Réunion de la Société de Toxicologie de Belgique et du Luxembourg (Assemblée générale de la BLT, 7 février 2006, VUB, Bruxelles, Belgique) : résumés des communications

Meeting of the Toxicology Society of Belgium and Luxembourg (BLT General Assembly, February 7, 2006, VUB, Bruxelles, Belgium): abstracts of the oral communications

1. Recent anti-epileptic drugs : safe in overdose ?

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New anti-epileptic drugs (AED) have become available over the last years. Anti-epileptic drugs are still common substances taken in intentional drug overdoses. The data collected from the US poison control centres show that the mortality and morbidity remain high after acute poisoning with the older anti-epileptics, and particularly with valproic acid. The objective of this short review is to discuss the safety profile of seven recent AED : topiramate, vigabatrin, tiagabine, gabapentine, levetiracetam, felbamate, lamotrigine and oxcarbazepine. *Topiramate* has been associated with at least one fatality. In contrast, ingestion of 4000 mg (10 times the daily therapeutic dose) did not result in significant complications. Metabolic acidosis may be partly due to carbonic anhydrase inhibition. A single case of *vigabatrin* overdose appeared in the literature (absence of symptoms following an ingestion of 60 g). *Tiagabine* is metabolised in the liver, with the possibility of microsomal induction. Altered consciousness is possible following tiagabine overdose, but also seizures occurrence. Several cases of *gabapentine* overdose have been published. Ingestion of large doses (49-91 g) has been followed usually by mild neurological complications. Three deaths have been reported in the 2000-2001 TESS database. *Levetiracetam* is not metabolised and is eliminated by the kidney. Acute poisoning is rare but may be complicated by coma. The elimination half-life is about 5 h and the symptoms are improving rapidly without specific treatment. *Felbamate* is structurally related to meprobamate but is devoid of significant cardiotoxicity. It is poorly metabolised in the liver. A mild CNS depression is possible following overdose. *Lamotrigine* exposure is not exceptional. In most of the cases, only mild to moderate symptoms were noted. Lamotrigine may influence the voltage dependent sodium channels not only into the brain but also in the heart. There is a possibility of an increased QRS dura-

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tion on the EKG. *Oxcarbazepine* is structurally related to carbamazepine. Mild CNS side effects have been observed, but the substance is also able to influence the voltage dependent sodium channels.

Conclusions: It appears from the recent case reports or from the limited number of case series, that acute poisoning with the newer AED results in an extremely low mortality and morbidity. As expected, a short-lasting central nervous system depression is a common finding, but few patients are requiring mechanical ventilation. Seizures are occasionally observed. Serious cardiovascular events have been reported in a very few cases. The treatment is mainly supportive.

2. Simultaneous determination of MDMA and analogs in hair by LC-MS/MS: application to the determination of MDMA after the absorption of a single Ecstasy tablet.

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Introduction: We present an application of LC-MS/MS, one of the most powerful analytical methods to be used for analysis of urine and hair to solve drug facilitated crimes in forensic expertise. Hair is a very useful sample matrix when the victim lodges a complaint several days after the suspected intake of the drug and when the drug involved has a short half-life, providing only short-term information when analyzing biological fluids. Moreover, hair analysis enables to show the victim's drug abstinence outside the period of the offence.

Materials and methods: To 20 mg of decontaminated and cut hair, 100 pg/mg of deuterated amphetamine and analogs (Promochem) were added as internal standards. Matrix dissolution was performed for 15 min in NaOH 1N at 80°C. Extraction was performed with hexane/ethyl acetate (2/1). After centrifugation, the organic layer was filtered with PTFE 0.2 µm then evaporated to dryness at ambient temperature. Urine was extracted with Toxi-tube A® (Varian) and with 1 ng/mL of deuterated analogs. The residues were reconstituted by 100 µl of MeOH and transferred in glass vials. Ten microliters were injected into the LC-ESI-MS/MS triple quadrupole TSQ Quantum Ultra (ThermoElectron). Separation was achieved on a C_{18}-column (Uptisphere ODB 150x2mm) at 30°C. Mobile phase (formate buffer 2mM pH 3 / ACN) was delivered in gradient mode for a total run time of 20 min. The detection was performed in positive and SRM mode and allowed the simultaneous detection of amphetamines and analogs. To each pseudomolecular ion 4 product ions were acquired at a scan time of 0.1 s with a width of 1.0 a.m.u. Detection limits ranged from 0.1 - 0.5 ng/mL in urine and from 5 - 20 pg/mg in hair.

