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Original Contribution

Effect of smoking reduction and cessation on the plasma levels of the oxidative stress biomarker glutathione – Post-hoc analysis of data from a smoking cessation trial

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ABSTRACT

Cigarette smoke contains high concentrations of free radical components that induce oxidative stress. Smoking-induced oxidative stress is thought to contribute to chronic obstructive pulmonary disease, cardiovascular disease and lung cancer through degenerative processes in the lung and other tissues. It is uncertain however whether smoking cessation lowers the burden of oxidative stress. We used data from a randomized controlled cessation trial of 434 current smokers for a post-hoc examination of the effects of smoking cessation on blood plasma levels of total glutathione (tGSH), the most abundant endogenous antioxidant in cells, and total cysteine (tCys), an amino acid and constituent of glutathione. Smoking status was validated based on serum cotinine levels. Multivariate linear mixed models were fitted to examine the association of smoking cessation and change in cigarette consumption with tGSH and tCys. After 12 months follow-up, quitters ($n=55$) had significantly increased levels of tGSH compared to subjects who continued to smoke ($P<0.01$). No significant change in tGSH was found for subjects who continued to smoke but reduced their intensity of smoking. No significant effect of smoking cessation or reduction was observed on levels of tCys. These results suggest that smoking cessation but not smoking reduction reduces levels of oxidative stress.

1. Introduction

Cigarette smoke is an abundant source of free radicals and aldehydes which cause oxidative stress and damage to the lung and other tissues in vivo. In clinical studies, long-term cigarette smoke exposure results in systemic lipid peroxidation and depletion of antioxidants such as vitamins A and C in plasma and elevation of inflammatory responses such as C-reactive protein, fibrinogen, and interleukin-6 [1]. Systemic oxidative stress observed in cigarette smokers can occur as a result of direct exposure to oxidants contained in cigarette smoke as well as indirectly through the activation of inflammatory responses resulting from exposure to cigarette smoke constituents [2]. Such effects in cigarette smokers may ultimately contribute to their increased risk of numerous diseases and disorders including chronic obstructive pulmonary disease (COPD), cardiovascular disease (CVD), and cancer [3].

As the major endogenous antioxidant, glutathione (GSH) is thought to play a prominent role in protecting against oxidative stress in the lung and other tissues. GSH in the epithelial lining fluid of the lung is depleted by both acute and chronic exposure to tobacco smoke [4]. Plasma levels of both GSH and its rate-limiting precursor amino acid cysteine (Cys) are decreased in association with smoking [5,6]. These decreases were due, in part, to oxidation as increases in the major oxidized forms of GSH (GSH disulfide, GSSG, and GSH-protein mixed disulfides, GSSP) and Cys (cystine and Cys-protein mixed disulfides, CSSP) were observed in smokers [7]. However, decreases in total (reduced+oxidized) GSH (tGSH) and Cys (tCys) were also observed.

Surprisingly, there have been relatively few studies on the effects of smoking cessation on short-term and long-term changes in oxidative stress and GSH status [8], and especially large longitudinal studies are lacking. Knowledge of the effects of smoking abstinence would help clarify the role of oxidative stress in the etiology of smoking-induced diseases, help determine whether

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differences in redox status in smokers are due to smoking itself or other host or environmental factors associated with smoking, and provide information regarding a potential usefulness of dietary or supplemental antioxidants in smokers. The current study used data from a physician-based smoking cessation trial from 2002 to 2004 [9] for a post-hoc examination of the effects of smoking cessation on tGSH and tCys levels.

2. Materials and methods

2.1. Study design and participants

The methods of the smoking cessation trial that was used for this study were previously described in detail [9,10]. In brief, a cluster-randomized smoking cessation trial was conducted in 82 general physician practices in south-west Germany from October 2002 through September 2004. The trial included 577 smokers randomized to one of 4 intervention arms. Patients aged 36–75 and who smoked at least 10 cigarettes per day were eligible for participation irrespective of their quit intentions. Participation in the study was conditional on written informed consent, and subjects were randomized to either a treatment-as-usual arm, a physician training and incentive intervention, a physician and training medication intervention, or a combined incentive and medication arm. A unique feature of the trial was that the interventions were physician-based. The training and incentive arm included a 2-h cost free tutorial in smoking cessation and financial remuneration for each successful quitter. The training and medication arm included the same tutorial and full patient-reimbursement for nicotine or buproprion pharmacotherapy prescriptions. The study protocol was approved by the ethics committees of the Medical Faculty of the University of Heidelberg and of the State Chamber of Physicians of the federal state of Baden-Wuerttemberg.

