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RESEARCH ARTICLE

Comparison of cyanide exposure markers in the biofluids of smokers and non-smokers

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Abstract

Cyanide is highly toxic and is present in many foods, combustion products (e.g. cigarette smoke), industrial processes, and has been used as a terrorist weapon. In this study, cyanide and its major metabolites, thiocyanate and 2-amino-2-thiazoline-4-carboxylic acid (ATCA), were analyzed from various human biofluids of smokers (low-level chronic cyanide exposure group) and non-smokers to gain insight into the relationship of these biomarkers to cyanide exposure. The concentrations of each biomarker tested were elevated for smokers in each biofluid. Significant differences ($p < 0.05$) were found for thiocyanate in plasma and urine, and ATCA showed significant differences in plasma and saliva. Additionally, biomarker concentration ratios, correlations between markers of cyanide exposure, and other statistical methods were performed to better understand the relationship between cyanide and its metabolites. Of the markers studied, the results indicate plasma ATCA, in particular, showed excellent promise as a biomarker for chronic low-level cyanide exposure.

Keywords: Chemical warfare agents, cyanide exposure, gas chromatography mass spectrometry, high performance liquid chromatography

Introduction

Cyanide is a rapidly acting, highly toxic chemical that is ubiquitous in nature and has several anthropogenic sources. The primary natural source of cyanide exposure is from plants (e.g. cassava, sorghum, bamboo, etc.) which contain cyanogenic glycosides that produce hydrogen cyanide upon hydrolysis (Hibbs, 1979; Conn, 1979, 1981; Nartey, 1980; Honig et al., 1983; Vetter, 2000; Knight and Walter, 2002; McGorum and Anderson, 2002). Cyanide-containing compounds are also produced by certain bacteria, algae and fungi (Solomonson, 1981; Way, 1984; Li et al., 2000). Although the primary source of cyanide poisoning is from edible plants, exposure can also occur by a number of anthropogenic sources, especially for those working in the industries where cyanide is used (Gossel and Bricker,, 1994; Vetter, 2000; Knight and Walter, 2002; Department of Health and Human Services, 2006; Eckstein, 2006). Anthropogenic sources of cyanide include automobile emissions, fires, and cigarette smoke (Nartey, 1980; Honig et al., 1983; Scherer, 2005, 2006). Additionally, about 1.4 million tons of cyanide are produced worldwide for use in mining (especially gold), metallurgy, plastic processing, electroplating, paints, etc. (Gossel and Bricker 1994; US Department of Health and Human Services, 2006; Eckstein, 2006). Cyanide has also been used by terrorists (Department of Health and Human Services, 2006; Eckstein, 2006; Schnepp, 2006) and in the capture of ornamental marine fish for aquarium trade (Hibbs, 1979; Honig et al., 1983).

The major pathway for cyanide metabolism is conversion of cyanide to thiocyanate (SCN^-) (Moriya and Hashimoto, 2001; Logue et al., 2010) (Figure 1). About 80% of an initial cyanide dose is converted to SCN^- (Weuffen et al., 1980; Fasco et al., 2007), which is subsequently excreted in the urine. The formation of SCN^- is enzymatically catalyzed by rhodanese

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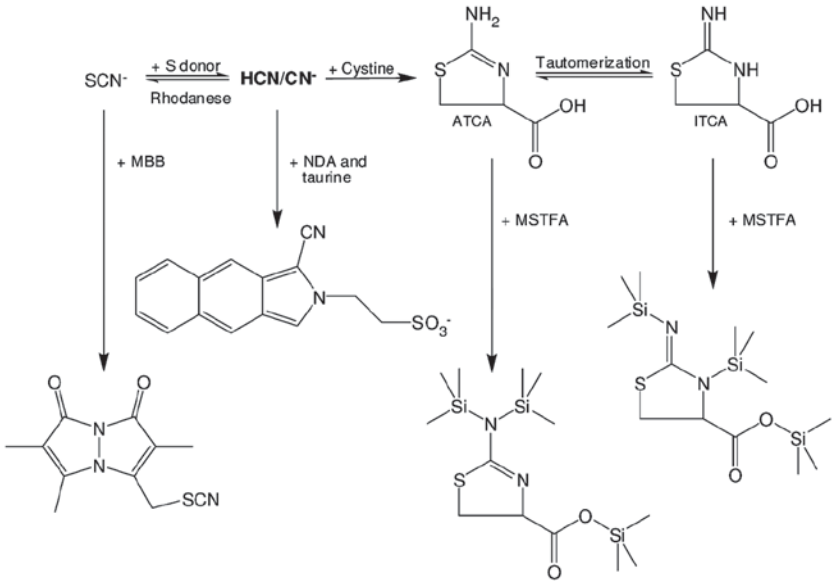

Figure 1. The metabolism of cyanide along with reactions utilized for the analysis of cyanide and its major metabolites (only those metabolites investigated in this study are shown). ATCA, 2-amino-2-thiazoline-4-carboxylic acid; ITCA, 2-iminothiazolidine-4-carboxylic acid; MBB, monobromobimane; NDA, naphthalene dialdehyde; MSTFA, N-methy-N-(trimethylsilyl) trifluoroacetamide.

