Angiogenic activity of human serum – dependence on sex

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Abstract

In our previous study we presented age-dependence of human serum angiogenic activity, evaluated in vivo after injection into mouse skin. We have found negative correlation between the age of serum donor and in vivo activity of serum, negative correlation between age and VEGF serum concentration and positive correlation between age and IL-18 level.

The aim of the present study was to evaluate in vivo angiogenic activity as well as concentration of VEGF and antithrombin-III in sera collected from 30 healthy women and 30 healthy men of various age (20 – 80 years old), and to analyze whether some relations exist between studied parameters and gender. In men, we have found : negative correlation between the age and serum in vivo activity, negative correlation between the age and serum VEGF concentration, positive correlation between the age and serum AT-III activity. In women, no such relations were observed.

Key words: *healthy people, sex, age, sera, angiogenesis, VEGF, AT-III.*

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Introduction

Angiogenesis, the growth of new blood vessels from the preexistent ones, is required for a variety of normal proliferative processes. Human serum contains substantial amounts of various angiostimulatory and angioinhibitory factors. Recently, we have reported age-dependence of human serum angiogenic activity [1]. Sera of older people were less effective in inducing neovascular response in mice cutaneous test, and contained lower concentration of important proangiogenic cytokine, vascular endothelial growth factor (VEGF), in comparison to the sera of those below the age of 50. In this paper we concentrate on sex differences in *in vivo* angiogenic activity of human serum. We also describe sex differences in the level of VEGF and in the activity of endogenous angiogenesis inhibitor, antithrombin-III (AT-III), in sera collected from men and women of various ages.

Material and Methods

Sera were collected from 60 people (30 men and 30 women), 20-80 years old, without immunological, inflammatory and neoplastic disorders. Informed consent was obtained from each person and the study was approved by local Ethics Committee.

Serum – (SIA) – induced cutaneous angiogenesis assay

Cutaneous angiogenesis assay was performed according to Sidky and Auerbach method [2] with own modifications [3, 4]. Studies have been performed in 2-month old, female inbred Balb/c mice. Mice have been of local laboratory breed, weighing ca 20 g each. The sera of healthy subjects were injected intradermally (0.05 ml per one injection, 3-6 injections per mouse) into regionally shaved, anaesthetized with chloral hydrate (POCH, Poland) groups of 3 or more mice. In order to facilitate the localization of injection sites

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later on, all injected samples were coloured with 0.1% of trypan blue. After 72 hours mice were killed with lethal dose of Morbital (Biowet, Poland). All newly formed blood vessels were identified and counted in dissection microscope in 1/3 central area of microscopic field, at 6 × magnification. Identification was based on the fact that newly-formed blood vessels differ from background vasculature by their small size, tortuosity and divarications. Mean number of newly-formed blood vessels was calculated from a dozen or so separate readings and designated as "angiogenic activity" of tested sample.

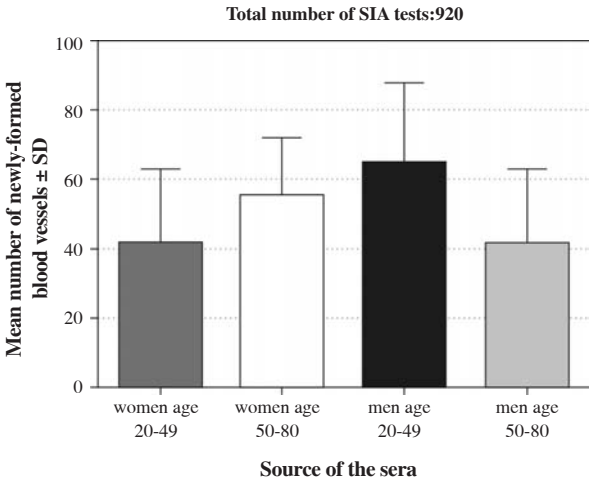


Fig. 1. Neovascular reaction induced in mice skin after injection of sera collected from 30 female and 30 male healthy human volunteers

Experiments were approved and supervised by the Local Ethics Committee.

Measurement of VEGF concentration

Cytokine levels in examined sera were determined using sandwich ELISA kits (R&D Systems, USA) for human VEGF, according to the producer instructions. Optical density was measured at 450 nm using spectrophotometric reader Elx800 (Biotek Instruments, Inc., USA). Cytokine concentration was expressed as pg/ml.

Measurement of AT-III activity

AT-III levels in plasma were assessed by the kinetic method using commercially available reagents (Dade Behring Marburg, Germany) according to manufacturer's instructions. Results were expressed as % of reference normal value.

