The ATP-binding cassette proteins ABCB1 and ABCC1 as modulators of glucocorticoid action

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Abstract

Responses to hormones that act through nuclear receptors are controlled by modulating hormone concentrations not only in the circulation but also within target tissues. The role of enzymes that amplify or reduce local hormone concentrations has become well-established for glucocorticoid and other lipophilic hormones; moreover, transmembrane transporters have proven critical in determining tissue responses to thyroid hormones. However, there has been less consideration of the role of transmembrane transport for steroid hormones. ATP-binding cassette (ABC) proteins were first shown to influence the accumulation of glucocorticoids in cells almost three decades ago, but observations over the past ten years suggest that differential transport propensities of both exogenous and endogenous glucocorticoids by ABCB1 and ABCC1 transporters provides a mechanism whereby different tissues are preferentially sensitive to different steroids. This Review summarises this evidence and the new insights provided for the physiology and pharmacology of glucocorticoid action, including new approaches to glucocorticoid replacement.

Introduction

Glucocorticoid hormones are vital for life; they confer diverse effects on multiple processes and systems. The adverse consequences of glucocorticoid excess are well demonstrated by the frank hypercortisolism of Cushing syndrome, but even subtle cortisol dysregulation has implications, contributing to cardiovascular disease, for example.[1] Over the past 30 years it has become clear that the concentration of glucocorticoid in the blood does not necessarily reflect that within tissues, as enzymes (such as 11β-hydroxysteroid dehydrogenase, which catalyses the interconversion of inert cortisone and active cortisol) and delivery mechanisms of corticosteroid binding protein can confer additional control over the absolute tissue levels.[2,3]

As lipophilic molecules, glucocorticoids can diffuse across cell membranes to interact with intracellular targets; however, they can also undergo active transmembrane transport. This process was first described for the ABCB1 transporter (of the ATP-Binding Cassette [ABC] protein family), which exports cortisol and a variety of synthetic glucocorticoids from 'sanctuary sites' including the brain.[4,5] Intriguingly, corticosterone is not readily exported by ABCB1, but we have discovered that the ABCC1 transporter, found in tissues including adipose, exports corticosterone but not cortisol.[6]

In this Review, we will explore the implications of this tissue-specific glucocorticoid transport in the central control of the hypothalamic–pituitary–adrenal (HPA) axis, adipose tissue metabolism and pregnancy. We will also consider whether the steroid specificity of ABCB1 and ABCC1 transport offers insights into the different roles of corticosterone and cortisol in humans and a potential opportunity for developing glucocorticoid therapies that are better targeted than those currently available to maximise efficacy and minimise toxicity.

[H1] The movement of lipophilic hormones

The 'free hormone hypothesis' determines that unbound lipophilic hormones move passively down a concentration gradient[7] and, indeed, steroids are taken up freely by cell types such as keratinocytes without the relevant membrane transporters.[8] Differences in tissue concentrations were previously attributed to differences in physicochemical properties, such as lipophilicity, until the discovery of the existence of specific thyroid hormone transporters challenged these traditional assumptions. In the case of triiodothyronine (T3), which is highly lipophilic owing to the iodinated aromatic ring, the level of hormone available to receptors not only depends on hormone synthesis and peripheral enzymatic conversion, but also on transport into and out of cells, notably by the monocarboxylate 8 (MCT8) transporter.[9] The uptake of T3 into neurons is critically impaired in the absence of MCT8, as occurs in the X-linked Allan-Herndon-Dudley syndrome of neurodevelopmental anomalies associated with abnormal thyroid function.[10]

The cellular uptake of glucocorticoids by membrane transporters has been demonstrated in Drosophila melanogaster, in which loss of the Ecdysone Importer (EcI) membrane transporter produces a phenotype that is identical to that resulting from the loss of ecdysone or the ecdysone receptor.[11] Organic anion transporting polypeptide transporters mediate the uptake of glucocorticoids in rat liver ex vivo; however, this uptake has not been reproduced in humans.[12,13] Furthermore, a saturable glucocorticoid uptake mechanism across the blood–brain barrier (BBB) and blood–cerebrospinal fluid barrier that was reported in mice was only discernible at supraphysiological concentrations, and so might not be physiologically relevant.[14]

Our increasing understanding of the importance of transporters for thyroid hormone function sets a biological precedent for a similar scenario for other lipophilic hormones; however, although the active cellular import of glucocorticoids in humans has not been shown, there is mounting evidence supporting the facilitated export of glucocorticoids from cells, particularly by two members of the ABC transporter family.

[H1] The ABC protein family

As members of one of the most highly conserved protein superfamilies, ABC proteins shuttle toxins, xenobiotics and signalling molecules across eukaryotic and prokaryotic cell membranes. These proteins are classified into seven subfamilies according to their structural similarity and sequence homology, and have been actively researched for decades, particularly in relation to multidrug resistance. The evolution and relevance of this transporter superfamily in the context of cancer drug efflux has been well reviewed;[15,16] however, of the over 50 human ABC proteins that have been identified, only ABCB1 and ABCC1 have recognised roles in glucocorticoid transport.[17]

The typical ABC transporter is a homodimer characterized by two transmembrane domains (TMDs) and two cytoplasmic nucleotide-binding domains (NBDs) (FIG. 1).[18] Each TMD domain contains between six and ten transmembrane α-helices, depending on the specific transporter, and is involved in substrate recognition. The cytoplasmic NBDs contain conserved motifs for ATP binding and hydrolysis, including the ABC signature motif (or C-loop motif), Walker A motif (P-loop) and Walker B motif.[17] Together, these dimeric NBDs act to hydrolyse ATP and provide energy to drive transport against concentration gradients.

Several models have been proposed to explain the relationship between ATP hydrolysis and TMD-mediated transport,[19] with most purporting that energy from ATP hydrolysis enables the TMDs to switch between inward- and outward-facing configurations (FIG. 1A). Individual ABC transporters are unidirectional: in eukaryotic cells, they are almost exclusively exporters, but both importers (of nutrients) and exporters (of toxins and cell wall substrates) exist in bacteria.[20] Consistent with this export function in eukaryotes, ABC transporters are typically found on the apical cell membrane at luminal surfaces to limit xenobiotic exposure.[17] Substrates range from ions to large proteins and there is a high degree of overlap between transporters, although the molecular basis for this overlap remains poorly documented.

[H1] ABCB1 and ABCC1 are steroid exporters

[H2] ABCB1 and steroid export

Initially named P-glycoprotein (P-gp) and later multidrug resistance protein 1 (MDR1), ABCB1 has been extensively studied as the archetypal multidrug transporter, exporting a broad array of xenobiotics including antineoplastics, antimicrobials and antidepressants from cells (reviewed in [15,21,22]). In humans, the *ABCB1* gene, located on chromosome 7q21.12, encodes a protein of 1280 amino acids (141.5 kDa) in size with 12 membrane-spanning α-helices distributed among two TMDs.[23] The polyspecificity of ABC transporters is often purported to result from the plasticity of the drug-binding pocket, both in terms of side chain and backbone arrangements. Numerous attempts have been made over the years to determine the 3D structure of ABC proteins in an effort to understand their transport mechanisms and their substrate specificity; however, their size and hydrophobicity pose significant challenges.[24] Advances in the use of cryo-electron microscopy have enabled structural insights into substrate binding.[25-27] Reconstitution of the structure of human ABCB1 in complex with chemotherapeutic drugs has revealed the drug-binding cavity to be globular in shape, with interactions contributed by all 12 membrane-spanning α-helices (FIG. 1B).[25] Substrate-induced structural changes in NBD2 are thought to confer changes in ATPase activity, which determines transport action.

A putative steroid-binding site has been identified in human ABCB1, but this is based upon a homology model of only the NBDs[28] and is not definitive. However, physiological data do support selective ABCB1-mediated transport of steroids. In the 1960s, murine fibroblasts were observed exporting steroids in an energy- and temperature-dependent manner, consistent with active transport.[29] Cortisol export was later (in 1992) demonstrated in a porcine renal tubular cell line (LLC-PK1) overexpressing human *ABCB1*.[30] Since then, several endogenous and synthetic steroids have been confirmed as ABCB1 substrates. Depending on the presence of hydroxyl groups at positions 11 and 17, steroids were stratified into three categories.[31] ABCB1-mediated efflux was highest for

steroids with both hydroxyl groups (including dexamethasone, cortisol and prednisolone), lowest for those with neither (deoxycorticosterone and progesterone), and intermediate in those with one hydroxyl group (including corticosterone and aldosterone). A-ring planarity and 6α- and 16α- methyl substitution were reported to enhance transport when compared to passive diffusion in the LLC-PK1 line, in keeping with the presence of a critical hydrophobic pocket in the steroid-binding region.[32] Methylprednisolone is the glucocorticoid most effectively exported by ABCB1, followed by prednisolone, betamethasone, prednisone, dexamethasone, cortisol and cortisone.[31-33] Aldosterone appears to be weakly transported, and there is no evidence that sex steroids or 11-deoxycorticosterone undergo ABCB1-mediated export,[31] although progesterone does bind avidly to ABCB1 with an inhibitory effect.[34] Corticosterone — the predominant glucocorticoid in rats and mice — was initially shown to be an ABCB1 substrate on the basis of efflux from murine macrophage-like cells,[35] and subsequent in vitro work in murine adrenocortical cells has demonstrated that pharmacological ABCB1 inhibition blocked the ability of these cells to secrete corticosterone.[36] However, this is in contrast to previous in vitro work showing that corticosterone was not exported in the murine LMCAT fibroblast line.[31,37-39] Studies of the human transporter have not shown corticosterone to be transported by ABCB1, so affinity might be species specific.[4,40] Importantly, studies in murine thymoma cells overexpressing *Abcb1* in which corticosterone and cortisol transport was compared showed a lower efflux of corticosterone compared with cortisol,[31] indicating an overall preference of this transporter for cortisol.

