THE EFFECTS OF SOOT PARTICULATE EXPOSURE ON TNF-α EXPRESSION IN THE MECHANISM OF CARDIOVASCULAR DISORDERS

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ABSTRACT

Air pollution exposure is related to increased cardiovascular morbidity and mortality. Factors that trigger this event are important for public health, whereas in the mean time epidemiological evidence implicates particulate matter (PM) as a cardiovascular risk factor. Still, the mechanism of this cardiovascular injury is still unclear. Oxidative stress is the main mechanism that explains biological process as a result of PM. On inflammation pathway there will be find increased TNF-a as a response to PM exposure.The aim of this study was to describe the effect of soot particulate exposure on TNF-a expression in mechanism of cardiovascular disorders. Experiments were performed on white female rats (Rattus novergicus) divided into 3 groups: control group (P0) (n=12), without exposure to soot particulate; treatment group 1 (P1) (n=12), exposed to soot particulate concentration of 532 mg/m3one hour/day for 30 days; and treatment group 2 (P2) (n=12), exposed to soot particulate concentration of 1064 mg/m3 one hour/day for 30 days. Immunohistochemical staining was used to quantify the expression of TNF-a in cardiac tissues. We quantified the expression of TNF-a by immunoreactive index score (IRS).Increased TNF-a expression on P2 is more significant (p=0.016) compared to P0. But there's no significant difference between P0 and P1 (p=0.660), and such with P1 compared to P2 (p=0.81).Exposure to soot particulate significantly increased expression of TNF-a in subjects. Our findings suggest the role of activation inflammatory pathways in response to soot particulate exposure in cardiovascular disorders. ***(FMI 2012;48:186-189)***

Keywords: *soot particulate, TNF-a, inflammatory pathways, oxidative stress*

ABSTRAK

Paparan polusi udara dihubungkan dengan meningkatnya morbiditas dan mortalitas kardiovaskular. Faktor-faktor yang memicu kejadian ini sangat menarik perhatian dan penting bagi kesehatan masyarakat, dimana saat ini bukti-bukti epidemiologis mengimplikasikan particulate matter (PM) sebagai sebuah faktor resiko kardiovaskular. Meski demikian mekanisme dari injuri kardiovaskuler ini belum jelas. Stres oksidatif merupakan mekanisme utama yang menerangkan proses biological akibat PM. Pada jalur inflamasi akan didapatkan peningkatan TNF-a untuk merespon paparan PM. Penelitian ini bertujuan untuk menjelaskan efek paparan partikulat jelaga terhadap ekspresi TNF-a pada mekanisme gangguan sistem penyakit kardiovaskular.Eksperimen dilakukan pada hewan coba berupa tikus betina (Rattus novergicus) yang dibagi menjadi 3 kelompok yaitu: kelompok kontrol (n=12), tanpa paparan partikulat jelaga; kelompok perlakuan 1 (n = 12), dipapar dengan partikulat jelaga konsentrasi 532 mg/m3 satu jam tiap hari selama 30 hari; kelompok perlakuan 2 (n=12), dipapar dengan partikulat jelaga konsentrasi 1064 mg/m3 satu jam tiap hari selama 30 hari. Ekspresi TNF-a pada jaringan jantung dinilai dengan pewarnaan immunohistokimia. Kami menilai ekspresi TNF-a berdasarkan indeks immunoreactive score (IRS).Peningkatan ekspresi TNF-a pada kelompok perlakuan 2 yang bermakna (p=0,016) dibandingkan dengan kelompok kontrol. Tetapi tidak didapatkan perbedaan bermakna antara kelompok kontrol dengan kelompok perlakuan 1 (p=0,660), demikian juga kelompok perlakuan 1 dibandingkan kelompok perlakuan 2 (p=0,81). Paparan terhadap partikulat jelaga secara signifikan meningkatkan ekspresi TNF-a pada hewan coba. Temuan kami menegaskan peran aktivasi jalur inflamasi sebagai respon paparan bahan partikulat pada penyakit kardiovaskular. ***(FMI 2012;48:186-189)***

