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Tissue-specific activation of cortisol in Cushing’s syndrome

Stuart A Morgan1,2, Zaki K Hassan-Smith1,2 and Gareth G Lavery1,2

1Institute of Metabolism and Systems Research, Institute of Biomedical Research, University of Birmingham, Birmingham, UK and 2Centre for Endocrinology Diabetes and Metabolism, Birmingham Health Partners, University of Birmingham, Birmingham, UK

Abstract

Glucocorticoids are widely prescribed for their anti-inflammatory properties, but have ‘Cushingoid’ side effects including visceral obesity, muscle myopathy, hypertension, insulin resistance, type 2 diabetes mellitus, osteoporosis, and hepatic steatosis. These features are replicated in patients with much rarer endogenous glucocorticoid (GC) excess (Cushing’s syndrome), which has devastating consequences if left untreated. Current medical therapeutic options that reverse the tissue-specific consequences of hypercortisolism are limited. In this article, we review the current evidence that local GC metabolism via the enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) plays a central role in mediating the adverse metabolic complications associated with circulatory GC excess – challenging our current view that simple delivery of active GCs from the circulation represents the most important mode of GC action. Furthermore, we explore the potential for targeting this enzyme as a novel therapeutic strategy for the treatment of both endogenous and exogenous Cushing’s syndrome.

Introduction

Due to their potent anti-inflammatory and immunosuppressive properties, estimates suggest that approximately 1–2% of the population of the UK and the USA take prescribed glucocorticoids (GCs) to treat a broad spectrum of autoimmune and inflammatory diseases (1, 2). Despite their effectiveness, the majority of these patients experience an adverse systemic side-effect profile including: visceral obesity, muscle myopathy, hypertension, insulin resistance, type 2 diabetes mellitus, osteoporosis, and hepatic steatosis (3, 4). Collectively, these ‘Cushingoid’ features contribute to increased cardiovascular morbidity and mortality (5). Endogenous GC excess (Cushing’s syndrome) is a rare diagnosis in comparison (incidence up to 2 per million per year) (6), but has devastating consequences if untreated (7). Although modern therapies, such as transsphenoidal surgery for Cushing’s disease, have dramatically improved prognosis, there is evidence that excess mortality persists.

Invited Author’s profile

Prof Gareth G Lavery is a Wellcome Trust Senior Research Fellow at the Institute of Metabolism and Systems Research, University of Birmingham. He has a research background in understanding the redox regulation of glucocorticoid metabolism and its impact upon metabolic physiology. His current interests include the tissue-specific management of glucocorticoid excess and delineating biosynthetic pathways that impact on the metabolic redox status of skeletal muscle.
even after disease remission (8). Furthermore, there is an inherent failure rate of first-line therapy with persistent and recurrent disease rates of between 10–24% and 5–22%, reported respectively in studies from large specialist centers (8, 9). Second-line therapies including radiotherapy and bilateral adrenalectomy are associated with lifelong hormonal deficiencies. Current medical therapeutic options that reverse the tissue-specific consequences of circulatory GC excess are limited by both efficacy and side effects, and new treatment strategies are urgently needed to improve long-term outcomes.

Recently, local GC metabolism via the enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) has been implicated in mediating the adverse metabolic complications associated with circulatory GC excess – challenging our current view that simple delivery of active GCs from the circulation represents the most important mode of GC action (10). In this article, we review the current evidence for the role of 11β-HSD1 in this regard, and explore the potential for targeting this enzyme as a novel therapeutic strategy for the treatment of both endogenous and exogenous Cushing’s syndrome.

11β-HSD1 and prereceptor GC metabolism

The availability of human cortisol (corticosterone in rodents) to bind and activate the GC receptor (GR) is controlled by 11β-hydroxysteroid dehydrogenases (11β-HSD1 and 11β-HSD2). These isozymes are products of separate genes and are members of the short-chain dehydrogenase/reductase superfamily. 11β-HSD2 is highly expressed in mineralocorticoid target tissues including the salivary gland, kidney, and colon (11). The cognate ligand of the mineralocorticoid receptor is aldosterone, however, cortisol has a similar binding affinity to this receptor. The role of 11β-HSD2 is to protect this receptor from unwanted activation by cortisol by inactivating it to cortisone.

