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Exposure evaluation of adult male Japanese smokers switched to a heated cigarette in a controlled clinical setting

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ABSTRACT

The objective of this clinical study was to investigate changes in levels of biomarkers of exposure (BOEs) in healthy Japanese male smokers who switched to a prototype heated cigarette (HC). This was a controlled, semi-randomized, open-label, residential study conducted in Japan. A total of 70 healthy Japanese male smokers were enrolled. Following enrollment, subjects smoked their usual brand of cigarette for 2 days and were subsequently randomized either to an HC group or a 10 mg tar conventional cigarette (CC10) group for four consecutive weeks. Levels of BOEs for ten selected cigarette smoke constituents (nicotine, carbon monoxide (CO), benzene, 1,3-butadiene, acrolein, hydrogen cyanide, crotonaldehyde, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone [NNK], pyrene, 4-aminobiphenyl), and urine mutagenicity were measured at several time points during the study period. At the end of the study period, except for blood carboxyhemoglobin, levels of BOEs for the other nine constituents and urine mutagenicity were significantly lower in the HC group compared to the CC10 group. These results suggest that exposure to most cigarette smoke constituents, except CO, can be reduced by switching from CC10 to HC.

1. Introduction

The mainstream cigarette smoke of a conventional cigarette (CC) contains thousands of chemical constituents and some of them have been reported to have various biological effects on the cells, tissues and biomolecules that constitute the human body (Public Health Service., 1964; IARC, 1986). The Institute of Medicine (IOM) reviewed the scientific basis for tobacco harm reduction (IOM, 2001). This report introduced the term 'potential reduced-exposure products' (PREPs), which substantially reduce the exposure to one or more smoke constituents. Consequently, some tobacco companies have been developing products that purport to reduce exposure to cigarette smoke constituents.

A number of modifications to conventional cigarette design have been reported to affect the yield of mainstream smoke constituents (MSCs) (Harris, 2011; O'Connor and Hurley, 2008). These studies revealed that modifications to agricultural practices (e.g., fertilizers, curing), plant characteristics (e.g., protein content, nicotine content), tobacco blending and cigarette design (e.g., additives, filters, paper) could reduce the yield of MSCs to some extent. Doolittle et al. (1990) and Borgerding et al. (1998) reported that the MSCs from a heated tobacco cigarette showed significant reductions in the number and mass of potentially genotoxic or cytotoxic materials compared to the conventional combustion cigarette. In addition, it was reported that the biological activities induced by smoke or its condensate from a heated tobacco cigarette were reduced both *in vitro* (Bombick et al., 1998a,b) and *in vivo* studies (Coggins et al., 1989).

During the course of time, Japan Tobacco Inc. has developed a novel heated tobacco product, a prototype heated cigarette (HC) to address the needs of a great variety of consumers. The HC consists of four consecutive functional parts, a heat source assembly (HSA), comprising a carbon heat source; the film substrate (FS), filled with tobacco leaf; a tobacco rod (TBR); and a filter part. This makes the HC structurally different from a conventional cigarette (Fig. 1). After igniting the HSA, a hot air flow generated by puffing warms the FS and passes through the TBR to generate smoke, and then the generated smoke is inhaled by puffing after passing through a filter. In conventional cigarettes, MSCs are generated by both combustion and heat degradation of the tobacco leaves. However, in the case of HC, MSCs are generated by thermal distillation and degradation, as the HC heats tobacco leaves at a lower temperature (a peak temperature during puffing in ISO condition: approximately 500 °C at the Film Substrate (FS).) than a conventional cigarette. With the lack of combustion, the production of smoke constituents through combustion and pyrolysis of tobacco leaves is reduced with HC. It was demonstrated by chemical analysis that mainstream smoke from HC had a markedly lower

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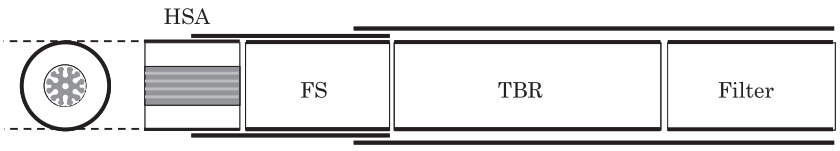

Fig. 1. Heated cigarette design. HSA, Heat source assembly part; FS, Film substrate part; TBR, Tobacco rod part.

amount of chemical constituents compared to conventional cigarettes. Thus it would be of interest to know the degree to which human exposure to cigarette smoke constituents is reduced by HC. In the present study, we investigated the changes in biomarkers of exposure (BOEs) when Japanese male smokers, who smoke 10–15 mg tar CC as their usual brand, were switched to either to HC or to a 10 mg tar CC (CC10) for four consecutive weeks in a controlled clinical setting.

