Steroid Hormone Synthesis Inhibition by Mineralocorticoid Receptor Antagonists

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Contributor: Xurong Mai , Mitsuhiro Kometani , Takashi Yoneda

Mineralocorticoid receptor antagonists (MRAs) have been found to concentration-dependently inhibit steroidogenesis in different degrees. However, many studies have proven the suppressive effects of MRAs on the activities of hormone synthase.

primary aldosteronism remission mineralocorticoid receptor antagonist steroidogenesis enzyme

1. Introduction

Primary aldosteronism (PA) is a leading form of secondary hypertension, a disease that presents with hypertension due to autonomous aldosterone hypersecretion from the adrenal glands. Compared to patients with essential hypertension (EHT), PA is associated with a three- to four-fold higher frequency of cardiovascular complications [1] and has a poor prognosis, requiring accurate diagnosis and qualified treatment. Furthermore, it is estimated that there are at least two million patients with PA in Japan, many of whom are misidentified as having EHT and are inappropriately treated [2][3]. PA can be divided into two subtypes, unilateral and bilateral lesions, depending on the site of lesion. The Endocrine Society and Japan Endocrine Society guidelines [4][5] recommend laparoscopic adrenalectomy on the affected side as the standard treatment for unilateral lesions. However, for bilateral PA (BPA), drug therapy with mineralocorticoid receptor antagonists (MRAs), such as spironolactone (SL) and eplerenone (EP), are recommended. Usually, drug therapy aims to exert an antihypertensive effect by suppressing the function of aldosterone. Remission of autonomous aldosterone production by MRAs in PA cases has been reported.

2. Inhibition of Steroid Hormone Synthesis by Mineralocorticoid Receptor Antagonists

SL has been widely used as an MRA for different types of hypertension since its discovery in the mid-1950s. SL is used for the oral treatment of PA as an MRA. SL has been reported to inhibit aldosterone synthesis in the adrenal cortex and aldosterone antagonism in the periphery. Inhibition of hormone synthesis has been reported for 11β-hydroxylase [6][7][8][9], 21-hydroxylase [8][9][10][11], 18-hydroxylase [6][7], and 17α-hydroxylase [10][11][12][13] (**Table 1**). The inhibitory effect of SL is thought to be due to the disruption of cytochrome P450 in steroid-synthesizing cells [9].

Menard et al. [14] reported that SL disrupted cytochrome P450 in adrenal and testicular microsomes, resulting in enzyme inhibition.

Table 1. Comparison of steroid hormone synthesis inhibition by MRAs.

References	Year	MRAs	Material	Steroid Hormone Synthesis & Production
[6]	1976	Spironolactone Canrenone Potassium carenoate	Adrenal Mitochondria from cows & humans	11β- ↓ 18- ↓
[7]	1977	Spironolactone	Human plasma	11β- ↓ 18- ↓
[10]	1979	Spironolactone	Adrenal and testicular microsomes from Sprague-Dawley rat & Guinea pig & dog	Cytochrome P-450 ↓ 17α- ↓ 21- ↓
[8]	1981	Spironolactone Canrenone	Adrenal mitochondria and microsomes from Guinea pig	11β- ↓ 21- ↓
[15]	1984	Spironolactone	Adrenal gland from rana ridibunda pallas frog	Aldosterone production ↓
[16]	2009	Spironolactone	Human adrenocorticoid H295R cells	Aldosterone production ↓ Cortisol production ↓
[17]	1990	Spironolactone 7α-thio-Spironolactone	Adrenocorticoid cells from Guinea pig	Cortisol production ↓
[12]	1991	Spironolactone 7α-thio-Spironolactone	Adrenocorticoid cells from Guinea pig	Cortisol production ↓ 17α- ↓
[9]	1978	Spironolactone Canrenone	Adrenal mitochondria and microsomes form Guinea pig	Cytochrome P-450 ↓ 11β- ↓ 21- ↓
[13]	1991	Spironolactone 7α-thio-Spironolactone	Adrenal microsomes from Guinea pig	Cytochrome P-450 ↓ 17α- ↓
[11]	1992	Spironolactone	Adrenal mitochondria and microsomes from Guinea pig	Cytochrome P-450 ↓ 17α- ↓ 21- ↓