(thiosulfate sulfurtransferase) and occurs when cyanide reacts with a sulfur donor (e.g. thiosulfate) (Salkowski and Penney, 1994; Lundquist et al., 1995, 1997; Isom and Baskin, 1997; Tsuge et al., 2000; Moriya and Hashimoto, 2001; Baskin et al., 2004; Logue et al., 2005). 2-Amino-2-thiazoline-4-carboxylic acid (ATCA) with its tautomeric form 2-imino-thiazolidine-4-carboxylic acid (ITCA; Figure 1) is formed by the reaction of cyanide with cystine (Wood and Cooley, 1956; Baskin et al., 2004) and may be predominant when sulfur donors and/or rhodanese are sparse (Moriya and Hashimoto, 2001). ATCA is not metabolized further (Baskin et al., 2004; Fasco et al., 2007) and therefore, may be a lasting signature of cyanide exposure. Other minor cyanide degradation pathways include conversion of cyanide to cyanocobalamine, one-carbon compounds, and protein adducts (Logue et al., 2010).

Verification of cyanide exposure (chronic or acute) is important in order to quickly administer cyanide antidote and reverse the toxic effects of cyanide poisoning. Currently, there are two main approaches used to verify cyanide exposure: i) direct analysis of cyanide (as CN^- or HCN), or ii) analysis of its major metabolite, thiocyanate. Although ATCA has been suggested as a possible marker of cyanide exposure, only a limited number of investigations have been completed to verify the connection between cyanide exposure and ATCA (Lundquist et al., 1995; Logue et al., 2005, 2009). If a strong relationship is established, forensic, clinical, military, scientific, environmental, and veterinary investigators could use this marker for diagnostic or forensic confirmation of cyanide exposure. The objective of the current study was to establish background concentrations of cyanide and its metabolites (SCN^- and ATCA) in common biological fluids (blood, urine, and saliva) of smokers and non-smokers and to evaluate possible correlations between these markers.

Methods

Materials

All solvents (HPLC grade) were obtained from Sigma-Aldrich (St. Louis, MO, USA). All aqueous solutions were prepared with distilled, deionized water (≥18.2 MΩ cm) obtained from a LabConco Water Pro PS system (Labconco, Kansas City, KS, USA). Sodium thiocyanate, sodium cyanide, sodium carbonate, tetrabutylammonium sulfate (TBAS), sodium hydroxide (NaOH), and isotopically-labeled potassium thiocyanate ($KS^{13}C^{15}N$) were obtained from Sigma-Aldrich. Potassium phosphate (KH_2PO_4), dipotassium phosphate (K_2HPO_4), boric acid tetrahydrate ($NaBO_2 \cdot 4H_2O$), sodium tetraborate decahydrate ($Na_2B_4O_7 \cdot 10H_2O$), sulfuric acid, and hydrochloric acid were obtained from Fisher Scientific (Fair Lawn, NJ, USA). Pentafluorobenzyl bromide (PFBBr) was obtained from Thermo Scientific (Hanover Park, IL, USA). Mixed mode cation exchange solid-phase extraction (SPE) columns were obtained from Waters Corporation (Milford, MA, USA). N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) was purchased from Pierce Chemical Company (Rockford, IL, USA). Phosphate/borate buffer was comprised of 0.05 M each of KH_2PO_4, K_2HPO_4 and $NaBO_2 \cdot 4H_2O$. Naphthalene-2,3-dicarboxaldehyde (NDA) solutions (2 mM) were prepared by dissolving NDA (TCI America, Portland, OR, USA) in 4 mL methanol, and diluting to 10 mL with phosphate/borate buffer. Taurine solutions (0.05 M) were made by dissolving taurine (Alfa Aesar, Ward Hill, MA, USA) in 10 mL phosphate/borate buffer. Tetraethylammonium acetate (TEAA)

