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Short communication

Tobacco and e-cigarette products initiate Kupffer cell inflammatory responses

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ABSTRACT

Kupffer cells are liver resident macrophages that are responsible for screening and clearing blood of pathogens and foreign particles. It has recently been shown that Kupffer cells interact with platelets, through an adhesion based mechanism, to aid in pathogen clearance and then these platelets re-enter the general systemic circulation. Thus, a mechanism has been identified that relates liver inflammation to possible changes in the systemic circulation. However, the role that Kupffer cells play in cardiovascular disease initiation/progression has not been elucidated. Thus, our objective was to determine whether or not Kupffer cells are responsive to a classical cardiovascular risk factor and if these changes can be transmitted into the general systemic circulation. If Kupffer cells initiate inflammatory responses after exposure to classical cardiovascular risk factors, then this provides a potential alternative/synergistic pathway for cardiovascular disease initiation. We aimed to elucidate the prevalence of this potential pathway. We hypothesized that Kupffer cells would initiate a robust inflammatory response after exposure to tobacco cigarette or e-cigarette products and that the inflammatory response would have the potential to antagonize other salient cells for cardiovascular disease progression. To test this, Kupffer cells were incubated with tobacco smoke extracts, e-cigarette vapor extracts or pure nicotine. Complement deposition onto Kupffer cells, Kupffer cell complement receptor expression, oxidative stress production, cytokine release and viability and density were assessed after the exposure. We observed a robust inflammatory response, oxidative stress production and cytokine release after Kupffer cells were exposed to tobacco or e-cigarette extracts. We also observed a marginal decrease in cell viability coupled with a significant decrease in cell density. In general, this was not a function of the extract formulation (e.g. tobacco vs. e-cigarette products or the formulation of the cigarette product). These results indicate that Kupffer cells are responsive to classical cardiovascular risk factors and that an inflammatory response is initiated that may pass into the general systemic circulation.

1. Introduction

Cardiovascular diseases remain to be the leading cause of death in the Western world (Heidenreich et al., 2011; Kochanek et al., 2011). Many risk factors have been identified that initiate and/or alter the progression of cardiovascular diseases. The effects of these risk factors on endothelial cells, platelets and white blood cells have been well established, however, the entire picture associated with cardiovascular disease progression is still missing. Many groups have shown that there are multiple competing interconnected pathways that promote cardiovascular disease development (reviewed in Barua and Ambrose, 2013; Berna-Erro et al., 2013; Ferroni et al., 2012; Messner and Bernhard, 2014), but yet initiating factors for disease development and their mechanisms are less clear. The effects of classical cardiovascular risk factors on Kupffer cells, which function to screen and clear blood within the liver, have not been investigated thoroughly. Under physiological conditions, the liver receives approximately 20% of the cardiac output and thus liver inflammation may play a role in cardiovascular disease initiation. We aimed to study how a classical cardiovascular risk factor alters Kupffer cell immune response.

Kupffer cells reside in the liver sinusoids and are primarily responsible for clearing invading pathogens and other foreign particle from the splanchnic circulation. However, recent work has illustrated that Kupffer cells interact with platelets and leukocytes

Abbreviations: gC1qR, receptor for the globular head of C1q; cC1qR, receptor for the collagen region of C1q.

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to mediate disease/inflammatory processes (Kotecha and Toledo-Pereyra, 2013; Pak et al., 2010; Sindram et al., 2001; Tamura et al., 2012; Wong et al., 2013). Furthermore, many groups have investigated the innate immune response of Kupffer cells that precede fatty-alcoholic liver disease (Cohen et al., 2010; Yokoyama et al., 1999). Since it has been established that Kupffer cells are responsive to foreign particles, Kupffer cells can interact with platelets (Wong et al., 2013) and leukocytes (Miyazaki et al., 2011; Wu et al., 2009) in a direct adhesion based mechanism or indirect cytokine-mediated manner, and Kupffer cells can release pro-inflammatory cytokines to initiate disease progression, we aimed to investigate if these processes occur in response to classical cardiovascular risk factor stimulation. These reactions may provide an alternative pathway for cardiovascular disease initiation, which may act in parallel with or activate other commonly investigated pathways.

It has been well documented that the exposure to tobacco products can lead to cardiovascular disease development by activating pro-inflammatory and/or pro-thrombotic function within the vasculature (Clair et al., 2013; Dong et al., 2010; Garrison et al., 1978; Holford et al., 2014; Monfrecola et al., 1998; Rubenstein et al., 2004; Zhu and Parmley, 1995). The current thought is that absorbed/dissolved fine-particulate matter, toxic chemicals and nicotine act to produce oxidative stress factors, induce the release of pro-inflammatory cytokines, and increase the myocardial demand for oxygen among many other effects. However, since these tobacco cigarette products are in the circulation, they will pass through the liver and possibly instigate effects on Kupffer cells. Furthermore, e-cigarettes, which are new "smoking" products, have been shown to produce fine-particulate matter and deliver nicotine at similar levels to traditional tobacco products (Czogala et al., 2014; Fuoco et al., 2014; Goniewicz et al., 2014). The effects of these products on cardiovascular health have not been thoroughly investigated and recent work has shown that the usage of these products is increasing in popularity, especially among adolescents and young adults (Choi and Forster, 2014; Sutfin et al., 2013). Since e-cigarettes deliver similar products to the cardiovascular system as traditional cigarettes and since the effects of traditional cigarettes on the liver inflammatory system have not been well documented, it is important to elucidate their effects.

