THEMATIC REVIEW

SULFATION PATHWAYS

Insights into steroid sulfation and desulfation pathways

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This paper is part of a thematic section on Sulfation Pathways. The guest editors for this section were Jonathan Wolf Mueller and Paul Foster. They were not involved in the peer review of this paper on which they are listed as authors and it was handled by another member of the journal's Editorial board.

Abstract

Sulfation and desulfation pathways represent highly dynamic ways of shuttling, repressing and re-activating steroid hormones, thus controlling their immense biological potency at the very heart of endocrinology. This theme currently experiences growing research interest from various sides, including, but not limited to, novel insights about phospho-adenosine-5′-phosphosulfate synthase and sulfotransferase function and regulation, novel analytics for steroid conjugate detection and quantification. Within this review, we will also define how sulfation pathways are ripe for drug development strategies, which have translational potential to treat a number of conditions, including chronic inflammatory diseases and steroid-dependent cancers.

Introduction

Steroid sulfation and desulfation pathways represent fundamental routes, which regulate steroid circulatory transport and action. While sulfated, almost all steroids are inert and unable to bind to and activate their specific nuclear receptors. Indeed, as they are no longer lipophilic, sulfated steroids require active transport into cells via organic anion transporters. Once intracellular, steroid conjugates can be desulfated, a process catalyzed by the ubiquitously expressed steroid sulfatase (STS) enzyme.

Over the past 50 years, scientific perspectives on why sulfated steroids exist have changed several times, from it being a mere solubilization step for subsequent renal secretion to sulfated steroids representing a dynamic pool of steroid precursors fueling peripheral steroid signaling (Reed et al. 2005). Such dynamic sulfation/desulfation processes are highly relevant in the endocrine communication between mother and fetus, a field that recently was reviewed elsewhere (Geyer et al. 2017). Another twist comes from recent evidence that sulfated steroids can still be substrates for steroidogenic enzymes, suggesting they may act as hormonal precursors for a wide range of steroids. We have previously provided a comprehensive review examining how sulfation and desulfation impacts steroid action in normal physiology and in a multitude of disease states (Mueller et al. 2015). Here, we aim to give an update on the key advancements in this rapidly moving field.

Different PAPS synthases for different sulfation pathways?

3-Phospho-adenosine-5′-phosphosulfate (PAPS) synthases and a subset of sulfotransferases work together to ensure
efficient sulfation of steroid hormones. PAPS synthases provide high-energy sulfate in the form of PAPS that is then used for sulfuryl transfer to hydroxyl- or aminogroups of acceptor molecules (Mueller & Shafqat 2013). Several recent cell-based studies investigated the function of PAPSS1. Small interfering RNA-mediated knockdown of PAPSS1 sensitizes non-small-cell lung cancer cells to DNA-damaging agents (Leung et al. 2015, 2017). PAPSS1 further seems to be essential for nuclear provirus establishment during retroviral (HIV) infection (Bruce et al. 2008). This was independent from tyrosine sulfation of the CCR5 co-receptor of HIV, but required the sulfotransferase SULT1A1 for HIV-1 minus-strand DNA elongation (Swann et al. 2016); however, the authors left open what SULT1A1 substrate was responsible for this effect.

A different picture emerges for the functionality of PAPSS2, the only other PAPS synthesize encoded in the human genome. Transcriptional co-regulation of the PAPSS2 genes with the SULT2A1 sulfotransferase gene has been reported in some cases (Sonoda et al. 2002, Kim et al. 2004). Generally, PAPS2 is believed to be an inducible gene (Fuda et al. 2002, Mueller et al. 2015); controlled by TGF-β via p38 kinase phosphorylating Sox9 (Coricor & Serra 2016). Rare compromising mutations in the PAPSS2 gene present clinically with bone and cartilage mal-formations and an endocrine defect (Noordam et al. 2009). By performing a DHEA challenge test, we established that inactivating PAPSS2 mutations cause apparent SULT2A1 deficiency (Oostdijk et al. 2015). DHEA could no longer be efficiently sulfated and was downstream converted to biologically active androgens, manifesting with undetectable DHEA sulfate, androgen excess and metabolic disease (Oostdijk et al. 2015).

