RESEARCH PAPER

Tobacco specific nitrosamines and potential reduced exposure products for smokers: a preliminary evaluation of Advance™

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Objective: To develop a method for evaluating the carcinogen delivery of potential reduced exposure products (PREPs) like Advance™, a PREP marketed to reduce smokers’ exposure to one tobacco specific nitrosamine (TSN), NNK, a potent lung carcinogen.

Design, setting, and participants: Latin square ordered, three condition, outpatient, crossover design with 12 smokers of light or ultra-light cigarettes (1.5 or more cigarettes/day). In each five day condition, participants either smoked own brand, Advance™, or no cigarettes. Also, on the first and last day of each condition, participants smoked one cigarette in the laboratory.

Main outcome measures: Subject rated measures of tobacco/nicotine withdrawal, expired air carbon monoxide, urine concentrations of cotinine and NNAL (one TSN biomarker), puff volume, duration, number, and interpuff interval.

Results: Relative to own brand, Advance™ produced similar withdrawal suppression, slightly lower carbon monoxide, equivalent cotinine, and 51% lower NNAL concentrations. The lowest cotinine and NNAL concentrations were observed in the no cigarette condition. Participants took fewer puffs when smoking Advance™.

Conclusions: Past experience with PREPs that failed to reduce smoking’s harm demonstrates the need for clinical methods in PREP evaluation. This study shows how assessing PREP induced changes in withdrawal and exposure to carbon monoxide, nicotine, and carcinogens may help predict PREP harm reduction potential. Adequate withdrawal suppression, slightly lower concentrations of carbon monoxide, and reduction of one TSN biomarker were observed for Advance™. In the future, clinical methods like those described here may be valuable for evaluating PREPs before they are marketed publicly.

Advance™ is a PREP marketed as a means to reduce exposure to tobacco toxicants, including TSNs. According to the manufacturer, “a patented tobacco curing process significantly inhibits the formation of tobacco-specific nitrosamines” in the tobacco used to make this PREP. No epidemiological data are available regarding Advance™. In a short term clinical study, Advance™ produced equivalent withdrawal suppression and tachycardia, 11% lower CO, and a 25% increase in plasma nicotine, relative to own brand cigarettes. No additional studies were conducted to examine if smokers only used Advance™ in a single, 2.5 hour session. Longer evaluations will be required to characterise PREP induced changes in TSN exposure. For example, one TSN thought to play a role in lung cancer in smokers is 4-(methylamino)-1-(3-pyridyl)-1-butanone (NNK). NNK is metabolised to 4-(methylamino)-1-(3-pyridyl)-1-butanol (NNAL). NNAL can be measured in human urine, is present in smokers, and declines during abstinence, with a distribution half life of 3.4 days. Thus, clinical evaluation of PREPs like Advance™ will likely involve measuring urinary NNAL concentration across several days of PREP use.

This preliminary study was designed to determine whether or not clinical methods can be used to measure PREP induced changes in urine concentrations of carcinogen biomarkers.

Abbreviations: FTC, Federal Trade Commission; IPI, interpuff interval; NNAL, 4-(methylamino)-1-(3-pyridyl)-1-butanol; NNK, 4-(methylamino)-1-(3-pyridyl)-1-butanone; PREP, potential reduced exposure product; TSN, tobacco specific nitrosamine; GSU, questionnaire of smoking urges, VAS, visual analogue scale
Because of the preliminary nature of the current study, smokers’ exposure to one carcinogen (NNK) was chosen for evaluation for three reasons: (1) NNK is a potent lung carcinogen; (2) several PREPs that are currently marketed in the US purport to reduce smokers’ exposure to NNK; and (3) measurement of NNAL, an NNK metabolite, is well validated. Participants completed three, five-day, outpatient conditions, in which they smoked no cigarettes, their own brand, or Advance™ cigarettes ad libitum. Urine samples were collected on days 1, 3, and 5 of each condition. An important goal of the study was to compare NNAL concentration in smokers’ urine across the three conditions; we also measured Advance™’s effects on CO, cotinine, tobacco/nicotine withdrawal symptoms, and, in a laboratory evaluation on the first and last day of each condition, puff topography.

