K35 A Case of Repeated Tramadol Poisoning in an Infant

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During this presentation, the participants will learn about the metabolism of tramadol and its incorporation into hair. The objective of this report is to show the potentials and pitfalls of interpreting results from segmental hair analysis and the use of toxicological analysis of several matrices to support an expert opinion.

In January 2001, a five-month female infant was admitted to the emergency room (ER) with lowered consciousness and convulsions. During early spring 2001, the infant had four additional admissions to ER with the same symptoms and also small pupils and respiratory depression. Besides toxicological screening at the hospital a neurological evaluation was also performed. The toxicological screening was negative and the neurological tests were normal. At this point, the lab was contacted and it was decided that in case of another ER visit, samples would be taken and sent to the National Board of Forensic Medicine for analysis. Nothing happened until November 2001 when the now 15-month-old girl was admitted to the ER with similar symptoms as before. Serum and urine samples were obtained and sent to the forensic laboratory in Linköping for analysis, and a police investigation was initiated. When the opioid tramadol together with its metabolites Ndesmethyltramadol (N-dm-T), and O-desmethyltramadol (O-dm-T) were identified in the girls' serum and urine, poisoning was suspected and because of the earlier ER visits a hair sample was obtained to find out if tramadol had been administered more than once. The girl's hair had never been cut. A search for other samples taken during the spring 2001 was also initiated.

Experiment

Hair was segmented (10 mm each), washed and weighed in screwcapped glass tubes. One mL of 1 M potassium hydroxide was added and the hair sample was heated at 80° C for 10 minutes with occasional shaking. After cooling to room temperature the sample was extracted with 3 ml of a mixture of dichloromethane:isopropanol (80:20) containing 20% pentane. To serum, spinal fluid, and urine 0.1 ml of potassium hydroxide was added before the extraction.

After centrifugation for 5 minutes at 4200 *g* 2.7 ml of the organic phase was aspirated and transferred to a new 10-mL screw-capped glass tube and the sample was evaporated under a gentle stream of nitrogen at room temperature. The sample was then reconstituted in 100 IL of mobile phase, and transferred to a vial. Liquid chromatography-tandem mass spectrometry with an electrospray interface was used for analysis. The transitions monitored were 264.1/58.1 for tramadol, 250.1/44.0 for N-dm-T, and 250.1/58.1 for O-dm-T. Calibration was performed as duplicates at 5, 10, 15, 20, 50, and 75 (ng) by addition of the analytes to 20 mg drug-free hair (obtained from a laboratory employee) or 0.1 ml donor serum or drug free urine.

Results and discussion

Results from body fluids are shown in the table below and the results from segmental hair analysis are shown in the figure.

Date	Matrix	Tramadol (Dg/mL)	O-dm-T (Dg/mL)	N-dm-T (Dg/mL)
01-01-14	spinal fluid	0.14	0.06	not detected
01-01-26	serum	0.56	0.14	0.07
01-11-19	serum	1.06	0.22	0.31
01-11-19	urine	present	present	present

The spinal fluid sample was taken during the first admission and the first serum sample was obtained during the second ER visit. Both were sent to the laboratory after the police investigation was initiated. Both samples had been stored in freezers at the hospital. The last serum and corresponding urine sample were taken during the latest ER visit and the samples were sent directly to the laboratory. All samples contained tramadol together with at least one metabolite but no other drugs (based on a neutral and a basic extraction followed by GC-NPD). Thus, tramadol might have been the cause of intoxication in all these three admissions to the ER.

During the investigation, one of the parents was suspected of having poisoned the infant on all six occasions. Before prosecuting for attempted murder, the prosecutor wanted to know if any other proof of tramadol administration could be obtained to include the three admissions in February-March when no samples were available. Hair samples from the girl were thus obtained in late February 2002, more than a year after the first ER visit.

The segmental analysis of hair showed the presence of tramadol in all segments, suggesting continuous administration of tramadol, though with changes in dose. The segments S3 and S4 represent October/November 2001 when the latest visit to ER occurred. The positive results from serum and urine taken at this time confirm tramadol intake. Also, the positive serum and spinal fluid specimens confirm the positive hair segments S13/S12 (January/February

2001). Still, the positive segments S11-S5 may indicate yet other intoxications after the last ER visit March 1st –6th. On the other hand, the effect of dormant hair may produce positive results even though the administration of drug had stopped several months earlier. Hair that continues to grow after termination of drug intake will push the positive segments farther out and leave behind drug free hair. However, hair that stops growing at any time during intake will stay positive in the proximal segments, thus causing a "lag time" for the hair to be totally negative. This can be illustrated by examining the segment S2 representing time when the girl was in protective care and could not possibly have been given tramadol. In conclusion, the different samples and matrices together with the symptoms complement each other to strengthen the opinion that tramadol was the cause of the intoxication on all six occasions but the positive hair segments S11-S5 does not necessary indicate additional intake of tramadol during this period. Finally, the close cooperation between clinical and forensic toxicology units is of paramount importance for quick and accurate diagnosis of poisoning.

Tramadol, Hair, LC-MS-MS

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