TECHNICAL NOTE

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DNA Fingerprinting from Tissues After Variable Postmortem Periods

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ABSTRACT: DNA typing is a useful tool in forensic cases for determining the identity of remains of humans who have been dead for various periods of time. DNA fingerprinting can be achieved only if high molecular weight DNA (HMWDNA) is extracted from the tissue samples of the bodies even after a long postmortem delay. Analyses were performed on various tissues collected during forensic autopsies of 24 bodies known postmortem ages. Tissues such as blood and kidney were found to be unsuitable for DNA fingerprinting because of a rapid degradation of the DNA after a period of one week. HMWDNA could be successfully extracted from brain cortex regardless of postmortem age.

KEYWORDS: forensic science, DNA fingerprinting, human identification

In forensic cases, questions of identity may concern remains of humans who have been dead for various periods of time.

Postmortem degradation of DNA is a very complex phenomenon, beginning with autolysis and followed by aerobic and bacterial destruction of cells. However, in spite of the development of the polymerase chain reaction (PCR) method, which today gives us good results with very small quantities (5 to 10 ng) of relatively low molecular weight DNA, we still continue to use the usual fingerprint method because the PCR technique has a low incrimination level yet.

The detection of DNA polymorphisms with single locus probes (SLP) for DNA fingerprinting consists of determining a restriction fragment pattern generated by hypervariable minisatellites located on specific chromosomes. This pattern is unique for each individual, except for monozygotic twins.

According to the Mendelian inheritance of DNA sequences, we may be able to reconstitute the familial relationship by comparing the fingerprints of the parents or close relatives with those of a proband. This analysis can be achieved only if high-molecular-weight DNA (HMWDNA) can be extracted from postmortem tissues.

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The aim of this study was to investigate the stability of HMWDNA in tissues after death. Previous studies [*1–3*] involved multilocus probes and other tissues such as bone for evaluating the DNA stability. We chose to study the DNA stability in other postmortem tissues and the ability of these tissues to yield HMWDNA for fingerprinting with SLP.

Materials and Methods

The tissue samples were collected, during forensic autopsies, of 24 bodies of known postmortem ages ranging from one day to one month. The tissue specimens were taken from skeletal muscle, heart, blood, lymph nodes, spleen, liver, kidney, and brain, then stored at −20°C prior to analysis.

The DNA purification method [*4*] consisted of a conventional procedure including incubation of the tissues in lysis buffer containing proteinase K (300 μg/ml) and SDS (2%), phenol/chloroform extractions and dialysis against Tris-EDTA buffer. At this step and for each specimen the DNA yield was estimated. The amount of recovered DNA was determined by comparison of the ultraviolet fluorescence of an aliquot of each sample with the fluorescence of known quantities of lambda DNA in a gel-stained with ethidium bromide. Samples and 20, 50, 100, 300, and 500 ng lambda DNA were loaded onto a 0.8% agarose gel and electrophoresis was carried out for 2 h at 100 V. After comparison with the range of lambda DNA quantities (20 to 500 ng) the final concentration of each DNA sample was determined. The molecular weight of the isolated DNA was estimated by comparison of its distance of migration with that of the 40 kb lambda DNA. HMWDNA was then digested, electrophoresed on an agarose gel, southern blotted and hybridized with two [32P]-labeled SLPs [YNH24 (D2S44), V1 (D17S79), Lifecodes Corp. NY] [*4*].

Autoradiographies of the hybridized filters were performed by exposure to Kodak XOMAT films at −80°C.

Results

The bodies used for this study were collected in the area of Strasbourg. At the autopsy, none of the bodies showed signs of infectious diseases. They were exposed to varying temperatures (5 to 25°C), humidity and light conditions. Exposure conditions could not be described precisely for each body.

Table 1 shows the quantity of HMWDNA extracted per gram of crude tissue analyzed at different postmortem ages in the case of blood, liver, kidney, lymph nodes, spleen, heart, muscles, and brain cortex, respectively. With blood samples, a good HMWDNA recovery from all the bodies investigated was obtained during the first week following the death. In each case, the quality and quantity of the DNA were good enough to realize DNA fingerprints with the SLP system (data not shown). In liver samples, only bodies of postmortem ages ranging from 1 to 7 days allowed us to obtain HMWDNA. HMWDNA was extracted from kidney samples up to one month after death, but the yield of DNA decreased rapidly, becoming very low beyond a period of one week. Past this period, we did not succeed in obtaining DNA fingerprints from DNA extracted. The extraction of DNA from lymph nodes was satisfactory up to one week. Over a postmortem period of 7 days, the DNA was degraded and unsuitable for blotting. HMWDNA was purified from spleen samples up to one week after death and the DNA present in heart tissue or in muscle cells was stable up to one month postmortem period, allowing us to perform DNA fingerprinting. Finally, out of all tissues tested, the brain cortex appeared to be the most stable tissue for DNA recovery, even after long postmortem periods. After 20 days, the DNA degradation was in each case less important in brain cortex than in liver, spleen, kidney, blood, muscles, lymph nodes and heart. From brain tissue we obtain in