eluent for HPLC was prepared by diluting 50 mL of 2 M TEAA (Transgenomic, Omaha, NE, USA) stock to 1 L with de-ionized water and filtering through a 0.45 μm nylon membrane filter (Millipore, Billerica, MA, USA). ATCA (L-2-amino-2-thiazoline-4-carboxylic acid) was obtained from Chem-Impex International (Wood Dale, IL, USA). A stock solution (100 μg/mL) was prepared in 0.1 M HCl and used throughout the current study. The ATCA internal standard, deuterated ATCA (ATCA-d2), was prepared in the lab of Dr. Herbert T. Nagasawa (Department of Veterans Affairs Medical Center, Minneapolis, MN, USA) by reaction of deuterated L-cysteine (3,3-d2) with cyanamide (Nagasawa et al., 2004). *(Note: Cyanide is extremely toxic and HCN is easily evolved. Care must be taken to avoid skin contact and inhalation/ingestion. The entire experimental setup, standard and sample preparation were conducted in a flow-monitored, well-ventilated hood. As HCN is volatile all solutions of CN^- were prepared in 0.1 M NaOH to ensure cyanide remained in ionized form).*

Biological fluids

Urine, saliva, plasma, and red blood cells (RBCs) were collected from a total of 100 human (*Homo sapiens*) subjects. Of these 100 subjects, 50 were regular smokers and 50 were non-smokers. Whole blood samples were collected and mixed with EDTA to prevent protein coagulation. The samples were initially gently mixed and then centrifuged to separate the plasma and RBC fractions. Cyanide was analyzed immediately on receipt of the biofluid samples and the remaining biofluids were stored in a freezer (approximately –80°C) until analysis. Eleven males and 39 females comprised the group of smokers, whereas, 13 males and 37 females comprised the group of non-smokers. All subjects completed an initial questionnaire that included questions pertaining to daily cigarette consumption and gender. Of the subjects, none were employed in an occupation where chronic cyanide exposure was considered likely. None of the female subjects were pregnant, none of the non-smokers indicated any significant exposure to second-hand cigarette smoke, and none were taking medication (e.g. sodium nitroprusside) that could contribute to cyanide exposure at the time the samples were collected. The plasma, saliva, urine, and RBC samples of the 100 subjects studied were divided into sub-groups based on gender (e.g. male smokers compared to female smokers) and smokers were divided into 5 subgroups based on the number of cigarettes consumed daily.

For some samples, the concentrations of certain biomarkers were below the detectable range. The inability to quantify biomarker concentrations was mainly limited to cyanide analysis. Although cyanide was analyzed quickly after the biological sample was procured, cyanide concentrations were difficult to determine for some samples. Difficulty in analyzing cyanide from biological fluids is most likely due to the low concentration of cyanide in biological fluids and the multiple ways in which cyanide is rapidly eliminated from the body, but it could also be due to the analysis technique utilized in this study. Interference with the analysis of ATCA from saliva appeared to correlate with discoloration for some samples, which could be due oral consumption of a substance (e.g. breath freshener or lozenge) before donating the samples and should be investigated further. Overall, the concentration of an individual marker of cyanide exposure could not be determined in 3.5% of the samples (i.e. 35 of 1000 samples).

Collection and analysis of human biological fluids was approved prior to this study by the Human Subjects Advisory Committee at South Dakota State University, the Avera Medical Institute (Sioux Falls, SD, USA), and the Human Research Protection Office of the US Army Medical Research and Materiel Command (Ft. Detrick, MD, USA).

Sample preparation and analysis

Cyanide was assayed by HPLC based primarily on the method of Felscher and Wulfmeyer (1998). Briefly, biofluid samples (100 μL) were added to 4 mL glass vials. An HPLC vial insert (300 μL), containing 0.1 M NaOH (100 μL) was placed into the vial and a screw-cap with septum was used to seal the vial. An aliquot of sulfuric acid (500 μL, 18 N) was injected into the vial using a 1 mL syringe to acidify the sample solution. HCN was then allowed to diffuse into the vial insert at room temperature. The vials were uncapped and the insert was removed and placed into a 2 mL HPLC auto-sampler vial. NDA (50 μL) and taurine (50 μL) solutions were added to the insert to create a fluorescent complex. For RBCs, the samples were weighed and 1 mL of deionized water was added to the samples. The samples were then shaken for several minutes and 1.5 mL of sample was transferred into a 2 mL microcentrifuge tube. The capped sample was centrifuged for 15 min at 15,700*g*. The supernatant was collected and samples were prepared by the microdiffusion method as previously described.

