension, hyperkalaemia, hyponatraemia and acidosis [9]. Even when appropriately treated with adrenal hormone replacement, the standardised mortality ratio of patients with primary adrenal failure is > 2:1, with cardiovascular deaths an important contributor to the excess [10]. Data from the European Adrenal Insufficiency Registry also have reflected these findings in patients with secondary adrenal insufficiency [11]. The reasons for this are hard to ascertain, although non-physiological GC replacement is thought by many physicians to be a contributory factor.

The treatment required for adrenal insufficiency is determined by the spectrum of hormonal deficiency in an attempt to restore physiological levels of each. In most cases of PAI, mineralocorticoid function needs to be replaced, but the data regarding the benefits of androgen replacement are less clear. Some evidence suggests that health-related quality of life can be improved in women with PAI through treatment with synthetic dehydroepiandrosterone [12].

GC replacement remains the most complicated aspect of treating adrenal insufficiency. There is no consensus as to the best available treatment other than the smallest dose of GC required to safely replace function is the key, with an additional aim of trying to mimic the peaks and troughs of the physiological circadian rhythm of cortisol levels. This review summarises the chronology of therapeutic GC replacement in adrenal insufficiency from its nascence in the 1930s, through modern-day options, to what options may exist in the future.

**GC replacement therapy**

Adrenal insufficiency was first described by Thomas Addison, working at Guy’s Hospital in London, in 1855 when he recognised the constellation of fatigue, loss of appetite and muscle weakness in a group of patients he felt to have an unexplained anaemia. At autopsy, he discovered ‘a diseased condition of the suprarenal capsules’ (Figure 1a) [13]. It is likely that at least one of the originally described patients had adrenal failure as a result of an autoimmune adrenitis as his own illustrations of patients show a demarcated rash with loss of pigment consistent with the autoimmune condition of vitiligo. Despite the recognition of the condition, it carried a 100% mortality rate until the development of therapeutic GCs [14] (see Figure 2).

**Past**

The first clinical evidence that human adrenal insufficiency could be treated with extracts from animal adrenal cortices was in 1930. Two biochemists from Princeton, Swingle and Pfiffner, demonstrated that infusions of bovine adrenal extract could transiently alleviate the symptoms of adrenal failure [15]. However, given the quantity of the animal adrenal required to produce small amounts of therapeutic cortin and difficulty in isolating said hormone from adrenaline, the widespread therapeutic use of such extract was not yet realised [16].

Later in that decade, two separate groups, Kendall et al [18] working at the Mayo Clinic and Reichstein [17] working in Zurich, identified that adrenocortical hormone was composed of up to 6 separate compounds, named A–F by Kendall et al [18]. Compounds A, B, E and F were found to be biologically active (A: 11-dehydrocorticosterone; B: corticosterone, E: cortisone; F: cortisol or hydrocortisone) (Figure 1b) [18]. Despite the ability to separate these crystalline compounds from bovine adrenal extract, it was not possible to generate sufficient quantities for the study or treatment in humans. Studies in small animals identified that structural differences in these compounds, namely, substitution of an oxygen molecule at C11 or C17 in the steroid skeleton, could divide these compounds into groups based on their physiological effects. Compounds E and F had a greater effect on carbohydrate metabolism and gluconeogenesis, and compounds A and B had a greater effect on electrolytes (GCs vs mineralocorticoids) [18].

The production of synthetic desoxycorticosterone acetate from deoxycorticosterone was a major step forward and was shown to be an effective treatment for patients with Addison disease [19,20]. This was noted to treat the electrolyte imbalance of the condition without affecting the associated hypoglycaemia. It was not until 1946 that the 36-step synthesis of cortisone for deoxycorticosterone acid was published by Sarett [21].

The first clinical use of compound E was in 1949. A rheumatologist, Hench, who had been working with Kendall at the Mayo Clinic, described a remarkable
improvement in symptoms in a patient who had been incapacitated with rheumatoid arthritis [22]. He shortened the name of the compound to cortisone from corticosterone, and it was started to be used more widely for other rheumatological conditions, demonstrating good anti-inflammatory effect.