References	Year	MRAs	Material	Steroid Hormone Synthesis & Production
[14]	1978	Spironolactone [6]	Dog plasma	Concentration of testosterone ↓ estradiol ↓ and cortisol ↓

nd bovine

(DOC) to

corticosterone and corticosterone to 18-hydroxy-corticosterone, although none of them interfered with CYP450 in mitochondria by themselves. Moreover, the potency and inhibition of aldosterone synthesis were found to be related to their affinity for cytochrome P450. This result is the same as that reported by Abshagen et al. [7]. A considerable increase in plasma DOC was detected after SL administration (100 mg three times a day) in five healthy male volunteers, suggesting inhibition of 11β- and 18-hydroxylase. In Menard et al.'s study [10], administration of SL in vivo at doses of 100 mg/kg caused a remarkable reduction in cytochrome P-450 in the adrenal glands and testes, 60% to 70% and 40% to 50%, respectively, indicating that steroids containing 7α-methylthio or 7α-methylsulfone groups could be the main reason for this destruction, because the destruction did not occur in the groups lacking these steroids. At the same time, through the rate of progesterone to 17α-hydroxyprogesterone and 21-hydroxyprogesterone, the activities of adrenal 17α-hydroxylase and 21-hydroxylase were detected, with a 50% to 80% loss, similar to the heme concentration of cytochrome P-450. Similarly, Colby's study [8] showed that both SL and canrenone could concentration-dependently inhibit microsomal 21-hydroxylation and mitochondrial 11β-hydroxylation, although the effect of canrenone was better when using mitochondria and microsomes derived from guinea pig adrenal glands.

Abbreviations: 11β-, 11β-hydroxylase; 21-, 21-hydroxylase; 18-, 18-hydroxylase; 17α-, 17α-hydroxylase; MRAs, mineralocorticoid receptor antagonists; "↓", decrease in steroid hormone synthesis activity, steroid hormone production or concentration.

In addition, another report focusing on aldosterone synthesis influenced by different MRAs, SL, prorenone, SC 19886, SC 26304, and SC 27169, by Netchitailo et al. [15] revealed that SL could inhibit 75% of aldosterone synthesis, but it would recover partially after drug infusion in frog adrenal glands. Furthermore, an experiment conducted by Ye et al. used human adrenocortical H295R cells [16]. After comparing the effects of SL and EP, there were several significant consequences: (1) SL could inhibit Ang II-stimulated aldosterone production by 80% and Ang II-stimulated cortisol production by 74%; (2) SL could inhibit pregnenolone metabolism to both aldosterone and cortisol by 67% and 74%, respectively; and (3) EP had no effect on basal, Ang II, or forskolin stimulation of aldosterone or cortisol production.

According to these studies, canrenone, one of the major active forms of SL, is often used to compare its effects with SL. SL is a prodrug with a short half-life (1.4 h), and the long half-life of canrenone (16.5 h) has also led to the conclusion that canrenone might be the main therapeutic form of SL treatment [6]. This can be proven in some way through the better inhibition of enzyme activity by canrenone and the inhibition of aldosterone synthesis in different human and animal experiments.

2.2. Peripheral Influence on Cortisol Secretion

In addition to aldosterone secretion from the zona glomerulosa, the production of cortisol from the zona fasciculata has begun to attract attention. A study designed by Rourke and Colby [17] used adrenal glands collected from guinea pigs to probe the inhibition of cortisol production by SL. Both SL and its intermediate, 7α-thio-SL, which is also one of the three major active forms of SL, decreased cortisol production in a time- and concentration-

dependent manner by nearly 50%, with 7α-thio-SL being far more potent. At the same time, Rourke et al. [12] focused on the mechanism of action of SL on cortisol production. In their experiment, they incubated adrenocortical cells derived from guinea pigs with three different agents: (1) the 17α-hydroxylase inhibitor SU-10′603, (2) 11β-hydroxylase inhibitor metyrapone, (3) cholesterol sidechain cleavage inhibitor aminoglutethimide, and SL or 7α-thio-SL. Eventually, they realized that the direct effects of SL and 7α-thio-SL on cortisol production resulted from the selective inhibition of 17α-hydroxylation, whereas metyrapone and aminoglutethimide did not change the efficacy of SL or 7α-thio-SL on cortisol production.

