Evaluation of Passive Smoking by Measuring Urinary Trans, trans-muconic Acid and Exhaled Carbon Monoxide Levels

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Abstract: No method has yet been established to evaluate the exposure to tobacco smoke in passive smoking (PS). We therefore conducted a study on the possibility that the levels of urinary trans, trans-muconic acid (MA) and the exhaled carbon monoxide (CO) could be indices of the passive exposure to tobacco smoke. The moderate correlation was observed between urinary MA levels and the number of consumed cigarettes per day in smokers. The mean urinary MA level of the PS (+) group was significantly higher than that with the PS (–) group. Among the PS (+) group, the mean MA level in the urine obtained in the afternoon was higher than that obtained in the morning. A high correlation was observed between the exhaled CO levels and the number of consumed cigarettes per day in smokers. Like the urinary MA level, the mean exhaled CO level in the PS (+) group, too, gave a significantly higher level than in the PS (–) group. Because the biological half life of MA (7.5 ± 0.85 h) was longer than that of CO (3.0 ± 0.36 h), the measurement of urinary MA level is recommended for evaluating the exposure of passive smoking. The measurement of exhaled CO levels is useful only for chain smokers and nonsmokers with PS just before measurement.

Key words: Trans, Trans-muconic acid, Passive smoking, Environmental tobacco smoke, CO concentration in expirium, Benzene

Introduction

The health risks of smoking and passive smoking (PS) have become clear in recent years, and control for smoking is now an important issue that must be solved in the management of occupational health^{1–8}. In 1996, the Japanese Ministry of Labor issued the “Guideline on the partition of workplaces between smokers and nonsmokers”.

At present, there is no established method of evaluating passive smoking. As indices for the exposure to tobacco smoke, attempts have been made until now to measure the

levels of urinary cotinine, urinary trans, trans-muconic acid (MA) and of carbon monoxide (CO) in the expirium^{6, 8, 9–20}.

The urinary MA is one of the metabolites of benzene. The measurement of MA has been reported to be useful as a biological index for the exposure to trace amounts of benzene^{21–24}. A small fraction of the urinary MA may be originated from the metabolism of sorbic acid, a customary additive in food, cosmetics and pharmaceutical products¹⁹. However, the amount of MA generated by the metabolism of ingested sorbic acid does not seem to be so significant as to discard MA as a benzene marker¹⁹. The cigarette smoke is known to emit benzene by pyrolysis of tobacco leaf¹⁵.

While exhaled CO levels are influenced by other sources

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of CO such as cooking, heating or vehicle exhaust, the measurement is very simple and convenient⁶⁾.

We therefore studied the possibility of evaluating the exposure to tobacco smoke especially in passive smoking by measuring the urinary MA level and the CO concentration in the expirium of workers having no chance of exposure to benzene or combustion in their work.

Subjects

Six students and personnels of the university were the subjects for the time course studies of MA and CO levels after smoking.

The subjects of the PS study were a total of 285 persons who were employees of a company and the university personnels. Among them, there were 47 persons who were nonsmokers and had no passive smoking (PS [-] group), 103 persons who were nonsmokers but had passive smoking (PS [+] group), and 135 smokers.

As shown in Table 1, the subjects included 243 males and 42 females, with their average age and standard deviation being 37.3 ± 11.1 for males and 27.5 ± 9.3 for females ($P < 0.001$). In nonsmokers, the average age of men was significantly higher than that of women ($P < 0.001$). In nonsmokers, the mean age of PS (+) group was higher than PS (-) group ($P < 0.001$). The number of cigarettes consumed per day ranged 1–50 with an average of 22.1 ± 11.3 .

Because the number of women was markedly smaller than that of men and sex difference of MA and CO data was negligible, the subjects for the following analysis were a total of men and women.

Methods

Measurement of urinary MA level

Urine samples were stored frozen at 20°C. On the day of measurement, the samples were thawed at room temperature before use.

The measurement of MA was performed by a modified method of Ghittori *et al.*¹⁸⁾. High performance liquid chromatography (HPLC) was used for the measurement. One ml of urine sample was mixed with 1 ml of citrate buffer (pH 5). After it was passed through a 0.2 μ m filter (GL Chromatodisk, Kurabo, Japan), one ml of the sample was treated with 3 ml of an anionic exchange cartridge (Bond Elut SAX, Valian Associate, USA). The cartridge was conditioned with 3 ml of methanol and 3 ml of distilled water before use. After the cartridge was washed with 6 ml of distilled water and 10 ml of 0.5 percent acetic acid, MA was eluted with 4 ml of 6 percent acetic acid.