[H2] ABCC1 and steroid export

First identified and cloned as multidrug resistance-associated protein 1 (MRP1), ABCC1 was also discovered in multidrug resistance studies where high levels of expression are poor prognostic indicators in certain malignancies.[41-43] Since then, ABCC1 has been shown to efflux a diverse range of conjugated xenobiotics and physiological organic anions.[44] Like ABCB1, ABCC1 demonstrates a

polarized distribution in epithelial cells, but is located on the basolateral rather than apical membrane.[45]

ABCC1 is encoded by the human *ABCC1* gene on the short arm of chromosome 16 (16p13.11). Strikingly, ABCC1 and ABCB1 share only 23% sequence identity, and differ substantially in their structural and physiological functions. To date, the structure of only bovine ABCC1 has been determined by cryo-electron microscopy.[45] The 190 kDa ABCC1 protein has 17 transmembrane α-helices distributed among three TMDs (TMD0, TMD1 and TMD2) rather than the two TMDs observed in ABCB1 (FIG 1C).[45]

The binding site between TMDs 1 and 2 is 'bipartite': it has a positively charged 'P pocket', which forms hydrogen bonds with glutathione residues, and a second 'H pocket', which interacts with hydrophobic moieties. This bipartite binding domain explains why glutathione coupling facilitates the transport of a wide range of compounds.[45]

ABCC1 substrates tend to be organic anions, whereas those for ABCB1 tend to be weak cations;[45] and ABCC1 uniquely exhibits affinity for phase II hepatic metabolites (endogenous and xenobiotic compounds conjugated with glutathione, glucuronide and sulphate to facilitate excretion). There are differences in substrate preference between human and other mammalian isoforms — for example, the glucuronide conjugate of 17β-oestradiol is a substrate only in humans.[46] It has also been shown in vitro, both in virally transfected mouse fibroblast LMCAT cells and subsequently in human adipocytes, that ABCC1 can export corticosterone and 11-deoxycorticosterone, but not cortisol, prednisolone or dexamethasone.[6,39]

Whilst ABCB1 is thought to transport substrates partitioning through the bilipid cell membrane (the 'hydrophobic vacuum'),[47] ABCC1 is only open to substates within the cytoplasm.[45]

[H1] ABCB1 and ABCC1 expression in tissues

The mRNA expression profiles of human ABCB1 and ABCC1 in various tissues are summarised in FIG. 2. ABCB1 is highly expressed (both at mRNA and protein level) in the adrenal gland, but also found at absorptive surfaces (for example, of the intestines), protective barriers (for example, testis, BBB and placenta) and in secretory tissues (for example, biliary canaliculi and renal tubule).[23] ABCC1 is widely expressed in almost all cell types, with highest levels in the thymus, parathyroid glands and skeletal muscle. It seems to be poorly expressed in the liver[48] and nervous system but, notably, is found in greater quantities than ABCB1 in adipose tissue and skeletal muscle.[23,49,50]

A model for the consequences of this tissue-specific transporter expression on the intracellular concentrations of different glucocorticoids is outlined in FIG. 3. Combining in vitro studies from three different laboratories, glucocorticoids can be separated into three groups depending on their relative propensity to be exported by ABCB1 and ABCC1.[6,31,39] According to this model, the intracellular concentrations of cortisol will be lower in tissues that predominantly express ABCB1 (including the central HPA axis negative feedback sites behind the blood-brain barrier), and those of corticosterone will be lower in tissues that predominantly express ABCC1, such as adipose tissue. We can use experimental data from animal and human studies to show how this may modulate the physiology of the HPA axis, influence lipogenesis within adipocytes and alter glucocorticoid transfer across the placenta.

[H2] ABCB1 and ABCC1 and the HPA axis

[H3] *Insights from murine models.*

Central control of the HPA axis depends on feedback from circulating glucocorticoids to the hypothalamus and pituitary, but to reach the brain the glucocorticoids must traverse the tightly packed endothelium of the BBB, where ABCB1 is found.[51] Murine models have been used extensively to assess ABCB1-dependent modulation of steroid concentrations within tissues, including the brain.

Importantly, rodents have two ABCB1 isoforms: ABCB1A (also known as MDR1A or MDR3) and ABCB1B (also known as MDR1B or MDR1),[52,53] which broadly share the characteristics of the human protein.[53] Indeed, *Abcb1a*-knockout mice accumulate 87 times more of the ABCB1 substrate ivermectin in brain than do wild-type animals,[54] while ABCB1 inhibition with tariquidar increases cerebral retention of labelled verapamil on PET imaging and demonstrates the role of ABCB1 at the human BBB.[55]

Abcb1a-knockout mice exhibit enhanced retention of cortisol and dexamethasone in the brain.[4,5,54,56] As seen in vitro, results for corticosterone export in vivo are varied, perhaps reflecting redundancy between the murine isoforms. One study reported no difference in the levels of infused radiolabelled corticosterone in the brains of adrenalectomised *Abcb1a*-knockout compared to wild-type mice.[4] However, the *Abcb1ab*-double knockout mouse retained an excess of cortisol and corticosterone in the brain;[57] this retention was greater for cortisol than for corticosterone, suggesting that, overall, ABCB1 activity in mice favours cortisol over corticosterone transport, as was also found in vitro. However, another group reported the opposite effect: a retention of both glucocorticoids in *Abcb1a*-knockout mice, and cortisol retention alone in the *Abcb1ab* double knockout mice.[58-60] The authors highlight methodological differences between the studies which might limit comparisons: for instance, in one study isotope radioactivity rather than intact steroid concentration was measured, and the use of labelled corticosterone in adrenally intact animals might have resulted in isotope dilution by endogenously secreted corticosterone.

From these findings we might predict that the HPA axis would be relatively suppressed by the accumulation of glucocorticoids in the brain if ABCB1 activity is reduced. Indeed, *Abcb1a*-knockout mice do show evidence of HPA axis suppression, with lower basal and stress-stimulated levels of corticosterone, adrenocorticotropic hormone (ACTH) and corticotrophin-releasing hormone than control animals, with the effect localised to the hypothalamic level.[61] Furthermore, mice treated with the ABCB1 inhibitor tariquidar show an attenuated corticosterone response to stressful stimuli.[62]

[H3] *Insights from dogs and humans.*

The ABCB1 protein is well conserved in larger, cortisol-dominant species, with a notable exception being in Collie-derived dogs. Like *Abcb1a*-knockout mice,[54] these animals are exquisitely sensitive to ivermectin owing to a 4-base pair deletion mutation (termed *Mdr1-1Δ)* for which 40–50% of this breed are homozygous.[63,64] This mutation results in a severely truncated protein (<10% of normal length), which is predicted to be non-functional. Anecdotally, Collie dogs are reported to recover relatively slowly from illness,[65] and animals with the MDR1$^{-/-}$ genotype showed chronic suppression of the HPA axis, with lower basal cortisol levels and greater ACTH suppression in response to dexamethasone than their wild-type counterparts. It has been hypothesised that enhanced brain retention of cortisol (the dominant canine glucocorticoid) leads to this HPA axis suppression, and predisposes the animals to a form of relative corticosteroid insufficiency.[65] This hypothesis has been supported by a metabolomics study demonstrating lower urinary cortisol metabolites in MDR1$^{-/-}$ dogs than controls [reaching significance for allotetrahydrocortisol (11.2 ± 3.4 ng/L versus 20.7 ± 14.9 ng/L, P=0.006) and β-cortol (105.5 ± 63.3 ng/L versus 221.0 ± 225.5 ng/L, P=0.025)].[66]

In a human study, the corticosterone:cortisol ratio in brain autopsy specimens was five times greater than the corresponding ratio in plasma in age- and sex-matched healthy controls.[4] Similarly, the ratio of corticosterone to cortisol in live subjects is 5-6 times higher in cerebrospinal fluid than in plasma.[67] Many drugs, including verapamil and cyclosporin A, inhibit ABCB1, but their experimental use to test ABCB1 physiology in humans is hampered by toxicity at levels that are too low to carry out meaningful studies of ABCB1 inhibition.[68]

[H3] *ABCB1 and ABCC1 modulate the HPA axis.*

These results are all consistent with the hypothesis that ABCB1 at the BBB exports cortisol and thereby modulates negative feedback of the HPA axis in cortisol-dominant species. The absence of ABCC1 in

the brain and BBB is consistent with corticosterone being retained to a greater extent than cortisol in brain. One additional complexity, however, is that the pituitary gland (which expresses both transporters)[69] lies outside the BBB but also contributes to the control of the HPA axis. We have demonstrated that administration of probenecid, an inhibitor of ABCC1, induces greater tonic negative feedback of the HPA axis in healthy subjects than placebo as judged by elevations in ACTH and cortisol during combined mineralocorticoid and glucocorticoid receptor antagonism.[70] This finding is consistent with ABCC1 also contributing to the export of corticosterone from the pituitary gland or other central feedback areas, and warrants further investigation in animal models.

[H2] ABCC1 transporters in adipose tissue

[H3] *Insights from mice and humans.*

In contrast with the BBB, where ABCB1 is more abundant than ABCC1, the reverse is true in adipose tissue. Glucocorticoids within adipose tissue induce lipogenesis; in particular, they stimulate the accumulation of lipids in visceral tissue and the production of adipokines.[71] *Abcc1*-knockout mice infused with corticosterone and cortisol showed an enhanced accumulation of corticosterone but not cortisol in adipose tissue, accompanied by the upregulation of both glucocorticoid-responsive and adipogenic genes.[6]

We have also demonstrated that human adipocytes preferentially accumulate cortisol over corticosterone, and that this accumulation was reversed in vitro after treatment with the ABCC1 inhibitors probenecid or MK-571.[6] It was also accompanied by activation of glucocorticoid-responsive and adipogenic genes (*PER1, ADIPOQ, ATGL, HSL*) and resulted in the increased accumulation of fatty acids in lipid droplets.[6] Moreover, during infusion of cortisol or corticosterone in vivo in patients with primary adrenal insufficiency, the induction of glucocorticoid-responsive gene expression (*PER1, LPL*) in adipose tissue was greater in response to cortisol than to corticosterone (achieved at plasma

glucocorticoid levels which were equipotent for ACTH suppression).[6] This suggests that corticosterone could have a more favourable metabolic profile than cortisol in glucocorticoid replacement, particularly when ACTH suppression is a target.

[H2] ABCB1 and ABCC1 in the placenta

As the interface between the mother and the fetus in pregnancy, the placenta functions both as a nutritive source and a barrier, including to glucocorticoid transport. The fetus is unable to synthesize cortisol until the third trimester, and therefore depends on maternal cortisol; however, although maternal cortisol levels increase by several-fold during pregnancy, this increase is not transferred to the fetus indiscriminately.[72] In early pregnancy, excessive glucocorticoids are detrimental to the fetus, so the placenta provides a glucocorticoid barrier,[73] but it confers a more facilitative role towards term for fetal organ maturation.[74]

[H3] *The placental glucocorticoid barrier: 11β-hydroxysteroid dehydrogenase 2.*

The enzyme 11β-hydroxysteroid dehydrogenase 2 is viewed as the main component of the placental glucocorticoid barrier, converting active cortisol to inactive cortisone.[75] However, the results of a study in which 11β-hydroxysteroid dehydrogenase 2 was inhibited during ex vivo perfusion of human placentas collected on ice immediately after delivery suggested that the enzyme might contribute only part of the glucocorticoid barrier, as cortisol transfer was restricted even at maximal inhibition of 11β-hydroxysteroid dehydrogenase 2.[76] The role of other mechanisms that are operating at the placental barrier, such as transmembrane transport, therefore warrants further consideration.

[H3] *The placental glucocorticoid barrier: ABCB1 and ABCC1.*

ABCB1 is located within syncytiotrophoblasts at the apical brush-border membrane**,** in direct contact with maternal blood.[77] It is highly expressed in early pregnancy and decreases towards term, consistent with the physiological role suggested above.[78] As occurs in other tissues, glucocorticoids have been shown to upregulate the expression of *ABCB1* in the placenta in the first trimester, which might enhance the barrier effect.[79] Data demonstrating low concentrations of ABCB1 substrates (antiretrovirals, for example) in the fetal circulation both at birth and in the ex vivo perfused placenta indicate that ABCB1-mediated export towards the maternal circulation is active in vivo.[80] ABCC1 is located on the fetal-facing placental surface and has been identified in cytotrophoblasts, syncytiotrophoblasts and the fetal endothelium.[81] This localization might be consistent with a role in transferring ABCC1 substrates such as folic acid to the fetus and, in contrast with *ABCB1, ABCC1* is upregulated towards term.[81,82] Studies of other ABCC1 substrates using the inhibitors probenecid and MK-571 have not demonstrated a clear effect on cross-placental transfer, so cannot be extrapolated to corticosterone transport.[83] It has been shown that the cortisol:corticosterone ratio is higher in the maternal circulation (15:1) than in the umbilical vein (7:1) at term,[84] which might be accounted for by fetal adrenal cortisol:corticosterone secretion rates or by the facilitated transport of maternal corticosterone by ABCC1 into the fetal circulation.

