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A Comparative Study on Different Effects and Exposure Duration Between Conventional Cigarette Smoke and Electronic Cigarette Vapor on Serum Interleukin-1β Level: An In Vivo Study

(PERBANDINGAN PENGARUH DAN PERBEDAAN DURASI PAPARAN ASAP ROKOK KONVENSIONAL DAN UAP ROKOK ELEKTRONIK TERHADAP KADAR INTERLEUKIN-1β PADA SERUM: SUATU STUDI IN VIVO)

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ABSTRACT

Smoking is known to have bad effects on health. People start using e-cigarettes with the assumption that e-cigarettes are safer, but the safety of e-cigarettes is still in doubt. Harmful substances from cigarettes can trigger an increase in free radicals and induce inflammatory process. The aim of this study was to evaluate and compare the increase of serum Interleukin-1β due to exposure to conventional cigarette smoke and e-cigarette vapor based on exposure time. This study was conducted experimentally on 30 male white rats Sprague Dawley strain. The rats were divided into five treatment groups (control group, two and four weeks of exposure to conventional cigarette smoke, and two and four weeks of exposure to electronic cigarette vapor). Smoking session was given once a day. The rats were sacrificed then necropsied and Interleukin-β levels were calculated using the enzyme linked immunosorbent assay (ELISA) Kit. The data obtained were analyzed using one-way analysis of variance (ANOVA) statistical test. Exposure to conventional cigarette smoke and e-cigarette vapor did not give any significant changes to Interleukin-1β level in rats, both at two weeks and four weeks of exposure. However, there was a tendency of increased Interleukin-1β levels with increasing time. This tendency is more obvious in groups with exposure to conventional cigarette.

Keywords: conventional cigarettes; electronic cigarettes; inflammation; Interleukin-1β, rats

ABSTRAK

Merokok diketahui memiliki efek buruk terhadap kesehatan. Dewasa ini, orang mulai menggunakan rokok elektronik dengan asumsi rokok elektronik lebih aman, namun keamanan dari rokok elektronik masih diragukan. Zat berbahaya dari rokok dapat memicu peningkatan radikal bebas dan menginduksi terjadinya proses inflamasi. Tujuan penelitian ini adalah mengevaluasi dan membandingkan peningkatan Interleukin-1β serum akibat paparan asap rokok konvensional dan uap rokok elektronik berdasarkan lama paparan. Penelitian ini dilakukan secara eksperimental pada 30 ekor tikus putih *(Rattus norvegicus)* strain *Sprague Dawley*. Tikus dibagi ke dalam lima kelompok perlakuan yaitu kelompok kontrol, paparan asap rokok konvensional dua dan empat minggu, serta paparan uap rokok elektronik dua dan empat minggu. Pengasapan dilakukan satu sesi per hari. Setelah pengasapan sesuai durasi kelompok, tikus dikorbankan nyawanya kemudian dinekropsi dan dilakukan perhitungan kadar Interleukin-1β menggunakan *enzyme linked immunosorbent assay* (ELISA) Kit. Data dianalisis menggunakan uji sidik

ragam satu arah atau *one-way analysis of variance* (ANOVA). Paparan asap rokok konvensional dan uap rokok elektronik tidak memberikan perubahan signifikan pada kadar Interleukin-1β serum tikus, baik pada paparan dua minggu maupun empat minggu. Terdapat kecenderungan peningkatan kadar Interleukin-1â seiring dengan bertambahnya waktu. Kecenderungan ini terlihat lebih jelas pada kelompok dengan paparan asap rokok konvensional.

Kata-kata kunci: rokok konvensional; rokok elektronik; inflamasi; Interleukin-1β; tikus

INTRODUCTION

There are more than 1.1 billion people above age of 15 who smoke tobacco in 2016 according to the World Health Organization. Indonesia is the top third country who consume cigarettes after China and India worldwide. Smoking is the main risk factor for cardiovascular diseases, respiratory diseases, various types of cancer, and many other diseases which deteriorate human functional ability. Two main types of cigarettes are conventional cigarette (tobacco product) and electronic cigarette. Nowadays, people starting to consume electronic cigarette as alternative to conventional cigarette with the assumption that e-cig is safer. But the safety of e-cig is still in doubt (Hajek *et al.,* 2019).