Kata kunci: *partikulat jelaga, TNF-α, jalur inflamasi, stres oksidatif*

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INTRODUCTION

Industrialization in the various regions of the world has been greatly associated with the emission of various substances which constitute air pollutants and increase air pollution. Air particulate matter (a-PM) otherwise known as aerosols is a major atmospheric pollutant which contains complex mixtures of chemical and/or biological components and is majorly made up of sulphate, nitrates, ammonia, sodium chloride, carbon, mineral dust and water. Although natural processes emit primary particles into the atmosphere, anthropogenic processes such as combustion from car engines; solid fuel; combustion in households and industrial activities

constitute the greater source of primary particles emitted into the atmosphere (Obinaju 2012). Epidemiological studies have shown that the largest portion of air pollution related mortality is due to cardiovascular diseases. Exposure to air pollution is associated with increased cardiovascular morbidity and mortality due to myocardial ischemia, arrhythmia and heart failure (Araujo 2011).

Simkhovich et al (2008) reported several causes of deaths and other medical fatalities thought to be realted to air pollution in Belgium, Pennsylvania and London. National Mortality and Morbidity Air Pollution Study (NMMAPS), based on data from 90 of the largest cities in the United States, estimated that the daily total and cardiopulmonary mortality increased in the short term by 0.31% for each 10 µg/m3 increase in PM10, measured over a 24-hour period. Gustavsson et al as cited in Brook et al (2004) reported increasing risks of myocardial infarction among 3000 Swedish workers with increasing cumulative exposure to products from non-vehicular combustion processes. Whereas Routledge & Ayres (2005) in their study, suggested myocardial infarction (MI) cases treated at London hospitals were triggered by outdoor air pollution. Furthermore, it has been showed that a reduction in 24 h average PM10concentration would result in reduction in cardiovascular admissions. Deaths due to heart failure, MI and stroke were all significantly higher in the polluted areas compared with the control areas, an effect more pronounced than that on respiratory mortality. In patients with implantable cardioverter defibrillators in Boston, USA, episodes of defibrillation were related to daily air pollution. In 2004, the American Heart Association(as cited in Brook et al 2004) stated that studies show an increased risk of cardiovascular events were consistently associated both short term and long term to the concentration of ambient particulate matter. The factors that trigger events are particular interest and importance to public health, where current epidemiological evidence implicates PM as a cardiovascular risk factor.

However the mechanism of cardiovascular injury is not yet clear. The evidence shows that air pollution contributes to acute cardiac events through various channels, including systemic inflammation, homeostasis pathway activation, vascular dysfunction, accelerated atherosclerosis, plaque instability, changes in autonomic control and cardiac arrhythmia (Link & Dockery 2010).In the experiments in-vitro, in-vivo and controlled human studies showed that interleukin 6 (IL-6) and tumor-necrosis-factor alpha (TNF-α) can arise in response to exposure to PM (Tsai et al 2012). Based on these facts, we are compelled study further the influence of particulate matter air pollutants exposure towards the risk of cardiovascular system disorders. The aim of this study was to investigate the effect of soot particulate exposure on TNF-α expression in mechanism of cardiovascular disorders.

MATERIALS AND METHODS

This research is an experimental study with a "post-test only control group design". Subjects of this study were female white rats (*Rattus novergicus*) weighed 100-200 gr, aged 4 months old and healthy. The eligible rats were then divided into 3 groups: 1) control group, 2) the first treatment group (P1), and the second treatment group (P2).This study is a numerical analytic unpaired (two-sided test). Calculation of the sample by using the type 1 error rate of 5% and type 2 error rate of 10%, and the mean differences were considered significant minimum is 1 then obtained n1=n2=10. To anticipate the possibility of drop out or damage to the unit experiment which resulted in loss to follow-up, then the correction is done by 20% of the sample size of the original calculation. Based on these calculations, the sample size or replication to be used in this study were 12 rats for each group. Carbon black powder was sprayed in an exposure box. The treatment is given in a different box to monitor exposure to the air temperature, flow rate of 5 to 7.5 km/h (light breeze) on local temperature and humidity with an atmospheric pressure are inhaled. The treatment is given in stages according to the group. Before the treatment, subjects were acclimatized to the exposure in the box. Subjects then enter the box and were exposed to treatment for a month. On day 31, subjects were sacrificed by dislocation of the atlas bone.