It was not until 1950 that it was determined that cortisol, compound F, was likely to be the terminal product of the adrenal cortex as application of large doses of hydrocortisone more closely resembled the clinical effects of large-dose administration of ACTH [23]. Shortly after this time, increased skill in manipulating the steroid skeleton structure led to the development of six further steroid medications for therapeutic use, all of which had different mineralocorticoid and GC effects. The wider therapeutic use of these medications led to an improvement in inflammatory conditions and also in treating adrenal insufficiency. It also led to the recognition of the plethora of side effects of supraphysiological corticosteroids, all of which had been described in 1960 [16].

Present
The imperative to not overdose patients receiving GC therapy has existed since these side effects were recognised. The classic side effects of gross over-treatment described in the 1960s carried significant morbidity and mortality. Subsequently, it has been discovered that less clinically overt over-treatment also carries risks of increased mortality and is associated with reduced natural killer cell function and increased cardiovascular risk [24–27].

Modern-day therapeutic aims are more focussed on attempting to replicate the circadian rhythm of cortisol secretion. It has been known since the 1970s that circulating cortisol levels are low while asleep before beginning to increase between 2 and 4 am, peaking approximately 1 h after waking before declining throughout the day except for small rises at meal times.
This rhythmicity occurs under the influence of the central suprachiasmatic nucleus in the hypothalamus. It acts as a central circadian pacemaker for a massive variety of cells in the body and requires daily adjustment through light exposure to synchronise the day/night cycle. In the case of cortisol, it drives corticotropin-releasing hormone secretion from the hypothalamus, leading to ACTH release from the pituitary and resulting in cortisol secretion from the adrenal. Cortisol in turn acts as a secondary messenger modulating peripheral clock genes in other cells.

The net effect of this intricate system is that it is hard to reproduce the physiological profile of cortisol levels in those patients on GC replacement. The physiological production of cortisol is $5-6 \text{ mg/m}^2$. The mainstay of treatment has for some time been giving 15-25 mg of oral hydrocortisone twice or three times daily with approximately half to two-thirds of the daily dose given on waking. Although this provides an approximation of the daily physiological demand, this regimen does not perfectly marry up with the physiological cortisol profile. It tends to result in peak cortisol levels that are supra-physiological and times of subtherapeutic levels pre-doses. Individual variability in the metabolism of tablets also means that replacement doses cannot be applied universally. As a result of this and the lack of consensus on appropriate biological markers of adequacy of replacement, regular clinical assessment of patients is paramount while treatment doses are being established and maintained.

The degree of variation in clinical practice is highlighted nicely in a global survey of more than 1200 patients with adrenal insufficiency. Treatments described included the following: hydrocortisone twice or thrice daily (75%), prednisone/prednisolone (11%), cortisone acetate (6%) and dexamethasone (4%) [33]. Prednisolone has the theoretical advantage of a smoother steroid profile over the course of the day as it has a prolonged binding time to the GC receptor. It also has the advantage of a once-daily tablet. Traditionally, a disadvantage of this treatment was the inability to measure the received corticosteroid dose, but prednisolone assays using liquid chromatography/mass spectrometry have now been developed, and some evidence suggests that this replacement regimen produces a more physiological replacement rhythm than other GCs [34]. Further studies looking into longer term outcomes are ongoing.

An alternative to prednisolone in terms of a once-daily tablet is dual-release hydrocortisone. This formulation of the tablet allows a morning spike of cortisol followed by a secondary slower release preparation throughout the day. Early studies found that in comparison with multiple-dose hydrocortisone or cortisone acetate, treatment with the dual-release hydrocortisone...
preparation Plenadren® may lead to improved weight, blood pressure and HbA1c in patients with concomitant diabetes mellitus [35]. The larger DREAM study suggested a benefit for Plenadren® in terms of improving natural killer cell maturity and lower rates of infection compared with a group on multiple-dose treatment (predominantly cortisol acetate) [36]. This study went on to look at mRNA expression of clock genes and found that some abnormalities seen at baseline between the cortisone acetate group and healthy subjects were corrected after switching to Plenadren® [37].

None of the preparations as yet described are able to mimic the slow rise in cortisol seen in the early morning at 4 am. Chronocort® is a preparation with delayed absorption, which is taken as the last thing at night and in the morning on waking. It has been shown to mimic physiological cortisol levels most closely [38]. It is not yet licensed, but phase II trials have shown findings suggestive of better adrenal androgen suppression in patients with congenital adrenal hyperplasia [39]. Further trials are ongoing.

