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A study to investigate changes in the levels of biomarkers of exposure to selected cigarette smoke constituents in Japanese adult male smokers who switched to a non-combustion inhaler type of tobacco product

Naoki Miura *, Dai Yuki, Naoki Minami, Aoi Kakehi, Yasuyuki Futamura

Product Science Division, R&D Group, Japan Tobacco Inc., Yokohama, Kanagawa, Japan

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ABSTRACT

In a clinical study, changes in 14 biomarkers of exposures (BOEs) from 10 tobacco smoke constituents and mutagens detected by the urine mutagenicity test were investigated using a non-combustion inhaler type of tobacco product (NCIT) by switching from a conventional cigarette. This study was conducted in 80 Japanese healthy adult males with a 4-week residential, controlled, open-label, parallel group design. After randomization, 40 smokers used NCIT with approximately 750 aspirations, other 20 smokers smoked approximately 20 pieces of an assigned 1-mg ISO tar conventional cigarette (CC1) every day. Twenty non-smokers (NS) did not use any tobacco product. Under this study condition, switching from cigarette to NCIT showed significant reduction in all BOEs measured. On day 29, the levels of these BOEs were almost the same as those in the NS group, except BOEs of nicotine and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). This suggested that the exposure to 8 constituents and mutagens in the NCIT group was similar to that in the NS group, while the exposure to nicotine was higher. Although the precise exposure level to NNK was not estimated because of the long half-life of its BOE, it would be substantially lower in the NCIT group than in the CC1 group.

1. Introduction

Many previous studies reported that cigarette smoking is a risk factor for serious diseases including lung cancer, coronary heart disease, emphysema, and chronic bronchitis. There are many types of tobacco products other than cigarette in the world, such as cigars, pipes, and nasal or oral snuff, and it has been reported that the users of such products are at a risk for serious diseases (International Agency for Research on Cancer, 2004, 2007). In addition to the various traditional tobacco products available, some non-traditional forms of tobacco products, such as electrically heated cigarettes (Buchhalter and Eissenberg, 2000) and dissolvable tobacco tablets (Rainey et al., 2011), have been recently introduced. Some of these were designed for reducing exposure to a series of selected harmful and potentially harmful constituents (Schorp et al., 2012). For the comprehensive evaluation of such emerging products, it is important to measure the users' exposure to chemical constituents derived from the products.

The non-combustion inhaler type of tobacco product (NCIT) is a new form of smokeless tobacco product that consists of a tapered mouthpiece and cartridge filled with finely cut tobacco leaves. The flavor components can be delivered from the tobacco leaves not by burning or heating but just by the air flow when users aspirate through its mouthpiece. We previously reported the results of a clinical study investigating the pharmacokinetics of nicotine when using prototype NCIT (Miura et al., 2013). That study results revealed that the bioavailability of nicotine delivered from prototype NCIT was similar to that delivered by conventional cigarette smoking, regardless of the form of the tobacco product. However, the degree of user's exposure to chemical constituents derived from the product, except nicotine, is unclear. The measurement of several chemical constituents delivered from NCIT by machine aspiration showed that many of these constituents were below the limit of detection (LOD), even when approximately 2000 aspirations were integrated (described in detail in this article). This result indicates a prospect of significant reduction in exposure to those constituents in NCIT users.

The measurement of biomarkers of exposure (BOE) is one of the effective methods to evaluate human exposure to smoke constituents. BOEs for tobacco smoke constituents are mainly the chemical constituents themselves and/or their metabolites, and at

* Corresponding author at: Corporate, Scientific and Regulatory Affairs Group, Japan Tobacco Inc., 2-2-1 Toranomon, Minato-ku, Tokyo 105-8422, Japan. Fax: +81 3 5572 1477.
E-mail address: naoki.miura@jt.com (N. Miura).

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present, some BOEs are validated and recommended to use for measuring the exposure of smoke constituents (Institute of Medicine, 2012). The advantage of monitoring BOEs is that they could reflect intra- and inter-individual differences in the tobacco smoking or using behavior. Moreover, the BOE monitoring method is applicable not only to the conventional cigarette but to the various other types of tobacco products, including the emerging products. The biomarker level must be background level if the exposure is zero. However, many of the validated BOEs are well known to be influenced by the other source of their corresponding chemicals existing in our living environment (Scherer, 2005). Therefore, for precise exposure evaluation, it is important to determine the degree of the influence of environmental factors other than tobacco on BOE levels.

The objective of this study was to evaluate changes in the selected BOEs with the use of NCIT in Japanese healthy adult male smokers, using a 1 mg ISO tar conventional cigarette (CC1) as a control. In addition, the changes in BOEs in Japanese healthy male non-smokers (NS) were evaluated for identifying the influence of factors other than tobacco on BOE levels in the same study environment. The BOEs monitored in this study were selected with reference to previously published information on harm reduction studies (Scherer, 2005) and the results of our preliminary BOEs exploratory study with smokers and non-smokers and our recent publication (Sakaguchi et al., 2014).

