A comparative study of the plasma level of arginase and rhodanese in smokers and non-smokers

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Summary: The purpose of this investigation was to determine and compare the activities of arginase and rhodanese in the blood plasma of cigarette smokers and non-smokers. The activity of arginase in the blood plasma of smokers was higher than arginase activity in the non-smokers (NS), however, in the smokers with diseases (SWD), the increase was significant (p<0.0007). The comparison between the activity of rhodanese in the SWD, smokers without diseases (SWOD) and NS blood plasma revealed a decrease in the activity of rhodanese in NS and no significant difference in the three groups with p<0.8677. This paper reported the enhancing effect of cigarette smoking could have on the disease state of smokers due to high arginase activity.

Keywords; Rhodanese, Arginase, Cigarette smoking, Plasma.

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Manuscript Accepted: November, 2010

INTRODUCTION

There are approximately 4000 chemicals in cigarettes, hundreds of which are toxic including over 60 carcinogens (Florini et al., 1980; Cerami et al., 1997; Taylor et al., 2001). The risk of developing smoking-related diseases, such as cancers, heart disease, stroke, and respiratory illnesses, increases with total lifetime exposure to cigarette smoke (Petruzzelli et al., 1997).

Rhodanese (EC 2.8.1.1) is found in both eukaryotes and prokaryotes (Westley, 1981). This mitochondrial enzyme is present in numerous tissues of mammals. Rhodanese is a sulphur transferase that catalyses the formation of thiocyanate from cyanide and thiosulphate or other suitable sulphur donors (Westley, 1981). Earlier study also indicated that rhodanese plays an important function in the regulation of mitochondrial respiration rate (Nagahara et al., 1995).

Arginase (EC 3.5.3.1) is the most widespread of the urea cycle enzymes in terms of tissue distribution (Kaysen and Strecker, 1973). Arginase activity has been observed in a variety of extrahepatic tissues, including, in particular, brain, kidney, small intestine and mammary gland (Jenkinson et al., 1996). The biological importance of arginase is related to its role in the control of cellular level of arginine and ornithine since these amino acids are required for various critical metabolic processes including protein synthesis, L-proline synthesis and production of polyamines (Jamshidzadeh et al., 2001). Several reports have shown that a higher activity of arginase is present in cancerous tissues (Jamshidzadeh et al., 2001). Furthermore, arginase has been found to be an index enzyme in cancer therapy (Jamshidzadeh et al., 2001; Singh et al., 2000). The main purpose of this study was to determine and compare the activities of rhodanese and arginase in blood plasma of SWD, SWOD and NS.

MATERIALS AND METHODS

All reagents used were of analytical grade and were either obtained from Sigma Chemical Company, St. Louis U.S.A or BDH Chemicals Limited, Poole, England.

Blood samples were collected from SWOD and NS around Ile-Ife town, South Western Nigeria. Blood samples of smokers with diseases SWD were collected from patients suffering from various pulmonary and heart diseases in Obafemi Awolowo University Teaching Hospital, Ile-Ife.
Collection and Treatment of Blood Samples
A total of 60 blood samples were collected from smokers and non-smokers around Ile-Ife. Blood samples were collected from 25 SWOD, 25 NS and 10 SWD using 5 ml disposable pyrogen free syringe and transferred into heparinised bottles containing lithium heparin. The blood samples were collected by venepuncture procedure and allowed to stand for 30 min to obtain supernatant (plasma) which were then used for enzyme assays and protein determination.

Enzyme assays
Arginase and rhodanese activities were determined by methods described previously by Kaysen and Strecker, (1973) and Agboola and Okonji (2004) respectively. The protein concentrations were determined as described previously (Bradford, 1976).

Statistical analysis
The results are presented as means ± SD. Data were analyzed by one-way ANOVA by using SAS/PC soft ware to examine whether there was any statistical difference among groups. If the difference evaluated with the ANOVA was significant, Duncan multiple range tests were used for paired comparisons. A P value less than 0.05 was considered statistically significant.

RESULTS
Table 1 shows the distribution of the enzymes; arginase and rhodanese in the smokers (healthy and disease) and the non-smokers. Duncan’s multiple range tests for protein (Table 1) shows that the protein level of the SWD was significantly higher than that of the SWOD and NS with p<0.0236. There was no significant difference in the activity of rhodanese in the SWD, SWOD and NS (Table 1); although, the rhodanese activity in the NS was found to be lower compared to the other two subject groups. Arginase activity in SWD was significantly higher with P<0.0007 than that of SWOD and not significantly different in the NS (Table 1). There was no significant correlation between the activity of plasma arginase of SWOD and NS.

