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Sutherlandia frutescens modulates adrenal hormone biosynthesis, acts as a selective glucocorticoid receptor agonist (SEGRA) and displays anti-mineralocorticoid properties

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ABSTRACT

Ethnopharmacological relevance: *Sutherlandia frutescens* is a traditional African medicinal plant used in the treatment of stress and anxiety, while also exhibiting anti-inflammatory properties.

Aim of study: The study aimed at linking anti-stress and anti-inflammatory properties of *S. frutescens* to its influence on glucocorticoid biosynthesis and the inflammatory response via steroid receptor interaction.

Materials and methods: The influence of *S. frutescens* extracts and sutherlandioside B (SUB),10 and 30 μM, on key steroidogenic enzymes was assayed in COS-1 cells. Effects were also assayed on basal and stimulated hormone levels in the adrenal H295R cell model. Agonist activity for transactivation and transrepression of the extract and SUB with the glucocorticoid- (GR) and mineralocorticoid receptor (MR) was subsequently investigated.

Results: Inhibitory effects of the extract towards progesterone conversion by CYP17A1 and CYP21A2 were significant. SUB inhibited CYP17A1 and 3β-HSD2, while not affecting CYP21A2. In H295R cells, SUB decreased cortisol and androgen precursors significantly. The extract decreased total steroid production (basal and stimulated) with cortisol and its precursor, deoxycortisol, together with mineralocorticoid metabolites significantly decreased under forskolin stimulated conditions. *S. frutescens* extracts and SUB repressed NF-κB-driven gene expression without activating GRE-driven gene expression and while neither activated MR mediated gene transcription, both antagonized the effects of aldosterone via the MR.

Conclusion: Data provide evidence linking anti-stress, anti-inflammatory and anti-hypertensive properties of *S. frutescens* to inhibition of steroidogenic enzymes and modulation of adrenal hormone biosynthesis. Findings suggesting *S. frutescens* and SUB exhibit dissociated glucocorticoid characteristics underline potential therapeutic applications in the treatment of inflammation and hypertension.

1. Introduction

Sutherlandia frutescens (S. frutescens) is a medicinal plant indigenous to South Africa and has customarily been used to treat a broad spectrum of ailments such as cancers, hence the common name Cancer bush, symptoms of anxiety and stress, inflammation as well as diabetes (van Wyk, 2008; Van Wyk, 2015). A number of secondary metabolites, including the sutherlandin flavonoids and triterpenoid compounds, have been isolated from *S. frutescens* extracts prepared from leaves (Fu et al., 2008), seed pods (Albrecht et al., 2012) and commercially available capsules (Avula et al., 2010). The triterpenoids identified in *S. frutescens* extracts include four complex cycloartane glycosides – sutherlandiosides (SU) A, B, C and D with SUB being the major triterpenoid present in plant material (Fu et al., 2008). The presence of the SU compounds were later also confirmed by liquid chromatography-mass spectrometry (LC-MS) (Albrecht et al., 2012; Avula et al., 2010). In the isoprenoid biosynthesis pathway of the triterpenoids, these molecules are oxidized by various cytochrome P450-dependent (P450) monooxygenases, after which glycosylation yields compounds containing a β-D-glucopyranose moiety at C24 (Lambert et al., 2011). Studies have shown that triterpenoids exhibit anti-cancer and anti-inflammatory properties with data indicating that many of these compounds target and downregulate nuclear factor-kappa B (NF-κB) (Bai et al., 2016; Chen et al., 2015; Yadav et al., 2010). Triterpenoids are furthermore widely used in Asian medicine in the treatment of chronic diseases which include amongst others, diabetes, obesity, cardiovascular atherosclerosis, arthritis and depression (Sanna et al., 2015; Venkatesha et al., 2016; Yadav et al., 2010).

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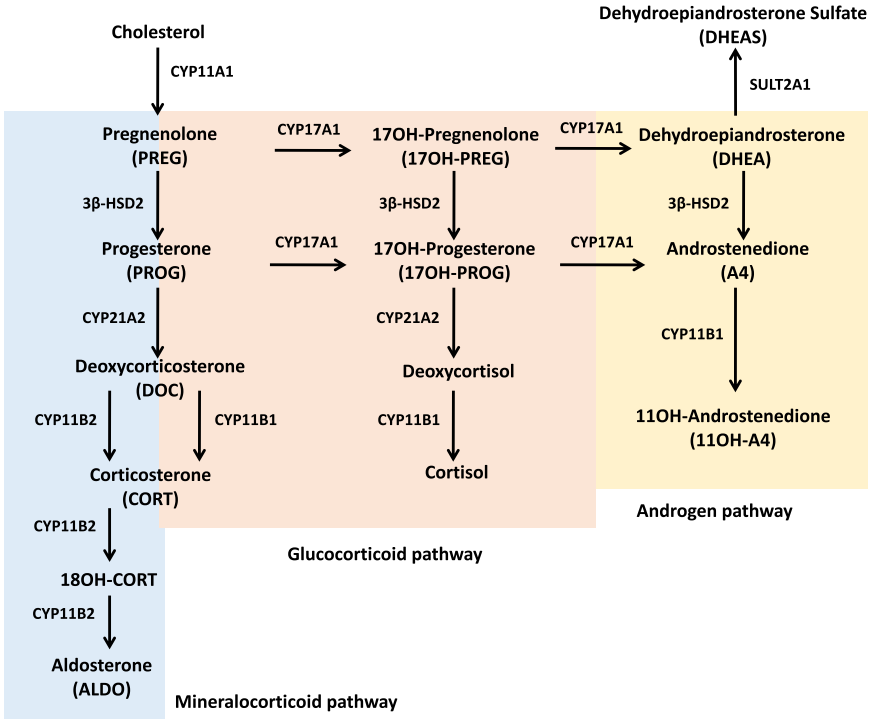

Fig. 1. Human adrenal steroid biosynthesis. The metabolism of cholesterol by the major steroidogenic enzymes yielding ALDO, cortisol and 11OHA4 in the mineralocorticoid-, glucocorticoid-, and androgen pathways, respectively.

