

Review Article

Alternative Strategies for the Treatment of Classical Congenital Adrenal Hyperplasia: Pitfalls and Promises

Karen J. Loechner,¹ James T. McLaughlin,² and Ali S. Calikoglu¹

¹*Division of Pediatric Endocrinology, Department of Pediatrics, University of North Carolina, CB#7039, 3341 MBRB, Chapel Hill, NC 27599-703, USA*

²*Department of Pharmacology, University of North Carolina, Chapel Hill, NC 27599-703, USA*

Correspondence should be addressed to Ali S. Calikoglu, asc@med.unc.edu

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Despite decades of different treatment algorithms, the management of congenital adrenal hyperplasia (CAH) remains clinically challenging. This is due to the inherent difficulty of suppressing adrenal androgen production using near physiological dosing of glucocorticoids (GC). As a result, alternating cycles of androgen versus GC excess can occur and may lead to short stature, obesity, virilization, and alterations in puberty. Novel therapeutic alternatives, including new and more physiological means of GC delivery, inhibitors at the level of CRH or ACTH secretion and/or action, as well as “rescue strategies”, such as GnRH analogs, antiandrogens, aromatase inhibitors, and estrogen receptor blockers, are available many of these agents, however, still require active investigation in CAH. Bilateral adrenalectomy is effective but it is also still an experimental approach. Gene therapy and stem cells, to provide functional adrenal cortical tissue, are at preclinical stage but provide exciting avenues for a potential cure for CAH.

1. Introduction

Appropriate treatment of children with congenital adrenal hyperplasia (CAH) is challenging because it requires the simultaneous and continuous control of a number of physiologic mechanisms. The molecular defect, usually in the enzyme 21-hydroxylase (21-OH), leads to a decreased production of cortisol that removes the normal negative feedback to pituitary corticotrophs [1, 2]. Consequently, adrenocorticotrophic hormone (ACTH) secretion is increased and drives the shunting of cortisol precursors to androgen synthetic pathways [3]. To control CAH, therefore, it is necessary both to adequately replace glucocorticoids (and mineralocorticoids in salt-wasting forms) and to prevent the production of excess androgens.

Adequate control of CAH requires life-long glucocorticoid (GC) replacement sufficient to maintain adrenal androgen suppression. Determining the “optimal level” of GC replacement, however, can be elusive. Undertreatment leads

to adrenal insufficiency and androgen excess, while over-treatment leads to obesity, iatrogenic Cushing’s syndrome, and osteoporosis [4–10]. Both under- and over-treatment of CAH can result in diminished adult height [11–14]. The final effect on bone age (BA) maturation will reflect the integrated effect of GCs and adrenal androgens over time, especially in the prepubertal child.

We present an overview of new/potential treatment strategies aimed at improving the outcome in patients with CAH. Included are strategies, such as GnRH analogs, antiandrogens, and aromatase inhibitors, for which there are clinical studies completed and/or ongoing to support their use in CAH. For other approaches, such as bilateral adrenalectomy and circadian hydrocortisone treatment, the treatment strategies have already been implemented in the clinical arena albeit in a small number of cases, but formal trials are lacking. Finally, we have included several regimens that have been utilized in other conditions and extrapolated for use in CAH.

2. Glucocorticoid-Sparing Strategies

2.1. Corticotropin Releasing Hormone (CRH) Antagonists. Corticotropin releasing hormone (CRH) is the primary trigger for ACTH release from pituitary corticotrophs, and thus, modulation of CRH actions offers an opportunity to dampen ACTH release. More specifically, inhibition at this higher level in the HPA (hypothalamic-pituitary axis) would be expected to result in a decrease in ACTH release. Inhibition of both CRH and ACTH, in turn, could permit lower GC requirements to maintain adrenal suppression while at the same time providing adequate replacement [15, 16]. Preclinical studies in rodents demonstrate that the CRH antagonist, antalarmin, blocks CRH1 receptor-induced increases both in adrenal size and behavioral responses [17, 18]. CRH analogues with more prolonged action such as astressin inhibit ACTH release [19], and alpha-helical CRF 9-41 reduces measures of stress in a sheep [20]. Trials using CRH (1) receptor antagonists in humans showed a decrease in CRH-associated signs of anxiety and depression. However, since the aforementioned studies in sheep and humans have not been clearly associated with decreases in CRH-induced ACTH release and/or cortisol levels, the direct clinical applicability of CRH antagonists to CAH remains unclear [20, 21].