Analysis of the fluorescent NDA complex was performed on an Agilent 1200 HPLC system consisting of a quaternary pump, auto-sampler, vacuum degasser, and fluorescence detector (Agilent Technologies, Wilmington, DE, USA). A Zorbax C-18 (150 mm × 4.6 mm i.d., 5 μm particle size; Agilent Technologies) column was used to separate the sample. The mobile phase was comprised of 0.1 M TEAA (pH 7.0) and methanol at a flow rate of 1.5 mL/min. A gradient elution was used: 20% methanol (held for 1 min), linearly increased to 80% over 15 min, then ramped to 100% over a minute, and held for 2 min. The fluorescent cyanide complex was excited at 418 nm and the emission was monitored at 460 nm.

2-Amino-2-thiazoline-4-carboxylic acid was analyzed from urine, saliva, and plasma according to the method described previously by Logue et al. (2005). The sample preparation of plasma samples was modified from the original procedure due to low recoveries

of the ATCA from the SPE columns used. Protein was initially precipitated from the plasma samples (100 µL) using 600 µL of 1% HCl in acetone, vortexed for 1 min, and centrifuged at 15,700*g* for 10 min. An aliquot (100 µL) of the supernatant was then used for sample preparation. The internal standard (100 µL of 680 nM ATCA-d_2) was added to each standard and sample. The analysis was carried out on an Agilent GC-MS system consisting of a 6890N series gas chromatograph, a 5973 series mass detector, and a 7683 autosampler (Agilent Technologies). A DB5-MS bonded phase column (30 m × 0.25 mm ID, 0.25 µm film thickness; J&W Scientific, Folsom, CA, USA) was used with helium as the carrier gas.

Free thiocyanate was analyzed from urine, saliva and plasma according to the method described by Youso et al. (2010) using the GC-MS instrument described above. Internal standard (30 µL of 1.0 mM $KS^{13}C^{15}N$) was added to each standard and sample prior to analysis.

Data analysis

Significant differences in cyanide, thiocyanate, and ATCA concentration levels of smokers and non-smokers were tested with a two-tailed *t*-test. Comparison of each sub-group of smokers and non-smokers (e.g. male and female subgroups and groups divided by the number of cigarette smoked per day by smokers) was accomplished by a one-way analysis of variance (ANOVA). Bonferroni and Tukey's multiple range tests were used to identify significant differences between the identified subsets of human subjects. All data are reported in the format mean ± standard deviation with the standard error of the mean (SEM) in parenthesis.

Results and discussion

Smoking status

Initially, the concentrations of cyanide and its metabolites (SCN^- and ATCA) in biofluids of smokers were compared to those of non-smokers. The mean concentrations of CN^- and its biomarkers for smokers and non-smokers in plasma, saliva, urine, and RBCs are reported in Table 1. The average concentrations of CN^-, SCN^- and ATCA were found to be higher for smokers compared to non-smokers in each biofluid, which is in agreement with the fact that smokers inhale small amounts of CN^- through cigarette smoke. Other studies have also reported elevated concentrations of CN^-, SCN^- and ATCA in biological fluids of smokers compared to non-smokers (Maliszewski, 1955; Ballantyne, 1977; Clark et al., 1981; Sano et al., 1989; Torano and van Kan, 2003; Logue et al., 2005, 2009). Although mean CN^- concentrations were higher in smokers for each biofluid, no significant differences (as defined by $p < 0.05$) were found among smokers and non-smokers due to the large variability in cyanide concentrations in each biofluid (Table 1). In fact, the average precision for cyanide analysis, as measured by percent relative standard deviation (%RSD), was 141% with no biofluid showing a precision of less than 100%. This large variability could be due to the rapid metabolism and short half-life of cyanide (0.34–1.00 h) in humans (Ansel and Lewis, 1970; Hartung, 1994; Lundquist et al., 1995; Sousa et al., 2004; Logue et al., 2010). Considering the short half-life of cyanide and the large variability in cyanide concentrations, determination of cyanide exposure from the direct analysis of cyanide from all biological matrices would be difficult in cases of acute exposure where significant time has passed and in cases of low-level chronic

Table 1. Mean and range of CN, SCN and ATCA concentrations of smokers and non-smokers in plasma, saliva, urine and RBCs.