By contrast, 11β-HSD1 expression is more widely distributed (12), with high expression detected in key metabolic tissues including adipose tissue, liver, and skeletal muscle. 11β-HSD1 activity is bidirectional, able to act as both an oxoreductase (activating GCs) and a dehydrogenase (inactivating GCs). However, in intact cells (13), oxoreductase activity predominates. This is supported by the higher affinity of 11β-HSD1 for the human inactive GC, cortisone (K_m = 0.3 μM), compared with cortisol (K_m = 2.1 μM) (14). 11β-HSD1 is tethered to the endoplasmic reticulum (ER) membrane, with the catalytic domain located within the lumen of the ER (15). A high concentration of NADPH within the ER lumen, generated by hexose-6-phosphate dehydrogenase (H6PDH), is thought to be responsible for maintaining the oxoreductase directionality of 11β-HSD1. In H6PDH knockout mice, 11β-HSD1 activity switches from reductase to dehydrogenase, underscoring the importance of this enzyme in maintaining the directionality of 11β-HSD1 (16).

Role of 11β-HSD1 in Cushing’s syndrome

The role of 11β-HSD1 in the development of Cushing’s syndrome first came into the spotlight with a clinical study performed by Tomlinson et al. (17) reporting on a rare case of a patient with pituitary-dependent Cushing’s disease who appeared to be protected from the classical Cushing’s phenotype. Specifically, this patient had normal fat distribution, absence of myopathy, and normal blood pressure. Subsequent investigation revealed a functional defect in 11β-HSD1 activity, as evidenced by serum and urinary biomarkers. Similarly, Arai et al. (18) described a patient with a cortisol-producing adrenocortical adenoma lacking the phenotype of Cushing’s syndrome, and again a defect in 11β-HSD1 activity was identified. These clinical observations appeared to suggest that tissue intrinsic 11β-HSD1 activity is the major determinant of the adverse metabolic manifestations of circulatory GC excess. Recently, we tested this premise using a mouse model of exogenous Cushing’s syndrome (10).

In this study, we demonstrated that 11β-HSD1 knockout mice were protected from the adverse metabolic side effects associated with circulatory GC excess including hypertension, hepatic steatosis, myopathy, and dermal atrophy. Additionally, these mice were protected from increased adiposity of both the omental and subcutaneous depots, paralleled by a blunted induction of lipolytic gene expression program in these tissues. In agreement with reduced lipid mobilization, these mice were spared from elevated serum free fatty acids. In an effort to pinpoint the 11β-HSD1 expressing tissue(s) involved in driving the metabolic side effects associated with GC excess, we generated tissue-specific 11β-HSD1 deletions in liver and adipose tissue. Whereas the liver-specific 11β-HSD1 knockout mice developed a full Cushingoid phenotype, the adipose-specific 11β-HSD1 knockouts were protected from the induction of lipolysis in adipose tissue, circulating fatty acid excess, and hepatic steatosis,

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demonstrating that GCs generated locally within adipose tissue are central in driving the hepatic manifestations of GC excess (i.e. through increasing adipose tissue lipid mobilization and oversupply to the liver) (10). However, in contrast to the global 11β-HSD1 knockout mice, the adipose-specific 11β-HSD1 knockouts were not protected against hypertension, increased adiposity, myopathy, or dermal atrophy induced by circulatory GC excess. This implies that GCs, reactivated by 11β-HSD1 within tissues other than the adipose tissue, is central in driving these extrahepatic Cushingoid features.

But how does 11β-HSD1 govern intracellular access to circulating GCs? We postulate that exogenously administered cortisol contributes to GR activation by three distinct mechanisms (Fig. 1). First, the direct effect of cortisol diffusing into the cell from the circulation, where it binds and activates the cytoplasmic GR. Secondly, circulating cortisol may be inactivated to cortisone by 11β-HSD2, largely in the kidneys, and once delivered to key metabolic target tissues is reactivated to cortisol by 11β-HSD1 to allow GR activation. Also, the stromal vascular fraction of adipose tissue expresses 11β-HSD2 (19), representing an additional source of substrate for adjacent adipocytes. Although, elevated urinary cortisone levels have been reported in patients diagnosed with endogenous/exogenous Cushing's syndrome and Cushing's disease (20), whether circulating levels are also elevated in these patients remains to be confirmed. Thirdly, GCs stimulate 11β-HSD1 expression and activity in a GR-dependent manner, further fuelling intracellular GC excess. We and others have demonstrated this feed-forward action in key metabolic tissues including adipose tissue (10, 21, 22, 23, 24). Despite this, Mariniello et al. (25) found no differences in adipose tissue 11β-HSD1 mRNA expression between patients with Cushing's syndrome and normal weight controls, although increased reactivation of GCs by 11β-HSD1 in this tissue was not investigated in this study.

