

Oxidative damage and arginase activity in tissues of rats exposed to cigarette smoke

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SUMMARY

Oxygen free radicals generated in biological systems by cigarette smoke (CS) inhalation can cause oxidative stress in tissues, resulting in lipid peroxidation. To investigate whether cigarette smoking at different periods affect peroxidation speed, we examined concentrations of malondialdehyde (MDA) and activities of the catalase (CAT) and arginase in various tissues of rats exposed to CS. A total of 30 Wistar-Albino male rats were divided into 3 equal groups (n = 10): the rats of the groups I and II have inhaled CS for 30 and 60 minutes per day respectively for a total period of 3 months, whereas the rats of the group III served as controls. Significant increases of plasma, liver, heart, vessel, kidney, testis and oesophagus MDA concentrations were observed in the groups I and II, whereas brain, muscle and spleen MDA concentrations did not change compared to controls. Stomach MDA contents were significantly lowered in both treated groups. No statistically significant difference in plasma and in tissue MDA concentrations was observed between the 2 experimental groups except for vessel and stomach contents which were significantly more elevated in the group I than in the group II. While CAT activities in erythrocyte and lung tissues of the groups I and II increased, they significantly decreased in heart and kidney tissues. The arginase activities were significantly increased in heart and spleen in the both CS exposed groups, and in testis only in the group I, whereas they were depressed in lungs for the 2 groups, in stomach only in the group I and in brain only in the group II. These results suggest that the increased production of oxygen free radicals by CS may be responsible for an enhanced risk of various diseases related to cigarette smoking (lung and kidney cancers, cardiovascular diseases...) amplified by the CAT inactivation, while arginase activities seemed to be independent of MDA fluctuations and of CS exposure.

Keywords: Cigarette smoke, plasma, tissue, malondialdehyde, catalase, arginase.

RÉSUMÉ

Stress oxydatif et activité en arginase dans les tissus de rats exposés à la fumée de cigarette

Les radicaux oxygénés libres générés dans les systèmes biologiques par l'inhalation de la fumée de cigarette peuvent causer un stress oxydatif dans les tissus, responsable d'une peroxydation lipidique. Afin de vérifier si la durée d'exposition à la fumée affecte l'intensité de cette peroxydation, nous avons mesuré les concentrations de malondialdéhyde (MDA) et les activités de la catalase (CAT) et de l'arginase dans différents tissus de rats exposés à la fumée de cigarette. Au total, 30 rats mâles Wistar albinos ont été répartis en 3 groupes égaux (n = 10) : les rats des groupes I et II ont inhalé la fumée de cigarette pendant 30 et 60 minutes par jour respectivement pendant une période de 3 mois, tandis que les animaux du groupe III ont servi de contrôles. Les concentrations de MDA dans le plasma ainsi que dans le foie, le cœur, les vaisseaux, les reins, les testicules et l'œsophage ont significativement augmenté dans les groupes I et II alors que les concentrations mesurées dans le cerveau, les muscles et la rate n'ont pas été significativement modifiées par rapport à celles observées chez les contrôles. Les teneurs stomacales de MDA ont été significativement abaissées dans les 2 groupes traités. Aucune différence significative des concentrations plasmatiques ou tissulaires de MDA n'a été mise en évidence entre les 2 groupes traités à l'exception des concentrations vasculaires et stomacales qui se sont avérées significativement plus élevées dans le groupe I que dans le groupe II. Alors que les activités de la CAT dans les érythrocytes et les poumons ont augmenté dans les groupes I et II, celles mesurées dans le cœur et les reins ont significativement diminué. Les activités en arginase ont significativement augmenté dans le cœur et la rate des rats appartenant aux 2 groupes traités, dans les testicules seulement chez les rats du groupe I, et elles ont diminué dans les poumons de tous les rats traités, ainsi que dans l'estomac des rats du groupe I et dans le cerveau des rats du groupe II. Ces résultats suggèrent que la fumée de cigarette provoque une production accrue de radicaux oxygénés libres responsable d'une augmentation du risque d'apparition de certaines maladies reliées au tabagisme (cancers des poumons et des reins, maladies cardiovasculaires...) d'autant plus forte que la CAT est inactivée alors que l'activité de l'arginase serait indépendante des fluctuations de MDA et de l'exposition à la fumée.

