[Environmental Health and Preventive Medicine 4, 71-74, July, 1999]

Original Article

Soluble Intercellular Adhesion Molecule-1 and Vascular Cell Adhesion Molecule-1 Concentrations, and Leukocyte Count in Smokers

Tsukasa NOGUCHI

Uegahara Hospital, Nishinomiya, Hyogo

Abstract

The leukocyte count and concentrations of sICAM-1 and sVCAM-1 in smokers were investigated. The subjects were 96 persons (31 smokers and 65 nonsmokers) hospitalized for a complete health checkup examination. There were no differences between the two groups for background factors (age (nonsmokers, 56.2 ± 9.2 vs smokers, 52.6 ± 11.3)gender ratio (m/f)(nonsmokers, 47/18 vs smokers, 24/7) and drinker ratio (+/-) (nonsmokers, 45/20 vs smokers, 26/5)). For smokers, the average number of cigarettes smoked daily, smoking period(ys) and Brinkman Index were 21.4 ± 10.4, 26.2 ± 14.5 and 584.2 ± 476.9, respectively. In smokers, total leukocyte count (/ μ l) (6371.0 ± 1303.4 vs 5063.1 ± 1279.1, p<0.0001), and sICAM-1 concentration (ng/ml) (202.6 ± 64.0 vs 163.0 ± 60.8, p<0.01) were higher than those in nonsmokers. No significant difference in the sVCAM-1 concentration was shown between the two groups. Items showing large correlation coefficients with the number of cigarettes smoked daily were the total leukocyte count (r = 0.45, p<0.0001) and sICAM-1 (r = 0.36, p<0.0001). In smokers, sICAM-1 showed correlation with the total leukocyte count (r = 0.42, p<0.05) . We conclude that the leukocyte count and sICAM-1 concentration in healthy smokers are higher than those in nonsmokers.

Key words: smoker, leukocyte, soluble, intercellular adhesion molecule-1, vascular cell adhesion molecule-1

Introduction

Smoking leads to chronic inflammatory changes and intercellular adhesion molecule-1 (ICAM-1) is expressed on the surface of vascular endothelial cells[1] in the lung. Neutrophils accumulate in the lung[2),3)] and express CD11/CD18[4)]. Free radicals or elastase released from neutrophils disturb the lung[5),6),7),8)]. Moreover, smoking has also been reported to increase the number of leukocytes in peripheral blood [9),10),11)]. Cigarette smoke condensate (CSC) induces the expression of CD11b on the surface of monocytes and ICAM-1 on endothelial cells[12)]. Recently, soluble forms of adhesion molecules were found. Soluble ICAM-1 (sICAM-1) and soluble vascular cell adhesion molecule-1 (sVCAM-1), hydrolytic products of the extracellular domain of cell membrane glycoprotein[13)], can now be measured [14),15)]. The concentration of these soluble adhesion molecules is believed to reflect the change of ICAM-1 or VCAM-1 expression on the cell surface. We decided to examine the concentrations of sICAM-1 and sVCAM-1 in smokers, and their relationship with leukocyte count.

Received Jun.3 1998/Accepted Mar.3 1999
Reprint requests to: Tsukasa NOGIUCHI,
Uegahara Hosoital, 1-85 Uegaharajuban-cho, Nishinomiya, Hyogo, 662-0884, Japan.
TEL: +81(798)52-2001
FAX: +81(798)52-2565

Materials and Methods

The subjects were 96 persons (71 males and 25 females) hospitalized for a complete health checkup examination. Patients who had fever, angina, increased erythrocyte sedimentation rate, cancer, collagen disease, angitis, hepatitis or any other diseases were excluded. The number of cigarettes smoked daily, smoking period and any drinking habit were obtained from a questionnaire. The Brinkman Index was calculated from (the number of cigarettes smoked daily) x (smoking period). Patient's blood was collected before breakfast (at about 9:00 a.m.). Examination items included blood pressure, electrocardiography, urinalysis, stool occult blood, complete blood cell count, erythrocyte sedimentation rate, biochemical examination (liver function, uric acid (UA), glycohemoglobin A1c (HbA1c), total cholesterol (TCHOL), triglyceride (TG), high density lipoprotein cholesterol (HDL) , 75g-oral glucose tolerance test (OGTT), chest X-ray, abdominal ultrasonography and gastrography.