[H1] Regulation and dysregulation

[H2] Regulation of ABCB1

The mechanisms underpinning the regulation of the expression of *ABCB1* have been reviewed thoroughly elsewhere.[85-87] The *ABCB1* promoter contains a number of areas of interest, including binding sites for the tumour suppressor p53, heat shock proteins and adopted orphan receptors, including the pregnane X receptor (PXR) and constitutive androstane receptor (CAR), which bind a number of xenobiotic ligands.[88] Xenobiotics, inflammatory mediators and cellular stress (such as irradiation, heat shock, hypoxia) typically upregulate *ABCB1* expression through common pathways

involving nuclear factor kappa B (NF-κB) and Y-box binding protein.[89,90] This upregulation appears to be a protective response, and polymorphisms in NF-κB are linked with increasing colon cancer risk, potentially owing to enhanced cellular exposure to toxins.[91]

Glucocorticoids modulate the expression of ABCB1 mRNA and protein in rodents and humans. This modulation has been demonstrated across multiple tissues using dexamethasone, prednisolone, cortisol, methylprednisolone and some inhaled glucocorticoids.[33,79,92-97] Although glucocorticoids predominantly induce the expression of *ABCB1*, this effect might be specific to some species or cell types, as there are also instances of *ABCB1* expression being downregulated.[98] This glucocorticoid effect is inhibited in the presence of the glucocorticoid receptor blocker RU486, indicating that this effect is at least partly mediated via the glucocorticoid receptor, but as no consensus glucocorticoid response element has been found in the human *ABCB1* promoter, it is assumed to be an indirect genomic effect. Dexamethasone-mediated upregulation of *ABCB1* in retinal pigment epithelium was reported to be abolished when the PXR receptor was silenced, implying that PXR (which does contain a consensus glucocorticoid response element) is either a co-regulator or a target of the glucocorticoid receptor.[97,99,100] This upregulation of expression raises concerns about increased drug efflux when glucocorticoids are used in combination with other ABCB1 substrates (as often occurs in chemotherapy protocols), and is theorised to be a cause of glucocorticoid resistance in conditions such as asthma;[33] however, this effect has also been exploited clinically — for example, methylprednisolone is used in the treatment of paraquat toxicity to increase excretion of the drug.[101]

However, the regulation of ABCB1 in inflammation is complex and potentially biphasic. Evidence from rodent studies indicates that, in the very early stages of inflammation, ABCB1 is functionally inhibited by lipopolysaccharides and inflammatory cytokines, despite mRNA levels remaining constant, perhaps owing to ABCB1 being trafficked away from the cell membrane; later in the evolution of inflammation, however, ABCB1 mRNA and protein levels are upregulated by the cytokines tumour necrosis factor and endothelin 1 converging on the NF-κB pathway.[89] Protein

turnover at the cell surface under normal conditions is relatively slow (the half-life of ABCB1 is estimated at just over 24 hours)[102] and there might be a role for post-translational and other mechanisms in modulating this turnover. Taken together, this evidence suggests that in times of increased physiological stress (for example, in response to illness or injury), ABCB1 can be upregulated both by stress-activated glucocorticoids and by signals released by cellular damage. This upregulation might result in positive feedback on cortisol production by further restricting glucocorticoid access to sites of higher negative feedback.

[H2] Regulation of ABCC1

Most research on factors affecting the expression levels of *ABCC1* and its protein activity relates to cancer biology and chemotherapeutics, whilst physiological regulation has been poorly studied to date. Basal transcription of *ABCC1* is stimulated by the SP1 transcription factor[103] which is, in turn, inhibited by the tumour suppressor protein p53.[104] It has not been clearly established whether PXR affects *ABCC1* transcription[105,106] and, although early mapping of the *ABCC1* promoter in a human leukaemic cell line did reveal a putative glucocorticoid response element, dexamethasone has not been shown to alter *ABCC1* expression in the human placenta or in lymphocytes.[94,107-109] Furthermore, we cannot clearly conclude whether ABCC1 is affected by acute inflammation in the same way that ABCB1 is, as both unchanged and increased mRNA expression have been reported in response to mediators such as lipopolysaccharide, tumour necrosis factor, IL-1 and IL-6.[110-112]

In vitro studies investigating the metabolic regulation of *ABCC1* have focused on endothelium, and have demonstrated that expression of the transcript is downregulated in a hyperglycaemic environment.[113] Metformin, a drug commonly used in the treatment of type 2 diabetes mellitus, is known to reduce *ABCC1* expression in a human hepatocellular carcinoma cell line through the AMP-activated protein kinase–hypoxia-inducible factor 1 pathway.[114]

Whilst limited, overall this evidence suggests that ABCC1 is regulated differently from ABCB1, and is predominantly responsive to metabolic and immunomodulatory signals rather than to mediators of acute stress or inflammation.

[H2] Pathological dysregulation

There have been few studies of variations in ABC transporter expression beyond the descriptions in various cancers mentioned above. A transcriptomic analysis utilising single-cell RNA sequencing showed upregulation of *ABCB1* in the adrenal cortex of patients with ACTH-dependent Cushing disease.[36] This upregulation probably reflects the effects of glucocorticoids on *ABCB1* expression, but might contribute to pathogenicity by further enhancing the export of cortisol from the gland. Hypothesizing that steroid retention in adipocytes due to low levels of ABCC1 could be a driving mechanism for obesity, we actually found that *ABCC1* mRNA levels were upregulated in the adipose tissue (subcutaneous and visceral) of individuals with obesity compared with lean individuals, which may reduce glucocorticoid concentrations in adipocytes, although this reduction might only be true for corticosterone.[6]

[H2 Lessons from human genetics

[H3] *Germline mutations in ABCB1***.**

Human germline mutations in *ABCB1* are rare. To our knowledge, there are only two publications of *ABCB1* mutations: twin girls with recurrent reversible toxic encephalopathy alongside febrile illness, [115] and a 13-year old boy with ivermectin sensitivity.[116] In both cases, the mutations were identified by whole exome sequencing and show compound heterozygosity. The twin girls were found to have a nonsense mutation (p.Pro1182X) combined with a splice variant (c.2786 + 1 G>T) and showed markedly enhanced CNS retention of ^{11}C-verapamil on PET imaging in comparison with their parents.

Their symptoms were suspected to be caused by retention of inflammatory mediators within the brain during acute illness, and it was shown in a mouse model by the authors that cytokines Tumour Necrosis Factor, Il-1, Il-6 and Ccl-2 were retained in brain at 24 hours after lipopolysaccharide injection in *Abcb1ab* knockout versus wild-type animals. The investigators estimated from studies in lymphocytes that only ~10% of functional ABCB1 protein was expressed. In the other case, the affected boy presented with severe neurological side effects after a single oral dose of ivermectin to treat scabies and was found to have inherited a nonsense mutation in ABCB1 from each parent (c.2380 C>T and c.3053_3056delTTGA), both of which are predicted to result in the loss of the carboxy-terminal nucleotide-binding domain. The boy and twin girls were otherwise healthy and growing normally in each case.

[H3] *Germline mutations in ABCC1.*

Similarly, there is only one published mutation of *ABCC1* of clinical significance: a heterozygous missense mutation (c.1769 A>G) identified as causing familial sensorineural deafness.[117] ABCC1 has been found within the rodent cochlea, where it could be protective against neurotoxins.[118] This mutation is thought to disrupt hydrogen bonds, and thus stability between the helices of the transmembrane domains in the proteins, but analysis of lymphoblastoid cell lines derived from affected family members showed loss of around 40–45% of ABCC1 mRNA expression when compared with those unaffected, suggesting an additional impairment in mRNA stability. Extrusion of SNARF-1, a known ABCC1 substrate, from lymphoblastoid cells as a measure of transport activity was subsequently shown to be slower.[117]

[H3] ***Polymorphisms in* ABCB1 *and* ABCC1.**

With nonsense and frameshift mutations being rare, there have been attempts to correlate common polymorphisms with clinically relevant outcomes (reviewed in [119]).

Three *ABCB1* variants are common in humans: c.2677 G>A/T, c.3435 C>T and c.1236 C>T. The c.3435 C>T allele is synonymous, but might affect mRNA stability;[120] c.1236 C>T is silent; and c.2677 G>A/T results in an amino acid substitution (alanine to serine or threonine), which could potentially result in substrate changes. There is marked variation in the frequency of these polymorphisms across different races: for example, c.3435 C>T is much less common in African populations (~80% of people from West Africa are homozygous for the C allele versus ~20% of individuals from western Europe).[120,121] However, it has not been convincingly demonstrated that these variants affect substrate transport, for instance levels of the ABCB1 substrate digoxin have been found to be increased, decreased and unchanged in the plasma of individuals with these polymorphisms. Subsequent attempts to correlate polymorphisms with response to chemotherapeutics, drug side effects, and resistance to anti-retroviral and anti-epileptic therapies have been similarly inconclusive.[122-124]

Studies of the HPA axis in individuals with *ABCB1* variants have unfortunately been inadequately powered. No differences were found in the levels of evening cortisol and ACTH in 30 Japanese men with C/C, C/T or T/T c.3435 genotypes (the variant associated with potentially reduced transporter mRNA stability); however, another study, of 51 women, reported lower levels of cortisol in the plasma, taken at 6 pm, of individuals with one or two copies of the T allele compared with C/C controls; these lower levels reached significance only in the follicular menstrual phase so an interaction with sex hormones is proposed.[125,126] In one candidate gene study of over 5,000 Japanese individuals, the c.2677 G>A/T variant was highly associated with increased body mass index, which could potentially reflect increased HPA axis activity, whilst in a study of 154 individuals with depression, the response of cortisol (but not ACTH) to corticotrophin-releasing hormone was lower in c.2677 TT homozygotes than in the major allele (GG) or heterozygous (TG) groups, which was taken to reflect reduced adrenal cortisol release.[36,127] However, neither plasma cortisol levels nor body mass index has been associated with any *ABCB1* polymorphisms in larger cohorts.

Genetic studies have also been undertaken in patients taking exogenous steroids. In a cohort of 171 patients requiring long-term treatment with glucocorticoids for adrenal insufficiency, those patients with the c.3435 TT genotype had lower bone density than CC or CT groups, suggesting greater systemic steroid absorption or enhanced bone retention.[128] There have been attempts to correlate glucocorticoid treatment outcomes in patients with rheumatoid arthritis, inflammatory bowel disease, immune thrombocytopenic purpura and nephrotic syndrome with ABCB1 polymorphisms.[129-132] Most, but not all, indicate a higher steroid response with the minor allele of the studied polymorphism, but studies are limited by sample size and a failure to control for multiple testing.

Documented polymorphisms for *ABCC1* are mostly rare and non-coding, and have not been assessed in the context of HPA axis activity or metabolism.[133] Three polymorphisms might predict the outcome of acute myeloid leukaemia, but any corresponding effect of these polymorphisms on transporter expression or function has so far not been established.[134]

[H1] Implications and future research

The observations that two ABC transporters influence the retention of glucocorticoids in tissues allow us to add membrane transporters to the list of factors that are involved in the metabolism of glucocorticoids at the pre-receptor level (FIG. 4). These observations provide insights into HPA axis physiology and how corticosterone and cortisol might carry out different functions in species that produce both steroids. These findings also provide therapeutic opportunities for anti-inflammatory and physiological replacement steroid therapies that might better target tissues mediating efficacy while avoiding those mediating toxicity.

