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Reduced exposure evaluation of an Electrically Heated Cigarette Smoking System. Part 3: Eight-day randomized clinical trial in the UK

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ABSTRACT

A randomized, controlled, open-label, parallel-group, single-center study to determine biomarkers of exposure to nine selected harmful and potentially harmful constituents (HPHC) in cigarette smoke and urinary excretion of mutagenic material in 160 male and female 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 using an Electrically Heated Cigarette Smoking System (EHCSS) smoking one of two EHCSS series-K cigarettes, the EHCSS-K6 cigarette (5 mg tar, 0.3 mg nicotine, and 0.6 mg CO) or the EHCSS-K3 cigarette (3 mg tar, 0.2 mg nicotine, and 0.6 mg CO), or switch to smoking *Philip Morris 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 determined HPHC including benzene and CO (the primary objectives), and urinary excretion of mutagenic material in the EHCSS-K6 (range −35.5 ± 29.2% to −79.4 ± 14.6% [mean ± standard deviation]), EHCSS-K3 (range −41.2 ± 26.6% to −83.1 ± 9.2%), and PM1 (range −14.6 ± 24.1% to −39.4 ± 17.5%) groups. The largest reductions in exposure occurred in the no-smoking group (range −55.4 ± 45.0% to −100.0 ± 0.0%).

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). 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) with the specially developed EHCSS cigarette has reduced levels of a wide range of toxicologically important cigarette smoke HPHC resulting in significantly lowered biological activity of mainstream smoke compared to conventional lit-end cigarettes in laboratory-based test systems (Werley et al., 2008; Zenzen et al., 2012). The third-generation EHCSS series-K puff-activated electrical heater can be used to smoke either non-menthol or menthol EHCSS series-K cigarettes. Two versions of the EHCSS series-K non-menthol cigarette are available which differ only in the construction of the filter. A more efficient filter is used in the EHCSS-K3 cigarette resulting in reduced delivery of cigarette smoke HPHC compared to the EHCSS-K6 cigarette when tested according to International Organization for Standardization (ISO) methods. The EHCSS heater cannot be used to smoke conventional lit-end cigarettes.

The current communication, the first in a series of five clinical evaluations describing data from investigations performed under both controlled (Tricker et al., 2012a,b,c) and real-life smoking conditions (Martin Leroy et al., 2012), reports a randomized, controlled, open-label, parallel-group, single-center study to compare biomarkers of exposure to selected cigarette smoke HPHC and excretion of mutagenic material in urine between two EHCSS cigarettes and two conventional cigarettes. Subjects normally smoking the *Marlboro* non-menthol cigarette (M6UK) were randomized into one of the five following groups: subjects either continued smoking the M6UK cigarette, or switched to smoke either the EHCSS-K3 or the EHCSS-K6 cigarette, or switched to smoke the *Philip Morris One* cigarette (PM1), or to no-smoking, for a duration of 8 days. The study was designed to examine changes in selected tobacco-specific and tobacco-related biomarkers of exposure to HPHC present in the gas–vapor phase (1,3-butadiene,

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acrolein, benzene, and crotonaldehyde, and CO) and the particulate phase (NNK, nicotine, pyrene, and *o*-toluidine) of mainstream cigarette smoke.

The primary objectives of the study was to compare exposure to CO, determined as carboxyhemoglobin concentration in blood at 17:00 h ($COHb_{17:00}$), and benzene, assessed as urinary excretion of *S*-phenyl mercapturic acid (*S*-PMA), between the study groups on Day 8. Exposure to CO and benzene were selected as the primary objectives of the study based on the reduction of both HPHC in mainstream smoke of the EHCSS compared to conventional cigarettes (Werley et al., 2008; Zenzen et al., 2012) and the previous observations that COHb and excretion of *S*-PMA are 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 smokers (19–50 years of age) with acceptable health conditions who had smoked 10–25 cigarettes per day (CPD) and the *Marlboro* non-menthol cigarette (6 mg tar, 0.5 mg nicotine, and 7.0 mg CO) as their exclusive brand for at least 4 weeks prior to screening were recruited. All subjects provided a signed informed consent 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, 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 as eligible for study inclusion; pregnant or lactating women were excluded. Other exclusion criteria included presence of a clinically significant disease, alcohol or drug abuse, <2% COHb (suggestive of being a non-smoker), <12.5 g/dl hemoglobin or <38% hematocrit, a positive test for human immunodeficiency virus (HIV) or hepatitis, and 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 of similar ISO tar and nicotine yields to the EHCSS-K6 and a representative CC with a low ISO tar and nicotine yield. The 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, 1995, 2000b,c). 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* (M6UK; 6 mg tar, 0.5 mg nicotine, and 7.0 mg CO), *Philip Morris One* (PM1; 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 ($N = 175$, 88 males and 87 females) completed a 7-day diary prior to check-in 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. On Day -2 the eligibility for study inclusion was re-confirmed. All subjects were confined to the clinic from Day -2 to Day 9 under medical supervision. Vital signs were measured and a physical examination performed (17:00). On Day -1, vital signs (07:00 and 21:00) 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 and 21:00), $COHb_{17:00}$ (17:00), and plasma cotinine ($COT\text{-}P_{17:00}$; 17:00). One hundred and sixty subjects (80 males and 80 females) were randomized into 1 of 5 parallel groups (EHCSS-K3, EHCSS-K6, M6UK, and PM1 cigarettes, and no-smoking; $N = 32$ subjects per group) using a stratification based on gender and median daily cigarette consumption (10–19 and 20–25 CPD). On randomization, subjects continuing to smoke CC (M6UK or PM1) were 'blind' to the identity of the test cigarettes. Subjects smoking EHCSS were blind to the tar and nicotine delivery of the test cigarette. 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 and 21:00), and determination of $COHb_{17:00}$ and $COT\text{-}P_{17:00}$ (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 the M6UK cigarette, on Day 1 through Day 8 subjects smoked their randomized study cigarette or stopped smoking if they were randomized to the no-smoking group. M6UK and PM1 cigarettes were lit using a blue flame gas lighter. EHCSS-K3 and EHCSS-K6 cigarettes were smoked using the EHCSS heater (Werley et al., 2008). To ensure study integrity, all M6UK and PM1 cigarette butts and smoked EHCSS-K3 and EHCSS-K6 cigarettes were collected. During

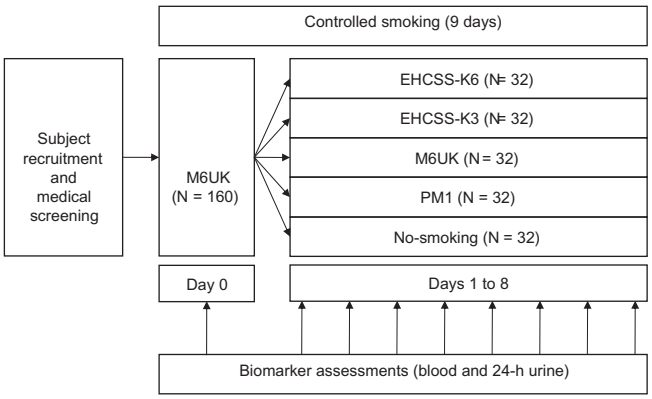

Fig. 1. Schedule of study events. Footnote: on Day 0 (baseline) all subjects smoked the M6UK cigarette prior to randomization into the five study groups. All cigarettes described in Table 2.