色の有無）をもとに、順次使用する検知管が指示されており、初心者でも使用可能と考えられる。ただし、高濃度のガスが存在しないと検知できないなど、検知感度が悪いことが最大の難点である。また、検知管の種類が多く、差し迫った状況下では作業効率の低下を招くことが危惧される。陽圧式化学防護衣を装着した状況下での作業は制限があるため、検知管を一本ずつ使用するのではなく、一度に数本の検知管を検査できるような治具が望まれる。

複合型ガス検知器は、酸素濃度、一酸化炭素濃度、硫化水素濃度が数値で表示されるため、検知活動や救助活動時のマスク装着の目安には有効である。また、近年事案の多かった硫化水素検知に有効であることから、検知の第一候補として考えて良いと思われる。

携帯型化学剤検知器は、諸外国でも初動隊に配備されている携帯器材で、全面のLEDが点滅すると同時に警報音が発せられる。表面弾性波検知器よりも偽陽性が少ないこととから危険区域での検知には有効であるとの報告もある。

ラマン分光検知器は、固体と液体のみの検知と制限があるものの、残存物がある場合には有効な検知器であると考えられる。

携帯型ガスクロマトグラフ/質量分析装置は、外気の妨害を削除して検索することも考えられるが、個々の事例によって外気の状態が異なることや検知可能となるまでに時間を要することから、実際の検知活動時には運用不可能と考えられる。

全国でも、エタノールや小麦粉などを使用した訓練は実施されているようであるが、今回実施した様な実際に使用される危険性の高い化学物質を用いての検知訓練は、全国的にも初めての事例であると考えられる。実際の事例に対応するためには、使用されうる化学剤を用いての検知訓練が必要であり、日常的な訓練の一環として取り組む必要があると考える。

3．他自治体での連携体制について

2001年9月11日に発生したアメリカ同時多発テロ事件に引き続き、粉末化した炭疽菌芽胞が郵便として送付されて肺炭疽が発生した。神奈川県においては、この炭疽菌事件をきっかけにテロに対応する大枠が決められた。そこで、化学災害発生後に化合物の特定を行う際の各検査機関の対応とその能力についてまとめた。

【消防】

特殊災害対応自動車が指定都市には備えられており、NBC災害のような放射性物質、生物剤、化学剤などの漏洩、飛散、流出による災害に対応する機材を積載している。この特殊災害に対応する専用機材を備えている他、質量分析装置や赤外分光分析装置が設置されて

いるため災害現場で起因化合物の分析が可能である。

【科学捜査研究所】

化学物質の特定を行うために必要な分析機器が十分に揃っている。基本的にテロあるいは化学災害時には化合物の特定を行うが被災者からの検体は行わない。

【衛生研究所】

当該研究所には化学物質の特定を行うために必要な分析機器が十分に揃っている。基本的に分析対象となる試料は健康危機管理に関連した検体であるが、ヒト由来の血液等はバイオハザードの観点から測定することはできない。具体的に分析する検体としては、死亡した野鳥中の農薬、食品全般、健康食品、飲料水中の農薬、洗剤、漂白剤、異臭、医薬品、混入した異物などである。

【労働基準監督署】

化学災害が発生した際、災害発生時の直接原因を分析し災害発生に対する危険有害要因を特定してそれらを取り除くことが目的であるため、直接化合物の分析を行うことはない。

原因物質の検査システムの観点から、化学災害の発生現場以外で分析を行う機関は、科捜研と衛研であり、以下の検査システムを有する。科捜研の検査は基本的には所轄警察署からの依頼によって鑑定が行われる。また、発生事例によっては所轄からの依頼が無い場合でも現場へ出向き検査を行う。しかし、基本的には、病院などの他機関からの検査は受け付けていない。衛研の検査は県民等から保健所への訴えによる。保健所から衛研への検査依頼が行われる。この際、検査の依頼者は県職員となり、食品の場合は監視員である。県民から直接、研究所へ依頼も可能であるが料金が発生する。しかし、一般依頼検査の費用は手数料条例で設定されているため、依頼検査を行うと衛研に赤字が発生するため現在は受け付けない方向である。