[H2] Revised glucocorticoid physiology

In rodents, the lack of steroid 17-hydroxylation necessitates that corticosterone is the sole endogenous glucocorticoid.[135] In humans and other species in which both glucocorticoids circulate, it is common to consider them interchangeable. Indeed, cortisol and corticosterone share similar metabolic pathways (for example, susceptibility to metabolism by 11β-hydroxysteroid dehydrogenase enzymes) and affinities for the glucocorticoid and mineralocorticoid receptors.[136-139] However, corticosterone does exhibit differences to cortisol, including more rapid clearance from the circulation, and a greater response to ACTH, such that the corticosterone:cortisol ratio rises under stress.[140-142]

The findings outlined in this Review further demonstrate that cortisol and corticosterone are not interchangeable with respect to glucocorticoid action. Specifically, in tissues where ABCB1 but not ABCC1 is present, such as the brain, cortisol concentrations are constrained by export back into the circulation and corticosterone can play a disproportionate role. Conversely, in tissues such as adipose where ABCC1 but not ABCB1 is expressed, corticosterone is exported and the response to cortisol can be disproportionate (FIG. 5). This observation raises the concept of a distinctive role for corticosterone in mediating HPA axis negative feedback. In the stressed state, the ability to restrict the high levels of circulating cortisol from accessing higher centres might prevent axis suppression and facilitate recovery, as demonstrated by the *Mdr1-1Δ* Collie dogs who lack this capacity.[65] It is recognised in other species that the ratio of cortisol to corticosterone and the peak levels of circulating glucocorticoids vary seasonally,[143] possibly in response to photoperiod length. If corticosterone is more accessible to negative feedback sites, and less peripherally anabolic than cortisol (in terms of effects on adipose tissue), then the energy-expending stress response might be restrained and access to vital adipose energy stores when food is scarce might be improved**.** Conversely, with a slower turnover than corticosterone in the circulation and adipose tissue in comparison with other tissues such as brain and liver,[144] cortisol might provide the option for medium-term adjustments, in comparison with the acute changes mediated by corticosterone.

Understanding the implications of the differential control and actions of cortisol and corticosterone in glucocorticoid physiology will require a detailed dissection of the dynamics of ligand availability for receptors within human target tissues in vivo. The increasing use of exome-wide sequencing in clinical as well as research settings might well identify further individuals or families with significant *ABCB1* and *ABCC1* mutations and offer new routes to addressing these key physiological issues.

[H2] Novel glucocorticoid therapies

A major limitation of current glucocorticoid therapies is their narrow therapeutic index. Despite extensive efforts, it has proved difficult to develop selective glucocorticoid receptor modulators with pharmacodynamic interactions that discriminate between efficacious and toxic gene transcription.[145] An alternative approach depends on the premise that efficacious and toxic effects are often mediated in different tissues, suggesting that the therapeutic index could be improved by modifying the pharmacokinetics of steroid drugs to 'target' them to the tissues where efficacy is mediated while avoiding tissues where toxicity is mediated. Could this be achieved by using steroids with different affinities for the ABCB1 and ABCC1 transporters?

When considering physiological replacement in patients with adrenal insufficiency, the challenges of this narrow therapeutic index are well documented, with adverse outcomes including, but not limited to, obesity, osteopenia and insulin resistance attributable to the steroid regime of these patients.[146,147] Such challenges are particularly evident in patients with congenital adrenal hyperplasia (CAH), in whom doses of glucocorticoid that achieve adequate adrenal androgen suppression are invariably associated with morbidity.[146] All glucocorticoids currently used to replace cortisol (hydrocortisone, prednisolone, dexamethasone, and the active metabolites of pre-drugs cortisone and prednisone) are substrates for ABCB1 but not ABCC1. Although pharmacokinetic adjustments, such as delayed release preparations, might confer some benefits,[148,149] they cannot

overcome the closeness of the dose-response relationship between efficacy and toxicity, and the prospect of choosing a glucocorticoid based on affinity for ABCC1 over ABCB1 is an intriguing therapeutic prospect.

As one such glucocorticoid, corticosterone is not currently available in an oral form, but our experimental work using intravenous corticosterone has provided proof-of-concept of the potential advantages of corticosterone in avoiding harmful metabolic effects mediated in adipose tissue. As described earlier, infused cortisol induced a greater response of glucocorticoid-responsive gene expression compared with infused corticosterone in the adipose tissue of patients with Addison disease.[6] In a similar study, 14 individuals with CAH also underwent ramped cortisol and corticosterone infusions; despite higher plasma levels of corticosterone being achieved, the amount of insulin released was greater in response to cortisol than to corticosterone – a marker of glucocorticoid effect on adipose to induce insulin resistance.[150]

The potential for glucocorticoid therapies that avoid toxicity in metabolic tissues deserves further investigation but would require the generation of an oral corticosterone preparation for practical administration to patients.

Conclusions

We have collated evidence from cell, animal and human studies that the ATP-binding cassette transporters ABCB1 and ABCC1 differentially export cortisol, corticosterone and synthetic glucocorticoids from tissues and contribute to pre-receptor glucocorticoid regulation. Differing transporter expression profiles in the brain, placenta and adipose confer different tissue sensitivities to these steroids, which might be important for optimising the responsiveness of the HPA axis, controlling fetal exposure to steroids throughout gestation, and optimising adipose fuel metabolism. Although much is known about these transporters in the context of multidrug resistance, their

physiological roles and regulation remain largely unexplored. The prospect of developing steroid therapies with transporter affinities that are tailored to give improved efficacy, without deleterious peripheral toxicity, offers new avenues for exploration for the management of inflammatory and endocrine diseases.

Box 1 - Multidrug Resistance

Multidrug resistance (MDR) is the ability of malignant cells to evade the actions of a broad range of chemotherapeutic agents. Tumours which are initially very sensitive can become resistant to multiple agents over the course of the disease, ultimately resulting in treatment failure and disease progression. There are several potential reasons for this, but the key mechanism is increased drug efflux out of malignant cells by membrane transporters, particularly those of the ABC family. Some tumours have innately high levels of transporter expression, but others develop this after exposure to chemotherapy.[151]

ABCB1 is the transporter most widely associated with MDR, particularly since alkylating agents, anthracyclines and vinca alkaloid drugs are all substrates.[21] ABCC1 and ABCG2 (aka Breast cancer Resistance Protein) are also implicated in MDR.

As examples, survival rates from lung cancer, multiple myeloma and acute myeloid leukaemia have been inversely associated with levels of ABCB1 expression.[152-154] High levels of ABCC1 expression are associated with poor outcomes in childhood neuroblastoma,[155] whilst over-expression of ABCG2 is a negative prognostic factor in pancreatic ductal adenocarcinomas.[156]

References

1 Walker, B. R. Glucocorticoids and cardiovascular disease. *Eur. J. Endocrinol.* **157**, 545-559, doi:10.1530/eje-07-0455 (2007).

2 Hammond, G. L. Plasma steroid-binding proteins: primary gatekeepers of steroid hormone action. *J. Endocrinol.* **230**, R13-25, doi:10.1530/joe-16-0070 (2016).

3 Seckl, J. R. & Walker, B. R. Minireview: 11beta-hydroxysteroid dehydrogenase type 1- a tissue-specific amplifier of glucocorticoid action. *Endocrinology* **142**, 1371-1376, doi:10.1210/endo.142.4.8114 (2001).

4 Karssen, A. M. *et al.* Multidrug resistance P-glycoprotein hampers the access of cortisol but not of corticosterone to mouse and human brain. *Endocrinology* **142**, 2686-2694, doi:10.1210/endo.142.6.8213 (2001).

5 Meijer, O. C. *et al.* Penetration of dexamethasone into brain glucocorticoid targets is enhanced in mdr1A P-glycoprotein knockout mice. *Endocrinology* **139**, 1789-1793, doi:10.1210/endo.139.4.5917 (1998).

6 Nixon, M. *et al.* ABCC1 confers tissue-specific sensitivity to cortisol versus corticosterone: A rationale for safer glucocorticoid replacement therapy. *Sci. Transl. Med.* **8**, 352ra109, doi:10.1126/scitranslmed.aaf9074 (2016).

7 Mendel, C. M. The Free Hormone Hypothesis: A physiologically based mathematical model. *Endocr. Rev.* **10**, 232-274, doi:10.1210/edrv-10-3-232 (1989).

8 Ponec, M. & Kempenaar, J. A. Biphasic entry of glucocorticoids into cultured human skin keratinocytes and fibroblasts. *Arch. Dermatol. Res.* **275**, 334-344, doi:10.1007/BF00417208 (1983).

9 Friesema, E. C. *et al.* Identification of monocarboxylate transporter 8 as a specific thyroid hormone transporter. *J. Biol. Chem.* **278**, 40128-40135, doi:10.1074/jbc.M300909200 (2003).

10 Friesema, E. C. *et al.* Association between mutations in a thyroid hormone transporter and severe X-linked psychomotor retardation. *Lancet* **364**, 1435-1437, doi:10.1016/s0140-6736(04)17226-7 (2004).

11 Okamoto, N. *et al.* A membrane transporter Is required for steroid hormone uptake in Drosophila. *Dev. Cell* **47**, 294-305.e297, doi:10.1016/j.devcel.2018.09.012 (2018).

12 Allera, A. & Wildt, L. Glucocorticoid-recognizing and -effector sites in rat liver plasma membrane. Kinetics of corticosterone uptake by isolated membrane vesicles--II. Comparative influx and efflux. *J. Steroid Biochem. Mol. Biol.* **42**, 757-771 (1992).

13 Bossuyt, X., Muller, M., Hagenbuch, B. & Meier, P. J. Polyspecific drug and steroid clearance by an organic anion transporter of mammalian liver. *J. Pharmacol. Exp. Ther.* **276**, 891-896 (1996).

14 Mason, B. L., Pariante, C. M., Jamel, S. & Thomas, S. A. Central nervous system (CNS) delivery of glucocorticoids is fine-tuned by saturable transporters at the blood-CNS barriers and nonbarrier regions. *Endocrinology* **151**, 5294-5305, doi:10.1210/en.2010-0554 (2010).

15 Juan-Carlos, P. M., Perla-Lidia, P. P., Stephanie-Talia, M. M., Mónica-Griselda, A. M. & Luz-María, T. E. ABC transporter superfamily. An updated overview, relevance in cancer multidrug resistance and perspectives with personalized medicine. *Mol. Biol. Rep.* **48**, 1883-1901, doi:10.1007/s11033-021-06155-w (2021).

16 Silverton, L., Dean, M. & Moitra, K. Variation and evolution of the ABC transporter genes ABCB1, ABCC1, ABCG2, ABCG5 and ABCG8: implication for pharmacogenetics and disease. *Drug Metabol. Drug Interact.* **26**, 169-179, doi:10.1515/dmdi.2011.027 (2011).

17 Vasiliou, V., Vasiliou, K. & Nebert, D. W. Human ATP-binding cassette (ABC) transporter family. *Hum Genomics* **3**, 281-290 (2009).

18 Wilkens, S. Structure and mechanism of ABC transporters. *F1000prime reports* **7**, 14-14, doi:10.12703/P7-14 (2015).