2.2. Strategies Directly Targeting ACTH Action/Secretion. Inhibition of either ACTH secretion or action, that is, targeting the next level of the HPA axis, represents another coherent strategy. The identification of selective melanocortin receptor subtypes (e.g., MC2-R for ACTH in the adrenal cortex) opens an avenue to blunt ACTH action at the adrenal gland and in turn reduce androgen production [22–24]. Again, this approach could lower GC dosing with the goal of a more physiologic range, such as that used in Addison's Disease (8–10 mg/m²/day; [25, 26]) rather than the “suppressive” regimens (12–15 mg/m²/day after infancy; [27]) usually required for CAH. Preclinical and clinical trials are needed to ascertain the safety and efficacy of ACTH receptor antagonists.

Blockade of ACTH release is another approach under investigation. *In vitro* studies demonstrated that ACTH release from corticotrophs is uniquely coupled to the dihydropyridine-sensitive subclass of voltage-dependent calcium channels, such as nifedipine or amlodipine, that are typically used to treat hypertension [28–30]. Clinical trials in Cushing's disease (another state of ACTH excess) reported acute decreases in ACTH levels with calcium channel blockers [31]. Amlodipine is a long-acting antagonist of DHP calcium channels that has been approved for pediatric hypertension, and it permits accurate daily dosing using a liquid suspension. We recently completed a placebo-controlled cross-over clinical trial in 13 children with either simple virilizing (SV) or salt wasting (SW) CAH (ages 6–12 years of age). We have found that addition of amlodipine (0.1 mg/kg daily bedtime dosing) can attenuate ACTH levels in children with CAH ([32]; Loechner, *unpublished observation*). A mild elevation in heart rate was observed in 2/13 children during change in position, but this was not associated with a drop

in blood pressure nor clinical symptoms with the exception of one child who exhibited mild symptoms of postural hypotension. It is noteworthy that this child had a SW CAH, suggesting that this approach may not be appropriate in such children. These data, however, suggest that the addition of the calcium channel antagonist, amlodipine, to CAH treatment regimen may be a safe and helpful adjuvant therapy in, at least, SV CAH. Longitudinal study is indicated to assess both effects of calcium channel blockade on long-term GC dosing, final adult height, and bone density.

Other potential agents to decrease ACTH secretion include cyproheptadine (a serotonin receptor antagonist), cabergoline (a dopamine agonist), and valproic acid (a GABA reuptake inhibitor). Each has been used with limited success in the treatment of Cushing's disease and Nelson's syndrome [33, 34], but their efficacy in the management of CAH remains to be studied.

3. Glucocorticoid-Delivery Strategies

3.1. Continuous Subcutaneous Hydrocortisone Infusions (CSHI). Studies of circadian cortisol therapy, using hydrocortisone (HC) infusions or modified-release oral formulations, show that these treatments can better mimic physiological cortisol rhythm and hence result in better GC replacement and ACTH suppression in patients with CAH. Merza and colleagues [35] showed that circadian intravenous infusions of HC delivered by a programmable pump that replicated the physiological cortisol circadian rhythm reduced ACTH and 17OHP levels in two patients with Addison's disease and two patients with CAH. In another study, continuous subcutaneous HC infusion (CSHI) was evaluated in seven patients with adrenal insufficiency and showed that morning ACTH levels were reduced to near normal levels, the HC doses were reduced in most patients, and all patients reported satisfaction with this modality [36]. In another recent report, a 4-year therapy with CSHI proved effective and well tolerated in a 14-year-old male with CAH. CSHI treatment produced rapid changes in CAH control and a 50% reduction in the daily HC dose within the first 3 months. In addition, the patient demonstrated normal adrenal androgen production and a normal progression through puberty [37].

