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Reduced exposure evaluation of an Electrically Heated Cigarette Smoking System. Part 5: 8-Day randomized clinical trial in Japan

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ABSTRACT

A randomized, controlled, open-label, parallel-group, single-center study to determine biomarkers of exposure to twelve selected harmful and potentially harmful constituents (HPHCs) in cigarette smoke and urinary excretion of mutagenic material in 128 male and female Japanese subjects smoking *Marlboro* cigarettes (6 mg tar, 0.5 mg nicotine, and 7.0 mg CO) at baseline. Subjects were randomized to continue smoking *Marlboro* cigarettes, or switch to the Electrically Heated Cigarette Smoking System (EHCSS) and smoke either the EHCSS-K6 (5 mg tar, 0.3 mg nicotine, and 0.6 mg CO) or the EHCSS-K3 (3 mg tar, 0.2 mg nicotine, and 0.6 mg CO) cigarette, or switch to smoking *Lark One* cigarettes (1 mg tar, 0.1 mg nicotine, and 2.0 mg CO), or to no-smoking. The mean decreases from baseline to Day 8 were statistically significant ($p \leqslant 0.05$) for all cigarette smoke HPHC including CO (the primary objective) and excretion of mutagenic material in the EHCSS-K6 (range: −14.6% to −75.6%) and EHCSS-K3 (range: −9.8% to −73.0%) groups. Statistically significant reductions (all $p \leqslant 0.05$) in exposure to ten cigarette smoke HPHC (range: −5.9% to −34.6%), but not urinary mutagenicity, were observed in the *Lark One* group. The largest mean reductions in exposure to HPHC (all $p \leqslant 0.01$ level) occurred in the no-smoking group (range: −13.7% to −97.6%).

1. Introduction

There is overwhelming medical and scientific consensus that cigarette smoking causes lung cancer, heart disease, emphysema, and other serious diseases in smokers (US Department of Health and Human Services, 2010; Ministry of Health, Labour and Welfare, 2008). In the US the Family Smoking Prevention and Tobacco Control Act (FSPTCA) (Family Smoking Prevention and Tobacco Control Act, 2009) has empowered the Food and Drug Administration (FDA) to evaluate and regulate modified risk tobacco products (MRTPs) (Deyton et al., 2010). The FDA, in consultation with the Institute of Medicine (IOM), has also been charged to issue guidance and regulations on the scientific evidence required for the assessment and ongoing review of MRTPs (Food and Drug Administration, 2012; Institute of Medicine, 2012).

The electrically heated cigarette smoking system (EHCSS) and EHCSS cigarette produces reduced levels of a wide range of toxicologically important cigarette smoke HPHC and significantly lowers the biological activity of mainstream smoke compared to conventional lit-end cigarettes in laboratory-based test systems (Werley et al., 2008; Zenzen et al., 2012). Electrical heating of the tobacco reduces pyrolysis, and produces smoke that contains lower amounts of most cigarette smoke HPHC. The current third-generation EHCSS series-K puff-activated electrical heater can only be used to smoke either non-menthol or mentholated series-K cigarettes. A more efficient filter is used in the EHCSS-K3 non-menthol cigarette resulting in reduced delivery of cigarette smoke HPHC compared to the EHCSS-K6 non-menthol cigarette when tested according to International Organization for Standardization (ISO) methods.

The current communication, the third in a series of five clinical evaluations of the EHCSS (Martin Leroy et al., 2012; Tricker et al., 2012a,b,c), reports a randomized, controlled, open-label, parallel-group, single-center study. Subjects normally smoking *Marlboro* non-menthol cigarettes with a 6 mg tar and 0.5 mg nicotine delivery (M6J) at baseline were randomized to continue smoking the M6J cigarette, or switched to using the EHCSS series-K heater to smoke either the EHCSS-K3 or the EHCSS-K3 cigarette, or switch to smoking the *Lark One* cigarette (Lark1), or switch to no-smoking, for a duration of 8 days. None of the study cigarettes contained menthol as a tobacco additive and all cigarettes were commercially

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available on the Japanese market. The study was designed to examine changes in selected tobacco-specific and tobacco-related biomarkers of exposure to HPHC known to be present in the gas–vapor phase (1,3-butadiene, acrolein, benzene, CO, and crotonaldehyde) and the particulate phase (2-naphthylamine, 4-aminobiphenyl, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone [NNK], acrylamide, nicotine, pyrene, and *o*-toluidine) of mainstream cigarette smoke. The use of suitable biomarkers of exposure to these HPHC offers one potential method to assess whether differences in exposure to cigarette smoke HPHC has occurred in smokers switching from one cigarette to another (Shields, 2002; Hecht, 2003; Hatsukami et al., 2005). Excretion of mutagenic material in urine was also measured.

The primary objective of the study was to compare exposure to CO, determined as carboxyhemoglobin concentration in blood at 17:00 h ($COHb_{17:00}$), between the study groups on Day 8. Exposure to CO was selected as the primary objective based on the reduction of CO in mainstream smoke compared to conventional cigarettes (Werley et al., 2008; Zenzen et al., 2012) and the previous observation that COHb is reduced in smokers after switching to the EHCSS (Frost-Pineda et al., 2008a,b).