An alternative to oral preparations of GC administration is through continuous subcutaneous hydrocortisone infusion (CSHI). This application has the benefit of being able to more closely replicate cortisol physiology. It also can be effective in patients with gastric absorptive issues for whom tablets are not well tolerated. Studies using continuous subcutaneous infusion of hydrocortisone in patients with PAI have demonstrated better normalisation of morning ACTH, but initially reported subjective health outcome benefits described have not stood up to a double-blind placebo-controlled trial conditions when compared with oral hydrocortisone [40–42].

Continuous hydrocortisone infusions fail to provide the ultradian pulsatility seen with physiological cortisol production. Hormonal production is in a constant homeostatic flux of small oscillating pulses within the daily profile described previously. Recently, a study looked at the importance in replicating this ultradian pulsatility in GC replacement in healthy young men [43]. They had their intrinsic GC production blocked with Metyrapone, and GC function was replaced with three-time daily oral hydrocortisone, a CSHI or a pulsatile ultradian profile of subcutaneous hydrocortisone. They found that pulsatility of infusion led to better quality of sleep and improved memory function. Pulsatility also affected neurological physiology independent of the cumulative GC dose. Long-term importance of ultradian pulses in patients receiving GC treatment for Addison disease is being studied in the PULSES trial (MR/R010919/1).

Future

Despite advancements in drug delivery of therapeutic GC replacement, the fact remains that no method is perfect. Inevitably, there are numerous subclinical consequences of current approaches that are not yet well defined.

Given this, the ideal solution would be an attempt at preservation of intrinsic adrenocortical function. In autoimmune adrenalitis, there may be a theoretical role for immunosuppression in preventing the destruction of the gland. However, this is not something that has been described. In an isolated case report of a girl with PAI secondary to autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy who received immunosuppression therapy with cyclosporin to improve pancreatic exocrine function, her adrenal function worsened over the course of treatment [44].

In the 1960s to 1970s, there was a surgical vogue for autotransplantation of adrenocortical tissue in patients undergoing bilateral adrenalectomy for Cushing disease. Although there are reports of success with patients avoiding therapeutic GC replacement in the short term, long-term follow-up data showed a variability in graft survival [45,46]. This practice has very much been replaced with more advanced techniques for localising and resecting the causative corticotroph pituitary tumours. The only current surgical technique in sparing cortical function is during bilateral resection of phaeochromocytoma in the context of inherited endocrine syndromes, but this is not widespread practice.

In the light of no clear evidence for preservation of intrinsic adrenal function, there is a growing interest in approaches for restoration. Reports of successful adrenal gland allotransplantation have been described with good functional outcome but are restricted to individual cases and occurred in the context of simultaneous kidney transplants [47–49]. The process for allotransplantation and xenotransplantation of adrenocortical cells has been refined in preclinical studies over the last 20 years with good results [50–54] and was reviewed in 2015 by Ruiz-Babot et al. [55]. However, were these approaches to near the stage of clinical trials, they would result in patients requiring lifelong immunosuppression which carries its own morbidity, for which there is no evidence in halting the autoimmune graft destruction in the context of Addison disease.

A potential solution to this may be in using increased understanding of the generation and reprogramming of inducible pluripotent stem cells. Several studies have shown the possibility of obtaining cells with steroidogenic properties, resembling adrenocortical cells from murine and human cell sources (reviewed in 2015 by Ruiz-Babot et al. [55]). Our laboratory has established a protocol for the generation of adrenocortical-like cells...
6 Adrenal cortex

via reprogramming of skin-, blood- and urine-derived cells in humans. Reprogramming was achieved via forced expression of steroidogenic factor 1 through lentiviral delivery, together with the activation of the protein kinase A pathway and in the presence of luteinising hormone–releasing hormone. These reprogrammed cells had ultrastructural features resembling steroid-secreting cells, expressed steroidogenic enzymes and secreted steroid hormones in response to physiological and pharmacological stimuli. They were viable when transplanted into the mouse kidney capsule and intra-adrenal (Figure. 1d) [56].

