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Immunocytochemical examinations of biological traces on expanding bullets (QD–PEP)

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ABSTRACT

When a crime victim has been injured with several different objects, it is of central importance for the forensic investigation to be able to show which object caused which injury, especially if one of the injuries was lethal. In cases of bullet penetration wounds it is often not possible to find such evidence. However, immunocytochemical investigations can accurately match a victim's injury to a particular bullet path through the body. In cases where expanding bullets have been used and the heart or liver has been struck by a projectile, it can be shown that the cells remaining on the bullet stem from those particular organs. In this case the specific cytological evidence was established by means of marking heart- and liver-specific tissue proteins with appropriate antibodies (cardiac troponin I and HepPar 1) followed by disclosure with an appropriate chromogen. Thus, in principle, cells can be used as evidence after being extracted from the projectiles by either damp cotton–wool swabs or adhesive trace evidence tape. Because of the specificity of the used immunocytochemical antibodies, finding evidence of an antigen on a particular projectile proves that it was the object that injured the organs.

1. Introduction

When a crime victim has been injured with several objects, it is of central importance for the forensic investigation to be able to accurately prove which object caused which injury, especially if one of the injuries was lethal. Through standard molecular tracing it is possible to match the cells remaining on an object with the injured person, but it is not possible to accurately match these cells to a particular organ. The insufficiency of the information thus attained is illustrated by the case where a victim was shot by two attackers, each with their own firearm. While one attacker shot the femoral muscles and the other lethally shot the heart and the liver, both confessed to having shot the femoral muscles, thereby making the other responsible for the lethal shot at the organs. However, an immunocytochemical examination of cells found on the bullets could provide the missing objective information. Organ-specific antigen determinants such as mitochondrial antigens in liver epithelial cells, and cardiac troponin I in heart muscle cells, to which antibodies bind, allow the allocation of cellular remnants to their organ of origin.

HepPar 1 is a monoclonal antibody which identifies a mitochondrial antigen in the liver [1,2]. In current immunohistopathology, HepPar I is the standard antibody used to differentiate hepatocellular carcinoma from other types.

Cardiac troponin I, a 23 kDa protein, together with the other subunits troponin C (18 kDa) and T (37 kDa), forms the regulatory troponin complex of striated muscles for the calcium-dependent interaction between the actin and myosin filaments. In contrast to troponin C, the cardiac isoforms of troponin I and T differ in their amino acid sequence from those of the skeleton muscle and can therefore be selectively detected. The cardio-specificity of cardiac troponin I is useful in the diagnosis of myocardial infarctions because troponin I blood levels are increased after ischemic damage to heart muscle cells [3,4]. According to Hansen and Rossen, cardiac troponin I seems to also be a sensitive parameter in the post mortem diagnosis of myocardial infarctions [5]. Other investigations came to the conclusion that while they do find increased levels of cardiac troponin I in the pericardial fluid and blood serum after lethal myocardial infarction, post mortem levels can also be increased after death by other causes and thus seem to be of limited specificity [6].

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We investigated the sensitivity and specificity of immunocytochemical troponin I and HepPar 1 staining for heart and liver tissue remnants, respectively, particularly on expanding bullets (QD–PEP bullets, manufactured by MEN).

2. Material and methods

Preliminary investigations were designed to prove the organ specificity of the used antibodies. To this aim, various human and pig organ tissues (kidney, lung, spleen, skeleton muscle, pancreas, and brain) were used as negative controls alongside the liver and heart tissues. After formalin fixation and embedding in paraffin, the tissues were stained with an antibody specific for liver tissue (HepPar 1, DCS) as well as an anti-troponin I-antibody (DAKO), the latter being specific for cardiac troponin I. The pig tissues served to uncover any cross reactivity between human and pig tissues as have been described for the used antibodies. After paraffin removal with xylol and decreasing alcohol series, the specimens were unmasked per microwave and the endogenous peroxidase activity was blocked with 3% H_2O_2 in distilled water. To reduce the unspecific binding at Fc-receptors, the specimens were treated with buffered goat serum (dilution 1:100 in 3% BSA buffer). A monoclonal liver-specific antibody from the mouse (HepPar1, DCS Company) in a dilution of 1:200 in BSA buffer served as primary antibody for detection of liver tissue. The primary antibody for the proof of cardiac troponin I was an anti-troponin I-antibody from the mouse (DAKO Company) in a dilution of 1:200 in BSA buffers. In both cases, a polymer solution made of horseradish peroxidase and anti-mouse-antibody (DAKO EnVision system) served as secondary antibody. Diaminobenzidine was used to detect the specific antibody binding. Finally, the specimens were stained with haematoxylin for 2 min.

In the next series of investigations, pig livers and hearts were placed individually into gelatin blocks to simulate the rubbing-off effect of the bodily tissues. In a parallel experiment, hearts and livers were placed together, one behind the other, into gelatine blocks so that both organs could be struck with a single projectile. Pig organs were obtained from a slaughterhouse where the animals had been slaughtered by electrocution in the usual way (independently of the experiment). A 2–4-h post mortem period elapsed before the shooting commenced.

