Int J Legal Med (2004) 118: 194–196
DOI 10.1007/s00414-004-0442-7

ORIGINAL ARTICLE

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Entomotoxicology for the forensic toxicologist: much ado about nothing?

Received: 11 July 2003 / Accepted: 22 March 2004 / Published online: 26 May 2004

Abstract We present a series of 29 necropsies in which organic compounds (including benzodiazepines, barbiturates, antidepressants, phenothiazine, opiates, cannabinoids, meprobamate, digoxin and nefopam) were detected in arthropod larvae sampled on human corpses. No correlation was observed between drug concentrations in the larvae versus human samples. When tested, inter-larvae and inter-site variations of drug concentrations (i.e., within larvae when analyzed separately, and within anatomic sites when larvae were grouped according to their site of sampling) were enormous and not reproducible from one case to another, confirming that arthropod larvae are unreliable for quantitative toxicological analysis. Since drugs identified in maggots are always detectable in the cadaver too, we conclude that larvae analysis is of almost no interest for practical forensic casework.

Keywords Entomology · Forensic entomology · Entomotoxicology · Diptera larvae · Toxicology · Analysis

Introduction

At the crossroads of zoology and chemistry, entomotoxicology ranks among the newest tools of the forensic armamentum. The two major problem areas are:

1. The identification and quantitation of xenobiotics in carrion-feeding arthopods, and their relevance for the toxicological assessment of the causes and circumstances of death (i.e. the toxicologist's point of view). According to the literature, drugs already detected in larvae include barbiturates (phenobarbital [1], secobarbital [2], amylobarbital and barbital [3]), benzodiazepines (diazepam [4], temazepam [5]), antidepressants (amitriptyline [5, 6, 7, 8, 9], nortriptyline [7], trimipramine [5]), opiates/opioids (morphine+codeine [10, 11, 12, 13, 14, 15, 16], propoxyphene [6]), amphetamine derivatives (amphetamine [3], MDMA +MDA [17]), cocaine and/or benzoylecgonine [18, 19, 20], trazodone [5], acetaminophen [3, 6], salicylates [3] and malathion [21].
2. The study of drug-induced changes in arthropod growth with respects to the estimation of the post-mortem interval (PMI) by entomological methods (i.e. the entomologist's point of view). Based on a near 15-year experience, the Medico-Legal Institute of Strasbourg (MLIS) was first in Europe to report on this topic [22], the present work aims to demonstrate that one cannot expect similar benefits from these two approaches.

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Material and methods

General procedure of analysis

All experiments were performed on blowfly larvae (maggots) sampled on human cadavers at various stages of putrefaction, during necropsies carried out at the MLIS. Briefly, 1–10 g larvae were pooled from multiple sites on/in the cadaver, weighed, then submitted to 2–3 cycles of washing (deionized H_2O) and drying (filter paper) to avoid external surface contamination. After mechanical homogenization (IKA Ultra-Turrax) in deionized water, 0.9% NaCl solution or specific buffer, the homogenate underwent liquid-phase or solid-phase extraction. Finally the dry extracts were resuspended in methanol then assayed, in most cases by gas-chromatography or liquid-chromatography coupled to mass spectrometry (GC/MS, LC/MS).

Results and discussion

Since 1988 we have done 29 necropsies in which organic drugs were detected in blowfly larvae sampled on the

corpses. The results are summarized in Table 1. Of course the sum of drug occurrences exceeds by far the number of autopsy cases, due to many multiple-drug fatalities, or because some compounds were present as metabolites of a parent drug (e.g., two of the three cases with oxazepam were associated with concomitant detection of nordiazepam). Our results clearly confirm that a wide range of compounds, especially psychotropics or drugs of abuse, may be detected in carrion-feeding larvae.

In all cases of our series, the drugs present in the larvae were also identified in the tissues from the cadaver on which the larvae had been sampled. For each definite compound, i) the concentrations in larvae were generally much lower than those in the cadaveric samples, ii) no correlation was observed between the concentrations measured in the larval compared to the human material. These results completely agree with those of another recent study [23]. As an example, in 4 fatalities of our series involving nordiazepam (always combined with other xenobiotics), blood-to-larvae ratios for this drug were in the range 1:23, and liver-to-larvae ratios in the range 3:38.

