Original Investigation

Effects of Cigarette Smoke on Tissue Trace Element Concentration of Rats Exposed to Second-hand Smoke

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Abstract

Trace elements have an important effect on and play a key role in a variety of the processes necessary for life. Studies have indicated a definite correlation between content of trace elements and many common diseases. It has been concluded that smoking may be a substantial source of intake of these hazardous elements, not only to the smoker, but to nonsmokers via passive smoke, as well. Even passive intake of such elements can change the metabolism of other trace elements and influence their concentrations. In order to assess their potential role in some human diseases, it is necessary to measure trace element concentrations in various tissues in experimental models. In this study, liver, kidney and spleen tissue samples from rats exposed to secondhand smoke were analysed for Fe, Cu, Zn, Cr, Mn and Co trace element levels by atomic absorption spectrophotometer. Cr, Mn, Fe and Co levels in the liver, Fe and Co levels in the kidney, and Zn, Cu, Mn and Co levels in the spleen were significantly lower than those of controls, but Cu levels in the kidney and Fe levels in the spleen were significantly higher than those of controls. Our data suggest that chronic exposure to cigarette smoke alters the trace element concentration of various tissues in rats exposed to secondhand smoke. These alterations may be attributable to oxidative stress produced by cumulative effect of inhaled smoke rather than the toxic effect of absorbed toxic metals. Low Mn levels in the liver and spleen, increased Cu levels in kidney and Fe levels in the spleen, and changes in the metabolism of Zn, Fe and Cu may be indicators of oxidative stress. Decreases in Co and Cr levels in rats exposed to secondhand smoke may also be related to the intake of the toxic trace elements present in cigarette smoke.

Key words: toxic elements, trace elements, cigarette smoke, oxygen free radical, oxidative stress

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Introduction

Essential trace elements have an important effect on and play a key role in a variety of the processes necessary for life. Various studies have indicated a definite correlation between trace element content and many common diseases [1-3]. Some carcinogens have been detected in tobacco and the particulate phase of tobacco smoke, and it is known that some toxic metals can cause cancer. Ni and Cr are carcinogenic agents [4]. There is an evidence in support of positive associations between As and risk of both lung and bladder cancers, and between Cd and lung cancer risk [5]. Human studies have shown an association between respiratory cancer and exposure [6]. In another work, it was demonstrated that genetic polymorphisms might provide relevant information to identify individuals with higher risk for lung cancer, due to arsenic exposure [7]. The ability of cadmium to produce neoplasms in the prostate and develop testicular tumors of rats were reported [8]. These elements compete for binding sites in the cell, change its enzymatic activity and exert direct or indirct action on the carcinogenic process accelerating the growth of tumors [9]. Metalloenzymes, which bind metals, control the chemical changes in tissue cells.

Previously, Sb, Br, As, Cd and Co (as cobalt carbonyl) have been shown to be toxic to the human biosystem, even at very low levels of intake [10-11]. Since As, B, Ba, Br, Cd, Cl, Cs, Cu, Hg, I, K, Li, Mn, Na, Pb, Rb, Sb, Sn, Tl and Zn are present in cigarette smoke [12]. It may be concluded that cigarette smoking is a substantial source of intake of these toxic elements; not only for the smoker, but to nonsmokers through passive smoking, as well**.**

Some of toxic metals especially Pb and Cd enter the body via inhalation. Inhalation absorption of Pb in adults varies between 30% and 85%, depending on respiratory rate, particle size, composition and solubility. Absorption of Pb via ingestion is lower, typically 10% to 15% in adults. Once absorbed, Pb can be found in the blood and soft tissues or the skeletal system. Exposures to Cd can occur through cigarette smoking and environmental exposures. Gastrointestinal absorption of cadmium is estimated to be around 5% to 8%. Inhalation absorption of Cd is generally higher, ranging from 15% to 30%. Cd absorption after inhalation such as from cigarette smoke can be as high as 50%. Once absorbed, Cd is highly bound to the metal binding protein “metallothionein” [13].