Mechanistically, it is difficult to explain why two highly conserved enzymes with an amino acid identity of 78% could not compensate for each other. Both enzymes have similar APS kinase catalytic activity (Grum et al. 2010), and they both shuttle between cytoplasm and nucleus, controlled by conserved nuclear localization and export signals (Schroder et al. 2012). However, PAPS synthases 1 and 2 differ markedly in their protein stability, with PAPSS2 being partially unfolded at physiological temperature (van den Boom et al. 2012). The natural ligand and substrate adenosine-5′-phosphosulfate (APS) stabilizes the enzyme, making APS an efficient modulator of sulfation pathways (Mueller & Shafqat 2013). For the sulfation pathways studied so far, the PAPS cofactor is always rate limiting (Kauffman 2004, Moldrup et al. 2011), but the question remains how specificity for one of the PAPS synthases is generated.

Substrate specificity and regulation of sulfotransferases

Sulfotransferases provide specificity to sulfation reactions by means of binding specific subsets of acceptor molecules (Coughtrie 2016). Our understanding of their structure, regulation and function within different sulfation pathways has significantly increased in recent years. The first crystal structure of a plant sulfotransferase in complex with substrate, Arabidopsis SULT18/AtSOT18 with the glucosinolate sinigrin bound to it, identified essential residues for substrate binding and demonstrated that the catalytic mechanism may be conserved between human and plant sulfotransferase enzymes (Hirschmann et al. 2017). Further, the core elements including the 5′-PSB and 3′-PB motifs, both involved in the binding of PAPS, are structurally conserved even in the distantly related tyrosine–protein sulfotransferases, human TPST1 and TPST2 (Teramoto et al. 2013, Tanaka et al. 2017). Protein substrates have to locally unfold and bind in a deep active site cleft to TPSTs and the vicinity of the acceptor tyrosine residues adopts an intrinsically unfolded conformation in order to facilitate this process (Teramoto et al. 2013, Tanaka et al. 2017). TPSTs were known to fulfill different biological functions; shear stress applied to primary cultures of human umbilical vein endothelial cells lead to downregulation of TPST1 via protein kinase C, but to upregulation of TPST2 via a tyrosine kinase-dependent pathway (Goettsch et al. 2002, 2006). However, there are no obvious differences in the substrate-binding site of TPST1 and 2; these need to be hidden in other non-conserved residues in the periphery. Similarly, substrate specificity may be controlled outside of the active center for Arabidopsis SULT18/SOT18 (Hirschmann et al. 2017). The substrate specificity of human SULT1A3, on the other hand, is well understood. A single amino acid substitution in the substrate-binding site (glutamic acid at position 146) makes SULT1A3 highly selective for catecholamines (both endogenous and xenobiotic) as Glu146 forms a salt bridge with the nitrogen on the catecholamine side chain (Dajani et al. 1999). With this one exception, the molecular understanding of the isoform specificity of sulfotransferases remains a challenge despite the wealth of structural information.

Recent insights into enzyme kinetics may be helpful here. It is well known that sulfotransferases can show substrate inhibition due to the formation of non-productive ternary complexes (Gulcan & Duffel 2011, Mueller et al. 2015). More recent is the view that sulfotransferases
may be allosterically regulated by their cofactor PAPS: This allosteric regulation extended the dynamic range of SULT1A1’s catalytic efficiency (Wang et al. 2014). Certainly, a new concept is that sulfotransferases might be allosterically regulated in an isozyme-specific manner; liver sulfotransferase SULT1A1 for example is modulated by catechins (naturally occurring polyphenols) and nonsteroidal anti-inflammatory drugs (Wang et al. 2016). All these modes of regulation of SULTs are illustrated in Fig. 1. A better understanding of sulfotransferase enzymes may have direct translational potential for drug development (Cook et al. 2016): Raloxifene is an approved selective estrogen receptor modulator that is quickly sulfated, and thus inactivated, in human cells. Modulating this compound in a way that prevented sulfation, but left its interaction with the estrogen receptor untouched, resulted in an enormous increase in estrogen receptor activation efficacy (Cook et al. 2016). It is likely that this approach could also work with other compounds.