These reports hold promise for significant benefits of CSHI that include: (1) good adrenal androgen suppression with decreased doses of glucocorticoid and (2) better management of stress and illness because of the pumps capacity to provide boluses readily and increased overall daily hydrocortisone dosing. Hydrocortisone infusions, however, are impractical for many patients and they pose the risk of site and pump failures. Development of oral modified-release formulations of hydrocortisone may be a more practical solution.

3.2. Dual-Timed/Sustained Release Formulations of HC. Although studies evaluating various GC preparations and their timing of administration have been ongoing for years, no clear-cut regimen has proved universally superior in

children with CAH. Two GC preparations that exploit the pharmacokinetic peak of a once daily, dual-timed HC in order to mimic the diurnal variation peak of ACTH [38, 39] and, in turn, facilitate more physiologic delivery are under evaluation. Chronocort, a once daily, dual-timed, sustained oral release HC appears to be promising. Johannsson and colleagues [40] reported a peak cortisol level whose onset is at one hour after oral intake with peak duration of approximately six hours, after that time cortisol levels decreased to a steady state for the remainder of the day. Chronocort was studied in an open label cross-over manner and compared with standard Cortef dosing in the control of CAH [38]. A single oral dose of Chronocort at 2200 compared to standard three daily doses of Cortef (with a higher evening dose of Cortef) showed that the Cortef regimen resulted in three peaks, whereas a single peak at 0600 occurred with Chronocort. 17OHP levels, androstenedione, and ACTH levels were all lower during this period with Chronocort compared to Cortef. However, afternoon “androgen control” was superior with Cortef because HC levels with Chronocort waned.

A second sustained preparation is that of Duocort, whose peak concentration is reached within one hour after intake. Consequently, the use of Duocort to suppress the morning ACTH and subsequent androgen surge in CAH would require taking the medication in the middle of the night. For children, this would be problematic and will result in decreased compliance.

These new drug-delivery technologies have a significant potential to improve and simplify GC replacement therapy by being more effective, having fewer adverse effects, and improving compliance. Both long-term efficacy and safety of these approaches needs to be established.

4. “Rescue” Strategies

When children with CAH are short and/or have a predicted adult height (PAH) that is significantly below that of their mid-parental target height (MPH), such that significant adult short stature is expected (e.g., as assessed by a markedly advanced bone age), measures beyond standard therapy are sometimes employed for the physical and psychosocial well-being of the child. Often, such strategies rely on experience in other conditions such as precocious puberty and McCune Albright syndrome in which stature has been compromised due to an advanced bone age driven by excessive androgens and/or estrogens and the resultant premature epiphyseal maturation. Moreover, chronically elevated adrenal androgens in children with CAH often associated with late onset of therapy or poor compliance can result in early puberty due to early maturation of the HPG axis [41].

4.1. GnRH Agonists with or without Growth Hormone (GH). As with central precocious puberty, the use of GnRH (gonadotropin releasing hormone agonists), alone or in combination with GH, has been shown to improve PAH [42–45]. The central precocity accompanying CAH has been shown to respond to GnRH agonists alone as well as in

