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A Dispensable Role for $P450_{scc}$ in the Overproduction of Aldosterone in Aldosterone-Producing Adenoma and Idiopathic Hyperaldosteronism in Patients with Primary Aldosteronism

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Abstract Our previous study suggests that cytochrome *P*-450 carbon 17α-hydroxylase/17,20-lyase ($P450_{c17\alpha}$) correlated with the overproduction of aldosterone in aldosterone-producing adenoma (APA) and idiopathic hyperaldosteronism (IHA) in patients with primary aldosteronism. To further investigate if cytochrome *P*-450 cholesterol side-chain cleavage enzyme ($P450_{scc}$) contributes to the overproduction of aldosterone in APA and IHA and if its mRNA expression differs in APA and IHA in patients with primary aldosteronism, we studied the expression of $P450_{scc}$ mRNA in APA and idiopathic hyperplastic nodules. Total RNA was extracted from APA of eight patients diagnosed as APA, idiopathic hyperplastic nodules of four patients diagnosed as IHA, seven normal adrenal glands and one normal muscle tissue. $P450_{scc}$ mRNA was examined by Northern blot analysis. No significant difference in $P450_{scc}$ mRNA was found among normal adrenal gland, APA or idiopathic hyperplastic nodules ($P>0.05$). These results suggest that $P450_{scc}$ contributes little to the overproduction of aldosterone in APA and IHA and cannot be considered as a marker to differentiate between them in patients with primary aldosteronism.

Keywords Aldosteronism · Adrenocortical adenomas · Idiopathic hyepraldosteronism · $P450_{scc}$

Abbreviations

APA	aldosterone-producing adenomas
IHA	idiopathic hyperaldosteronism
$P450_{c17\alpha}$	cytochrome *P*-450 carbon 17α-hydroxylase/17,20-lyase
$P450_{scc}$	cytochrome *P*-450 cholesterol side-chain cleavage enzyme cytochrome
$P450_{aldo}$	cytochrome *P*-450 aldosterone synthase

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Introduction

In adrenal gland, cytochrome *P*-450 carbon 17α-hydroxylase/17,20-lyase ($P450_{c17\alpha}$) and cytochrome *P*-450 cholesterol side-chain cleavage enzyme ($P450_{scc}$) are two important enzymes associated with the biosynthesis of aldosterone [1–3]. The biosynthesis of aldosterone from cholesterol needs the catalyzation of $P450_{scc.}$ Pregnenolone is produced when cholesterol is catalyzed by $P450_{scc}$ and pregnenolone is the substrate for production of aldosterone. Pregnenolone is also the substrate for both cortisol and sex

hormone where $P450_{c17\alpha}$ plays an important role in this process. Thus, the activity of $P450_{c17\alpha}$ may affect the contents of aldosterone by controlling the contents of the prognenolone in adrenal glands [1–3].

Our previous study suggests that deceased $P450_{c17\alpha}$ correlated with the overproduction of aldosterone in aldosterone-producing adenoma (APA) and idiopathic hyperaldosteronism (IHA) in patients with primary aldosteronism [4], but $P450_{c17\alpha}$ mRNA was expressed comparably in APA and IHA. To further investigate if the other important enzyme $P450_{scc}$ contributes to the overproduction of aldosterone in APA and IHA and if its expression differs in APA and IHA in patients with primary aldosteronism, we studied the expression of $P450_{scc}$ mRNA in APA and IHA in patients with primary aldosteronism.

Subjects and Methods Between July 1996 and July 1997, 12 patients diagnosed clinically as primary aldosteronism due to "APA" were admitted to Pekin Union Medical College Hospital for surgery. Clinical data from the patients are shown in Table 1. All of these patients had typical findings for primary aldosteronism, such as hypertension, hypokalemia, suppressed plasma rennin activity and elevated plasma aldosterone. Plasma ACTH levels were also normal in all patients. Computered tomography revealed a solid mass in adrenal gland in each patient. After surgery, eight out of 12 patients were diagnosed as APA, whereas, four out of 12 were adrenal nodular hyperplasia by pathologic examination. This study also included seven adrenal glands harvested from normal adults from brain-dead patients for renal transplantation. All tissues were carefully dissected to avoid from the possible medulla contamination. All tissues harvested were snap-frozen and stored in liquid nitrogen. This study was approved by appropriate ethical committee and each subject gave informed consent.

Northern blot The method for Northern blot has been described in details previously [4]. Briefly, pUC19 plasmid containing human $P450_{scc}$ cDNA was a gift from Dr. Morohashi (Kyushu University, Japan). The pUC19 plasmid was amplified and digested by EcoR I and Sal I (Friendship Corp. of PUMCH, Beijing). A 1.75 kb probe was obtained, purified and labeled with α-^{32}P-dATP (Yahui Corp. Beijing) using the kit from Promega company. Total RNA was extracted using guanidine isothiocyanate-phenol-chloroform [5]. The concentration of RNA was measured by absorbances at 260 nm and 280 nm. Twenty μg of denatured RNA was loaded into each well of the agarose gel. Total RNA was separated using formaldehyde 1% agarose gels, and transferred overnight to nylon membrane by capillary blotting. Blots were baked for 3 h at 70°C, prehybridized for 4 h at 42°C, and then hybridized with the ^{32}P-labeled dATP probe in hybridization solution at 42°C for 24 h. Membranes were washed as previously described [4]. Equivalent amount of total RNA loaded in each gel lane was assessed by stripping the membranes and reprobing for β-actin RNA, separately. Blots were then exposed to Kodak film at −70°C with an intensifying screen for 3 days. Hybridization intensities were quantitated using a laser scan densitometer (Pharmacia).

