Acute effects of electronic and tobacco cigarette smoking on complete blood count

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A B S T R A C T

The World Health Organisation called for research assessing the safety of electronic cigarette (e-cigarette). We evaluated the acute effect of active and passive e-cigarette and tobacco cigarette smoking on complete blood count (CBC) markers in 15 smokers and 15 never-smokers, respectively. Smokers underwent a control session, an active tobacco cigarette smoking session, and an active e-cigarette smoking session. Never-smokers underwent a control session, a passive tobacco cigarette smoking session, and a passive e-cigarette smoking session.The results demonstrated that CBC indices remained unchanged during the control sessionand the active and passive e-cigarette smoking sessions ($P > 0.05$). Active and passive tobacco cigarette smoking increased white blood cell, lymphocyte, and granulocyte counts for at least one hour in smokers and never smokers ($P < 0.05$).It is concluded that acute active and passive smoking using the e-cigarettes tested in the current study does not influence CBC indices in smokers and never smokers, respectively. In contrast, acute active and passive tobacco cigarette smoking increase the secondary proteins of acute inflammatory load for at least one hour. More research is needed to evaluate chemical safety issues and other areas of consumer product safety of e-cigarettes, because the nicotine content in the liquids used may vary considerably.

1. Introduction

Evidence collected during the past four decades have unanimously demonstrated that both active and passive tobacco cigarette smoking increase morbidity and the risk for premature death and generate adverse acute and long-term health effects in nearly all systems of the human organism (Flouris et al., 2010b; World Health Organisation, 2004, 2006). Despite the global initiatives and the implementation of smoke-free measures, smoking still kills nearly 6 million people every year (Flouris, 2009; World Health Organisation, 2011). In this light, alternative smoking strategies may contribute towards reducing the threat to public health caused by the tobacco epidemic (Flouris and Oikonomou, 2010). One such strategy is the electronic cigarette (e-cigarette), an electronic nicotine delivery system that was introduced in the global market during the past five years (Polosa et al., 2011). These devices have become popular in spite the dearth of research on their safety and efficacy (Etter et al., 2011; Flouris and Oikonomou, 2010). The most recent World Health Organisation regulatory consultation on the safety of electronic nicotine delivery devices called for intensified research efforts assessing the health effects of their use (World Health Organisation, 2010).

Currently, there is a severe lack of published data regarding the potential toxic effects of the natural and/or synthetic chemicals incorporated in e-cigarettes. Researchers have primarily been focused on e-cigarette efficacy towards reducing nicotine withdrawal symptoms, but they have neglected chemical safety issues and other areas of consumer product safety. Indeed, very few studies have examined parameters related to the health effects of e-cigarette use. One study reported that e-cigarettes yield only

Abbreviations: AS_{CON}, active tobacco cigarette smoking control session; AS_{TOB}, active tobacco cigarette smoking session; $AS_{E\text{-}CIG}$, active e-cigarette smoking session; CBC, complete blood count; CO, exhaled carbon monoxide; e-cigarette, electronic cigarette; PS_{CON}, passive tobacco cigarette control session; PS_{TOB}, passive tobacco cigarette smoking session; $PS_{E\text{-}CIG}$, passive e-cigarette smoking session; ppm, parts per million.

10% of the nicotine concentration in blood plasma compared to tobacco cigarettes (Bullen et al., 2010).This was in line with another study demonstrating that e-cigarettes do not affect plasma nicotine, exhaled CO, or resting heart rate (Vansickel et al., 2010). However, more recent studies by the same group reported that e-cigarettes can increase heart rate, deliver clinically significant amounts of nicotine, and reduce cigarette abstinence symptoms (Vansickel and Eissenberg, 2012; Vansickel et al., 2012). However, there is no information on more routinely-performed haematology laboratory tests such as the complete blood count (CBC) which is one of the most commonly ordered blood tests in medicine providing an overview of an individual's general health status as well as information for infection, inflammation and inflammatory disease, deficiencies in the immune system, bone marrow disease and other health-related conditions (Michota and Frost, 2004). Acute and chronic active tobacco cigarette smoking has been known to increase white blood cell count (Bridges et al., 1993). Moreover, previous epidemiological studies reported that chronic passive tobacco cigarette smoking can increase white blood cell count (Panagiotakos et al., 2004; Ronchetti et al., 1990). Therefore, the purpose of this randomized crossover study was to evaluate the acute effect of active and passive e-cigarette and tobacco cigarette smoking on CBC markers.