Finally, it is population genetics influencing steroid sulfation pathways and the interindividual variability in drug response. Several coding SNPs in SULT genes influence an individual’s sulfation capacity (Louwers et al. 2013), but also gene number variations have been reported for SULT2A1 (Ekstrom & Rane 2015) and other sulfotransferases (Marto et al. 2017). In fact, the SULT2A1 gene seems to be more evolvable than, for example, PAPS syntheses (Mueller et al. 2015); Ensembl (https://www.ensembl.org) lists various expansions of this gene in different lineages with an eight-genes-comprising gene cluster in mice (Zerbino et al. 2018), while a set of two PAPS synthase genes is highly conserved in vertebrates (van den Boom et al. 2012). A reverse approach using metabolomics and pharmacogenomics indicated that acetaminophen use phenocopied the effect of genetic variants of SULT2A1 on sulfated metabolites of androstenediol, pregnenolone and DHEA (Cohen et al. 2018). This study also challenges views on the mechanism of action of acetaminophen in pain management as sulfated sex hormones can function as neurosteroids and modify nociceptive thresholds.

### Analytics of steroid conjugates

From the very beginning of steroid metabolomics, steroid mixtures were de-conjugated before analysis, mainly by gas-chromatography-mass-spectrometry (Shackleton 2010). However, measuring both free and conjugated steroids may give complementary information. Quantification of conjugates could be laboriously carried out using biochemical separation techniques (Shackleton et al. 1968) or in multi-step differential de-conjugation measurements (Hill et al. 2010). Experimentally, detection of intact steroid conjugates was reported already in 1982 (Shackleton & Straub 1982), using particle beam ionization; however, this technique did not become standard in analytical labs.

Only recently, more and more reports describe the targeted measurements of steroid sulfates and glucuronides using LC-MS/MS. Galuska et al. (2013) reported a combined targeted method for intact steroid sulfates and unconjugated steroids. Six steroid sulfates were quantified by ESI-MS-MS in negative mode and, separately, 11 unconjugated steroids were analyzed by atmospheric pressure chemical ionization (APCI)-MS-MS in positive mode. This combined method could be used for different biological matrices including aqueous solutions, cell lysates and serum (Galuska et al. 2013). Validated targeted LC-MS/MS assays for different sex steroid sulfates from human serum are becoming available (Dury et al. 2015, Sanchez-Guijo et al. 2015b, Poschner et al. 2017). Nevertheless, all these assays require separate runs for the conjugated and free steroids. An integrated method for quantifying free and sulfated steroids in a single LC-MS/MS run was recently described (Lee et al. 2016). It used both SIM and MRM modes as well as polarity switching and was capable of detecting eight free steroids and four sulfated ones. All methods described so far were targeted assays.

![Figure 1](image)

**Figure 1**

Different modes of regulation of sulfotransferase enzymes. (A) Human SULT1A3 contains a unique glutamate ‘E’ in the substrate-binding site, specifically binding catecholamines. (B) Substrates can bind in non-productive conformations, causing substrate inhibition. (C) Dissociation of PAP from the sulfotransferase may be rate-limiting, causing product inhibition. (D) Allosteric protein–protein contacts may regulate SULT function. (E) Non-substrate molecules may allosterically activate sulfotransferases. Please refer to the main text for further explanation.
Noteworthy, low-energy collision-induced dissociation may be a way to discover new sulfo-conjugates. Maekawa et al. (2014) used this technique not only to detect sulfate adducts (−97 m/z), but also glycine (−74 m/z) or taurine conjugates. The group of Oscar Pozo developed a modification of this idea to monitor disulfates (McLeod et al. 2017); these doubly sulfated steroids will be discussed further below. Constant ion loss monitoring of one of the sulfates (−97 m/z) allowed untargeted detection of potentially all soluble disulfates; with the caveat that phosphates could also cause this signal (McLeod et al. 2017). This method was recently applied in prenatal diagnostics (Pozo et al. 2018).

Further developments in steroid conjugate analytics may involve ultra-high-performance supercritical fluid chromatography linked to mass spectrometry (Doue et al. 2015) and mass spectrometry imaging as established for sulfated gluco-lipids (Marching et al. 2014) or for testosterone (Shimma et al. 2016), allowing for spatial resolution of sulfation ratios.