Marker	Smoking status	Matrix	N	Concentration (µM)*	%RSD**	Range (µM)
Cyanide	Smoker	Plasma	50	0.12 ± 0.18 (SEM = 0.02)	150	0.0020–0.84
		Saliva	45	0.25 ± 0.66 (SEM = 0.09)	260	0.0054–4.0
		Urine	42	0.24 ± 0.28 (SEM = 0.04)	110	0.0053–1.3
		RBCs	45	0.70 ± 0.75 (SEM = 0.10)	110	0.022–3.1
	Non-smoker	Plasma	49	0.08 ± 0.08 (SEM = 0.01)	100	0.0026–0.29
		Saliva	47	0.11 ± 0.14 (SEM = 0.02)	140	0.0038–0.84
		Urine	46	0.19 ± 0.23 (SEM = 0.04)	130	0.0086–1.6
		RBCs	45	0.46 ± 0.67 (SEM = 0.01)	140	0.022–3.9
SCN	Smoker	Plasma	50	97 ± 47 (SEM = 7)	49	12–220
		Saliva	50	1100 ± 1100 (SEM = 200)	93	48–3800
		Urine	50	100 ± 88 (SEM = 12)	86	8.6–400
	Non-smoker	Plasma	50	27 ± 23 (SEM = 3)	85	4.6–130
		Saliva	50	960 ± 900 (SEM = 130)	93	170–4200
		Urine	50	34 ± 15 (SEM = 2.1)	44	16–110
ATCA	Smoker	Plasma	50	0.26 ± 0.05 (SEM = 0.01)	19	0.19–0.45
		Saliva	49	0.37 ± 0.33 (SEM = 0.047)	88	0.034–1.4
		Urine	50	0.90 ± 0.82 (SEM = 0.10)	91	0.11–3.0
	Non-smoker	Plasma	50	0.21 ± 0.03 (SEM =0.003)	12	0.15–0.27
		Saliva	47	0.20 ± 0.16 (SEM = 0.02)	79	0.03–0.73
		Urine	50	0.79 ± 0.93 (SEM = 0.13)	120	0.078–5.0

*The values are reported as mean ± standard deviation. **%RSD is the percent relative standard deviation of the biomarker concentration.

cyanide exposure. Conversely, during the initial minutes following exposure, direct CN^- analysis may be the only biomarker capable of indicating cyanide exposure (Hartung, 1994; Calafat and Stanfill, 2002; Sousa et al., 2004; Logue et al., 2010).

Although no significant differences were found for CN^-, significant differences were observed for SCN^- in plasma and urine (Table 1; Figure 2A and 2B show the SCN^--biofluid pairs that demonstrated significant differences between smokers and non-smokers). Similar studies conducted to determine plasma SCN^- concentrations from smokers and non-smokers by Jacob et al. (1984) and Glatz et al. (2001) yielded similar results. The average precision for SCN^- analysis was 75% RSD, which is considerably lower than for cyanide determination. Concentrations of SCN^- were very high in each biofluid compared to CN^- and ATCA, especially salivary SCN^- concentrations (Table 1). This is due to the fact that SCN^- is naturally produced in saliva as an antibacterial agent (Clem and Klebanoff, 1966; Tsuge et al., 2000). Although large concentrations of SCN^- are advantageous in terms of analysis, this large background level may make it difficult to discern relatively small differences in SCN^- concentrations, especially salivary, for smokers and non-smokers. Besides being produced in saliva, SCN^- is also involved in other biological processes. For example, thiocyanate has been found to be oxidized to sulfate *in vitro* (Wood et al., 1947).

Significant differences ($p < 0.0001$) for ATCA were observed in plasma and saliva, but no significant differences were found in urine concentrations (Figure 2C and 2D show the ATCA–biofluid pairs that showed significant differences between smokers and non-smokers). The average precision of ATCA analysis for the biofluids studied was 68% RSD. The precision of ATCA analysis was better than SCN^- analysis and much better than CN^- analysis. It should also be noted the excellent precision of analysis of ATCA from plasma (Table 1; 19% for smokers and 12% for non-smokers). The %RSD was less than half of the next closest biofluid-biomarker pair. This excellent precision between individuals indicates that plasma ATCA concentrations are very consistent and should, at the very least, serve as an excellent marker of low-level chronic cyanide exposure.

The absence of significant differences in urinary ATCA concentrations is most likely due to inherent inconsistencies in urine samples compared to plasma samples due to dilution, diet, etc. (Logue et al., 2009, 2010). In an earlier study, Logue et al. (2005) did find significant differences in the urinary concentrations of ATCA between smokers and non-smokers. The earlier study was a more limited study, but the urine collection was accomplished in one day in the morning hours. Therefore, the large error found in this study may have been caused by inconsistencies in sample collection compared to the Logue et al. (2005) study and indicates that determination of ATCA from urine may not be capable of verifying cyanide exposure consistently.