Mots-clés : Tabagisme, plasma, tissus, malondialdéhyde, catalase, arginase.

Introduction

Cigarette smoke (CS) contains a large variety of compounds including many oxidants and free radicals that are capable of initiating or promoting oxidative damage. These include various compounds, which are capable to cause increases of the generation of various reactive oxygen species (ROS) such as superoxide anion (O₂⁻), hydrogen peroxide (H₂O₂), hydroxyl radical (OH), peroxy radical (ROO). Oxidative

damage may result from ROS generated by the activated phagocytes following cigarette smoking. In general, ROS are compounds characterized by an unpaired electron, which makes them very highly reactive towards proteins, lipids and nucleic acids, thus initiating peroxidation reactions that cause irreversible cell damage in the different tissues. These ROS in turn are capable of initiating and promoting oxidative damage in the form of lipid peroxidation [18, 23]. *In vitro* studies are generally supportive of the hypothesis that CS can initiate or

promote oxidative damage [7]. Cigarette smoking may thus be associated with an increase of the incidence and severity of various diseases like cancer, chronic obstructive lung disease, arteriosclerosis and cardiovascular disease [42, 45]. The reason may be that free radicals exist in CS [48]. Evidence suggests that the free radicals in CS contribute to the adverse effects of smoking cigarettes. CS causes lipid peroxidation, oxidation of protein thiols and alterations of plasma protein carbonyls [10, 36].

H₂O₂ is a product of cellular metabolism that must be removed efficiently in order to avoid its cytotoxic effects on living cells or its reduction in the presence of Fe²⁺ (Fenton reaction) into other ROS, such as HO·, which is a particularly damaging free radical generated *in vivo*. To protect cells from the presence of H₂O₂, there are two enzymatic scavenging systems: catalase (CAT) and glutathione peroxidase (GSH-Px). The CAT (EC 1.11.1.6) is a haemoprotein characterized by its ability to rapidly metabolize relatively high concentrations of H₂O₂ by reducing it to water and oxygen [9, 15]. The deleterious effects of the free radicals are kept under check by a delicate balance between the rates of their production and their elimination by the different antioxidant systems. Any shift can result in an increase of the peroxidative stress and may lead to cellular damage. As smokers are being subjected to oxidative stress resulting from oxidants and free radicals present in smoke, as well as ROS generated by increased activated phagocytes, their antioxidants status is likely to be adversely affected [8, 43, 46].

Arginase (L-arginine amidinohydrolase, EC 3.5.3.1) catalyses the hydrolysis of L-arginine into L-ornithine and urea in the final reaction of the urea cycle [34]. The main source of the arginase enzyme is the mammalian liver which provides the urea cycle. Lower arginase activities are also found in extra-hepatic tissues such as the kidney, heart muscle, intestine, lung, spleen, brain, and skeletal muscle [2, 41]. It is important to understand the inter-relationships between arginase and nitric oxide synthase (NOS), since both enzymes are able to utilize arginine as substrate: arginase which catalyzes the hydrolysis of arginine into urea and ornithine and NOS which oxidizes arginine into citrulline and NO, considered as a ROS. Consequently, an increase of arginase activity would indirectly reduce NO₂ production and promote the antioxidant response [29].

This study was designed to investigate the effects of CS inhalation at the different times on the lipid peroxidation and antioxidant defence systems, including arginase activity in rat tissues.

Material and Methods

ANIMALS AND PROTOCOL DESIGN

Totally 30 Wistar-Albino male, 8 week old rats weighing of 200-250 g were obtained from a local Veterinary Research Institute. All protocols in the present study were approved by the local ethics committee in the College of Veterinary Medicine at the Firat (Euphrates) University, Elazig, Turkey. Upon arrival, the animals were allowed to acclimatise for 2

weeks. Rats were housed in a temperature-controlled room (22-25 °C) with a 12:12 light: dark cycle; water and food were given *ad libitum*. The rats were divided into three groups of 10 animals. Two groups inhaled CS in an inhalation cabin for a period of 3 months (group I: for 30 minutes per day / group II: for 60 minutes per day). The third group served as the control group, and inhaled clean air. A glass cabin was prepared and insulated with silicone (dimension: 50x35x36 cm, thickness 0.5 mm, inner volume 0.060 m³). A short plastic pipe, with one end left outside, was inserted into the cabin.

