Regulatory Toxicology and Pharmacology 77 (2016) 109–116

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

Impact of e-cigarette refill liquid exposure on rat kidney

Narges El. Golli [a, *], Aicha Jrad-Lamine [a], Hajira Neffati [a], Houssem Dkhili [a], Dalila Rahali [a], Yosra Dallagi [a], Michele V. El May [b], Saloua El Fazaa [a]

[a] LMBA, Department of Biology, Faculty of Sciences, University of Tunis El Manar, Tunis, Tunisia
[b] Laboratory of Histology-Embryology and Cell Biology, Faculty of Medicine of Tunis, University of Tunis El Manar, Tunis, Tunisia

ARTICLE INFO

Article history:
Received 27 October 2015
Received in revised form
21 February 2016
Accepted 22 February 2016
Available online 27 February 2016

Keywords:
e-liquid
Kidney injury
Oxidative stress
Histopathology
Rat

ABSTRACT

Electronic-cigarettes (e-cigarette), the alternative to classic cigarettes are becoming extremely popular but their safety is not still established. Recent studies have showed cytotoxic effects of the electronic cigarette and its recharge e-liquid, *in vitro*. The present study was designed to evaluate e-cigarette liquid nephrotoxicity in rats. For this purpose, 32 rats were treated for 28 days as follows: Control group was injected intraperitoneally with NaCl 9 g/l; e-cigarette 0% treated group received an intraperitoneal injection of e-liquid without nicotine diluted in NaCl 9 g/l, e-cigarette treated group, received an intraperitoneal injection of e-liquid containing 0.5 mg of nicotine/kg of body weight/day diluted in NaCl 9 g/l and nicotine-treated group received an intraperitoneal injection of 0.5 mg of nicotine/kg of body weight/day diluted in NaCl 9 g/l. In nicotine group, creatinine level was increased, whereas urea and acid uric levels were decreased. In e-liquid-exposed groups, levels of uric acid and mainly urea were lower. Interestingly, after e-liquid exposure, oxidative stress status showed increased total protein and sulfhydril content, whereas superoxide dismutase and catalase activities were decreased. However, the levels of lipid peroxides were not increased after e-liquid exposure. Histological studies identified excess of cells with reduced and dark nuclei exclusively located in the renal collecting ducts. Thus, e-liquid seems to alter anti-oxidant defense and to promote minor changes in renal function parameters. This preliminary study raises some flags about possible nephrotoxicity of e-cigarette liquids in rats. As some features observed in rats may not be observed in human smokers, additional studies are needed to further qualify conclusions that might be applicable to actual users of e-cigarettes.

1. Introduction

Because of the thousands of toxic chemicals contained in cigarette smoke, more than 480,000 persons die every year (USDHHS, 2014). Cigarette smoking induces numerous adverse human health outcomes (Willis et al., 2012), and constitutes an important risk factor for several cancers, as well as lung, vascular and kidney diseases (Fagerstrom, 2002). Indeed, it is now well established that tobacco smoking accelerates the progression of chronic kidney disease (Orth et al., 1997). Nicotine, one of the major compounds of tobacco plays an important role in the pathogenesis of renal diseases, as it exacerbates acute renal ischemic injury (Arany et al., 2011).

Smoking induced renal oxidative stress is also an important factor in the pathogenesis of renal injury (Halimi and Mimran, 2000; Muthukumaran et al., 2008; Pryor and Stone, 1993). And once more, nicotine is responsible, since it induces the production of Reactive Oxygen Species (ROS) and leads to a decrease in the endogenous antioxidant status (Muthukumaran et al., 2008).

As a replacement to classic cigarettes, electronic cigarettes also called e-cigarettes (ECs) are becoming extremely popular. ECs are electronic devices produced in order to provide a sensation of traditional smoking without the harmful effects of conventional cigarettes. EC contains an e-cig refill (e-fluid or e-liquid), which mainly contains humectant (propylene glycol (PG) and/or vegetable glycerin (or VG) and/or polyethylene glycol 400 (PEG400)), concentrated flavor and optionally variable doses of nicotine (Besaratinia and Tommasi, 2014).

In contrast to the conventional cigarette, studies on ECs are still lacking. A systematic review of the existing literature on health consequences of vaporing of electronic cigarettes was recently published by Pisinger and Dossing (Pisinger and Dossing, 2014). They concluded that “no firm conclusions could be drawn on the

* Corresponding author.
E-mail address: nelgolli@yahoo.fr (N.El. Golli).

http://dx.doi.org/10.1016/j.yrtph.2016.02.012
0273-2300/

safety of ECs". Two other studies have reported *in vitro* e-liquid cytotoxicity in human embryonic stem cells, mouse neural stem cells (Bahl et al., 2012) and human pulmonary fibroblasts cells (Williams et al., 2013). Recently, Lerner and collaborators showed that e-liquids aerosols (produced after heating) exhibit oxidant reactivity in lung cells by generating ROS (Lerner et al., 2015). But, to the best of our knowledge, e-liquid nephrotoxic potential has not been yet carried out. Furthermore, it is not known whether e-liquid can promote renal oxidative stress by generating free radicals or altering antioxidants response.

Based on these reports, this study was designed to investigate the impact of e-liquid on rat kidney by determining biochemical parameters and performing histological examination.

2. Methods

2.1. Chemicals

We used certified ISO 9001 electronic cigarette refill bottles with tobacco flavor, with 18 mg/ml of nicotine or without nicotine. E-cigarette refill liquid is composed of propylene glycol (50%), vegetal glycerin (40%), distilled water (5–10%), flavorings (1–5%) and nicotine (0–1.8%).