The intake of trace elements through passive smoking can change the metabolism of other essential trace elements and influence their concentrations [14]. It is therefore necessary to

measure these elements in various tissues in order to assess their potential role in various diseases. In humans, trace elements are mainly investigated in sera, not tissue; however, the presence of trace elements in blood may not accurately represent the degree of exposure [15]. Since an animal model is necessary in order to investigate trace elements' metabolism, we planned our study to focus on specific tissues in rats exposed to secondhand smoke, a group in which data would otherwise be difficult to determine. Unlike previous studies, we aimed to analyze the effect of cigarette smoke on the levels of Cr, Mn, Co, Fe, Cu and Zn in the tissues of the liver, kidney and spleen. We chose to study these elements since they are influenced by toxic metals and plays a role in antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT).

Materials and Methods

Sample collection

Sixteen Wistar Albino adult male rats, weighing from 250-300 g, comprised the experimental and control groups. Kocaeli University Medical Faculty Ethic Committee approved the experimental design. All rats were fed tap water and ad libitum, and had free access to standard laboratory rat chow. Wood shavings were used for bedding. The model of passive and sidestream smoking (collected from the smouldering end of a lighted cigarette) was conducted by modifying from Xie et al. [16] as follows: The rats in the secondhand smoke group (n = 8) were placed in a glass chamber with a capacity of 150 dm^3. The chamber had 3 holes, each 3 cm in diameter; 2 were placed in opposing walls, and the third was connected to a standard smoking machine (Bogwaldt, Germany). The machine was programmed to replicate standard human puff duration and frequency. A national brand of filtered cigarettes, containing 12 mg tar and 0.9 mg nicotine, was used. Each rat was exposed to 4 cigarettes per day for 30 minutes, twice, for 10 weeks at the same time each day (10.00-10.30 and 16.00-16.30; "smoking rats"). The interval between smoking periods was 2 hours. The animals did not have access to food in the inhalation chamber, but water was available. Two cigarettes were lit in the morning, at 10.00 and in the evening at 16.00, for 30 minutes each. At 11.30 and 16.30, the animals were removed from the inhalation chamber. Experimental and control rats were housed separately during the study and non-exposure intervals. The last smoking period was 18 hours before the experiments were conducted, and the rats were then sacrificed. The control rats (n = 8) were also placed in the chamber, but they inhaled fresh air which did not contain cigarette smoke.

Analysis of trace elements

Liver, kidney and spleen tissue samples were taken and weighed and transferred into metal-free glass tubes for Fe, Cu, Zn, Cr, Co and Mn analyses, immediately frozen and kept at a temperature of -20^0C until analysis. Samples were dried in the furnace at 120^0C for one hour, and the dry weights of tissue samples were determined. The samples were first digested with 3 ml of concentrated nitric acid at 100^0C in the furnace for 1 hour, and 3 ml of perchloric acid (60%) was added to the cooled materials. The samples were then heated at 120^0C until their total volume was reduced by half. Digested materials were diluted with deionised water to 5 ml. Last dilutions of samples were mixed in a shaker for 15 minutes just prior to measurement. A blank solution of deionized water and the acids used for tissue digestion was prepared by keeping the water/acid ratio in prepared tissue samples to analyze. Zn and Fe were analyzed by Analytik Jena Vario 6 Flame atomic absorption spectrophotometer, based on comparison with external standards. The spectral light sources were Zn and Fe hollow cathode lamps [17-18]. A Perkin-Elmer Zeeman Z/3030 atomic absorption spectrophotometer with Zeeman-effect background correction, equipped with a HGA-600 graphite furnace and AS-60 auto sampler, was used to determine levels of Cr, Cu, Mn and Co. The spectral light sources were Cr, Cu, Mn and Co hollow cathode lamps. Pyrolytic graphite coated tubes without or with special dual cavity platforms (Rinsgdorff Werke GmbH, Bonn, Germany, RW0553/4), which had two cavities instead of one, and were made from solid pyrolitic graphite, were used. These were inserted into pyrolytic graphite-coated tubes (Ringsdorff Werke GmbH, Bonn, Germany, RW 0555/9-HD-Pyc) in which the dosing holes were enlarged to form a slot to facilitate pipetting into the two separate cavities. All solutions were pipetted manually using an Eppendorf pipette [19]. Calibration curves for studied elements were linear up to the highest working concentrations. Limit of quantification was 0.001 μg/g. Results were calculated as μg/g wet weight of tissues.

