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Study of androgen and atherosclerosis in old-age male*

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Abstract: Objective: To observe the difference of androgen and inflammatory cytokines level in atherosclerosis and analyse their relations. Method: Both carotid arteries and arteries of lower extremity were subjected to ultrasonic examination by Doppler's method. Those with much atheromatous plaque formation were ranged into case group, and those with normal result formed control group. Total, free testosterone and estradiol were assayed by radioimmunoassay. C reactive protein (CRP) was assayed by nepheloturbidity. Tumor necrosis factor-α (TNF-α), Interleukin-6 (IL-6), Interleukin-8 (IL-8), Interleukin-10 (IL-10), Interleukin-18 (IL-18), soluble intercellular adhesion molecule-1 (sICAM-1) and soluble vascular cell adhesion molecule-1 (sVCAM-1) were assayed by ELISA. The mean difference between two groups and the correlation between free testosterone and cytokines were analysed. Results: Free testosterone was (6.337±3.371) pg/L in case group and (11.375±4.733) pg/L in control group, $P<0.01$. No differences were found in total testosterone and estradiol. CRP was (27.294±10.238) mg/L in case group and (12.843±6.318) mg/L in control group, $P<0.01$. IL-6 was (41.700±31.385) pg/L in case group and (25.396±20.772) pg/L in control group, $P<0.05$. IL-8 was (89.249±58.357) pg/L in case group and (67.873±31.227) pg/L in control group, $P<0.05$. sICAM-1 was (470.491±134.078) pg/L in case group and (368.487±97.183) pg/L in control group, $P<0.01$. sVCAM-1 was (537.808±213.172) pg/L in case group and (457.275±157.273) pg/L in control group, $P<0.05$. There were no differences in TNF-α, IL-10 and IL-18. Correlation analysis showed that FT (free testosterone) had negative correlation with CRP, IL-6 and sICAM-1. Among them FT had well correlation with CRP, correlation index was −0.678. Conclusion: Free testosterone was in negative correlation with atherosclerosis in old-age male. Free testosterone may have the role of anti-atherosclerosis, and this effect was not achieved by its transformation to estradiol. Low free testosterone level was followed by increased level of inflammatory cytokines. Low free testosterones coexist with inflammation and they both affect the process of atherosclerosis in old-age male.

Key words: Atherosclerosis, Old-age male, Androgen, Free testosterone, Inflammatory cytokine
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INTRODUCTION

The relation of androgen and atherosclerosis (AS) is always a hot spot in medical study. Most studies consider that high physiological level of androgen can protect the endothelium and inhibit AS. de Pergola *et al*.(2003) pointed out that hypotestosteronemia may accelerate the development of atherosclerosis and increase CHD (coronary heart disease) risk in obese men. Makinen *et al*.(2005) found that normal testosterone levels may offer protection against the development of atherosclerosis in middle-aged men. Inflammation obtained much attention as an important risk factor in AS and coronary heart disease. George *et al*.(2003) suggested that AS was a kind of chronic inflammatory disease and that autoimmunity played an important role in AS. Huittinen *et al*.(2003) suggested infection, immunity and inflammation coexist,

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and that they increased the risk of AS. Study of androgen and inflammation at the same time is few. In this work, we observed the difference of free testosterone (FT), total testosterone (TT), estradiol (E_2), CRP, TNF-α, IL-6, IL-8, IL-10, IL-18, sVCAM-1 and sICAM-1 in different degree atherosclerosis. We studied the action of androgen and inflammation in the process of atherosclerosis, and analysed simply the correlation of FT and inflammatory cytokines.

MATERIALS AND METHODS

Patients

1. Criterion of atherosclerosis

Both carotid arteries and arteries of lower extremity received ultrasonic examination by Doppler's method. Those with much atheromatous plaque formation formed the case group, and those with normal result formed the control group.

2. Selection of case group

Older-age males who underwent health examination in the VIP ward in our hospital from Nov. 2002 to June 2004 were included. Gonad function disorder, disease of prostate, atrial fibrillation, severe valve disfunction, obvious coronary heart disease, diseases of liver and kidney and cerebrum, tumour, severe hypertension (*BP* (blood pressure)>180/110 mmHg or complications) and diabetes mellitus were excluded. Taking of aspirin and Tatin (>15d) was not allowed and body temperature did not exceed 37.1 °C. Doppler's method was adopted to ensure people selected were correctly placed in the case group.

3. Selection of control group

The condition was the same as that for the selection of the case group. Doppler's method was adopted to make sure that people selected followed the criteria of the control group.