Table 1. One-way ANOVA results

Number of groups	4			
F	4.583			
R squared	0.1971			
Bartlett's test for equal variances				
Bartlett's statistic (corrected)	1.480			
p value	0.6869			
p value summary	ns			
Do the variances differ signif. (p < 0.05)	No			
ANOVA Table	SS	df	MS	
Treatment (between columns)	6025	3	2008	
Residual (within columns)	24540	56	438.2	
Total	30560	59		
Bonferroni's Multiple Comparison Test	Mean Diff.	t	Significant? p<0.05?	Summary
Women age 20-49 vs. Women age 50-80	-13.61	1.777	No	ns
Women age 20-49 vs. Men age 20-49	-23.03	3.242	Yes	*
Women age 20-49 vs. Men age 50-80	-0.3100	0.03781	No	ns
Women age 50-80 vs. Men age 20-49	-9.420	1.278	No	ns
Women age 50-80 vs. Men age 50-80	13.30	1.577	No	ns
Men age 20-49 vs. Men age 50-80	22.72	2.865	Yes	*

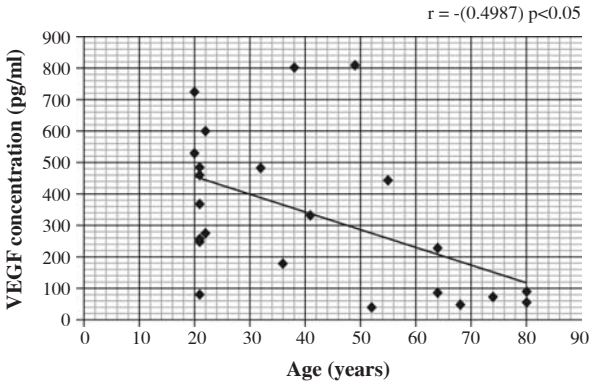


Fig. 2. Age-dependence of VEGF concentration in healthy men sera (negative correlation between the age of serum donor and VEGF serum concentration)

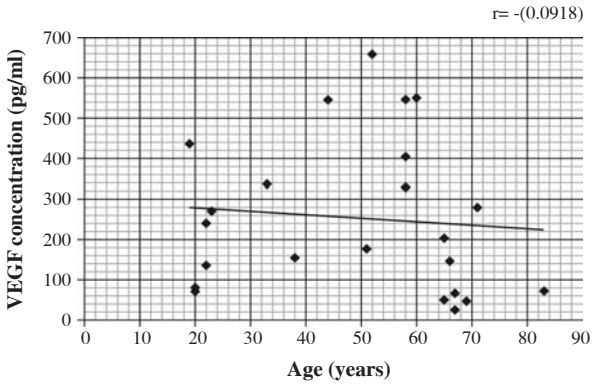
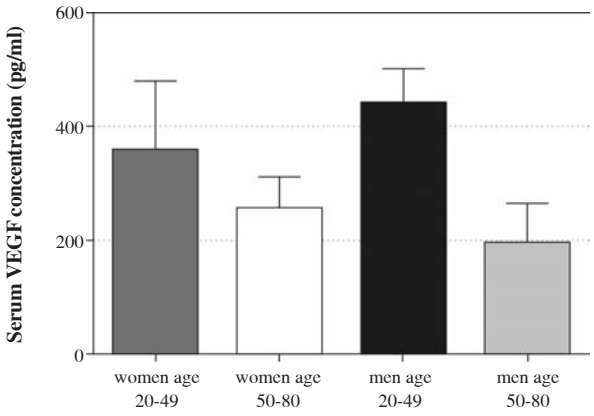
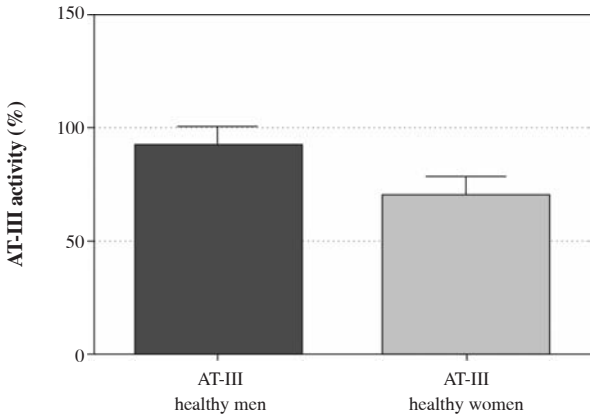


