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DNA: POSTMORTEM STABILITY IN VARIOUS HUMAN ORGAN TISSUES

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INTRODUCTION

The detection of DNA polymorphisms considerably expanded the means of discrimination of individuals based on small tissue samples. So-called "DNA-fingerprints", a banding pattern generated by hypervariable minisatellites are unique for every individual, except for monozygotic twins (Jeffreys et al., 1985a, 1985b). The bands are inherited according to the law of Mendel, hence familial relationship is reconstructible by comparing fingerprints of the parents or close relatives with the one of the unknown (Jeffreys, 1986). Questions of identity in forensic cases often concern remains of humans who have been dead for a shorter or longer time. Since high-molecular-weight DNA is a prerequisite to obtain reliable RFLP-patterns, it is important to investigate the postmortem stability of DNA under various conditions. Reports of Pääbo in 1985 on isolation of DNA from mummies of 2000 years of age amplified the enthusiasm to "raise (genetically) the dead and buried" as Jeffreys expressed it 1984. However, only short length DNA-fragments or none at all were recoverable so far (Huges et al., 1986). Postmortem decay of human bodies is an extremely complex and not yet fully understood process which consists of an aerobic and bacterial decomposition of organic material. It starts with autolysis followed by putrefaction. Autolysis is a nonbacterial autodigestion of tissue by enzymes liberated from the lysosomes whereas putrefaction is an anaerobic bacterial decomposition of proteins which is often accompanied by production of gas. However, the two processes usually cannot be clearly separated and both show a maximum of activity at temperatures between 34-40 ° Centigrade. In autolysis, loss of enzyme regulation and lactic acidosis enhance the activity of some enzymes, e.g of the hydrolases, whereas other's activity is reduced. Surprisingly, most of these enzymes are remarkably resistant to autolysis itself. But they are usually rapidly destroyed by bacteria. DNA in dead cells itself is degraded by nucleases which belong to the large enzyme group of the "hydrolases". Two major groups exist: endonucleases and exonucleases. Endonuclease decompose the DNA from inside the DNA-strand by randomly shearing it into smaller fragments whereas exonucleases cut single nucleotides one after another from one of the terminal ends, thus gradually shortening the fragment. We have investigated the yields of undegraded DNA extracted of human tissue specimens. We examined tissue of brain, lymph nodes, liver, spleen, psoas muscle, kidneys and blood of human bodies of various postmortem age. Nonspecific degradation of highmolecular DNA by random fragmentation could lead to the nonpredicable appearance of extra-bands in DNA-fingerprints.To study possible postmortem changes of the banding patterns, DNA-fingerprinting was done on all the samples . Finally, preserving agents other than freezing for the storage of tissue specimen until DNA extraction were tested in a few cases.

MATERIAL AND METHODS

Tissue specimen of brain cortex, lymphatic node, liver, spleen, psoas muscle, kidney and blood of bodies of known postmortem age were collected from autopsy-cases of the University Institute of Forensic Medicine of Zurich. A maximum of 29 cases were examined. The bodies were "naturally" aged cases recovered in the county of Zurich and were not exposed to artificial ageing. The postmortem periods ranged from 0.2 to 19 days. The causes of death were heterogeneous. Since the amount of nuclear mass varies considerably in different organ tissues from very high in lymphatic tissue to low quantities in skeletal muscle, four blocks of 50 to 400 mg of tissue were separately collected, weighted and kept at 4° C if handled the same day or frozen at -20°C until homogenizing in a solution of 1 ml of 10 mM TRIS-HCl (pH 7.6), 10 mM

