DISASTER VICTIMS IDENTIFICATION BY USING THE DNA TECHNOLOGY ON DENTAL PULP: preliminary results.

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INTRODUCTION

On March 31st, 1995 a Roumanian Airbus A310 flying to Brussels crashed in Balotesti near Bucarest. The Disaster Victims Identification (DVI) teams identified 52 of the 60 victims. We were requested to identify 8 victims by DNA typing for which insufficient morphological data was available.

The human tooth plays an important role in victim identification when the corpse is extremely decomposed, as dentine and enamel provide a protective enclosure for genomic and mitochondrial DNA. DNA typing assays were first performed on selected clinical samples obtained through both the conservative (by sampling of dental pulp) and destructive (by crushing the entire tooth) approaches. 26 unidentified jaw fragments were then submitted to analyses.

MATERIALS & METHODS

- 4 undiseased teeth from 3 patients from the dental clinic, with a bloodstain from each patient.
- 15 unidentified jaw fragments were taken during the week after the aircraft disaster (A series) They were stored in non hermetically closed plastic bags during 6 weeks at 0 - 10 ° C, mixed with unidentified corpse fragments, then at -20 ° C in our laboratory.
- A second series (B series) of 11 jaw fragments were found 6 weeks later on the site of the accident, then kept at -20 ° C in our laboratory.
- Blood samples on EDTA or bloodstains from 18 relatives of 6 victims were collected.

DNA Extraction

- For the clinical teeth, the pulp was sampled immediately after extraction, the teeth without pulp were kept during a week at room temperature.
- 2 clinical teeth without pulp were crushed in fine power with a mixer mill type MM 2000 (Retsch, Germany) and recovered with 1,5 ml 8 M Urea - 300 mM NaCl - 10 mM Tris-HCl, pH 8 - 5 mM EDTA, pH8 - 2 % SDS. Organic extraction was done and the extracted DNA resuspended in 300µl 10 mM Tris-HCl, pH 7,5 - 1 mM EDTA, pH 8 ($T_{10}E_1$).
- The dental pulp of 51 teeth from the 26 jaw fragments was sampled after selection by radiographic examination by the odontologist. The pulp was extirpated from the coronal pulp chamber and radicular canals, after horizontal section of the tooth at the cementoenamel junction (Smith et al. 1993).

These pulpes were put either in 700 µl homogenization solution with urea (remaining non dissolved fragments were further digested with Proteinase K), either in 700 µl 10 mM Tris-HCl, pH 8 - 10 mM EDTA, pH 8 - 100 mM NaCl - 0.5 % SDS with 20 µl of Proteinase K (10 mg/ml) and 10 µl of β-Mercaptoethanol. After over night incubation room temperature (urea) or at 50 ° C (Proteinase K), organic extraction was performed and the extracted DNA resuspended in 50 to 150 µl T_{10} E_1 or 10 mM Tris-HCl, pH 7,5 - 0.1 mM EDTA, pH 8 (T_{10} $E_{0.1}$).

DNA Quantification

- *Dental pulp and crushed teeth*: First the quantity and quality of total DNA was estimated by loading 1/30 to 1/10 of the resuspended DNA on 0.8 % agarose gels, followed by ethidium bromide staining. A more accurate estimate of the amount of human DNA was then obtained by using the "human DNA quantitation system" (GIBCO BRL, USA) based on the hybridization

to the human D17Z1 probe (Waye et al. 1989).
- *Blood Samples*: the DNA yield was determined by U.V. absorption at 260, 270 and 280 nm and by hybridization with the D17Z1 probe.

Analysis methods: By RFLPs and by PCR amplification

RFLPs method:

Briefly, DNA from blood samples and teeth were restricted by HinfI, then loaded on 1 % agarose gels in 40 mM Tris acetate - 1 mM EDTA buffer (60 volts - 20 hours) . After transfer on nitrocellulose membranes, the samples were successively hybridized with the radioactive probes pH30 and pLH1 (GIBCO BRL, USA) (Milner et al. 1990, Eisenberg et al. 1993). Radiographies were obtained with intensifying screens at - 70 ° C.

PCR methods:

Amplification conditions:

- ***D1S80 locus*** (AmpliFLP™ D1S80, PCR amplification kit, Perkin Elmer, USA) (Kasai et al. 1990, Budowle et al. 1991)

The amplification reaction occured in a total volume of 50 µl (20 µl mix with AmpliTaq DNA Polymerase, 10 µl 5 mM Mg Cl and 20 µl of a 0.5ng/µl DNA dilution.
96 °C - 3 min; 29 cycles [95 °C - 15 sec, 66 ° C - 15 sec, 72 ° C - 40 sec]; 72 ° C - 10 min (GeneAmp PCR System 9600, Perkin Elmer, USA).