2. Materials and methods

2.1. Subjects and procedures

The experimental protocol was approved by the Ethics Committee at the University of Thessaly. Two groups of adults volunteered and provided written consent: 15 smokers (⩾15 cigarettes/day; 8 males; 7 females; 36.8 ± 9.9 years; body mass index 25.6 ± 4.1 kg/m^2) and 15 never-smokers (8 males; 7 females; 28.87 ± 1.5 years; body mass index 23.6 ± 3.0 kg/m^2). Exclusion criteria included pregnancy, signs of acute illness, abnormal spirometry and/or other evidence of pulmonary disease, other chronic conditions or use of medication known to influence lung function. Smokers reporting previous use of e-cigarettes were also excluded for ethical reasons (i.e., possible relapse into tobacco cigarette smoking) as previously (Eissenberg, 2010; Vansickel et al., 2010). All women participants were premenopausal with regular menstruation and were tested during the late luteal phase of their menstrual cycle.

2.2. Experimental design

Subjects in each of the two groups participated in three experimental sessions assigned in a random order and separated by a minimum of seven days of wash-out. The group of smokers underwent a control session (AS_{CON}), an active tobacco cigarette smoking session (AS_{TOB}), and an active e-cigarette smoking session ($AS_{E\text{-}CIG}$). The group of never smokers underwent a control session (PS_{CON}), a passive tobacco cigarette smoking session (PS_{TOB}), and a passive e-cigarette smoking session ($PS_{E\text{-}CIG}$). All subjects participated in each experimental session once. Blood samples were collected prior to, immediately after, as well as one hour after the active and passive smoking sessions. Prior to each experimental session, each subject was assessed for exhaled carbon monoxide (CO) (Bullen et al., 2010). Values of CO > 15 ppm or reporting by the subject of active smoking or excessive passive smoking in the previous 10 h led to rescheduling of the said session (Bullen et al., 2010).

2.3. Active smoking protocol

In the AS_{CON} session, smokers were asked to "smoke" an unlit-cigarette of their own brand for 30 min. In the AS_{TOB} session, smokers were asked to smoke two tobacco cigarettes of their own brand within 30 min. Finally, in the $AS_{E\text{-}CIG}$ session, smokers were asked to smoke a number of puffs on an e-cigarette (device: Giant, Nobacco G.P., Greece) within 30 min. The e-cigarette liquid used (Nobacco USA Mix, Nobacco G.P., Greece) had a "tobacco taste" and, according to the manufacturer, incorporated nicotine at 11 mg/ml. Extensive information regarding the e-cigarette device and liquid used is available at the manufacturer's website (Nobacco G.P., 2012). They were selected for this study because the specific liquid is the only one available in the Greek market that has been analysed by an independent publicly-funded research institute (Leondiadis, 2009). This analysis, reviewed in detail elsewhere (Flouris and Oikonomou, 2010), demonstrated that the liquid used incorporates >60% propylene glycol, <10% nicotine, <5% linalool, <5% tobacco essence, and <1% methyl vanilyn (Leondiadis, 2009). It is assumed that the composition of the vapor phase that is finally inhaled is similar. The number of e-cigarette puffs for each participant during the $AS_{E\text{-}CIG}$ session was calculated as: [(mg of nicotine in own brand of tobacco cigarettes × 1.5 × 50)/11] × 2.

