

Segmental hair testing to disclose chronic exposure to psychoactive drugs

Análisis segmentario del pelo para detectar la exposición crónica a drogas psicoactivas

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Abstract

This study presents the case of a 4-year-old healthy child admitted to the paediatric ward for suspected accidental intoxication due to ingestion of narcoleptic drugs (methylphenidate, sertraline and quetiapine), taken on a regular basis by his 8-year-old brother affected by Asperger syndrome.

Intoxication can be objectively assessed by measurements of drugs and metabolites in biological matrices with short-term (blood and urine) or long-term (hair) detection windows.

At the hospital, the child's blood and urine were analysed by immunoassay (confirmed by liquid chromatography-mass spectrometry), and sertraline and quetiapine and their metabolites were identified. The suspicion that the mother administered drugs chronically prompted the analysis of six, consecutive 2-cm segments of the child's hair, using ultra-high performance liquid chromatography-tandem mass spectrometry, thereby accounting for ingestion over the previous 12 months. Quetiapine was found in the first four segments with a mean concentration of 1.00 ng/mg \pm 0.94 ng/mg hair while sertraline and its metabolite, desmethyl-sertraline, were found in all segments with a mean concentration of 2.65 \pm 0.94 ng/mg and 1.50 \pm 0.94 ng/mg hair, respectively. Hair analyses were negative for methylphenidate and its metabolite (ritalinic acid).

Biological matrices testing for psychoactive drugs disclosed both acute and chronic intoxication with quetiapine and sertraline administered by the mother.

Keywords: Segmental hair testing; Children; Antidepressants; Antipsychotics; Ultra-high performance liquid chromatography-tandem mass spectrometry.

Resumen

Se presenta el caso de un niño sano de 4 años de edad que ingresa en la sala de hospitalización pediátrica por la sospecha de una intoxicación accidental debido a la ingesta de fármacos narcolépticos (metilfenidato, sertralina y quetiapina), que tomaba de forma pautada su hermano de 8 años de edad que padecía un síndrome de Asperger. La evaluación objetiva de la intoxicación se puede realizar con la determinación de los fármacos y sus metabolitos en matrices biológicas con una ventana de tiempo corta (sangre y orina) o larga (pelo).

En el hospital se realizó un análisis de sangre y orina mediante inmunoanálisis (confirmado mediante espectrometría líquida-cromatografía de masas) y se identificó la presencia de sertralina y quetiapina y sus metabolitos. Con la sospecha de administración crónica de fármacos al niño, se procedió al análisis del pelo con cromatografía líquida de ultra-alto rendimiento-espectrometría de masas en tándem. El pelo se dividió en 6 segmentos consecutivos de 2 cm de longitud, de forma que permitieron estudiar la ingesta de los fármacos durante los últimos 12 meses. En los primeros 4 segmentos se encontró quetiapina con una concentración media de 1,00 ng/mg \pm 0,94 ng/mg de pelo y en todos los segmentos se encontraron sertralina y su metabolito, desmetil-sertralina, con una concentración media de 2,65 \pm 0,94 ng/mg y 1,50 \pm 0,94 ng/mg de pelo, respectivamente. El análisis de pelo resultó negativo para metilfenidato y su metabolito (ácido ritalínico).

La detección en matrices biológicas de fármacos psicoactivos demostró la intoxicación aguda y crónica por quetiapina y sertralina, administradas por la madre.

Palabras clave: Análisis segmentario del pelo; Niños; Antidepresivos; Antipsicóticos; Cromatografía líquida de ultra-alto rendimiento-espectrometría de masas en tándem.