19 Seeger, M. A. & van Veen, H. W. Molecular basis of multidrug transport by ABC transporters. *Biochim. Biophys. Acta* **1794**, 725-737, doi:10.1016/j.bbapap.2008.12.004 (2009).

20 Kang, J. *et al.* PDR-type ABC transporter mediates cellular uptake of the phytohormone abscisic acid. *Proc. Natl. Acad. Sci. U. S. A.* **107**, 2355-2360, doi:10.1073/pnas.0909222107 (2010).

21 Hodges, L. M. *et al.* Very important pharmacogene summary: ABCB1 (MDR1, P-glycoprotein). *Pharmacogenet. Genomics* **21**, 152-161, doi:10.1097/FPC.0b013e3283385a1c (2011).

22 Sissung, T. M. *et al.* Pharmacogenetics of membrane transporters: an update on current approaches. *Mol. Biotechnol.* **44**, 152-167, doi:10.1007/s12033-009-9220-6 (2010).

23 Uhlén, M. *et al.* Proteomics. Tissue-based map of the human proteome. *Science* **347**, 1260419, doi:10.1126/science.1260419 (2015).

24 Esser, L. *et al.* Structures of the multidrug transporter P-glycoprotein reveal asymmetric ATP binding and the mechanism of polyspecificity. *J. Biol. Chem.* **292**, 446-461, doi:10.1074/jbc.M116.755884 (2017).

25 Alam, A., Kowal, J., Broude, E., Roninson, I. & Locher, K. P. Structural insight into substrate and inhibitor discrimination by human P-glycoprotein. *Science* **363**, 753-756, doi:10.1126/science.aav7102 (2019).

26 Lusvarghi, S., Robey, R. W., Gottesman, M. M. & Ambudkar, S. V. Multidrug transporters: recent insights from cryo-electron microscopy-derived atomic structures and animal models. *F1000Res* **9**, doi:10.12688/f1000research.21295.1 (2020).

27 Nosol, K. *et al.* Cryo-EM structures reveal distinct mechanisms of inhibition of the human multidrug transporter ABCB1. *Proc. Natl. Acad. Sci. U. S. A.* **117**, 26245-26253, doi:10.1073/pnas.2010264117 (2020).

28 Mares-Sámano, S., Badhan, R. & Penny, J. Identification of putative steroid-binding sites in human ABCB1 and ABCG2. *Eur. J. Med. Chem.* **44**, 3601-3611, doi:10.1016/j.ejmech.2009.02.027 (2009).

29 Gross, S. R., Aronow, L. & Pratt, W. B. The active transport of cortisol by mouse fibroblasts growing in vitro. *Biochem. Biophys. Res. Commun.* **32**, 66-72 (1968).

30 Ueda, K. *et al.* Human P-glycoprotein transports cortisol, aldosterone, and dexamethasone, but not progesterone. *J. Biol. Chem.* **267**, 24248-24252 (1992).

31 Bourgeois, S., Gruol, D. J., Newby, R. F. & Rajah, F. M. Expression of an mdr gene is associated with a new form of resistance to dexamethasone-induced apoptosis. *Mol. Endocrinol.* **7**, 840-851, doi:10.1210/mend.7.7.8105374 (1993).

32 Yates, C. R. *et al.* Structural determinants of P-glycoprotein-mediated transport of glucocorticoids. *Pharm. Res.* **20**, 1794-1803 (2003).

33 Crowe, A. & Tan, A. M. Oral and inhaled corticosteroids: differences in P-glycoprotein (ABCB1) mediated efflux. *Toxicol. Appl. Pharmacol.* **260**, 294-302, doi:10.1016/j.taap.2012.03.008 (2012).

34 Yang, C. P., DePinho, S. G., Greenberger, L. M., Arceci, R. J. & Horwitz, S. B. Progesterone interacts with P-glycoprotein in multidrug-resistant cells and in the endometrium of gravid uterus. *J. Biol. Chem.* **264**, 782-788 (1989).

35 Wolf, D. C. & Horwitz, S. B. P-glycoprotein transports corticosterone and is photoaffinity-labeled by the steroid. *Int. J. Cancer* **52**, 141-146 (1992).

36 Lopez, J. P. *et al.* Single-cell molecular profiling of all three components of the HPA axis reveals adrenal ABCB1 as a regulator of stress adaptation. *Sci Adv* **7**, doi:10.1126/sciadv.abe4497 (2021).

37 Medh, R. D., Lay, R. H. & Schmidt, T. J. Agonist-specific modulation of glucocorticoid receptor-mediated transcription by immunosuppressants. *Mol. Cell. Endocrinol.* **138**, 11-23 (1998).

38 Pariante, C. M. *et al.* Antidepressants enhance glucocorticoid receptor function in vitro by modulating the membrane steroid transporters. *Br. J. Pharmacol.* **134**, 1335-1343, doi:10.1038/sj.bjp.0704368 (2001).
39 Webster, J. I. & Carlstedt-Duke, J. Involvement of multidrug resistance proteins (MDR) in the modulation of glucocorticoid response. *J. Steroid Biochem. Mol. Biol.* **82**, 277-288 (2002).
40 Peng, R., Zhang, H., Zhang, Y. & Wei, D. Y. Impacts of ABCB1 (G1199A) polymorphism on resistance, uptake, and efflux to steroid drugs. *Xenobiotica* **46**, 948-952, doi:10.3109/00498254.2016.1138249 (2016).
41 Pajic, M., Norris, M. D., Cohn, S. L. & Haber, M. The role of the multidrug resistance-associated protein 1 gene in neuroblastoma biology and clinical outcome. *Cancer Lett.* **228**, 241-246, doi:10.1016/j.canlet.2005.01.060 (2005).
42 Paprocka, M. *et al.* MRP1 protein expression in leukemic stem cells as a negative prognostic marker in acute myeloid leukemia patients. *Eur. J. Haematol.* **99**, 415-422, doi:10.1111/ejh.12938 (2017).
43 Cole, S. P. *et al.* Overexpression of a transporter gene in a multidrug-resistant human lung cancer cell line. *Science* **258**, 1650-1654 (1992).
44 Cole, S. P. Multidrug resistance protein 1 (MRP1, ABCC1), a "multitasking" ATP-binding cassette (ABC) transporter. *J. Biol. Chem.* **289**, 30880-30888, doi:10.1074/jbc.R114.609248 (2014).
45 Johnson, Z. L. & Chen, J. Structural basis of substrate recognition by the multidrug resistance protein MRP1. *Cell* **168**, 1075-1085.e1079, doi:10.1016/j.cell.2017.01.041 (2017).
46 Stride, B. D. *et al.* Pharmacological characterization of the murine and human orthologs of multidrug-resistance protein in transfected human embryonic kidney cells. *Mol. Pharmacol.* **52**, 344-353, doi:10.1124/mol.52.3.344 (1997).
47 Higgins, C. F. & Gottesman, M. M. Is the multidrug transporter a flippase? *Trends Biochem. Sci.* **17**, 18-21, doi:https://doi.org/10.1016/0968-0004(92)90419-A (1992).
48 Nishimura, M. & Naito, S. Tissue-specific mRNA expression profiles of human ATP-binding cassette and solute carrier transporter superfamilies. *Drug Metab. Pharmacokinet.* **20**, 452-477, doi:10.2133/dmpk.20.452 (2005).
49 Flens, M. J. *et al.* Tissue distribution of the multidrug resistance protein. *Am. J. Pathol.* **148**, 1237-1247 (1996).
50 Cole, S. P. Targeting multidrug resistance protein 1 (MRP1, ABCC1): past, present, and future. *Annu. Rev. Pharmacol. Toxicol.* **54**, 95-117, doi:10.1146/annurev-pharmtox-011613-135959 (2014).
51 Cordon-Cardo, C. *et al.* Multidrug-resistance gene (P-glycoprotein) is expressed by endothelial cells at blood-brain barrier sites. *Proc. Natl. Acad. Sci. U. S. A.* **86**, 695-698, doi:10.1073/pnas.86.2.695 (1989).
52 Gruol, D. J., Vo, Q. D. & Zee, M. C. Profound differences in the transport of steroids by two mouse P-glycoproteins. *Biochem. Pharmacol.* **58**, 1191-1199 (1999).
53 Su, L. *et al.* Abcb1a and Abcb1b genes function differentially in blood–testis barrier dynamics in the rat. *Cell Death Dis.* **8**, e3038-e3038, doi:10.1038/cddis.2017.435 (2017).
54 Schinkel, A. H. *et al.* Disruption of the mouse mdr1a P-glycoprotein gene leads to a deficiency in the blood-brain barrier and to increased sensitivity to drugs. *Cell* **77**, 491-502 (1994).
55 Wagner, C. C. *et al.* A pilot study to assess the efficacy of tariquidar to inhibit P-glycoprotein at the human blood-brain barrier with (R)-11C-verapamil and PET. *J. Nucl. Med.* **50**, 1954-1961, doi:10.2967/jnumed.109.063289 (2009).
56 Schinkel, A. H., Wagenaar, E., van Deemter, L., Mol, C. A. & Borst, P. Absence of the mdr1a P-Glycoprotein in mice affects tissue distribution and pharmacokinetics of dexamethasone, digoxin, and cyclosporin A. *J. Clin. Invest.* **96**, 1698-1705, doi:10.1172/jci118214 (1995).