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EDTA, 100 mM NaCl (pH 8) and 40 mM DTT with a mechanical homogenizer of Popper. After adding 2% SDS and 40µg/ml Proteinase K the homogenized tissue was incubated at 37°C overnight . DNA-extraction was done three times for 10 minutes in equal volumes of a 1:1 mixture of phenol and methylene chloride and in 1 volume of methylene chloride for again 10 minutes. DNA was precipitated by adding 1/10 volume of 3 M Na-acetate and 2.5 volumes of absolute ethanol followed by freezing at -80°C for a least 2 hours. Recovery of low quantities of DNA necessitated centrifugation for 10 to 15 minutes at 13000 rpm. After vacuum drying for 20 minutes, the pellets were dissolved in appropriate volumes—usually in 20 to 400 µl— of TE-buffer of pH 7.6 . The yields of DNA were estimated semi-quantitatively on testgels for each specimen by direct comparison with ethidium-bromide stained λ-DNA-markers of 50, 100, 200 and 500 ng DNA. The mean value of these 4 estimates per organ and case and its standard deviation were calculated and expressed in microgram of recovered DNA per milligram of crude tissue. Theses values were plotted against the postmortem period for all organ tissues. Approximately 0.2 to 1 µg of DNA was sheared with 20 units of the restriction enzyme Hinf I at 37°C overnight, electrophoresed in a 22 cm 1.2 % agarose gel (SIGMA II A 8677) for 48-65 hrs and Southern blotted onto nitrocellulose filters. The ^{32}P-labelled probe was prepared from the human minisatellite M13 recombinant 33.15 after insert preparation of replicative forms by double digestion with EcoRI and HindIII and random oligolabling according to Feinberg and Vogelstein using the commercially available Multiprime System of Amersham (RPN.1601). Southern blots were hybridized overnight at 42°C in the presence of 50µg/ml denatured salm sperm, 0.1% SDS, 6% polyethylene glycol 6000, 45 % formamid , 10x Denhardt´s and 1xSSC. After washing the filters with 1xSSC for 10 minutes at room temperature and for 1 hr at 65°C, they were autoradiographed for 1 to 5 days at -80°C using 2 intensifying screens.

RESULTS

Quantitative Yields of DNA

Brain Cortex: The meanvalues of DNA of the 4 extractions varied considerably and ranged from 0.004 µg to 0.14 µg per milligram of crude tissue in 24 cases. A steady exponential decline of the yields of DNA in relation to the postmortem age was observed. The correlation coefficient of these two variables was acceptable (R=0.62).
Lymphatic tissue: Do to the abundance of cell nuclei in lymphatic tissue, DNA yields per milligram of tissue were about 10-15 times greater than those of brain cortex (0.09 to 4.6 µg of highmolecular DNA per mg crude tissue). In 17 cases of cervical lymph nodes, a steady decline of the DNA yields in relation to the postmortem age was again observed. However, the correlation coefficient of these two variables was lower (R=0.55).
Psoas Muscle: In 24 cases, the meanvalues of the yields of 4 DNA extractions of psoas muscle were of the same order of magnitude as the ones of brain cortex and varied between 0.003 to 0.100µg.
Blood: Yields of highmolecular DNA of postmortem blood samples showed a considerable variation and ranged between 0.0005 to 0.113 µg per microliter blood in 23 cases investigated. A direct correlation of the yields to postmortem age was not found. It was shown that blood samples drawn from vessels were not homogeneous. Presence of small clots in the sample increased the DNA yields considerably and was found to be responsible for the great quantitative variations of DNA yields. DNA extractions of blood clots that are often present in decayed bodies gave in fact the best results.
Spleen: DNA yields of splenic tissue per milligram tissue were about 2 times lower than those of lymph node in 18 cases and varied between 0 to 2.2 µg per mg crude tissue. Decrease of highmolecular DNA was exponential with a good correlation to postmortem age (R=0.84).
Kidney: Tissue of renal cortex was extracted in 13 cases. DNA yields ranged between 0 and 0.54 µg and dropped exponentially with a good correlation to postmortem age (R=0.84) with one exception.
Liver: Liver tissue always showed a high proportion of degraded DNA independant of the postmortem period and therefore yields could not properly be estimated.