Here, we chose to investigate the effects of tobacco products and e-cigarette products on Kupffer cell innate immune responses that have been previously correlated to cardiovascular disease progression. We hypothesized that Kupffer cells would initiate an innate immune response after the exposure to physiologically relevant levels of tobacco cigarette or e-cigarette extracts. Additionally, these responses may initiate or promote traditional cardiovascular disease responses that have been identified in previous studies.

2. Material and methods

2.1. Tobacco smoke extracts and e-cigarette vapor extracts

Tobacco smoke extracts were prepared as previously described (Rubenstein et al., 2004, 2010). Briefly, Marlboro 100s (16 mg tar and 1.2 mg nicotine, Philip Morris) were used to prepare mainstream (smoke that passes through the filter during normal inhaling; referred to as "MS") and sidestream (smoke from the smoldering tip, which does not pass through the filter; referred to as "SS") extracts. A modification of this smoke extraction apparatus was used to produce extracts from e-cigarettes (illustrated in Fig. 1). Since the atomizer only activates when negative pressure is applied, metered vacuum pressure was applied to the e-cigarette. This would activate the atomizer and initiate the vaporization procedure. Two brands of e-cigarettes were used for preparing extracts. One brand comes as a complete e-cigarette

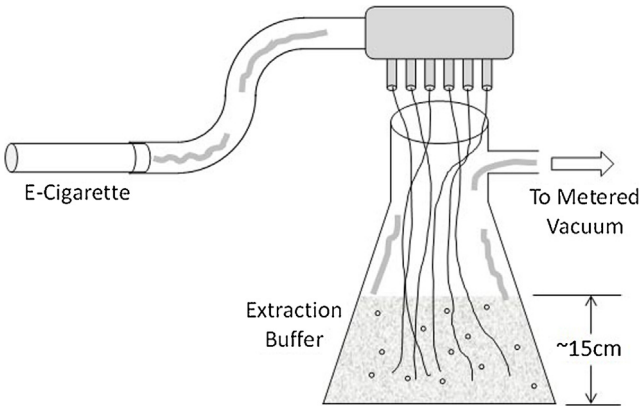

Fig. 1. Schematic of e-cigarette extraction methods, in which all of the e-vapor passes through a step-down manifold into a HEPES buffer with a height of approximately 15 cm. Other details are given in Section 2.

and no modifications by the user can be made (NJoy, OneJoy Traditional Flavor, 1.2% Nicotine by volume and 1.8% Nicotine by volume). For the second brand, e-juice is added to the atomizer by the user (eGo, OKC Vapes, Desert Sands Flavor with 0 mg, 12 mg or 18 mg nicotine). For all extracts, puffing was mimicked by alternating the airflow rate every 30 s, from 200 mL/min for 25 s to 600 mL/min for 5 s. For the e-cigarette extracts, the e-cigarette was removed from the apparatus when the flow rate was alternated to 200 mL/min. This procedure was followed for 5 min, which is the time it takes to "smoke" an entire traditional cigarette. Smoke or e-vapor was distributed by a step-down manifold (Small Parts Inc.) to six 0.9 mm capillary tubes submerged into a HEPES buffered saline (130 mM NaCl, 20 mM HEPES-NaOH, pH 7.4) with a depth of ~15 cm. Extracts were aliquoted and stored at −20 °C until use. The atomizer for eGo extracts was extensively cleaned between extract preparations. Note that all chemicals and reagents were purchased from Sigma Aldrich Co. LLC. (St. Louis, MO) unless noted otherwise.

2.2. Kupffer cells

Immortalized Kupffer cells (from Sprague–Dawley Rats) were purchased from Applied Biological Materials Inc. (Richmond, BC, Canada). Kupffer cells were maintained in Prigrow II medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin and 100 μg/mL streptomycin (all supplied by Applied Biological Materials) at 37 °C and 5% CO_2. Kupffer cells were cultured on flasks and plates that were pre-coated with 0.5 mg/mL type 1 collagen (37 °C for 1–2 h). Plates were washed 2× with PBS (pH 7.4) prior to passaging cells to the coated plates. At confluence, Kupffer cells were passaged with trypsin digestion for approximately 3 min at a 1:4 or 1:5 ratio by area. For experiments, 1 day after passing, tobacco smoke extracts (at a final concentration of 1 cigarette/5 L), e-cigarette vapor extracts (at a final concentration of 1 cigarette/5 L), 50 nM pure nicotine or lipopolysaccharide (LPS, 1 μg/mL) was added to Kupffer cells for 48 h. All experiments included an internal paired negative control consisting of Kupffer cells exposed to normal media only. For statistical purposes the seeding density of all experiments was maintained at approximately 1000 cells/cm^2.

2.3. Complement deposition onto Kupffer cells and C1q receptor expression

After exposure to tobacco smoke extracts or e-cigarette vapor extracts, the activation/deposition of complement proteins onto Kupffer cells was quantified, as a means to monitor the progression of innate immune responses. C1q, C4d and C3b are deposited activation products of C1, C4 and C3. Additionally, C5b-9, the