2.2. Data and specimen collection

Participants were asked to fill in self-administered questionnaires both at baseline and at 12-months follow up, asking for socio-demographic information as well as for smoking-related information such as current smoking status and smoking intensity (in cigarettes per day). The follow-up questionnaire additionally asked for information on smoking cessation in those who had quit in the meantime. At both time points, the general practitioners collected blood samples (serum and EDTA plasma) from participants, and provided further medical diagnoses and anthropometric data (such as prevalent coronary heart disease, diabetes, hypertension and cancer, as well as height and weight). Blood samples were sent to the study center where plasma was stored at −80 °C.

2.3. Laboratory measurements

Subjects provided a blood specimen at baseline and at 12-months follow-up. Samples were analyzed for cotinine to determine and validate self-reported smoking status at baseline and follow-up. Serum cotinine levels were determined by radioimmunoassay at a commercial laboratory (Immundiagnostik, Bensheim, Germany).

Plasma samples were available from all subjects and were analyzed for plasma tGSH (GSH+GSSG+GSSP) and tCys (Cys+cystine+CSSP). The different redox forms of GSH and Cys were not measured individually because of the likelihood of oxidation occurring with long-term storage at −80 °C. Prior to analysis, 200 μl of blood plasma was added to 200 μl 8 M Urea in 1 mM EDTA (pH 7.5) and incubated for 10 min at 40 °C. After addition of 20 μl of octanol, 200 μl of aqueous 1.3 M potassium borohydride was added slowly to prevent foaming. This mixture was incubated at 40 °C for 1 h, before cooling in an ice bath and slowly adding 600 μl of 20% MPA. The samples were centrifuged, and the resultant supernatants were removed and analyzed for GSH or Cys.

GSH was determined using a 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB)/enzymatic recycling procedure previously described [11]. Briefly, to each well of a flat-bottomed 96-well microtiter plate (Greiner Bio-one, Frickenhausen, Germany) we added 50 μl of each calibrator or processed plasma sample, followed by 50 μl each of 0.5 mg/ml DTNB and 2.5 units/ml glutathione oxidoreductase (Sigma Aldrich, St. Louis, MO). Change in absorbance at 540 nm was monitored for up to 5 min and quantitated by comparison with a calibration curve. tGSH concentrations were expressed in μM (μequiv GSH/L).

Measurements of Cys were performed using a modification of the acid ninhydrin spectrophotometric method of Gaitonde [12]. Briefly, processed plasma was reacted with ninhydrin and glacial acetic acid followed by incubation in boiling water for 10 min, cooling to room temperature, and addition of 95% ethanol. The resulting Cys-ninhydrin conjugate was measured spectrophotometrically at 560 nm and quantified by comparison with a calibration curve. tCys concentrations in blood were expressed in μM (μequiv Cys/L).

2.4. Statistical analyses

Of 577 patients participating in the intervention, 574 had provided a blood sample at baseline. Of these, 437 both took part in the blood sample collection at 12 months-follow-up and provided self-reported smoking status at follow-up. Three subjects who reported being quit at follow-up had to be excluded because they indicated use of nicotine replacement therapy, which precluded a clear cotinine-based validation of their self-reported abstinence. Thus, 434 subjects formed the basis of this study.

Determination of quit status at follow-up was based on cotinine-validated self-reported smoking status, i.e. quitters were defined by self-reported quit status and a serum cotinine level <15 ng/ml at follow-up. Self-reported continuing smokers or participants with a serum cotinine level ≥ 15 ng/ml were categorized as continuing smokers [13]. Smoking intensity at baseline was based on three categories of cigarettes per day (10–19, 20–29 and ≥ 30). Smoking intensity at follow-up was based on four categories of cigarettes per day (1–9, 10–19, 20–29 and ≥ 30).

Descriptive statistics included means and standard deviations. Cross-sectional differences between groups such as quitters versus continuing smokers were assessed with ANOVA, and within-group changes over time with paired *t*-tests. For multivariate analyses of change in tGSH and tCys values, linear mixed models were fitted with smoking-related variables (quit smoking versus continuing smoking, difference in cigarette consumption) as explanatory variables, controlling for the clustering of patients by general practitioner (random effect) as well as for sex, education and change in body mass index (BMI). The models were also adjusted for the baseline-level of the respective parameter in order to correct for autocorrelation.

All statistical tests were two-sided, with an alpha level of 0.05. SAS v9.3 was used throughout.

3. Results

Table 1 shows the basic demographic characteristics of the study sample. About 56% were women and 79% had less than 12 years of formal education. About 71% of study participants smoked