The cigarette was lit during the experiment and placed at the end of this pipe and the entire cigarette was puffed. During this study, cigarettes without a filter tip (Bitlis-Tekel) were used. Rats were decapitated on the day after following their last cigarette, then blood and tissue samples were taken from the animals. Blood samples were collected into tubes containing EDTA. The samples were centrifuged at 2 000 g for 5 min at 4°C, and then the plasma was immediately harvested and kept for the assay of plasma malondialdehyde (MDA) concentration. The remaining erythrocytes were washed three times with 5 ml of 0.9% NaCl, and erythrocyte lysates were used for determination of CAT and arginase activities as well as haemoglobin concentration.

Tissue specimens were removed rapidly and utilized for biochemical analysis, as described below. Tissue samples were kept at -20°C until analysed. Tissue specimens were weighed, rinsed with ice-cold deionised water, cut into small pieces and then dried on a filter paper. The tissues were homogenized and diluted to a concentration of 1:10 using the appropriate buffer, depending upon the measured variable. In this way, with the use of Teflon tips, tissue samples (w/v) were homogenized in an Elvehjem-Potter homogeniser (Dupont Instruments, Sorwall Homogenizer, USA). The homogenates were centrifuged at 1 000 g for 15 min at 4°C and the supernatants were used for MDA and CAT assays. The homogenates were centrifuged at 18 000 g for 30 min at 4°C and the supernatants were used for the arginase assay.

BIOCHEMICAL ANALYSIS

Plasma MDA concentrations (the end product of lipid peroxidation) were measured according to the method of SATOH [39]. MDA contents of tissue homogenates were assayed spectrophotometrically according to the method of OHKAWA *et al.* [30]. MDA concentrations in plasma and tissue were expressed as nmol/ml and nmol/g tissue, respectively. CAT activity was estimated by measuring the breakdown of H₂O₂ at 240 nm according to the method of AEBI [1]. CAT activity was expressed as k/g Hb in erythrocytes and k/g protein in tissues. Arginase activity was spectrophotometrically measured by the thiosemicarbazide diacetylmonoxime urea (TDMU) method of GEYER AND DABICH [16]. One unit of arginase activity was expressed as the amount of enzyme catalysing the formation of 1 µmole of urea during 1 hour at 37°C. The results were given as unit/mg of protein. Haemoglobin (Hb) concentration was determined according to DRABKIN [13] whereas tissue protein contents were determined by the method of LOWRY [27].

STATISTICAL ANALYSIS

Results were expressed as mean \pm standard errors (SEM). ANOVA test was used for statistical analysis. The 5% level of significance was used to establish differences. Simple correlation analysis was performed.

Results

Plasma MDA concentrations were significantly increased in the 2 experimental groups compared to the control group ($p < 0.05$). In the same way, significant increases ($p < 0.05$) of MDA contents in liver, heart, vessel, kidney, testis and oesophagus were evidenced for the 2 groups of CS-inhaled rats. Curiously, trachea and lung MDA contents were significantly enhanced in the group I (CS for 30 min/day) compared to the group III (control) ($p < 0.05$) and not in the group II (CS for 60 min/day) (Table I). By contrast, stomach MDA contents were significantly lowered in both treated groups ($p < 0.05$) and no significant alteration of tissue MDA concentrations was noted in brain, muscle and spleen. Although no statistically significant difference in plasma and in tissue MDA concentrations was observed between the 2 experimental groups (except for vessel and stomach contents which were significantly more elevated in the group I than in the group II), the rats of the group I seemed to produce more ROS than animals of the group II.

CAT activities of all groups are summarized in Table II. While CAT activity in erythrocyte and lung tissue of experimental groups significantly increased compared to the control group, it decreased in the heart and kidney ($p < 0.05$), but it did not change in spleen. No statistical difference in

CAT activities was evidenced between the 2 experimental groups.