combination with GH [14, 41, 46–48]. While effective in stalling central puberty, growth velocity often decreases upon initiation of GnRH agonist therapy, because it suppresses the effects of sex steroids on linear growth. GH-induced improvement in growth velocity occurs in central precocious puberty as well as in CAH [43, 49]. Quintos et al. [14] reported that GH treatment alone ($n = 12$) or in combination with leuprolide acetate ($n = 8$), a GnRH agonist, improved growth velocity after 1 (5 ± 1.9 to 7.8 ± 1.6 cm/yr) and 2 (6 ± 1.6 cm/yr) years of treatment. A similar improvement in the height SD score for bone age was found in the treatment groups. Lin-Su et al. [47] demonstrated, in a subsequent trial in which fourteen children with CAH were followed to final adult height, that combination treatment with leuprolide acetate and GH can result in an improved final adult height by approximately 1SD compared to matched control children who received standard therapy. The effect of these agents, and their duration of use on both the pubertal growth spurt and on bone accretion/peak bone mineral density are areas that require more study in children with CAH.

4.2. Antiandrogens. Blockade of excess androgen production and/or their action is an essential component of CAH management. The aforementioned strategies have targeted attenuation of adrenal androgen production through manipulation of physiologic feedback loops involving GCs and the hypothalamus and pituitary corticotroph. An alternative approach is to suppress the adrenals less rigidly and to address directly androgen synthesis or actions by using agents that alter (1) synthesis of androgens, (2) conversion of testosterone to its more active metabolites, (3) aromatization of androgens to estrogens, (4) androgen receptor action, and (5) estrogen receptor action.

4.2.1. Inhibitors of Adrenal Androgen Synthesis. Of the adrenal androgen synthesis inhibitors, ketoconazole has been the most widely studied [50]. An azole antifungal drug, ketoconazole at high doses is capable of lowering both testosterone and DHEA (dihydroepiandrosterone) by inhibiting the cholesterol side chain cleavage enzyme, 17- α hydroxylase, and 17, 20 lyase. While effective in lowering adrenal androgen production, the use of ketoconazole in clinical practice is complicated with its dose-limiting side effects such as fatigue, neurotoxicity, hepatotoxicity, and nausea. Its absorption is erratic and it can interfere with the P450-dependent metabolism of many drugs. In addition, (although not critical to patients with CAH) ketoconazole can reduce cortisol synthesis sufficient to necessitate the need for corticosteroid replacement in most patients [51]. Ketoconazole, in more modest doses used for other indications in children, has a relatively benign side-effect profile. However, the dose to suppress adrenal steroid synthesis would be higher and require close monitoring for severe side effects such as hepatotoxicity. Nevertheless, the use of ketoconazole as an adjuvant therapy in CAH warrants investigation.

Abiraterone acetate is an orally available, potent, selective, high affinity, irreversible inhibitor of the 17α -hydroxylase and C17, 20-lyase (CYP450c17) enzymes [52]. Abiraterone acetate is approximately six to seven times more potent in inhibiting CYP450c17 than ketoconazole [53]. Preclinical and phase I studies in patients with prostate cancer showed that abiraterone effectively suppresses testosterone production in the adrenal gland, and doses of up to 2000 mg/d were tolerated [54]. Adverse effects (e.g., hypertension, edema, and hypokalemia) associated with abiraterone use are related to the increased production of nonandrogenic steroids as a result of downstream defect on mineralocorticoid secretion created by inhibition of 17α -hydroxylase activity [55]. Although such an enzymatic blockade could be problematic in a number of conditions, it should not be a clinically significant problem in patients with classical 21-hydroxylase deficiency because aldosterone synthesis is already impaired. Abiraterone is a promising agent for the management of CAH, but it requires studies of safety and efficacy in children with CAH.

4.2.2. Androgen Receptor Antagonists. Flutamide, an androgen-receptor blocker, has been used to treat children with CAH as part of a four-drug regimen, including testolactone (an aromatase inhibitor), low-dose hydrocortisone, and fludrocortisone. Short-term results are encouraging and showed improved control of height velocity and bone maturation at reduced glucocorticoid dosage [56].

