Int J Clin Exp Pathol 2015;8(4):3742-3747
www.ijcep.com /ISSN:1936-2625/IJCEP0006374

Original Article

Expression of androgen receptor in coronary artery in the cases of sudden coronary death

Kaiyi Liu[1,2], Chunyu Shen[1,3], Xinshan Chen[1]

[1]Department of Forensic Pathology, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China; [2]Department of Pathology, Meizhou People's Hospital, Meizhou 514031, China; [3]Department of Molecular Pharmacology and Physiology, University of South Florida, Tampa, 33620 FL, USA

Received January 26, 2015; Accepted March 23, 2015; Epub April 1, 2015; Published April 15, 2015

Abstract: To study the expression of androgen receptor (AR) in the cases of sudden death caused by coronary heart disease (CHD) and relationship between AR and sudden coronary death (SCD) to explore the mechanism of the development of coronary atherosclerosis and provide references for the prevent and treatment of CHD and medicolegal identification of SCD. 53 cases selected from the autopsied cases in our department from 2011 to 2012 were divided into 3 groups: 18 case of SCD, including 11 males and 7 females, as experimental group, another 18 cases, including 11 males and 7 females, with CHD but died of mechanical injuries and poisoning as control group I, and 17 cases without CHD who also died of mechanical injuries and poisoning including 10 males and 7 females, as control group II. After HE-stained and immunohistochemistry-stained (SP) for the slices, the expression of AR in coronary arteries were observed and the average optical density (AOD) of positive signal in each case were detected by using Image-Pro Plus 6.0 software. We found strongly positive expression of AR in control group II, weakly positive expression in control group I and a little weaker in the experimental group. The expression of AR in coronary artery was reduced in CHD patients, which suggested that androgen may have favorable effects on the cardiovascular system.

Keywords: Androgen receptor, sudden coronary death, immunohistochemistry, coronary atherosclerosis

Introduction

In the practice of forensic pathology, the sudden death cases caused by coronary atherosclerotic heart disease (CAD) were the most common. Most of them were males. Although more men seemed to be involved in the crime and disputes compared with women, the truth was that there were significant gender differences in the incidence of CAD according to the epidemiologic investigation. CAD was mostly happened in the older over 40 years old especially in men. But the incidence rose sharply in the postmenopausal women and got close to that in the men of the same age. Therefore, it was widely considered that estrogen had favorable effects on the cardiovascular system. In recent years, there was a different understanding of the role of androgen playing in the cardiovascular system. Just like estrogen, androgen can also induce coronary vasodilatation and suppression of inflammatory factors [1, 2]. In a certain concentration, androgen may have protective effects on the cardiovascular system. Since androgen can be changed into estrogen by aromatase, it was of great significance that how androgen affect the development of CAD and acute coronary syndrome. In fact, while clinical study objects were living people, forensic pathology almost study the corpses. So it was difficult to detect the blood concentration of androgen directly. But we can study the expression of the androgen receptor (AR) in the coronary arteries which make more sense to reflect the exposure to androgen of tissues during its lifetime indirectly, for it played its biological effects mainly through AR.

Material and methods

Samples and groups

All coronary artery formalin-fixed and paraffin-embedded (FFPE) samples were selected from

Table 1. Basic data of these 3 groups

Age	Experimental		Control I		Control II	
	Male	Female	Male	Female	Male	Female
31~40	1	1	1	1	1	1
41~50	4	1	4	1	5	2
51~60	3	3	3	3	2	2
61~70	2	1	2	1	2	1
71~80	1	1	1	1	0	1
Total	11	7	11	7	10	7
	18		18		17	

Table 2. AOD value of AR in three groups

	Experimental	Control I	Control II	*F*	*P*
AOD_{AR}	0.071±0.038	0.074±0.269	0.162±0.895	13.985	<0.0001

Table 3. Dunnett's T3 test results

Groups	I-J absolute value	*P*
Experimental vs. Control I	0.003	>0.05
Experimental vs. Control II	0.091	<0.05
Control I vs. Control II	0.088	<0.05

the autopsied cases in the Department of Forensic Medicine, Tongji Medical College, Huazhong University of Science and Technology from 2011 to 2012, filtered by the following conditions.

① Cases of sudden cardiac death as experimental group. Cases with CHD but died of mechanical injuries and poisoning as control group I. And cases without CHD who also died of mechanical injuries and poisoning as control group II.

② All cases were drawn materials within 48 hours and immobilized with 4% formalin.

