Activity of Cytochrome p450 as a Steroidogenesis and Oxidation Catalyst of Cholesterol in Experimental Animals Exposed to Cigarette Smoke

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Abstract

BACKGROUND: Basically, body regulates various harmful substances, including nicotine and tar which were carried by cigarette smoke. Nicotine which accumulated in blood was transported to liver to be metabolized and excreted in kidneys. The metabolism of nicotine into a harmless substance to body is closely related to the role of cytochrome P450 enzyme. In liver, nicotine was catalyzed by cytochrome P450 to intermediate nicotine-Δ-1′ (5′) - iminium ion. The elevation of cholesterol leads the P450 to discard overage cholesterol to keep cholesterol homeostasis and atheroprotection.

AIM: The aim of the study was to determine the activity of cytochrome P450 to cigarette smoke exposure and to cholesterol level.

MATERIALS AND METHODS: A total of 32 Rattus norvegicus male Wistar albino strain was exposed to cigarette smoke with a different number of cigarettes for 57 weeks (2, 3, and 4 cigarette/day group, with a group of control). In the 58th week, the blood of rats was collected for enzyme-linked immunosorbert assay analysis.

RESULTS: Both cytochrome P450 activity and cholesterol level affected to cigarette smoke exposure (p > 0.05, 0.00 for cholesterol levels and 0.04 for p450 levels).

CONCLUSIONS: Exposure of cigarette smoke significantly has an impact on increasing cholesterol levels and decreasing the activity of P450 enzyme. The elevating of cigarette number had a significant impact between the smoker and non-smoker groups.

Introduction

The Southeast Asia Tobacco Control Alliance report entitled The Tobacco Control Atlas, Association of Southeast Asian Nations (ASEAN) Region showed that Indonesia was the country with the highest number of smokers in ASEAN (65.19 million people), equivalent to 34% of the total population of Indonesia (2016). Around 79.8% of smokers bought cigarettes at kiosks, stalls, or minimarkets, and 17.6% bought cigarettes from supermarkets. There were 2.5 million outlets of cigarette retailers in Indonesia [1].

Basic Health Research (RISKESDAS) [2] released data that 85% of households in Indonesia were exposed to cigarette smoke. Approximately eight smokers died due to active smoking and one passive smoker died from smoke cigarette exposure of active smokers. Based on this ratio, at least 25,000 deaths in Indonesia occurred due to others smoke cigarette. About 4000 chemicals were detected in cigarette smoke, and nicotine was active chemical with the highest amount. Nicotine was responsible for various physiological changes that lead to patho-physical occurrence such as malignancy, hypoxia, and other systemic disorders. In other reports, pure nicotine was also known to have very high levels of toxicity.

Not only nicotine, other substances in cigarette smoke that potentially as strong toxin for humans included carcinogenic N-nitrosomornicotine, nitrosamine (specifically due to fermentation and drying of tobacco leaves) [3]. Specifically, more than 43 carcinogens were found in cigarettes, cigarette smoke had a high concentration of naphthalene and polycyclic aromatic hydrocarbons, which were known to have active carcinogenic activity and have detrimental effects in the body [4].

Basically, body regulates various harmful substances, both drugs, and other substances, including nicotine and tar, contained cigarette smoke. Nicotine which accumulated in blood was metabolized and excreted through kidneys. The metabolism of nicotine became a friendly substance in the body was related to the role of cytochrome P450 enzyme. In liver, nicotine entered two metabolic pathways. First, nicotine was catalyzed by cytochrome P450 to intermediate nicotine-Δ-1′ (5′)
Results

Assumption test

The results of the normality test for P450 activity and cholesterol levels variable showed that the data were normally distributed (p > 0.05; 0.067 for cholesterol levels and 0.173 for P450 activity).

Hypothesis test

The result of the one-way ANOVA test was presented in Table 1.

| Cholesterol activity | n  | F    | Sig     |
|----------------------|----|------|---------|
| P450 activity        | 32 | 359.232 | 0.000* |

Both cholesterol level and P450 activity have significant results (p > 0.05; p = 0.000 and p = 0.044, respectively). Data were then analyzed using the post-hoc LSD test to discover the different effects which received by each group.

The result in Tables 2 and 3 showed that the more number of cigarettes given, the more severe impact to the body. The activity of P450 showed that there was a significant difference between control and treatment groups, while in cholesterol variable, a significant difference was showed only in the control group toward treatment 2 and 3.

Table 2: Post-hoc LSD test of cholesterol level inter groups

| Dependent variable | (1) group | (J) group | Sig. 95% confidence interval |
|--------------------|-----------|-----------|-----------------------------|
| Cholesterol        | K         | P1        | 95% confidence interval     |
|                    | P2        | P3        | Lower bound                |
|                    | 0.000     | 16.1083   | 11.5888                   |
|                    | 0.000     | 22.2512   | 16.8388                   |
|                    | 0.000     | 36.7069   | 32.1874                   |
| P1                 | 0.000     | 11.5888   | 16.1083                   |
|                    | 0.000     | 12.2512   | 6.7431                    |
|                    | 0.000     | 22.2512   | 16.8388                   |
| P2                 | 0.000     | 21.5917   | 16.1083                   |
|                    | 0.000     | 7.7431    | 12.2512                   |
|                    | 0.000     | 12.2512   | 6.7431                    |
| P3                 | 0.000     | 36.7069   | 32.1874                   |
|                    | 0.000     | 32.1874   | 26.7069                   |
|                    | 0.000     | 18.3388   | 22.8583                   |
|                    | 0.000     | 8.3359    | 12.8550                   |

Activity of cytochrome P450 was analyzed by enzyme-linked immunosorbent assay method. The data which resulted in from histopathological observations were analyzed using SPSS version 20.0. Normality test by Shapiro–Wilk test was done to determine normal distribution (n ≤ 50). Normally distributed data were continued to one way ANOVA parametric test method. Conversely, Kruskal–Wallis nonparametric test was used if data were not normally distributed. Hypothesis was considered as significant if p < 0.05 and then followed by a post-hoc least significant difference (LSD) analysis to determine the differences between treatment groups.