Control sample material containing known amounts of the elements of interest was analyzed to validate the accuracy of the methodology. The standards were freshly prepared from standard stock metal solutions (Titrisol 1000±0.002mg/1, Merck, Darmstadt, Germany) just prior to analysis, and were used for initial calibration for each substance. These solutions were also used as internal quality standards. Hollow Cathode Lamp and Background Correction (with Deuterium Lamp) modes were selected to element analysis. Each result was corrected for the appropriate reagent used and matrix blanks. For the accuracy of trace element

concentrations, each sample was measured two times for each element analysis. Deionized water was used to clean the chamber and zero control for each analysis. Stock standard metal solutions for every metal (1,000mg/1, Merck) were used for positive control, and were tested every 25 samples to ensure reliability of measurement.

SPSS for Windows version 13.0 (SPSS Inc.) was used to analyze statistical data. Data normality was analyzed by the Shapiro-Wilks normality test. Because not all of the variables followed a normal distribution ($p<0.05$), the Mann-Whitney U tests were used to compare variables between two groups, and $p < 0.05$ was regarded to be statistically significant.

Results

Zn, Fe, Cu, Cr, Mn and Co levels in the liver, kidney and spleen tissues of rats in the control and secondhand smoke groups are shown in Table 1, statistical significances (*P* values) are also indicated.

In the secondhand smoke group Cr, Mn Fe and Co levels in the liver, Fe and Co levels in the kidney, and Zn, Cu, Mn and Co levels in the spleen were significantly lower than those of controls; however, Cu levels in the kidney and Fe levels in the spleen were significantly higher than those of controls.

Of the all possible ratios of the trace elements studied, some ratios were found significantly different. The ratios were higher than those of control rats in the smoking group for spleen are as follows: Fe/Zn, Fe/Mn, Fe/Cu, Fe/Co, Cr/Cu, Cr/Co, Cr/Mn. In the control group, ratios of Fe/Zn and Fe/Cu in the kidney, and Fe/Cu in the liver were higher than those of rats in the smoking group (Table 2).

Discussion

Some studies on trace element distribution in human and animal tissues have been published previously [20]. Cu concentrations have been found to be highly variable in the tissues of all species; levels of Zn, Fe, and Ca carbonate in the diet also influence Cu retention in the liver and other tissues. Zn on Fe and Cu metabolism appears to be the result of interference with Fe and Cu: both utilization at the cellular level and the increased blood level of Fe and Cu and their excretion are affected [21].

Table 1. Trace element levels in control and secondhand smoke groups (± SE, μg/g wet weight)

		Control (n = 8)	Secondhand smoke group (n = 8)	*P*
Liver	Zn	19.87±1.7	14.44±1.65	NS
	Fe	60.58±12.59	16.86±1.57	0.0238
	Cu	1.52±0.27	1.86±0.01	NS
	Cr	3.42±0.64	0.69±0.05	0.038
	Mn	3.06±0.62	0.61±0.03	0.016
	Co	0.57±0.02	0.22±0.02	0.019
Kidney	Zn	15.01±1.54	12.71±1.11	NS
	Fe	62.34±7.15	10.64±0.67	0.0095
	Cu	0.68±0.11	1.73±0.13	0.019
	Cr	0.81±0.12	1.02±0.09	NS
	Mn	1.54±0.17	1.87±0.16	NS
	Co	0.72±0.09	0.12±0.02	0.038
Spleen	Zn	22.91±2.63	10.82±0.63	0.01
	Fe	40.89±2.8	77.41±5.91	0.03
	Cu	1.62±0.13	0.64±0.04	0.016
	Cr	1.38±0.07	3.11±0.22	NS
	Mn	1.02±0.08	0.48±0.01	0.0095
	Co	0.42±0.05	0.49±0.004	0.016

P, Statistical significance; NS, not significant.