Results: We applied this method to the analysis of urine and hair of 16-year-old girl for the determination of MDMA and metabolite after a suspected intake of a single Ecstasy tablet in an iced tea. Twelve hours after, the victim went in a first laboratory where urine #1 was collected and analyzed by immunoassay (amphetamines positive). After her complaint 8 days post ingestion, urine #2 and blood were collected for forensic purposes. All biological fluids were kept frozen. Hair was collected 2 months after the offence. Analysis showed the presence of MDMA (37 µg/mL) and MDA (4 µg/mL) in urine #1 by LC-DAD. Urine #2, blood and hair were analyzed by LC-ESI-MS/MS : blood was tested negative but MDMA was detected in urine #2 at 0.42 ng/mL and in hair at 22 pg/mg (LOD ~5 pg/mg). MDA was not detected in urine #2. A detection limit of 0.1 ng/mL in urine allowed the detection of a single Ecstasy tablet up to 8 days.

Conclusion: These results demonstrate the power of LC-MS/MS as well as hair analysis for the elucidation of drug facilitated offences or crimes. To our knowledge, it is the first description of the determination of MDMA in hair after the absorption of a single Ecstasy tablet.

3. Acute cocaine intoxication of a nine month-old infant: a proof of chronicity by hair analysis.

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A nine month-old girl was brought to the emergency department of ZNA Stuivenberg with convulsions and respiratory insufficiency, suggesting an acute intoxication. There were no physical signs of child abuse, and no injection marks were present. Because of the need for intubation and sedation she was transferred to the intensive care unit of the Queen Paola children's hospital. Toxicological screening of the child's urine revealed a positive result for benzoylecgonine (BZE), later confirmed as cocaine and its metabolites. Although morphine and pholcodine in urine were also positive and were determined at 14 ng/mL and 18 ng/mL, the child successfully recovered. Since the

mother denied giving her child any drugs, or using drugs herself, a toxicological analysis of the child's hair was performed.

For the analysis a five point calibration curve was made with a standard mixture of amphetamine and derivates, cocaine, morphine, methadone, codeine and their metabolites. The method involved decontamination of the hair sample (cut in pieces) with dichloromethane, the addition of deuterated internal standards to the sample and calibrators followed by overnight incubation in a mixture of phosphate buffer (pH = 6.0) and methanol (1:1), solid phase extraction, derivatisation with hexafluoroisopropanol and trifluoroacetic acid anhydride and analysis by gas chromatography coupled to mass spectrometry in the electron impact mode of detection.

A hair string of 8 cm was cut in two equal segments. In the most distal (oldest) segment we found 81 ng/mg hair of cocaine, 12 ng/mg hair of BZE and 7,8 ng/mg hair of 6-monoacetylmorphine (6-MAM). In the proximal (youngest) segment, values of 61 ng/mg hair of cocaine, 8.3 ng/mg hair BZE and 5.1 ng/mg hair 6-MAM were found.

The high concentrations of cocaine and BZE seen in this case, combined with the inversed ratios cocaine/BZE compared to blood and/or urine (6.8 and 7.3 for the two parts, respectively), indicate that the child has been given cocaine probably since the day she was born. Since the dichloromethane wash solution was negative for cocaine, BZE and 6-MAM, external contamination could be excluded. The presence of 6-MAM indicates also a possible heroin administration. After a month the analysis was repeated on a new 4 cm hair sample. Three segments of about 1 cm were analysed. The results were in accordance with the previous analysis: beginning from the oldest segment cocaine was 64, 19.9, and 17.7 ng/mg hair, BZE was 11.8, 2.4 and 2.7 ng/mg hair. 6-MAM could only be found in the oldest segment in a concentration of 8.8 ng/mg hair. With these confirming results child protective issues were installed.

Conclusion: In addition to the initial urine testing, hair analysis is a useful tool to diagnose repeated exposure to drugs of abuse.

4. A fatal intoxication with the antimalarial drug chloroquine

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Due to the recent trend of frequent travelling to tropical destinations, the prescription of antimalarial drugs like chloroquine for instance, has increased continuously.

Nevertheless, fatal accidental intoxications or fatal suicide attempts with this drug are rarely seen in Belgium. The toxicity of these compounds however, should never be underestimated.

We describe the case of a 47-year-old man who committed suicide with chloroquine and was found dead at home by his children. No empty blisters or tablet fragments were found.

After initial screening on an alkaline urine extract by HPTLC with iodoplatinate detection and GC-MS analysis, chloroquine was identified as the possible cause of this fatal intoxication. Chloroquine and its two major metabolites, desethyl- and bisdesethylchloroquine, were quantified in heart blood plasma, femoral blood plasma and urine by HPLC-DAD. The concentrations of chloroquine is these samples were respectively 23.4 mg/L, 6.3 mg/L and 61 mg/L.

The literature shows that plasma concentrations higher than 0.6 mg/L may produce toxic effects and that concentrations higher than 3.0 mg/L may be fatal due to acute cardiotoxicity. Besides a whole blood ethanol concentration of 0,97 g/L, no other drugs than chloroquine were detected.