③ Coronary lesions in experimental group and control group I were up to III grade that plaque lesions accounted for more than 51% of coronary artery cross sectional area of the main branches. In control group II, we drew materials from the left anterior descending artery which was 1.5 cm away from the left main coronary artery branch.

④ All cases in experimental group were in line with the WHO (ICD-10) diagnostic criteria of sudden cardiac death (I46.1, I96). And cases in the control groups were proved to be non-SCD by forensic pathology examination.

Immunohistochemical staining

After baking, dewaxing and washed by PBS, paraffin sections were put into EDTA solution to undergo the microwave repairing. Then we added 3% hydrogen peroxide solution and incubated at room temperature for after washing with PBS again. 10 min later, we used PBS washing 3 times and 5% BSA blocking for 20 min. When washing out BSA, each slice was added 50 μl of 1:100 rabbit anti-human AR antibody and incubated at 4°C overnight. After washing with PBS, we added 50 μl-100 μl secondary antibody and incubated at 4°C again for 50 min each slice. Using PBS washing 3 times and then removing PBS, every slice was added 50 μl-100 μl freshly prepared DAB and controlled the color by microscope. Then all slices were counterstained with hematoxylin, dehydrated through graded alcohol and cemented with neutral gum. Negative control was using PBS instead of primary antibody.

Result judge: coronary artery endothelial cells within the brown particles AR distribution area (positive).

Image analysis and statistics

When excluding false positive immunohistochemical staining, each slice randomly selected non-overlapping, equal area of five horizons, using Image-Pro Plus 6.0 software to measure the average optical density (AOD) of positive signal per field. All data were statistically analyzed by SPSS 19.0. One way ANOVA was used to compare the difference among these 3 groups. The comparison of every two groups was using Dunnett's T3 test. And the comparison between males and females was using t test. A P value < 0.05 is considered signific-ant.

Results

Basic data

In the experimental group, there were totally 18 cases, while the youngest was 37 years old and the eldest was 79 (53.78±11.26). 11 of these cases were males, and the rest cases were females, and no significant statistical differ-

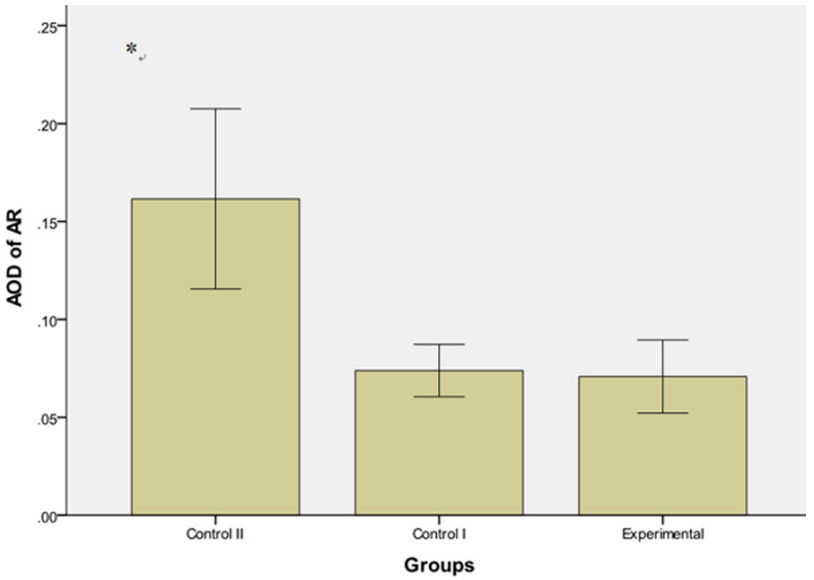

Figure 1. AR expression in three groups. Compared with control group II, AR expressed in coronary arteries were significantly reduced in experimental group and control group I ($P<0.05$). There was no significant statistical difference between experimental group and control group I ($P>0.05$).

Table 4. AOD value of AR in males and females

	Males	Females	T	*P*
AOD_{AR}	0.095±0.077	0.102±0.058	-0.754	0.454

ence between the age of males and females ($P=0.982$). The average survival time after onset was 0.57±0.59 hours, which was in line with the WHO (ICD-10) diagnostic criteria of sudden cardiac death (I46.1, I96). 4 cases which have been found dead were identified as coronary death after forensic pathology examination to rule out other causes of death, and unable to determine the incentive; 9 cases fell to the ground after quarrelling, tussle, catch-up or treatment, existing mental stimulation, physical activity, infection obvious incentive; 5 cases occurred vomiting, irritability, sweating and other clinical manifestations of acute coronary syndrome before death (**Table 1**).