Arginase activities (Table III) in heart and in spleen were significantly increased in the 2 treated groups compared to the control group ($p < 0.05$). In the heart, the enzyme activity was significantly higher in the group II ($p < 0.05$) whereas spleen arginase activity was similar in the both 2 groups. Furthermore, testis activity only enhanced in rats of the group I (CS exposition: 30 min/day) ($p < 0.05$). By contrast, arginase activity was depressed in lung in the 2 experimental groups ($p < 0.05$), in stomach only in the group I ($p < 0.05$), in brain only in the group II ($p < 0.05$), and did not significantly vary in liver, trachea and in kidney compared to the controls.

In all rats, heart and kidney CAT activities negatively correlated with the corresponding tissue MDA contents ($r = -0.64$ and $r = -0.82$, $p < 0.01$ respectively) whereas this enzyme activity was positively associated with MDA content ($r = 0.47$, $p < 0.01$) in lung (Table IV). When only the population of CS exposed rats was taken into consideration, highly significant negative correlations were found between these 2 parameters in kidney and in spleen ($r = -0.91$ and $r = -0.72$, $p < 0.01$ respectively) and a positive correlation was observed in heart ($r = 0.62$, $p < 0.01$) (Table IV). Arginase activities were positively correlated with MDA contents in the spleen, the heart and the testis ($r = 0.44$, $p < 0.05$; $r = 0.55$ and $r = 0.51$, $p < 0.01$, respectively) in all rats (Table V). In the population of CS exposed rats (groups I and II), these 2 parameters were positively associated in the trachea ($r = 0.56$, $p = 0.01$) and negatively in the liver and the stomach ($r = -0.48$, $p < 0.05$ and $r = -0.73$, $p < 0.01$ respectively) (Table V). No correlation (positive or negative) between CAT and arginase activities was evidenced in any analyzed tissues.

| MDA concentrations | Group III (Control) | Group I (30 min/day) | Group II (60 min/day) |
|----------------------------|------------------------------|-------------------------------|-------------------------------|
| Plasma (mmol/L) | 1.54 \pm 0.13 ^a | 4.28 \pm 0.23 ^b | 5.23 \pm 0.78 ^b |
| Vessel (nmol/g tissue) | 3.10 \pm 0.17 ^a | 7.51 \pm 0.96 ^c | 4.87 \pm 0.14 ^b |
| Spleen (nmol/g tissue) | 3.90 \pm 0.37 | 4.24 \pm 0.44 | 4.44 \pm 0.32 |
| Heart (nmol/g tissue) | 3.57 \pm 0.19 ^a | 6.61 \pm 0.44 ^b | 7.65 \pm 0.57 ^b |
| Muscle (nmol/g tissue) | 5.95 \pm 0.14 | 5.21 \pm 0.39 | 6.36 \pm 0.73 |
| Liver (nmol/g tissue) | 9.62 \pm 0.28 ^a | 13.24 \pm 1.00 ^b | 12.19 \pm 0.63 ^b |
| Trachea (nmol/g tissue) | 10.5 \pm 1.38 ^a | 12.00 \pm 2.08 ^b | 9.70 \pm 0.64 ^a |
| Lung (nmol/g tissue) | 3.50 \pm 0.41 ^a | 7.05 \pm 0.80 ^b | 5.24 \pm 0.44 ^{ab} |
| Oesophagus (nmol/g tissue) | 3.15 \pm 0.70 ^a | 7.19 \pm 0.87 ^b | 6.83 \pm 0.86 ^b |
| Stomach (nmol/g tissue) | 9.32 \pm 1.14 ^a | 4.56 \pm 0.91 ^b | 2.08 \pm 0.16 ^c |
| Kidney (nmol/g tissue) | 6.28 \pm 0.81 ^a | 16.96 \pm 0.81 ^b | 17.38 \pm 1.24 ^b |
| Brain (nmol/g tissue) | 4.12 \pm 0.21 | 4.64 \pm 0.55 | 4.53 \pm 0.35 |
| Testis (nmol/g tissue) | 6.11 \pm 0.26 ^a | 9.93 \pm 0.51 ^b | 8.43 \pm 0.78 ^b |

Different superscripts a,b,c in the same row indicate statistically significant changes ($p < 0.05$).