The BOEs examined in this study were chosen based on information obtained from available publications on harm reduction studies (Scherer, 2005) and our preliminary BOEs exploratory study with smokers and non-smokers. These BOEs included urinary nicotine and its 5 major metabolites, expressed as nicotine equivalent (NE); whole blood carboxyhemoglobin (COHb), a BOE for carbon monoxide (CO); trans, trans-muconic acid (TMA) and S-phenylmercapturic acid (S-PMA), metabolites of benzene; monohydroxybutenyl-mercapturic acids (MHBMA), a metabolite of 1,3-butadiene; 3-hydroxypropylmercapturic acid (3-HPMA), a metabolite of acrolein; 3-hydroxyl-1-methylpropyl mercapturic acid (HMPMA), a metabolite of crotonaldehyde; thiocyanate (SCN), a BOE for hydrogen cyanide; 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and its glucuronide, metabolites of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK); 4-aminobiphenyl (4-ABP), unchanged 4-ABP; 1-hydroxypyrene (1-OHP), a metabolite of pyrene, and urine mutagenicity, reflective of compounds that are active in a modified Ames assay.

2. Materials and methods

2.1. Subjects

Healthy Japanese male smokers aged 21–49 years and who reported smoking at least 20 conventional cigarettes (10–15 mg tar value, printed on the package) per day for more than one year and the same brand for at least 8 weeks preceding screening were recruited. The health of subjects was checked before their entry into the trial by physical examination, medical history, vital signs, 12-lead electrocardiogram and laboratory tests. The serum cotinine level was measured to prevent the entry of non-smokers into the trial, and smokers whose serum cotinine level exceeded 29 ng/mL were enrolled based on the data from our preliminary BOEs exploratory study with smokers and non-smokers. All volunteers were paid for participating and provided written informed consent before enrollment. Subjects were free to leave the study at any time including when they chose to quit smoking.

2.2. Overall study design

This study used a controlled, semi-randomized, open-label, parallel group, residential, 4-sites design. A total of 70 healthy Japanese male smokers were enrolled. Following enrollment, subjects were admitted to the clinical study site for 2 days (baseline period), and stayed for four consecutive weeks (investigation period). The study design is shown in Fig. 2. To minimize the effect of diet on BOEs (e.g. urine mutagenicity), meals omitting grilled and fried foods were served from the previous day of the 24-h urine collection to the completion of 24-h urine collection. A 4-week investigational period was set to eliminate the carry-over effect from usual brand smoking because the elimination half-lives of NNAL [alpha phase: 3–4 days, beta phase: 40–45 days (Hecht et al., 1999)] and SCN [6.4 days (Junge et al., 1985)] have been reported to be long in comparison with those of other BOEs measured in this study.

On Day-1 and Day 0 (baseline period), the subjects were asked to smoke their usual brand of cigarettes in a manner similar to their routine smoking. On Day-1, participants were allowed to smoke at any time until bedtime (11:00 p.m.). On Day 0, subjects were allowed to smoke from the completion of medical checkup and blood and urine sampling in the morning to bedtime (11:00 p.m.). During the baseline period, the number of cigarette smoked per day was approximately the same as their routine use.

On Day 1 (first day of the investigation period), the subjects were allocated randomly either to the HC group (47 smokers) or the CC10 group (23 smokers) such that the ratio of sample size in the HC group and the CC10 group was approximately 2:1. Assignment to study groups was stratified by age (21–30, 31–40 and 41–50 years) and BMI (<18.5, ⩾18.5 to <25.0, ⩾25.0) so that the HC group and CC10 group were evenly matched for these two parameters. Subjects were to smoke their assigned cigarettes in the smoking room. In the HC and the CC10 groups, each subject was to smoke in a prescribed controlled manner (i.e., smoking approximately 20 HCs or CC10s per day, eight puffs per cigarette) These puff count were set from the results of the average puff count (approximately 8) in the smoking behavior survey under *ad libitum* conditions by in-house examination and the average puff count (approximately 8) in the ISO or Tobacco Institute of Japan (TIOJ) machine smoking condition described above.

Blood samples were collected into blood collection tubes containing EDTA-2Na, and then centrifuged at 3000 rpm for 10 min. at 4 °C. Plasma was transferred into plastic tubes and stored at −80 °C until analysis. For SCN measurement, plasma was collected between 9:00 and 11:00 a.m. on Day 0 (baseline period), Day 7, Day 14, and Day 28. For measurement of COHb, subjects smoked one test cigarette at 4:00 p.m. and blood samples were taken between 30 and 45 min after the completion of smoking on Day 0 (baseline period), Day 6, Day 13, and Day 27. Twenty-four hour urine collection for BOEs measurement was started between 9:00 and 11:00 a.m. on Day 0 (baseline period), Day 7, Day 14 and Day 28. The 24-h cumulative urine sample from each subject was refrigerated at 2–8 °C during the collection, and then stored at −70 to −90 °C until analysis.

This study was conducted in accordance with Good Clinical Practice (GCP) and the principles that have their origin in the Declaration of Helsinki. The protocol was approved by the Institutional Review Board of Japan Tobacco Inc. and each of the external medical institutions.

2.3. Reference and test cigarettes

The reference cigarette, CC10, was a commercially available cigarette brand in Japan with the values of 10 mg tar and 0.8 mg nicotine printed on the package. The test cigarette, HC, was a prototype of a heated tobacco product prepared by Japan Tobacco Inc. (Fig. 1).

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