現在のところ、科捜研や衛研などの検査機関へ他の医療機関等から検査を依頼することは不可能である。科捜研は多くの鑑定機材を有しているため化学災害の分析には最適と考えられる。しかし、科捜研への検査依頼は所轄の警察署から鑑定処分許可状を添えて行うのが一般的である。これに従えば、病院などで採取された検体に関しても所轄が一定の手続きを執る必要がある。しかし、警察が鑑定処分許可状を請求するのは基本的に事件性がある場合だけなので、事件として認定されなければ検査される可能性は極めて低い。従って、偶然発生した化学災害に関しては犯罪性が無いと判断されれば、検査対象にはならないであろう。

一方、衛研に関しては分析技術と機器を有しているものの基本的には健康被害に対して

の原因を特定することが目的である。更に、バイオハザードの観点から生体由来の試料の検査は受け付けていないことからも医療機関からの検査依頼は困難である。

大規模災害となれば、科捜研や衛研では対処不可能な場合が考えられる。このような災害時には地方自治体の長が自衛隊を要請し、自衛隊が対応にあたる。従って、このような大災害時は対応は上記と異なるかもしれないが明確ではない。緊急時に限り科捜研や衛検などへ検査依頼が行えるシステムを構築するのが最も設備投資が少ない解決方法であろう。

別添４

研究成果の刊行に関する一覧表

書籍

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Brief Communication

Preliminary screening method for the determination of inorganic arsenic in urine

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ABSTRACT

A simple and rapid method was developed for the routine determination and classification of inorganic arsenic based on its clinical and forensic properties. Inorganic arsenic was isolated from urine by using copper granules, which was then made to react with ammonium molybdate in order to detect its presence with the naked eye. Based on studies of extraction and reaction conditions, e.g., reaction temperature and time, a colorimetric screening method was established. The reaction mixture was measured by a spectrophotometer, and there was linearity from 0.05 to 2.0 μg/ml and the correlation coefficients of the calibration curves were greater than 0.99. The coefficients of intra-day variation at 0.2 and 2.0 μg/ml of inorganic arsenic in urine were 9.6 and 4.2%, respectively (n = 5). The minimum detectable level in urine is 0.03 μg/ml, and it is possible to detect the lowest level of poisoning according to the published reports. The proposed method was applied to a poisoning case wherein the patient ingested NEOARSEN BLACK® with alcohol, which contained 45% of arsenic trioxide. This method produced positive results in all the urine samples tested, and this method is useful for the screening of inorganic arsenic based on its clinical properties because it enables the detection of inorganic arsenic in urine without expensive equipment.

1. Introduction

Arsenic is widely distributed in the environment and biological materials. Although only a small amount of arsenic exists in the human body, it is thought to be an essential element for humans. Medicines that contain arsenic are still used in dental treatments, although there is a poor impression on arsenic compounds because they have been used in homicides and as components of chemical weapons. It is well known that groundwater pollution of inorganic arsenic is global occurrence such as South Asia, Southeastern Asia and South American countries. Recently, it has been focused that arsenic decomposed chemical weapons and these ingredients pollute soil and water, and this health hazard has occurred in Japan and China. Therefore, a simple and rapid method is required for the routine determination and classification of arsenic based on its clinical properties. Many methods have been reported for the determination of arsenic in the environmental and in biological materials [1–5]. Form separation analysis by high-performance liquid chromatography with inductively coupled plasma mass spectrometry (HPLC–ICP–MS) is a current trend in biological analysis because the toxicity of arsenic depends on its chemical species and forms. However, it is difficult for everyone to use this equipment.