This study has been approved by Ethics Committee of Medical Faculty, Andalas University number: 490/KEP/FK/2019.
Table 3: Post-hoc LSD test of P450 inter groups

| Dependent variable | (I) group | (J) group | Sig. | 95% confidence interval |
|--------------------|-----------|-----------|------|-------------------------|
| P450 cytochrome K  | P1        | 0.112     | 0.3484 | 3.1344                  |
|                    | P2        | 0.010     | 0.6273  | 4.1101                  |
|                    | P3        | 0.021     | 0.3512  | 3.8340                  |
| P1                 | K         | 0.112     | -3.1344 | 0.3484                  |
|                    | P2        | 0.259     | -0.7057 | 2.7171                  |
|                    | P3        | 0.415     | -1.0418 | 2.4410                  |
| P2                 | K         | 0.010     | -4.1101 | -0.6273                 |
|                    | P1        | 0.259     | -2.7171 | 0.7657                  |
|                    | P3        | 0.746     | -2.0175 | 1.4652                  |
| P3                 | K         | 0.021     | -3.8340 | -0.3512                 |
|                    | P1        | 0.415     | -2.4410 | 1.3418                  |
|                    | P2        | 0.74      | -1.4652 | 2.0175                  |

Discussion

**Cigarette smoke exposure and cholesterol levels**

Cigarette smoke exposure in humans had a fairly strong correlation, especially with the increase of lipid and homocysteine profiles, which were the main triggers for atherosclerosis [7]. In line with these findings, there was a significant effect of cigarette smoke exposure to the increasing of cholesterol levels in rats. It could be due to high lipolysis activity by nicotine metabolic activity in body. Haragopal and Aruna [8] found that nicotine in cigarette tobacco-induced the adrenal medulla to release catecholamine. High levels of catecholamine in blood directly influenced the increase of lipolysis of triglycerides stored in adipose and trigger an elevation of free fat acid secretion into blood.

There was still a debate about the components of lipid profile as a marker of the presence of nicotine response in body. Alharbi [9] found that there was an increase in the average of cholesterol level in smokers and non-smokers. Another study conducted by Hassan et al. [10] revealed that an increase in the period of exposure (years) increased the incidence of dyslipidemia and cholesterol levels and could be a determinant of various pathological incidence such as atherosclerosis which causes coronary heart disease.

The increase of pathological incidence such as atherosclerosis, increased cholesterol levels, and coronary heart disease experienced by individuals exposed to cigarette smoke was closely related to the length of exposure time and intensity of smoking cigarettes. This study was also found that the more number of cigarettes given to rats, the higher cholesterol levels. Similar finding published Digiacomo et al. [11] who found that the more intensity and duration of smoking, the heavier impact on the body. Negative effects on multiple tissues and increased the risk of various pathological occurrence and inflammatory symptoms were found. The pathogenesis findings occurrence reflected a series of lipolysis and the induction of free radical enzymes such as Nitrogen Oxides (NOX) and oxide of carbon (COX) which increased free radical levels (reactive oxygen species) [12].

**Cigarette smoke exposure and P450 activity**

The lowering of various metabolic enzyme levels included P450, was evidence of the effect of nicotine regulation in body. A decrease level of an enzyme indicated an imbalance work of system, and it found in this study. There was a different activity found in the decrease of P450 enzyme in the treatment group.

P450 enzyme has an important role in the metabolism of nicotine. Bao et al. [3] found that P450 played a role in the metabolic pathway of nicotine and changed it into a low toxic substance. aromatase P450 acted on the catalytic pathway to convert nicotine into intermediate nicotine-Δ1 (5') - iminium ion. The increase of nicotine in the body induced various free radical pathways as a negative onset of liver function which resulted in a decrease in P450. Another study conducted by Hukanen et al. [13] reported that brief exposure to nicotine had no significant effect on P450 activity, but this probably due to the differences in body’s ability to accommodate toxic types entered a body system. A different finding was reported by Kwiecień et al. [5] found that P450 played a role in regulating nicotine into a material that was easy to eliminate from the body.

In another study, Li et al. [14] reported that from various enzyme groups, P450 group had an important role in metabolizing nicotine products, especially those found in cigarettes. P450 was also active as a catalyst in the steroidogenesis pathway or steroid hormone metabolism and drug metabolism.

The higher the nicotine level, the lower the P450 level, as a result of decreased liver function due to the accumulation of various free radical enzymes such as NOX and COX. In regulating cigarette smoke exposure to both individual smokers and non-smokers, various imbalances occurred in the body system. As a form of real physiological manifestation, namely an increase in various free radicals in the body, resulted in a state of oxidative stress in cells [6].

The decrease of P450 function directly caused an increase of lipolysis as a result of a decrease in various hormone functions, especially steroid hormones and an increase in lipid secretion in blood. In this is, of course, the function of various organs will be hampered, the formation of embolism and atherosclerosis which leads to impaired heart function. Furthermore, the incidence of hypogonadism and infertility can be clinical manifestations as a result of the decline in function of p450 [15].
Conclusion

This study concluded that cigarette smoke exposure significantly has an impact on increasing serum cholesterol levels and decreasing P450 enzyme activity in rats. Increasing the number of cigarettes affected significantly both smoker and non-smoker groups.

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