The cause of death of this man could be attributed to the intake of a massive amount of chloroquine. The much higher concentration of chloroquine in heart blood in relation to venous blood is suggestive for the absorptive state as far as no post mortem redistribution of the drug has taken place. At these concentrations fatal outcome is a consequence of cardiac conduction disturbances followed by asystolia in the terminal phase.

5. A LC-MS/MS assay for tacrolimus in liver biopsies after orthotopic liver transplantation: correlation with rejection.

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This study was designed to validate a specific assay to quantify tacrolimus (TAC) in liver biopsies after hepa-

tic transplantation and to compare the predictive value of either tissue or blood TAC concentrations for rejection (as determined by BANFF, score 0-9). 250 liver biopsies were performed in adult population after orthotopic liver transplantation (OLT), under TAC monotherapy, after 7 days, 6 and 12 months post-transplantation. Samples were stored at -80°C until analysis. The assay was developed by LC-MS/MS, using human liver tissue spiked with TAC for calibration. Samples were dried under nitrogen, weighted, homogenized with 1mL of phosphate buffer, then extracted with 5mL of ethyl acetate after addition of 100µL of Ascomycin (50ng/mL) as internal standard. Samples were mixed 30min, centrifuged 5min, before separation of the organic layer subsequently evaporated to dryness. Dry residue is reconstituted with 50µL of methanol. 20µL are injected in the LC-MS/MS. Chromatographic conditions include a cartridge column C18 Phenomenex 4x3 mm maintained at 55°C, a 0.3mL/min flow rate of a mobile phase (30% buffer ammonium acetate 2mM/70% methanol-ammonium acetate 2mM). TAC and Ascomycin were monitored by detecting specific product ions resulting of the fragmentation of their precursor ions using MRM acquisition mode (821.4>768.2m/z, and 809.6>756.6m/z, respectively). Analytical performances were: linearity ranging from 6-500pg/mg dry tissue, LOD 1.9pg/mg, LOQ 6pg/mg, intra- and inter-assay reproducibility ranging from 2.9-17.3% and 5.1-13.0%, respectively. TAC concentrations found in these liver biopsies ranged from <6pg/mg up to 298pg/mg, and displayed better correlation with liver histologic rejection score than blood concentrations. The cut-off concentration corresponding to moderate or severe rejections (BANFF scores > 6), was around 30pg/mg. Rejections according to BANFF > 6 were characterized by mean TAC tissue and blood concentrations of 10.7pg/mg and 7.2ng/mL, respectively, whereas corresponding values for BANFF ≤ 6 were 71pg/mg ($p<0.05$) and 7.3ng/mL (NS).

6. Surface detection of environmental contamination with cytotoxic drugs to prevent occupational exposures

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Contamination of personnel while handling cytotoxic agents has been clearly identified during occupational exposures through the detection of urinary trace concentrations of drugs. Surface detection of drugs e.g. on work surface, floors, desks, doors... could be considered as an optimal way of prevention, easier because not involving urine extraction and not involving drug metabolites production. However, the surface detection method must be properly standardized.

The purposes of this work are

1. to validate a standard procedure to collect and measure cyclophosphamide (CYP) and ifosfamide (IFO) from work surface
2. collect 20 surface samples and measure the drugs concentrations in the hospital pharmacy at St Luc University Hospital
3. provide a tool to improve quality and security for occupational professionals

Wipe sampling is performed using deionised water soaked 8-folded (Kimberly Clark) Kleenex (2 mL). Surfaces (25 x 25 cm) were wiped with these tissues before to be placed into 30 mL vials containing 28 mL of water. Vials were treated during 10 min by ultrasonication and 30 min shaking. One mL of solution is thereafter transferred into Eppendorf vials for automated injection into LC-MS/MS system (MicroQuattro Waters-Micromass Ltd, UK). Analyses were performed by MRM using as transition mass m/z: 261/91.8 and 261/140 for IFO and CYP respectively. Calibration curves ranged from 1-100 ng/mL. Limit of quantification (LOQ) of both CYP and IFO are 1.8 ng/mL. Recovery yield and coefficient of variation were evaluated to 68.9% and <7.4% respectively. 20 different surfaces were tested in our hospital pharmacy: vertical laminar airflow cabinet, desks, phones, refrigerator, floor, water tap, door handle, glass wall and bench. After wiping, all samples were stored at -20°C until analysis. Even though strict cautions are applied in our Hospital Pharmacy, three areas were detected positive (2 for CYP and 3 for IFO) at concentrations ranging from 3.9-292.6 ng/dm_. The origin of the contaminations are under investigation but could be due either to a secondary release out the airflow cabinet filter, initial contaminations (box or container) from the manufacturer or inadequate decontamination procedure.

Surface cytotoxic drugs detection could be used as a