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Detection of Antidepressant and Antipsychotic Drugs in Postmortem Human Scalp Hair

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ABSTRACT: The presence of therapeutic drugs in postmortem human scalp hair was investigated. Hair samples from 21 cadavers known to have taken antidepressant and antipsychotic drugs were solubilized in 1 M sodium hydroxide. Drugs were extracted using solvent extraction procedures and analyzed by gas chromatography-mass spectrometry (GC-MS) and high performance liquid chromatography (HPLC). Antidepressant drugs detected were amitriptyline, dothiepin, doxepin, imipramine, trimipramine, and mianserin. Antipsychotic drugs detected were haloperidol, chlorpromazine and thioridazine. Concentrations of these drugs and their metabolites ranged from 1.3 to 242 ng/mg hair. Segmental analysis demonstrated that the drug concentrations detected were either consistent with the known dosing regime of the deceased, or were able to provide an indication of drug use within the last few months prior to death. This study reinforces the potential of hair as a useful tissue in forensic investigations, in establishing a history of past exposures to therapeutic drugs.

KEYWORDS: toxicology, drug detection, hair, psychotropic drugs

Measurements of therapeutic drug concentrations have most commonly been determined from bodily fluids such as blood and urine. However, concentrations in such specimens only reflect drug use within a few days prior to sampling, and therefore cannot distinguish between acute or chronic drug use. The application of hair analysis for the detection of drugs in the human body is now being developed as an alternative, as it offers the potential for the detection of drug exposures over extended periods of time.

While the occurrence of illicit drugs and drugs of abuse in hair is well documented, little is known on the presence of therapeutic drugs, namely antidepressant and antipsychotic drugs, in hair. The studies that have been performed on antidepressant drugs to date have been limited, and have targeted a few individual drugs. Amitriptyline, nortriptyline and imipramine have been detected in the hair of psychiatric patients taking these antidepressant drugs [*1*]. Amitriptyline, nortriptyline, and clomipramine have also been detected in the hair of postmortem cases [2]. The antipsychotic drug haloperidol has also been detected in hair [*3,4*]. To our knowledge, haloperidol is the only antipsychotic drug reported to have been detected in hair.

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This study was designed to determine whether a wide range of antidepressant and antipsychotic drugs are incorporated into human scalp hair following their use, and whether segmental analysis can assist in obtaining histories of drug use.

Methods

Reagents and Materials

Methanol and hexane (Mallinckrodt, Aust.), and acetonitrile (Millipore/Waters, Aust.) were of HPLC grade. Trizma base was obtained from Sigma (USA) and diethylamine was obtained from Fluka (Aust.). Butyl chloride (BDH Chemicals, Aust.) was redistilled prior to use. All other chemicals were of analytical reagent grade (Ajax Chemicals, Aust.).

Glass extraction tubes were silanized with a 5 % solution of Surfasil® (Pierce, IL,) in toluene for 1 h, then rinsed twice in methanol prior to drying.

Drug Standards

Pure standards of all drugs were obtained either from Sigma Chemical Co. (USA) or from the curator of standards from the Australian Government Analytical Laboratories.

Stock solutions for all drugs (1 mg/mL) were prepared daily in methanol. Working standards were prepared by diluting stock solutions with distilled water to give concentrations of 100 μg/mL and 10 μg/mL. For calibration curves, working standards were added to drug-free hair samples to give drug concentrations ranging from 0.1 to 10.0 μg/mL.

Sample Collection and Washing Procedure

Selection of postmortem cases was based on knowledge of the medical histories of the deceased persons. Dosing information was either obtained from police reports or from the treating medical practitioner. Specimens of as many strands of hair as possible were pulled from the vertex posterior or the nape of the neck in each case. Tape was placed around the root ends of the hair strands so the area of most recent growth could be distinguished from that of past growth. Control hair samples were also obtained from postmortem cases and living subjects known to be drug free.

All hair samples were soaked in deionized water for five min to eliminate traces of blood. This was followed successively by three brief rinses in methanol to remove any other surface contaminations. Hair samples were subsequently dried and weighed.

GC Extraction Procedure

An alkaline digestion procedure was used to extract drugs from hair. One mL of sodium hydroxide was added to hair samples of

approximately 20 to 100 mg. One μg of cyclizine (internal standard) was added and the tubes incubated in a shaking water bath at 70 °C for 30 min. Concentrated hydrochloric acid (HCl) was added to all samples until a pH of between 9.5 and 10 was obtained. Control hair samples and calibration curves for all suspected drugs were incubated under the same conditions as the case hair samples in every assay.