2.4. Passive smoking protocol

In the PS_{CON} session, participants were exposed to normal room air for one hour inside a 60 m^3 controlled chamber. In the PS_{TOB} session, participants were exposed to air polluted with tobacco cigarette smoke at a stable CO concentration adjusted at bar/restaurant levels (23 ± 1 ppm; CO90 CO–CO_2 analyzer, Martindale Electric Ltd., Watford, UK), for 1 h inside the same chamber, as previously described (Flouris et al., 2010a; Flouris et al., 2009; Flouris et al., 2008; Metsios et al., 2007). Mainstream smoke was generated from cigarettesby combustion of cigarettes from various popular brands using an air pump (DYN, Volos, Greece) set at an air flow rate of 4 l/min. Cigarettes were half smoked using the air pump and then were left lit for 2 min to generate sidestream smoke, and then the rest of the cigarettes were smoked. An average of 29.2 ± 0.9 cigarettes were smoked in order to achieve the required level of CO in the exposure chamber. In the $PS_{E\text{-}CIG}$ session, participants were exposed to air polluted with e-cigarette vapor for one hour in the same chamber. In this case, a simulated a bar/restaurant e-cigarette smoking environment was achieved by smoking e-cigarettes via the same air pump set at an air flow rate of 4 l/min for the same time as in the PS_{TOB} session.

2.5. Complete blood count measurements

Blood samples were collected by a certified phlebotomist from an antecubital vein into plain evacuated test tubes. A total of 3 ml of whole blood was used to assess: white blood cells, red blood cells, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red blood cell distribution width, platelet count, mean platelet volume, platelet hematocrit, and platelet distribution width. Moreover, different types of white blood cells – specifically, lymphocytes, monocytes, and granulocytes – were measured as a total count and as a percentage. All blood samples were tested using a Mythic 18 (Orphée S.A., Geneva, Switzerland) autoanalyser.

2.6. Statistical analysis

A priori calculation for the determination of the minimum required sample size was conducted based on previously-published FEV_1 values before and after (4.9 ± 0.4 vs. 4.5 ± 0.3 in men and 3.7 ± 0.4 vs. 3.2 ± 0.3 in women) a similar 1-hour passive smoking session (Flouris et al., 2009). The resulting minimum required sample size was 8 participants for 2-tailed type 1 and type 2 errors of 5%. Friedman tests followed by post hoc Wilcoxon signed-rank tests were used to assess changes over time (i.e., prior to, immediately after, and one hour after active or passive smoking) during AS_{CON}, AS_{TOB}, $AS_{E\text{-}CIG}$, PS_{CON},PS_{TOB}, and $PS_{E\text{-}CIG}$ on all CBC variables. The level of significance was set at $P < 0.05$.

3. Results

In smokers, no changes were observed during the control session (Fig. 1). Active tobacco smoking increased white blood cell count, lymphocyte count, and granulocyte count for at least one hour ($P < 0.05$; Fig. 1), while the remaining CBC variables were not influenced. Specifically, Friedman tests revealed that white blood cell count ($\chi^2 = 20.81$; $P < 0.001$), lymphocyte count ($\chi^2 = 7.65$; $P = 0.022$), and granulocyte count ($\chi^2 = 14.16$; $P = 0.001$) increased significantly over time. Post hoc Wilcoxon signed-rank tests demonstrated that white blood cell count ($z = -2.9$, $P = 0.004$) and granulocyte count ($z = -2.1$, $P = 0.039$) were increased immediately following active tobacco cigarette smoking. One hour following active tobacco cigarette smoking, white blood cell count ($z = -3.1$, $P < 0.001$), lymphocyte count ($z = -2.5$, $P = 0.013$), and granulocyte count ($z = -2.8$, $P = 0.005$) remained significantly higher than normal. In contrast to these findings, e-cigarette smoking did not affect the complete blood count indices studied ($P > 0.05$) (Fig. 1).