2. Materials and methods

2.1. Study design

This study was conducted at 4 medical institutions in Japan using a permuted-block, randomized (for smokers), controlled, forced-switching, open-label, parallel group design. All subjects who passed the screening test stayed at one of the medical institutions throughout the study period, except several times of outings accompanied by the clinical staff. The study period of 31 consecutive days was set in consideration of the elimination half-lives of some BOEs. The outing, such as bowling, seeing a movie, and art appreciation, was about 2 h, and was set once a week.

All smoking subjects smoked their usual brand of a 1 mg ISO tar cigarette within ±10% of their usual daily consumption for the first 2 days (day −1 and 0, baseline period). Following the baseline period, the smokers were randomly assigned to 2 groups: NCIT and CC1, as described in Fig. 1. Subjects in the NCIT group used 2 NCIT cartridges per day, and those in the CC1 group smoked an assigned CC1 within ±10% of their usual daily consumption from day 1 to day 28. All non-smokers were automatically assigned to the NS group and did not smoke throughout the study period.

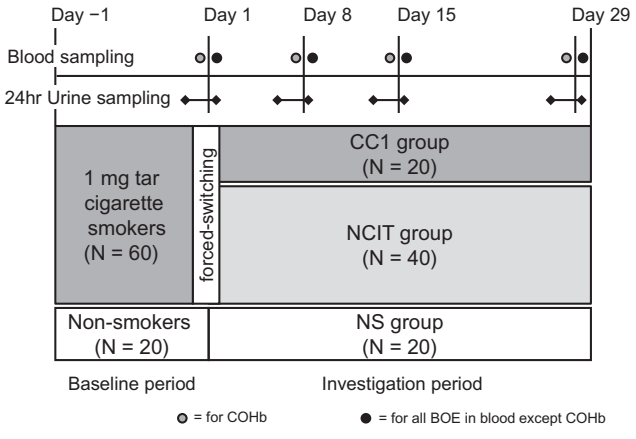

Fig. 1. Study design. All smoking subjects smoked their usual brand of 1 mg ISO tar cigarette on day −1 and 0, and then the smokers were randomly assigned to the NCIT and CC1 groups. All non-smokers were automatically assigned to the NS group and did not smoke throughout the study period. NCIT: non-combustion inhaler type of tobacco product. CC1: 1 mg ISO tar conventional cigarette. NS: non-smokers. BOE: biomarker of exposure. COHb: carboxyhemoglobin.

All four medical institutions had approximately 20–30 subjects each, and the number of subjects assigned to each group were set as equal (CC1–NCIT–NS, 1:2:1) in order to avoid the institution-specific effect on the particular group.

The clinical staff observed and recorded any adverse events. They also ensured that the subjects abstained from using nicotine-containing products except for the tobacco products specified for the study.

The study was conducted at the Maruyama hospital (Hamamatsu, Japan), OCROM clinic (Osaka, Japan), Sumida hospital (Tokyo, Japan), and Hakata clinic (Fukuoka, Japan) in accordance with Good Clinical Practice (GCP) and the principles that have their origin in the Declaration of Helsinki. This study protocol was approved by the Institutional Review Board of Japan Tobacco Inc. and the medical institutions.

2.2. Subjects

Eighty Japanese healthy males, 60 smokers and 20 non-smokers, aged 21–49 years and having a body mass index (BMI) in the range of 18.5–25.0 kg/m^2 were enrolled. The health of the subjects was checked before their entry into the trial by physical examination, examination of medical history and vital signs, 12-lead electrocardiography and laboratory tests.

Eligible smokers for the study had smoked a 1 mg ISO tar conventional cigarette without regard to menthol or non-menthol with a daily consumption of at least 20 cigarettes for at least 1 year prior to screening and their serum cotinine levels had exceeded 14 ng/ml at the screening (Pérez-Stable et al., 1992). Eligible non-smokers had no experience of routinely smoking the conventional cigarette and had not used any tobacco products including the conventional cigarette for at least 1 year before screening. Non-smokers with their serum cotinine levels lower than 14 ng/ml at the screening were enrolled.

All volunteers were paid for participating and provided written informed consent before the enrollment.

2.3. Study procedure

All smoking subjects smoked or used their assigned test tobacco product whenever they wanted from 7 to 23 o'clock throughout the study period, except for some constraints on the timing of tobacco smoking or usage; the subjects were not allowed to use NCIT or smoke the conventional cigarette from the time of awakening to the completion of clinical examination in the morning on day −1, 1, 8, 15, 22, and 29. During outings, the subjects were not allowed to use test tobacco because accurate checking of each subjects' tobacco use was difficult for the clinical staff. In addition, the subjects used NCIT with 15 aspirations or smoked 1 piece of the conventional cigarette at approximately 16 o'clock, and then 30–45 min after use or smoking, blood sampling was performed on day 0, 7, 14, and 28 to measure carboxyhemoglobin (COHb) levels. The subjects abstained from using or smoking the test tobacco product until the completion of this blood sampling.

Subjects who smoked the conventional cigarette (smoking subjects on day −1 and 0 and the CC1 group subjects from day 1 to day 28) received the cigarette from the clinical staff in each case and were allowed to smoke only in the designated smoking room. Subjects in the NCIT group received 2 pieces of NCIT cartridge daily from the clinical staff at a set time and were forbidden to enter