Although this study shows that the determination of ATCA from plasma is promising for determination of cyanide exposure, there have been only few studies performed to evaluate the relationship between cyanide exposure and ATCA (Logue et al., 2010). Logue et al. (2009) provided evidence that ATCA can be used to determine chronic cyanide exposure. Other studies have also shown that ATCA is stable in biological fluids for months and does not metabolize further (Wood and Cooley, 1956; Lundquist et al., 1995; Baskin et al., 2004; Logue et al., 2005). However, further studies are required to validate the use of ATCA as a marker of cyanide exposure.

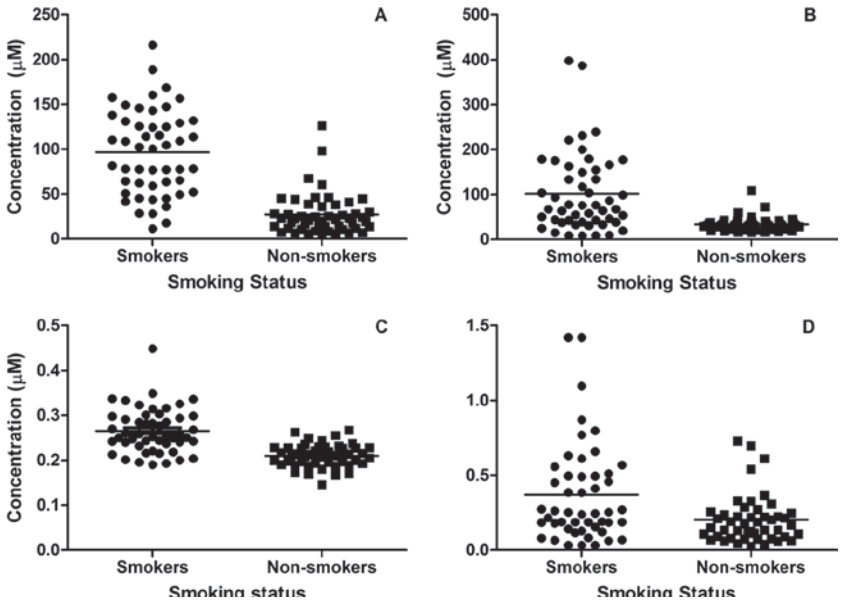

Figure 2. Concentrations of cyanide exposure biomarkers in biological fluids of smokers and non-smokers. Concentrations of SCN in A) plasma B) urine, and ATCA concentrations in C) plasma D) saliva. These cyanide exposure markers showed significant differences.

Gender

The plasma, saliva and urine concentrations of CN^- biomarkers were grouped based on gender and smoking status (e.g. male smokers compared to female smokers, male non-smokers compared to female non-smokers, etc.). The number of participants in each group and the mean concentration of CN^-, SCN^- and ATCA for each gender group are reported in Table 2. Although no significant differences were found when comparing CN^- concentrations among gender groups, some thiocyanate and ATCA concentrations were significantly different. Lack of significant differences between gender groups for CN^- was generally expected considering no significant differences were found for comparisons of smokers and non-smokers in aggregate.

Significant differences were found for some gender groups when comparing SCN^- concentrations that generally mirrored the significant differences found based on smoking status. Specifically, these groups were male smokers (MS) compared with male non-smokers (MNS), female smokers (FS) compared to female non-smokers (FNS), MS compared with FNS, and FS compared to MNS for both plasma and urine. Among smokers, the urinary SCN^- concentrations were significantly higher for MS compared with FS. This may be due to a number of factors, including differences in cigarette consumption (male smokers reported slightly higher daily cigarette consumption), cigarette preference, and diet. However, as generally expected, most significant differences between the groups were based on smoking status and not gender.

Significant differences were found for ATCA in plasma and saliva that were also generally based on smoking status (i.e., MS/MNS, FS/FNS, MS/FNS, FS/MNS) and not gender. Although in saliva, the only significant difference was between FS and FNS. This is most likely due to the limited number of male participants in the study.

The general lack of significant differences between genders within the same smoking group indicates that cyanide behaves similarly in both males and females. Therefore, the same marker of cyanide exposure could be used for both males and females without concern about gender.