Table 2. Meaningful trace element ratios in control and secondhand smoke groups

	Control	Secondhand smoke group	*P*
Spleen			
Fe/Zn	1.78	7.15	0.019
Fe/Cu	25.25	120.95	0.0095
Fe/Mn	40.09	161.27	0.0095
Fe/Co	97.36	157.98	0.0095
Cr/Cu	0.85	5.18	0.0381
Cr/Co	3.29	6.35	0.0095
Cr/Mn	1.35	6.48	0.0381
Kidney			
Fe/Zn	4.15	0.84	0.038
Fe/Cu	91.68	6.15	0.0095
Liver			
Fe/Cu	39.9	9.07	0.0095

P, Statistical significance.

In rats, it has also been demonstrated that a high intake of Zn depresses absorption of both Cu and Fe, and there is a highly significant inverse correlation between hepatic Fe and Cu concentration [22]. While the liver and spleen tissues usually contain the highest amounts of Fe, among the tissues of the body, Zn appears to be abundant in the liver, kidney, bone, retina, prostate, and muscle [23].

Cigarette smoke contains significant amounts of both Cd and Pb [14]. It has been shown that smoking tobacco results in an increased intake and accumulation of cadmium in humans [24-26]. Previous studies have identified a correlation between Pb, Cd, and nutritional trace elements. It was observed that an increase in Pb concentration in hair causes a decrease in Fe and Ca concentrations and changes the ratios of Fe/Cu, Fe/Zn, and Ca/Zn and it was proposed that Pb alters Ca and Fe concentrations not only in blood but also in the hair [22]. Significant inverse correlations were observed between Cd and Zn in the blood, whereas significant positive correlations were noted between Cd and Zn in seminal plasma [27]. Increasing Pb concentrations in the blood correlate with elevated levels of Mn and Zn in whole blood, erythrocytes, and plasma. These results indicated that Zn and Fe status might influence the kinetics of Pb [28-30]. Finally, it was shown that Cu and Zn concentrations increase in rat tissues when exposed to Pb and Cd [29]. In this study, we found a significantly decreased Fe/Zn ratio in the kidney of rats exposed to secondhand smoke, possibly influenced by the decreased Fe levels in the kidney.

In a study of Thai women, Cd body burden was found to be inversely correlated with serum ferritin and those with low iron stores. In our study, Fe levels in the liver were significantly lower than those of control group, possibly due to inhalation of Pb and Cd. The metal transporter protein Nramp2, known also as DMT1, was shown to be involved in Cd absorption [31]. Moreover, increased expression of the intestinal DMT1 was found in iron deficiency [32]. Generally, increased expression of the metal transporter protein is responsible for absorbing iron and possibly other divalent metallic ions, including Cd. This could shed a light on why increases in Cd may be associated with iron deficiency. Se concentrations and many toxic metals have also been shown to correlate [33]. In a cell culture study in which cells were grown in media containing various metal ions, Ni and Cu depressed Mn transport substantially [34]. Our finding that Mn is decreased in the liver may be the result of Ni in cigarettes; other research suggests that there are no significant increases in response to cigarette smoke exposure for some of the elements discussed [35]. The presence and amount

of trace elements were not determined in the cigarette smoke itself in this study, so the presence of effects of some toxic trace elements (e.g., Pb, Cd or Ni) cannot be truly confirmed. Still, the effects of all of these trace elements may also be attributed to the cumulative effect of cigarette smoke, which can stimulate trace element distribution.

Some toxic trace elements and other biochemically important elements (Al, As, Br, Cd, Cl, Cr, Cs, Cu, Hg, I, K, Li, Mn, Na, Ni, Pb, Rb, Se, Sn, TI, Po-210, and Zn) are linked with smoking [14,15]. Significantly lower serum concentrations of retinal, alpha-tocopherol, selenium and zinc, and increased concentrations of copper, have been identified in the sera of smokers. Antioxidants, such as alpha-tocopherol (vitamin E), scavenge free radicals and reduce the toxicity of oxygen [36].