In order to satisfy the demand for arsenic detection, we developed a colorimetric method for clinical and forensic use. In the first stage, we examined the colorimetric detection of inorganic arsenic in drinks or gelled foods by commercially available test kits (Pack Test and Merckoquant produced by Kyoritsu Rikagaku Kenkyusho, Japan, and Merck, Germany, respectively). The results revealed that these kits can detect inorganic arsenic in drinks and gelled foods [6,7]. Their detection limits were 0.5–25 μg/ml; however, they could not identify whether the inorganic arsenic is trivalent or tetravalent. In order to confirm inorganic arsenic poisoning, it is expected to detect concentrations as low as 0.1 μg/ml because the normal concentration range of arsenic in urine was from 0.01 to 0.3 mg/l and the fatal poisoning concentration range was from 0.1 to 0.4 mg/l [8]. However, it is impossible to detect the inorganic arsenic concentration in urine by using these kits. In this study, we attempt to utilize previously existing knowledge to develop a simple and rapid method through which a routine determination and classification of inorganic arsenic based on its clinical and forensic properties can be carried out. In biological materials, inorganic arsenic is adsorbed on to the surface of copper. Following desorption from copper, inorganic arsenic is then detected by a colorimetric reagent.

2. Material and methods

2.1. Materials

Inorganic arsenic standard solution (As(III), 1 mg/ml and pH 5) of analytical grade for atomic absorption spectrometry and distilled water were purchased from Wako Pure Chemicals (Osaka, Japan). Disodium hydrogen arsenate (As(V)) and other chemicals were of analytical grade. Hydrochloric acid (35.0–37.0%) used

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was of inorganic arsenic analytical grade. Copper granules (purity 99.99%, 12–16 mesh) were purchased from Mitsuwa Pure Chemicals (Osaka, Japan). The granules were washed with hydrochloric acid and distilled water before use.

Drug-free urine was collected from healthy adult males and used to make urine samples containing inorganic arsenic and for use as blank urine controls. Urine samples collected from a case of inorganic arsenic poisoning were frozen at −20 °C and stored until analysis.

2.2. Sample preparation

We put 5 ml of urine, 3 g of copper granules, and 3 ml of hydrochloric acid in a polypropylene bottle (10 ml) and heated it at 100 °C for 20 min to facilitate adsorption of inorganic arsenic to the copper surface. After cooling to room temperature, the solution was poured and the copper granules were washed three times by distilled water. The adsorbed inorganic arsenic was then removed with 0.5 ml of sodium hydroxide solution (0.1 M) and the procedure was repeated three times. The combined eluate was neutralized with hydrochloric acid and subsequently used for the colorimetric detection.

2.3. Colorimetric detection

The method of colorimetric detection used was a modified version of that had reported previously [9]. Briefly, the eluate and 50 μl $KMnO_4$ (10^{-4} M) were added to a glass tube. After 5 min, 50 μl of l-ascorbic acid (0.1 g/ml) and 100 μl of ammonium molybdate (13 g $[(NH_4)_6Mo_7O_{24}\cdot 4H_2O]$/100 ml 9 M H_2SO_4) were added into the tube and the mixture was heated at 50 °C for 20 min. The absorbance (840 nm) of the reaction mixture was measured using a spectrophotometer (Shimadzu, UV-200S double beam spectrophotometer).

2.4. Optimal conditions

To obtain an optimal condition for adsorption of inorganic arsenic in urine, the amount of copper granules (0.5, 1.0, 3.0, 5.0, and 10 g), the added volume of hydrochloric acid (1.0, 2.0, and 3.0 ml), and the heating times (10, 20, and 30 min) were examined in triplicates using urine samples spiked with 2 μg/ml of inorganic arsenic. After achieving optimal conditions for the adsorption of inorganic arsenic, the effects of NaOH concentration and number of repetitions were determined to remove inorganic arsenic from the surface of the copper granules.