Measuring sulfation ratios of different enzymes precisely might help to expand what has been called the ‘sulfated steroid pathway’ (Sanchez-Guijo et al. 2016). The concept that sulfation does not prevent downstream conversion of steroids, but modulates it, is based on the side-chain-cleaving activity of cytochrome P450 CYP11A1 toward cholesterol sulfate (Tuckey 1990). This observation was then extended to CYP17A1 that bound and metabolized pregnenolone sulfate (Neunzig et al. 2014). It is STS that can then convert sulfated steroids to biologically active steroids (Sanchez-Guijo et al. 2016). Steroid analysis of patients with steroid sulfatase deficiency suggests that other enzymes partially can complement STS (Sanchez-Guijo et al. 2016). In such a pathway, the sulfo-group acts as protection group, allowing downstream biochemical conversions on one side of the steroid molecule, but not on the other.

Selected steroid species in sulfo-focus

Several steroid conjugates have been known for decades, but only recently have these forms been thought to be biologically meaningful and worth studying. Here, we briefly review knowledge about vitamin D sulfates, steroid disulfates and 11-oxo-androgens.

Vitamin D

25-Hydroxy-vitamin D3-3-sulfate (25-OH-D3-3-S) is a major metabolite of vitamin D3 found in the systemic circulation (Axelson 1985). As circulating concentrations of 25OH-D3-3-O-sulfate seem not to be rapidly secreted by the kidney, there is the possibility that this sulfate metabolite may serve as a reservoir of 25OH-D3 in vivo, contributing indirectly to the biological effects of vitamin D (Wong et al. 2018). Sulfotransferase SULT2A1 was identified as the major vitamin D3-sulfating enzyme (Kurogi et al. 2017, Wong et al. 2018). SULT2A1 showed activity toward several vitamin D3-related compounds, whereas SULT1A1 and SULT2B1a/SULT2B1b only showed sulfating activity for, respectively, calcitriol and 7-dehydrocholesterol (Kurogi et al. 2017).

The relationship between vitamin D and sulfation pathways is reciprocal. The vitamin D receptor also induces transcription of the steroid sulfotransferases SULT2A1 (Echchgadda et al. 2004) and SULT2B1b (Seo et al. 2013) as well as the phase I monooxygenase CYP3A4 (Ahn et al. 2016), among other genes. Interestingly, the induction of STS by vitamin D3 and retinoids was reported in HL60 promyeloid cells (Hughes et al. 2001). As net effect, vitamin D transcriptional regulation results in androgen inactivation (Ahn et al. 2016) and elevated sulfation activity that might increase the levels of vitamin D sulfate metabolites.

Several analytical methods have been reported to detect and quantify vitamin D3 sulfoconjugates (Higashi et al. 2014, Gao et al. 2017, Abu Kassim et al. 2018). Axelson reported values of 35±14 nM for 25-hydroxy-D3-3-sulfate in plasma from 60 patients (Axelson 1985). Gao measured 56±24 nM for 25-OH-D3-3-sulfate in serum from six healthy volunteers (Gao et al. 2017) and Abu Kassim found a range of 9.52–43.8 nM for 25-OH-D3-3-sulfate in serum of ten volunteers (Abu Kassim et al. 2018). Concentrations of this vitamin D3 sulfoconjugate were consistently higher than its glucuronidated counterparts. More importantly, the reported circulating concentrations for vitamin D3-3-sulfate reach up to what is regarded as the normal level of circulating 25-OH-vitamin D3, 80–250 nM (Holiss 2010). Early studies described vitamin D3-3-sulfate as less biologically active than free vitamin D3 in rodents (Nagubandi et al. 1981, Cancela et al. 1987). Considering the high circulating concentrations of 25-OH-D3-3-sulfate in the human circulation, it should be taken into account when determining a person’s vitamin D status – it could be a reservoir for local generation of 25-OH-D3 and the active 1,25-di-OH-D3.