One mL of 2 M tris buffer was added to the 1 mL of hair digests, and the tubes mixed. Eight mL of distilled butyl chloride was added and the tubes extracted on a rotating wheel for 20 min. Tubes were then centrifuged for 5 min (2000 rpm). The extraction solvent was transferred to clean tubes containing 1 mL of 0.1 M HCl and back-extracted for 20 min. Tubes were centrifuged and the solvent layer discarded. One mL of 2 M tris buffer and 8 mL of distilled butyl chloride were added and the tubes re-extracted for 20 min. The extraction solvent was transferred to clean tubes and evaporated to dryness in a sample concentrator (#SVC 200H, Savant Industries, Melb., Aust.). The residue was reconstituted in 100 μL of methanol and transferred into 200 μL glass inserts. Two μL of the extracts were injected directly into the GC.

GC Instrumentation and Conditions

Hair samples were initially screened on a gas capillary column using nitrogen/phosphorous detection. Chromatography of drugs was achieved on a non-polar, BP-5, fused silica column, 12 m × 0.53 mm I.D, 1.0 micron film thickness (#054197, S.G.E., Melb., Aust.). The temperature program was set at 100°C for 2 min, increasing at 7.5°C/min until 310°C, then held for 10 min. The carrier gas was helium and the flow rate was approximately 3 mL/min.

Mass Spectral Confirmation

The same extract was transferred to plastic vials, and 1 μL was injected into a GC (Hewlett Packard 5890A) attached to a Mass Selective Detector (HP 5970A). The capillary column was a 25 meter Ultra-2, cross linked 5% phenyl methyl silicone column, 0.2 mm I.D, 0.33 μm liquid coating (HP, #19091B-102). The temperature program was set at 90°C for 2 min, increasing at 15°C/min until 310°C, then held for 10 min. Peaks were identified using a full scan GC-MS analysis of samples, scanning over a mass range of m/z 29 to 495. The drugs were identified based on a comparison of retention times and the relative abundance of confirming ions based on a percentage match from "WILEY" and/or "PMW-TOXR.1" mass spectral libraries. Confirmations were obtained if the percentage match of the spectra with the library was at least 75%. Confirmations were also based on metabolite patterns and comparison with standards.

Quantitation Procedures

For quantitation of antidepressant and antipsychotic drugs, a HPLC method was used as previously described [5]. In brief, 95% hexane: 5% butanol was the solvent used to extract antidepressants and haloperidol, while butyl chloride was used to extract chlorpromazine and thioridazine. The solvent layers were back-extracted in 0.2% orthophosphoric acid. The solvent layer was discarded and an aliquot of the acid layer was injected into the HPLC system.

Results

The drug content of postmortem hair samples was established through initial screening by GC with confirmation by GC-MS. Antidepressant and antipsychotic drug concentrations were then quantitatively measured by HPLC. Inter assay coefficients of variance data for the HPLC procedure ranged from 3.6–6.4% at 0.25 μg/mL and from 9.3–13% at 1.00 μg/mL, for all drugs. Recoveries of antidepressant and antipsychotic drugs in hair samples were calculated by measuring peak heights of unextracted amounts of drugs and comparing this to peak heights obtained from extracted standards containing the same concentration. Recovery data ranged from 72 to 94%, and the detection limit for all drugs was 0.1–0.25 ng/mg hair.

Overall, 21 case hair samples were analyzed for antidepressant and antipsychotic drugs by GC and HPLC (Table 1). No drugs were detected in all six control hair samples taken from cases in which no drugs were expected. Root sheath cells or other skin-related appendages were not seen in any of the cases.

In total, six of the most commonly prescribed antidepressant drugs (in Australia) were detected in the postmortem hair specimens. Six cases were positive for dothiepin, and the concentrations measured ranged from 6.7 to 137 ng/mg hair. In all six cases, a metabolite of dothiepin, northiaden, was also detected. Six cases were positive for amitriptyline, with concentrations ranging from 3.5 to 34 ng/mg hair. In 5 of these cases a metabolite of amitriptyline, nortriptyline, was also detected, with concentrations ranging from 3.8 to 9.2 ng/mg hair. Two cases were positive for doxepin and the concentrations detected were 7.7 and 87 ng/mg hair. Imipramine and its metabolite, desipramine, were detected in 1 case at concentrations of 104 and 88 ng/mg hair, respectively. Mianserin was detected in 1 case at a concentration of 9.2 ng/mg hair, while trimipramine and its metabolite, desmethyltrimipramine, were also detected in one case.

