Cigarette Smoking and Urinary Organic Sulfides

JIAN LE AND CAO WEN-JUN

School of Public Health, Zhejiang University, Hangzhou 310031, China

In order to observe how cigarette smoking influences levels of thio-thiazolidine-4-carboxylic acid (TTCA), high performance liquid chromatography (HPLC) was used to detect TTCA in urine from 18 healthy male volunteers. At the same time, the total amount of urinary organic sulfides was determined by the iodine azide test (IAT). Nine of the volunteers had smoking histories (5 to 10 cigarettes per day, as the smoking group), and the rest only occasionally smoke (1 to 2 cigarettes per month, as the control group). Samples were collected in the early morning (limosis) and 90 minutes after smoking a cigarette. Results showed that smoking a single cigarette could elevate the level of urinary organic sulfides both in the smoking and control groups, while a smoking habit appeared to have no significant influence on the urinary organic sulfide level. No significant cumulative effect of cigarette smoking on urinary organic sulfides was found. The influence of cigarette on urinary organic sulfides was temporary. The results suggest that cigarette smoking might be a confounding factor in biomonitoring the levels of carbon disulfide in exposed workers.

INTRODUCTION

Although biomonitoring of carbon disulfide (CS_2) exposure is performed by determining the levels of urinary 2-thio-thiazolidine-4-carboxylic acid (TTCA) and/or the total amount of urinary organic sulfides, the coexisting confounding factors that may cause overestimation of CS_2 exposure are still not very clear. Simon *et al*. (1994) reported that TTCA could be detected in the urine of individuals who were not exposed to CS_2, especially after the consumption of the brassica vegetables. The presence of TTCA was confirmed after extraction and separation by capillary electrophoresis, supercritical fluid chromatography, and mass spectrometry, and it was suggested that diet should be considered as a non-negligible source of the overestimation of CS_2 exposure. In the present paper, reversed-phase HPLC of urinary TTCA and IAT for urinary sulfides were performed to determine whether the cigarette smoking has some influence on excretion of urinary organic sulfides.

MATERIALS AND METHODS

Subjects and Urine Collection

Eighteen healthy male undergraduates were studied as volunteers. Their ages ranged from 21 to 24 years. Nine of them had smoking histories for 2—5 years and smoke 5—10 cigarettes per day (as the smoking group), and the rest only occasionally smoke (1—2 cigarettes per month, as the control group). They had no histories of administration of any drugs in the two weeks before sampling. The eating habits of both groups were similar, and their diet was controlled strictly from the day before

0895-3988/2000
CN 11-2914

sampling until the end of the last collection. Volunteers in the control group had not smoked at least one week before sampling. Early morning limosis urine and 90 min post smoking urine samples were collected in polyethylene bottles. After detecting concentrations of urine creatinine (Cr), the urine samples were frozen to −20℃ until analysis.

Chemicals and Reagents

Methanol was of chromatographic grade, pure water was double distilled and other chemicals were of analytical reagent grade. Chemicals for mobile phase in HPLC were filtered with a 0.3 nm microporous filtering membrane before use. TTCA was originally synthesized and purified following a method described by Kopecky et al. (1984).

Methods

Cr determination in urine. The concentration of Cr in urine of the volunteers was determined immediately after collection according to the method of Jaffe (Larsen 1972).

Biomonitoring. Urine TTCA was measured in a HPLC (UV-272 nm; Ultrasphere ODS C18 column with 5 μm particles, 4.6×250 mm; mobile phase: methanol/glacial acetic acid/pure water: 14.5/1.5/84.5 v/v/v; flow rate 1.5 ml/min). The calibration curve was linear over the range of 0−200 mg/L, the detection limit was 0.008 ng and the coefficient of variation (CV) for different levels of TTCA concentration (0.2 to 40 mg/L) were 2.06%−7.62%. For more detailed information about the method, see the previous paper (Jian 1995).

The assay for urinary IAT was performed based on the following reaction:

$$2NaN_3 + I_2 \rightarrow 3N_2 + 2NaI.$$

The reactioin between sodium azide and iodine proceeds slowly at room temperature but is apparently catalyzed by the addition of a number of organic sulfide compounds such as dithiocabamates, 2-mercaptothiazolidone-5 and thiourea. The method was performed with some modifications, specifically, in controlling the reaction temperature at 25℃ in a water bath, and the total reaction time was 4 h. The end point was determined with starch filter paper. Because the total urinary organic sulfide levels were influenced by urine flow, an indirect index for evaluating-exposure index E ($E = Cr \times \log t$) was proposed (Djuric *et al.*, 1965), where t was the time in seconds for the disappearance of pale blue color, Cr was creatinine concentration in g per L urine, and the higher the levels of urine organic sulfides the lower the values of index E. If the highest Cr value was 30 mmol/L (3.4 g/L) and the end point of decoloration was not reached within 4 h (14 400 seconds), the test was considered negative and the exposure index E was recorded as 14 ($\log 14\,400 \times 3.4 \approx 14$).