Steroid disulfates

Several steroid-diols like estradiol or androstenediol can be doubly sulfated, most likely by the same steroid
sulfotransferases due to the pseudo-symmetry of those steroids (Mueller et al. 2015) and a high degree of plasticity in the substrate-binding sites (Berger et al. 2011). As early as in 1962, steroid disulfates (also referred to as bis-sulfates) were described as a constituent of human urine (Pasqualini & Jayle 1962). Falany and coworkers established for 24-hydroxycholesterol-3,24-disulfate that double sulfation leads to a terminal product that is resistant to reactivation by STS (Cook et al. 2009). This fueled the idea that a second sulfation step represented a further regulatory step or an irreversible step toward inactivation (Mueller et al. 2015). Double sulfation also changes affinity for organic anion transporters. While estradiol-3-sulfate and estradiol-17-sulfate both were substrates for the sodium-dependent organic anion transporter SOAT (SLC10A6), estradiol-3,17-disulfate no longer was cargo for this transporter (Grosset et al. 2018); depending on where the second sulfation step may occur within the cell, a steroid disulfate may be confined to that cellular compartment.

11-Oxo androgenic steroids

The C19 steroid 11β-hydroxy-androstenedione is produced by the adrenal in significant amounts; it has however long been regarded as a dead-end product of adrenal steroidogenesis (Pretorius et al. 2017). In recent years, evidence has accumulated that this steroid could be converted to potent androgenic 11-oxygenated steroids, 11-keto-testosterone and 11-keto-dihydrotestosterone, which have similar potency to testosterone and dihydrotestosterone to activate the human androgen receptor (Storbeck et al. 2013). Sulfated 11-oxo-steroids have not been reported until now, analogous to other androgens (Schiffer et al. 2018). Interestingly, 11-oxo-steroids seem to be resistant to glucuronidation in various cancer cell lines (du Toit & Swart 2018) and 11-keto-testosterone and 11-keto-dihydrotestosterone are metabolized at a slower rate than testosterone and dihydrotestosterone (Pretorius et al. 2016). It seems that the 11-oxo modification prevents conjugation, making these steroids to exert prolonged androgenic effects.

STS action and regulation

STS is a membrane-bound protein with its active site located in the lumen of the endoplasmic reticulum (Thomas & Potter 2013). It catalyzes the hydrolysis of sulfate ester bonds from many chemical structures, and it is heavily involved in the desulfation of steroids. STS’s main hormone substrates are estrone sulfate, DHEAS, pregnenolone sulfate and cholesterol sulfate. Thus, STS action represents a major intracrine route in regenerating biologically active steroids. The crystal structure of STS has been determined (Hernandez-Guzman et al. 2003) showing a domain consisting of two antiparallel α-helices that protrude from the roughly spherical structure; this gives it a ‘mushroom-like’ shape. Despite this, very little is known on what factors regulate STS activity. STS undergoes post-translational modifications, the key one being the generation of C-alpha formylglycine (FGly), the catalytic residue in the active site of STS, from a cysteine by sulfatase-modifying factors 1 and 2 (SUMF1 and SUMF2). Furthermore, STS contains four potential N-glycosylation sites, however, only two (Asn47 and Asn259) are used (Stein et al. 1989, von Figura et al. 1998) and only mutations at these sites decrease activity (Stengel et al. 2008).

Most recent studies have focused on directly measuring STS activity in a range of diseases and conditions in order to shed some light on how this enzyme is molecularly controlled (Fig. 2). Evidence from chronic liver disease and pre-osteoblastic cells suggests inflammatory mediators, in particular, TNFα (Newman et al. 2000) can regulate STS expression and activity most likely through NF-κB signaling (Dias & Selcer 2016, Jiang et al. 2016), with activity depressed by glucocorticoid treatment (Dias & Selcer 2016). Interestingly, estrogens have also been shown to influence STS activity in leukocytes taken from pregnant patients where STS activity is increased in the 3rd trimester (Miyakawa et al. 1994). In support of this, Gilligan et al. have shown estradiol (E2) treatment can increase STS activity in colorectal cancer cells via G-protein-coupled estrogen receptor (GPER) action (Gilligan et al. 2017a). These studies suggest a potential positive feedback mechanism by which elevated local estrogen synthesis can further drive estrogen desulfation and activity. How this system is controlled by downstream GPER mediators remains unknown. However, it is of interest that many steroids, including estrogens, are anti-inflammatory and thus local sulfation/desulfation regulation may represent a mechanism by which steroids control the local influence of an inflammatory insult.