57 Uhr, M., Holsboer, F. & Muller, M. B. Penetration of endogenous steroid hormones corticosterone, cortisol, aldosterone and progesterone into the brain is enhanced in mice deficient for both mdr1a and mdr1b P-glycoproteins. *J. Neuroendocrinol.* **14**, 753-759 (2002).
58 Mason, B. L., Pariante, C. M. & Thomas, S. A. Changes in the brain accumulation of glucocorticoids in abcb1a-deficient CF-1 mice. *J. Neuroendocrinol.* **24**, 1440-1446, doi:10.1111/j.1365-2826.2012.02353.x (2012).
59 Mason, B. L., Pariante, C. M. & Thomas, S. A. A revised role for P-glycoprotein in the brain distribution of dexamethasone, cortisol, and corticosterone in wild-type and ABCB1A/B-deficient mice. *Endocrinology* **149**, 5244-5253, doi:10.1210/en.2008-0041 (2008).
60 Pariante, C. M. The role of multi-drug resistance p-glycoprotein in glucocorticoid function: studies in animals and relevance in humans. *Eur. J. Pharmacol.* **583**, 263-271, doi:10.1016/j.ejphar.2007.11.067 (2008).
61 Muller, M. B. *et al.* ABCB1 (MDR1)-type P-glycoproteins at the blood-brain barrier modulate the activity of the hypothalamic-pituitary-adrenocortical system: implications for affective disorder. *Neuropsychopharmacology* **28**, 1991-1999, doi:10.1038/sj.npp.1300257 (2003).
62 Thoeringer, C. K., Wultsch, T., Shahbazian, A., Painsipp, E. & Holzer, P. Multidrug-resistance gene 1-type p-glycoprotein (MDR1 p-gp) inhibition by tariquidar impacts on neuroendocrine and behavioral processing of stress. *Psychoneuroendocrinology* **32**, 1028-1040, doi:10.1016/j.psyneuen.2007.08.001 (2007).
63 Mealey, K. L., Bentjen, S. A., Gay, J. M. & Cantor, G. H. Ivermectin sensitivity in collies is associated with a deletion mutation of the mdr1 gene. *Pharmacogenetics* **11**, 727-733 (2001).
64 Tappin, S. W. *et al.* Frequency of the mutant MDR1 allele in dogs in the UK. *Vet. Rec.* **171**, 72, doi:10.1136/vr.100633 (2012).
65 Mealey, K. L., Gay, J. M., Martin, L. G. & Waiting, D. K. Comparison of the hypothalamic–pituitary–adrenal axis in MDR1-1Δ and MDR1 wildtype dogs. *J. Vet. Emerg. Crit. Care (San Antonio)* **17**, 61-66, doi:doi:10.1111/j.1476-4431.2006.00196.x (2007).
66 Gramer, I. *et al.* Urinary cortisol metabolites are reduced in MDR1 mutant dogs in a pilot targeted GC-MS urinary steroid hormone metabolome analysis. *J. Vet. Pharmacol. Ther.* **45**, 265-272, doi:10.1111/jvp.13050 (2022).
67 Raubenheimer, P. J., Young, E. A., Andrew, R. & Seckl, J. R. The role of corticosterone in human hypothalamic-pituitary-adrenal axis feedback. *Clin. Endocrinol. (Oxf.)* **65**, 22-26, doi:10.1111/j.1365-2265.2006.02540.x (2006).
68 Thomas, H. & Coley, H. M. Overcoming multidrug resistance in cancer: an update on the clinical strategy of inhibiting p-glycoprotein. *Cancer Control* **10**, 159-165, doi:10.1177/107327480301000207 (2003).
69 Bernstein, H. G. *et al.* Vascular and extravascular distribution of the ATP-binding cassette transporters ABCB1 and ABCC1 in aged human brain and pituitary. *Mech. Ageing Dev.* **141-142**, 12-21, doi:10.1016/j.mad.2014.08.003 (2014).
70 Kyle, C. J. *et al.* ABCC1 modulates negative feedback control of the hypothalamic-pituitary-adrenal axis in vivo in humans. *Metabolism* **128**, 155118, doi:10.1016/j.metabol.2021.155118 (2022).
71 Lee, M. J., Gong, D. W., Burkey, B. F. & Fried, S. K. Pathways regulated by glucocorticoids in omental and subcutaneous human adipose tissues: a microarray study. *Am. J. Physiol. Endocrinol. Metab.* **300**, E571-580, doi:10.1152/ajpendo.00231.2010 (2011).
72 Rainey, W. E., Rehman, K. S. & Carr, B. R. Fetal and maternal adrenals in human pregnancy. *Obstet. Gynecol. Clin. North Am.* **31**, 817-835, x, doi:10.1016/j.ogc.2004.08.006 (2004).
73 Michael, A. E. & Papageorghiou, A. T. Potential significance of physiological and pharmacological glucocorticoids in early pregnancy. *Hum. Reprod. Update* **14**, 497-517, doi:10.1093/humupd/dmn021 (2008).

74 Larry, C. G. Effect of corticosteroids for fetal maturation on perinatal outcomes. NIH Consensus Development Panel on the Effect of Corticosteroids for Fetal Maturation on Perinatal Outcomes. *JAMA* **273**, 413-418, doi:10.1001/jama.1995.03520290065031 (1995).
75 Brown, R. W., Chapman, K. E., Edwards, C. R. & Seckl, J. R. Human placental 11 beta-hydroxysteroid dehydrogenase: evidence for and partial purification of a distinct NAD-dependent isoform. *Endocrinology* **132**, 2614-2621, doi:10.1210/endo.132.6.8504762 (1993).
76 Stirrat, L. I. *et al.* Transfer andmetabolism of cortisol by the isolated perfused human placenta. *J. Clin. Endocrinol. Metab.* **103**, 640-648, doi:10.1210/jc.2017-02140 (2018).
77 Iqbal, M., Audette, M. C., Petropoulos, S., Gibb, W. & Matthews, S. G. Placental drug transporters and their role in fetal protection. *Placenta* **33**, 137-142, doi:10.1016/j.placenta.2012.01.008 (2012).
78 Sun, M. *et al.* Expression of the multidrug resistance P-glycoprotein, (ABCB1 glycoprotein) in the human placenta decreases with advancing gestation. *Placenta* **27**, 602-609, doi:10.1016/j.placenta.2005.05.007 (2006).
79 Lye, P. *et al.* Glucocorticoids modulate multidrug resistance transporters in the first trimester human placenta. *J. Cell. Mol. Med.* **22**, 3652-3660, doi:10.1111/jcmm.13646 (2018).
80 Nanovskaya, T. *et al.* Role of P-glycoprotein in transplacental transfer of methadone. *Biochem. Pharmacol.* **69**, 1869-1878, doi:10.1016/j.bcp.2005.03.030 (2005).
81 Nagashige, M. *et al.* Basal membrane localization of MRP1 in human placental trophoblast. *Placenta* **24**, 951-958, doi:10.1016/s0143-4004(03)00170-x (2003).
82 Pascolo, L. *et al.* Effects of maturation on RNA transcription and protein expression of four MRP genes in human placenta and in BeWo cells. *Biochem. Biophys. Res. Commun.* **303**, 259-265, doi:https://doi.org/10.1016/S0006-291X(03)00327-9 (2003).
83 Rahi, M. M., Heikkinen, T. M., Hakala, K. E. & Laine, K. P. The effect of probenecid and MK-571 on the feto-maternal transfer of saquinavir in dually perfused human term placenta. *Eur. J. Pharm. Sci.* **37**, 588-592, doi:https://doi.org/10.1016/j.ejps.2009.05.005 (2009).
84 Sippell, W. G., Becker, H., Versmold, H. T., Bidlingmaier, F. & Knorr, D. Longitudinal studies of plasma aldosterone, corticosterone, deoxycorticosterone, progesterone, 17-hydroxyprogesterone, cortisol, and cortisone determined simultaneously in mother and child at birth and during the early neonatal period. I. Spontaneous delivery. *J. Clin. Endocrinol. Metab.* **46**, 971-985, doi:10.1210/jcem-46-6-971 (1978).
85 Johnson, R. A., Ince, T. A. & Scotto, K. W. Transcriptional repression by p53 through direct binding to a novel DNA element. *J. Biol. Chem.* **276**, 27716-27720, doi:10.1074/jbc.C100121200 (2001).
86 Labialle, S., Gayet, L., Marthinet, E., Rigal, D. & Baggetto, L. G. Transcriptional regulators of the human multidrug resistance 1 gene: recent views. *Biochem. Pharmacol.* **64**, 943-948, doi:10.1016/s0006-2952(02)01156-5 (2002).
87 Scotto, K. W. Transcriptional regulation of ABC drug transporters. *Oncogene* **22**, 7496-7511, doi:10.1038/sj.onc.1206950 (2003).
88 Yan, J. & Xie, W. A brief history of the discovery of PXR and CAR as xenobiotic receptors. *Acta Pharmaceutica Sinica B* **6**, 450-452, doi:https://doi.org/10.1016/j.apsb.2016.06.011 (2016).
89 Miller, D. S. Regulation of P-glycoprotein and other ABC drug transporters at the blood-brain barrier. *Trends Pharmacol. Sci.* **31**, 246-254, doi:10.1016/j.tips.2010.03.003 (2010).
90 Zhou, S. F. Structure, function and regulation of P-glycoprotein and its clinical relevance in drug disposition. *Xenobiotica* **38**, 802-832, doi:10.1080/00498250701867889 (2008).
91 Andersen, V., Christensen, J., Overvad, K., Tjønneland, A. & Vogel, U. Polymorphisms in NFkB, PXR, LXR and risk of colorectal cancer in a prospective study of Danes. *BMC Cancer* **10**, 484, doi:10.1186/1471-2407-10-484 (2010).

92 Demeule, M., Jodoin, J., Beaulieu, E., Brossard, M. & Béliveau, R. Dexamethasone modulation of multidrug transporters in normal tissues. *FEBS Lett.* **442**, 208-214, doi:10.1016/s0014-5793(98)01663-9 (1999).
93 Iqbal, M., Gibb, W. & Matthews, S. G. Corticosteroid regulation of P-glycoprotein in the developing blood-brain barrier. *Endocrinology* **152**, 1067-1079, doi:10.1210/en.2010-1227 (2011).
94 Manceau, S. *et al.* Expression and induction by dexamethasone of ABC transporters and nuclear receptors in a human T-lymphocyte cell line. *J. Chemother.* **24**, 48-55, doi:10.1179/1120009x12z.00000000010 (2012).
95 Narang, V. S. *et al.* Dexamethasone increases expression and activity of multidrug resistance transporters at the rat blood-brain barrier. *Am. J. Physiol. Cell Physiol.* **295**, C440-450, doi:10.1152/ajpcell.00491.2007 (2008).
96 Perloff, M. D., von Moltke, L. L. & Greenblatt, D. J. Ritonavir and dexamethasone induce expression of CYP3A and P-glycoprotein in rats. *Xenobiotica* **34**, 133-150, doi:10.1080/00498250310001630215 (2004).
97 Zhang, Y. *et al.* Expression and activity of p-glycoprotein elevated by dexamethasone in cultured retinal pigment epithelium involve glucocorticoid receptor and pregnane X receptor. *Investig Ophthalmol Vis Sci* **53**, 3508-3515, doi:10.1167/iovs.11-9337 (2012).
98 Nishimura, M. *et al.* Comparison of inducibility of multidrug resistance (MDR)1, multidrug resistance-associated protein (MRP)1, and MRP2 mRNAs by prototypical microsomal enzyme inducers in primary cultures of human and cynomolgus monkey hepatocytes. *Biol. Pharm. Bull.* **31**, 2068-2072, doi:10.1248/bpb.31.2068 (2008).
99 Pascussi, J.-M., Drocourt, L., Fabre, J.-M., Maurel, P. & Vilarem, M.-J. Dexamethasone induces pregnane X receptor and retinoid X receptor-α expression in human hepatocytes: synergistic increase of CYP3A4 induction by pregnane X receptor activators. *Mol. Pharmacol.* **58**, 361-372, doi:10.1124/mol.58.2.361 (2000).
100 Pascussi, J. M., Busson-Le Coniat, M., Maurel, P. & Vilarem, M. J. Transcriptional analysis of the orphan nuclear receptor constitutive androstane receptor (NR1I3) gene promoter: identification of a distal glucocorticoid response element. *Mol. Endocrinol.* **17**, 42-55, doi:10.1210/me.2002-0244 (2003).
101 Lin, J. L., Lin-Tan, D. T., Chen, K. H. & Huang, W. H. Repeated pulse of methylprednisolone and cyclophosphamide with continuous dexamethasone therapy for patients with severe paraquat poisoning. *Crit. Care Med.* **34**, 368-373 (2006).
102 Katayama, K. *et al.* Revealing the fate of cell surface human P-glycoprotein (ABCB1): The lysosomal degradation pathway. *Biochim. Biophys. Acta* **1853**, 2361-2370, doi:10.1016/j.bbamcr.2015.06.001 (2015).
103 Zhu, Q. & Center, M. S. Evidence that SP1 modulates transcriptional activity of the multidrug resistance-associated protein gene. *DNA Cell Biol.* **15**, 105-111, doi:10.1089/dna.1996.15.105 (1996).
104 Wang, Q. & Beck, W. T. Transcriptional suppression of multidrug resistance-associated protein (MRP) gene expression by wild-type p53. *Cancer Res.* **58**, 5762-5769 (1998).
105 Kauffmann, H. M. *et al.* Influence of redox-active compounds and PXR-activators on human MRP1 and MRP2 gene expression. *Toxicology* **171**, 137-146, doi:10.1016/s0300-483x(01)00570-4 (2002).
106 Magnarin, M. *et al.* Induction of proteins involved in multidrug resistance (P-glycoprotein, MRP1, MRP2, LRP) and of CYP 3A4 by rifampicin in LLC-PK1 cells. *Eur. J. Pharmacol.* **483**, 19-28, doi:10.1016/j.ejphar.2003.10.010 (2004).
107 Gu, X. & Manautou, J. E. Regulation of hepatic ABCC transporters by xenobiotics and in disease states. *Drug Metab. Rev.* **42**, 482-538, doi:10.3109/03602531003654915 (2010).