TABLE I: Plasma and tissues (vessel, spleen, heart, muscle, liver, trachea, lung, oesophagus, stomach, kidney, brain and testis) MDA concentrations in rats experimentally exposed to cigarette smoke (CS) and controls (group III). Rats of the group I and II were exposed to cigarette smoke inhalation for 30 min/day and 60 min/day for 3 months, respectively. MDA values are expressed as means \pm standard errors (SEM).

| CAT activity | Group III (Control) | Group I (30 min/day) | Group II (60 min/day) |
|-----------------------|----------------------------|----------------------------|----------------------------|
| Erythrocyte (k/g Hb) | 0.028 ± 0.002 ^a | 0.053 ± 0.008 ^b | 0.051 ± 0.001 ^b |
| Heart (k/mg protein) | 0.92 ± 0.03 ^a | 0.39 ± 0.06 ^b | 0.42 ± 0.04 ^b |
| Lung (k/mg protein) | 3.14 ± 0.45 ^a | 8.42 ± 0.18 ^b | 9.12 ± 0.21 ^b |
| Kidney (k/mg protein) | 5.20 ± 0.92 ^a | 3.68 ± 0.16 ^b | 3.66 ± 0.04 ^b |
| Spleen (k/mg protein) | 0.55 ± 0.08 | 0.58 ± 0.04 | 0.58 ± 0.04 |

Hb: Blood haemoglobin concentration.

Different superscripts a,b,c in the same row indicate statistically significant changes ($p < 0.05$).

TABLE 2: CAT activities in erythrocytes and tissues (heart, lung, kidney and spleen) of rats experimentally exposed to cigarette smoke (CS) and controls (group III). Rats of the groups I and II were exposed to cigarette smoke inhalation for 30 min/day and 60 min/day for 3 months, respectively. Values are expressed as means ± standard errors (SEM).

| Arginase activity | Group III (Control) | Group I (30 min/day) | Group II (60 min/day) |
|------------------------|----------------------------|----------------------------|-----------------------------|
| Spleen (U/mg protein) | 0.21 ± 0.01 ^a | 1.02 ± 0.12 ^b | 0.80 ± 0.17 ^b |
| Heart (U/mg protein) | 0.07 ± 0.006 ^a | 0.12 ± 0.004 ^b | 0.19 ± 0.004 ^c |
| Liver (U/mg protein) | 63.35 ± 5.74 | 94.00 ± 10.72 | 93.10 ± 12.63 |
| Trachea (U/mg protein) | 1.30 ± 0.87 | 0.31 ± 0.24 | 0.17 ± 0.03 |
| Lung (U/mg protein) | 0.61 ± 0.03 ^a | 0.13 ± 0.03 ^b | 0.18 ± 0.03 ^b |
| Stomach (U/mg protein) | 1.80 ± 0.09 ^a | 0.72 ± 0.11 ^b | 1.56 ± 0.11 ^a |
| Kidney (U/mg protein) | 16.28 ± 2.33 | 16.75 ± 0.73 | 15.20 ± 1.02 |
| Brain (U/mg protein) | 0.150 ± 0.004 ^a | 0.130 ± 0.011 ^a | 0.040 ± 0.004 ^b |
| Testis (U/mg protein) | 0.080 ± 0.003 ^a | 0.240 ± 0.036 ^b | 0.130 ± 0.009 ^{ab} |

Different superscripts a,b,c in the same row indicate statistically significant changes ($p < 0.05$).

TABLE 3: Arginase activities in tissues (liver, heart, lung, brain, trachea, kidney, testis, stomach and spleen) of rats experimentally exposed to cigarette smoke (CS) and controls (group III). Rats of the groups I and II were exposed to CS inhalation for 30 min/day and 60 min/day for 3 months, respectively. Results are expressed as means ± standard errors (SEM).

Discussion

In the present study, increases of plasma and tissue MDA concentrations were observed in CS-exposed rats, particularly in kidney, vessel, oesophagus, lung, heart, testis and liver, and these alterations were roughly independent of the duration of inhalation. CS has been implicated as a risk factor in many diseases such as pulmonary and cardiovascular pathologies [48]. According to epidemiological studies, about 30% of cancer-caused deaths were due to smoking. The mortality rate of smoking lung cancer patients is about 10 times higher than that of non-smoking patients [48]. The risk of throat cancer in smoking population is 20-30 times higher than in non-smoking people. Moreover 30-40% of bladder and kidney cancers are related to smoking. The incidence of oesophageal cancer in the smoking population is 10 times higher than in non-smoking people. The risk of heart attack is directly related to the heaviness of smoking [37]. We can observe that tissues able to greatly generate ROS under CS exposure were also potential targets of tobacco-induced cancers.