The three most common antipsychotic drugs were also detected. Haloperidol was detected in two cases, and the concentrations measured were 17 and 242 ng/mg hair. Two cases were positive for chlorpromazine, at concentrations of 1.3 and 29 ng/mg hair, and thioridazine was detected in 3 cases at concentrations ranging from 4.5 to 71 ng/mg hair. Two of the three cases positive for thioridazine were also positive for its 2 major metabolites, mesoridazine and sulforidazine. The concentrations measured for these metabolites were 15 to 45 ng/mg and 2.8 to 8.8 ng/mg hair, respectively. In addition, methadone and propoxyphene were detected in one case each, while nicotine was detected in 11 cases.

TABLE 1—*Prevalence and concentration of drugs detected in 21 postmortem cases.*

Drug (metabolite)	Number of cases	Concentration (ng/mg hair)
Amitriptyline	6	3.5–34 (18 ± 10)
(nortriptyline)	5	3.8–9.2 (6.5 ± 2.3)
Doxepin	2	7.7–87
Dothiepin	6	6.7–137 (66 ± 55)
(northiaden)	6	detected
Imipramine	1	104
(desipramine)	1	88
Mianserin	1	9.2
Trimipramine	1	detected
(desmethyltrimipramine)	1	detected
Haloperidol	2	17–242
Chlorpromazine	2	1.3–29
Thioridazine	3	4.5–71 (28 ± 37)
(mesoridazine)	2	1.5–45
(sulforidazine)	2	2.8–8.8

Mean concentrations ± S.D. are given in parentheses.

Segmental analysis was performed on several hair samples positive for either antidepressant or antipsychotic drugs. Hair specimens from each of these cases were cut into two segments corresponding to the available medical history.

In one case, a 63-year-old female had been prescribed 120–140 mg/day of Tolvon (mianserin) from October 1991 until her death in May 1992. Medical records indicated that she had not taken mianserin prior to this. The hair sample collected was approximately 12 cm in length and was cut into two segments: from 0–8 cm, representing the 6.5 months the deceased had been prescribed mianserin, and from 8–12 cm, representing the drug-free period. Mianserin was detected in the 0–8 cm segment of hair at a concentration of 9.2 ng/mg hair (Fig. 1*a*). No mianserin was detected in the 8–12 cm segment.

Similarly, in a second example, a 37-year-old female had been prescribed 100 mg/day of Prothiaden (dothiepin) from November 1990 until her death in June 1992. No medical records could be obtained prior to November 1990. The hair sample collected was 40 cm in length. However, since only a few strands were available, the hair was only cut into two segments: from 0 to 25 cm, approximately representing the 18 months the deceased had been prescribed dothiepin, and from 25 to 40 cm, representing the dothiepin-free period. Dothiepin was measured in the 0 to 25 cm segment of hair at a concentration of 37 ng/mg hair (Fig. 1*b*). In addition, the metabolite northiaden was also detected. However, neither dothiepin nor its metabolite were detected in the 25 to 40 cm segment. Unexpectedly, amitriptyline was detected in the 25 to 40 cm segment at a concentration of 34 ng/mg hair, but was not detected in the 0 to 25 cm section of hair, suggesting previous treatment with amitriptyline.

In a third case, a 46-year-old male had been admitted to hospital for an overdose of antidepressant drugs. He later died. Toxicological analysis on antemortem blood samples revealed the presence of amitriptyline and nortriptyline. However, since there were no recent medical records available prior to the overdose, it was not known whether the deceased had been on long-term treatment with amitriptyline, or if he had just taken a single high dose. The hair sample collected was 8 cm in length and was cut into two sections: from 0 to 3 cm and 3 to 8 cm. Amitriptyline was detected at concentrations of 23 and 21 ng/mg hair in the 0 to 3 cm and the 3–8 cm segments, respectively (Fig. 1*c*). Nortriptyline was also detected in both segments.