TABLE 1—*Quantity of HMWDNA extracted per g of crude tissue analyzed.*

DNA yields in μg HMWDNA/g tissue	1 Day; N^b = 6		2–7 days; N = 9		8–30 days; N = 5		More than 36 days N = 3		85 days N = 1
	Average ± SD[a]	Range	Average ± SD	Range	Average ± SD	Range	Average ± SD	Range	
Brain	78 ± 37	45–469	68 ± 39	19–135	76 ± 75	1–152	60 ± 37	32–114	36
Liver	131 ± 49	43–154	177 ± 151	32–506	NR[c]	NR	NR	NR	NR
Spleen	521 ± 251	58–800	433 ± 395	5–1014	NR	NR	NR	NR	NR
Kidney	911 ± 145	625–1100	252 ± 210	50–762	4 ± 1	1–6	1 ± 0.5	1–2	NR
Lymph nodes	958 ± 754	97–5752	752 ± 548	0–1875	25 ± 5	20–30	NR	NR	NR
Blood	20 ± 15	0.5–44	19 ± 12	5–40	NR	NR	NR	NR	NR
Muscle	50 ± 37	17–45	48 ± 28	18–96	54 ± 3	51–57	NR	NR	NR
Heart	60 ± 53	11–140	65 ± 32	6–128	23 ± 21	1–45	NR	NR	NR

[a]SD = standard deviation.
[b]N = number of specimens studied.
[c]NR = no HMWDNA recovered.

each case, even after 85 days, reliable RFLP patterns using 1 μg of HMWDNA of the total yield obtained from only 1 g of crude brain tissue sample.

We also found an important variation in the yield of the DNA extraction from a same organ at the same postmortem period: the DNA yield ranged from 0 to 1875 μg/g of crude tissue (Table 1). No significant correlation could be established between the recovery of the amounts of DNA and the postmortem age of the tissues. For a given body there is no roughly correlation between the relative recovery in each tissue; the yields can be high in kidney and lymph nodes and low for all the other tissues.

Discussion

In forensic science, the generation of individual DNA fingerprints opens new possibilities for identifying bodies by reconstituting a familial relationship. Therefore the postmortem stability of DNA becomes an important point and has already been studied by a few authors [*1–3,5,6*]. Pääbo [*7*] reported the DNA recovery from Egyptian mummies, but only DNA of low molecular weight (<3.5 kb) could be obtained. Gill [*2*] successfully achieved sex determination from degraded DNA samples of blood stains that were up to 4 years old, nevertheless they purified once again only low molecular weight DNA. However, in the forensic field our aim is not to isolate genes from archeological human remains but to isolate HMWDNA from bodies in order to determine their identity. In forensic cases the identification is performed by comparing the DNA fingerprint of the discovered body with those of close relatives. Therefore after a long period of time, it becomes very difficult to still be able to find a parent for the comparison.

Bär et al. [*1*] studied different tissue samples at various postmortem periods ranging up to 19 days, and concluded to find a good stability of the DNA in brain cortex, lymph nodes and psoas muscle. In another study, Hagelberg [*5*] showed DNA patterns obtained after PCR amplification of DNA extracted from bone tissue.

We performed DNA extractions on tissue specimens of human organs of postmortem ages ranging from 1 to 85 days (Table 1). During the first week, HMWDNA was recovered in high quantities from various organs as well as from blood. After three weeks, a good DNA stability was only found in brain cortex.

DNA fingerprinting using two single locus probes (YNH24, V1), was performed on all samples. One μg of DNA was used to obtain a pattern. Identical banding patterns were observed for different tissues taken from the same individual of a determined postmortem age. After two weeks, a smear of DNA appeared when the DNA was extracted from organs, such as liver, spleen and kidney, showing a higher degree of autolysis (data not shown).

No correlation was found for a given body at a specific postmortem period between the quantities of DNA recovered and the type of tissue used. But, we could only establish that for a postmortem period between 1 and 7 days the yield of DNA is higher in lymph nodes and kidneys than in other tissues.

Our data are in accordance with those of Bär [*1*]. We observed a slower degradation of the HMWDNA extracted from brain cortex than from other tissue. These observations may be due to the brain's location within the skull, preserving it from early environmental bacterial contamination.

The amount of extracted HMWDNA could not be correlated either with macroscopic changes in the specimen or the duration of the postmortem period.

Conclusion

DNA fingerprinting is a powerful method that may be used for identifying human remains even after long postmortem periods—up to 85 days. The DNA extraction and

analysis can be performed by using samples of brain cortex, which seems to be the tissue of choice even in the presence of macroscopic putrefaction. Furthermore, the gross appearance of the samples does not necessarily preclude a successful analysis.

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