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Analysis of nerve agent metabolites from nail clippings by liquid chromatography tandem mass spectrometry

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ABSTRACT

While several methods for the bioanalysis of nerve agents or their metabolites have been developed for the verification of nerve agent exposure, these methods are generally limited in the amount of time after an exposure that markers of exposure can be detected (due to rapid metabolism from biological matrices). In this study, a method for the analysis of nerve agent hydrolysis products from nail clippings was developed to allow evaluation of nails as a long-term repository of these markers. Pinacolyl methylphosphonic acid (PMPA) and isopropyl methylphosphonic acid (IMPA) were extracted from nail samples with *N*,*N*-dimethylformamide and subsequently analyzed by liquid chromatography-tandem mass spectrometry. Limits of detection for PMPA and IMPA were 0.3 μg/kg and 7.5 μg/kg and linear ranges were 0.75–300 μg/kg and 30–1500 μg/kg, respectively. Precision was within 10% and 8% for PMPA and IMPA, respectively, and accuracy was 100 ± 12% for both analytes. The approach presented here is complementary to current methods for nerve agent exposure verification, and should allow for long-term determination of nerve agent poisoning.

1. Introduction

Long-term, retrospective analysis of nerve agents is important for several scenarios. First, a low-dose exposure may cause symptoms that are easily misdiagnosed as something other than nerve agent exposure, such as pupil constriction, rhinorrhea, and mild breathing difficulties. If it is later suspected that nerve agent exposure caused these symptoms, perhaps months or even years following the exposure, the opportunity to definitively verify nerve agent exposure may be lost. This is because most agents and metabolites are eliminated from the body days after expected exposure. Another scenario necessitating long-term, retrospective detection of nerve agent exposure is the occurrence of a potential exposure in a remote area. Soldiers, contractors, and civilians in remote areas would not necessarily have access to medical treatment for weeks or months if an exposure were to occur. Moreover, medical personnel nearest the event may be ill-equipped to diagnose the cause of the symptoms, especially if patients have difficulty describing the details of the incident.

Both of these scenarios were exemplified by the unknowing demolition of chemical weapons in an open pit at Khamisiyah, Iraq during the first Iraq war [1]. It has been estimated that more than 120,000 soldiers may have encountered a vapor cloud created by the demolition [1]. Soldiers were in a remote area and those who reported to medical personnel were potentially misdiagnosed. Therefore, once nerve agent exposure was suspected, biomarkers of nerve agent exposure were lost to normal metabolism and elimination.

Several approaches have been explored to verify nerve agent exposure, including measuring the decrease in acetylcholinesterase (AChE) activity in blood [2–4], analysis of hydrolysis products of the parent agents [5–9], fluoride reactivation of bound agent [10–13], and analysis of nerve agent adducts [14–21]. A decrease in AChE activity occurs when the nerve agent binds with the active site of the enzyme (Fig. 1, Reaction A; AChE denoted by R'-OH). AChE activity is generally measured spectrophotometrically using the Ellman procedure [2,22]. Measuring AChE activity as an indicator of nerve agent exposure has several disadvantages such as the inability to identify the parent agent, its lack of specificity for nerve agents (i.e., chemicals other than nerve agents can cause AChE inhibition), and person-to-person variability of baseline AChE concentrations. Another drawback is that *de novo* synthesis of the enzyme makes the procedure less suitable for retrospective detection of exposure [18,22].

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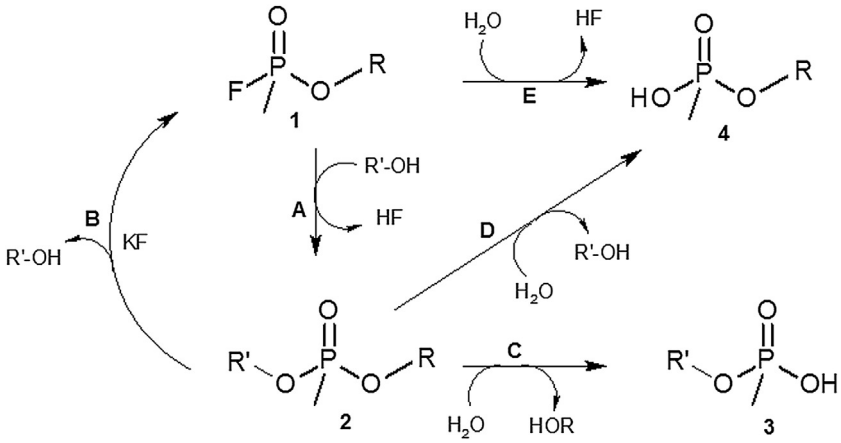

Fig. 1. Biological reactions of nerve agents and species used to verify exposure. Reaction A indicates the reaction of the nerve agent with the active site on the enzyme (designated R'-OH). Reaction B depicts fluoride reactivation of the enzyme and reformation of the parent agent. Ageing of the agent-enzyme complex is shown in Reaction C. Cleavage of the agent from the enzyme via hydrolysis and direct hydrolysis of the nerve agent are shown in Reactions D and E, respectively.