These studies challenge the idea that simple delivery of active GCs from the circulation represents the most important mode of GC action, and raises an intriguing question: Is Cushing's a cortisone disease? This concept makes physiological sense, in terms of individual tissues regulating precisely their GC availability – rather than it being dictated by a distant gland. In support, a negative association between circulating cortisone levels and bone mineral density and osteocalcin has been reported, independent of circulating cortisol levels, implying that 11β-HSD1 activity within osteoblasts regulates bone mineral density (26).

Selective 11β-HSD1 inhibition – therapeutic implications for Cushing’s syndrome

Transsphenoidal surgery is the first-line therapy for the treatment of Cushing’s disease; however, there are scenarios where alternative treatment options are required, such as in persistent/recurrent hypercortisolism, in preparation for operative intervention, while awaiting effects of radiotherapy, or where surgery is not an option. Recent outcome studies suggest that although mortality has greatly improved in Cushing’s disease, there appears to be persisting excess risk in spite of control of
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Clinical, GCs are used for their powerful immunosuppressive effects in a broad range of medical applications. They are, however, limited by their adverse metabolic effects, and this has been an area of great recent research interest. The development of selective GR agonists (27) that target the transrepressive effects of GCs over the transactivating actions is a rational approach, but has yet to deliver a clinical drug (28, 29).

Current pharmacological strategies for Cushing’s disease are either pituitary directed (dopamine agonists, somatostatin analogs, and retinoic acid) or focused on blocking cortisol secretion and effect (steroidogenesis inhibitors and GR antagonists) (30). Current therapies have low to modest rates of urinary free cortisol or clinical normalization as monotherapy. Furthermore, they are associated with side effects including hyperglycemia (pasireotide, a somatostatin analog), gastrointestinal symptoms, and symptoms associated with adrenal insufficiency (steroidogenesis inhibitors, GR antagonists). Recent studies suggest that combination therapy is associated with improved efficacy (31, 32). Overall, there is a need for larger studies of current and new agents with long-term assessment of associated morbidity and mortality. Since prereceptor GC metabolism by 11β-HSD1 may play a critical role in driving the Cushingoid features associated with circulatory GC excess, therapeutically targeting this enzyme may offer an alternative, potentially more efficacious, approach in the treatment of both exogenous and endogenous Cushing’s syndrome (Fig. 2).

To date, a number of selective 11β-HSD1 inhibitors have been developed, although none has been tested in the setting of circulatory GC excess. Previous studies using this class of drug have found them to have beneficial effects upon glucose tolerance, insulin sensitivity, and dyslipidemia when administered to rodent models of obesity, type 2 diabetes, and the metabolic syndrome, including db/db, ob/ob, KKAy, ApoE–/–, and Ldlr 3KO mice (33, 34, 35, 36, 37). Furthermore, clinical studies using compounds developed by both Incyte Corporation (Wilmington, DE, USA) and Merck have since been administered to patients with type 2 diabetes and those ‘failing’ metformin therapy. In agreement with the rodent studies, these compounds modestly improve blood glucose control, insulin sensitivity, as well as lipid profiles (38, 39, 40, 41). However, their further commercial development for their use in this context has been precluded by the small magnitude of their glucose-lowering effect.

If therapeutically targeting 11β-HSD1 were to be used to abrogate the Cushingoid side effects associated with circulatory GC excess, then adipose tissue (rather than liver) would need to be the primary target, specifically to limit the detrimental hepatic manifestations of GC excess (10). Importantly, selective compounds have already been developed, which are pharmacologically active in this tissue (42). It is likely that targeting 11β-HSD1 in other key metabolic tissues such as the skeletal muscle would also be highly beneficial, specifically to relieve the extrahepatic manifestations of circulatory GC excess such as myopathy.