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Quality of the recovered DNA

The overall quality of the extracted DNA was estimated as the amount of slow migrating high-molecular-weight DNA to faster moving "smearing" DNA of lower molecular weight for each organ. Increasing amounts of low molecular DNA in direct relation to postmortem age were obvious for all organ tissues but a few cases were especially marked by this DNA degradation independent of the postmortem period. Elevated room temperature as environmental factor and/or infectious disease before death were the common main causes for the advanced autolysis in these cases.
Stability of DNA in brain cortex was excellent for a period of 3 weeks postmortem. The quality of the DNA of lymph node extracts was at least as good as the one of brain. The stability of DNA of psoas muscle was also similar to brain and lymph node tissue. Some rare exceptions were probably a consequence of the differences of postmortem body cooling do to the quite different topographical localization of these organs in the human body. DNA degradation of splenic tissue was more rapid than in lymph nodes in some of the cases. A liquefying consistency of the splenic tissue was typical in these cases and autolysis was usually also a prominent feature. In our experience, high-molecular-weight DNA was found in only small amounts in splenic tissue after 4-5 days postmortem.
Degradation of DNA of kidney tissue was almost total after 5 days in the 11 cases examined.

Storage of tissue specimen in absolute ethanol or waterfree aceton preserved high-molecular-weight DNA. After thorough washing of the tissue samples in sodium chloride, no influence of the fixatives on the quality of DNA was observed. However, preservation of the tissues in a solution of 4%-formalin resulted in negative yields of DNA.

DNA-FINGERPRINTING ON POSTMORTEM TISSUE SPECIMEN

DNA-fingerprinting using the minisatellite probe 15.1 was performed on all the samples. Basically identical banding patterns were found for different tissues of the same individual. Gradual disappearance - fainting to complete loss - of the long fragments in the 15-20 kb range was sometimes noticed. This alteration could not strictly be correlated to the postmortem age of the samples but appeared to be a consequence of the degree of autolysis. Organs commonly showing a higher degree of autolysis like liver, spleen and kidney sometimes showed this fainting of the long fragments after only a few hours postmortem. Nonspecific fragmentation of DNA seems to primarily affect the longer fragments. But since the resulting fragments are all of shorter but never identical length, no extrabands are to be expected on the fingerprints and therefore false exclusions do not occur. However, the evidentiary value of the fingerprint is lower. Cases with complete loss of high-molecular-weight DNA never showed recognizable banding-patterns after hybridisation. An amount of 1 to 60 mg of crude tissue was usually sufficient to produce a DNA-fingerprint.

CONCLUSIONS

We found that in principal high-molecular-weight DNA could be recovered postmortem in large quantities from various human organ tissues as well as from blood, but not all organs were equally well suitable. Excellent DNA stability was found in brain cortex, lymph nodes and psoas muscle over a period of three weeks postmortem. Degradation was relatively slow. Spleen, kidney and thyroid gland showed good DNA stability up to 5 days postmortem but after longer periods, very rapid degradation was usually observed. Yields of DNA of blood were very variable do to inhomogeneous samples. Blood clots gave very good results. Generally, the degradation of highmolecular DNA correlated directly to the length of the postmortem period (Tab.). However in some cases; DNA degradation was already prominent after a very short period and all organs were equally touched. Case histories showed that high environmental temperature at the place of death and/or infectious disease prior to death were the main factors for the occurrence of rapid autolysis.Tissue specimen can be stored frozen at -20°

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or in absolute ethanol or waterfree aceton for longer periods without notable deterioration of high-molecular-weight DNA.

Tab.:
Summary of the quantitative and qualitative DNA yields of different human organ tissues

Tissue	Slope of post-mortem DNA-Degradation	Quality of DNA (highmolecular)	DNA-Yields µg DNA per mg tissue max	min
Brain Lymph Node Psoas Muscle	DNA / p.m.	+ + + + + + + + +	0.145 4.6 0.148	0.005 0.09 0.002
Spleen Kidney	p.m.	++ +	2.2 0.548	0.005 0.0002
Blood	p.m.	+ +++ (clots)	0.110	0.0005

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