

Visualization of Epigenetic Toxicological DNA Changes

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Introduction

In the field of forensic medicine, DNA analysis methods, which include DNA profiling (Jeffreys et al. 1985) and the polymerase chain reaction (PCR) (Saiki et al. 1985), have been used extensively for identification in criminal cases and in paternity testing with excellent results. One principle of forensic identification is that DNA analysis from the same body must always show the same pattern. However, it is known that the development of malignant disease such as carcinoma, may alter the DNA profile (Saito et al. 1991) and the same possibility may exist where carcinogen or other toxic agents have been active. No study has been published on DNA analysis of forensic toxicological cases as regards identification. Thus DNA typing methods may not be reliable in the case of toxicological victims. Any changes in the DNA, jeopardises possibility of using DNA analysis for the forensic identification of intoxicated individuals. This is a preliminary report on DNA changes caused by poisons. Their effect on well-known DNA analysis methods has been examined.

Materials and methods

In this experiment, we evaluated epigenetic toxicological DNA changes using five methods indicated in Table 1. Southern hybridization with multi-locus probes and with single-locus probe, AmpFLP for minisatellite loci and for microsatellite and HLADQ α were carried out according to the manufacturer's instructions.

Table 1 Experimental procedures

Chronic methamphetamine toxication: rabbit experimental case

Materials: 5 - 10 mg/kg methamphetamine intravenous toxication each two days

Methods: 1. southern blotting with MLP (33.15, 33.6)
with SLP (MS31, MS43a, MS1, g3)

2. PCR (AmpFLP by minisatellite): D1S80 (MCT118)

3. PCR (AmpFLP by microsatellite): TH01, TPOX, CSF1PO

4. PCR: HLADQ α

Results and discussion

DNA changes caused by methamphetamine could be evaluated by southern hybridization with a multi-locus probe, but not with a single-locus probe. DNA profiles produced bands with probe 33.15 in the high molecular area where no bands exist in humans. In this area the DNA changes were clearly observed. Two

pre-intoxication bands disappeared and one pre-intoxication band was discolored. With HLADQ α DNA typing, pre-intoxication positive spots became negative after intoxication and pre-intoxication negative spots became positive after intoxication. With AmpFLP on D1S80 locus, a band appeared in an area where no bands exist in humans. This band appeared clearly before intoxication but disappeared after intoxication. Besides, the pre-intoxication negative band changed to post-intoxication positive band. This also happened at the TH01, TOPX and CSF1PO. Sensitivity to detect the changeability of DNA was highest in AmpFLP of microsatellite loci. There were any changes in 7 cases among total 8 cases in AmpFLP of microsatellite loci.

From the above results, epigenetic DNA changes caused by methamphetamine intoxication were detected in the same individuals, the results of well-established forensic DNA analysis methods showing changes which could confuse identification procedures.

Acknowledgment

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References

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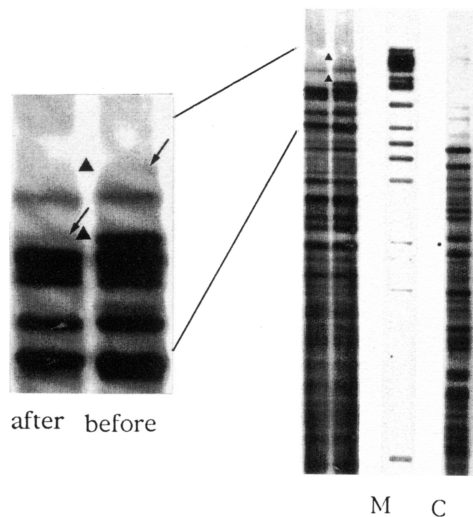
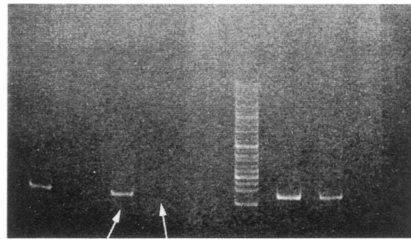


Fig. 1 DNA fingerprint with MLP33.15 before and after methamphetamine intoxication.

1	2	3	4	C	1.1	1.2	1.3	1.4	1.5	1.6	1.7	1.8	1.9	2.0	2.1	2.2	2.3	2.4	2.5	2.6	2.7	2.8	2.9	3.0	3.1	3.2	3.3	3.4	3.5	3.6	3.7	3.8	3.9	4.0	4.1	4.2	4.3	4.4	4.5	4.6	4.7	4.8	4.9	5.0	5.1	5.2	5.3	5.4	5.5	5.6	5.7	5.8	5.9	6.0	6.1	6.2	6.3	6.4	6.5	6.6	6.7	6.8	6.9	7.0	7.1	7.2	7.3	7.4	7.5	7.6	7.7	7.8	7.9	8.0	8.1	8.2	8.3	8.4	8.5	8.6	8.7	8.8	8.9	9.0	9.1	9.2	9.3	9.4	9.5	9.6	9.7	9.8	9.9	10.0
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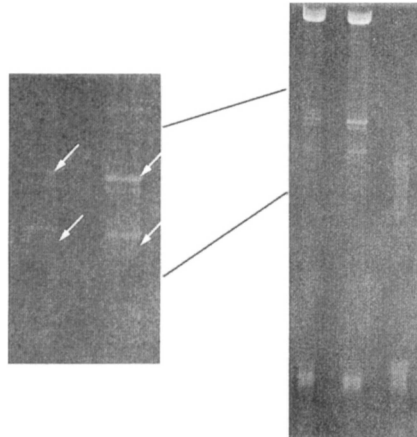
before
after (2w)
after (death)

Fig. 2 HLADQ α type before and after methamphetamine toxication.



before after M

Fig. 3 D1S80 DNA fragments before and after methamphetamine toxication.



before after

M

Fig. 4 TH01 DNA fragments before and after methamphetamine toxication.