In the context of exogenous Cushing’s syndrome, cotreatment with a selective 11β-HSD1 inhibitor is likely to benefit patients prescribed GCs with similar kinetics to those of endogenous cortisol. This includes prednisolone and prednisone widely prescribed in Europe and the USA, respectively. These synthetic GCs have similar binding affinities to 11β-HSD1 and 2 as cortisol and cortisone, and are metabolized by these enzymes in an...
analogous manner (43). As such, selectively targeting the reactivation aspect of this inactivation/reactivation loop has the potential to limit intracellular prednisolone levels, which may be central in driving the systemic side effects associated with commonly prescribed GCs.

There are hypothetical concerns that 11β-HSD1 inhibition may be associated with hypothalamic–pituitary–adrenal axis activation and androgenic side effects may have failed to materialize in clinical studies. Increases in adrenocorticotrophic hormone and DHEAS within the normal range have been reported, but these were off-set by increases in sex hormone-binding globulin and were not associated with symptoms (39). Although these compounds appear to be well tolerated in short-term studies, the consequences of long-term 11β-HSD1 suppression in humans are unknown, and further studies are necessary to ensure that symptoms suggestive of tissue-specific GC deficiency are not encountered.

An important consideration that might influence their therapeutic potential is their impact on both acute and chronic inflammation. During an acute inflammatory response, 11β-HSD1 expression is increased in macrophages, and the local increased GCs availability is thought to have a beneficial anti-inflammatory effect. Based on this premise, it was anticipated that 11β-HSD1 would worsen acute inflammation. Indeed, this is what is seen in 11β-HSD1 knockout mice following the induction of sterile peritonitis. However, the peritonitis was actually found to resolve at the same rate as the wildtype animals (44). Similarly, cardiac function was much better preserved in 11β-HSD1 knockout mice following myocardial infarction, despite initially having comparatively higher levels of local inflammation (45, 46). Although these studies imply that loss of 11β-HSD1 may not have an adverse impact on an acute inflammatory response, a study using a mouse model of arthritis and carrageenan-induced pleurisy found 11β-HSD1-deficiency to result in the converse (47).

Chronic inflammation, on the other hand, arises from acute inflammation that fails to resolve, and this has been postulated to play a role in the development and propagation of type 2 diabetes, visceral obesity, as well as other aspects of the metabolic syndrome (48). As discussed above, selective 11β-HSD1 inhibitors have been shown to have beneficial effects on these ‘chronic inflammatory diseases’. As such, although there is evidence that 11β-HSD1 inhibition impedes the resolution of certain acute inflammation conditions, their impact on chronic inflammatory diseases is beneficial. However, this aspect of their efficacy needs urgent testing in a clinical setting.

Conclusions

Taken together, current data suggest that GC reactivation by 11β-HSD1 is key to the development of the adverse metabolic profile associated with circulatory GC excess – underscoring 11β-HSD1 as a potentially novel therapeutic target in the treatment of both endogenous and exogenous Cushing’s syndrome (Fig. 2). If these rodent findings translate into clinical studies in man, then there is no doubt that this class of agent will add significantly to the repertoire of drugs available to the clinician to limit the adverse side effects experienced by patients taking prescribed GCs. Certainly, clinical studies of subjects with hypercortisolism protected from Cushingoid features, due to loss of 11β-HSD1 function are encouraging (17, 18). However, there remain important issues that need to be clarified, such as the consequences of long-term 11β-HSD1 suppression, the influence of these compounds on the immunosuppressive properties of prescribed GCs, and their effects on the endogenous control of acute inflammation. As such, clinical studies are urgently needed to fully evaluate the use of this class of compound in the context of circulatory GC excess. These studies could be focused on Cushing’s disease patients with persistent or recurrent disease following first-line therapies or in those who are not surgical candidates, where it is clear that there is real clinical need. It is also interesting to speculate that selectively targeting 11β-HSD1 may be of great benefit, as an adjunctive therapy in 1–2% of the population taking prescribed GCs, where Cushingoid side effects remain a major burden; however, questions regarding their effects on underlying inflammatory disease need to be answered.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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