Conventional cigarette can be classified by the packaging material, raw materials or contents of the cigarettes, manufacturing process, or the usage of filter (Haris *et al.,* 2012). Scientists have found more than 7.537 chemical substances from different classes in cigarette smoke. Some of the dangerous chemical substance found in conventional cigarette smoke are carbon monoxide, nicotine, benzo[a]pyrene, N-Nitrosamine, acrolein, acetaldehyde, formaldehyde, 1,3-Butadiene, and many other compounds such as pyridine, ammoniac, carbon dioxide, ketone, cadmium, nickel, and nitrogen oxide (CDC, 2010). High level of carbon monoxide in the blood leads to tissue hypoxia (Guzman, 2012). Tissue hypoxia will increase the production of pro-inflammatory cytokine Interleukin-1β through NF-êB pathway. The hypoxia condition limits selective targeting of pro-IL-1β (inactive IL-1β) towards autophagy degradation. At the same time, increasing the expression of inflammasome NLRP3 which will activate caspase-1 and increase the secretion of IL-1β (Folco *et al.,* 2014; Zhang *et al.,* 2018). Other compound that can be found in both conventional and electronic cigarette is nicotine (FDA, 2020). Nicotine is soluble in water and has a similar molecule shape as acetylcholine (important neurotransmitter in the brain) which makes it addictive (Tiwari *et al.,* 2020)

Electronic cigarette is famous among Nicotine Replacement Therapy (NRT) choices. gum, inhaler lozenges, nasal spray, and skin patch are other examples of NRT (Tanuwihardja and Susanto, 2012; Stead *et al.,* 2018). E-cig vapor is produced from the heating of e-liquid. common ingredients in e-liquid are nicotine, propylene glycol, glycerin, essence, and other compounds that don't have the toxic property as tar and tobacco in conventional cigarette. All of which can induce the production of free radicals (Aini *et al.,* 2018). A study by Al-Aali *et al.* (2018), showed a significant increase of pro-inflammatory cytokine TNF-β and IL-1β in groups of people who smoke e-cig compared to non-smoker.

Harmful substances from cigarettes can trigger an increase in free radicals and induce an inflammatory process (Lopez and Brough, 2011; Sumanasekera and Waingeh, 2016; Wang *et al.,* 2018). At the time harmful substances of cigarette smoke enter the body, it causes free radicals like Reactive Oxygen Species (ROS) to accumulate resulting in oxidative stress. This state will further trigger an inflammatory reaction (Yang *et al.,* 2013). The inflammatory reaction is a result of signaling pathway activation such as NF-êB pathway, MAP Kinase pathway, and histone modification (Barnwal *et al.,* 2018; Gordon *et al.,* 2013; Noerager *et al.,* 2015).

Interleukin-1â is a potent pro-inflammatory cytokine secreted by macrophage, NK cell, B cell, endothelium, fibroblast, and astrocyte as innate immunity response (Vaillant *et al.,* 2020). Interleukin-1β is also called a master regulator of inflammatory reaction because of its role in regulating innate immune system. The secretion of IL-1β will activate lymphocyte, stimulate macrophage, increase the adhesion of leukocyte to endothelium, change the hypothalamus set point, and cause the release of acute phase protein by the liver (Kaneko *et al.,* 2019; Vaillant *et al.,* 2020).

As a response to exposure of conventional cigarette smoke and electronic cigarette vapor, oxidative stress will activate NF-êB pathway.

Cigarette smoke causes degradation of inhibitor kappa B-alpha) IêB-á and activate nuclear factor-kappa B (NF-êB) on lymphocyte and other cells by increasing nuclear translocation. This activation will stimulate alveolar epithelium to secrete IL-1β further recruiting leukocyte to the inflammation site (Qiu *et al.,* 2017; Cao *et al.,* 2021). *Functio laesa* is one of the cardinal signs of inflammation marked by decreased or loss of function of the tissue. Particularly in smokers, the repeated exposure to cigarette smoke will cause damage to the respiratory system exposed locally (Lee *et al.,* 2012).

Previous study conducted by Sumanasekera and Waingeh (2016) showed that cigarette smoke exposure increases IL-1β secretion on cardiac stem cell. Increase of IL-1β secretion is also found in serum and bronchoalveolar lavage together with other pro-inflammatory cytokines as a result of cigarette smoke exposure (Wang *et al.,* 2018). According to Husari *et al.* (2016), IL-1β secretion increased along with the addition of conventional cigarette smoke extract and exposure duration. This finding is incompatible compared to increase curve by e-cig exposure (Glynos *et al.*, 2018). Thus, the aim of this study was to evaluate and compare the increase of serum Interleukin-1β due to exposure to conventional cigarette smoke and e-cigarette vapor based on exposure time.