Nerve agents can be released from the AChE via hydrolysis (Fig. 1, Reaction D), or directly hydrolyzed (Fig. 1, Reaction E), to form the corresponding alkylmethylphosphonic acid (AMPA; Compound 4). AMPAs have been analyzed from several biological matrices using mainly gas chromatography (GC) or liquid chromatography (LC) with mass spectrometry (MS) or tandem-MS detection. As shown in Fig. 2, metabolites formed by hydrolysis are detectable for a maximum of 1 and 2 days from blood and urine [8,23], respectively, while AMPAs in hair are detectable for at least 30 days [24].

Fluoride reactivation of enzyme bound agent (Fig. 1, Reaction B) uses excess fluoride ions to cleave the phosphorus-enzyme bond, and reform the phosphorus-fluorine bond [10,12,25], to produce the parent nerve agent (Compound 1). Although this process is simplistic, it is highly dependent on how quickly the adduct (Compound 2) undergoes ageing (Reaction C to compound 3), after which, the adduct can no longer be reactivated [13,15]. This process works well for analysis of sarin-esterase adducts due to relatively slow ageing (hours) [12,13]. However, reactivation does not work particularly well for other agent adducts which age more rapidly (minutes), such as the soman-esterase adduct [4]. In either case, ageing of the bound nerve agent precludes the use of this technique for long-term verification of exposure.

Nerve agent adducts, such as phosphylated AChE or butyrylcholine esterase (BuChE) (Compound 2), are mainly analyzed by MS methods [14,15,18]. If the agent adduct is hydrolyzed (Fig. 1, Reaction D), long-term verification of nerve agent exposure via this technique is not possible. Although esterase adducts cannot be used to verify exposure after hydrolysis, aged AChE/BuChE adducts can still be used to verify exposure [18,22]. Although it is currently unclear, aged adducts may offer a means of long-term verification while the adducted protein is circulating (i.e., in mammals, the half-

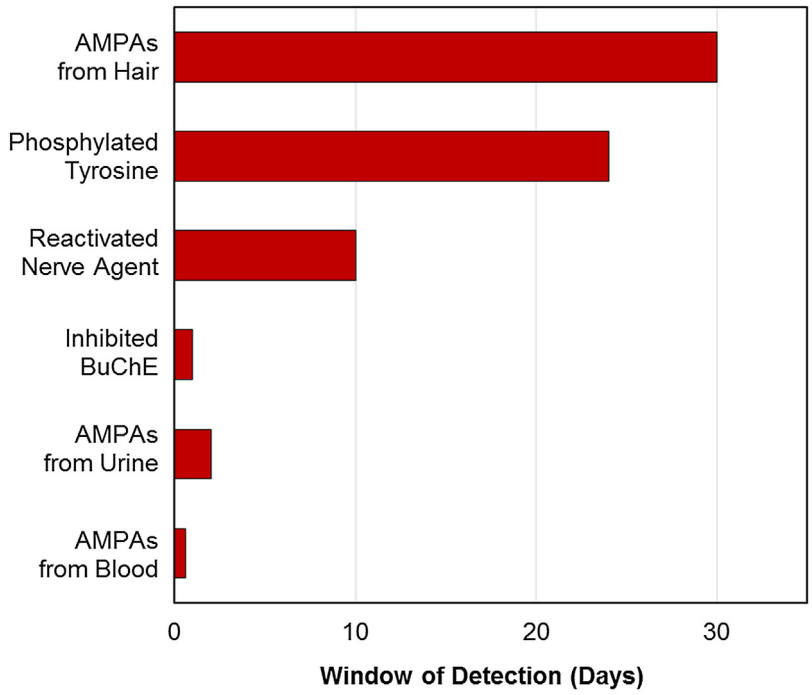

Fig. 2. Longest window of detection, to date, of AMPAs from hair [24], phosphylated tyrosine [20], reactivated nerve agent [10,12], inhibited BuChE [19], and AMPAs from urine [8,23] and blood [23].