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ORIGINAL ARTICLE

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DNA typing of cellular material on perforating bullets

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Abstract DNA typing of cellular debris from perforating bullets was investigated following shooting experiments. A total of 14 perforating gunshots were fired into 9 calves. PCR typing of tissue fragments was done using bovine-specific primers flanking a 247 bp segment within the bovine lactoglobulin gene. Positive amplification results were obtained for all 9 hollow point (HP) and all 5 full metal jacket (FMJ) bullets. In contrast to HP bullets the smooth surfaces of the FMJ bullets did not have visible biological material, which resulted in weaker bands in the DNA analysis compared to HP bullets. Tissue seemed to accumulate at the base of the projectiles. Due to the lack of a suitable marker in bovines, only a species identification was carried out on the DNA from tissue on the bullets. The small amount of DNA extract (up to 5%) required for specification is promising for the successful application of a set of short tandem repeat (STR) systems for individualization in humans. By individualizing tissue on perforating bullets, the bullet and the victim it passed through can be linked. This can assist the investigation of gunshot deaths, especially when several persons are involved in a gun fight.

Key words Ballistics · DNA typing · Perforating bullets · Polymerase chain reaction · Bovine lactoglobulin gene

Introduction

Shooting incidents can pose a number of problems but one of the most important is to identify the person who fired the gun. Among the methods applicable, the determination of gunshot residues on the hand (e.g. Nesbitt et al. 1976; DeGaetano and Siegel 1990) can lead to the identification of the person shooting. But in cases where more than one person fired a gun or where several persons were injured or killed, it is extremely difficult to determine who shot whom, especially when the bullets have perforated and therefore exited from the victim. Wound ballistics can assist this task by a detailed investigation, provided the evidence at the scene has been carefully collected (e.g. Sellier 1982; DiMaio 1985).

In cases of gun fights involving several persons, the detection of a perforating bullet is of major importance and can lead to the identification of the person who fired the weapon by a comparison of the bullet and the firearms involved. But the bullet usually cannot be linked to the person through whom it has passed. A technique capable of demonstrating and simultaneously individualizing biological material on bullets is necessary to determine which bullet injured or killed whom. Apart from the detection of inert material on bullets (DiMaio et al. 1987; Smith and Harruff 1987; Petraco and De Forest 1990), cytological investigations of bullets after perforation of human victims have been conducted (Nichols and Sens 1990, 1991; Knudsen 1993). In the literature available to us, no method has been published for DNA typing of biological material from bullets.

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Materials and methods

The detailed experimental set-up is described elsewhere (Karger et al. 1996). Briefly, calves, 5–6 months old and all destined for slaughter, were sacrificed by gunshots to the right temple by a veterinary surgeon experienced in handling experimental animals and in accordance with regulations governing their use. Immediately after these fatal shots, additional shots were fired into the abdomen and into the hind legs of the animals. The firearms used were a 9 mm SIG P210 pistol and a Heckler und Koch MP5 9 mm submachine gun. Two kinds of ammunition were fired: a conventional 9 × 19 mm Luger (= Parabellum) full metal jacket (FMJ) round produced by Ammunitionsarsenalet and a 9 × 19 mm Luger Action-1 round produced by Dynamit Nobel. A total of 14 perforating shots were fired into 9 animals. All perforating bullets were retained in a bullet collector made of stapled cardboard. Each bullet was collected in a separate plastic tube. 3–5 days after the experiment, the bullets were stored at –20° C until examination.

The bullets were screened under a macroscope for blood, hair and tissue fragments. These were removed from the bullets using fiberglass applicators (3 × 5 mm; Pharmacia, Sweden) which had been soaked with sterile Aqua bidest. Handling of the applicators was done with a forceps. If no material was visible the whole surface of the bullet was swabbed with moistened fiberglass applicators which were subsequently transferred to 1.5 ml screw cap test tubes. DNA extraction was carried out with Chelex 100 and Proteinase K (Wiegand et al. 1993) with the modification that the Proteinase K lysis at 56° C was extended to 4 h.

A bovine-specific 247 bp fragment of the b-lactoglobulin gene was amplified by the polymerase chain reaction (PCR) using the following primers (Medrano and Aguilar-Cordova 1990):

JBLG2 5′-TGTGCTGGACACCGACTACAAAAAG-3′
JBLG3 5′-GCTCCCGGTATATGACCACCCTCT-3′

From the DNA extracts (total volume 200 μl) 1–5% (2–10 μl) were subjected to 30 cycles of PCR using a Trio-Thermoblock thermal cycler (Biometra, Germany). Each PCR mix (25 μl) contained 2 μl 10 X reaction buffer (100 m*M* Tris/HCl pH 8.8, 500 m*M* KCL, 1% Triton X-100, 0.1% gelatine), 20 pmol each primer, 200 μ*M* dNTPs, 25 m*M* $MgCl_2$ and 5 μg bovine serum albumin (BSA). Amplification parameters were: 94° C – 1 min; 60° C – 1 min; 72° C – 1 min, 30 cycles.

Non-denaturing gel electrophoresis of the amplification products was performed using a discontinuous buffer system (Allen et al. 1989) on 8% polyacrylamide gels at 1000 V, 20 mA, 10 W until the bromophenol blue front had reached the anode (separation distance: 10 cm). DNA fragments were visualized by silver staining according to Budowle et al. (1991).