The plasma MDA concentrations were higher in the rats exposed to CS than in non-smoke-exposed rats. It was previously found that serum MDA concentrations were

higher in smokers than in non-smokers [23] and it is well known that CS induces oxidative stress in plasma [10]. During the development of oxidative stress, oxygen-derived free radicals such as O_2^- are generated into the cells. This O_2^- forms H_2O_2 by dismutation, which finally undergoes an iron-catalysed reaction to form cytotoxic OH. The breakdown of membrane phospholipids and lipid peroxidation demonstrated in many diseases may be free-radical mediated [4, 33]. Oxygen free radicals have been suggested to exert their cytotoxic effect by causing peroxidation of membrane phospholipids, which could result in alterations in membrane fluidity, increasing permeability and loss of membrane integrity [14].

KOUL *et al.* [25] reported that in the first phase of investigation, where mice were subjected to CS inhalation and/or fed with α -tocopherol for 2 weeks, no significant change in pulmonary lipid peroxidation, in reduced glutathione content or in any of the enzymatic antioxidants was observed, except for glutathione reductase. It seems that the treatment of CS inhalation for 2 weeks was not enough to induce any significant oxidative stress and thereby no increase of lipid peroxidation. As the exposure of CS to the mice was extended from 2 to 4, 6 and 8 weeks, significant increases of the lipid peroxidation

| Tissues | Correlations between CAT All rats | activities and MDA contents Groups I and II |
|-------------|--------------------------------------|--|
| All tissues | r = -0.23 | r = -0.37 |
| Heart | r = -0.64** | r = 0.62** |
| Lung | r = 0.47** | r = 0.07 |
| Kidney | r = -0.82** | r = -0.91** |
| Spleen | r = -0.01 | r = -0.72** |

* p < 0.05 and ** p < 0.01

TABLE 4: Correlation coefficients between tissue MDA concentrations and CAT activities in rats experimentally exposed to cigarette smoke (CS) and controls (group III). Rats of the groups I and II were exposed to CS inhalation for 30 min/day and 60 min/day for 3 months, respectively.

| Tissues | Correlations between CAT arginase All rats | activities and MDA contents Groups I and II |
|-------------|---|--|
| All tissues | r = 0.20 | r = 0.18 |
| Spleen | r = 0.44* | r = 0.02 |
| Heart | r = 0.55** | r = 0.11 |
| Liver | r = 0.09 | r = - 0.48* |
| Trachea | r = 0.20 | r = 0.56** |
| Lung | r = - 0.35 | r = 0.20 |
| Stomach | r = 0.06 | r = - 0.73** |
| Kidney | r = - 0.04 | r = 0.10 |
| Brain | r = 0.06 | r = 0.09 |
| Testis | r = 0.51** | r = 0.01 |

* p < 0.05 and ** p < 0.01

TABLE 5: Correlation coefficients between tissue MDA concentrations and arginase activities in rats experimentally exposed to cigarette smoke (CS) and controls (group III). Rats of the groups I and II were exposed to CS inhalation for 30 min/day and 60 min/day for 3 months, respectively.

intensity was observed, and were attenuated by dietary vitamin E supplementation. Moreover, they observed that the antioxidant enzymes like CAT, glutathione peroxidase (GSH-Px) and glutathione reductase increased significantly in the smoked groups, but also in α -tocopherol + smoked groups after 4, 6 and 8 weeks of CS exposure where antioxidant vitamin E still could not prevent the increase of lipid peroxidation. These increases of the enzyme activities would be a non-specific response to the treatment or would be not sufficient to efficiently reduce CS exposure-induced oxidative stress.