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Given the severity of paediatric mood and anxiety disorders and their psychosocial and functional consequences, clinical practice demands the development of complementary approaches for difficult cases, such as combining antidepressants with other psychotropic drugs (Díaz-Caneja, Espliego, Parellada, Arango and Moreno., 2014). Paediatric psychiatric polypharmacy, defined as the receipt of multiple daily psychiatric medications for the same or for different conditions, has been increasing (Hilt et al., 2014). Antidepressants have become one of the drug classes most frequently prescribed in combination and are commonly co-prescribed with stimulants and antipsychotics in children and adolescent affected by psychiatric disorders (Díaz-Caneja et al., 2014). Concerns about this include a lack of research evidence supporting the effectiveness of most medication combinations, poorly defined side effects from medication combinations (Hilt et al., 2014). Side effects were particularly more common in medication combinations including either selective serotonin reuptake inhibitors (SSRIs) or antipsychotics (Hilt et al., 2014).

Non compliance, but specially or excessive consumption and also criminal forced parental administration, mainly in combination, has been demonstrated in some cases and may lead to potential severe side effects (Binchy, Molyneux and Manning, 1994; Gaillard et al., 2011; Pawłowicz, Wasilewska, Olanski and Stefanowicz, 2013).

The objective assessment of intoxication can be disclosed by drug and metabolites measurement in biological matrices for short-term (blood and urine) or long-term (hair) time window, with hair testing particularly successful in paediatric chronic intoxications (García-Algar et al., 2005; Joya et al., 2009; Joya et al., 2011; Papaseit, García-Algar, Simo, Pichini and Farre, 2013; Pichini et al., 2006; Pichini et al., 2014a; Pichini et al., 2014b; Pichini et al., 2014c).

In addition, segmental hair analysis can disclose a month to month (considering 1 cm segment cuts) eventual repeated chronic exposures and, in some instances, identify patterns of drug use/administration (Pichini et al., 2006; Thieme, Baumer, Sachs and Teske, 2013).

We report a case of a child admitted to the hospital for suspected psychoactive drugs intoxication and found chronically intoxicated by segmental hair testing.

Method

Case report

A 4 years old child was admitted to the paediatric ward of the hospital with lower extremity pain and clumsiness. The day before his admission the family noticed increasing drowsiness and gait alterations. The initial exam on the emergency room he was exhausted, drowsiness, afebrile and presented cough and thick nasal discharge. The child was admitted with the diagnosis of encephalitis versus drug

intoxication (two years ago he had been admitted for an episode of encephalitis that resolved over a three months period). A blood cell count, liver and renal function test including the determination of lactates and ammonia and cerebrospinal fluid analysis were done with normal results a head CT was also performed being normal. Specific gas chromatography mass spectrometric analysis of blood and urine revealed the presence of sertraline and quetiapine, two of the three psychoactive drugs (the other was methylphenidate) taken by the 8 years old brother affected by Asperger syndrome.

Once hospitalized, the child presented a wide range of symptoms such as persistent drowsiness, generalized weakness, slurred speech, urinary incontinence and constipation. During the first four days he experienced miosis, lacrimation and blepharospasm. During four days clinical symptoms changed gradually: no deep reflexes from the second day, an erythematous flush in the upper third of the trunk, head and neck, dystonic movements of the extremities, extrapyramidal signs and generalized tonic seizures with mydriasis scarcely reactive to light that resolved with diazepam. He was then admitted to the intensive care unit (ICU) for further management and monitoring. Few hours after the transfer to the ICU the child experienced a gradual recovery of vigilance and muscle tone, mydriasis resolved, he was able to sit alone, to grasp objects, to move them from hand to hand, and to lift them. An improvement in the language, including the comprehension of simple commands was observed. After 24 hours the child was able to walk.

The severe symptomatology, a previous similar episode, and the two drugs present in blood and urine samples prompted to ask for a segmental hair testing with the high suspicion of chronic non accidental administration by the mother of methylphenidate, sertraline and quetiapine administered also to his treated brother.