2.5. HPLC–ICP–MS

The Agilent 1100 series HPLC model was used for separating inorganic arsenic species, and the Agilent 7500i ICP–MS was used for inorganic arsenic-specific detection. Chromatographic separation was performed on an L-column ODS (250 mm; internal diameter (i.d.), 4. mm; 5 μm; CERI, Tokyo, Japan) with water as the mobile phase. The sample was diluted with ultrapure water. The injection volume was 100 μl, and separation was carried out at 30 °C. The eluate from the HPLC system was introduced online to the ICP–MS. Signals at m/z 35, 75, 77, and 78 were monitored. Inorganic arsenic species in the samples were identified by matching their retention time with those of the inorganic arsenic standards.

2.6. A case of poisoning

A 47-year-old male drank NEOARSEN BLACK® with alcohol. NEOARSEN BLACK® contains 45% of arsenic trioxide and is commonly used in dental clinics. The estimated amount of the preparation consumed was 1 g. Three hours later, he was sent to the hospital in an ambulance. He felt nausea and suffered from consciousness disorder. Diarrhea continued as a typical symptom of inorganic arsenic poisoning. Urine samples were collected at 3, 6, 12, 24, and 36 h after he was admitted to the hospital.

3. Results and discussion

Although the Reinsch test has been performed for more than a hundred years, a detailed examination with this test did not provide definite results [10,11]. To achieve optimal conditions for inorganic arsenic adsorption in urine and its' desorption from the surface of copper granules were examined using urine samples spiked with 2 μg/ml of inorganic arsenic. Different types of copper, such as small thin strips, coils, and wires were examined for use in inorganic arsenic adsorption. Copper granules were used in this study because of the large surface area of each granule as compared to the other types of the same weight. The effect of the amount of the copper granules is illustrated in Fig. 1. The mixture was heated at 100 °C for 20 min. The absorbance of the reaction mixture was dramatically enhanced in the amount of copper up to 1 g, but this increase was not observed beyond 3 g of copper. The effect of heating time and the added volume of hydrochloric acid are illustrated in Fig. 2. The volume of hydrochloric acid did not affect the absorbance, however, the absorbance was maximal for 20 min heating. Therefore, the following conditions were adopted as the optimal condition for inorganic arsenic adsorption; 5 ml of urine, 3 g of copper granules, and 3 ml of hydrochloric acid were heated at 100 °C for 20 min.

With these conditions for inorganic arsenic adsorption, the effects of NaOH concentration and number of repetitions on the removal of inorganic arsenic from the surface of copper granules were examined. When inorganic arsenic was removed from the surface of copper granules with 0.1 M NaOH, there was a frequency-dependent increase in the absorbance; it was maximized at a three-time removal. Furthermore, the effect of NaOH concentration on the removal of inorganic arsenic was examined. Differences in the NaOH concentration (0.01, 0.05, 0.1, 0.5, and 1.0 M) did not result in any differences in the absorbance. Therefore, the above conditions were adopted as the optimal condition for inorganic arsenic elution.

The urine samples spiked with inorganic arsenic were prepared at the inorganic arsenic concentrations of 0, 0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, and 5.0 μg/ml and analyzed by three times of each point using the above procedure. The calibration curves were obtained by plotting the absorbance against the inorganic arsenic concentration. Linearity was observed from 0.05 to 2.0 μg/ml and the corre-

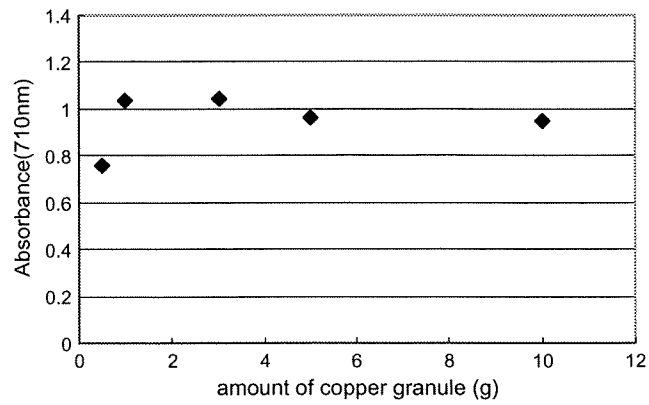

Fig. 1. Effect of an amount of copper granule on the recovery of inorganic arsenic in urine. Each point represents the mean of three samples.