In control group I, there were also totally 18 cases, while the youngest was 32 years old and the eldest was 79 (53.11±12.71). Just like the experimental group, 11 of these cases were males, and the rest cases were females, and no significant statistical difference between the age of males and females ($P=0.765$) (**Table 1**).

In control group II, there were 17 cases, including 10 males and 7 females. The youngest of them was 33 years old and the eldest was 78 (53±12.04). And there was also no significant statistical difference between the age of males and females ($P=0.385$) (**Table 1**).

Statistical analysis showed that there was no significant statistical difference among the 3 groups ($P=0.979$) and between the age of males and females ($P=0.471$).

Immunohistochemical staining results

The brown fine particles can be seen deposited in coronary endothelial cells in experimental group. The similar results can be found in control group I, but slightly higher. In control group II, there were obvious brown particles deposited in coronary endothelial cells (**Table 2**; **Figures 3**-**5**).

Statistically analysis

Data from microscopic image analysis for each slice were statistically analyzed and results were as following: ① Compared with control group II, AR expressed in coronary arteries were significantly reduced in experimental group and control group I ($P<0.05$). ② There was no significant statistical difference between experimental group and control group I ($P>0.05$) (**Table 3**; **Figure 1**); ③ There was no significant statistical difference between males and females ($P>0.05$) (**Table 4**; **Figure 2**).

Discussion

Androgen synthesized from cholesterol. In plasma, it mostly bound with albumin and sex hormone binding globulin (SHBG). Only 2% testosterone was in free form. Normally, the testosterone concentration reached to the highest during young age of men but decreased after 50. Androgen receptor (AR) belonged to the

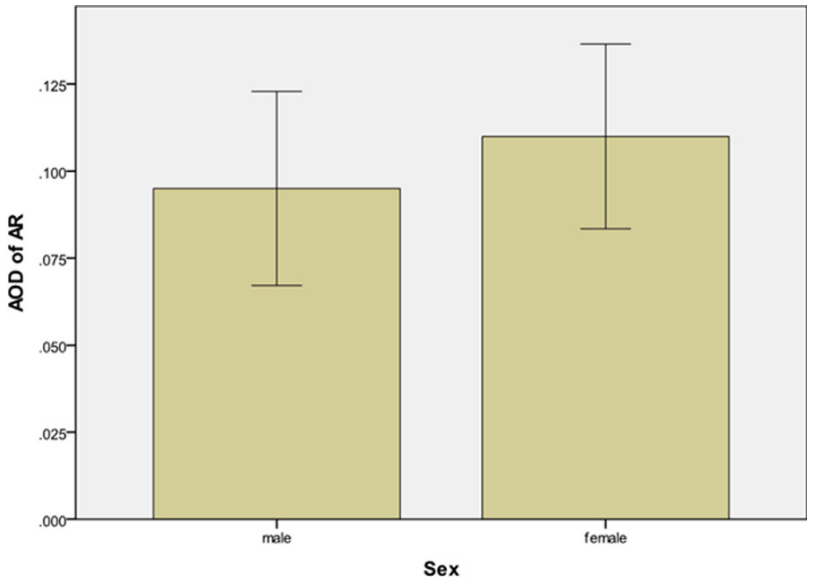

Figure 2. Gender difference of AR expression. There was no significant statistical difference between males and females (P>0.05).

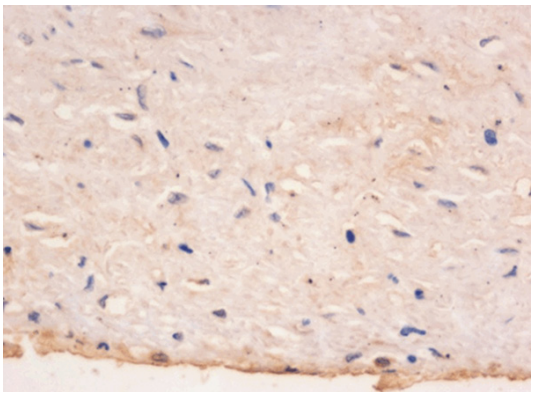

Figure 3. AR expressed in control group II (SP×400).

nuclear receptor superfamily. It can mediate both gene and non-gene effects of androgen. AR was widely distributed in the nervous system, cardiovascular system. Immunohistochemistry has been confirmed that AR existed in myocardium, arteries, of course, including coronary arteries and its branches in rat. And the smaller the artery was, the higher positive rate of AR was [3].

