

Department of Forensic Medicine

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General Summary

Our main research projects in 2008 have focused on sudden unexpected infant death due to milk aspiration, diagnosis of drowning by detection of specific DNA fragments of aquatic bacteria from blood samples, the identification of war-dead remains by DNA analysis, the objective evaluation of the limits of DNA typing based on the intensity of ninhydrin treatment, age and skewed X chromosome inactivation in autopsy specimens, and quantitative analyses of medicines and poisonous substances in forensic autopsy cases.

Research Activities

Forensic pathology

1. Sudden unexpected infant death due to milk aspiration

An experimental study was performed in a murine model to examine longitudinal changes in the pathological findings of the lungs and other organs in cases of milk aspiration. Immunostaining with an anti-human α lactalbumin antibody showed positive reactions against the antibody over time in the lungs, kidneys, and spleen. Detection of aspirated milk in organs other than the lung would be clear evidence of intravital milk aspiration and would suggest previous or recurrent milk aspiration.

2. Diagnosis of drowning by detection of specific DNA fragments of aquatic bacteria

In general, death by drowning is diagnosed when diatoms are detected in organs other than the lungs. We speculate that bacteria are more useful markers than are plankton for the diagnosis of death by drowning. In preserved blood samples from 30 cases of freshwater drowning, specific DNA fragments of *Aeromonas sobria*, a common aquatic bacteria, were examined with the polymerase chain reaction (PCR). The DNA fragments of the bacterium were detected with nested PCR in most cases.

DNA analysis

1. Identification of war-dead remains with DNA analysis

We performed identification of war-dead remains buried in the former Soviet Union by means of DNA analysis as part of the war-dead remains return project of the Ministry of Health, Labour and Welfare. For genetic markers we used single nucleotide polymorphisms of hypervariable regions of mitochondrial DNA and short tandem repeats of nuclear DNA.

2. The objective evaluation of the limit of DNA typing based on the intensity of ninhydrin treatment

Shed epithelial cells on a sheet of paper were stained with ninhydrin reagent, and DNA typing was performed. We studied the relationship between the intensity of purple

staining after ninhydrin treatment and the limit of DNA typing as mitochondrial DNA polymorphisms, and we attempted to perform an objective evaluation to determine the target of the staining area for DNA analysis.

3. Age and skewed X chromosome inactivation in autopsy specimens

We studied the association of age and skewed X chromosome inactivation in autopsy specimens from female subjects. Two X chromosomes were differentiated with a methylation-sensitive enzyme and the human androgen receptor PCR assay. A weak correlation was found between age and the frequency of skewed X chromosome inactivation, and we attempted to apply this technique to forensic age estimation.

Forensic toxicology

1. Quantitative analyses of medicines and poisonous substances

Medicines and poisonous substances (abuse drugs, alcohol, carbon monoxide, cyanide, and agricultural chemicals) suspected to have caused deaths were quantitatively analyzed by gas chromatography (GC), GC/mass spectrometry (MS), and spectrum photometry in samples obtained at autopsy.

2. Qualitative and quantitative analyses of hydrogen sulfide

Analysis of hydrogen sulfide and its metabolite thiosulfate was attempted with GC/MS in cases of poisoning by inhalation of hydrogen sulfide. We detected, lethal doses of sulfide in all cases. In addition to samples of blood and urine, samples of cerebrospinal fluid were useful for analysis.

Establishment of age estimation

We studied the estimation of date of birth from the quantity of radioactive carbon isolated from tooth enamel. We examined the application of this technique to age estimation for forensic medicine.

Publications

Okuno S, Sakurada K, Ohta H, Ikegaya H, Kazui Y, Akutsu T, Takatori T (Natl Inst Police Sci), Iwadate K. Blood-brain barrier penetration of novel pyridinealdoxime methiodide (PAM)-type oximes examined by brain microdialysis with LC-MS/MS. *Toxicol Appl Pharmacol* 2008; **227**: 8-15.

Iwadate K, Aoyagi M, Sakai K, Ochiai E, Abe S, Maebashi K, Nakamura M, Fukui K. Feasibility of immunohistochemical examination of lungs to distinguish intravital milk-aspiration from post-mortem "aspiration": a experimental study using a murine model. *Res Pract Forens Med* 2008; **51**: 233-7.