The aforementioned conditions are closely associated with an impaired endocrine system and as such, the triterpenoids which have molecular structures closely related to the cyclopentanoperhydrophenanthrene structure of steroid hormones, may also interact with the steroidogenic P450 enzymes which catalyze steroid biosynthesis. In addition, the triterpenoids may mimic or antagonize the downstream actions of endogenous steroid hormones. While we have shown that *S. frutescens* extracts inhibit the catalytic activity of the adrenal steroidogenic P450 enzymes, P450 17α-hydroxylase/17,20-lyase (CYP17A1) and P450 21-hydroxylase (CYP21A2) (Prevoo et al., 2004, 2008), a more recent *in vivo* study by Smith and van Vuuren, 2014, showed that *S. frutescens* extracts affected steroidogenesis increasing plasma corticosterone levels in male Wistar rats (Smith and van Vuuren, 2014). Although the inhibition of several hepatic P450 enzymes by *S. frutescens* extracts has also been shown (Fasinu et al., 2013), reports on the interaction of the SU compounds with adrenal steroidogenic P450 enzymes are limited.

Adrenal P450 enzymes catalyze the biosynthesis of the steroid hormones in the mineralocorticoid, glucocorticoid and androgen pathways (Fig. 1). These steroids are all derived from the precursor steroid, cholesterol, with P450 side-chain cleavage (CYP11A1) catalysing its conversion to pregnenolone (PREG). In the mineralocorticoid pathway, limited to the zona glomerulosa of the adrenal cortex, PREG is converted by 3β-hydroxsteroid dehydrogenase type 2 (3β-HSD2) to progesterone (PROG) and subsequently to deoxycorticosterone (DOC) by CYP21A2. DOC is converted to aldosterone (ALDO) via corticosterone (CORT) and 18-hydroxycorticosterone (18OH-CORT) by aldosterone synthase (CYP11B2). In the glucocorticoid pathway, 17-hydroxypregnenolone (17OH-PREG), a product of the CYP17A1 conversion of PREG, is converted to cortisol via 17-hydroxyprogesterone (17OH-PROG) and deoxycortisol, catalyzed by 3β-HSD2, CYP21A2 and P450 11β-hydroxylase (CYP11B1) respectively. In the androgen pathway, dehydroepiandrosterone (DHEA), the product of 17OH-PREG, is converted to androstenedione (A4) by 3β-HSD2. We recently showed that A4 is also a substrate for CYP11B1 which catalyzes the formation of 11-hydroxyandrostenedione (11OHA4) (Swart et al., 2013). Although the adrenal P450 enzymes may catalyze the hydroxylation of more than one steroid, these enzymes are reported to be highly specific for their natural substrates, and compounds inhibiting the binding of steroid substrates to their respective enzymes will modulate subsequent hormone biosynthesis.

In adrenal steroidogenesis CYP17A1, together with 3β-HSD2 and CYP21A2, play a key role in channeling PREG into the three respective pathways (Goosen et al., 2013). We have previously reported that an aqueous extract of *S. frutescens* inhibited both PREG and PROG conversion by CYP17A1 significantly with the inhibition of PROG conversion being greater. The extract was also able to bind to microsomal P450 enzymes eliciting a reverse type I spectrum, suggesting that compounds bind to sites other than the active pocket. In addition, we showed that a methanolic extract and a mixture of the triterpenoid compounds inhibited the binding of PREG and PROG to adrenal microsomal P450 enzymes (Prevoo et al., 2008). Interference of the binding of PROG and PREG and the modulation of their metabolism by CYP21A2 and CYP17A1 may thus impact on the downstream production of glucocorticoids and mineralocorticoids, and in so doing also impact on the expression of various genes influenced by adrenal hormone levels via the glucocorticoid receptor (GR) and mineralocorticoid receptor (MR) in target tissue. The GR and MR are generally associated with the regulation of inflammatory (Nixon et al., 2013) and hypertensive processes (Messaoudi et al., 2012), involving gene transcription which is influenced by many other factors such as co-regulators and genetic modifiers (Bosscher et al., 2016; Griekspoor et al., 2007; Harris et al., 2013).

Conventional drugs used in the treatment of inflammation and hypertension target the GR and MR, respectively. Considering that *S. frutescens* is used as a traditional medicine to treat an array of conditions and has been shown to elicit immunomodulating effects (Zhang et al., 2014), the question arises whether *S. frutescens* and the triterpenoids can act via these receptors. Although a previous report has shown that *S. frutescens* extracts down-regulate lipopolysaccharide-induced NF-κB activity (Ajit et al., 2016), to our knowledge, no previous studies have investigated the effects of *S. frutescens* or the SUs on any members of the steroid receptor family. Compounds such as ginsenosides, which are classed as triterpene saponins with an