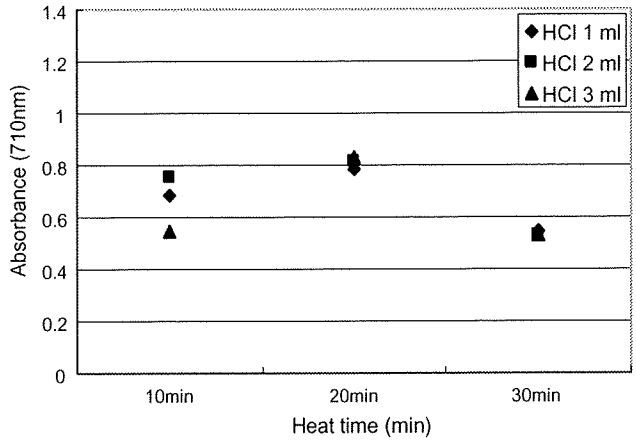

Fig. 2. Effect of a volume of hydrochloric acid and heating time on the recovery of inorganic arsenic in urine. Each point represents the mean of three samples.

lation coefficients of the calibration curves were more than 0.99. The coefficients of intra-day variation at 0.2 and 2.0 μg/ml of inorganic arsenic in urine (n = 5) were 9.6 and 4.2%, respectively. The minimum detectable levels in urine were 0.03 μg/ml and it is possible to detect the lowest level of poisoning according to published reports. In the case of inorganic arsenic poisoning in humans, urine inorganic arsenic concentrations are 1.9–51.4 μg/ml [12–15]. Intoxication with inorganic arsenic mixed in curry occurred in Wakayama, Japan, in July 25, 1998; 4 people died and 63 people were injured. The inorganic arsenic concentrations in the 3 survived children were 9.6, 12.3, and 21.2 μg/ml [16]. These concentrations are measurable by this proposed method. The absolute recovery was 50% at the optimal condition, which seems acceptable as compared to that by the published report [17].

When normal and inorganic arsenic-spiked urine samples were directly examined by commercially available kits, false negative and positive results were observed. These commercially available kits can be used for the detection of inorganic arsenic in urine by pretreatment of the proposed method. Organic arsenic such as dimethylarsinate and diphenylarsinate were not determined by this method; therefore, this method can distinguish between the organic and inorganic forms of arsenic.

The proposed method was applied to a poisoning case wherein the patient ingested 1 g of NEOARSEN BLACK® with alcohol, which contained 45% of arsenic trioxide. This method produced positive results in all the urine samples collected at 3, 6, 12, 24, and 36 h after admission and the concentrations were 0.72, 0.22, 0.48, 0.14, and 0.10 μg/ml, respectively. A tendency of correlation was obtained between these results and results obtained using the HPLC–ICP–MS method. The proposed method is useful for the screening of inorganic arsenic in clinical and forensic sciences because it is easy to detect inorganic arsenic in urine without the use of expensive equipments and also to diagnose the possibility of poisoning. However, it is difficult to calculate the accurate inorganic arsenic concentration in urine at this stage. Although the proposed method was applied to only one case of arsenic poisoning in this study, we are planning to assess the correlation between arsenic levels obtained by this method and those by the instrumental method in more poisoning cases.

4. Conclusion

A simple colorimetric method combined with Reinsch test was developed for use in the clinical and forensic laboratory. This method detects as low as 0.03 μg/ml of inorganic arsenic in urine and is useful for the pre-screening to perform the form separation analysis by HPLC–ICP–MS based on its clinical properties. In addition, inorganic arsenic in various fluids can be purified with our extraction method; therefore, it can be potentially used as a sample preparation procedure for other apparatus.

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