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^{1}H NMR spectroscopic identification of a glue sniffing biomarker

Bobae Kwon [a], Siwon Kim [a], Sosun Kim [a], Dong-Kye Lee [b], Yu-Jin Park [b], Myung-Duck Kim [c], Jae-Shin Lee [b], Suhkmann Kim [a,*]

[a] Department of Chemistry and Chemistry Institute for Functional Materials, Pusan National University, 30 Jangjeon-dong, Geumjeong-gu, Busan 609-735, Republic of Korea
[b] National Forensic Service, Dongsam-dong Youngdo-gu, Busan 606-801, Republic of Korea
[c] National Forensic Service, Shinwol 7-dong Yangchon-gu, 158-097, Republic of Korea

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ABSTRACT

Organic solvent abuse typically involves sniffing organic solvents to experience the mind-altering conditions they induce. In Republic of Korea, organic solvent abuse is a serious social problem, especially among teenagers. Several studies have addressed the effects of organic solvent abuse on mind and body, but there are no simple methods by which such abuse can be positively identified. In this report, we describe a method for analyzing toluene metabolites (toluene is the main ingredient of glue) in glue-sniffers' urine using ^{1}H NMR spectroscopy. Toluene is a commonly used solvent in the rubber, paint, plastics, leather, printing, and chemical industries. Inhaled toluene is metabolized to hippuric acid in the liver and excreted in the urine. Hippuric acid is known as a good biomarker for biological monitoring of toluene exposure. We have scanned hippuric acid and other toluene metabolites by NMR spectroscopy and performed statistical multivariate analysis of the data. Based on this analysis, we sought to determine parameters by which glue-sniffing (toluene inhalation) behavior may be verified. We also demonstrate the use of a pattern recognition method for accurate and efficient analysis of NMR data. In comparison to conventional methods, such as mass spectroscopy coupled with liquid chromatography or gas chromatography, nuclear magnetic resonance spectroscopy has several advantages, including simple sample preparation, non-destructive sampling, accuracy, short acquisition time, and reproducibility in the determination of urinary hippuric acid.

1. Introduction

Organic solvents are commonly used in Korean industries. These organic solvents are toxic, either through occupational or intentional exposure [1–3]. Guidelines which regulate working environments and the use of personal protective devices can reduce occupational exposure, but organic solvent abuse among teenagers who sniff industrial glue is easily concealed and can occur over long periods of time, leading to excessive exposure.

Organic solvent abuse is a serious social problem, especially among adolescents between the ages of 13 and 18. Glue sniffing is a common form of organic solvent abuse and frequently causes sudden death in adolescents and young adults [4]. The behavior is becoming popularized among adolescents in the Republic of Korea, with some teenagers abusing organic solvents nearly to the point of addiction. Many organic solvents are abused among teenagers have become dependent on them. Glue is inexpensive, easily obtained, legal to purchase, and simple to sniff, all of which contribute to its common use as a hallucinogen. It is thought that organic solvent abuse is related to behavioral and personality disorders. Medical poisoning could be induced by organic solvent abuse in teenagers, and users should be required psychiatric evaluation and appropriate treatment.

A 2003 survey by the Korean National Youth Commission studied nationwide rates of glue sniffing and the results of the survey showed that 96% of teen boys and 66% of teen girls have sniffed glue at least once ($p < 0.01$) (the survey included 100 adolescents in a young offender institution, aged 10–19, 50 males and 50 females). The development of an analytical method for the identification of solvent abuse will aid in the treatment and management of adolescent offenders [5,6].

The glues in use within the Republic of Korea are comprised of approximately 74–88% organic solvents including acetone, methyl ethyl ketone, methyl cyclopentane, cyclohexane, toluene, and cyclohexanone. Toluene is the main solvent constituent of glue, although it can be as low as 10.8% in low-toluene glues [7]. Toluene is a mono-substituted benzene derivative and a lipophilic aromatic hydrocarbon, typical of organic solvents and the main ingredient of lacquer, thinner, and glue. As toluene has very low water solubility, it must be metabolized prior to elimination via the urine and perspiration. Toluene is inhaled and absorbed through the lungs; 18% is excreted in the expired air and the remainder is metabolized

* Corresponding author. Tel.: +82 51 510 2240; fax: +82 51 516 7421.
E-mail address: suhkmann@pusan.ac.kr (S. Kim).

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to benzyl alcohol by the liver cytochrome P-450 system [8]. Benzyl alcohol is dehydrogenated to benzaldehyde by alcohol dehydrogenase and to benzoic acid by alcohol oxygenase [9]. Benzoic acid conjugates with glycine to form hippuric acid, which is water soluble. Thus toluene is mainly excreted in the urine as hippuric acid and benzoic acid [10]. We report the observation of hippuric acid in urine samples using NMR spectroscopy. The concentration of hippuric acid was strongly elevated in the urine of glue sniffers; other identified metabolites included citrate and creatinine. The results provide definitive evidence of toluene inhalation.

2. Method and experimental design

2.1. Sample collection

A total of 9 urinary samples were available for this study. Four samples were collected from people who self-identified as glue-sniffers (toluene concentration was over 0.1 μg/mL by gas chromatography using urine; data not shown). One sample was collected within 2 weeks of last use; however, no physical evidence was available to verify this assertion (“2W glue sniffer”). The remaining samples were from 4 healthy volunteers with no history of glue sniffing. Collected samples were stored at 4 °C prior to analysis.

2.2. Preparation of urinary samples for NMR analysis

Urine samples were prepared for ^{1}H NMR spectroscopy by adding 50 μL of D_2O containing 20 mM TSP (sodium trimethylsilyl [2,2,3,3-$^2H_4$]propionate) to 450 μL urine to a final TSP concentration of 2 mM. TSP was used as a chemical shift reference with a δ 0.0 singlet peak and the deuterium oxide (D_2O) was used as the lock signal. All samples were placed in 5-mm NMR tubes for analysis and were stored at 4 °C except during analysis.

