Forensic Science International 245 (2014) 45–50

Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

The utilization of stable isotope analysis for the estimation of the geographic origins of unidentified cadavers

Stuart J. McLean [a], Hiroshi Ikegaya [a,*], Pekka J. Saukko [b], Huang Yung Zheng [c], Kyoko Itoh [d], Shinji Fushiki [d]

[a] Department of Forensic Medicine, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, 602-8566, Japan
[b] Department of Forensic Medicine, University of Turku, 20520, Finland
[c] Department of Urology, Fujian Medical University Union Hospital, 350001, China
[d] Department of Pathology and Applied Neurobiology, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, 602-8566, Japan

ARTICLE INFO

Article history:
Received 13 March 2014
Received in revised form 23 June 2014
Accepted 5 October 2014
Available online 14 October 2014

Keywords:
Stable isotope analysis
Identification
Geographic origin
Urine

ABSTRACT

The number of unidentified cadavers is increasing worldwide and the effective methods which reveal their geographic origin are not well known. This study reports on the utilization of $\delta^{18}O$, $\delta^{13}C$, $\delta^{2}H$ and $\delta^{15}N$ ratios gained through stable isotope analysis of urine samples collected from eight locations: Chiba, Japan; Fuzhou, China; and Denpasar, Indonesia in our pilot study with data from healthy volunteers from five further locations from healthy volunteers: Melbourne and Perth, Australia; Qingdao, China; Turku, Finland and Oklahoma, USA. This study posits that the utilization of $\delta^{18}O$ and $\delta^{2}H$ is more feasible than $\delta^{13}C$ and $\delta^{15}N$ stable isotope ratios in differentiating or estimating the origin of human samples. Secondly, this study demonstrated that the $\delta^{18}O$ and $\delta^{2}H$ stable isotope ratios of urine samples from eight locations differed significantly.

1. Introduction

Partly as a result of increasing globalization, the number of unidentified cadavers is increasing in Europe and Japan [1,2]. Particularly, in the case of acts of terrorism and large natural disasters, the determination of a cadaver's geographic origin is extremely important to facilitate later DNA identification, yet is problematic. However, to determine a corpse's or living human's geographic origin, forensic science requires effective methods to accompany and support ordinal anthropological methods [3]. It has been shown that genotypes of the JC virus within urine or renal tissue are closely related with the geographic origin of the human source of the samples [4]. In addition, JC virus genotyping has been effective in cases where soft tissue remained on unidentified cadavers [5,6]. However, the JC virus is not always detectable. Thus, the detection of $\delta^{13}C$ virus genotypes was utilized to estimate the geographic origin of other cadavers and to estimate more accurately the geographical origin of cadavers by analyzing the distribution of various viruses [7–9]. However, where viruses are not detected, further methods of establishing the geographical origin of cadavers are also necessary, especially methods to find the recent- to medium-term origin of corpses or living humans.

Stable isotope analysis is a sub-field of archeometry, geochemistry and hydrology, and is an increasingly frequently utilized technique in determining the origin of unidentified cadavers. Hydrogen (H), sulphur (S), carbon (C), oxygen (O) and nitrogen (N) are present in nature in variable ratios. Local isotopic signatures are present in flora and fauna, making their way up through the food chain, and into plants and animals. There is a large body of literature documenting the utilization of stable isotope analysis through the analysis of biogenetic tissue in determining the origin and authenticity of beverages, food and food ingredients [10–20]. In Japan the stable isotope of strontium is used to establish where rice was produced [21], distinguishing among imports from Australia, China, or California, for example.

As a result of the food chain passing along isotopic signatures of the environment through the relative presence of isotope ratios of light elements in human tissue and body fluids [22–25] it is possible to estimate the geographic and climatic conditions ($\delta^{2}H$, $\delta^{18}O$) of habitat, and the dietary habits of organisms ($\delta^{13}C$, $\delta^{15}N$,

* Corresponding author at: Department of Forensic Medicine, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, 456 Kajiicho, Kamigyo, Kyoto 602-8566, Japan. Tel.: +81 75 251 5343; fax: +81 75 251 5343.
E-mail address: ikegaya@koto.kpu-m.ac.jp (H. Ikegaya).

http://dx.doi.org/10.1016/j.forsciint.2014.10.011
0379-0738/

$\delta^{34}S$). Recently, within the field of forensic science, stable isotope analysis has been used to determine the origins of drugs [26], banknotes [27], gunshot [28], humans [29–32] and a cadaver [33].

In 2007, despite different criminalistic and forensic methods (e.g. tooth status, fingerprint or DNA analysis) German authorities were unable to identify a male murder victim found on an expressway. However, stable isotope analysis assisted in identifying the origin of the man and the capture of the perpetrators of the crime [33]. This demonstrated the potential value of stable isotope analysis to forensic science.

In 2013, the effectiveness of utilizing $\delta^{18}O$ and $\delta^{2}H$ when determining the origin of urine samples from Chiba, Japan; Fuzhou, China; and Denpasar, Indonesia from healthy volunteers was investigated. Statistically significant differences in $\delta^{2}H$ and $\delta^{18}O$ values from the three locations were found [32].