Daily cigarette consumption

Smoking participants were additionally grouped according to the average number of cigarettes consumed daily. Five groups were created that allowed for a similar number of participants in each group: <10 ($N = 8$), 10–14 ($N = 14$), 15–19 ($N = 10$), 20–24 ($N = 9$), >25 ($N = 9$). Although no correlation could be drawn between any of the consumption groups for CN^- and SCN^- concentrations in any matrix, salivary ATCA concentrations in the 20–24 group did show significant differences with all other consumption groups. Some previous studies have also found no correlation between daily cigarette consumption and concentrations of cyanide and thiocyanate (Levine and Radford, 1978; Jarvis, 1989; Demkowska et al., 2008). Conversely, other studies have found a correlation between these two parameters (Yamanaka et al., 1991; Tsuge et al., 2000; Connolly et al., 2002; Hassan et al., 2009). The lack of correlation in the current study may be due to the increase in apparent half-life of cyanide with chronic exposure that is likely due to depletion of sulfur donors in people smoking a relatively high number of cigarettes (Lanno and Dixon, 1993; Okolie and Osagie, 1999; Garcia, 2005). With an increase in the apparent half-life of cyanide, significant differences among groups that smoke a large number of cigarettes per day (e.g. 10–25) may not be as easily observed as smokers of a relatively small number of cigarettes (e.g. <10 per day).

Biomarker concentration ratios

Strong significant differences between groups of smokers do not necessarily offer insight into the type of correlation between a marker and toxic agent exposure. Therefore, Logue et al. (2009, 2010, 2011) previously suggested using the biomarker concentration ratio (BCR) of a high-level exposure group (e.g. smokers) to a low-level exposure group (e.g. non-smokers) to determine marker correlations and to reduce inter-study variability. This ratio helps correct for inconsistencies in sampling, storage, and analysis methods when analyzing markers of cyanide exposure. A similar BCR for the parent agent and the proposed marker indicates a strong relationship between the two compounds. In order to better understand the relationship between cyanide exposure and metabolites of cyanide, BCRs for smokers compared to non-smokers were calculated for CN^-, SCN^-, and ATCA in each biofluid. Table 3 shows the calculated BCRs for CN^-, SCN^-, and ATCA in the biofluids analyzed along with the values reported in the literature (Logue et al., 2010).

Overall, the BCRs for CN^-, SCN^-, and ATCA found in the current study were within the ranges found in the literature. The BCR for CN^- was easily within the range of reported values, but the variability of cyanide concentrations from the biofluids in this study makes it difficult to draw significant conclusions. The blood, saliva and urine BCRs of SCN^- in this study are inconsistent compared to ATCA, but were within the literature range of SCN^- BCR (Table 3). The range of BCRs for SCN^- from literature studies are much larger than those obtained for CN^- or ATCA (Table 3). This is most likely because SCN^- is produced and consumed in processes other than cyanide exposure (Wood et al., 1947; Wood and Williams, 1949; Sano et al., 1989; Logue et al., 2010).

Relatively few studies have been performed on ATCA and only limited data is available. Even so, the ATCA BCRs found in this study are in good agreement with the ratios found for cyanide (in this study and others) and those found for ATCA from other studies, including the relative magnitude of the BCRs between biofluids (i.e. salivary BCRs are higher than the plasma BCRs, and urinary BCRs are the lowest).

Table 2. CN, SCN and ATCA concentrations in plasma, saliva, urine, and RBCs of each group and sub group of participants.

Marker	Matrix	Gender Group	N	Sub-group	N	Concentration (μM)*
Cyanide	Plasma	Male	23	Smokers	11	0.13 ± 0.23 (SEM = 0.07)
				Non-smokers	12	0.084 ± 0.084 (SEM = 0.02)
		Female	76	Smokers	39	0.12 ± 0.17 (SEM = 0.02)
				Non-smokers	37	0.072 ± 0.075 (SEM = 0.01)
	Saliva	Male	21	Smokers	9	0.16 ± 0.16 (SEM = 0.05)
				Non-smokers	12	0.097 ± 0.085 (SEM = 0.02)
		Female	71	Smokers	36	0.28 ± 0.73 (SEM = 0.10)
				Non-smokers	35	0.11 ± 0.16 (SEM = 0.03)
	Urine	Male	22	Smokers	10	0.30 ± 0.25 (SEM = 0.08)
				Non-smokers	12	0.14 ± 0.073 (SEM = 0.02)
		Female	66	Smokers	32	0.23 ± 0.28 (SEM = 0.05)
				Non-smokers	34	0.20 ± 0.27 (SEM = 0.05)
	RBCs	Male	23	Smokers	10	0.66 ± 0.62 (SEM = 0.20)
				Non-smokers	13	0.59 ± 0.56 (SEM = 0.20)
		Female	67	Smokers	35	0.72 ± 0.79 (SEM = 0.10)
				Non-smokers	32	0.41 ± 0.70 (SEM = 0.10)
SCN	Plasma	Male	24	Smokers	11	100 ± 70 (SEM = 21)
				Non-smokers	13	27 ± 16 (SEM = 4)
		Female	76	Smokers	39	95 ± 40 (SEM = 6)
				Non-smokers	37	27 ± 25 (SEM = 4)
	Saliva	Male	24	Smokers	11	1300 ± 1000 (SEM = 300)
				Non-smokers	13	820 ± 670 (SEM = 190)
		Female	76	Smokers	39	1100 ± 1100 (SEM = 200)
				Non-smokers	37	1000 ± 970 (SEM = 160)
	Urine	Male	24	Smokers	11	150 ± 140 (SEM = 41)
				Non-smokers	13	34.5 ± 8.2 (SEM = 2.3)
		Female	76	Smokers	39	88 ± 64 (SEM = 10)
				Non-smokers	37	34 ± 17 (SEM = 3)
ATCA	Plasma	Male	24	Smokers	11	0.290 ± 0.060 (SEM = 0.020)
				Non-smokers	13	0.210 ± 0.026 (SEM = 0.007)
		Female	76	Smokers	39	0.260 ± 0.041 (SEM = 0.007)
				Non-smokers	37	0.210 ± 0.024 (SEM = 0.004)
	Saliva	Male	24	Smokers	11	0.30 ± 0.39 (SEM = 0.10)
				Non-smokers	13	0.180 ± 0.082 (SEM = 0.02)
		Female	72	Smokers	38	0.39 ± 0.30 (SEM = 0.05)
				Non-smokers	34	0.21 ± 0.18 (SEM = 0.03)
	Urine	Male	24	Smokers	11	0.75 ± 0.57 (SEM = 0.17)
				Non-smokers	13	0.78 ± 1.3 (SEM = 0.40)
		Female	76	Smokers	39	0.95 ± 0.88 (SEM = 0.10)
				Non-smokers	37	0.79 ± 0.78 (SEM = 0.10)