More potent antiandrogens are now available that are in use for the treatment of prostate cancer. Nilutamide, a derivative of flutamide, has pharmacokinetic properties allowing once-daily dosing. Bicalutamide, also derived from flutamide, has two-times increased affinity for the androgen receptor, as compared with flutamide and nilutamide, a longer half-life (one week), and significantly decreased toxicity, most notably in hepatotoxicity. Both nilutamide and bicalutamide have been used effectively in the treatment of prostate cancer [57, 58] and have potential in the management of CAH, although the safety and efficacy in the treatment of CAH is not known.

MDV3100 is the most recently developed antiandrogen agent. It binds androgen receptors with an eight-fold higher affinity than does bicalutamide. While other antiandrogens show androgen-receptor agonist properties under some experiment conditions, MDV3100 appears to be a true androgen-receptor antagonist because it both impairs androgen receptor nuclear translocation and blocks DNA binding without apparent agonist properties. The phase 1/2 trials of MDV3100 in patients with prostate cancer show promising results [50]. Again, the role in the management of CAH has yet to be studied.

4.2.3. 5- α Reductase Inhibitors. Inhibitors of the enzyme 5- α reductase include finasteride and dutasteride [59]. They block the conversion of testosterone to its active form, dihydrotestosterone (DHT). 5- α reductase inhibitors reduce androgen action on peripheral tissues and may have a role in alleviating the consequences of hyperandrogenism in

patients with CAH. These drugs, however, are contraindicated in females of child-bearing age due to their capacity to cross the placenta and induce ambiguous genitalia in the fetus.

4.3. Aromatase Inhibitors (AIs). Aromatase Inhibitors (AIs) act by inhibiting P450 aromatase, the enzyme that catalyzes the aromatization of C19 androgens (androstenedione, testosterone) to C18 estrogens (estrone, estradiol). The initial uses for AIs included estrogen-dependent breast cancer [60] and gynecomastia [61]. The expansion of AI use to other conditions has evolved with the development of selective inhibitors of aromatase. The capacity of AIs to decrease estrogen, as well as their effect on the maturation of growth plates, led to the study of the capacity of AIs to prolong linear growth in children with short stature of a variety of etiologies, including CAH [62, 63].

Evidence for the efficacy began with investigation of the nonselective first generation AI, testolactone with positive effects on growth, and PAH in male central precocity (reviewed in [64]). Examination of testolactone therapy in girls with McCune Albright syndrome also showed an improved PAH [65]. Moreover, a study of testolactone in CAH suggested a positive effect when used in conjunction with an androgen receptor antagonist although three-times-daily dosing proved a hindrance with compliance [56, 66]. After 2 years, the group that is receiving the experimental treatment regimen had elevated blood androgen levels, but linear growth rate and bone maturation were normal. No significant long-term adverse effects were seen.

Third generation AIs appear to be better tolerated and require only once-daily dosing. Selective third-generation AIs have been used in several short stature conditions, including constitutional delay of growth and puberty [67–69], idiopathic short stature, and growth hormone deficiency [70, 71]. These studies reported that treatment with the AIs letrozole or anastrozole result in improved PAH. The role of these selective AIs warrants controlled longer-term studies in CAH. Further studies of AIs also are warranted in conditions more comparable to CAH such as central precocious puberty and in gonadotropin-independent precocity, such as in McCune Albright syndrome. As in CAH, these disorders are associated with advancement in bone age (BA) that truncates the time of linear growth and leads to diminished adult stature. A significant goal of AI therapy is to permit a child's chronologic age (CA) to "catch-up" with their BA, such that PAH is improved [64, 72].

Study of anastrozole in male rats showed an increase in testosterone and decrease in estrogen levels, and an elevation in FSH is likely due to diminished E2 levels. Inhibin B showed no significant change in the rats and spermatogenesis appeared grossly normal [73]. In contrast, in a study of boys with delayed puberty, letrozole resulted in increased LH concentrations and LH pulse amplitude [67]. Dunkel [62] reported no adverse effects on testicular size or inhibin B levels in boys. The rise in LH, in addition to elevation in testosterone levels, has raised the theoretical concern that letrozole may result in a PCOS-like picture in girls. In

contrast, the use of AIs is currently under investigation to improve fertility in women with PCOS [74]. The potential teratogenic effects of AIs are, however, unknown [75, 76].