2. Materials and methods

2.1. Subjects

Adult male and female Japanese smokers (19–50 years of age) with acceptable health conditions who had smoked 10–30 cigarettes per day (CPD) were recruited. Subjects were to have smoked the *Marlboro* non-menthol cigarette (6 mg tar, 0.5 mg nicotine, and 7.0 mg CO) as their exclusive brand for at least 2 weeks prior to study confinement. All subjects signed an informed consent form prior to screening procedures. Subjects were compensated for study participation and were free to withdraw from the study at any time. Screening was performed within 4 weeks prior to in-clinic study confinement and included medical history, physical examination, vital signs, electrocardiogram (ECG), pulmonary function tests, clinical laboratory tests, urine pregnancy test, blood COHb, and the Fagerström Test for Nicotine Dependence (FTND) (Heatherton et al., 1991). Women of childbearing potential who used a reliable method of contraception were considered eligible for study inclusion; pregnant or lactating women were excluded. Other exclusion criteria included clinically significant disease, alcohol or drug abuse, <1.5% COHb (suggestive of being a non-smoker), <12.5 g/dl hemoglobin or <38% hematocrit, body mass index (BMI) $\leqslant$ 17.6 and $\geqslant$26.4 kg/m^2, a positive test for human immunodeficiency virus (HIV) or hepatitis, and the use of a nicotine-containing product other than cigarettes within 3 months prior to screening. The use of any medication with the exceptions of hormonal contraceptives for female subjects and occasional use of paracetamol (up to 1 g/day) to treat headache was prohibited in the week before the study.

2.2. Cigarette products

Conventional cigarette (CC) brands were selected to include a leading market share cigarette on the Japanese market of similar ISO tar and nicotine yields to the EHCSS-K6 and a representative CC with a low ISO tar and nicotine yield. Both cigarettes also had a similar tobacco blend to that used in the EHCSS test cigarettes. Study cigarettes were analyzed for tar and nicotine according to ISO methods. All study cigarettes were conditioned according to ISO standard 3402 (International Organization for Standardization, 1991). Conventional cigarettes were smoked on a smoking machine according to ISO standard 3308 (International Organization for Standardization, 2000a). Tar, nicotine and CO were determined according to ISO standards 4387, 10315, and 8454, respectively (International Organization for Standardization, 2000b,c; International Organization for Standardization, 1995). These methods are essentially similar to methods used by the Tobacco Institute of Japan for declaration of tar and nicotine levels on cigarette packaging. Mainstream smoke from EHCSS cigarettes was generated on a modified smoking machine with a carousel adapted to use the EHCSS series-K lighter. The EHCSS smoke generation conformed to ISO standard 3308; some slight technical deviations were required. The ISO yields as declared on the cigarette packaging were as follows: *Marlboro* (M6J; 6 mg tar, 0.5 mg nicotine, and 7.0 mg CO), *Lark One* (Lark1; 1 mg tar, 0.1 mg nicotine, and 2.0 mg CO), EHCSS-K6 (5 mg tar, 0.3 mg nicotine, and 0.6 mg CO), and EHCSS-K3 (3 mg tar, 0.2 mg nicotine, and 0.6 mg CO).

2.3. Study design and conduct

All recruited subjects (N = 131; 91 males and 40 females) completed a 7-day diary prior to admission to the clinic during the morning on Day −2 (Fig. 1). The median daily cigarette consumption according to the 7-day diary was used to individually determine the maximum number of cigarettes that the subject could smoke per day during the study (120% of the median, rounded to the nearest whole number, with a maximum of 30 CPD if the median was greater than 25 CPD). All subjects were confined to the clinic from Day −2 to Day 9 under medical supervision. On Day −2, the eligibility for study inclusion was re-confirmed. Assessments included COHb (13:00), vital signs (15:00), and a physical examination. On Day −1, vital signs and a 12-lead ECG were measured (07:00) and blood samples drawn for clinical laboratory tests. On Day 0 (baseline), assessments included determination of biomarkers of exposure in a 24-h urine sample (combined urine voids starting at 07:00), vital signs (07:00), COHb ($COHb_{07:00}$ and $COHb_{17:00}$; 07:00 and 17:00), plasma cotinine and nicotine (COT-$P_{17:00}$ and NIC-$P_{17:00}$; 17:00). One hundred and twenty-eight subjects (89 males and 39 females) were randomized into 1 of 5 parallel groups (EHCSS-K3, EHCSS-K6, M6J, Lark1, and no-smoking; N = 28 per smoking group, and N = 16 in the no-smoking group) using a stratification based on gender and median daily cigarette consumption (10–19 and 20–30 CPD). On randomization, subjects continuing to smoke conventional cigarettes (M6J or Lark1) were 'blind' to the identity of the test cigarettes. Non-randomized subjects were released from the study center after completing all scheduled assessments. Subjects withdrawing from the study or those removed by the Investigator after baseline were not replaced. From Day 1 through Day 8, subjects participated in their assigned study groups. Assessments included determination of biomarkers of exposure in 24-h urine samples (starting at 07:00), vital signs (07:00), $COHb_{07:00}$ (Days 1, 5, and 8), $COHb_{17:00}$, COT-$P_{17:00}$, and NIC-$P_{17:00}$. On Day 9 (end of study), vital signs, ECG, clinical laboratory tests, and a physical examination were performed at 07:00 prior to release of subjects from the study center.

On Day −2 through Day 0, subjects were only permitted to smoke M6J cigarettes. On Day 1 through Day 8 subjects smoked their randomized study cigarette or stopped smoking if they were randomized to the no-smoking group. M6J and Lark1 cigarettes were lit using a blue flame gas lighter. EHCSS-K3 and EHCSS-K6 cigarettes were smoked using the EHCSS series-K heater (Werley et al., 2008). To ensure study integrity, all M6J and Lark1 cigarette butts and smoked EHCSS-K3 and EHCSS-K6 cigarettes were collected. Smoking was permitted only at designated smoking times from 07:30 to 23:00 and subjects were neither encouraged nor forced to smoke at any time during the study. All subjects received a dietician-designed low-mutagen diet (Smith et al., 1996). Identical menus were served on Days −1, 4 and 7 (days