Mutations in the STS gene and X-linked ichthyosis

Mutations or deletions of the STS gene result in X-linked ichthyosis (XLI), a condition associated with hyperkeratosis (Ballabio et al. 1989). XLI is also termed STS
Steroid sulfation

Steroid sulfation deficiency and represents a common inherited metabolic disorder, with 1:6000 live births and no geographical or ethnic variation (Fernandes et al. 2010). Patients with XLI have no sulfatase activity and thus cholesterol sulfate breakdown is impaired. The subsequent cholesterol sulfate accumulation physiologically stabilizes cell membranes (Williams 1992) and builds-up in the stratum corneum causing partial retention hyperkeratosis with visible scaling (Williams & Elias 1981, Elias et al. 1984). With this loss of desulphation, it is reasonable to assume XLI patients would also exhibit depleted circulating desulfated steroid concentrations, which would subsequently effect their hormone-related development. However, in healthy adult men, STS has no significant impact on systemic androgen reactivation from DHEAS (Hammer et al. 2005), thus suggesting STS loss has less physiological effects than anticipated. Indeed, in XLI patients, a compensatory mechanism has been identified through the upregulation of 5α-reductase which, the authors suggest, maintains peripheral androgen activation despite reduced androgen availability (Idkowiak et al. 2016). Along with changes in androgen metabolism, XLI patients also have elevated plasma concentrations of 27-hydroxycholesterol-3-sulfate compared to healthy males (Sanchez-Guijo et al. 2015a). The effects of this increased oxysterol sulfate remains unknown.

Greater than 90% of XLI patients harbor complete deletions of the STS gene. However, there have been 14 point mutations within the STS gene previously reported; three nonsense mutations and 11 missense mutations (Mueller et al. 2015). More recently, a mutation in exon 3 of the STS gene was shown to cause a complete loss of STS activity in the affected patient (del Refugio Rivera Vega et al. 2015). Furthermore, two unrelated Japanese patients with ichthyosis are known to have two different point mutations in exon 7 (Oyama et al. 2016). A novel indel mutation in exon 5 of the STS gene has also been reported leading to a frameshift causing a premature stop codon 81 codons downstream from the substitution site (Takeichi et al. 2015). Intriguingly, this frameshift did not affect the reported active site of STS; thus, the encoded transcript may be spared if a truncated mutant protein was synthesized.

STS and cancer

Breast cancer

The most exciting advancements in steroid desulphation research have come through two recently completed clinical trials of the STS inhibitor Irosustat (STX64, 667Coumatate). The IPET trial examined Irosustat in treatment of naive ER+ early breast cancer patients (Palmieri et al. 2017b) and the Phase II IRIS trial examining the clinical benefit rate of Irosustat combined with aromatase inhibition in advance and metastatic ER+ breast cancer (Palmieri et al. 2017a). Although patient recruitment numbers were relatively low (IPET n=13; IRIS n=27), both trials demonstrated some clinical benefit for STS inhibition. In the IPET trial, breast tumors were assessed for the effects of Irosustat on tumor growth as measured by 3'-deoxy-3-[^18F]-fluorothymidine uptake measured by PET scanning (FLT-PET) and Ki67 immunohistochemistry. STS inhibition significantly reduced Ki67 scores and the tumor uptake of FLT as measured by PET. Furthermore, Irosustat also decreased tumor STS expression, with this effect also observed in other estrogen-metabolizing enzymes and ERα expression. This suggests STS inhibition may have beneficial effects with regards to dampening down tumor estrogen synthesis.