Curatola et al considered that DHEA produced PSA-NCAM to inhibit monocyte adhesion to endothelial cells through AR [4]. For vessel wall, androgen-AR system was against to the vascular remodeling such as medial thickness and perivascular fibrosis of arteries caused by angiotensin II [5]. Therefore, AR may play a protective role in the development of coronary atherosclerosis process. In this study, the expression of AR were significantly lower in experimental and control group I than that in control group II, which was further confirmed the protective effect of AR on the cardiovascular system.

When a series of factors, such as inflammation, blood flow shear stress increasing, acted on the coronary plaque, it can cause plaque unstable, local endothelin-1 (ET-1) concentrations abnormally elevating, coronary spasm, plaque rupture and, the severest, sudden death. In the inflammatory environment, dihydrotestosterone (DHT) can reduce cyclooxygenase-2 (COX-2) level in coronary artery smooth muscle cells so as to keep plaque stable. In contrast with ET-1, Nitric oxide (NO) had a strong vasodilator effect. AR can upregulate NOS through PI3K/Akt pathway to increase production of NO [6]. In vitro experiments in pigs, testosterone -inhibited the flow of Ca^{2+}. AR can not only make high-conductance calcium-activated potassium channel on vascular smooth muscle open, but also adjust the abundance and activity of delayed rectifier potassium (I_{kr}) and ATP-sen-sitive potassium channel (K_{ATP}). The increase of NO, the decline of intracellular calcium concentration and the open of potassium can make coronary artery vasodilatation and improving myocardial ischemia. Androgen receptor regulated vasodilatation mainly through non-gene effect. Although reaction time was short, effect produced rapidly. Therefore, it played an important in the acute coronary lesions, especially in the event of coronary spasm.

But Navarro-Dorado et al considered that testosterone-induced arterial relaxation directly did not depend on endothelium, NO, AR and potassium channel, but depended on the L-type calcium channel. In this study, there was no significant statistical difference between experimental group and control group I [7]. It may be concerned with AR activity and androgen not completely acting via AR.

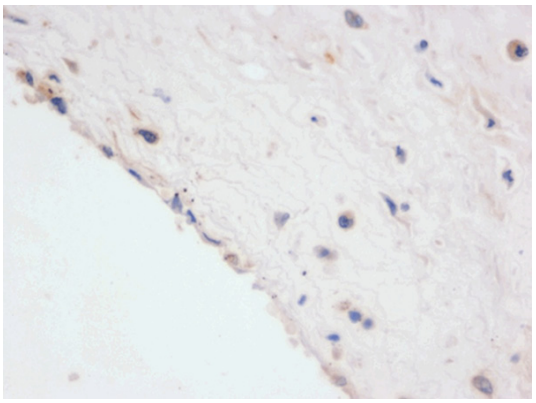

Figure 4. AR expressed in control group I (SP×400).

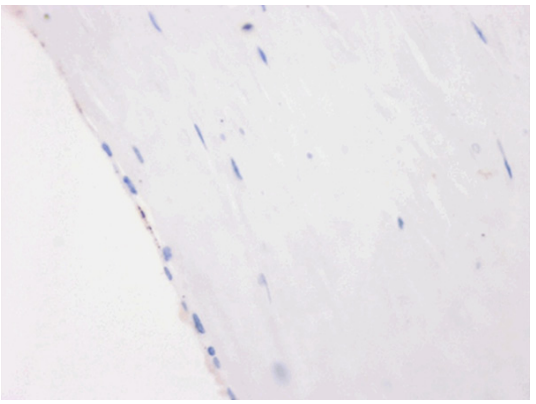

Figure 5. AR expressed in experimental group (SP×400).

In addition to the abundance of receptor expression, the strength of its activity can also affect the sensitivity of organization to androgen. Research showed that AR exons $(CAG)_n$ short repeats was positively correlated with the risk of men coronary heart disease [8]. A study on AR exon 1 CAG repeat polymorphism for 2878 middle-aged men in European Male Aging Study (EMAS) [9] found that the repeat length of CAG was significantly positively correlated with testosterone and E_2, suggesting that $(CAG)_n$ repeat length related to the activity of AR. The longer the CAG repeat length was the lower AR activity. And androgen compensatorily rose. Extremely long repeats (n>40) may cause Kennedy's syndrome, which characterized by varying degrees of androgen insensitivity [10]. No matter the activity of AR was too high or too low, it was positively correlated with the risk of men coronary heart disease. The result was similar to the 12 years follow-up study for 639 postmenopausal women by Laughlin [11] et al, and there may be a U-shape contact between testosterone and coronary heart disease.