108 Manceau, S. *et al.* ABC drug transporter and nuclear receptor expression in human cytotrophoblasts: influence of spontaneous syncytialization and induction by glucocorticoids. *Placenta* **33**, 927-932, doi:10.1016/j.placenta.2012.07.016 (2012).

109 Zhu, Q. & Center, M. S. Cloning and sequence analysis of the promoter region of the MRP gene of HL60 cells isolated for resistance to adriamycin. *Cancer Res.* **54**, 4488-4492 (1994).

110 Cherrington, N. J., Slitt, A. L., Li, N. & Klaassen, C. D. Lipopolysaccharide-mediated regulation of hepatic transporter mRNA levels in rats. *Drug Metab. Dispos.* **32**, 734-741, doi:10.1124/dmd.32.7.734 (2004).

111 Evseenko, D. A., Paxton, J. W. & Keelan, J. A. Independent regulation of apical and basolateral drug transporter expression and function in placental trophoblasts by cytokines, steroids, and growth factors. *Drug Metab. Dispos.* **35**, 595-601, doi:10.1124/dmd.106.011478 (2007).

112 von Wedel-Parlow, M., Wölte, P. & Galla, H. J. Regulation of major efflux transporters under inflammatory conditions at the blood-brain barrier in vitro. *J. Neurochem.* **111**, 111-118, doi:10.1111/j.1471-4159.2009.06305.x (2009).

113 Neuser, J., Fraccarollo, D., Wick, M., Bauersachs, J. & Widder, J. D. Multidrug resistance associated protein-1 (MRP1) deficiency attenuates endothelial dysfunction in diabetes. *J Diabetes Complicat* **30**, 623-627, doi:10.1016/j.jdiacomp.2016.02.002 (2016).

114 Ling, S. *et al.* Metformin reverses multidrug resistance in human hepatocellular carcinoma Bel-7402/5-fluorouracil cells. *Mol Med Rep* **10**, 2891-2897, doi:10.3892/mmr.2014.2614 (2014).

115 Seo, J. *et al.* Biallelic mutations in ABCB1 display recurrent reversible encephalopathy. *Ann Clin Transl Neurol* **7**, 1443-1449, doi:10.1002/acn3.51125 (2020).

116 Baudou, E. *et al.* Serious Ivermectin Toxicity and Human ABCB1 Nonsense Mutations. *N. Engl. J. Med.* **383**, 787-789, doi:10.1056/NEJMc1917344 (2020).

117 Li, M. *et al.* Extrusion pump ABCC1 was first linked with nonsyndromic hearing loss in humans by stepwise genetic analysis. *Genet. Med.* **21**, 2744-2754, doi:10.1038/s41436-019-0594-y (2019).

118 Saito, T. *et al.* Expression of multidrug resistance protein 1 (MRP1) in the rat cochlea with special reference to the blood-inner ear barrier. *Brain Res.* **895**, 253-257, doi:10.1016/s0006-8993(01)02020-0 (2001).

119 Leschziner, G. D., Andrew, T., Pirmohamed, M. & Johnson, M. R. ABCB1 genotype and PGP expression, function and therapeutic drug response: a critical review and recommendations for future research. *Pharmacogenomics J.* **7**, 154-179, doi:10.1038/sj.tpj.6500413 (2007).

120 Hoffmeyer, S. *et al.* Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo. *Proc. Natl. Acad. Sci. U. S. A.* **97**, 3473-3478, doi:10.1073/pnas.050585397 (2000).

121 Cascorbi, I. *et al.* Frequency of single nucleotide polymorphisms in the P-glycoprotein drug transporter MDR1 gene in white subjects. *Clin. Pharmacol. Ther.* **69**, 169-174, doi:https://doi.org/10.1067/mcp.2001.114164 (2001).

122 Dumond, J. B. *et al.* A phenotype-genotype approach to predicting CYP450 and P-glycoprotein drug interactions with the mixed inhibitor/inducer tipranavir/ritonavir. *Clin. Pharmacol. Ther.* **87**, 735-742, doi:10.1038/clpt.2009.253 (2010).

123 Hu, W. *et al.* MDR1 gene polymorphism correlated with pathological characteristics and prognosis in patients with primary hepatocellular carcinoma receiving interventional therapy. *Anticancer Drugs* **30**, 233-240, doi:10.1097/cad.0000000000000680 (2019).

124 Skalski, D. *et al.* Associations between MDR1 C3435T polymorphism and drug-resistant epilepsy in the Polish population. *Acta Neurol. Belg.* **117**, 153-158, doi:10.1007/s13760-016-0690-6 (2017).

125 Suzuki, A. *et al.* C3435T polymorphism of the MDR1 gene is not associated with blood levels of hypothalamus-pituitary-adrenal axis hormones in healthy male subjects. *Genet. Mol. Res.* **16**, doi:10.4238/gmr16019447 (2017).
126 Nakamura, T. *et al.* Effects of ABCB1 3435C>T genotype on serum levels of cortisol and aldosterone in women with normal menstrual cycles. *Genet. Mol. Res.* **8**, 397-403 (2009).
127 Ichihara, S. *et al.* Association of a polymorphism of ABCB1 with obesity in Japanese individuals. *Genomics* **91**, 512-516, doi:10.1016/j.ygeno.2008.03.004 (2008).
128 Lovas, K. *et al.* Glucocorticoid replacement therapy and pharmacogenetics in Addison's disease: effects on bone. *Eur. J. Endocrinol.* **160**, 993-1002, doi:10.1530/eje-08-0880 (2009).
129 Cuppen, B. V. *et al.* Polymorphisms in the multidrug-resistance 1 gene related to glucocorticoid response in rheumatoid arthritis treatment. *Rheumatol. Int.* **37**, 531-536, doi:10.1007/s00296-017-3653-1 (2017).
130 Pawlik, A., Wrzesniewska, J., Fiedorowicz-Fabrycy, I. & Gawronska-Szklarz, B. The MDR1 3435 polymorphism in patients with rheumatoid arthritis. *Int. J. Clin. Pharmacol. Ther.* **42**, 496-503 (2004).
131 Yang, Q. F. *et al.* Contribution of MDR1 gene polymorphisms on IBD predisposition and response to glucocorticoids in IBD in a Chinese population. *J. Dig. Dis.* **16**, 22-30, doi:10.1111/1751-2980.12205 (2015).
132 Han, S. S., Xu, Y. Q., Lu, Y., Gu, X. C. & Wang, Y. A PRISMA-compliant meta-analysis of MDR1 polymorphisms and idiopathic nephrotic syndrome: Susceptibility and steroid responsiveness. *Medicine (Baltimore)* **96**, e7191, doi:10.1097/md.0000000000007191 (2017).
133 Conseil, G., Deeley, R. G. & Cole, S. P. Polymorphisms of MRP1 (ABCC1) and related ATP-dependent drug transporters. *Pharmacogenet. Genomics* **15**, 523-533, doi:10.1097/01.fpc.0000167333.38528.ec (2005).
134 Kunadt, D. *et al.* Multidrug-related protein 1 (MRP1) polymorphisms rs129081, rs212090, and rs212091 predict survival in normal karyotype acute myeloid leukemia. *Ann. Hematol.* **99**, 2173-2180, doi:10.1007/s00277-020-04163-7 (2020).
135 Koren, L. *et al.* Cortisol and corticosterone independence in cortisol-dominant wildlife. *Gen. Comp. Endocrinol.* **177**, 113-119, doi:10.1016/j.ygcen.2012.02.020 (2012).
136 Arriza, J. L. *et al.* Cloning of human mineralocorticoid receptor complementary DNA: structural and functional kinship with the glucocorticoid receptor. *Science* **237**, 268-275, doi:10.1126/science.3037703 (1987).
137 Dunn, J. F., Nisula, B. C. & Rodbard, D. Transport of steroid hormones: binding of 21 endogenous steroids to both testosterone-binding globulin and corticosteroid-binding globulin in human plasma. *J. Clin. Endocrinol. Metab.* **53**, 58-68, doi:10.1210/jcem-53-1-58 (1981).
138 Giannopoulos, G. & Keichline, D. Species-related differences in steroid-binding specificity of glucocorticoid receptors in lung. *Endocrinology* **108**, 1414-1419, doi:10.1210/endo-108-4-1414 (1981).
139 Mani, O., Nashev, L. G., Livelo, C., Baker, M. E. & Odermatt, A. Role of Pro-637 and Gln-642 in human glucocorticoid receptors and Ser-843 and Leu-848 in mineralocorticoid receptors in their differential responses to cortisol and aldosterone. *J. Steroid Biochem. Mol. Biol.* **159**, 31-40, doi:10.1016/j.jsbmb.2016.02.017 (2016).
140 Nishida, S., Matsumura, S., Horino, M., Oyama, H. & Tenku, A. The variations of plasma corticosterone/cortisol ratios following ACTH stimulation or dexamethasone administration in normal men. *J. Clin. Endocrinol. Metab.* **45**, 585-588, doi:10.1210/jcem-45-3-585 (1977).
141 Peterson, R. E. Plasma corticosterone and hydrocortisone levels in man. *J. Clin. Endocrinol. Metab.* **17**, 1150-1157, doi:10.1210/jcem-17-10-1150 (1957).
142 Peterson, R. E. & Pierce, C. E. The metabolism of corticosterone in man. *J. Clin. Invest.* **39**, 741-757, doi:10.1172/jci104091 (1960).