Sample collection, preparation and analysis

Child hair, measuring 12 cm, was cut in 6 segments of 2 cm, representing a time window of approximately 2 months per segment for as total of 12 months. Hair samples were analyzed for the presence of methylphenidate, sertraline and quetiapine and their metabolites (ritalinic acid and desmethyl-sertraline) and any other eventual drug of abuse. At the time of analysis, the metabolite of quetiapine: 7-hydroxy-quetiapine standard was not available. Briefly, hair samples (20 mg) were reduced in short cuts and after decontamination with dichloromethane and methanol they were added with 10 µl internal standard (promethazine 2 µg/ml) and treated with 500 µl M3 buffer reagent (Comedical, Trento, Italy) for an hour at 100 °C. Then, the treated samples were cooled at room temperature and 100 µl of the M3 extract was diluted with 900 µl of water before a sample volume of 10 µl was analysed by ultra-high performance liquid chromatography-tandem mass spectrometry. Chromatography was carried out

in reversed phase using a Acquity UPLC HSS C18 column (2.1 mm × 150 mm, 1.8 μm) using a linear gradient elution with two solvents: 0.1% formic acid in acetonitrile (solvent A) and 0.1% formic acid in waters (solvent B). Solvent A was maintained 10% for the first 0.50 min. It was increased to 55% from 0.50 to 4.00 min, held at 55% from 4.0 to 6.00 min, and then decreased back to 10% from 6.00 to 6.10 min and held at 10% from 6.10 to 10.00 min for re-equilibration. The flow rate was kept constant at 0.40 mL/min during the analysis. The separated analytes were detected with a triple quadrupole mass spectrometer operated in multiple reaction monitoring (MRM) mode via positive electrospray ionization (ESI). The applied ESI conditions were the following: capillary voltage

3.0 kV, desolvation temperature 600 °C, source temperature 150 °C, cone gas flow rate 60 L/h, desolvation gas flow rate 1100 L/h and collision gas flow rate 0.13 mL/min. Cone energy voltages, MRM transitions, and collision energy voltages were established for each analyte and the values are listed in Table 1. The method was validated as elsewhere described (Pichini et al., 2014a) and applied with limit of quantification at 0.1 ng/g, and limit of detection at 0.04 ng/g. Linearity ranged from 0.1 to 10 ng/g. Imprecision was lower than 10%, analytical recovery ranged between 70.1% and 95.3% and process efficiency was 80.9%. All analytes under investigation showed no significant ion suppression/enhancement (less than 10% analytical signal suppression due to matrix effect).

Table 1. Ultra-performance liquid chromatography tandem mass spectrometry parameters for the multiple reaction monitoring (MRM) acquisition mode

Analytes	Retention time (min)	MRM transitions					
		Quantification			Confirmation		
		m/z	CV (V)	CE (eV)	m/z	CV (V)	CE (eV)
Ritalinic acid	2.99	220.3 > 84.2	30	16	220.3 > 174.2	30	20
Methylphenidate	3.51	234.3 > 84.1	26	18	234.3 > 56.1	26	32
Quetiapine	4.16	384.2 > 221.2	26	36	384.2 > 253.2	26	20
Desmethyl-sertraline	5.48	292.2 > 159.0	8	28	292.2 > 275.1	8	16
Sertraline	5.68	306.2 > 159.1	14	30	306.2 > 275.1	14	14
Promethazine (IS)	4.84	285.2 > 198.1	22	28			

Results

The results of hair testing on six 2.0 cm length strands collected after child admission to the hospital are shown in Table 2. Methylphenidate and its metabolite, ritalinic acid were absent in all the examined segments while quetiapine was present in the hair segments corresponding to the past eight months and sertraline and its metabolite desmethyl-sertraline were present in all segments representing the past twelve months.

Quetiapine concentration decreases from 2.29 ng/mg in the proximal segment to 0.10 ng/mg to the distal segment.

Sertraline and its metabolite, desmethyl-sertraline, were found in all segments. However, sertraline concentration was quite stable in the distal segments and increasing just in the segments corresponding to the past four months while

metabolites concentration were decreasing from the proximal to the distal strand.

