Plasma Concentrations of Reputed Tumor-associated Soluble CD44 Isoforms (v5 and v6) in Smokers Are Dose Related and Decline on Smoking Cessation

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Abstract

There is some evidence to suggest that smoking may affect circulating levels of CD44 (sCD44) molecules. Therefore, we investigated the effect of smoking on the circulating level of sCD44 by comparing the change in total sCD44, sCD44v5, and sCD44v6 concentrations over 1 year in a group of people who quit smoking (n = 30) and a control group of people who continued to smoke (n = 30). Smoking status and compliance were monitored by analysis of plasma cotinine and expired CO levels and also by self-reported tobacco use. We show a dose-dependent relationship between smoke intake and baseline plasma concentrations of reputed tumor-associated CD44 variant isoforms (sCD44v5 and sCD44v6) in smokers (n = 60). There was a significant decline in the level of both sCD44v5 and sCD44v6 in quitters as compared with continuing smokers [−13.2 (95% confidence interval, −7.6 to −18.8; P < 0.001) and −62.2 ng/ml (95% confidence interval, −33.9 to −90.6; P < 0.001), respectively], but not in the total sCD44 concentration. These results show that the increased concentrations of sCD44v5 and sCD44v6 in smokers are dose related and reversible and suggest that the attributed diagnostic specificity and prognostic value of sCD44 molecules in malignant and inflammatory disease may be affected by smoking status.

Introduction

Aberrant expression of variant CD44 isoforms has been identified in certain premalignant lesions and carcinomas and has been associated with tumor growth, metastatic potential, and poor prognosis (1–8). Furthermore, circulating levels of total sCD44 and specific soluble CD44 isoforms have been shown to correlate with tumor metastasis in some malignancies, including non-Hodgkin’s lymphoma and breast, gastric, and colon carcinomas (9–13). The level of soluble CD44 is also known to be higher in the body fluids of subjects with particular inflammatory conditions, such as rheumatoid arthritis (14, 15), pouchitis and colitis (16), and bronchitis (17). Recent evidence suggests that tobacco smoking might lead to increased concentrations of circulating total CD44 (sCD44) and specific tumor-associated sCD44 isoforms (18–20). Therefore, we examined the influence of smoking and smoking cessation on plasma levels of total CD44 and the reputed inflammation- and tumor-associated isoforms sCD44v6 and sCD44v5 (10, 13, 15, 19–26). Because it has been suggested that circulating levels of sCD44v5 and sCD44v6 may have prognostic significance in specific malignancies (13, 22–24, 26), this issue is of primary importance.

Materials and Methods

Subjects. Thirty subjects known to have stopped smoking completely for 52 weeks (quitters) and a control group of 30 subjects who continued to smoke (continuing smokers) were sampled retrospectively from the database of a large smoking cessation treatment trial (27). Pulse and blood pressure were taken at baseline. Otherwise, subjects attending the smoking cessation clinics were not examined medically but were apparently fit and healthy. Those with a recent history of heart or malignant diseases were excluded. As described previously, quitters were those who reported not smoking at all treatment follow-ups (weeks 2, 4, 8, 12, 22, 26, and 52) and registered as nonsmokers on analysis of expired air CO concentration (<10 ppm) at each visit and on analysis of plasma cotinine concentration (<15 ng/ml) at 52 weeks, whereas continuing smokers were those who reported having failed to remain abstinent from tobacco smoking throughout the year and registered >9 ppm carbon monoxide in expired breath and a plasma cotinine concentration of ≥50 ng/ml at 2 weeks (27). Plasma was sampled at baseline, when all 60 subjects were smoking normally just prior to their quit attempt, and 52 weeks later, when 30 subjects had been abstinent from smoking for 1 year.

Measurement of Plasma Levels of Total sCD44, sCD44v5, and sCD44v6. sCD44 concentrations were determined blind to smoking status at 52 weeks by ELISA [total sCD44, monoclonal antibody BU52 (BU52 recognizes the standard/hematopoietic form of sCD44); R&D Systems, Abingdon, United Kingdom]; sCD44v5 and v6, monoclonal antibodies VFF8 and VFF18, respectively (Bender Medsystems, Vienna, Austria)]. Each assay was performed in duplicate. Absorbance was measured at 450 nm using a Dynatech MR 700 automated microplate reader.
reader. The concentration of sCD44 was calculated from a calibration curve using duplicate standard concentrations of CD44 standard, CD44v5, and CD44v6.

**Statistical Evaluation.** The relation between baseline measures of smoke intake (expired CO, plasma cotinine, and daily cigarette consumption) and concentrations of sCD44 molecules was modeled by bivariate linear regression. The response due to smoking cessation was measured as the change in sCD44 concentration between baseline and 1 year. The t-test was used to examine the difference in change between quitters and continuing smokers.