Statistical analysis All values are expressed as mean ± SEM. Statistical analysis of data was performed using an unpaired two-tailed Student's t test. A p value<0.05 was considered significant.

Results

APA, Idiopathic Hyperplastic Nodule and Normal Adrenal Gland Expressed Comparable $P450_{scc}$ mRNA

The intensities of β-actin and $P450_{scc}$ mRNA were analyzed by laser optical density scanner and the results are shown in Table 2. As a negative control, no $P450_{scc}$ signal was detected in skeletal muscle by Northern blot (data not shown). The average intensities of $P450_{scc}$ mRNA in APA, idiopathic hyperplastic nodule and normal adrenal gland

Table 1 Clinical data of the patients recruited in this study

Tissue	APA	Nodular hyperplasia	Normal adrenal	Muscle
Number	8	4	7	1
Age range (years)	26–40	27–41	20–30	40
Sex	7F/1 M	3F/1 M	6 M/1F	M
Mass	18–40 g	20–38 g	5–9 g	8 g
Hypertension	+	+	N/A	N/A
Hypokalemia	+	+	N/A	N/A
Plasma rennin activity	Low	Low	N/A	N/A
Plasma aldosterone	High	High	N/A	N/A
ACTH	Normal	Normal	N/A	N/A
10 years' follow-up	Disease free	Medicine	N/A	N/A

Table 2 The relative mRNA expression level of $P450_{scc}$ in APA, idiopathic hyperplastic nodule and normal adrenal gland

APA	Sample No.	1	2	5	6	7	8	10	11
	Ratio(%)	28	17	17	24	32	29	42	14
	Average(%)	25±9							
Idiopathic hyperplastic nodule	Sample No.	3	4	9	12				
	Ratio(%)	22	8	46	34				
	Average(%)	28±16							
Normal adrenal gland	Sample No.	13	14	15	16	17	18	19	
	Ratio(%)	17	28	31	23	31	47	28	
	Average(%)	29±9							

groups were 25±9, 28±16, 29±9 (percentage compared to β-actin) respectively. No significant difference was found between each group ($P>0.05$, Table 2). These results suggest that $P450_{scc}$ is not a critical contributor to the overproduction of aldosterone in APA and IHA. Therefore it cannot be further considered as a marker to differentiate between them in patients with primary aldosteronism.

Discussion

The purpose of this study is trying to identify a cytochrome *P*-450 enzyme which contributes to the overproduction of aldosterone in patients with primary aldosteronism and from there to further investigate if this molecule can be used as a marker to differentiate between APA and IHA. APA and IHA (including diffused adrenal hyperplasia and nodular hyperplasia) are the two main causes for primary aldosteronism [1–3]. It is very important to differentiate the cause of primary aldosteronism because the treatment and prognosis are quite different [1–4]. Patients with APA can be cured by surgery, while medicine is the only effective treatment for patients with IHA. Single idiopathic hyperplastic nodule causing IHA usually mimics APA and sometime unnecessary surgery is performed to these patients [1–3]. This study included four patients with IHA (single hyperplastic nodule) who were all misdiagnosed as APA and were performed unnecessary surgeries. Presently pathologic examination from samples after surgery is the only reliable method to differentiate nodular idiopathic hyperplasia from APA. In some cases, if the pathologic characteristics are not typical, it is a hard for a pathologist to give a definite diagnosis [1–4]. Therefore, a method which can provide further supporting evidence to differentiate nodular idiopathic hyperplasia from APA is necessary.

This study is a continuation of our previous study which suggests that $P450_{c17\alpha}$ correlated with the overproduction of aldosterone, although its expression was comparable in APA and idiopathic hyperplastic nodule in patients with primary aldosteronism. This study suggests that different from $P450_{c17\alpha,}$ $P450_{scc}$ contributes little to the overproduction of aldosterone in APA and IHA in patients with primary aldosteronism. Therefore, together with our previous studies, neither of the mRNA expression of $P450_{c17\alpha}$ or $P450_{scc}$ can be used as a marker to differentiate APA and IHA due to their comparable mRNA expression in the two type tissues [4]. This study is unique since, to the best of our knowledge, this is the first study to directly compare mRNA expression level of *P*-450_{scc} in three different adrenal tissues: APA, idiopathic hyperplastic nodule and normal adrenal gland.

The gene coding human $P450_{scc}$ locates in 15q23-24 [6–9]. There are 20 k base pairs with 9 exons and 8 introns. $P450_{scc}$ mRNA contains only 2 k base pairs coding a protein with 521 amino acids. After a signal peptide cut, the truncated $P450_{scc}$ is the active form for $P450_{scc}$. Although $P450_{scc}$ is a rate-limiting enzyme in the biosynthesis of aldosterone [1–3], it is not the major enzyme contributing to the overproduction of aldosterone. Our results are consistent with the report by Dr. Ogo and his colleagues [10] who showed that similar expression level of mRNA in APA (three samples) and normal tissues (three samples). These results are also consistent with the study by Shibata and colleagues who showed that $P450_{scc}$ protein was similar in APA and normal adrenal tissues [11]. Since the study by Bassett and colleagues suggested that APA had higher cytochrome *P*-450 aldosterone synthase ($P450_{aldo}$) mRNA expression by realtime PCR [12], it might be interesting to directly compare the $P450_{aldo}$ mRNA expression levels in APA, idiopathic hyperplastic nodule and normal adrenal gland using Northern blot in our future studies.

In this study, all patients are followed up for 10 years. Eight patients with APA all have no clinical, biochemical or radiological recurrence and remain disease free. Four patients with IHA are still treated medically.

In short, $P450_{scc}$ is not a key contributor to the overproduction of aldosterone in APA and IHA. Therefore, it is not a good candidate molecule to be considered as a marker to differentiate IHA from APA in patients with primary aldosteronism.

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