In never smokers, CBC indices did not fluctuate significantly ($P > 0.05$) throughout the control session (Fig. 1). Passive tobacco cigarette smoking caused a significant increase in white blood cell count, lymphocyte count and granulocyte count for at least one hour ($P < 0.05$; Fig. 1), whereas the rest of the CBC indices studies did not show significant changes. Specifically, Friedman tests revealed that white blood cell count ($\chi^2 = 16.04$; $P < 0.001$), lympho-

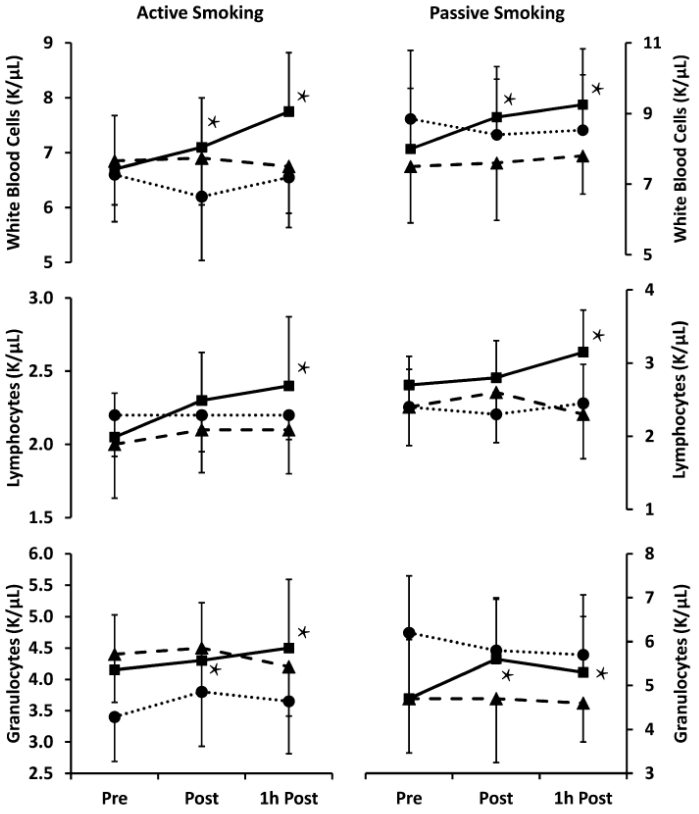

Fig. 1. White blood cell count, lymphocyte count, and granulocyte count prior to, immediately following, as well as 1 h following active (left graphs) and passive (right graphs) smoking in smokers and never smokers, respectively. Results are presented as median ± mean absolute deviation. Squares with solid lines represent tobacco cigarette smoking, triangles with dashed lines represent e-cigarette smoking, while circles with dotted lines indicate the control session. Asterisks indicate statistically significant change from baseline (i.e., pre) values.

cyte count ($\chi^2 = 7.51$; $P = 0.023$), and granulocyte count ($\chi^2 = 9.91$; $P = 0.007$) increased significantly over time. Post hocWilcoxon signed-rank tests demonstrated that white blood cell count ($z = -2.2$, $P = 0.03$) and granulocyte count ($z = -2.0$, $P = 0.045$) were increased immediately following active tobacco cigarette smoking. One hour following passive tobacco cigarette smoking, white blood cell count ($z = -2.9$, $P = 0.003$), lymphocyte count ($z = -2.6$, $P = 0.011$), and granulocyte count ($z = -2.4$, $P = 0.018$) remained significantly higher than normal. In contrast to these findings, passive e-cigarette smoking did not affect complete blood count ($P > 0.05$) (Fig. 1).