This study aims to be the first study to investigate the relative usefulness of $\delta^{18}O$, $\delta^{13}C$, $\delta^{2}H$ and $\delta^{15}N$ values from urine to determine the origin of its source, because of the ease with which samples can be collected. In addition, this study aims to build upon our pilot study [32] by adding data from five further locations—Melbourne and Perth, Australia; Qingdao, China; Turku, Finland and Oklahoma, USA—to the data from samples collected from Chiba, Japan; Fuzhou, China; and Denpasar, Indonesia in our pilot study.

2. Materials and methods

2.1. Urine samples

Two milliliters of urine were collected from six individuals living in each of the eight study locations: Chiba, Fuzhou, Qingdao, Melbourne, Perth, Turku, Oklahoma and Denpasar. One sample from Qingdao and two samples from Perth were lost while in transit. All samples were sealed and stored at −80 °C until analysis. Samples were collected from volunteers who had not traveled outside the immediate region within the last 6 weeks. Volunteers were not advised to keep to any special diet prior to sample collection, and some of the volunteers reported drinking bottle water regularly, without knowing the locational origin of the water. All samples were collected under the informed consent of healthy volunteers and the research was approved by the Ethical Committee of Kyoto Prefectural University of Medicine (ERB-C-133).

3. Stable isotope analysis

3.1. Sample preparation

Prior to analysis, samples were centrifuged at 1500 × *g* to settle out any sediment to ensure that only the liquid component of the samples was analyzed.

3.2. Deuterium analysis

Deuterium analysis of the samples was performed in duplicate using the equilibration technique. In brief, sample aliquots were pipetted into Exetainer tubes and an insert vial containing 5% platinum on alumina was added. The tubes were sealed and then filled with pure hydrogen. Samples were left to allow complete equilibration of the water with the hydrogen gas. Analysis was undertaken using continuous-flow isotope ratio mass spectrometry using a Europa Scientific ANCA-GSL and GEO 20-20 IRMS.

3.3. A brief outline of the calibration method

The samples were measured against three reference standards, the first standard being IA-R054 with δ^{2}HV-SMOW = +4.93%, the second being IA-R052 with δ^{2}HV-SMOW = −157.12% and the third being IA-R053 with δ^{2}HV-SMOW = −61.97%. All three standards are traceable to the primary reference standards V-SMOW2 (Vienna-Standard Mean Ocean Water) and V-SLAP2 (Vienna-Standard Light Antarctic Precipitation) distributed by the International Atomic Energy Agency (IAEA).

The IA-R054 standard was used as the reference to which the samples and other standards were measured. The IA-R052 standard was used for calibration of the $\delta^{2}H$ values and the IA-R053 standard was used as a check of this calibration.

4. Oxygen-18 analysis

After deuterium analysis, the vials were flushed with pure CO_2 and left to allow complete equilibration of the water with the CO_2 gas. Reference waters (including a quality control standard) were prepared in the same manner. The samples and references were then analyzed using continuous-flow isotope ratio mass spectrometry using a Europa Scientific ANCA-G and 20-20 IRMS.

4.1. A brief outline of the calibration method

The samples were measured against three reference standards, the first standard being IA-R054 with δ^{18}OV-SMOW = +0.56%, the second being IA-R052 with δ^{18}OV-SMOW = −19.64% and the third being IA-R053 with δ^{18}OV-SMOW = −10.18%. All three standards are traceable to the primary reference standards V-SMOW2 (Vienna-Standard Mean Ocean Water) and V-SLAP2 (Vienna-Standard Light Antarctic Precipitation) distributed by the IAEA.

The IA-R054 standard was used as the reference to which the samples and other standards were measured. The IA-R052 standard was used for calibration of the $\delta^{18}O$ values and the IA-R053 standard was used as a check of this calibration.

4.2. Nitrogen-15 and carbon-13 analysis

The technique used for this analysis was EA-IRMS (Elemental Analyzer Isotope Ratio Mass Spectrometry). In this technique, samples and reference materials are pipetted and dried into tin capsules, sealed, and then loaded into an automatic sampler on a Europa Scientific Roboprep-CN sample preparation module. From there they were dropped in a furnace held at 1000 °C and combusted in the presence of oxygen. The tin capsules flash combust, raising the combustion temperature of the samples to 1700 °C. The combusted gases were swept in a helium stream over a combustion catalyst (Cr_2O_3), copper oxide wires (to oxidize hydrocarbons), and silver wool to remove sulphur and halides. The resultant gases (N_2, NO_x, H_2O, O_2, and CO_2) are sucked through a reduction stage of pure copper wires held at 600 °C. This removes any oxygen and converts NO_x species to N_2. A magnesium perchlorate chemical trap removes any water. Nitrogen and carbon dioxide are separated using a packed column of gas chromatograph held at an isothermal temperature of 65 °C. The resultant chromatographic peaks enter the ion source of the Europa Scientific 20-20 IRMS, where they are ionized and accelerated. Gas species of different mass are separated in a magnetic field and then simultaneously measured on a Faraday cup universal collector array. For N_2, masses 28, 29, and 30 were monitored and for CO_2, masses 44, 45, and 46 are monitored.

Both references and samples were converted to gases and analyzed in the same manner. The analysis proceeded in a batch process, whereby a reference was analyzed followed by a number of samples and then another reference.

The reference material used during analysis of all samples was a mixture of IA-R045 (iso-analytical working reference standard ammonium sulphate with a $\delta^{15}N$ value of −4.71% vs. air) and