Importantly, hypocortisolism observed in cells derived from patients with adrenal insufficiency due to congenital adrenal hyperplasia could be rescued by expressing the wild-type version of the defective disease-causing enzymes (21-hydroxylase). This study provided for the first time an effective tool, with many potential applications to study adrenal biology and pathobiology in a personalised manner, and opened up avenues for the development of novel precision therapies for the treatment of adrenal insufficiency [56].

Conclusion
In 164 years, since Thomas Addison’s original description of adrenal insufficiency, clinical outcomes have dramatically improved. The catalyst for this change was the Nobel Prize–winning work of Hench, Kendall and Reichstein, which enabled the production of synthetic GC replacement for the first time. The development of widely available hydrocortisone and cortisone acetate tablets resulted in these being the mainstay of GC replacement used by clinicians in the past 60 years. There is increasing evidence that patients on GC replacement still carry higher mortality and morbidity than previously thought. These effects mostly have been attributed to the effects of excess GC on the cardiovascular and immune system, but it is likely there are many other subclinical contributors [10,11]. Better understanding of the circadian rhythm of cortisol levels and the role of cortisol as a second messenger in regulating other clock genes has focussed the intention of recent research in generating GC replacement options that mimic physiological rhythmicity.

There are limited data that Plenadren®, prednisolone and Chronocort® may more closely replicate innate GC levels and resultantly improve clinical outcomes, but long-term follow-up is required to generate proof and change. CSHIs that are programmed to most closely mimic physiological cortisol variation have not yet provided the best clinical results, highlighting the importance of other factors potentially such as replicating ultradian pulsatility. The outcomes of the PULES trial will be informative in this regard.

There is a compelling case for stem cell regeneration therapy in adrenal insufficiency. Adrenal insufficiency has received less focus for research in this regard than other endocrinological disorders such as type 1 diabetes mellitus partly due to its low incidence but also likely due to the relatively late recognition of the true scale of imperfection in current therapeutic options. Adrenal insufficiency can benefit by following in the tracks of work carried out in diabetes. The US-based company ViaCyte is carrying out a trial of an implantable device containing pancreatic beta cells engineered from embryonic stem cells as a treatment option in type 1 diabetes (ViaCyte, Inc; https://viacyte.com). The membrane of the device prevents autoimmune or alloimmune reaction to the cells but allows glucose sensing and hormone secretion. Similar strategies could be used in patients with autoimmune PAI. The promise of being able to reprogramme differentiated cells of an individual to become functioning adrenocortical cells obviates the need for such immune protection. This technology, together with gene-editing techniques, offers huge hope for people with adrenal insufficiency in the context of inherited enzyme defects. It is likely that the first human trials for such technology are still someway off, but the future of GC replacement therapy is very exciting.

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Papers of particular interest, published within the period of review, have been highlighted as:
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8 Adrenal cortex


Spin off from the DREAM study above. In the same groups, the authors used reverse transcription and quantitative PCR to assess the mRNA expression of a series of clock genes in peripheral blood mononuclear cells. They showed that patients with AI on standard therapy had dysregulated clock genes compared to controls. Switching to once-daily administration reconditions peripheral tissue gene expression to levels close to controls. This again enforces the importance of the subclinical sequela of potential GC overtreatment and supports further longer-term study into modified-release hydrocortisone.


A randomised, double-blind, placebo-controlled crossover study of three different modes of hydrocortisone replacement in healthy males. Subjects were given metyrapone to clock endogenous cortisol production and were split into GC replacement regimens or continuous subcutaneous hydrocortisone infusion, pulsatile subcutaneous hydrocortisone infusion and standard oral thrice daily hydrocortisone regimen. Subjects receiving pulsatile infusions reported better subjective quality of sleep and displayed better working memory. This group also had improved regulation of cerebral response under emotional stimulation as assessed by functional MRI. Important study highlighting further subclinical sequence of GC replacement therapy and suggestive of the importance of pulsatility in replacement in improve neuro-psychological outcomes.


Preclinical study in which the authors generated human induced ste-roidogenic cells from fibroblasts, blood-, and urine-derived cells through direct reprogramming. This produced functional phenotypically adrenocortical cells which were viable on orthotopic transplantation in mice. This study introduces the future of potential therapies for AI using regenerative medicine.