Through further studies, we can clearly see that SL has the ability to reduce the production of aldosterone and cortisol in several ways. On the one hand, the enzyme activities of 21-hydroxylase, 11β-hydroxylase, and 18-hydroxylase will be affected by SL, leading to the reduction in aldosterone; on the other hand, the selective inhibition of 17α-hydroxylase will certainly decrease the production of cortisol. SL, canrenone, and 7α-thio-SL were potent inhibitors of aldosterone synthesis and cortisol production.

2.3. Interrelation with Cytochrome P-450

The in vitro experiment by Menard et al. [10] showed that in the presence of NADPH, incubation of testicular microsomes with SL caused a sharp destruction of >80% of the amount of cytochrome P-450, but the decrease was not obvious without NADPH in adrenal and testis tissues. The studies performed in this study, in vivo and in vitro, indicated that SL could reduce the concentration of cytochrome P-450 in the adrenal gland or testes, not directly by itself, but probably in one of its metabolite forms instead. Greiner et al. [9] similarly reported that the incubation of adrenal microsomes with SL plus NADPH resulted in the decline of cytochrome P-450, but this phenomenon did not occur in the metabolite of SL, canrenone, with or without NADPH, considering that canrenone was neither a reactive metabolite nor could it be mediated by adrenal microsomes, although both agents were responsible for inhibiting the binding of steroid substrates to cytochrome P-450 and reducing the activities of adrenal mitochondria (11β-hydroxylase) and microsomes (21-hydroxylase). However, a subsequent study by Kossor et al. [13] implied that 7α-thio-SL was an obligatory intermediate in the SL-induced CYP450 decrease. The initial step of SL activation is the deacetylation of SL to 7α-thio-SL, but the subsequent pathways require the destruction of CYP450. Incubation of guinea pig adrenal microsomes with 7α-thio-SL plus NAPDH resulted in a significant decline in CYP450 and 17α-hydroxylase by >50%. In contrast, after adding antisera to 17α-hydroxylase, no change in CYP450 degradation occurred, which showed that the activation of 7α-thio-SL required 17α-hydroxylase.

Kossor and Colby [11] used a high dose (100 mg/kg) and low dose (25 mg/kg) of SL to determine dose-dependent effects on the outer (zona glomerulosa plus zona fasciculata) and inner (zona reticularis) zones in guinea pigs. The results showed that high doses of SL significantly decreased 17α-hydroxylase and 21-hydroxylase, accompanied by the degradation of mitochondrial P450 and heme concentrations. However, for low doses of SL, microsomal P450 activities were inhibited, whereas 21-hydroxylase declined in the inner zone only, and the amount of mitochondrial P450 did not change in either zone. Additionally, a high dose of SL altered the gross appearance of the adrenal glands, which has also been found with low doses of SL, indicating that a high dose of SL may have a

number of unclear, or even toxic, effects on adrenal glands. A high dose of SL (100 mg/kg) is widely used in experiments, and Menard et al. [14] used this dose of SL in male dogs.

Interestingly, they found that a high dose of SL could decrease the concentrations of testosterone in testicular and peripheral venous plasma, and the concentration of cortisol in adrenal venous plasma, by 60–75% and 50–65%, respectively. In contrast, canrenone and potassium canrenoate did not cause any significant changes. Although some of the positive results were meaningful and helpful for the study of SL, the difficulty between practical usage and experimental results is worth noting. Ye et al. [16] reported that the inhibitory effects of SL occurred at concentrations far higher than those needed to block mineralocorticoid receptors: up to 400 mg per day.

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