After disinfection with 70% alcohol, the subjects were immediately dissected and subject's heart was collected and fixed in formalin buffer for examination of TNF-α by immunohistochemistry methods. Immunohistochemical staining with avidin-biotin complex method was used to determine the expression of TNF-α in the rat cardiovascular system. Samples of subject's heart were prepared on an object glass. It was subsequently rehydrated with graded alcohol, washed with phosphate buffered saline (PBS), and then immersed in 3% hydrogen peroxide H2O2 (in DI water) for 20 minutes, 1% bovine serum albumin (BSA) in PBS for 30 minutes at room temperature. Primary Antibody (Anti- TNF-α) 1:1000 was applied and left overnight in temperature 4^{o}C. The following procedure was application of biotin-labeled secondary antibody (Anti Rat IgG biotin labeled) and primary antibody anti- TNF-α for 1 hour at room temperature, the SA-HRP (Sterp Avidin-Hoseradish Peroxidase) for 60 minutes at room temperature, Chromogen DAB (3,3-diaminobenzidine

tetrahydrochloride) for 20 minutes at room temperature. Samples were then counterstained (Aceto orcein) for 3 minutes at room temperature and then examined under a microscope. In each turn of the stages, slides were always washed with PBS to clear the rest of the material attached. TNF-α expression reading was done with 400X magnification on 10 field view with a microscope.

RESULTS

Descriptive data are presented as mean ± SD or median and frequency is displayed as a percentage. To test the normality of the data distribution used one sample Kolmogorov-Smirnov test. If the normal distribution of data obtained, then tested using different statistical parametric One Way ANOVA, and if found significant differences, then followed by statistical test Post Hoc (Tukey HSD). Whereas if the obtained data distribution is not normal, then tested the Kruskal-Wallis non-parametric statistics, and if the results of the statistical tests was no significant difference, then followed by Mann-Whitney test statistic U. The results of the expression of TNF-α due to exposure to particulate soot for 30 days obtained mean difference between groups. In the control group gained a mean 1.4 ± 1.350, treatment group 1, mean 2.13 ± 2,031, while treatment group 2, mean 3.8 ± 2.426.Examination of the expression of TNF-α was assessed by the IRS index test data normality by Kolmogorov-Smirnov test. Normal distribution of data obtained with p = 0.016. Statistical tests were performed by using one way ANOVA. Obtained mean difference of TNF-α expression between groups were significant with p = 0.016. Furthermore, analysis performed using the Post Hoc Test (Tukey HSD) which found significant differences between the control group and treatment group 2 (p=0.019), whereas the control group than treatment group 1 not found significant differences (p=0.660), as well as between treatment group 1 than treatment group 2 was not found significant differences (p=0.660).

DISCUSSION

CoLaus studies involving 6183 adult participants who are in Lausanne, Switzerland, analyzed the relationship between short-term exposure to PM10 (on the day of the inspection visit) with increased expression of hs-CRP, interleukin 1-β (IL-1β), interleukin 6 (IL-6), and TNF-α. Each showed elevated levels of PM10 by 10 μg/m3 would be an increase of TNF-α was 0.024 (0.013-0.035) pg/mL. This study concludes that there is a positive relationship between short-term exposures to PM10 with TNF-α and increased cardiovascular risk. Mukae et al (2001) concluded in their study the biological effects of PM on systemic inflammation are involved in the cardiovascular response. They showed an increase in cytokines, including TNF-α released by macrophages due to exposure to PM10. Soukup & Becker also reported an increase in cytokines (IL-6 and TNF-α) as a pro-inflammatory mediators after exposure to PM2.5 and PM10. Seagrave et al. in their study concluded that the PM is removed by burning diesel can increase the production of pro-inflammatory mediators such as cytokines TNF-α.In our study found an increased expression of TNF-α in the treatment group 2 compared with the control group (p=0.016). Whereas the treatment group 1 not found significant differences (p=0.660) when compared with the control group. Likewise found no significant difference (p=0.81) between treatment groups 1 and 2 treatment groups. These findings support the hypothesis that exposure to particulate soot can increase the expression of TNF-α in cardiovascular disease.

CONCLUSION

Our study of soot particulates get exposure for 30 days can increase the expression of tumor necrosis factor (TNF-α) in the animal heart tissue. Significant increased expression of TNF-α was obtained in the treatment group 2 when compared with the control group. Increased expression of TNF-α is linear with increasing dose exposure to particulate matter are given. These findings suggest a possible role of oxidative stress and activation of pro-inflammatory pathways in response to soot particulate exposure. The findings in our study are important in explaining how the soot particulate matter can contribute to cardiovascular events.

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