Measurement of sICAM-1 and sVCAM-1 concentrations

As described elsewhere, sICAM-1 concentrations in sera were measured by enzyme linked immunosorbent assay (ELISA) using two types of monoclonal antibodies, HA 58 and MoAb CL207[14]. sVCAM-1 concentrations in sera were measured by human VCAM-1 · ELISA kit (R&D Systems, Abingdon, UK) [15].

Difference of mean values between smoking and non-smoking groups

Age, gender ratio, height, body weight, body mass index (BMI), the number of cigarettes smoked daily, smoking period, Brinkman Index and drinking habit were examined as background factors. The other examination items were as follows: systolic blood pressure (SBP), diastolic blood pressure (DBP), UA, fasting blood sugar (FBS), TCHOL, TG, HDL, total leukocyte count, sICAM-1 and sVCAM-1.

Chi-square test was used to detect the difference of the ratio of genders or drinkers between smokers and non-smokers. The Wilcoxon rank sum test was used to detect the difference of mean values of each examination item between the two groups.

Correlation between the number of cigarettes and leukocytes or sICAM-1 or sVCAM-1

Spearmann's correlation coefficient was used to investigate the correlation between the number of cigarettes smoked daily, smoking period or Brinkman Index and leukocytes, sICAM-1 or sVCAM-1.

Correlation between concentration of sICAM-1 and leukocytes

Spearmann's correlation coefficient was used to investigate the correlation between the sICAM-1 concentration and leukocytes.

Results

Difference of mean values between smoking group (n=31) vs non-smoking group (n=65)

There were no differences between the two groups for age, ratio of gender, height, BW, BMI, and ratio of drinkers (Table 1). In smokers, total leukocyte count and sICAM-1 concentration were higher than those in nonsmokers (Table 2). No difference in sVCAM-1 concentration was found between the two groups.

Correlation between the number of cigarettes, smoking period or Brinkman Index and leukocytes, sICAM-1 or sVCAM-1 (Table 3)

Table 1 Comparison of background factors between smokers and nonsmokers

	Nonsmoker	Smoker	P
Age (yr)	56.2 ± 9.2	52.6 ± 11.3	NS
Gender (m/f)	47 / 18	24 / 7	NS
Height (cm)	161.4 ± 8.2	165.2 ± 7.1	NS
BW (kg)	61.5 ±11.5	63.0 ± 9.7	NS
BMI (kg/m2)	22.7 ± 3.3	23.0 ± 2.9	NS
Cigarettes (no./day)	0	21.4 ± 10.4	n/a
Smoking period (yr)	0	26.2 ± 14.5	n/a
Brinkman Index	0	584.2 ±476.9	n/a
Alcohol (+/-)	45 / 20	26 / 5	NS

BW: body weight, BMI: body mass index, m: male, f: female.
Brinkman index was calculated from (the number of cigarettes smoked daily) x (smoking period). NS indicates not statistically significant. n/a indicates nor applicable. All values are presented as mean ± SD, except gender and alcohol.

For the relationship with total leukocyte count or sICAM-1, the item with the largest coefficients of correlation was the number of cigarettes smoked daily. No correlation was found between the sVCAM-1 concentration and the number of cigarettes smoked daily, smoking period or Brinkman Index.

Correlation between concentration of sICAM-1 and leukocytes (Table 4)

In smokers, sICAM-1 showed a correlation with the total leukocyte count. There was no correlation between sVCAM-1 and leukocyte count.

Discussion

In smokers, the total leukocyte count was higher than that in nonsmokers. As we had excluded patients who had acute inflammatory diseases, this leukocytosis may have been caused by constituents of cigarette smoke. In smokers, alveolar macrophages (AM) accumulate in the respiratory tract[16]. Macrophages obtained from a randomly selected subset of smokers but not of nonsmokers exhibited increased production of M-CSF in response to lipopolysaccharide (LPS)[17]. Bronchial epithelial cells markedly increase the survival of human neutrophils in vitro via the release of granulocyte-colony stimulating factor (G-CSF) and granulocyte-macrophage colony stimulating factor (GM-CSF)[18]. This cytokine release from lung tissue or the immune system in the lung may be related to the increase of neutrophils in peripheral blood. Other factors may also contribute to neutrophil