The consensus statement by Lawson Wilkins Pediatric Endocrine Society [77] outlined the following concerns on the use of AIs in short stature: (1) all of the data derived from interventions are limited to male patients; (2) only two randomized, double-blinded, placebo-controlled trials were performed, and relatively small number of subjects were treated with AIs; (3) very few patients have been followed to near-adult height, and adult heights from the controlled trials are not available; (4) short-term safety data are reassuring; however, long-term data are lacking. Therefore, AIs are not recommended to be used in females. Long-term studies addressing concerns on their efficacy and safety in both prepubertal and pubertal boys and girls are needed before AI use becomes more widespread.

4.4. Estrogen Receptor Blockers. Estrogen receptor blockers, like aromatase inhibitors, may have a place in the management of CAH with the goal of delaying androgen-induced rapid skeletal maturation. Many selective estrogen receptor modulators (SERMs) are available. Triphenylethylenes (Tamoxifen, Droloxifene, Idoxifene, Toremifene, and Ospemifene), Benzothienopyrenes (Raloxiphen, Arzoxifene), and Benzopyrans (Ormeloxifene, levormeloxifene) are effective in blocking estrogen action in breast cancer but have agonistic actions in other tissues including skeletal system [78, 79]. Although their effect on skeletal maturation has not been studied, it is likely that those agents will have no or little effect in delaying bone age maturation. Fulvestrant, the only steroidal antiestrogen, is unique among this category of drugs in that it is a powerful antiestrogen that binds with high affinity to the estrogen receptor but initiates no apparent estrogen agonist activity [80, 81]. As might be expected, therefore, fulvestrant has been reported to block the estradiol-accelerated skeletal maturation in prepubertal mice [82]. Although its effect on compromising the androgen-accelerated skeletal maturation in the same model was mild [83], fulvestrant seems to be the best candidate among estrogen receptor blockers to prevent rapid androgen-induced acceleration in skeletal maturation in children with CAH.

5. Bilateral Adrenalectomy

The rationale for bilateral adrenalectomy in patients with CAH is similar to other conditions with hormonal overproduction such as hyperthyroidism, acromegaly, hyperprolactinoma, primary hyperaldosteronism, or Cushing's disease in which the hyperfunctioning endocrine organ is removed, and a replacement therapy is started for the resulting hormonal deficiency. In CAH, bilateral adrenalectomy aims to eliminate androgen excess. Other supportive arguments for adrenalectomy are the observations that the management of adrenal insufficiency is less problematic compared to CAH because lower GC doses are necessary, and salt-losing crisis are less likely. Potential problems with bilateral

adrenalectomy include surgical risk and higher risk of adrenal crisis because the majority of patients with CAH make some endogenous cortisol and aldosterone, potential reduction in ability to respond to stress due to epinephrine deficiency resulting from removal of the adrenal medulla and the potential for Nelson syndrome and adrenal rest tumors if ACTH suppression is not maintained.

Experience with bilateral adrenalectomy in CAH is limited to relatively small case series and individual case reports. Van Wyk and Ritzen [84] reported follow-up studies in 18 patients who underwent bilateral adrenalectomy for CAH. A total follow-up of 90 patient-years was available and showed that adrenalectomy is a safe procedure in CAH patients with a good quality of life and health benefits. Signs of androgen excess decreased with much lower doses of glucocorticoid, and obesity was a less common problem. No serious peri- or postoperative morbidity was reported. While most patients had high ACTH levels and hyperpigmentation initially after adrenalectomy, particularly when the HC dose was reduced to $<10 \text{ mg/m}^2$, adequate dose adjustment solved this problem. Adrenal rest tumor was found in one case, but eight patients had high postoperative elevations of steroid precursors suggesting the presence of adrenal rest tumors. Since this initial report more cases have been documented with similar results including its use to successfully treat infertility in two females with CAH [85–87]. Laparoscopic adrenalectomy has reduced perioperative morbidity; however, adrenal rest tumors and corticotrophin hypersecretion in association with a pituitary microadenoma have been reported [88]. In contrast to legitimate concerns, no problems with epinephrine deficiency are reported and may be explained by the findings that patients with CAH on GC replacement already have greatly reduced secretion of epinephrine [89].