**DESIGN AND METHODS**

**Participants and setting**

Eight women (one non-white) and four men (one non-white) completed this institutional review board (IRB) approved study. Participants were included if they were 18–50 years old (mean (SD) 24 (8.4) years), provided a breath sample ≥ 15 parts per million (ppm) CO at screening (mean 25.3 (9.7) ppm), and smoked ≥ 15 king sized, non-mentholated, “light” or “ultra-light” cigarettes/day (mean 18.8 (2.7) cigarettes/day). They were moderately nicotine dependent, as indicated by the Fagerstrom™ nicotine tolerance questionnaire (mean 4.9 (0.9)). Exclusion criteria included past or current cardiovascular disorders and current pregnancy, breastfeeding, or smoking cessation or reduction efforts.

**Materials**

Depending upon condition, participants smoked own brand (Own), Advance™ (Adv), or no cigarettes. Participants’ Own cigarettes were Marlboro Lights (seven participants), Camel Lights (three participants), Doral Lights (one participant), and Marlboro Ultra Lights (one participant). By the Federal Trade Commission (FTC) method, on average, Own yielded 0.75 mg nicotine, 11.3 mg CO, and 10.3 mg tar. Similarly, by the FTC method, Adv yielded 0.8 mg nicotine, 9.1 mg CO, and 9.8 mg tar. In all cases, Adv and Own were equal length (85 mm). However, as described, the tobacco used in Advance™ cigarettes is cured such that, according to the manufacturer, formation of TSNEs is inhibited. According to the FTC method, smoke from Advance™ cigarettes contains 80% less NNK than smoke from normally marketed cigarettes. All cigarettes used in this study were purchased by laboratory staff and re-packaged into containers with no product labelling.

**Compliance measures**

Because the study involved five days of no smoking, compliance with all study conditions was monitored using a combination of daily expired air CO (BreathCO; Vitalograph, Lenaxa, Kansas, USA) and thrice weekly semi-quantitative analysis of cotinine (a nicotine metabolite) in urine (using Nicalert® test strips; Nymox, Maywood, New Jersey, USA).

**Main outcome measures**

Daily computerised withdrawal measures consisted of visual analogue scales (VAS) and the questionnaire of smoking urges (QSU). VAS items were presented above a horizontal line with anchors on the left (“not at all”) and right (“extremely”). Subjects moved a mouse controlled cursor and clicked to produce a vertical mark on the horizontal line. The score was the distance of the vertical mark from the left anchor, expressed as a percentage of line length. VAS items describe tobacco/nicotine withdrawal symptoms: “Urges to smoke”, “Irritability/frustration/anger”, “Anxious”, “Difficulty concentrating”, “Restlessness”, “Hunger”, “Impatient”, “CRAVING a cigarette/nicotine”, “Insomnia/disturbed sleep”, “Increased eating”, “Drowsiness”, “Depression/feeling blue”, and “Desire for sweets”. The QSU consists of 32 multiple choice items, and yields two empirically derived factors: factor 1 (intention to smoke); and factor 2 (anticipation of relief from withdrawal).

Each day, while the participant rested quietly, heart rate and skin temperature were recorded every 20 seconds for 30 minutes (Monitor 507E, Criticare Systems, Waukesha, Wisconsin, USA). Thrice weekly urine samples were stored at ~70°F for later analysis of cotinine (GC/MS; LOQ = 1 ng/ml) and NNAL total (that is, NNAL and NNAL-glucuronide). NNAL total was measured using LC-MS/MS (LOQ = 50 pg/ml; the assay was performed by MDS Pharma Services of Lincoln, Nebraska, USA, and is a modified version of a recently reported method).

Puff topography was assessed while participants smoked a single cigarette on days 1 and 5. Cigarettes were smoked through a mouthpiece connected to a pressure transducer and pressure changes were amplified and digitised. Software (Plowshare Technologies, Baltimore, Maryland, USA) converted signals to airflow (ml/s) and integrated the data over time for each puff, producing measures of puff number, volume, duration, and interpuff interval (IPI).

**Procedure**

Participants completed three, Latin square ordered, five day conditions (Monday to Friday) in which they smoked Own, Adv, or no cigarettes; in all other respects, conditions were identical. Weekends, when participants smoked Own, were washout periods. On each study day, subjective withdrawal, CO, heart rate, and skin temperature were assessed. In addition, a urine sample was obtained on days 1, 3, and 5. Semi-quantitative urine cotinine was assessed immediately, and separate 3 ml aliquots of urine were stored for later quantitative analysis of cotinine and NNAL. On days 3 and 5, CO and semi-quantitative urine cotinine data were used to assess compliance with condition smoking restrictions. For example, when participants were in the no smoking condition, compliance was verified with decreases in CO and semi-quantitative cotinine, relative to day 1, and was reinforced monetarily ($30 on day 3 and $70 on day 5) in each condition. Participants who failed to comply with condition restrictions once were offered another chance to complete the condition (two participants repeated the no smoking condition). Participants who failed to comply more than once were withdrawn (one participant was withdrawn for repeated non-compliance in the Adv condition; this participant’s data are excluded from all analyses). On days 1 and 5, puff topography was assessed as participants smoked one cigarette (ad libitum) after daily assessments were completed (Own, Adv, or, in the no smoking condition, participants smoked Own). Participants were paid an additional $100 for study completion, and thus could earn a total of $400 in this three week study.