Table 1 Organic compounds identified in arthropod larvae (Medico-Legal Institute of Strasbourg 1988–2002)

Compound	Number of cases	Concentrations (pg/mg)
Benzodiazepines		
Nordiazepam	4	228, 776, 125, 21
Oxazepam	3	44, 153, 200
Lorazepam	1	155
Bromazepam	1	810
Alprazolam	1	27
Triazolam	1	204
Barbiturates		
Phenobarbital	3	761, 2,250, 500
Amobarbital	1	1,540
Antidepressants		
Amitriptyline	1	133
Clomipramine	1	28
Dothiepin	1	280
Fluoxetine	1	16
Venlafaxine	1	59
Phenothiazine		
Chlorpromazine	2	551, 16
Cyamemazine	2	103, 489
Levomepromazine	1	45
Alimemazine	1	22
Opiates/Opioids		
Morphine	3	137, 182, 90
Codeine	3	22, 59, 12
Pholcodine	1	13
Propoxyphene	1	867
Miscellaneous		
THC-COOH	2	16, 39
11-Hydroxy-THC	1	11
Meprobamate	2	4,439, 718
Digoxin	1	21
Nefopam	1	880

Depending on the anatomic site of sampling, variations of drug concentrations (inter-site variations) appeared to be enormous and not reproducible from one case to another; in a 31-year-old man who died from an overdosage of heroin and nordiazepam, PMI ca. 15 days, the average nordiazepam levels measured in 5 groups of maggots sampled from different anatomic sites were: perioral area (n=17 larvae) 837 ng/g, upper digestive tract (n=24) 359 ng/g, trunk (n=21) 358 ng/g, upper limbs (n=18) 24 ng/g, lower limbs (n=22) 76 ng/g. In a similar study performed on a 22-year-old female who died from a mixed overdose of cyamemazine and ethanol (PMI 5–7 days), the mean cyamemazine levels in 4 groups of maggots were: perioral area (n=31 larvae) 67 ng/g, trunk (n=48) 129 ng/g, upper limbs (n=39) 178 ng/g, lower limbs (n=44) 82 ng/g.

When tested inter-larvae variations (i.e., within larvae analyzed separately) of drug concentrations were also found to be very large. For instance, in a 47-year-old man who died from a meprobamate + ethanol overdose (PMI ca. 7 days, blood meprobamate 87 μg/ml, blood ethanol 3.11 mg/ml), meprobamate was separately assayed by LC/MS on 30 larvae removed from the external surface of the trunk: the mean±S.D. concentration was 718±748 pg/mg (range: not detected in 6 cases to 3412 pg/mg). In another case, a 29-year-old female who died from a massive ingestion of nordiazepam, cyamemazine and codeine, concentrations of nordiazepam in 30 larvae collected from the external surface of whole body were 776±1081 pg/mg (range: not detected in 8 cases to 4416 pg/mg). A similar observation was made by Sadler et al. in 45 groups of larvae fed on the same foodstuff spiked with a known amount of amitriptyline: drug levels measured in larvae ranged from not detected to 148 pg/mg (mean 42.6 pg/mg) [8].

From our experience it seems hardly expectable to find a quantitative relationship, even tenuous, between the concentration of a drug in an individual's biofluids at the time of death, and that in larvae sampled weeks to months later on the cadaver. This is due to a wide array of influencing factors, most of them completely unpredictable or largely unexplored: i) the postmortem redistribution of drugs in the human body (the longer the PMI, the greater its extent); ii) the stability of drugs in human remains, especially at the body's surface where larvae are generally sampled (it depends on temperature, humidity, pH, exposure to UV-radiation and strongly varies from one drug to another); iii) the pharmacokinetics of each drug in maggots (which is likely to depend on the nature of the drug, the species of the arthropod, its stage of growth, ambient temperature, etc.), without forgetting drug interactions in case of mutiple-drug fatalities that are by far the most frequent. Considering these uncertainties, fly larvae clearly appear to be unreliable samples for quantitative toxicological analyses, thus any attempt to estimate the cause and circumstances of death by this method is highly

questionable and scientifically dishonest. Moreover, since maggots can provide only qualitative results (which implies that only positive results should be considered: absence of drugs in the maggots does not mean absence of drugs in the cadaver!), there is absolutely no advantage in analyzing carrion-feeding larvae instead of samples from the carrion itself. Modern extraction procedures (e.g. solid-phase) and recent advances in detection systems (LC/MS, GC/MS/MS) have considerably improved both the sensitivity and specificity of toxicological determinations, and made the formerly difficult analysis of putrefied samples, a routine task.

In conclusion, contrasting with the major interest of studying drug-induced changes on arthropod development with respects to the PMI estimation (see recent advances in this journal issue [24, 25]), the determination of drugs in larvae for forensic-toxicological purposes does not meet the expectations it aroused a decade ago: unless enormous advances occur in the knowledge of factors influencing drug concentrations in larvae, such results have almost no interest in practical casework and will remain at best a laboratory curiosity—at worst, a scientific imposter.

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