Statistical analysis. The software SPSS, version 6.0 for windows, was used for analysis. Urine concentrations of TTCA and E values of IAT between the smoke group and nonsmoke group were compared using the group t-test. Comparing data from individuals before and after smoking was performed with the paired t-test.

RESULTS

Urinary Excretion of TTCA Before and After Smoking

The results of the paired t-test showed that after smoking one cigarette, urinary TTCA levels both in the smoking group and control group were significantly higher than those collected before smoking (see Table 1).

TABLE 1

Levels of Urinary TTCA (mg/g Cr) Before and After Smoking

Group	Time of Sampling	Cases	TTCA (median)	Range	P value
Smoking	Before smoking	9	0.155	0.077—0.233	
	After smoking one cigarette	9	0.752	0.092—1.412	0.027
Control	Before smoking	9	0.146	0.017—0.225	
	After smoking one cigarette	9	0.496	0.029—0.993	0.037

Urinary Excretion of Total Organic Sulfides Before and After Smoking

After smoking, the urinary level of organic sulfides was increased in both groups compared to those samples collected before smoking. The index E values were decreased (Table 2).

TABLE 2

Levels of Urinary Index E Before and After Smoking

Group	Time of Sampling	Cases	Index E ($\bar{x} \pm s$)	P value
Smoking	Before smoking	9	9.637 ± 1.939	
	After smoking one cigarette	9	4.309 ± 1.385	0.000
Control	Before smoking	9	11.073 ± 2.775	
	After smoking one cigarette	9	6.162 ± 2.452	0.000

Urinary Excretion of TTCA and Other Organic Sulfides in Two Groups

In order to ascertain whether smoking habit influenced the excretion of organic sulfides, group t-test was used to compare TTCA concentration and index E between the smoking group and the control group, both before and after smoking. There were no signifcant differences in the TTCA and index E values in the two groups either before or after smoking ($P > 0.05$) although it appears that the values of organic sulfides in the smoking group were somewhat higher than those in the control group (Figs. 1 and 2).

DISCUSSION

Noninvasive urinary monitoring in workers exposed to CS_2 is an attractive option, if a stable exogenous metabolite can be measured and the confounding factors can be detected with adequate sensitivity and specificity. In this study we sought to determine whether cigarette smoking was confounding factor that could influence the excretion of urinary organic sulfides.

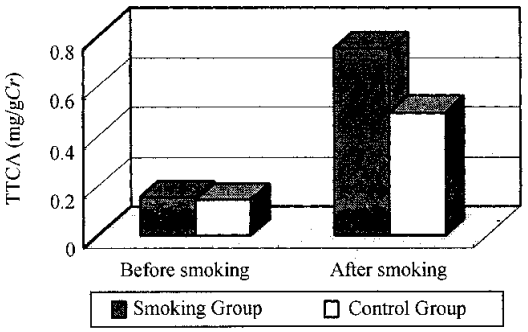

FIG. 1. TTCA levels between two groups both before and after smoking.

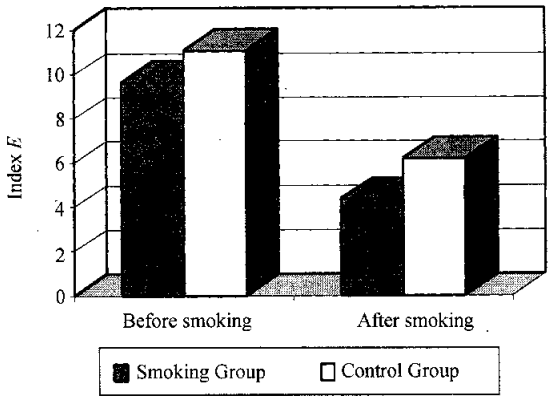

FIG. 2. Index *E* levels between two groups both before and after smoking.