Methods

1. Sample collection and assay

Five milliliter venous blood was taken in the early morning before breakfast. No anticoagulant was used. The sample was centrifugated for 10 min at a speed of 2000 r/min. The supernatant was collected and frozen at −70 °C. Total testosterone (TT), free testosterone (FT) and estradiol (E_2) were assayed by radioimmunoassay (SN-682B Gamma counter), and the reagents were purchased from Biosource Europe SA (FT) in Belgium and from Orion Co. (TT and E_2) in Finland. CRP was assayed by nephroturbidity (Automatic Analyzer 7600-110), and the reagent was purchased from Kehua Co. in Shanghai (China). TNF-α, IL-6, IL-8, IL-10, IL-18, sVCAM-1 and sICAM-1 were assayed by ELISA (enzyme immunity autoanalyzer from Human Co. in Germany), and the reagent was purchased from Jingmei Co. in Shenzhen (China). All the samples were assayed twice at the same time, average values were adopted.

2. Body mass index (BMI) determination

This task was done by one nurse. Body height and body weight were measured. Body mass index (BMI)=weight (kg)/square of height (m^2).

3. Statistical analysis

All data were expressed in the form of $\overline{\chi} \pm s$, and software SPSS11.0 was used to analyse the data. *t* test by Pearson's method and correlation analysis of normal distribution by Spearman's method.

RESULTS

Comparison of case group and control group: The mean ages were (66.2±7.3) and (64.6±9.1) years, respectively. BMI were (24.8±3.2) and (25.3±2.9) kg/m^2, respectively. The absence of apparent differences in age and BMI showed that the different level of androgen was not caused by differences in age or weight. The hypertension patients mainly took drugs such as ACEI, β-blocker and calcium antagonists to control their blood pressure. Because the hypertension numbers were similar in the two groups, we could omit the effect of drugs such as ACEI (angiotensine converting enzyme inhibitors) and β-blocker. For the same reason, we could neglect the effect caused by smoking. The body temperature and blood routine examination results were all in normal range, so we could preclude the possibility of CRP level increasing due to obvious general infection. And we also could preclude the error caused by drugs such as aspirin and statins (Table 1).

Free testosterone in the case group was obviously lower than that in the control group, while no differences were found regarding total testosterone and estradiol. Inflammatory cytokines that we selected, such as CRP, IL-6, IL-8, sVCAM-1 and sI-

CAM-1 in the case group were obviously higher than those in the control group, while there were no significant difference in TNF-α, IL-10 and IL-18 (Table 2). Correlation analysis showed that free testosterone had negative correlation with CRP, IL-6, and sICAM-1. The correlation index of CRP was is −0.678 (Table 3).

DISCUSSION

Androgen is the generic name of C-19 steroid substance. The main active component of androgen in the male is testosterone. About 80% of testosterone in plasma is combined with sex hormone binding globulin, a small part of it is combined with albumin and only 2% is in free form, namely free testosterone (FT). The latter two kinds of testosterone have bioaction, and only FT has regulatory action in the body. Testosterone has close relationship with age regarding generation and secretion, it is secreted much after male adolescence and reaches the highest level in 20~29 years old males. Then its level decreases with age. After 50 years old its level decreases rapidly and FT declines most obviously (Lv and Wu, 2001).

The specific relation between androgen and atherosclerosis is not clear up to now. Most clinical studies and animal experiments showed that normal level testosterone can dilate the aorta of mouse and the coronary artery of human and animals (canine and rabbit), and increase blood stream velocity (Webb *et al*., 1999). Microdosage of testosterone can inhibit the adhesion of monocytes to the vascular wall, and inhibit the proliferation of vascular smooth muscle cells (Somjen *et al*., 1998). Taking testosterone can relieve symptom of angina pectoris in older-age male patients with coronary heart disease and extend the interval time of exercise evoking myocardial ischemia (Webb *et al*., 1999; English *et al*., 2000a). In this study, FT in the case group was obviously lower than

Table 1 The basic characteristics of case group and control group

	Case group (n=38)	Control group (n=32)
Age (year)	66.2±7.3	64.6±9.1
BMI (kg/m^2)	24.8±3.2	25.3±2.9
Hypertension	20	17
Years<5	8	9
Years between 5~10	7	6
Years>10	5	2
Smoking information		
Never smoke	12	14
Smoke occasionally	15	11
Smoking index number<100	7	5
Smoking index number>100	4	2

Table 3 Correlation analysis of free FT and cytokines

	Correlation index	P
FT and TT	0.157	0.536
FT and E_2	−0.216	0.644
FT and CRP	−0.678	0.022
FT and TNF-α	−0.142	0.475
FT and IL-6	−0.427	0.024
FT and IL-8	−0.206	0.732
FT and IL-10	0.221	0.356
FT and IL-18	0.181	0.087
FT and sICAM-1	−0.368	0.035
FT and sVCAM-1	0.169	0.538