There were significant increases of the MDA contents in trachea and lung of CS exposed animals. Active oxygen species, probably derived from the CS, played a role in smoke-mediated fibber transport into tracheobronchial epithelium [43]. Similarly, CHURG and CHERUKUPOLLI [8] reported that smoke inhalation for 30 days caused slight trachea and lungs damage in rats. They concluded that i) CS rapidly produced lipid peroxidation in tracheal segments *in vitro*, ii) the severity of the process was directly related to the amount of smoke exposure, and iii) inflammatory cells are not required for this effect. Lipid peroxidation in this system appears to be mediated by active oxygen species, because aqueous extract of CS directly induced an increased formation

of lipid peroxidation products [26]. In a previous study [31], we have reported that the proliferation of bronchus and bronchiole epithelia and the installation of pulmonary fibrosis and emphysema were correlated with the duration of smoking in rats. Furthermore, the increase of macrophage number and their subsequent activation were particularly marked in subjects that have inhaled CS for long periods. It was stated that the free radicals of CS caused tissue damage. In spite of an increase of lung CAT in this study, MDA concentrations in this tissue remained increased in CS exposed rats. This indicates that the CS exposure increased ROS generation in the lung and that antioxidant defence mechanisms in the lung did not sufficiently protect the respiratory system from smoke mediated oxidative injury. Because CS is a complex mixture of numerous reactive substances, cigarette smoking can elicit complicated physiological and pathological responses including inflammatory-immune system activation. Therefore, oxidative damage of cell components can be result not only from a direct reaction with reactive substances in the smoke but also from the smoke-induced secondary events such as activation and infiltration of phagocytes into the lung [32].

No significant difference in brain MDA contents between CS-exposed rats and controls was obtained. Similarly,

BASKARAN *et al.* [5] reported that brain MDA concentrations did not significantly differ between animals exposed to CS and control animals, whereas MDA contents enhanced in lung, liver and kidney, suggesting that CS did not cause free radical-mediated tissue damage in the hippocampus. However, knowledge of the effects of CS on lipid peroxidation, antioxidant enzymes in the hippocampus is lacking [11].

We observed a large increase of testis and vessel MDA concentrations after 3 months of exposure to CS. RAJPURKA *et al.* [35] stated that the MDA contents of the treated rat testis increased in comparison with controls. Evidence has accumulated that CS destroyed the endothelial structure of the blood vessels, increased the cholesterol, lipoprotein and endothelin-1 concentrations in serum and could cause arteriosclerosis [44]. In the same way, inhalation of CS resulted in a profound increase of liver MDA content. The enhanced liver MDA concentration is indicative of oxidative injury of the hepatic membrane after exposure to CS.

A not significant increase of spleen MDA concentrations was observed after 3 months of exposure to CS, but MDA concentrations significantly decreased in stomach. These results were in agreement with those of KIM *et al.* [24] which have reported that chronic environmental tobacco smoke exposure can increase lipid peroxidation in red blood in lung and in spleen tissues, but not in stomach tissue. Higher indices of lipid peroxidation in spleen can be related to decrease immune functions caused by acute and chronic exposure to nicotine and other toxic components. It has been proposed that benzopyrene and CS condensates produce cytogenetic damage, which is mediated by signalling of aryl hydrocarbon receptors and antigen-mediated T cells, and that they also deplete IP3-sensitive calcium stores [12]. The other hypothesis for the increased vulnerability of spleen is presumed to be reduced systemic blood flow [40]. The general mechanisms of the patho-physiological events resulting from CS-induced oxidative damage of biological macromolecules and tissues have been mainly centred around the unstable free radicals and ROS present in the CS including O₂, H₂O₂, alkoxyl radicals, ROO., NO and NO₂ for quite a long period [32].

HOIDAL *et al.* [21] found that cigarette smoking did not lead to a general induction of antioxidant enzymes. Increased activities of superoxide dismutase (SOD) and CAT, which play important roles in scavenging the toxic intermediates of incomplete oxidation, were commonly found, but GSH-Px was not changed in pulmonary alveolar macrophages from smokers or in hamsters that inhaled CS [19, 28, 44]. TOTH *et al.* [44] reported that erythrocyte GSH-Px and CAT activities were increased in smokers compared to non smokers. However, the relationship between red blood cells and lung cells for antioxidant capacity *in vivo* was not clear. HUSAIN *et al.* [22] have demonstrated that the chronic administration of nicotine significantly decreased CAT activity in liver, as well as significantly increased CAT activity in lung and testis. It was also reported that CAT activities in kidney and heart decreased [3, 20]. In agreement with these studies, we observed marked decreases of CAT activities in heart and kidney, while MDA contents were greatly increased in these tissues: taken all rats (controls and CS-exposed) into consideration, a significant negative correlation was obtained