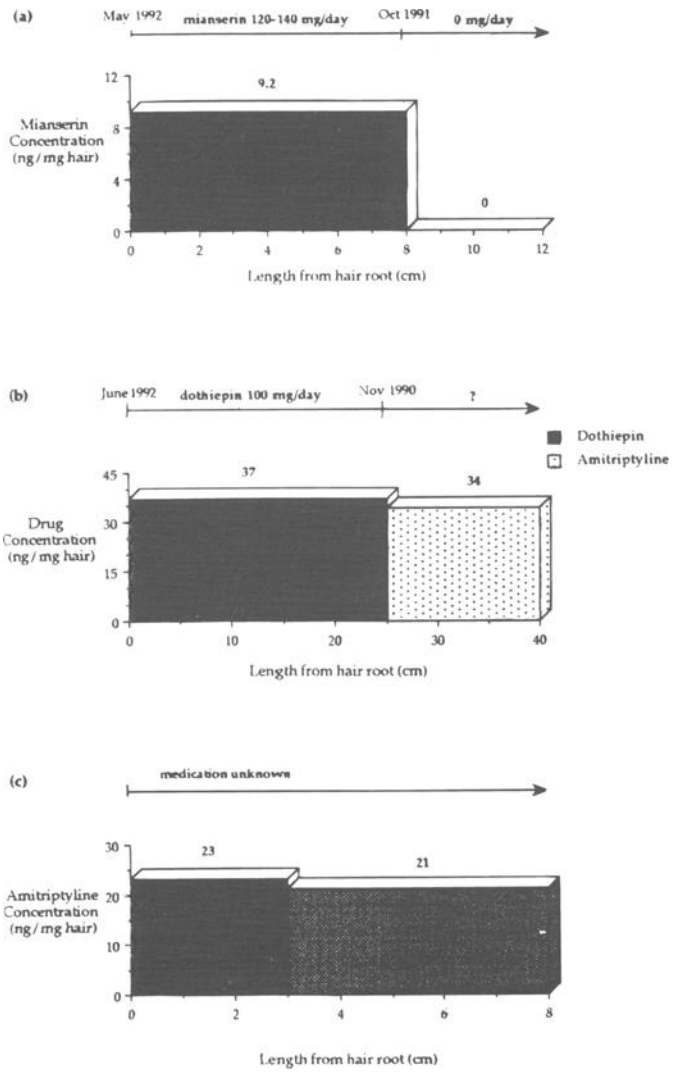

FIG. 1—*Concentration-time profiles in hair in* (a) *a person starting mianserin 6.5 months prior to death,* (b) *a person switching from amitriptyline to dothiepin approximately 18 months prior to death, and* (c) *a person found to be taking amitriptyline for at least 6 months prior to death.*

Discussion

A wide range of antidepressant and antipsychotic drugs have been shown to be incorporated into human scalp hair during their therapeutic use. Metabolites of dothiepin, amitriptyline, imipramine, trimipramine and thioridazine were also detected in hair. The concentrations of various drugs detected compare favorably with reported studies. Haloperidol has been detected in human scalp hair at concentrations of 2.3 to 245 ng/mg hair after the administration for four months of 3 to 30 mg/day of haloperidol [*3*]. These concentrations correlated with daily doses. In the present study, haloperidol was detected at concentrations of 17 and 242 ng/mg hair in persons who had been prescribed 5 and 30 mg/day of haloperidol, respectively.

Amitriptyline has been detected at concentrations of 13 and 24 ng/mg hair after the long-term treatment of 25 to 50 mg/day of amitriptyline to psychiatric patients [*1*]. This compares with amitriptyline concentrations of 3.5 to 34 ng/mg hair after the administration of similar doses. Imipramine has been detected at concentrations of 16 and 69 ng/mg hair after the long-term treatment of 25 to 50 mg/day of imipramine [*1*]. In this study, a person had been prescribed 200 mg/day of imipramine for 11 months, and the imipramine concentration detected in the deceased's hair was 104 ng/mg hair. These results suggest that there may be a dose-proportionality of antidepressant and antipsychotic drugs in hair.

Individual dosage histories of haloperidol can be obtained by measuring the distribution of this drug along the length of hair strands [*3,4*]. Segmental analysis has also been used in human scalp hair to establish drug histories of heroin, cocaine and phencyclidine users [*6*]. Similar histories were obtained with mianserin, dothiepin, amitriptyline and also chlorpromazine. These observations confirm that segmental analysis of hair may therefore provide a useful technique in establishing patterns of therapeutic drug use in the weeks to months prior to death.

The amount of detail obtained from segmental analysis of hair strands will depend on the length of the segments. While smaller (1 cm) lengths may provide a more accurate pattern of drug use,

more work is required to investigate incorporation of drugs in hair from other mechanisms such as sweat. Given the lack of cross-over of drugs in the segments of the cases investigated here, it is unlikely that sweat may have caused substantial incorporation of drugs over extensive lengths of hair. Drugs associated with root sheath cells and other skin-related appendages were not seen at any of the root ends of hairs. Consequently, drugs associated with skin were unlikely to have influenced our results.

In conclusion, hair analysis offers the potential for the detection of exposures to antidepressant and antipsychotic drugs, and would therefore provide a useful history of past drug use. Further controlled studies investigating the influence of various factors on the drug content in hair will need to be performed before interpretations of the drug concentrations in hair can be made.

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