RESEARCH METHODS

This experimental study was conducted in the Animal Laboratory of School of Medicine and Health Sciences Atma Jaya Catholic University of Indonesia and Integrated Laboratory of the Faculty of Medicine, University of Indonesia for sample examination. This study has received its approval from the Ethics Committee of School of Medicine and Health Sciences Atma Jaya Catholic University of Indonesia (No: 15/10/KEP-FKIKUAJ/2020).

Animals

A total 30 adult male white rats (*Rattus norvegicus*) Sprague-Dawley strain age 10-12 weeks old (150-250 g) was housed in cages of 3 to 4. The cages were maintained at a constant 23-25°C with a 12 hour-light/dark cycle. All animals were acclimated for one week prior to initiating conventional cigarette smoke and electronic cigarette vapor. All the rats are healthy with no signs of any macroscopic abnormality. The rats were then randomly divided into five groups with each group consisting of six rats determined by degree of freedom analysis of variance/ANOVA formula. The fifth groups are control group, group with two weeks and four weeks of exposure to conventional cigarette smoke, and group with two weeks and four weeks of exposure to electronic cigarette vapor.

Smoking Session

Smoking exposure session was done once per day for 11.5 minutes each session. The rats will be put in a smoking chamber individually. The chamber is 15 cm x 15 cm x 18 cm in size. Then, the cigarettes were heated and connected to a 20 mL syringe with 3-way infusion valves. An 20 cc bolus of conventional cigarette smoke or e-cig vapor was injected into the chamber every 30 seconds. Both conventional cigarette and electronic cigarette has the same dose, and was given in a total of 12 puffs using 20 mL syringe each session accumulating a concentration of 0.2 mg nicotine from conventional cigarettes for rats. This level of daily exposure resembles the consumption of adult normal cigarette smoker who smoke approximately 12 cigarette sticks per day.

Serum Preparation

The rats were sacrificed and then necropsied according to their duration group allocation (two weeks or four weeks) using an overdose of injected anesthetic of ketamine + xylazine combination. Thoracotomy was done and blood sample was draw using cardiac puncture method. Blood sample was then centrifuge on 2894 rpm for 15 minutes. The serum was kept at a constant 2-8°C before examined. IL-1â were examined using the Rat IL-1â (Interleukin 1 Beta) ELISA Kit.

Statistical Analysis

Data were expressed as the mean and standard deviation. Obtained data was compared using the one-way ANOVA statistical test with Tamhane's post-hoc multiple comparison. A p-value of <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The mean and standard deviation of IL-1â serum level of each group is shown in Table 1. The table shows no significant increase of IL-1â in groups with e-cig vapor (ECV) or conventional

Table 1. Mean (±SE) of Interleukin-1â concentration in blood serum

Treatment groups	Mean of Interleukin-1â (pg/mL) ± SD
Control	4.3400±0.20518[a]
E-cig Vapor (2 Weeks)	4.8660±0.47030[a]
E-cig Vapor (4 Weeks)	4.8660±0.54054[a]
Conventional Cigarette (2 Weeks)	4.9480±0.90256[a]
Conventional Cigarette (4 Weeks)	5.1760±0.80541[a]

[a, b]Different superscripts within the same column indicate significant differences (P<0.05)

cigarette smoke (CCS) compared to control group. However, both treatment groups show higher concentration of IL-1β compared to control group. The mean of conventional cigarette smoke was higher than that of e-cigarette vapor both at week two and week four. Interleukin-1β levels in conventional cigarette smoke exposure increased along with increasing duration of exposure. This is different from e-cigarette vapors where the difference in duration of exposure to e-cigarettes does not show a mean difference.

The result doesn't correspond to the literature and previous studies that show significant increase of Interleukin-1β after exposure to CCS or ECV. This finding can be due to the relatively short half-life of IL-1β which is 2.5 hours (Hazuda *et al.,* 1988). The secretion of IL-1β is continuous for the first two hours after induction of pathogen (refers to nicotine and other dangerous chemical substances in CCS or ECV). In this study, last smoking session and necropsy was more than 18 hours apart. The use of anesthesia combination ketamine + xylazine can also influence the expression of IL-1β. An experimental study conducted by Erdem *et al.* (2014), showed that the use of ketamine + xylazine combination as anesthetics gives significant decrease to inflammatory reaction and edema scoring on rats with acute pulmonary injury. But, all the rats in this study used the same anesthetic procedure.