2.1.1. Analysis of e-liquids composition by gas Chromatography-Mass Spectrometry (GC–MS)

30 μl of e-liquid were diluted in 470 μl of methanol and analyzed on a Trace-GC Ultra gas chromatograph with mass detection performed on an ITQ900®(Thermo Scientific). The injector was set with a split ratio of 1:10 at 250 °C. Compounds were separated with an Agilent Technologies DB5HT column (30 m × 0.250 mm × 0.1 μm) and carrier gas was high-purity helium at 1.1 ml min^{-1} flow. The oven temperature was initially held at 100 °C for 2 min, then raised to 320 °C at a rate of 15 °C min^{-1} and held for 1 min. Compounds were detected by electronic impact ionization, with the source temperature set at 220 °C. Data analysis was performed with Xcalibur™ software using NIST and a home-made database.

2.2. Animals

Adult male Wistar rats weighting 160 ± 20 g were purchased from SIPHAT (Tunis, Tunisia). Before beginning experiments, all animals were acclimated for 1 week under well-controlled conditions of temperature (22 ± 2 °C), relative humidity (70 ± 4%), and a 12/12 h light–dark cycle with 07:30–19:30 being light phase. Animals were housed as 2 in a polypropylene cage. They were fed with standard pellet diet (SISCO, Sfax, Tunisia) and given free access to water *ad libitum* all along the experiment. Procedures involving the animals and their care followed the Guidelines for Ethical Control and Supervision in the Care and Use of Animals.

2.3. Study design

A total of 32 rats were randomized into 4 groups of 8 animals each as follow: Group 1: Control group, were injected intraperitoneally with NaCl 9 g/l (total volume = 500 μl). Group 2: E-cigarette 0% treated group, received an intraperitoneal injection of electronic cigarette refill liquid without nicotine (less than 10 μl) diluted in NaCl 9 g/l (total volume = 500 μl). Group 3: E-cigarette treated group, received an intraperitoneal injection of electronic cigarette refill liquid containing 0.5 mg of nicotine/kg of body weight (bw)/day (less than 10 μl) diluted in NaCl 9 g/l (total volume = 500 μl). Group 4: NICOTINE treated group, received an intraperitoneal injection of 0.5 mg of nicotine/kg of bw/day diluted in NaCl 9 g/l (total volume = 500 μl). Rats were treated daily for 4 weeks and sacrificed by decapitation 24 h after the last injection.

2.4. Dose and exposure mode

Intraperitoneal (i.p.) route was chosen because it allows successful delivery of e-liquid directly to the kidneys, decreasing other pathways of metabolization. Moreover, the dose of e-liquid containing 0.5 mg/kg of bw/day did not show any sign of toxicity in rats. Higher doses caused paralysis of the back limbs of the animals, diarrheas and increased heart rate. Finally, a previously published report suggested that daily i.p. administration of 0.5 mg of nicotine/kg of bw was a lower dose than a smoking comparable dose (Matta et al., 2007).

2.5. Blood and tissue sampling

After decapitation, arteriovenous blood was quickly collected and centrifuged at 1000 g for 10 min at 4 °C. Plasma aliquots were stored at −80 °C until use. Kidneys were carefully dissected out, made free from adherents, weighed and stored at −80 °C until biochemical analysis.

2.6. Biochemical assays

Kidneys were excised and homogenized in phosphate-buffered saline (PBS). After centrifugation at 10,000 g for 10 min at 4 °C, supernatants were used for biochemical determination.

2.6.1. Oxidative stress assessment

Lipid peroxidation was evidenced by measuring the formation of malondialdehyde (MDA) using the method of Buege and Aust (Buege and Aust, 1978). Briefly, 0.1 ml of tissue supernatant and 1.9 ml of 0.1 M sodium phosphate buffer (pH 7.4) were incubated at 37 °C for 1 h. After the incubation, the mixture was precipitated with 10% TCA (trichloroacetic acid) and centrifuged (2,300 g for 15 min at room temperature) to collect supernatant. Then, 1 ml of 1% TBA was added to the supernatant and placed in boiling water for 15 min. After cooling to room temperature, absorbance was taken at 532 nm and was converted to MDA and expressed in nmol per mg protein using molar extinction coefficient of $1.56 \times 10^5\ M^{-1}\ cm^{-1}$.

For determination of sulfhydril groups level, 50 μl of tissue supernatant was mixed with 1 ml of the Tris base (0.25 M)-EDTA (20 mM) buffer, pH 8.2, and absorbance was measured at 412 nm. To this, was then added 20 μl of 10 mM DTNB. After 15 min at ambient temperature, absorbance was measured again with a DTNB blank (Hu, 1994). Results were expressed in mM.

Superoxide dismutase (SOD) was assessed according to Misra and Fridovich (1972). The assay procedure involves the inhibition of epinephrine auto-oxidation in an alkaline medium (pH 10.2) to adrenochrome, which is markedly inhibited by the presence of SOD. Epinephrine was added to the assay mixture, containing tissue supernatant and the change in extinction coefficient was followed at 480 nm in a spectrophotometer. The unit of enzyme activity is defined as the enzyme required for 50% inhibition of auto-oxidation of epinephrine and expressed as nmol per minute per mg of protein.

Catalase activity was assayed by the method previously described by Aebi (1984). Briefly, the assay mixture consisted of 0.05 M phosphate buffer (pH 7.0) and 0.019 M H_2O_2, in a total volume of 0.3 ml. Changes in absorbance were recorded at 240 nm. Catalase activity was calculated in terms of μmol of H_2O_2 consumed per minute (U) per mg of protein.

Glutathione S-transferase (GST) catalyzes the conjugation