- ***HUMTHO1 locus*** (Geneprint™ STR THO1 System, Promega, USA) (Polymeropoulos et al. 1991, Edwards et al. 1992)

The amplification reaction occured in a total volume of 25 µl (22.5 µl mix (with Taq DNA Polymerase (Promega), and 2.5 µl of a 10 ng/µl DNA dilution.
96 ° C - 3 min; 28 cycles [95 ° C - 60 sec, ramp to 60 ° C - 120 sec, 60 ° C - 60 sec]; 72 ° C - 10 min (GeneAmp PCR System 9600, Perkin Elmer, USA).

Electrophoretical separation of amplified fragments:

It was realised on gels 32 cm long, for both loci (GIBCO BRL Model SA-32).
- ***D1S80 locus***: instructions of manual from the kit (Perkin Elmer) were strictly followed.
- ***HUMTHO1 locus***: the separation of the amplified products was done on denaturing 6% Hydrolink Long Ranger gels (FMC, USA).

The visualisation of the bands was performed by *silver staining* according to the given instructions of the two kits, respectively.

RESULTS AND DISCUSSION

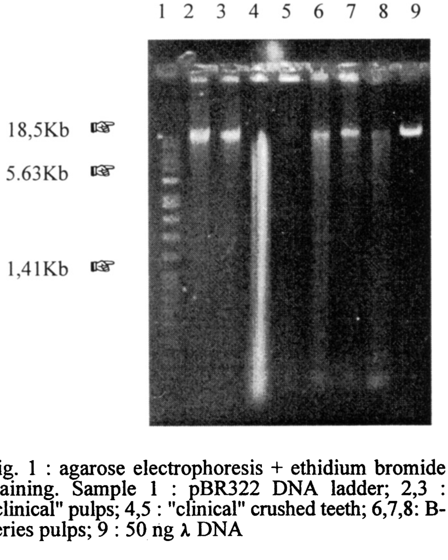

Fig. 1 : agarose electrophoresis + ethidium bromide staining. Sample 1 : pBR322 DNA ladder; 2,3 : "clinical" pulps; 4,5 : "clinical" crushed teeth; 6,7,8: B-series pulps; 9 : 50 ng λ DNA

For the clinical teeth, high amounts of intact DNA (from 900 to 2000 ng, mean 1570 ng) were obtained from the pulps. The powdered remaining teeth yielded higher amounts of DNA (6000 ng), but it was much more degraded (Fig. 1). This confirms the observations of Smith et al. (1993).

For the aircraft samples, 2 or 3 teeth per jaw fragment were analysed, except for 3 of the 26 fragments which contained only 1 tooth. Each tooth of the same fragment gave similar DNA yield, except for 3 of the 23 fragments.

For all the B series samples, the amount of DNA extracted from the pulp was high (from 100 to 10000 ng, mean 3500 ng) and only partially degraded (Fig. 1). This result was obtained for only 5 jaw fragments of the A series (from 0 to 4800 ng, mean 1880 ng); all other A fragments yielded hardly any DNA (from 0 to 60 ng, mean 14 ng).

The quality of the DNA recovered from the clinical teeth, the 11 aircraft B-teeth analysed and only 3 of the 15 jaw fragments of the A series was sufficient to use the RFLP typing method with success. These results show that although teeth provide a protective environment, storage conditions still remain important. However, air dried storage seems to be a simple and efficient procedure to keep relatively intact DNA containing source.

The RFLP results were confirmed by D1S80 and HumTHO1 typing for the clinal teeth and for 11 jaw fragments. We never observed more than 2 alleles per sample, which indicate that we do not seem to have contamination problems. At this stage of the study, compared to the RFLP technology, the PCR methods has not allowed us to type more degraded samples. And we have not obtained more results for STR (HUMTHO1) typing than for VNTR (D1S80) typing, as could be expected from the size difference between these markers. However, the amplifications should be repeated after purification of the DNA by Microcon 100 microconcentrator. Several dilutions of DNA template should also be tried, indeed the amount of human DNA could be underevaluated for severely degraded samples (Prinz et al. 1993).

We were able to determine the genetic profile of jaw fragments from 14 different persons and 4 of these jaws correspond to 4 of the missing victims.

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