Figure 2

The regulation of STS activity. Many factors are known to either increase or decrease STS activity. To increase STS activity, sulfatase-modifying factors 1 and 2 (SUMF1 and SUMF2) generate C-alpha formylglycine (FGly), the catalytic residue in the active site of STS, from a cysteine. Estrogens, in particular estradiol, have been shown to increase STS activity in leukocytes in the third trimester of pregnancy and in colorectal cancer cells, with this effect potentially regulated by G-protein-coupled estrogen receptors (GPER). Inflammation, mediated by TNFα through NF-KB signaling, also increases local STS activity. Many cancers, in particular breast, prostate, and colorectal cancer, have all been shown to have higher STS activity compared to non-malignant tissue. Factors that decrease STS activity include mutations in the SUMF1 gene leading to failure of the formation of FGly and thus reduced catalytic activity. Drugs, such as Irosustat, that target STS activity have been developed. Interestingly, glucocorticoids, including dexamethasone, can reduce STS activity in various cell lines. Inherited STS deficient (X-linked ichthyosis) patients have loss of STS activity.
Previous pre-clinical studies have shown combining aromatase inhibitors with STS inhibition was a viable strategy to treat MCF-7 xenografts in mice (Foster et al. 2008a). Thus, the IRIS trial testing this strategy in breast cancer patients who had lapsed while on aromatase therapy. Clinical benefit rate was seen in 18.5% (95% CI 6.3–38.1%) of patients with a median progression-free survival of 2.7 months (95% CI 2.5–4.6). Considering the difficulty of treating advanced and metastatic breast cancer, these results are encouraging for the future of STS inhibition in breast cancer treatment. Furthermore, MCF-7 cells resistant to letrozole treatment have been shown to have higher STS mRNA expression and greater expression of organic anion-transporting polypeptides, which mediate estrone sulfate transport into the cell (Higuchi et al. 2016). This provides some molecular insight into aromatase resistance and how STS inhibition may be beneficial to patients who relapse on aromatase inhibitors. However, more clinical data are still required to examine whether Irosustat, or indeed other STS inhibitors, would be beneficial for ER+ aromatase-resistant breast cancer patients.

**Gynecological cancers**

Along with new evidence suggesting the importance of STS and SULT1E1 expression in endometriosis (Piccinato et al. 2016), there are new insights into how desulfation impacts endometrial (Sinreih et al. 2017) and ovarian (Ren et al. 2015, Mungenast et al. 2017) cancers. This work represents a growing interest in local estrogen metabolism and action in gynecological conditions (Rizner 2016, Rizner et al. 2017). Indeed, these studies show a lack of aromatase activity and expression in these cancers, implicating STS activity as the most likely pathway through which local estrogen synthesis occurs (Ren et al. 2015, Sinreih et al. 2017). Indeed, high SULT1E1 protein expression is positively associated with better-differentiated epithelial ovarian cancers compared to grade 3 epithelial ovarian cancers (Mungenast et al. 2017). This suggests estrogen sulfation, and thus inactivation, limits estrogen tissue availability reducing the potential mitogenic effects of non-sulfated estrogens. Thus, targeting desulfation (i.e. via STS inhibition) may be an important strategy in treating ovarian and endometrial cancer. Pre-clinical mouse xenograft studies have previously demonstrated that STS inhibition blocks estrone sulfate-stimulated growth of endometrial tumors (Foster et al. 2008b), although this theory remains to be tested clinically. Furthermore, and if the STS pathway dominates estrogen synthesis, then these studies may go some way to explain the clinical failure of aromatase inhibitors to treat endometrial cancer (Boglioio et al. 2016).

**Gastrointestinal cancers**

A growing body of evidence on gastrointestinal cancers now implicates sex steroids and their desulfation as important drivers of proliferation (Barzi et al. 2013, Foster 2013, Ur Rahman & Cao 2016). Most research has focused on colorectal cancer (CRC) as previous work has shown a potential prognostic role for STS and SULT1E1 protein expression in CRC (Sato et al. 2009), implicating a high STS and low SULT1E1 expression as indicative of a poor outcome. More recently, over-expression of STS in the CRC cell line HCT116 increases proliferation in vitro and in vivo xenograft mouse models, with these effects blocked by STS inhibition by STX64 (Gilligan et al. 2017b). These actions were shown to be through increased estrogen desulfation and activation of the GPER, a finding further supported by evidence these effects may be modulated by a hypoxic environment (Bustos et al. 2017). Indeed, it is of interest to note STS activity can increase hypoxia inducible factor HIF1A expression in cervical and prostate cancer cells, suggesting STS action may be further regulated by hypoxic conditions (Shin et al. 2017). Furthermore, E2 treatment increases both STS activity (Gilligan et al. 2017a) and GPER expression in CRC (Bustos et al. 2017), suggesting a novel positive feedback loop through which E2 can drive CRC proliferation.

