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Original Contribution

Biomarkers of oxidative damage in cigarette smokers: Which biomarkers might reflect acute versus chronic oxidative stress?

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ABSTRACT

Cigarette smoking predisposes to the development of multiple diseases involving oxidative damage. We measured a range of oxidative damage biomarkers to understand which differ between smokers and nonsmokers and if the levels of these biomarkers change further during the act of smoking itself. Despite overnight abstinence from smoking, smokers had higher levels of plasma total and esterified F_2-isoprostanes, hydroxyeicosatetraenoic acid products (HETEs), F_4-neuroprostanes, 7-ketocholesterol, and 24- and 27-hydroxycholesterol. Levels of urinary F_2-isoprostanes, HETEs, and 8-hydroxy-2′-deoxyguanosine were also increased compared with age-matched nonsmokers. Several biomarkers (plasma free F_2-isoprostanes, allantoin, and 7β-hydroxycholesterol and urinary F_2-isoprostane metabolites) were not elevated. The smokers were then asked to smoke a cigarette; this acute smoking elevated plasma and urinary F_2-isoprostanes, plasma allantoin, and certain cholesterol oxidation products compared to presmoking levels, but not plasma HETEs or urinary 8-hydroxy-2′-deoxyguanosine. Smokers showed differences in plasma fatty acid composition. Our findings confirm that certain oxidative damage biomarkers are elevated in smokers even after a period of abstinence from smoking, whereas these plus some others are elevated after acute smoking. Thus, different biomarkers do not measure identical aspects of oxidative stress.

Cigarette smoking causes damage to the cardiovascular, pulmonary, and many other systems [1–5]. Cigarette smoke is rich in reactive oxygen species (ROS), such as oxides of nitrogen, alkoxyl and peroxyl radicals, and peroxynitrite [2,6], which contribute to disease development [2].

Previous studies assessing oxidative damage in cigarette smokers have mostly used single biomarkers of such damage [3,7,8]. Few studies have examined multiple biomarkers of oxidative damage in the same cohort of smokers and if their levels are chronically elevated or rise only during (or shortly after) the act of smoking itself. Several reports showed higher urinary F_2-isoprostane (F_2-IsoP) levels in smokers than in nonsmokers [8–10]. The increase in urinary F_2-IsoPs seems to be related to the amount of cigarettes smoked: higher in heavy smokers (>30 cigarettes per day) compared with moderate smokers (15–30 cigarettes per day) [9]. However, cessation of smoking decreased urinary F_2-IsoP levels significantly within 1 week.

Cigarette smoke can induce free radical damage to DNA and generate DNA base oxidation products such as 8-hydroxy-2′-deoxyguanosine (8OHdG) and 5-hydroxymethyl-2′-deoxyuridine. Urinary 8OHdG was elevated in smokers in one study [11] but not in another [12]. In another report, urinary 8OHdG was higher in ex-smokers compared to nonsmokers but not in current smokers [3].

In this study, we measured a range of oxidative damage markers, namely, plasma F_2-IsoPs, F_4-neuroprostanes (F_4-NeuroPs), cholesterol oxidation products (COPs), allantoin, urinary F_2-IsoPs and their metabolites (2,3-dinor-5,6-dihydro F_2-isoprostanes and 2,3-dinor F_2-isoprostanes), and 8OHdG. We also measured hydroxyeicosatetraenoic acid products (HETEs) in plasma and urine.

A wide range of biomarkers of lipid peroxidation is available for use in human samples [13–15]. However, the prostaglandin-like F_2-isoprostanes are formed in vivo through free radical-induced peroxidation of arachidonic acid and are considered by many to be the best indicators of lipid peroxidation, and they are not confounded by diet [15,16]. Their levels are increased in many human diseases [15,17,18] and in smokers, as reviewed above [7–9]. Arachidonic acid can also be oxidized by enzymes and by reactive oxygen species to generate HETEs [19]. Several HETE isomers are found (5-, 12-, 15-, and 20-HETEs) in cancer cells, plasma, and urine and even in saliva of smokers [19–21]. Most HETEs are claimed to be associated with

Abbreviations: ROS, reactive oxygen species; F_2-IsoP, F_2-isoprostane; 8OHdG, 8-hydroxy-2′-deoxyguanosine; COPs, cholesterol oxidation products; F_4-NeuroP, F_4-neuroprostanes; HETE, hydroxyeicosatetraenoic acid product; GC/MS, gas chromatography–mass spectrometry.

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inflammatory disorders and have vasoactive effects [19]. Recently, products of peroxidation products of docosahexaenoate, in particular, F_4-neuroprostanes, have been found to be present in neuronal membranes and cerebrospinal fluid [22], and elevated levels in plasma of patients after ischemic and hemorrhagic stroke have been reported [23,24].

Oxidation products can also arise from cholesterol (COPs). Cholesterol can be oxidized by enzymatic (cytochrome P450-dependent) reactions (to give 24- and 27-hydroxycholesterol and 7α-hydroxycholesterol) or by oxidative damage (to give 7β-hydroxycholesterol and 7-ketocholesterol) [25]. Several of these COPs have been implicated in diseases such as coronary artery disease, dengue, and Parkinson disease [25–28]. Moreover, it has been suggested that plasma 7β-hydroxycholesterol levels may predict the development of lung cancer, because elevated levels were found in smokers with lung cancer compared to those without [29].