Fig. 3. Lack of correlation between the age of women and VEGF content of their sera



Kruskal-Wallis test	
p value	0.0281
Exact or approximate p value?	Gaussian Approximation
p value summary	*
Do the medians vary signif. (p < 0.05)	Yes
Number of groups	4
Kruskal-Wallis statistic	9.091

Fig. 4. Serum VEGF concentration of healthy women and healthy men



Mann Whitney test	
p value	0.0433
Exact or approximate p value?	Gaussian Approximation
p value summary	*
Are medians signif. different? (p < 0.05)	Yes
One- or two-tailed p value?	Two-tailed
Sum of ranks in column A, B	334.5. 193.5
Mann-Whitney U	73.50

Fig. 5. Antithrombin-III activity in sera of healthy men and women

Statistical analysis

Statistical evaluation of the results was done by: Pearson's correlation test, ANOVA, Mann-Whitney and Kruskal-Wallis tests, Bonferroni Multiple Comparison Test (GraphPad Prism software).

Results

The results of angiogenic activity of sera from men and women in age groups 20-49 and 50-80 are shown on Fig. 1. Young men sera presented higher activity than sera of both women groups, and this activity decreased with age- as low

Fig. 6. Positive correlation between the age of healthy men and AT-III activity of their sera

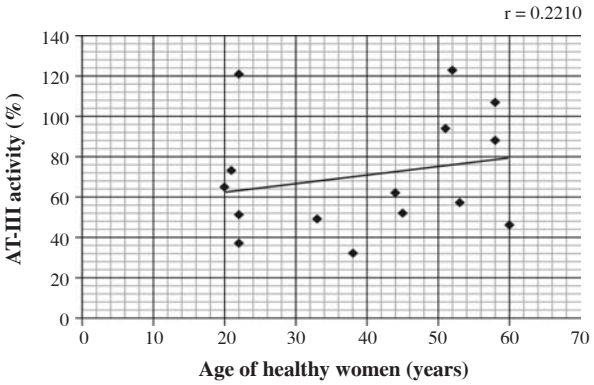


Fig. 7. No correlation between the age of healthy women and AT-III activity of their sera

as to the values characteristic for women (Table 1). In men, we have found negative correlation between the age and serum VEGF concentration (Fig. 2). In women, no such relationship was observed (Fig. 3). VEGF content was the highest in young men group and significantly decreased with age (Fig. 4). AT-III serum activity was higher in men than in women (Fig. 5)Positive correlation between the age and AT-III activity was seen in men only (Figs. 6 and 7).

Discussion

The results obtained in this study reveal differences between some parameters of angiogenic activity of sera collected from healthy men and healthy women. Malamitsi et al. [5] reported controversial results concerning VEGF – they observed higher serum VEGF levels in females than in males. However, their study group included fetuses, neonates, children, and pregnant women, what may explain controversy. The same authors [6] in other paper reported no difference between males and females in respect to the serum levels of basic fibroblast growth factor (bFGF), other important proangiogenic cytokine. Bruserud et al. in their study performed on young athletes and elderly individuals discovered, that serum levels of some pro-angiogenic factors (bFGF, angiogenin and leptin) and of one antiangiogenic (endostatin) were higher in elderly individuals [7].

There is some controversy concerning the effect of testosterone on VEGF production and vascular reactivity. Ray et al. [8] reported deleterious effects of endogenous and exogenous testosterone on mesenchymal stem cell VEGF production in vitro. Shao et al. , however, reported in rats with prostatic hypertrophy that testosterone increased microvessel density and multiplication of vascular endothelial cells [9]. According to Liu et al. [10] testosterone can stimulate prostatic growth probably by upregulating the protein expression of VEGF and FLK-1. Jones et al. reported positive influence of testosterone upon vascular reactivity [11]. In the present study we observed the highest serum angiogenic activity and VEGF level in the group of men below 50 years old, presumably being good testosterone producers. So, it may partly explain decrease of serum angiogenic activity and VEGF content in older men. We also observed in men another age-dependence: positive correlation with antithrombin level.

Antithrombin is a member of the serpin family of proteins and functions as an inhibitor of thrombin. The cleaved and latent forms of antithrombin also inhibit angiogenesis and tumor growth in vivo, acting selectively upon endothelial cells [12]. It was described, that antiangiogenic antithrombin blocks the heparan sulfate-dependent binding of proangiogenic growth factors to their endothelial cell receptors [13]. Then, the other explanation for decreasing angiogenic activity in elderly men might be the increase of AT-III activity in their sera.

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