DISCUSSION
Cigarette smoking has been reported to be one of the main causes of cancer and endothelial dysfunction (a major player in cardiovascular and respiratory diseases). Investigation on the pattern of distribution of enzymes in different tissues is of importance as this type of information can help localize certain biochemical processes that are unique to a tissue. Such information might also provide a basis for developing diagnostic and therapeutic approaches when these tissues are damaged.

In this study, there was no significant difference in the activity of rhodanese among the groups as compared to the results obtained for arginase. The high arginase activity could be consistent with the role of this enzyme in ornithine and proline biosynthesis. From the data, the arginase level in the blood plasma of SWOD is comparable with those of NS. This is expected because the NS that is passive not to be smoking could have been exposed to cigarette smoke (passive/secondary smokers). However, smoking cigarette in a disease state could have an enhancing effect on the disease as seen in the high arginase activity.

High arginase activity observed in the blood plasma of SWD may also reflect the functions of the enzyme in plasma. These include synthesis of precursors of polyamines and proline which are metabolites required for normal cell differentiation and collagen formation, respectively. It can also be speculated that the increased arginase activity may lead to less nitric oxide production, consequently increases the susceptibility to infections (Maarsingh et al., 2008).

Report has shown that free radicals contained in cigarette smoke may trigger oxidation reactions and modify biologic membranes and also modifies plasma proteins (Petruzelli et al., 1997). Reznick et al. (1992) have shown that cigarette smoke is rich in free radicals that can accelerate the production of reactive oxygen species.

Table 1:
Arginase and rhodanese activities of smokers (healthy and disease) and non-smokers (mean±SD) with Protein concentration and Age distribution

<table>
<thead>
<tr>
<th></th>
<th>Arg Activity (µmol/ml)</th>
<th>Rho Activity (RU)</th>
<th>Protein (mg)</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-smokers(n=25)</td>
<td>43.108±4.32 B</td>
<td>11.578±1.46 A</td>
<td>20.935±0.37 B</td>
<td>24.880±4.07 B</td>
</tr>
<tr>
<td>Smokers(n=25)</td>
<td>38.864±1.04 B</td>
<td>17.550±1.37 A</td>
<td>32.846±0.43 A/B</td>
<td>23.720±1.51 B</td>
</tr>
<tr>
<td>Smokers with infections(n=10)</td>
<td>205.198±14.23A</td>
<td>16.089±5.37 A</td>
<td>37.961±0.84 A/B</td>
<td>39.700±11.07 A</td>
</tr>
<tr>
<td>P values</td>
<td>&lt;0.0007</td>
<td>&lt;0.8677</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Arg= Arginase; Rho= Rhodanese; RU= Rhodanese unit; n= no of subjects; SD= Standard Deviation.
It is also possible that the free radicals in cigarette smoke must have resulted in oxidative damage to the enzyme, arginase in SWOD (Table 1) thereby reducing the activity of the enzyme considerably as compared to the arginase activity of the non-smokers.

Rhodanese activity in smokers (SWOD and SWD) was a little more than its activity in non-smokers even though there was no significant difference among the groups. This could be explained based on the content of cigarette smoke as reported by Reis et al. (2004); that when cigarette is burnt, it releases cyanide in form of hydrogen cyanide. However, Nakajima et al. (2008) has shown that rhodanese can be induced, which could possibly explain the high activity of rhodanese in smokers; that is, cyanide present in cigarette smoke could be an inducing agent for rhodanese, since the primary role of rhodanese is cyanide detoxification (Westley, 1981).

However, the activity of rhodanese in blood plasma which showed no significant difference in the three groups might indicate other function(s) for the enzyme other than cyanide detoxification. Such functions include energy metabolism through its participation in regulation of mitochondrial electron transport (Lee et al., 1995), regulation of antioxidative proteins such as superoxide dismutase (1 and 2) (Nakajima et al., 2008) and in the biosynthesis of iron-sulphur centres (Westley, 1981).

Within the limit of the present study, it can be concluded that plasma arginase activity is enhanced in SWD in contrast to SWOD and NS subjects; therefore, suggesting a possible mechanism by which cigarette smoking may lead to diseases associated with smoking cigarette. Therefore analysis of plasma arginase activity of smokers may represent an important marker enzyme in the diseases and the drugs targeting arginase pathway could have therapeutic potentials in these diseases.

Acknowledgement
The Authors wish to thank Dr. A. Adewole of Department of Human Anatomy and Cell Biology for his assistance in the collection of blood samples from SWD.

REFERENCES

Rhodanese and arginase activities in smokers