Urate is produced as an end product of human purine metabolism. It has been proposed to exert antioxidant capabilities in vivo and, in the presence of reactive oxygen species, it is degraded to allantoin [30,31]. Allantoin has therefore been suggested as an additional biomarker of oxidative stress and its levels are elevated in several diseases in which oxidative stress is involved [24,30–33]. These include some diabetic patients [33] and ischemic stroke patients [24,32]. No data have been published on the effects of smoking on the levels of allantoin. However, another oxidation product of urate, triuret, is found at elevated urinary levels in smokers [34].

Few studies have examined multiple biomarkers of oxidative damage in the same cohort or if they are chronically elevated (i.e., they remain high even when the subject is not smoking) or rise only during the act of smoking itself. Most studies that assess the reliability of markers of oxidative damage have been performed in animals [35,36], and fewer in humans [8,18,37,38]. Markers of acute oxidative damage (useful for acute interventional studies) might perhaps differ from markers of chronic oxidative damage (perhaps useful for chronic intervention and epidemiological risk exploratory studies).

The aims of this study were (i) to assess the changes in a selected range of biomarkers of oxidative damage after cigarette smoking and (ii) to determine the ability of these biomarkers to detect rapid changes in oxidative damage using cigarette smoking as a model of oxidative challenge in humans.

Methods

Subject recruitment

Community-based subjects were recruited through advertisements and referrals. Smokers were defined as otherwise healthy individuals age 21 years and above who had smoked for at least 5 years before their study participation, and controls were age-matched individuals who had never smoked, nor were they exposed to cigarette smoke at work or at home. The mean ± SD age of smokers ($n = 119$) was 32 ± 11 years and of controls ($n = 59$) 31 ± 10 years. Information on demographic characteristics, body mass index, blood pressure, and the amount and duration of cigarette smoking was obtained. Smokers and controls were required to fast overnight for at least 8 h before blood and urine samples were collected the following morning. They were then asked to smoke one cigarette in an open area and return for a repeat collection of blood and urine samples one hour later. The brand of cigarette smoked was not standardized, to reflect the "real world" situation and because requesting smokers to change to a standard brand would be difficult and sometimes unethical (e.g., if the proposed "standard" brand had a higher tar content). The study participants, recruited in a ratio of 2 smokers:1 control, provided written informed consent before their involvement in this study. The study protocol was reviewed and approved by the Domain-Specific Review Board of the National Healthcare Group, Singapore.

A total of 178 healthy subjects (119 smokers and 59 controls), comprising 81 Chinese, 27 Malays, and 11 Indians, were included in this study. Both groups were comparable for age and gender (Table 1). On average, cigarette smokers smoked 9 ± 5 cigarettes per day for a duration of 14 ± 10 years. After collection, the blood and urine samples, processed as described below, were immediately stored at −80 °C until analysis. Information on BMI, blood pressure, glucose, lipids (cholesterol, HDL, LDL, triglycerides), and liver (γ-glutamyl transferase, alanine aminotransferase) function (measured using COBAS c111; Roche, Switzerland) is summarized in Table 1.

Biomarkers of oxidative damage

We measured a range of oxidative damage biomarkers in plasma and urine of all subjects to address differences in oxidative damage

Table 1
Clinical and laboratory characteristics of the subjects.

	Controls	Smokers	
		Before smoking	After smoking
Age (years)	31 ± 10 (range 21–70)	32 ± 11 (range 21–57)	–
Male (%)	81	84	–
BMI (kg/m^2)	23 ± 5	25 ± 5	–
Cigarettes smoked per day	–	9 ± 5	–
Number of years smoking	–	14 ± 10	–
Systolic blood pressure (mm Hg)	122 ± 12	123 ± 15	125 ± 13
Diastolic blood pressure (mm Hg)	80 ± 11	79 ± 10	81 ± 9
Cholesterol (mmol/L)	5.2 ± 0.9	5.3 ± 1.1	5.3 ± 1.1
LDL (mmol/L)	3.5 ± 0.9	3.5 ± 1.0	3.5 ± 1.0
HDL (mmol/L)	1.6 ± 0.4	1.3 ± 0.2^{+}	1.3 ± 0.3
Triglycerides (mmol/L)	0.9 ± 0.6	1.5 ± 1.6	1.4 ± 1.4
Glucose (mmol/L)	4.8 ± 0.6	4.5 ± 1.1	4.3 ± 0.8
γ-Glutamyl transferase (U/L)	35 ± 48	42 ± 38	40 ± 36
Alanine aminotransferase (U/L)	30 ± 28	36 ± 30	36 ± 30
Plasma nicotine (ng/ml)	11 ± 5	31 ± 8^{+++}	37 ± 11***
Plasma cotinine (ng/ml)	3 ± 3	14 ± 10^{+++}	32 ± 26***
Urinary nicotine (ng/mM Cr)	0.04 ± 0.07	9 ± 11^{+++}	52 ± 94***
Urinary cotinine (ng/mM Cr)	0.05 ± 0.07	88 ± 94^{+++}	547 ± 849***

Values are expressed as means ± SD. BMI, body mass index; LDL, low-density lipoprotein; HDL, high-density lipoprotein; Cr, creatinine.
$^{+}p<0.01$, $^{+++}p<0.0001$ smokers (before) vs nonsmokers; unpaired t test.
$^{***}p<0.0001$ before vs after smoking; paired t test.