between MDA concentrations and CAT activities in heart and in kidney ($p < 0.01$) suggesting that free radicals present in smoke roughly inactivate this enzyme. Nevertheless, as in exposed rats these 2 parameters were positively associated ($p < 0.01$) in the heart and strongly negatively associated in the kidney, the enzyme inactivation may be partial in the heart and total in the kidney. The increased lipid peroxidation may be amplified by a decrease of the antioxidant system: a decrease of this enzyme activity can lead to the excessive availability of superoxides and ROO., which in turn generate OH. radicals resulting in the initiation and propagation of lipid peroxidation [38]. These results suggest that the risk of cigarette smoking for heart and kidney diseases might be related to an increased production of oxygen free radicals amplified by depression of antioxidant systems.

However, increased CAT activities in lung and erythrocyte after exposure to CS are indicative of an efficient elimination of toxic H₂O₂ in these respective tissues and lung CAT activities positively correlated with pulmonary MDA concentrations ($p < 0.01$) in all rats. The antioxidant enzyme activity was enhanced in order to protect these tissues against the deleterious effect of the oxygen derived free radicals. MCCUSTER and HOIDAL [28] have already shown that the activities of SOD and CAT from alveolar macrophages of smokers and smoke-exposed hamsters increased two-fold compared to the control group, but there was no change in the activity of GSH-Px. Some studies have shown that the administration of CAT resulted in protection against H₂O₂-mediated lipid peroxidation [3, 44, 47]. In this way, in the present study, MDA concentrations and CAT activities in spleen were markedly negatively correlated ($p < 0.01$) in CS exposed rats, suggesting that even a slight activation of the enzyme may neutralize a weak ROS overproduction. In spite of the induction of defence enzymes such as CAT, the antioxidant capacity seems to be swamped by enhanced active oxygen radicals. It was shown that myocardial tissue was attempting to detoxify oxygen free radicals; however, the attempt was insufficient and the defence system was overwhelmed. The antioxidant activities were not sufficient to cope with the oxidative stress produced and did not prevent the lipid peroxidation [42, 44]. Furthermore, a study by HARATS *et al.* [17] on smokers smoking five to seven cigarettes during a 90 minute interval reported significant increases of lipid peroxidation. Protection against this increase was achieved by dietary supplementation of vitamin C or E for several weeks before their experiment. Vitamin C, which can facilitate both vitamin E recycling and vitamin A stability, is an important part of antioxidant defence under conditions of smoke exposure [6, 43]. Consequently, it would be probable that accumulation of other antioxidants (E and C vitamins) in lungs would partially protect the CAT enzyme for ROS inactivation.

No significant difference in the MDA concentrations and in CAT activities was noted according to the duration of daily CS exposure (30 min vs. 60 min). Even, it seems that CS exposure for 30 min/day produced more ROS than for 60 min, thereby increased the lipid peroxidation. It may be possible that 30 min/day of CS was enough to tilt the balance between the oxidative stress due to CS and the efficiency of endogenous antioxidants.

Our literature review found no study on the effect of CS on arginase in rat tissues. The present study shows that arginase activities in heart, spleen and testis tissues increased significantly during CS inhalation and positively correlated with the tissue MDA contents in all rats, whereas they were lowered in lung, stomach (group I), and brain (group II) and they were not altered in kidney, trachea and liver. Except for the brain, the highest increases of arginase activities were recorded in tissues where MDA contents were similar to control values (spleen). The variations of arginase activities in the population of CS exposed rats were diversely correlated (positively or negatively) with the MDA contents of the liver, trachea and stomach which were weakly increased or even decreased (stomach) compared to controls. Furthermore, no correlation between CAT and arginase activities was evidenced in any analyzed tissues. Taken together, these results suggest that arginase activity would not be directly linked to CS-induced oxidative stress or to antioxidant systems.

The results of our study suggested that CS induced lipid peroxidation in liver, lung and kidney and that CAT activities were enhanced in lungs but not in kidney and heart in order to protect these tissues against the deleterious effects of the ROS whereas arginase activities seemed independent of tissue ROS accumulation. CS may cause oxidant/antioxidant imbalance and it may cause peroxidation in tissues. We also observed that passive CS was independent of the daily duration of the CS exposure.

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