Discussion

Hair testing in the four years child hospitalized with neurological symptoms of possible intoxication revealed chronic intoxication with two psychoactive drugs: quetiapine and sertraline, due to criminal administration by the mother of drugs prescribed to his brother.

Published data on quetiapine and sertraline concentration in hair are scarce. Only one published paper could be found that reported quetiapine concentration in hair of adults treated with dosages between 200 and 1200 mg (Binz, Yegles, Schneider, Neels and Crunelle, 2014). Queti-

Table 2. Psychoactive drugs in hair sample from intoxicated child

Segment (cm)	Methylphenidate (ng/mg)	Ritalinic acid (ng/mg)	Quetiapine (ng/mg)	Sertraline (ng/mg)	Desmethyl-sertraline (ng/mg)
0-2	nd	nd	2.29	4.28	3.79
2-4	nd	nd	1.23	3.28	1.89
4-6	nd	nd	0.40	2.07	0.87
6-8	nd	nd	0.10	2.25	1.66
8-10	nd	nd	nd	1.88	0.41
10-12	nd	nd	nd	2.13	0.39

pine concentrations ranged from 0.35 to 10.21 ng/mg hair with 7-hydroxy-quetiapine concentrations from 0.02 to 3.19 ng/mg hair in two cm or longer hair segments. Individuals showed a trend in quetiapine concentration that matches to our one: a linear decrease from proximal to distal segment. The authors hypothesized the effect of cosmetic hair treatments (eg. shampoos and other products) as a reason for concentration decrease, more plausible than a change in compliance or dosage. This was in accordance with the internationally accepted effect of cosmetic hair treatments and sweat influence in decreasing xenobiotic concentration from proximal to distal hair segments (Jurado, Kintz, Mendez and Repetto, 1997). Similarly, only one paper reported sertraline concentration in human post-mortem hair with sertraline concentrations ranging from 0.6 to 1.6 ng/mg hair with desmethyl-sertraline concentrations ranging from 0.5 to 2.6 ng/mg hair of death person (Wille et al., 2009).

Although data on hair testing for quetiapine and sertraline in children are lacking, from obtained results we could conclude that child was treated repeatedly with the two drugs. The clinical manifestations of antipsychotic drug toxicity generally include varying degrees of central nervous system depression, anticholinergic effects, pupillary changes, seizures, hypotension, and cardiac conduction abnormalities (Cobaugh et al., 2007). In addition, many patients who overdose on selective serotonin reuptake inhibitors are asymptomatic (Sarko, 2000). Symptoms, when they do occur, are usually self-limited and consist of tachycardia, drowsiness, tremor, nausea, agitation, visual hallucinations, diaphoresis, flushing and vomiting (Grenha, Garrido, Brito, Oliveira and Santos, 2013; Myers, Dean and Krenzelo, 1994; Pao and Tipnis, 1997).

In our case report, infant symptoms resulting from ingestion of psychotropic drugs and chronic exposure was established on the basis of the presence of quetiapine and sertraline in hair. The peak in drug concentration occurs in the 0 to 2-cm section of hair for both quetiapine and sertraline, suggesting that these drugs were accidentally or intentionally or forcedly ingested in a non negligible amount before acute intoxication and consequent hospitalization.

The main clinical implications of this case are related to the usefulness of hair analysis to disclose chronic consumption or exposure to drugs of prescription (and also to drugs of abuse) related to overdose, combination or polypharmacy.

Conclusion

Hair testing is complimentary to blood and urine testing in disclosing suspected chronic intoxication to toxic xenobiotics in presence and/or absence of acute one. Furthermore, segmental hair analysis can provide information as to whether the substance was taken regularly before the alleged incident or if the substance had been ingested only in

a short timeframe that corresponded to the moment of the incident.

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Conflicts of interests

The authors declare that there are no conflicts of interests.

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