2.3. NMR spectroscopy

^{1}H NMR spectral data were acquired on a 500 MHz Varian Unity-Inova (Varian Inc., Palo Alto, CA) and 298 K operating at a proton NMR frequency of 499.789 MHz. Water signals were suppressed by using a presaturation pulse sequence. All measurements were processed with an ID/PFG 5 mm probe. 512 transitions were accumulated and the ^{1}H NMR spectral acquisition time was about 30 min per sample. All data were calibrated to TSP at δ 0.00 ppm and an exponential function using a line broadening of 0.2 Hz was applied to each free induction decay prior to Fourier transformation.

2.4. Statistical analysis of NMR spectra

All NMR spectra were manually phased and baseline-corrected by MestreNova Suite 5.3.1 (Mestrelab Research, USA). Full-resolution NMR data were imported into Chenomx NMR Suite 6.01 software (Chenomx Inc., Canada) and regions corresponding to water/HDO and urea (δ 4.7–6.0) and TSP (δ −0.25–0.25) were excluded from statistical analyses. All remaining regions of the spectra were reduced to ppm spectral buckets and were normalized to the region of 0.5–9.5 ppm by MestreNova Suite 5.3.1.

A spectral assignment and estimated concentration of each metabolite was generated by Chenomx NMR Suite 6.01 software. Principal component analysis (PCA) was applied to all samples using Simca-P+.

3. Results

3.1. ^{1}H NMR spectral analysis of urine

A single spectrum was selected from each test group as examples for detailed signal assignment. Assignments were made using the Chenomx 500 MHz library database and a literature compilation [11–13]. Fig. 1 displays the chemical shift information for several metabolites. All spectra were normalized to the TSP peak; however, the water and urea peaks (from δ 4.7 ppm to δ 6.0 ppm) were excluded from whole spectra. The major identified compounds were hippuric acid, creatinine, dimethylamine, alanine, 3-hydroxybutyrate, citrate, and TMAO (Trimethylamine N-oxide). In the middle frequency region, δ 2.7–6.3 ppm, citrate, hippuric acid, TMAO, and creatinine were observed. In the high frequency region, δ 6.3–8.5 ppm, hippuric acid and other aromatic compounds were observed.

Fig. 1 shows the assigned urine spectrum of a control, a glue sniffer and the 2W glue sniffer with TSP and water resonances

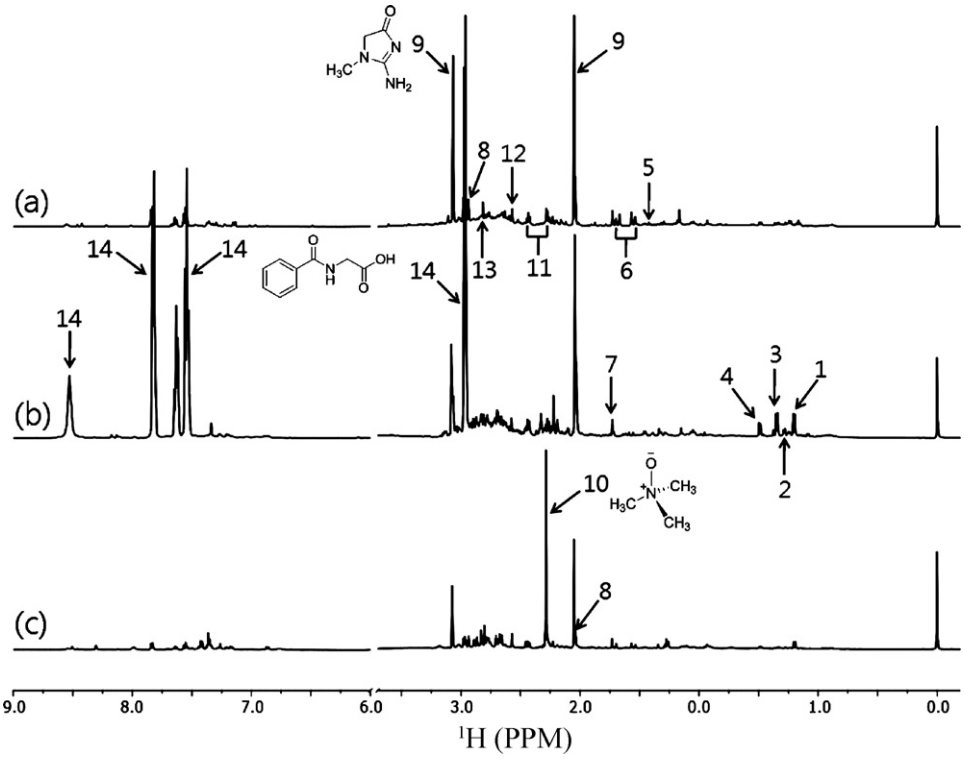

Fig. 1. Representative ^{1}H NMR assigned spectra of controls, glue sniffers and the 2W glue sniffer with TSP (δ 0.00 ppm) and water resonances removed. All spectra were normalized to the TSP peak (from δ −0.026 ppm to δ 0.023 ppm). The following metabolites were identified with Chenomx. (a) Control, (b) glue sniffer, (c) 2W glue sniffer. Assigned list: (1) 3-hydroxybutyrate, (2) 3-hydroxyisovalerate, (3) lactate and threonine, (4) alanine, (5) succinate, (6) citrate, (7) dimethylamine, (8) creatine, (9) creatinine, (10) TMAO (Trimethylamine N-oxide), (11) taurine, (12) glycine, (13) guanidoacetate, (14) hippuric acid.