**Data analysis**

The final 15 minutes of heart rate and skin temperature data were averaged to produce a single value for each day. Topography data were treated as in previous work, yielding average puff number, volume, duration, and IPI for each cigarette. Data were entered into a within-subject condition (Own, Adv, no smoking) by day analysis of variance, which allowed the simultaneous testing of questions related to main effects of condition (that is, “Independent of study day, did outcome measures differ when participants smoked Own, Adv, and/or no cigarettes?”), main effects of day (that is, “Independent of condition, did outcome measures differ across days?”), and the interaction between these two factors (that is, “Did differences in outcome measures observed during each condition depend upon the particular day on which the data were...
A complete description of the theory and computation of the analysis of variance (ANOVA) statistical procedure can be found in several authoritative texts and is beyond the scope of this work. For CO, subjective withdrawal, heart rate, and skin temperature there were five levels of day (day 1–5); for urine cotinine and NNAL, there were three levels of day (days 1, 3, and 5); and for puff topography there were two levels of day (days 1 and 5). Significance levels were adjusted for violations of the sphericity assumption using Huynh-Feldt corrections. Paired t tests were used to examine mean differences between conditions on each day for measures with significant interactions and/or a condition main effect; because these comparisons were planned in this preliminary evaluation, results with a probability value of p < 0.05 are reported as significant.

RESULTS
Statistical analysis results for main effects of condition, day, and the interaction of condition and day are shown in table 1. A significant effect of condition indicates that outcome measures differed, depending on condition (Own, Adv, or no smoking). A significant effect of day indicates that outcome measures changed significantly over time (from day 1 to day 5). A significant condition by day interaction indicates that differences in outcome measures among conditions depended on the day the outcomes were assessed. Main effects of condition and interactions of condition by day are of greatest interest, as these results indicate outcomes that depended upon Own, Adv, and/or no smoking.

Compliance measures
Table 1 shows a significant condition by day interaction for CO and semi-quantitative urine cotinine. The mean CO concentrations obtained on each day of each condition (± SEM) are displayed in fig 1 (top panel). Mean CO concentrations on days 2–5 of the no smoking condition were significantly lower than Own and Adv. Interestingly, relative to Own, mean (SD) CO concentrations were significantly lower in the Adv condition on day 2 (Own 22.0 (10.0) ppm; Adv 15.2 (10.8) ppm), day 4 (Own 20.6 (11.2) ppm; ADV 16.6 (8.3) ppm), and day 5 (Own 21.3 (9.2) ppm; Adv 15.0 (10.8) ppm). For semi-quantitative urine cotinine (values range from 0–6), mean (SD) concentrations on day 3 (5.1 (1.1)) and day 5 of the no smoking condition (3.0 (1.4)) were significantly lower as compared to Own (Own day 3, 5.9 (0.3); Own day 5, 6.0 (0.0); Adv day 3, 5.9 (0.3); Adv day 5, 5.9 (0.3) data not shown). No differences in semi-quantitative urine cotinine were observed between Own and Adv.

Main outcome measures
Regarding subjective withdrawal, scores increased in the no smoking condition relative to Own and Adv across study days. For example, for the “Desire for sweets” VAS (the measure with the largest interaction F value), scores in all three conditions were similar on day 1, but on day 2 scores in the no smoking condition (mean (SD) 31.4 (33.0)) were significantly greater than for Own (14.3 (29.0) ppm) and Adv (16.3 (28.8) ppm). For this measure on day 5, mean scores in the no smoking condition (37.2 (39.5)) were significantly higher relative to Own.
and both factors of the QSU.)