**Steroid sulfation pathways, the brain and behavior**

XLI patients have an association with behavioral disorders, which include attention deficit hyperactivity disorder (ADHD), autism and social communication deficits (Davies et al. 2009, Stergiakouli et al. 2011). A study examining 384 patients with ADHD identified two SNPs in the STS gene significantly associated with this condition (Brookes et al. 2008). Indeed, the polymorphism rs17268988 within the STS gene is associated with inattentive behavior in males with ADHD (Humby et al. 2017). More recently, XLI patients have been shown to be at a significantly increased risk of developmental conditions and psychiatric illness (Chatterjee et al. 2016). The hormonal implications in these conditions remains ill-defined, although researchers have hypothesized disturbed neuronal DHEA-DHEAS metabolism might result in altered neurotransmitter function contributing
to the observed abnormalities. There is some support for this theory, albeit in a different disease context. Evidence suggests declining concentrations of neurosteroids, such as DHEA and DHEAS, are closely associated with increased risk of Alzheimer’s disease (AD) (Wojtal et al. 2006, El Bitar et al. 2014). STS inhibition attenuated cognitive deficits in spatial learning and memory and in hippocampal synaptic plasticity in rats with amyloid β protein-induced AD (Yue et al. 2016). The authors suggest STS inhibition elevated brain DHEAS concentrations with this accounting for the neuroprotective effects, although neuronal DHEAS levels were not measured. Thus, definitive proof that DHEAS is the key neurosteroid linked to STS action within the brain remains to be seen.

Another sulfated steroid, pregnenolone sulfate, is known to inhibit GABA neurotransmission in the brain. Two new studies shed light on the effect of this and other neurosteroids on GABA(A) receptor function. The stimulating neurosteroids tetra-hydrodeoxycorticosteron (THDOC) and pregnanolone bind to the very same site within the transmembrane domain (Laverty et al. 2017, Miller et al. 2017). Inhibitory pregnenolone sulfate on the other hand binds to another site within the transmembrane domain and fosters pore opening, which corresponds to the desensitized state (Laverty et al. 2017).

Conclusion

Despite this review only covering the past few years of steroid sulfation and desulfation research, it highlights the now strong evidence supporting the importance on sulfation and desulfation pathways in controlling steroid action. Most importantly, early clinical trials in hormone-dependent breast cancer of the STS inhibitor Irosustat are encouraging and suggest inhibiting desulfation as a viable strategy. Thus, targeting steroid desulfation in other cancers and conditions remains of significant interest. Furthermore, improvements in measuring both sulfated and non-sulfated steroids via mass spectrometry should allow for more sensitive quantification and thus a greater ability to tease out how the balance between sulfation and desulfation is regulated.

However, there is still much we do not know. Defining which PAPS synthase interacts with which SULT would lead to a greater understanding on steroid sulfation pathways and may lend itself to specific inhibitory strategies (Mueller et al. 2018). Most researchers in this area focus on sulfated estrogens and androgen precursors (e.g. DHEAS); however, we have little grasp of whether other sulfated steroids, such as vitamin D, represent biologically relevant reservoirs for local desulfation and subsequent action. Furthermore, we are only beginning to understand about disulfated steroids, and at present, we do not know how these are formed and whether they possess biological function. Finally, we still do not clearly understand what factors regulate STS activity, although inflammation seems most likely to play a role.

Declaration of interest

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

Funding

This work did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

Acknowledgements

The authors acknowledge Martin Hewison and Karl Storbeck for reading and commenting on some parts of this manuscript. Research in the area of this review was funded by the European Commission (Marie Curie Fellowship SUPA-HD 625451, to J W M), the Wellcome Trust (ISSF award, to J W M), the MRC (Proximity-to-Discovery, to P A F and J W M) and the Society for Endocrinology (Early Career Grants, to J W M and P A F; Themed Scientific Meeting Grant, to P A F and J W M).

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http://jme.endocrinology-journals.org
https://doi.org/10.1530/ME-18-0086
© 2018 Society for Endocrinology
Published by Bioscientifica Ltd.
Printed in Great Britain
Steroid sulfation

Shin S, Im HJ, Kwon YJ, Ye DJ, Baek HS, Kim D, Choi HK & Chun YJ


Received in final form 11 May 2018
Accepted 15 May 2018
Accepted Preprint published online 15 May 2018