*The values are reported as mean ± standard deviation.

Table 3. Calculated concentration ratios of smokers to non-smokers for CN, SCN, and ATCA from biofluids. Reported literature values are also shown (Logue et al., 2010).

	Saliva		Urine		Blood	
Marker	Obtained	Reported	Obtained	Reported	Obtained	Reported
CN^-	2.4	1.7	1.3	1.5-5.6	1.6 (Plasma) 1.5 (RBC)	1.2–3.1 (Blood)
SCN^-	1.2	1.1–30	3.0	1.1-44	3.6 (Plasma)	1.7–7.1 (Blood)
ATCA	1.8	NR	1.1	2.7	1.2 (Plasma)	1.5 (Plasma)

NR, Not reported.

Biomarker correlations

Correlations were evaluated based on each biofluid (e.g. the correlation of urinary and salivary thiocyanate for female smokers) and individual marker of cyanide exposure (e.g. the correlation of urinary ATCA and SCN^-) for each participant group of smokers (e.g. female smokers). Although most correlations between participant groups were weak and inconsistent, the correlation coefficients of plasma

and urinary SCN^- ranged from 0.23 to 0.73 for cigarette consumption groups. Moreover, the three moderate consumption groups (10–14, 15–19, and 20–24 cigarettes per day) showed the greatest correlation ($R^2 > 0.44$). The correlation between plasma and urinary SCN^- was generally expected because SCN^- is carried in the plasma and excreted in urine (Gupta, 2009; Logue et al., 2010).

Some other correlations were also found when separating participants by gender. The plasma and salivary concentrations of SCN^- in aggregate correlated for both males ($R^2 = 0.46$) and females ($R^2 = 0.47$) with remarkably similar slopes (29.0 and 26.4, respectively). The salivary and urinary ATCA concentrations of male smokers also showed a strong positive correlation ($R^2 = 0.78$, slope = 1.26). There were a number of strong correlations for cyanide concentrations between biofluids within the smoking groups, but the biofluid identities were not consistent. Compared to cyanide, there were relatively few strong correlations for ATCA, but there was a negative correlation between ATCA concentrations in plasma and saliva for the <10 and the 15–19 cigarettes per day groups.

Conclusions

This study helped establish the range of endogenous CN^-, SCN^-, and ATCA concentrations in human biofluids and better understand the relationship between CN^- and its metabolites, SCN^- and ATCA. From the study, ATCA and SCN^- may allow determination of low-level chronic cyanide exposure, and the analysis of plasma ATCA, in particular, showed excellent promise as a biomarker for cyanide exposure. No previous studies were carried out to analyze ATCA from saliva and the data obtained in this study for saliva indicate that salivary ATCA concentrations may also be used as a marker for chronic cyanide exposure. However, studies must evaluate the behavior (i.e. formation, distribution, and excretion) of ATCA during and after controlled cyanide exposures. A relationship should also be established between the dose of cyanide and the formation of ATCA.

Declaration of interest

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