Sudden coronary death played an important role in the practice of forensic pathology. Since coronary heart disease had significant gender differences, investigating the relationship between androgen and sudden coronary death can further explore the occurrence and development of coronary atherosclerosis, and provide references for the prevention of coronary heart disease and identification of sudden coronary death in forensic pathology.

Acknowledgements

This work was partly supported by operating research grants from Hubei Province Science Research Foundation.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Xinshan Chen, Department of Forensic Pathology, Tongji Medical College, Huazhong University of Science and Technology, 13 Hangkong Rd, Qiaokou District, Wuhan 430030, China. E-mail: xschen@mails.tjmu.edu.cn

References

[1] Osterlund KL, Handa RJ and Gonzales RJ. Dihydrotestosterone alters cyclooxygenase-2 levels in human coronary artery smooth muscle cells. Am J Physiol Endocrinol Metab 2010; 298: E838-845.

[2] Chou TM, Sudhir K, Hutchison SJ, Ko E, Amidon TM, Collins P and Chatterjee K. Testosterone induces dilation of canine coronary conductance and resistance arteries in vivo. Circulation 1996; 94: 2614-2619.

[3] Bentmar Holgersson M, Giwercman A, Bjartell A, Wu FC, Huhtaniemi IT, O'Neill TW, Pendleton N, Vanderschueren D, Lean ME, Han TS, Finn JD, Kula K, Forti G, Casanueva FF, Bartfai G, Punab M and Lundberg Giwercman Y. Androgen receptor polymorphism-dependent variation in prostate-specific antigen concentrations of European men. Cancer Epidemiol Biomarkers Prev 2014; 23: 2048-2056.

[4] Crews JK and Khalil RA. Antagonistic effects of 17 beta-estradiol, progesterone, and testosterone on $Ca2^+$ entry mechanisms of coronary vasoconstriction. Arterioscler Thromb Vasc Biol 1999; 19: 1034-1040.

[5] Ikeda Y, Aihara K, Yoshida S, Sato T, Yagi S, Iwase T, Sumitomo Y, Ise T, Ishikawa K, Azuma H, Akaike M, Kato S and Matsumoto T. Androgen-androgen receptor system protects ag-

ainst angiotensin II-induced vascular remodeling. Endocrinology 2009; 150: 2857-2864.
[6] Meyer MR, Prossnitz ER and Barton M. The G protein-coupled estrogen receptor GPER/GPR-30 as a regulator of cardiovascular function. Vascul Pharmacol 2011; 55: 17-25.
[7] Navarro-Dorado J, Orensanz LM, Recio P, Bustamante S, Benedito S, Martinez AC, Garcia-Sacristan A, Prieto D and Hernandez M. Mechanisms involved in testosterone-induced vasodilatation in pig prostatic small arteries. Life Sci 2008; 83: 569-573.
[8] Mosaad YM, Shahin D, Elkholy AA, Mosbah A and Badawy W. CAG repeat length in androgen receptor gene and male infertility in Egyptian patients. Andrologia 2012; 44: 26-33.
[9] Huhtaniemi IT, Pye SR, Limer KL, Thomson W, O'Neill TW, Platt H, Payne D, John SL, Jiang M, Boonen S, Borghs H, Vanderschueren D, Adams JE, Ward KA, Bartfai G, Casanueva F, Finn JD, Forti G, Giwercman A, Han TS, Kula K, Lean ME, Pendleton N, Punab M, Silman AJ and Wu FC. Increased estrogen rather than decreased androgen action is associated with longer androgen receptor CAG repeats. J Clin Endocrinol Metab 2009; 94: 277-284.
[10] Rajender S, Singh L and Thangaraj K. Phenotypic heterogeneity of mutations in androgen receptor gene. Asian J Androl 2007; 9: 147-179.
[11] Laughlin GA, Goodell V and Barrett-Connor E. Extremes of endogenous testosterone are associated with increased risk of incident coronary events in older women. J Clin Endocrinol Metab 2010; 95: 740-747.