The Hitachi L-400 UV Detector and Hitachi L-6300 Intelligent Pump (Hitachi, Ltd., Japan) were used for HPLC. The urinary MA level was expressed in μ g/g · creatinine for adjusting creatinine of urine. The urinary creatinine was measured by the alkaline picric acid method.

Time course of excretion of MA in the urine after smoking

The smokers were asked to smoke 2 cigarettes (Seven Stars Light, Japan Tobacco Industry) in 10 minutes with the informed consent. Urinary MA levels were measured before smoking and 1, 2, 3, 4, 5, 6, 7, 8 and 10 hours after smoking. They were asked not to smoke other than for the experiments on the test day from the time of waking up to the last urine sampling.

Table 1. The average age of the subjects according to sex and smoking

	Men		Women	
	n	age	n	age
Nonsmokers	118 (67)	38.6 ± 11.7	31 (25)	25.4 ± 6.9
PS (-) group	32 (28)	25.2 ± 7.7	15 (12)	25.4 ± 7.4
PS (+) group	86 (39)	42.6 ± 9.6	16 (13)	25.1 ± 6.3
Smokers	125 (38)	37.4 ± 10.2	11 (3)	34.0 ± 12.6
1~19/day group	35 (9)	32.4 ± 9.6	9 (2)	33.1 ± 11.6
20~/day group	90 (29)	39.3 ± 9.8	2 (1)	38.0 ± 13.0
Total	243 (105)	37.7 ± 11.1	42 (28)	27.5 ± 9.3

Data are given in mean $\pm$ S.E. (): number of the subjects who were measured for exhaled CO levels. PS: passive smoking.

Measurement of CO concentration in the expirium

The 133 subjects were measured for CO concentration in their expirium in the afternoon of the same day of urine sampling, using New Micro Smoker Lyzer (Bedfont Company, England). As instructed in the manual of the apparatus, the subjects were asked to inhale a maximum of air, to stop breathing for 15 sec., and then to exhale slowly to the completion of expirium.

Time course of CO in the expirium after smoking

The smokers were asked to smoke one cigarette (Seven Stars Light, Japan Tobacco Industry) in 10 minutes. They were measured for the CO concentration in their expirium before smoking, 3, 5, 10 minutes after smoking, and thereafter at an interval of 10 minutes until 90 minutes.

Statistical evaluations

We computed geometric mean values for MA because the values for this level showed a normal distribution when they had been logarithmically converted. Arithmetic means were computed for CO levels because they showed normal distribution. The difference of means in three or more groups was evaluated by an analysis of variance and Scheffe's analysis for multiple comparison.

Results

Time course of excretion of MA in the urine after smoking

Fig. 1 shows the changes with time in urinary MA levels

Fig. 1. Time course of urinary MA levels after smoking.

The urinary MA levels in 6 subjects are plotted. Values for the typical subject are connected. The urinary MA level started to rise in 2.8 ± 0.48 hours and reached the peak in 4.8 ± 0.60 hours after smoking. The biological half life was 7.5 ± 0.85 (5.5–10.7) hours.

in 6 smokers after smoking. We calculated the apparent elimination rate (K_e) and the corresponding half life ($t_{1/2}$) by the way of Boogaard and Sittert²³. The following formula was used:

$$\ln (C_2 - C_1) = -K_e \times (t_2 - t_1),$$

$$t_{1/2} = -\ln (2)/K_e,$$

where C_1 denotes the concentration of MA in the urine sample collected at the peak time t_1 and C_2 the concentration of MA at the last sampling time t_2 . The urinary MA level started to rise in 2.8 ± 0.84 hour, and reached the peak in 4.8 ± 0.60 hours after smoking. The biological half life was 7.5 ± 0.85 (5.5–10.7) hours.

Time course of CO in the expirium after smoking

Fig. 2 shows the CO concentration with time in the expirium of 6 smokers after smoking. We calculated corresponding half life ($t_{1/2}$) by the same way of the calculation for urinary MA. The CO in the expirium started to rise immediately after smoking, and reached the peak in 3 minutes. The biological half life was 3.0 ± 0.36 (1.5–4.1) hours.