Pucheau et al. [37] showed that the activity of antioxidant enzymes increased or decreased significantly in a diet supplemented with, or deficient in, trace elements, thus illustrating their protective effect. A positive correlation was found between Zn and Cu in both blood and serum [38] and they are known to interact in the body [39]. Smoking tobacco produces deficiencies in vitamin B6, Mg, Co and Cr and interferes with the body's metabolism of normal fat and essential fatty acids [40,41]. In addition to increasing body Cd smoking is also known to cause an excessive body Cu burden [42]. In a study by Dubick et al. [43], serum Cu and Zn concentrations were significantly higher in smokers than in nonsmokers. Nicotine treatment also resulted in significantly lower liver glutathione concentrations and higher Cu, Zn superoxide dismutase activity than in controls. Zn and Cu both bind to Cu/Zn superoxide dismutase in erythrocytes [44] and to metallothionein (MT) in erythrocytes and serum [45]. In our study, we found decreased levels of Zn, Cu and increased level of Fe in the spleen, where blood cell destruction occurs. Zn plays an important role in the immune system, via its prevention of membrane peroxidation by Cu/Zn SOD (Suzuki 1993). It is also very likely that enhanced Zn concentrations play an important part in the body's tissue repair mechanisms.

Changes in Zn, Cu and Fe metabolism can also lead to disorders in the antioxidant defence system [47,48]. In a study of the livers and kidneys of rats, exposure to 50 mg Cd/1 led to a decrease in the activities of SOD in the liver and CAT in the liver and kidney, and an increase in the kidney activity of SOD and malondialdehyde (MDA) concentration in both these organs [49]. We found that Cu levels were increased in the kidney, while Mn and Fe levels were decreased in liver. In addition to being the cofactor of hydrolase, decarboxylase and

transferase enzymes, Mn is involved in glycoprotein and proteoglycan synthesis, and is found in mitochondrial SOD which protects against toxicity from oxygen (i.e., superoxide). Our findings of decreased levels of Mn and Fe in the liver and increased Cu in the kidney are consistent with SOD activity in these organs and the CAT activity in the liver in the aforementioned study bearing in mind that Cu and Mn are the cofactors of SOD, and CAT has one heme that carries Fe.

Co is partly or completely volatized in cigarette smoke, and is inhaled or absorbed via smoking. In a study conducted on Egyptian cigarettes, Cr, Zn, Fe and Co element concentrations ranged from 0.06 to 6.3 μg/g, 76.8 to 180 μg/g, 2864 to 7854 μg/g and 1.70 to 6.66 μg/g in tobacco, 1.12 to 2.13 μg/g, 47.8 to 120 μg/g, 4621 to 4639 μg/g and 2.4 to 2.71 in wrapping paper, 1.82 to 18.97 μg/g, 234 to 1189 μg/g, 5657 to 24349 μg/g and 3.67 to 36.8 μg/g in ash, respectively. Substrating the percentage retained in the filter and recovered in ash from the total elemental input (source) gives the percentage of the element in the smoke (sink). Total input source (μg/g) versus smoke sink (%) for Cr ranged from 0.347 to 4.7 μg/g versus 4% to 46%; for the Zn, 65.3 to 137.2 μg/g versus 4% to 10,9%; for the Fe, 2443.8 to 6338.4 μg/g versus 36.1% to 39.5%; for the Co, 1.44 to 5.2 μg/g versus 9.2% to 42.0% (Nada et al. 1999).

We found significantly decreased Co levels in the liver and spleen and decreased Cr level in liver of rats in the smoking group. Wolfsperger et al. compared the analysis of hair samples of smokers to those of non-smokers, and found decreased Co in smokers and increased Cr levels in non-smokers, possibly due to increased excretion of Cr. The geometric means (μg/g) of smokers versus non-smokers were Cd 0.075 versus 0.038 ($P < 0.05$), Co 0.025 versus 0.010 ($P < 0.05$), Cr 0.84 versus 0.72 ($P < 0.05$), Pb 3.42 versus 1.47 ($P < 0.001$), and Ni 0.64 versus 0.32 ($P < 0.005$) [50].

Low Mn levels in the liver and spleen, increased Cu levels in the kidney and Fe levels in the spleen, and changes in the metabolism of Zn, Fe and Cu may indicate oxidative stress. Decreases in Co and Cr levels in rats exposed to secondhand smoke may also be related to the intake of toxic trace elements present in cigarette smoke.

Conclusions

Our data indicate that chronic exposure to cigarette smoke alters the concentration of some trace elements in the various tissues of rats exposed to secondhand smoke. Alterations in trace element concentrations may be related to the oxidative stress produced through the cumulative effect of inhaled smoke as well as the interaction of absorbed toxic metals with essential trace elements.

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