Other factors that could influence this result is the dual role of nicotine towards IL-1β. Nicotine was shown to be both an upregulator or downregulator of NF-êB signaling pathway. In healthy individuals, cigarette smoke causes increase in inflammatory reactions. This is a different case on infected individual or in individual with chronic inflammation, where CSS or ECV will lower the immunity and decrease inflammatory reaction (Moeintaghavi *et al.,* 2017; Qiu *et al.,* 2017). This factor can be eliminated because all the rats that were in this study were healthy and weight measurement was done every week.

Conventional Cigarette to IL-1β by Exposure Duration

The exposure of CCS didn't give any significant changes compared to the control group (p>0.05). However, this study showed increasing IL-1β level along with increasing duration of exposure. This finding is in line with an *in vitro* study conducted by Husari *et al.* (2016) that showed increased IL-1β along with increase CCS extract. Interleukin-1β is a pro-inflammatory cytokine produced by cells in non-specific immunity such as macrophages. Interleukin-1β will be produced in an inactive form in response to the presence of pathogens through Pathogen Associated Molecular Patterns (PAMPs). The presence of this first pathogen alone is not sufficient to activate Pro-Interleukin-1β. Pro-IL-1β will only be active after the next encounter with the pathogen either through PAMPs or Danger Associated Molecular Pattern (DAMPs). The pro-inflammatory protease caspase-1 then cleaves and activates Pro-IL-1β, followed by rapid secretion of Interleukin-1β. From this secretory process, we can see that the secretion of Interleukin-1β is strongly influenced by the type and intensity of pathogen stimulation (Lopez and Brough, 2011). This explains the tendency of increasing levels of Interleukin-1β on exposure to conventional cigarette smoke week two to week four.

Electronic Cigarette to IL-1β by Exposure Duration

The different exposure duration of ECV did not give any significant changes to IL-1β serum levels (p>0.05). There was a tendency of increased IL-1β at two weeks of exposure but no further increase until four weeks of exposure. This tendency is in line with a study by Glynos

et al. (2018) that showed an increase of IL-1β in three days (acute) of exposure to ECV but not at four weeks of exposure. Interleukin-1β plays an important role in non-specific immunity so that it provides an acute response (Lopez and Brough, 2011). Along with the increase in the duration of exposure, adaptation can occur to the body so that the same dose of exposure does not give further effect. This adaptation was demonstrated by a study conducted by Kendrick *et al.* (1976) where continuous exposure slowly led to adaptation by decreasing the sensitivity of mice to cigarette toxicity. Based on this study, adaptation was seen more clearly in the e-cigarette vapor exposure group, characterized by the same Interleukin-1â levels at week two and week four. This may occur because conventional cigarettes contain higher levels of harmful substances and toxicity compared to electronic cigarettes (Goniewicz *et al.,* 2018; Maruqes *et al.,* 2021).

Conventional Cigarette and Electronic Cigarette

This study demonstrates a higher tendency of increased IL-1β in CCS compared to ECV. The tendency was found both in two weeks and four weeks of exposure. Although the increase of IL-1β was not significant statistically, but this finding is in line with previous studies that showed higher increase of IL-1β in group with CCS exposure compared to group with ECV exposure along with longer duration of exposure (Glynos *et al.*. 2018; Husari *et al.,* 2016).

In correspond to literature and previous studies, cigarette causes inflammation by increasing oxidative stress. A study by Husari *et al.* (2016) found that acute lung tissue damage due to exposure to CCS was associated with secondary increase of oxidative stress caused by increased free radical. In sharp contrast to that, there is no significant evident of oxidative stress on exposure to ECV even with longer duration compared to the CCS (Husari *et al.,* 2016). This could explain the findings found in this study that CCS exerts a higher inflammation reaction than ECV. However, this does not mean that the use of e-cig is a safe use. Research regarding the chronic effects of e-cig is scarce and needed (Marques *et al.,* 2021).

CONCLUSION

Exposure to conventional cigarette smoke and e-cigarette vapor did not give any significant changes to the serum levels of Interleukin-1â in rats, both at two weeks and four weeks of exposure. However, there was a tendency of increased Interleukin-1â levels with increasing time. This tendency is more obvious in groups with exposure to conventional cigarette.

SUGGESTION

Further research is suggested to give higher dosage and exposure duration of CSS and ECV. The size of smoking chamber could be adjusted to suit the size of rats better. A more modern smoking devices could also be used to minimalized bias. Smoking session could also be divided into morning, afternoon, or evening session to represent better human smoking pattern.

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