**Results**

There were no significant differences in mean age [44 (SD = 10) and 43 (SD = 8) years], gender (15 females and 14 females), or smoke intake at baseline [mean cigarette consumption, 26 (SD = 10) and 25 (SD = 7) cigarettes/day] between the group who subsequently succeeded in quitting and those who failed to quit, respectively. Pulse and blood pressure were taken at baseline, but neither correlated with CO or other measures of smoke intake, CD44v5/CD44v6, or cessation outcome.

The change in sCD44v5 and sCD44v6 concentrations over 1 year from baseline in quitters and continuing smokers is presented in Fig. 1. The reductions in both sCD44v5 and sCD44v6 levels were significant when analyzed in the quitters alone at 52 weeks compared to baseline (−16.2 and −79.4 ng/ml respectively; both P < 0.001) and when comparing quitters with continuing smokers [mean difference in change in sCD44v5 = −13.2 ng/ml (95% confidence interval, −7.6 to −18.8); mean difference in change in sCD44v6 = −62.2 ng/ml (95% confidence interval, −33.9 to −90.6); both P < 0.001]. The difference in the change in total CD44 levels over 52 weeks in the quitters (−19.9 ng/ml) compared with the change in continuing smokers (−11.5 ng/ml) was not significant (P = 0.380).

At baseline, there was no significant difference in sCD44, sCD44v5, or sCD44v6 levels between those who subsequently quit and those who continued smoking. In regression analyses at baseline, expired CO showed a linear dose-response relationship to sCD44v5 and sCD44v6 concentration. The increase in sCD44v5 concentration was estimated to be 0.95 ng/ml per 1 ppm expired CO [t(1,58) = 3.0; r = 0.370; P = 0.004]. The increase in sCD44v6 concentration was estimated to be 4.2 ng/ml per 1 ppm expired CO [t(1,58) = 4.3; r = 0.490; P < 0.001]. Expired CO also correlated with the plasma concentrations of CD44v5 (r = 0.27) and CD44v6 (r = 0.35) at 52 weeks in the continuing smokers. There was a significant correlation between cotinine and CD44v6 at baseline (r = 0.32; P = 0.01), but not between cotinine and CD44v5 (r = 0.18; P = 0.16). Correlations of CD44 variant concentration with cigarette consumption were small and nonsignificant (CD44v5, r = 0.07; CD44v6, r = 0.16). A positive correlation between the plasma concentration of sCD44v5 and sCD44v6 was noted [r = 0.870; P < 0.001]. However, there was no correlation between several measures of smoke intake (expired CO, plasma cotinine, and reported cigarette consumption) and total sCD44 concentration. As expected, the correlation between CO and cotinine at baseline was strong (r = 0.49; P < 0.001).

**Discussion**

We have previously shown a small but significant elevation of total sCD44 in smokers (18), but we found no dose-dependent relationship between serum cotinine level and total CD44 concentration. In contrast, Kittl et al. (19) reported that the mean concentration of sCD44v5 in smokers was double that of non-smokers. We hypothesized that the effect of smoking on sCD44 was not the result of a blanket effect of tobacco smoking on all CD44 gene products.

In the present study, there was no dose-dependent relationship noted between any smoke intake measure (plasma cotinine, expired CO, and daily cigarette consumption) and...
total sCD44 level, and no reduction in the plasma concentration of total sCD44 was seen in quitters 1 year after smoking cessation as compared with the level in continuing smokers. The fact that there was also no evidence of significant correlations between sCD44v5 or sCD44v6 levels and the number of cigarettes smoked daily is not surprising. Self-reported consumption is not only subject to recording error but is also an unreliable measure of smoke intake, given wide variations in smoking styles. It should also be noted that only those smoking 15 or more cigarettes/day were included in the trial, which restricted the range of values for consumption.

In contrast, by using biochemical markers as dependable indices of tobacco smoke exposure, we have provided strong evidence that plasma concentrations of CD44v5 and CD44v6 in smokers are dose related and that levels of sCD44v5 and sCD44v6 significantly decline after smoking cessation. This suggests that the elevated levels of sCD44v5 and sCD44v6 are attributable to the smoking experience. Therefore, we suggest that the ascribed diagnostic specificity and prognostic value of sCD44v5 and sCD44v6 in inflammatory and malignant disease may be altered. It has been previously reported, for example, that circulating levels of sCD44v5 and sCD44v6 were 81 and 237 ng/ml, respectively, in subjects with rheumatoid arthritis in comparison to 33 and 81 ng/ml, respectively, in a control group with miscellaneous subjects with rheumatoid arthritis in comparison to 33 and 81 ng/ml, respectively, in a control group with miscellaneous subjects with rheumatoid arthritis.

References


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