In addition to its use in patients who are intractable to conventional treatment, bilateral adrenalectomy as a “prophylactic” measure in young children with double null mutations of their CYP-21 gene is under evaluation. The rationale is to prevent potential virilization due to inadequate suppression of androgens and/or iatrogenic glucocorticoid excess before they occur in severe cases of CAH. Two patients underwent prophylactic adrenalectomy younger than 5 years of age. The youngest patient who was adrenalectomized at 16 months had two episodes of hypoglycemia on low dosage of HC (8 mg/m^2), which did not recur after HC dosage was increased ($11\text{--}13 \text{ mg/m}^2$) throughout her 6-year follow-up after adrenalectomy. The first patient who had prophylactic adrenalectomy at 3 years of age is now 16 years old. She has reached her midparental height and has normal menses and no signs of hyperandrogenism. She, however, continues to have elevated ACTH levels while on replacement dose of glucocorticoid and mineralocorticoid [90].

Bilateral adrenalectomy appears to be a safe and efficacious method of managing patients with severe forms of CAH. Long-term studies comparing this approach with other treatment modalities, however, are lacking. Undoubtedly, adrenalectomized patients should be monitored closely with meticulous management of glucocorticoid and mineralocorticoid replacement and prompt institution of stress

dosages at the onset of illness. Prophylactic adrenalectomy of young patients may be a viable option in the future if its long-term safety is established but currently should be limited to academic centers with established research protocols.

6. Gene Therapy

As other disorders caused by a single gene defect, gene therapy is theoretically possible for all forms of CAH. The development of gene therapy requires an animal model to test the strategy, and a specific, effective, and safe vector to deliver the gene. Both are available for 21-hydroxylase deficiency. The mouse strain H-2aw18, a recombinant mouse line that has a deletion spanning the C4 gene and one of the two 21-hydroxylase gene, shows typical characteristics of 21-hydroxylase deficiency. Introducing a recombinant DNA fragment containing the murine genomic gene for 21-hydroxylase rescued the lethal mutation and showed that this mouse line is a valid model to study gene therapy [91]. Adenoviruses have selective adrenotropism in animals making them a good vector for gene therapy [92, 93]. Indeed, intra-adrenal injection of adenoviral vector encoding the 21-hydroxylase gene has restored the impaired adrenocortical function in these 21-hydroxylase deficient mice [94], and bovine adrenocortical cells have shown functional and morphological changes in the adrenal cortex after transfection with recombinant adenoviruses [95]. The experience with adenoviruses to target 21-hydroxylase to the adrenal cells is encouraging, but overall safety and specificity in targeting the adrenal gland remain unclear. There may be a need to develop new vectors (viral or non-viral) to improve the specificity and efficiency of gene transfer in adrenal gland and to ensure long-term DNA integration. In recent years, other recombinant viruses such as retrovirus, lentivirus, adeno-associated virus, and herpes simplex virus have been widely utilized as vectors for gene transfer. Nonviral gene delivery methods that use synthetic or natural compounds or physical forces to deliver DNA to a cell have become available. While gene therapy is likely to become an option for the treatment of CAH with these advances, the research specific to the gene therapy in CAH appears to be sparse.