4. Discussion

In this study we present the first comprehensive data regarding the acute effect of active and passive e-cigarette and tobacco cigarette smoking on CBC markers. Our results suggest that active e-cigarette smoking in smokers and passive e-cigarette smoking in never smokers do not affect markers of CBC. In contrast, active tobacco cigarette smoking in smokers and passive tobacco cigarette smoking in never smokers increase white blood cell count, lymphocyte count, and granulocyte count for at least one hour. The results on active tobacco cigarette smoking are in line with published evidence showing an increased number of leukocytes and granulocytes following acute smoking (Hockertz et al., 1994; Morrison et al., 1999; Winkel and Statland, 1981). With respect to passive tobacco cigarette smoking, some (Panagiotakos et al., 2004; Ronchetti et al., 1990), but not all (Husgafvel-Pursiainen et al., 1987; Sochaczewska et al., 2010; Venn and Britton, 2007), studies suggest that chronic exposure to passive tobacco cigarette smoking leads to increased white blood cell count. The systemic inflammation observed following acute passive tobacco cigarette smoking is also in line with results from the main proteins of acute inflammatory load. Specifically, interleukins 4, 5, and 6 as well as interferon gamma show a prolonged increase following tobacco cigarette smoke inhalation (Flouris et al., 2009), while levels of C-reactive protein are higher in individuals passively exposed to tobacco cigarette smoke on a daily basis (Panagiotakos et al., 2004).

Circulating white blood cells, which are exposed to the systemic environment are directly involved in low-grade inflammation related to atherosclerosis. As such, our results suggests that the increase in circulatory inflammation markers – observed even during acute active and passive tobacco cigarette smoking – may be implicated in the pathophysiological mechanism that underlies the biological effects of tobacco smoking. In this light, it is important to stress that the e-cigarettes tested produced no statistically significant impact on the indices of CBC – at least in the acute phase as assessed in this study. Although it is essential to investigate the impact of long-term e-cigarette use, exploring the acute

phase of e-cigarette vapor inhalation on CBC, which is one of the most commonly ordered blood tests in healthcare is crucial and represents an essential first step in the germane research agenda (Etter et al., 2011).

The adopted protocols for active and passive smoking have been standardized by our group (Flouris et al., 2010a; Flouris et al., 2009; Flouris et al., 2008; Metsios et al., 2007) and/or others (Bullen et al., 2010; Vansickel et al., 2010) and did not result in extreme and/or prolonged tobacco cigarette or e-cigarette inhalation. Concentrations of CO as high as 33 ppm have been recently reported at bars (Goniewicz et al., 2009), while CO concentrations of up to 29 ppm have been previously reported in workplace environments (White and Froeb, 1980). In addition, a number of studies on the acute health effects of passive smoking have used CO concentrations between 30 and 40 ppm (Giannini et al., 2007; Kato et al., 1999; Leone and Balbarini, 2008), while exposures at 24 ppm are considered moderate (Scherer et al., 1990). Nevertheless, it is important to underline that the present results apply to the specific e-cigarette device and liquid tested and may not be reproducible in other devices and/or liquids. Moreover, the present results should be further confirmed by assessing the response of the main proteins of acute inflammatory load to the e-cigarette vapor.

In light of the above, it is concluded that, for the e-cigarettes tested in the current study, acute active and passive smoking do not appear to influence CBC indices in smokers and never smokers, respectively. In contrast, acute active and passive tobacco cigarette smoking increase white blood cell count, lymphocyte count and granulocyte count for at least one hour. More research is needed to evaluate chemical safety issues and other areas of consumer product safety of e-cigarettes, especially because the nicotine content in the liquids used may vary considerably. Moreover, appropriate regulations must be designed for the implementation and commercialization of this technology in order to ensure consumer product safety (Etter et al., 2011; Flouris and Oikonomou, 2010).

Conflict of Interest

Andreas Flouris' salary is paid by the Centre for Research and Technology Thessaly. He has served as an expert consultant for the World Health Organization regarding electronic nicotine delivery systems. All authors report no financial or personal relationships with other people or organisations that could inappropriately influence (bias) their actions.

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