Table 2 Comparison of examinnation items between smokers and nonsmokers

		Nonsmoker	Smoker	P value
SBP	(mmHg)	131.0 ± 16.6	126.8 ± 17.8	NS
DBP	(mmHg)	85.8 ± 11.0	80.1 ± 10.2	NS
UA	(mg/dl)	5.6 ± 1.5	5.8 ± 1.3	NS
FBS	(mg/dl)	107.6 ± 12.7	112.0 ± 29.4	NS
TCHOL	(mg/dl)	211.0 ± 36.7	204.2 ± 31.5	NS
TG	(mg/dl)	120.7 ± 55.9	146.9 ± 76.7	NS
HDL	(mg/dl)	57.4 ± 12.2	53.2 ± 15.7	NS
Leukocytes	(/μ l)	5063.1 ± 1279.1	6371.0 ± 1303.4	<0.0001
sICAM-1	(ng/ml)	163.0 ± 60.8	202.6 ± 64.0	<0.01
sVCAM-1	(ng/ml)	683.4 ± 115.6	687.7 ± 145.0	NS

SBP: systolic blood pressure, DBP: diastolic biood pressure,
UA: uric acid, FBS: fasting blood sugar,TCHOL: total cholesterol, TG: triglyceride, HDL: high density lipoprotein-cholesterol, sICAM-1: soluble vascular cell adhesion molecule-1, sVCAM-1: soluble vascular celladhesion molecule-1. NS indicates not statistically significant.
All values are presented as mean ± SD.

Table 3 Correlations of the number ofcigarettes, smoking Period and Brinkman Index with leukocytes, sICAM-1 and SVCAM-1.

	No. / day	Period(yr)	Brinkman Index
Leukocytes	**** 0.45	*** 0.36	*** 0.38
SICAM-1	**** 0.36	** 0.28	** 0.30
SVCAM-1	0.01	-0.01	-0.03

All values are correlation coefficients
P value for statistical significance: **, <0.01; ***, <0.001;****, <0.0001

Table 4 Correlation between sICAM-1 and leukocytes

	Nonsmoker	Smoker	Total
Correlation Coefficient	0.17	0.42	0.32
P value	0.40	<0.05	<0.01

increase. For example, the concentration of serum catecholamine increases with smoking[19].

Catecholamine, similar to corticosteroid, is one of the factors which increases neutrophils in peripheral blood[20]. There are also some components of cigarette smoke which may directly cause an increase of neutrophils in peripheral blood. For instance, nicotine prolongs neutrophil survival in vitro by the suppression of apoptosis[21]. As for the change in lymphocytes, the increase of natural killer (NK) cells has been observed as an acute effect of smoking[22]. Tobacco-glycoprotein (TGP) is a potent immunostimulator that has been isolated from cured tobacco leaves and CSC. It acts as a mitogen in vitro, stimulating proliferation of human peripheral blood T-lymphocytes, but it also stimulates B-cell differentiation and immunoglobulin production[23),24]. These observations may be related to the increase of leukocytes.

The serum concentration of sICAM-1 in smokers was higher than that in nonsmokers in our investigation. As ICAM-1 can be expressed on leukocytes, epithelial cells, fibroblasts and keratinocytes as well as endothelial cells, the cell type or organ from which sICAM-1 separates is not known. However, current smokers with chronic bronchitis display an increased number of macrophages, very late antigen (VLA)-1-positive cells, and ICAM-1-positive vessels in the lung compared with normal nonsmoking subjects[1]. Therefore, chronic bronchitis may be a cause of sICAM-1 increase by smoking.

Smoking causes accumulation of cells, especially AM, within the alveolar space[16]. Adherence of AM to human umbilical vein endothelial cells (HUVEC) was higher for smokers than for nonsmokers. The increased binding of smoker AM to endothelial cells could be inhibited by treating the HUVEC with monoclonal antibody against ICAM-1[25]. Thus, the adherence of AM to endothelial cells in the lung is thought to be supported by ICAM-1 molecules. This may be one of the reasons for the sICAM-1 increase observed in smokers. Further investigation is needed to clarify these mechanisms.

In normal lungs, ICAM-1 is weakly expressed on endothelial cells, while VCAM-1 is not expressed[26]. VCAM-1 is expressed on the surface of activated vascular endothelial cells[27),28] and CSC induces its expression on endothelial cells[12]. Smoking may then cause VCAM-1 to be expressed on the surface of vascular endothelial cells in the lung or other organs. However, in our investigation, the sVCAM-1 concentration did not differ between smokers and nonsmokers. Thus, the expressibility of VCAM-1 by smoking may be weaker than that of ICAM-1. This may be related to the difference of molecular mechanisms (activation of protein kinase C, signaling pathways, binding of NF-kappa B to specific DNA sequences).

Acknowledgments

We thank Judy Noguchi for her helpful editing.

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