Analysis of DNA Damage in Forensic Blood Evidence Exposed to Swimming Pool Gel Electrophoresis

Joseph Batte, Master of Forensic Science, Molecular biology concentration

Introduction

Investigation of forensic cases in which the victim and/or evidence were exposed to swimming pool water can be challenging. This is in part due to inconclusive forensic DNA typing results, obtained from DNA being fragmented or damaged in another way. Swimming pools are usually disinfected using chlorine or bromine derivatives, which are chemicals known to hydrolyze, oxidize, destroy and/or modify DNA bases. Often, insufficient amounts of disinfectants are added into pools, resulting in incomplete binding and inactivating of contaminants, such as ammonia excreted by swimmers. This results in formation of disinfectant byproducts, (DBPs), for example trihalomethane, which are genotoxic, carcinogenic and mutagenic, altering and/or damaging DNA. Given the effects of disinfectants and DBPs on DNA, we hypothesize that trace amounts of chlorine, bromine and their corresponding DBPs in swimming pool water, could interfere with forensic DNA analysis, by inducing double and/or single strand DNA breaks. We subjected bovine blood and blood stains to swimming pool or control purified water and assessed dsDNA and ssDNA breaks by neutral and alkaline electrophoresis, respectively.

Materials and Methods

200µL of EDTA-anticoagulated bovine whole blood or blood stains were exposed to 0.2mL, 0.4mL, 0.6mL, 0.8mL, 1mL, 1.2mL, 1.4mL, 1.6mL, 1.8mL or 2mL of swimming pool water, or Millipore water at room temperature for 24hrs. DNA was extracted using phenol-chloroform, followed by ethanol precipitation, and rehydrated in TE buffer. Aliquots were analyzed using neutral or alkaline agarose gel electrophoresis to detect double and single strand DNA breaks, respectively. DNA digested with Alu I restriction enzyme, and bovine whole blood exposed to 100% (v/v) bleach were used as positive, and severe DNA damage controls respectively. Resulting gel images were visualized using a UV Bio Doc-It Imaging System.

Results

As shown in figure 1, all samples display dsDNA breaks. Compared to controls, we observed more prominent DNA double strand breaks in pool water-treated blood, with most samples displaying laddering, consistent with cellular apoptosis. However, the laddering pattern observed in pool water-treated samples disappears in samples to which larger volumes of pool water were added, indicating that resulting DNA fragmentation is possibly not induced cellular apoptosis, but as a result of chemicals in pool water interacting directly with DNA.

Discussion and Conclusions

We observed dsDNA and ssDNA breaks in blood treated with pool water, indicating DNA damage. This could potentially result in decreased quality or failure of subsequent DNA analysis and inconclusive DNA profiles derived from blood or other tissue evidence. The observed prominent DNA breaks, at least the dsDNA breaks, are probably a result of trace amounts of disinfectants and DBPs present in swimming pool water. Our detection method is not sensitive enough to detect differences in ssDNA breaks between pool water-treated samples and controls. Swimming pool water used in this study was collected from an indoor swimming pool, with an automatic chemical dispenser, indicating that chemical levels were well regulated. However, the effect of pool water on DNA evidence could vary, for example in a home pool, where the disinfecting procedure is typically manually maintained and not performed as regularly. The resulting irregularities in trace amounts of disinfectant and DBPs, in conjunction with other damaging factors, such as UV light, in outdoor pools, potentially enhance DNA fragmentation, severely affecting forensic DNA typing. Future studies will further characterize and quantify the effects of individual compounds on DNA integrity and address the impact of these damaging agents on DNA typing results.

References


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