The organs embedded in gelatine were fired at with Pistols (Sig Sauer P 226/Walther P 5) with a 9 mm calibre Para/Luger. All experimental shots were carried out at a 2 m shooting range. Expanding bullets specially developed for police use (QD–PEP) were used. Made out of tombac ($CuZn_5$), the bullet weighs 5.9 g. The hollowed out bullet tip is 5.3 mm in diameter and 8 mm deep and contains a hard plastic pellet insert, 5.6 mm in diameter. Upon impact and penetration of the target matter, the plastic pellet insert is forced further into the hollow cavity, thus freeing the opening of the bullet tip for the 'mushrooming' expansion. On account of these special ballistic features, the PEP bullet can be categorised between a round-nose full jacketed bullet and a hollow point bullet. According to the manufacturers, depth of penetration of the gelatine should be a minimum of 20 cm and a maximum of 30 cm. Particularly characteristic of the PEP bullet is controlled expansion (mushrooming of the projectile to between 9 and 15 mm diameter maximum) and deformation stability which prevents fragmentation within the target matter (e.g. shattering or splintering). Both types of organ tissue were subsequently subjected to immunocytological testing using double staining because in reality a bullet often hits several organs. After the specifically targeted cells were found on all bullets, in the final step of the process the organs were wrapped in pig skin before being transferred into the gelatine blocks. In this way the rubbing-off effect on the skin should be most effectively simulated. The skin was placed both in front of and behind the organ so that analogous to an actual penetration wound, the projectile first had to pierce the skin, then the gelatin and the organ, and finally to exit the simulated body through the skin. At this point the bullets were once again collected from the gelatin. All experimental shots (111 shots with expanding bullets, i.e. QD–PEP bullets) were carried out under standard conditions at a 2 m shooting range. After firing, the bullets were carefully removed from the gelatin blocks. The fired projectiles were stored at 4 °C until the cells could be secured and examined immunocytochemically. The time between shooting the organs and extraction of cells was from 4 to 10 h.

During the experiments cells were extracted in a number of different ways for the purposes of further laboratory investigation. The swabbing method yielded good end results with negligible contamination. In the first procedure the cells were extracted from the bullets with adhesive trace evidence tape usually used by the police. The tape was then secured onto specimen slides using double-sided tape and after drying, the cells were stained. Before staining, the area of the tape assumed to contain cells should be outlined with a Dako-Pen® in order to guarantee a trouble-free staining process. Otherwise the materials may run off the slides.

In the second variant, cells were retrieved by swabbing. The bullets were smeared with cotton swabs that had already been soaked in 200 μl PBS-buffer and these were then re-immersed in a buffer solution. The cells, now dissolved in the emulsion, were sucked up into a pipette and transferred onto specimen slides. A small variation of this method would be to simply soak and wash the bullet or bullet parts in the PBS-buffer solution and then similarly retrieve the cells (dissolved in the emulsion) using a pipette and transfer them onto adhesive specimen slides for drying. Also here it is recommended that the area of cells on the specimen slide should be outlined with a Dako-Pen®. The swabbing method enables cells and cell remnants to be concentrated on a small area of the specimen slide, thus considerably simplifying the subsequent analyses. Once the cells had been extracted from the bullets, transferred onto the specimen slides and dried, the staining took place.

To determine the temporal delimitation of the tissue antigen stainability, bullets were evaluated in regular intervals (2, 3, 5, 7, 10, 13, 16, 20, and 30 days) after being stored at normal ambient conditions. All staining was accompanied by positive controls and negative controls as well as omission controls, in which the primary antibody was missing.

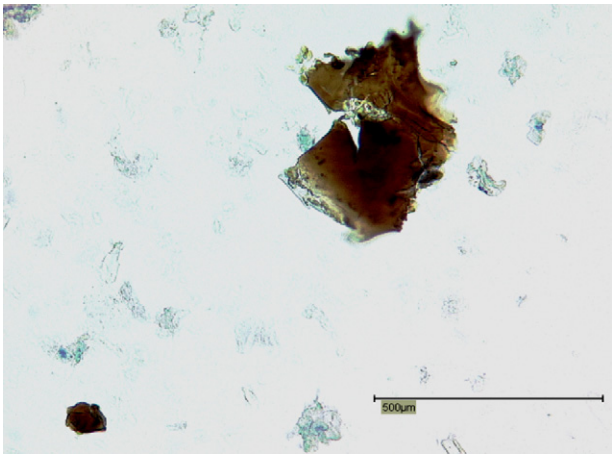

Fig. 1. Positive immunoreaction to anti-troponin I-antibodies staining after heart shot.

3. Results

An analysis of the findings from the trial gunfire at the pig hearts using expanding bullets showed clear evidence of heart cells in all cases (Fig. 1). In most cases the results were immediately visible, although whole cell clusters – as partly observed after the livers were fired at with expanding ammunition – were seldom present. Individual cells or cell remnants as well as intact pieces of tissue taken from the bullets were often found on the specimen slides. An analysis of the trail gunfire at pig livers also yielded a 100% evidence rate. Mostly the very large number of detectable cells (Fig. 2) and tissue fragments was noteworthy. Following the designated staining process, whole cell clusters could frequently

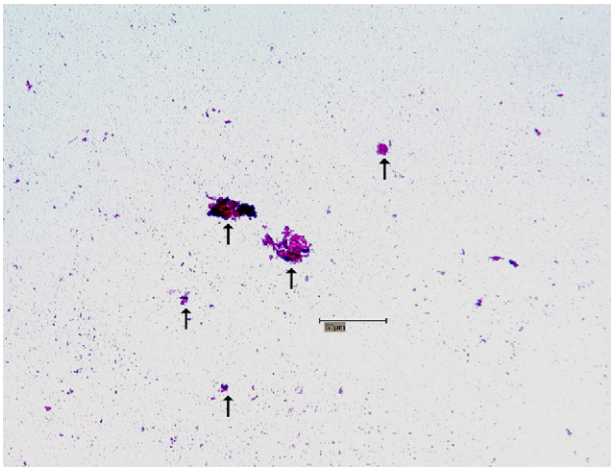

Fig. 2. Positive immunoreaction to HepPar 1-antibodies staining after liver shot.