143 Vera, F., Antenucci, C. D. & Zenuto, R. R. Cortisol and corticosterone exhibit different seasonal variation and responses to acute stress and captivity in tuco-tucos (Ctenomys talarum). *Gen. Comp. Endocrinol.* **170**, 550-557, doi:10.1016/j.ygcen.2010.11.012 (2011).
144 Hughes, K. A., Reynolds, R. M., Andrew, R., Critchley, H. O. & Walker, B. R. Glucocorticoids turn over slowly in human adipose tissue in vivo. *J. Clin. Endocrinol. Metab.* **95**, 4696-4702, doi:10.1210/jc.2010-0384 (2010).
145 Sundahl, N., Bridelance, J., Libert, C., De Bosscher, K. & Beck, I. M. Selective glucocorticoid receptor modulation: New directions with non-steroidal scaffolds. *Pharmacol. Ther.* **152**, 28-41, doi:https://doi.org/10.1016/j.pharmthera.2015.05.001 (2015).
146 Arlt, W. *et al.* Health status of adults with congenital adrenal hyperplasia: a cohort study of 203 patients. *J. Clin. Endocrinol. Metab.* **95**, 5110-5121, doi:10.1210/jc.2010-0917 (2010).
147 Bergthorsdottir, R., Leonsson-Zachrisson, M., Oden, A. & Johannsson, G. Premature mortality in patients with Addison's disease: a population-based study. *J. Clin. Endocrinol. Metab.* **91**, 4849-4853, doi:10.1210/jc.2006-0076 (2006).
148 Johannsson, G. *et al.* Improving glucocorticoid replacement therapy using a novel modified-release hydrocortisone tablet: a pharmacokinetic study. *Eur. J. Endocrinol.* **161**, 119-130, doi:10.1530/eje-09-0170 (2009).
149 Mallappa, A. *et al.* A phase 2 study of Chronocort, a modified-release formulation of hydrocortisone, in the treatment of adults with classic congenital adrenal hyperplasia. *J. Clin. Endocrinol. Metab.* **100**, 1137-1145, doi:10.1210/jc.2014-3809 (2015).
150 Kyle, C. *et al.* Proof of concept that corticosterone has a higher therapeutic index than hydrocortisone in patients with Congenital Adrenal Hyperplasia. *JES* **3(1)**, doi:10.1210/js.2019-SAT-009 ((Abstract SAT 009) 2019.).
151 Grem, J. L. Recent insights into the molecular basis of intrinsic resistance of colorectal cancer: new challenges for systemic therapeutic approaches. *Cancer Treat. Res.* **98**, 293-338, doi:10.1007/978-1-4615-4977-2_11 (1998).
152 Byrgazov, K. *et al.* Up-regulation of multidrug resistance protein MDR1/ABCB1 in carfilzomib-resistant multiple myeloma differentially affects efficacy of anti-myeloma drugs. *Leuk. Res.* **101**, 106499, doi:https://doi.org/10.1016/j.leukres.2020.106499 (2021).
153 Shaffer, B. C. *et al.* Drug resistance: still a daunting challenge to the successful treatment of AML. *Drug Resist Updat* **15**, 62-69, doi:10.1016/j.drup.2012.02.001 (2012).
154 Triller, N., Korosec, P., Kern, I., Kosnik, M. & Debeljak, A. Multidrug resistance in small cell lung cancer: expression of P-glycoprotein, multidrug resistance protein 1 and lung resistance protein in chemo-naive patients and in relapsed disease. *Lung Cancer* **54**, 235-240, doi:10.1016/j.lungcan.2006.06.019 (2006).
155 Haber, M. *et al.* Association of high-level MRP1 expression with poor clinical outcome in a large prospective study of primary neuroblastoma. *J. Clin. Oncol.* **24**, 1546-1553, doi:10.1200/jco.2005.01.6196 (2006).
156 Yuan, Y. *et al.* The clinical significance of FRAT1 and ABCG2 expression in pancreatic ductal adenocarcinoma. *Tumour Biol.* **36**, 9961-9968, doi:10.1007/s13277-015-3752-0 (2015).

Highlighted References

Alam, A., Kowal, J., Broude, E., Roninson, I. & Locher, K. P. Structural insight into substrate and inhibitor discrimination by human P-glycoprotein. *Science* **363**, 753-756, doi:10.1126/science.aav7102 (2019).
This paper provides novel insights into the structure and mechanism of human ABCB1.

Johnson ZL, Chen J. Structural basis of substrate recognition by the multidrug resistance protein MRP1. *Cell.* 2017;168(6):1075-1085.e1079.
This paper on bovine ABCC1 provides the best insight into the structure and function of the protein.

Leschziner, G. D., Andrew, T., Pirmohamed, M. & Johnson, M. R. ABCB1 genotype and PGP expression, function and therapeutic drug response: a critical review and recommendations for future research. *Pharmacogenomics J.* **7**, 154-179, doi:10.1038/sj.tpj.6500413 (2007).
A detailed overview of the significance of *ABCB1* polymorphisms in humans and their clinical applications.

Lopez, J. P. *et al.* Single-cell molecular profiling of all three components of the HPA axis reveals adrenal ABCB1 as a regulator of stress adaptation. *Sci Adv* **7**, doi:10.1126/sciadv.abe4497 (2021).
The authors use a variety of approaches to show the role of ABCB1 in humans and rodents in modulating steroid secretion and the HPA axis. Single cell RNA profiling details expression of Abcb1a and Abcb1b across the axis.

Mealey KL, Gay JM, Martin LG, Waiting DK. Comparison of the hypothalamic–pituitary–adrenal axis in MDR1-1Δ and MDR1 wildtype dogs. *J Vet Emerg Crit Care (San Antonio).* 2007;17(1):61-66.
The importance of ABCB1 in modulating the HPA axis in a cortisol-dominant species is highlighted by these affected canines.

Nixon M, Mackenzie SD, Taylor AI, Homer NZ, Livingstone DE, Mouras R, Morgan RA, Mole DJ, Stimson RH, Reynolds RM, Elfick AP, Andrew R, Walker BR. ABCC1 confers tissue-specific sensitivity to cortisol versus corticosterone: A rationale for safer glucocorticoid replacement therapy. *Sci Transl Med.* 2016;8(352):352ra109.
The specificity of ABCC1 for transport of corticosterone over cortisol in humans and rodents adipose is shown here.

Ueda K, Okamura N, Hirai M, Tanigawara Y, Saeki T, Kioka N, Komano T, Hori R. Human P-glycoprotein transports cortisol, aldosterone, and dexamethasone, but not progesterone. *J Biol Chem.* 1992;267(34):24248-24252.
The original study showing that ABCB1 is a steroid transporter.

Webster JI, Carlstedt-Duke J. Involvement of multidrug resistance proteins (MDR) in the modulation of glucocorticoid response. *J Steroid Biochem Mol Biol.* 2002;82(4-5):277-288.
This study in a murine line shows the specificity of Abcb1 for transport of cortisol over corticosterone, and of Abcc1 for transport of corticosterone over cortisol in vitro.

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Related links

The Human Protein Atlas: https://www.proteinatlas.org/

Key points

- Humans have two circulating glucocorticoid hormones, cortisol and corticosterone, which diffuse into cells to become transcription factors when bound to their intracellular receptors.
- The availability of glucocorticoids to interact with their receptors depends not only on their plasma concentration but also on their intracellular concentration, which is modulated by intracellular enzymes and by transmembrane transporters.
- Glucocorticoids are susceptible to cellular export by membrane transporters from the ABC (ATP-binding cassette) transporter family: cortisol is a substrate for the ABCB1 transporter, and corticosterone for ABCC1.
- Tissues expressing ABCB1 (such as the brain) might be relatively sensitive to corticosterone over cortisol; those expressing ABCC1, such as adipose, might be more sensitive to cortisol.
- In future, therapeutic glucocorticoids could be selected on the basis of lower tendency to be exported from sites of efficacy and higher tendency for export from sites where harmful side effects occur.

Glossary terms:

sanctuary sites – areas within the body that are relatively protected from access by drugs (e.g. anti-cancer agents) and toxins

α-helices – a form of secondary protein structure formed by hydrogen bonding between amine and carbonyl groups of amino acids 4 apart, and resulting in a stable rod shape

luminal surfaces – the lining surfaces of body channels, such as the intestines or blood vessels

polyspecificity – the capacity to bind multiple unrelated substrates

glutathione coupling – conjugation with the tri-peptide glutathione

phase II hepatic metabolites – conjugation of a substance to another molecule, such as glutathione or glucuronide, in the liver to make it more water soluble and thus facilitate excretion

syncytiotrophoblasts – cells forming the outer layer of the placenta, and the major site of gas and nutrient exchange between mother and fetus

cytotrophoblasts – the inner stem cell layer of the placenta villi – cellular precursors to syncytiotrophoblasts

adopted orphan receptors – an orphan receptor is a receptor whose ligand has not been identified. It can later be termed an “adopted orphan receptor” when a ligand is discovered.

compound heterozygosity – the presence of two different mutant alleles at a genetic locus

lymphoblastoid cell lines – immortalised cells which are derived from, and closely resemble, peripheral blood lymphocytes

synonymous – a silent genetic mutation where a change in DNA sequence does not result in a change in the amino acid sequence of the protein produced

therapeutic index – the margin between the desirable and undesirable effects of a drug. The narrower the margin, the more likely it is that side effects will occur at a therapeutic dose.

Figure legends

Figure 1: Action and structure of ABCB1 and ABCC1. a| In general, most ABC transporters are comprised of two transmembrane domains (TMDs) and 2 nucleotide binding domains (NBDs). In the proposed model of action, binding of ATP dimerises the NBDs and induces a conformational change within the TMDs, resulting in the switch between 'inward' and 'outward' facing configurations.[17,18] Subsequent hydrolysis of ATP returns the transporter to baseline status. b| Ribbon diagram of human ABCB1 (Protein Data Bank ID 6QEX) and c|ribbon diagram of bovine ABCC1 (Protein Data Bank ID 5UJA). The amino (N)- and carboxyl (C)- terminal halves are coloured magenta and blue, respectively. NBD1 and NBD2 are coloured green and yellow, respectively, with the drug-binding pocket highlighted.

Figure 2: Tissue-specific expression of *ABCB1* and *ABCC1*. Human expression of *ABCB1* and *ABCC1*, as derived from data from the Human Protein Atlas, is shown. Expression is normalised to an Nx (normalised expression) value based on outputs from the Human Protein Atlas, the genotype-tissue expression (GTEx) project and FANTOM5 transcriptomic analyses (data available online from The Human Protein Atlas).[23] Tissues are ranked in order of *ABCB1:ABCC1* ratio, such that those towards the top of the Y axis have greater *ABCB1* expression, and those at the bottom have higher *ABCC1* expression.

Figure 3: Tissue ABC transporter expression determines glucocorticoid sensitivity. The influence of ABCB1 and ABCC1 on the retention of common glucocorticoids within human target tissues according to transporter affinity is depicted. Steroids in red are predominantly substrates for ABCB1, those in dark blue are predominantly substrates for ABCC1, and those in light green are substrates for neither transporter. Passive diffusion is indicated by double-headed arrows.

Figure 4: Intracellular glucocorticoid regulatory pathways. After diffusing into cells (double-headed arrows), the glucocorticoids cortisol and corticosterone might (1) be exported by the membrane-bound ATP transporters ABCB1 and ABCC1; (2,3) might undergo enzymatic metabolism by 11β-hydroxysteroid dehydrogenase (11β-HSD), 5α reductase or carbonyl reductase enzymes; or (4) might become incorporated in the intracellular steroid pool. These processes restrict access to the nuclear glucocorticoid and/or mineralocorticoid receptors (GR and MR), which mediate the cellular response (5).

Figure 5: Modulation of the hypothalamic–pituitary–adrenal (HPA) axis by ABCB1 and ABCC1. Glucocorticoids are secreted from the adrenal cortex upon stimulation by signals from the hypothalamus and pituitary . They act peripherally on sites throughout the body, and feed back to the hypothalamus, pituitary and higher centres to maintain homeostasis. ABCB1 present at the blood–brain barrier might act to restrict the access of cortisol to feedback sites. Conversely, ABCC1, which is found without ABCB1 in adipose and skeletal muscle, exports corticosterone but not cortisol. The activity of the adrenal enzyme CYP17 (17-hydroxylase) determines the ratio of secreted cortisol:corticosterone**.**

Text for Table of Contents

This Review discusses the ATP-binding cassette (ABC) proteins ABCB1 and ABCC1 and their preferential cellular export of cortisol and corticosterone, respectively, as well as exploring the potential to select therapeutic glucocorticoids on the basis of their different tendencies for export to avoid harmful side effects.