Evaluation of exposure to tobacco smoke by urinary MA level

As shown in Fig. 3, the PS (+) group gave a significantly higher mean value than the PS (–) group ($P < 0.01$). The 20 cigarettes/day or more smoking group gave a significantly higher mean value than the 1–19 cigarettes/day group ($P < 0.001$). However, there was no significant difference

Fig. 2. Time course of CO concentration in expirium after smoking.

The exhaled CO levels in 6 subjects are plotted. Values for the typical subject are connected. The CO concentration in the expirium started to rise immediately after smoking and reached the peak in 3 minutes. The biological half life was 3.0 ± 0.36 (1.5–4.1) hours.

Fig. 3. Geometric mean urinary MA levels by the types of smoking.

SE is expressed in barr. The PS (+) group gave a higher mean value than the PS (-) group. P.S., passive smoking; N.S., not significant; **, $P<0.01$; ***, $P<0.001$.

between the 1-19 cigarettes/day smoking group and the PS (+) group, although the former gave a slightly higher value than the latter.

Fig. 4 shows a comparison among the PS (-) group and two divided PS (+) groups according to the time of sampling, the AM group and the PM group. There was no significant difference between the PS (-) group and the PS (+) AM group. The PS (+) PM group gave a significantly higher value than the PS (+) AM group ($P<0.01$). All samples of PS (-) were obtained in the afternoon.

Fig. 5 shows the relationship between the urinary MA level and the number of consumed cigarettes per day. A significant and moderate correlation was observed between them ($r=0.651$; $P<0.001$).

Evaluation of exposure to tobacco smoke by exhaled CO concentration

As shown in Fig. 6, the PS (+) group and the 1-19 cigarettes/day smoking group gave significantly higher mean values than the PS (-) group and the PS (+) group, respectively ($P<0.001$). The 20 cigarettes/day or more smoking group gave a significantly higher value than the 1-19 cigarettes/day group ($P<0.05$).

Fig. 4. Geometric mean urinary MA levels for nonsmokers with passive smoking (-) group and the passive smoking (+) groups.

The passive smoking (+) group is divided into two groups according to the sampling time such as AM (morning) and PM (afternoon). SE is expressed in barr. P.S., passive smoking; N.S., not significant; **, $P<0.01$; ***, $P<0.001$.

Fig. 5. Correlation of urinary MA level with the number of consumed cigarettes per day.

The logarithmically scaled levels of urinary MA on the vertical axis were plotted against the number of cigarettes per day on the horizontal axis. A moderate and significant correlation was observed between them. $r=0.651$; $n=183$; $P<0.001$.

Fig. 7 shows the relationship between the CO concentration in the expirium and the number of cigarettes smoked. A highly significant correlation was observed between them ($r=0.863$; $P<0.001$).

Fig. 6. Arithmetic means of CO concentration in the expirium by the types of smoking.

SE is expressed in barr. P.S., passive smoking; *, $P < 0.05$; ***, $P < 0.001$.

Fig. 7. Correlation of CO concentration in the expirium with the number of consumed cigarettes per day.

The CO levels in the expirium on the vertical axis were plotted against the number of cigarettes on the horizontal axis. A highly and significant correlation was observed between them. $r = 0.863$; $n = 81$; $P < 0.001$.

Correlation of exhaled CO concentration with urinary MA level

Fig. 8 show the correlation of exhaled CO level with urinary MA level for PS (-), PS (+), and smoker groups respectively. No significant correlation was observed in all groups.

Discussion

It has already been pointed out that MA was effective as an index for exposure to benzene in the working environment⁽²¹⁻²⁴⁾. Because MA can be used to evaluate the

Fig. 8. Correlation of urinary MA level with exhaled CO levels.

The logarithmically scaled values for urinary MA levels on the vertical axis were plotted against the exhaled CO concentration on the horizontal axis. a) In passive smoking (-) group, no significant correlation was observed between them. $r = 0.07$; $n = 40$; $P = 0.669$. b) In passive smoking (+) group, no significant correlation was observed between them. $r = 0.057$; $n = 52$; $P = 0.688$. c) In smokers, mild but no significant correlation was observed between them. $r = 0.265$; $n = 41$; $P = 0.941$. P.S., passive smoking.

exposure to trace amounts of benzene, it has been applied to the evaluation of exposure to tobacco smoke^{6, 12, 15, 17, 18, 20}.