"Hunger", "Impatient", "Increased eating", "Depression/
observed on the other subjective withdrawal measures with
days 2–5 for no smoking relative to Own and Adv) was
p < 0.08). A similar pattern of results (higher ratings on study
(15.1 (29.0)) and were elevated relative to Adv (18.2 (28.2);
p < 0.08). A similar pattern of results (higher ratings on study
days 2–5 for no smoking relative to Own and Adv) was
observed on the other subjective withdrawal measures with
significant main effects or interactions (that is, “Irritability/
frustration/anger”, “Anxious”, “Difficulty concentrating”,
“Hunger”, “Impatient”, “Increased eating”, “Depression/
feeling blue”, and both factors of the QSU.)

Figure 1 (bottom panel) shows a somewhat different
pattern for NNAL. There were no between condition differences
on day 1, but on days 3 and 5, NNAL concentrations
were significantly lower in the no smoking and Adv
conditions, relative to Own (for day 3: no smoking mean (SD)
213.2 (183.6) pg/ml; Adv 394.6 (338.0) pg/ml; Own 588.8
(398.5) pg/ml; for day 5, no smoking 182.2 (131.5) pg/ml; Adv
298.2 (183.6) pg/ml; Own 603.9 (319.0) pg/ml).

Participants smoked a single cigarette in the laboratory on
days 1 and 5 of each condition. For puff number, a significant
effect of condition was observed. Participants took fewer
puffs, on average, from Adv (mean (SD) for day 1, 9.6 (2.8)
puffs; day 5, 9.2 (2.9) puffs) than from Own (day 1, 11.7 (4.2)
puffs; day 5, 10.8 (3.0) puffs). No significant effects were
observed for puff volume, puff duration, or interpuff interval,
indicating that decreased puff number was the only behav-
ioural change observed when participants smoked Adv,
relative to when they smoked Own.

DISCUSSION

Cessation is the only proven method for tobacco users to
decrease their exposure to tobacco related carcinogens, and
thus reduce their likelihood of tobacco related disease and
death. Advance™ is intended to reduce smokers’ exposure to
tobacco delivered toxicants that smokers inhale may limit the
development of tobacco related diseases. However, the link
between reduced TSN cause and reduced disease is uncertain,
and there are no accepted methods for demonstrating that Advance™ (or any cigarette-like PREP)
actually reduces smokers’ carcinogen exposure. This study
was designed to examine clinical methods of measuring PREP
induced changes in carcinogen exposure, by comparing TSN
metabolite concentrations (that is, urine NNAL) when smok-
ers used their own brand, Advance™, or no cigarettes for five
days.

As might be expected, smoking own brand cigarettes main-
tained urine NNAL concentrations (that is, a 2.1% increase in
mean NNAL was observed) across study days. Relative to own
brand, by the fifth day, Advance™ use was associated with 51% lower
mean NNAL concentrations, while not smoking was
associated with 70% lower concentrations (fig 1, bottom
panel). These data demonstrate that PREP induced changes in
carcinogen exposure can be measured in a five day clinical
study, and suggest that Advance™ reduces NNK exposure sig-
ificantly (with longer study periods, even greater reductions
in toxicant exposure may be observed). To the extent that
TSNs are associated with tobacco related mortality, incorporat-
ing low nitrosamine tobacco in other products may be an
important public health goal. However, the many other
tobacco delivered toxicants that smokers inhale may limit the
reduction in death and disease that may be attributable to TSN
exposure reduction.

Some other results observed in this study (small but reliable
CO reduction, equivalent withdrawal suppression relative to
own brand) are consistent with those reported in a previous
short term clinical evaluation of Advance™. Also in that short
term study, four, eight puff smoking bouts using Advance™
produced significantly increased plasma nicotine concentra-
tions (that is, urine NNAL) when smokers smoked their own brand, by the fifth day, Advance™ use was associated with 51% lower
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What this paper adds

The tobacco industry has been marketing potential reduced exposure products (PREPs) that purport to reduce smokers’ tobacco delivered carcinogen exposure (for example, Brown and Williamson’s Advance™). Few studies describe the effects of PREPs in smokers. Careful evaluation of these and other PREPs is essential, given that previous industry sponsored PREPs (that is, low yield cigarettes) reduced neither smokers’ exposure to tobacco delivered carcinogens nor tobacco associated mortality.

This study shows how assessing PREP induced changes in withdrawal and exposure to carbon monoxide, nicotine, and carcinogens may help predict PREP harm reduction potential. Adequate withdrawal suppression, slightly lower concentrations of carbon monoxide, and reduction of one tobacco specific nitrosamine biomarker were observed for Advance™. In the future, clinical methods like those described here may be valuable for evaluating PREPs before they are marketed publicly.

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