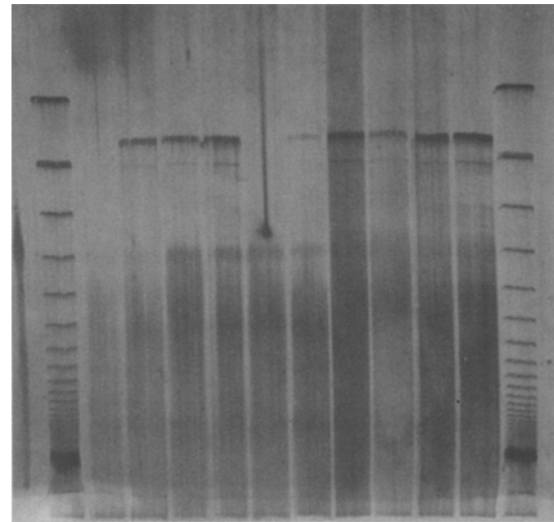

Fig. 2 Silver-stained polyacrylamide gel of PCR amplified 247 bp segments located within the bovine lactoglobulin gene. DNA was isolated from material on perforating bullets as described. 1 + 12: 123 bp ladder; 2: negative control (no DNA); 3: positive control (bovine DNA); 4: Full Metal Jacket (FMJ) bullet 1, tip/sides; 5: FMJ bullet 1, base; 6: FMJ bullet 2, tip/sides; 7: FMJ bullet 2, base; 8: Action-1 (A-1) bullet 1, tip/sides; 9: A-1 bullet 1, base/channel; 10: A-1 bullet 2, tip/sides; 11: A-1 bullet 2, base/channel

Results

Abundant organic and inert material could be seen with the naked eye on most of the Action-1 bullets. Due to a special design very similar to hollow points, Action-1 bullets deform during penetration of tissue and usually show a cavity in the tip and a small central channel from tip to base (Sellier and Kneubuehl 1994). The resulting mould, edges and irregularly shaped surfaces are ideal for the retention of tissue (Fig. 1). The FMJ bullets, on the other hand, did not deform or fragment at all. The surfaces were smooth except for the usual firing marks (Fig. 1). Thus a meticulous search of all 5 FMJ bullets with the macroscope only resulted in the detection of one single and extremely small particle only, presumably consisting of soft tissue. The remaining 4 FMJ bullets were clean under the macroscope.

Fig. 1 The 2 types of 9 mm bullets used after recovery from the bullet collector. On the left, there is an Action-1 bullet after perforation of a hind leg. The firearm used was a H + K MP 5 submachine gun, impact velocity approximately 480 m/s. The cavity in the tip and the opening of the central channel can be seen. Part of the deformation zone has broken off. On the right, there is a FMJ bullet without deformation or fragmentation after perforation of a head. The pistol used was a SIG P210, impact velocity approximately 370 m/s. Contrary to the Action-1 bullet, the FMJ bullet does not show any prominent parts or cavities

Organic material was collected from 2 separate regions of the bullets: 1. tip and sides, 2. base and, in the cases of Action-1 bullets, central channel. PCR products were obtained from all 9 Action-1 bullets. No amplification product was obtained in 2 cases from the base/channel and in one case from the tip/sides. For the 5 FMJ bullets, the PCR also showed positive reactions. However, the bands were weaker compared to Action-1 bullets (Fig. 2). In 2 cases, the PCR reaction showed no results for the tip/sides and in 2 more cases, the results were definitely weaker for the tip/sides than for the base of the same bullet. For the remaining FMJ bullet, both results were similar.

Discussion

Using cytological methods, Nichols and Sens (1991) demonstrated cellular material on one perforating bullet (and on 59 that did not exit) and Knudsen (1993) was able to identify tissue on 4 out of 36 perforating FMJ bullets. The smooth surface of non-deforming FMJ bullets resulted in a small number of positive cytological results.

With DNA typing, positive results were obtained for every single bullet due to the high sensitivity of PCR. Very small amounts of biological material and even scattered cell debris not visible with 30 X magnification or degraded tissue is sufficient for DNA typing. In an effort to determine preferential locations of cells on bullets, we differentiated between base/central channel and tip/sides. For Action-1 bullets, the results showed no dependence on the location from which the material was collected. For FMJ bullets, however, more positive results were obtained from the base of the bullet and the results were more intense. Although the small number of FMJ bullets ($n = 5$) restricts the significance of this statement, it appears logical because the base forms a depression protecting the tissue inside from being stripped off after exiting from the body. Additionally, cells can accumulate at the base of the bullet as a result of aerodynamic forces. In any case, no meticulous search is necessary. Instead, "blind" wiping of the entire FMJ bullet including the base appears to be the most promising way of collecting material for DNA analysis.

We did not individualize DNA due to the lack of appropriate polymorphic markers in bovines. But only up to 5% of the total 200 μl of DNA extract were required for specification/species identification in this experiment. Considering the similarities in sensitivity and size of the amplification product, the successful application of a set of STR (short tandem repeat) systems for the individualization of tissue from bullets after perforation of a human body is promising. Consequently, identification of the person injured or killed by the perforating bullet at hand will be possible.

Conclusions

Providing the technical equipment and the necessary skill and experience are available, DNA-typing of tissue from bullets is an excellent technique for the detection and individualization of biological material on bullets which have perforated a body. The investigation can be widely used and the results are reliable and extremely sensitive. DNA could be recovered and specified in every case, even if no material was visible on FMJ bullets using 30 X magnification.

Methods that are able to identify a person who fired a gun and to link a spent bullet to a firearm are well established in forensic science. In cases when it is important to know which victim was injured or killed by which perforating bullet, DNA typing can be carried out as an additional method. The decision whether DNA typing is to be performed should be made as soon as possible because the bullets at the scene must be protected from contamination. Also, material for DNA typing has to be collected before any further investigation of the bullet. This will not cause any delay because the recovery of biological material can be done at the scene with a minimum of equipment within a few minutes.

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