The results prosented in Table 1 and Table 2 indicate that single cigarette smoking could influence the excretion of urinary organic sulfides, as represented by urine TTCA levels and index *E* values. These results suggest that TTCA could be derived from a source other than CS_2 biotransformation, and the IAT reaction which could also reflect the excretion of urinary organic sulfides, had comparable results to that of TTCA. There are some sulfide components in cigarette (Zhu, 1989). It was suggested that during smoking these components could be inhaled and supplied as the materials to form TTCA and organic sulfides. However, it is not clear whether the sulfide components in cigarette could induce the catabolism of sulfide substances *in vivo*. On the other hand, some studies found low amounts of CS_2 (0.7-2 μg/cigarette) in the main stream of cigarette smoke, and similar concentrations of CS_2 in the exhaled air of smokers and non-smokers (Phillips, 1992). Therefore, it is possible that trace amounts of CS_2 exists in ambient air as well as in cigarette smoke, which was inhaled by normal volunteers and caused the increased level of urinary organic sulfides (including TTCA).

To determine the cumulative effect of cigarette smoking on urinary elimination of organic sulfides, samples of early morning limosis urine (before smoking) from members of the smoking group and the control group were compared, and were also compared with those collected after smoking. The results indicate that the increased level

of organic sulfides in urine is not be caused by a cumulative effect, but as a direct result of cigarette smoking. It is still not clear whether cigarette smoking has similar effects on workers exposed to CS_2.

Some authors confirmed (Rosier *et al*., 1984; Simon *et al*., 1994) that various sulfur containing drugs and diets with foods such as cabbage also could catalyze the IAT reaction and give false positive results or cause higher values of TTCA and therefore overestimation of CS_2 biotransformation. So, it was important to control the influence of these items mentioned above. Considering the relative insensitivity of the original method (Poucke *et al*., 1990; Krstev *et al*., 1993), a modified IAT was conducted to improve the sensitivity. Because the decolorization time was influenced by temperature, a suitable constant temperature (25℃) was required to both minimize the bias caused by temperature and promote the process of decolorization. Moreover, the total reaction time was extended from 3 h to 4 h, which also improved the sensitivity of the test. The modified IAT had similar results in comparison with those of the TTCA test. It can be concluded from this study that although the specificity of the IAT reaction was lower than the TTCA, it does not require expensive equipment (HPLC) and the operation process is simple, and therefore the IAT reaction still has some practical value for evaluating urinary organic sulfides, especially in a rapid exposure screening.

ACKNOWLEDGEMENT

It is a pleasure to acknowledge the help of Professor Hu Di-Sheng for advice on this work.

REFERENCES

Djuri, D., Surdu, K. N., and Berkeš, I. (1965). Iodine-azide test on urine of persons exposed to carbon disulphide. *Bri. J. Ind. Med.* **22**, 321-323.

Jian Le (1995). Determination of Urinary 2-thio-thiazolidine-4-carboxylic acid by High Performance Reversed-phase Liquid. Chromatography. *J. Hyg. Res.* **24**(5), 257-259. (in Chinese)

Kopecky, I. and Smejkal, J. (1984). Simple preparation of pure urinary 2-thio-thiazolidine-4-carboxylic acid (TTCA) as a reference standard for carbon disulfide exposure tests. *Bull. Soc. Chim. Belg.* **93** (3), 231-232.

Krstev, S., Perunicic, B., Farkic, B., and Varagic, M. (1993). Environmental and biological monitoring in carbon disulfide exposure assessment. *Med. Lav.* **84**(6), 473-481.

Larsen, L. (1972). Creatinine assay by a reaction-kinetic principle. *Clin. Chim. Acta* **41**, 209-217.

Phillips, M. (1992). Detection of carbon disulfide in breath and air: a possible new risk factor for coronary artery disease. *Int. Arch. Occup. Environ. Heath* **64** (2), 119-123.

Poucke, L. Van., Peteghem, C. Van., and Vanhoorne, M. (1990). Accumulation of carbon disulphide metabolites. *Int. Arch. Environ. Health* **62**, 479-482.

Rosier, J., Billemont, G., Peteghem, C. V., Vanhoorne, M., Grosjean, R., and Walle, V. D. (1984). Relation between the iodine azide test and the TTCA test for exposure toi carbon disulphide. *Bri. J. Ind. Med.* **41**, 412.

Simon, P., Nicot, T., and Dieudonne, M. (1994). Dietary habits, a non-negligible source of 2-thio-thiazolidine-4-carboxylic acid and possible overestimation of carbon disulfide exposure. *Int. Arch. Occup. Environ. Health* **66**, 85-90.

Zhu Chuan (1989). Hazard of Smoking, pp. 24-29. Shen Yang Press, Shen Yang. (in Chinese)

(*Received September* 22, 1999 *Accepted December* 23, 1999)