7. Stem Cells and CAH

Adult stem cells are derived from specific tissues and have been studied in many venues, particularly that of the hematopoietic system. Limitations of adult stem cells include (1) lack of pluripotency, (2) difficulty to maintain *in vitro*, and (3) HLA-limitations for transplantation, thus requiring concomitant immunosuppression [96]. Studies using marrow-derived mesenchymal stem cells (MSCs) have provided an additional avenue in that they can be harvested from adults yet are still considered pluripotent [97]. More recent studies have suggested general methods for preparation of induced pluripotent stem cells (iPSCs) from other somatic cell lineages [98]. Note that any cells that are harvested from patients with CAH, either MSCs or iPSCs, could be used as allografts (avoiding issues with HLA

compatibility) but would still harbor a genetic deficiency in steroidogenesis and thus would only be useful as cellular vectors for gene therapy. Despite this, use of adult stem cells benefits from a longer track record of investigation and also circumvents ongoing political obstacles regarding the use of fetal tissue.

In contrast, embryonic stem cells (ESCs) provide an approach that could bypass many of the restrictions of adult stem cells [96]. ESCs obtained during the blastocyst stage are logistically easier to maintain and cultivate *in vitro* and can remain for a longer period in the pluripotent stage. In principal, ESCs could be triggered to differentiate into regulated, steroidogenic tissue using protocols derived from adult stem cell studies [99]. However, as with adult tissue transplants, HLA-matching is required with potential for graft rejection and necessitates immunosuppressive agents. The next generation of ESCs, however, is those that are HLA matched and, therefore, bypass immunosuppressive regimens after transplantation.

Yazawa et al. [97] showed that human MSCs transfected with SF-1, necessary for both gonadal and adrenal steroidogenesis, differentiated into GC-producing (versus testosterone-producing) cells when transplanted into murine testis. Gondo et al. [100] also used SF-1 to produce steroidogenic cells from MSCs; moreover, the cells were responsive to stimulation by ACTH. The authors then reported that a differential hormonal profile was synthesized by transformed stem cells derived from bone marrow than from those derived from adipose stem cells, suggesting that site of stem cell origin is important in determining which steroidogenic pathways can be induced [101].

An additional approach is that of Hammer and colleagues, who have characterized a subpopulation of adrenal cells that are typically undifferentiated or “dormant” [102, 103]. Stimulation of these cells to differentiate into mature adrenal cells under various physiologic or pathophysiologic conditions may be possible, and studies aimed at harvesting this quiescent population for both differentiation and/or regeneration of adrenal tissue are targets for both CAH and for adrenal insufficiency of other etiologies [101–103].

The potential use of stem cells to provide functional adrenal cortical tissue would, if realized, represent a cure for CAH. A key to the success of such an approach would be to provide adrenal tissue that responded in a physiological fashion to both positive and negative regulators.

8. Conclusion

Although introduction of glucocorticoid and mineralocorticoid treatment markedly decreased mortality and morbidity of patients with CAH, management of CAH remains challenging. Clinicians are often frustrated by the difficulties in maintaining satisfactory adrenal suppression without producing an unacceptable degree of hypercortisolism. Shortened adult stature and progressive obesity are common. Novel treatments with great potential to improve outcomes are available, but their use is hampered by the lack of studies in the CAH population. Most clinical trials to date

suffer from relatively small sample sizes and/or retrospective approach. Prospective, randomized, placebo-controlled multicenter trials are required to interpret the appropriate use of these promising agents in children with CAH. Unfortunately, funding is sparse, and the pharmaceutical industry has little interest in investing such a small population of patients. It is critical, therefore, that pediatric endocrinologists, patients, and their families, in addition to advocacy groups, continue to promote collaborative efforts to permit such research endeavors.

Abbreviations

ACTH: Adrenocorticotrophic hormone
 AI: Aromatase Inhibitor
 CAH: Congenital Adrenal Hyperplasia
 CRH: Corticotropin Releasing Hormone
 GC: Glucocorticoid
 GnRH: Gonadotropin Releasing Hormone
 HC: Hydrocortisone.

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