In this study, the urinary MA levels were measured for 285 subjects by a modified method of Ghittori *et al.*¹⁸. A moderate and significant correlation was observed between the number of cigarettes smoked and the urinary MA levels. When the urinary MA levels for the PS (+) group were divided into two groups according to the time of sampling, the PM group gave a significantly higher mean urinary MA level than did the AM group. This suggests that nonsmokers inhale tobacco smoke in non-partitioned workplaces and the effects of PS are more remarkable when the urine samples are obtained in the afternoon than in the morning.

No significant difference of mean urinary MA levels was observed between the PS (+) group and the 1–19 cigarettes/day smoking group. It can be predicted that nonsmokers working in non-partitioned workplaces and light smokers are exposed to similar amount of tobacco smoke.

We studied the time course of excretion of urinary MA after smoking and found that the half life of MA was about 7.5 ± 0.85 hours. The results suggest that smokers have low urinary MA levels in the morning, because inhaled benzene in the tobacco smoke was metabolized and excreted into urine during night. This seems to be proved by a significant difference between the PS (+) AM group and the corresponding PM group. If nonsmokers have been exposed to tobacco smoke before work shift in their home, the effects of passive smoking persist in the morning. The MA level in the urine sample obtained in the morning does not indicate the tobacco exposure at the workplace.

In the measurements of CO concentration in the expirium, a significant difference was observed between the PS (–) and the PS (+) group. A significant difference was also observed even between the 1–19 cigarettes/day group and the PS (+) group. These results were different from the results of the measurement of urinary MA levels.

The correlation is very high between the exhaled CO concentration and the number of consumed cigarettes. Because CO has a peak in 3 minutes after smoking and the biological half life is as short as 3.0 ± 0.36 (1.5–4.1) hour, the exhaled CO levels in nonsmokers are greatly affected when exposed to environmental tobacco smoke just before the measurement. Therefore, the CO concentration in the expirium is a good index only for smokers who are frequently and periodically exposed to tobacco smoke, but is not a suitable index for evaluating passive smokers who irregularly inhale environment tobacco smoke⁶. Therefore, significant correlation was not observed between the MA level in urine

and CO levels in the expirium.

Urinary cotinine is the main metabolite of nicotine, the principal alkaloid of the tobacco plant. The urinary cotinine has been measured to evaluate the effects of passive smoking^{6, 16}. The nicotine yield of cigarette main stream smoke (MS) is about 1 mg/cigarette, and the side stream smoke (SS)/MS ratio amounts to 2.6–3.3. Urinary cotinine has a biological half life of as long as 16–20 hours^{6, 16}. The urinary cotinine level is affected with passive smoking at places other than workplace, because of its long half life^{6, 8}.

MA is one of the metabolite of benzene. Benzene was emitted 30–50 μg /cigarette in MS and 345–653 μg /cigarette in SS⁶. The SS/MS ratio of benzene is more than 10 times that of nicotine. MA excretion reaches the peak in 4.8 ± 0.60 hours after smoking, and MA has the biological half life of 7.5 ± 0.85 hours. It is possible to exclude the effects of passive smoking at home to some extent by taking urine samples at the end of work shift in the afternoon.

Urinary MA is also detected following dietary intake of food preservative sorbic acid. Estimated daily intake of sorbic acid was 29.0 mg per day based on the results of the “National Nutrition Survey” published by the Ministry of Health and Welfare, Japan²⁵. Under the experimental conditions the estimated conversion of sorbic acid to urinary MA was 0.12% (0.08–0.19%)²⁰. Thus, urinary excretion of MA due to dietary sorbic acid is calculated to be $2.48 \mu\text{g/g} \cdot \text{creatinine}$. This is small enough to disregard the effect of ingested sorbic acid.

To evaluate the passive exposure to tobacco smoke at workplaces, the method should be sensitive enough, easy to test, and as inexpensive as possible. In evaluating the passive exposure to tobacco smoke, we consider that the measurement of urinary MA level is superior to the measurement of urinary cotinine level, because the SS/MS ratio of benzene was reported higher than nicotine, and urine samples taken once in the afternoon are sufficient to evaluate the exposure to tobacco smoke at workplaces.

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