Table 2 Comparison of sex hormone and inflammatory cytokines between two groups

	Case group	Control group	P
TT (nmol/L)	15.128±3.276	14.867±6.289	0.350
FT (pg/L)	6.337±3.371	11.375±4.733	0.000
E_2 (pmol/L)	71.059±27.371	62.374±31.078	0.104
CRP (mg/L)	27.294±10.238	12.843±6.318	0.000
TNF-α (pg/L)	510.684±179.674	495.566±203.545	0.152
IL-6 (pg/L)	41.700±31.385	25.396±20.772	0.037
IL-8 (pg/L)	89.249±58.357	67.873±31.227	0.029
IL-10 (pg/L)	65.235±37.836	70.348±52.378	0.198
IL-18 (pg/L)	71.754±36.301	67.628±41.852	0.105
sVCAM-1 (pg/L)	537.808±213.172	457.275±157.273	0.013
sICAM-1 (pg/L)	470.491±134.078	368.487±97.183	0.007

Normal level (according to Test Kit): TT: 8.2~34.6 nmol/L; FT: 11.5~42.5 pg/L; E_2: 0~228 pmol/L

that in the control group, while there was no significant difference of TT in the two groups. We also found that TT value in the two groups was in normal range set by the kit we adopted. FT value in the case group was obviously lower than the lower limit of normal range of the kit, while FT value in the control group was almost in the normal range of the kit. All these phenomenon showed that androgen might have beneficial effect on the process of atherosclerosis. And the key substance was free testosterone instead of the total testosterone. Our viewpoint was in line with that of English *et al*.(2000b). Lastly we found that there was no obvious difference of E_2 in the two groups and that FT had no obvious correlation with E_2. We postulated that instead of changing to E_2, FT fulfilled its action of inhibiting the progress of AS with a special mechanism (English *et al*., 2000b; Giuseppe *et al*., 1999). But this mechanism was not very clear to us.

Ever since Ross *et al*.(1974) proposed the theory of "damage response" in the process of atherosclerosis, more and more investigations suggested the process of atherosclerosis is actually an inflammatory reaction after vessel injury. Inflammation exists in the whole process of atherosclerosis. Ross (1999) emphasized in the New England Journal of Medicine that atherosclerosis was a process of chronic inflammation. Signorelli *et al*.(2003) discovered that inflammatory cytokines rose after a treadmill test in patients with peripheral arterial disease. So he proposed that white blood cell activation is characteristic of systemic atherosclerosis and those inflammation markers, such as IL-6, TNF-α, sVCAM-1, sICAM-1 and selectins, increase in conditions of hemodynamic stress. Li (2004) considered that inflammation plays an important role in AS and in the occurrence and development of atherosclerosis's complications. In this study, CRP in the case group was obviously higher than that in the control group, and that CRP in the control group also was higher than the normal level. This is in line with the patient's condition in two groups, and supports the result of Blake and Ridker (2001)'s study. Our not finding obvious difference of TNF-α between the two groups did not accord with others' experiment results. As for the four interleukin markers, IL-6 and IL-8 levels in the case group were significantly higher than those in the control group, but no difference were found in IL-10 and IL-18 levels. Guan *et al*.(1998) considered that IL-6 and IL-8 can promote the phagocytosis of macrophage to low density lipoprotein (LDL), accelerate the deposition of lipid and activate macrophage to secrete monocyte chemoattractant protein (MCP) and also can promote the proliferation of smooth muscle cell by autocrine. Most people consider that IL-10 is an anti-inflammatory marker. Waehre *et al*.(2002) proposed that IL-10 may have beneficial effects on mechanisms that are important in plaque rupture and thrombus formation. IL-18 is a proinflammatory cytokine. Mallat *et al*.(2002) discovered that IL-18's level rise in acute coronary syndrome patients, and that the concentration of IL-18 had negative correlation with the left ventricle's ejection function. There were obvious difference between sICAM-1 and sVCAM-1, and this was in line with Chen *et al*.(2001)'s study result. In brief, our experiment showed that inflammation exists in the early period of AS when serious consequences such as myocardial infarction and cerebral infarction are not present, and that some inflammatory cytokines' level has relation with the degree of AS.

Study on the correlation between androgen and inflammation is not adequate. In our experiment, we found that FT had obvious negative correlation with CRP, IL-6 and sICAM-1, especially the CRP. We suspect that low level of FT might be associated with a certain degree of inflammation. The chronic inflammatory reaction is more obvious in low FT people. Compared with normal level FT population, those with low level of FT has a more unstable internal environment, and their degree of AS is much more serious. A recent study of Malkin *et al*.(2004) support our suspicion. After therapeutic supplement of androgen, they found that the level of TNF-α and IL-1β decreased obviously and that the level of IL-10 increased obviously. So Malkin *et al*.(2004) suggested testosterone replacement to shift the cytokine balance to a state of reduced inflammation.

Study of androgen, inflammation and atherosclerosis is not sufficiently thorough up to now and many problems are not clear to us. Further study of the relation between them can help us understand better the background of coronary heart disease and discover new therapeutic methods.

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