

Short communication

Hair analysis to monitor abuse of analgesic combinations containing butalbital and propyphenazone

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Abstract

Butalbital, a barbiturate, is present in analgesic combinations used by headache sufferers. Overuse/abuse of these combinations may cause dependence, chronic migraine, and medication-overuse headache (MOH). MOH is difficult to manage: it improves interrupting analgesic overuse, but requires monitoring, because relapses are frequent. A gas chromatography-mass spectrometry (GC-MS) method for hair analysis has been developed and validated to document abuse of an analgesic combination containing butalbital and propyphenazone by a patient with MOH. For over ten years the patient managed her headache using eight suppositories/day of an analgesic combination containing butalbital 150 mg, caffeine 75 mg, and propyphenazone 375 mg per suppository. An outpatient detoxification treatment was carried out. After three weeks, the patient reduced the consumption to one suppository/day. At the first control visit, after three months from the beginning of detoxification, the patient increased the use of the combination to four suppositories/day and at the second control visit, after seven months from the beginning of detoxification, she was back to eight suppositories/day. At the two control visits, a hair sample was taken for determination of butalbital and propyphenazone. Moreover blood and urine samples for determination of butalbital were drawn at the beginning of detoxification treatment and at the two control visits. With the segmental analysis of two hair samples the medication history of ten months could be estimated. In the first hair sample, collected at the first control visit, in the distal segment, butalbital and propyphenazone concentrations were ~~respectively~~, respectively, 17.5 ng/mg and 56.0 ng/mg, confirming the prolonged abuse; in the proximal segment, concurrently with the detoxification treatment, butalbital and propyphenazone concentrations had reduced respectively to 5.45 ng/mg and 11.1 ng/mg. The second hair sample, collected at the second control visit, proved the fair course of the detoxification treatment in the distal segment and signalled relapse in the abuse of the analgesic combination in the proximal segment. In the clinical context, hair analysis can be advantageously used to monitor the abuse of analgesic combinations with butalbital, common among headache patients. The validation data showed that GC-MS method developed for determination of butalbital and propyphenazone was rapid, highly sensitive, specific and selective.

Abbreviations: MOH, medication-overuse headache; GC-MS, gas chromatography-mass spectrometry; CNS, central nervous system; GABA, gamma-aminobutyric acid; GGT, gamma-glutamyl transpeptidase; IS, internal standard

Keywords: Butalbital; Propyphenazone; Hair analysis; GC-MS; Drug monitoring; Medication-Overuse Headache

1 Introduction

Barbiturates are central nervous system (CNS) depressants that act by enhancing the action of gamma-aminobutyric acid (GABA), the major inhibitory neurotransmitter. Over time, the use of barbiturates in medicine has been reduced because of their narrow therapeutic index and high potential for abuse [1]. The use of analgesic combinations containing butalbital, a short to intermediate-acting barbiturate, along with caffeine and analgesics (e.g., codeine, aspirin or acetaminophen or propyphenazone) is instead still common for acute treatment of primary headaches [2]. Butalbital-containing analgesic combinations are the most frequently prescribed therapy for migraine, used by 36% of subjects taking prescription medicines for their headaches [3]. Migraine patients who suffer from recurrent attacks are a population at risk of abuse and dependence on these combinations, which, in addition, have the highest probability, together with opioid analgesics, to cause chronic migraine and medication-overuse headache (MOH) [4]. MOH is a chronic headache which is precisely due to analgesics overuse and improves only by analgesic medications withdrawal [5].

In clinical setting, to monitor drug addicts' detoxification treatments, given the limitations of self-reports on drug use, toxicological analysis plays a fundamental role. Short-term monitoring is supported by toxicological analysis of urine samples. In recent years, hair matrix has been shown to be a fundamental biological specimen for long-term drug monitoring since hair analysis can provide information on therapy compliance and drug use over a period of weeks-to-months before sample collection [6].

Here, we presented the use of hair analysis to monitor detoxification treatment and document a ten months' history of abuse of an analgesic combination containing butalbital, caffeine, and propyphenazone by a patient suffering from chronic migraine.

2 Materials and methods

2.1 Case history

The patient was a Caucasian woman aged 74 years who began to suffer from migraine with puberty. Since she was a girl, the patient had begun to manage her migraine attack with an analgesic combination containing butalbital 150 mg, caffeine 75 mg, and propyphenazone 375 mg by rectal route, and continued to use it, more and more frequently, with the worsening of migraine. When she came to the headache centre, for over ten years the patient was using eight suppositories per day of this combination, in total: butalbital 1200 mg/day, caffeine 600 mg/day, and propyphenazone 3000 mg/day. Chronic migraine complicated by medication-overuse headache according to the International Headache classification, ICHD-3 beta [4] was diagnosed, and hospital admission was offered to discontinue analgesic abuse and treat headache. However, the patient refused hospitalization and an outpatient treatment was therefore started.

To prevent withdrawal symptoms and treat headache, during analgesic abuse discontinuation, delorazepam at decreasing dosages was prescribed. After the first week, the patient had reduced the use of the analgesic combination to two suppositories per day. Seven days later, the patient had further reduced the use of the analgesic combination to one suppository per day. At the end of the third week, she was very pleased with the way her migraine was controlled by only one suppository of the analgesic combination and wanted to stop the treatment.

After two months, at the first control visit, the patient reported that she had re-increased the use of the analgesic combination to four suppositories per day. Nevertheless, she continued to refuse hospital admission. To the second control visit, after four months, the patient had returned to use every day eight suppositories of the analgesic combination with butalbital. Only over the last five days she had reduced the use of the analgesic combination to four suppositories per day.

Written informed consent was obtained from the patient for publication of this case report.

2.2 Samples collection and preparation

At the first control visit (after 3 months from the beginning of detoxification treatment), a first hair sample of 6 cm in length was collected and at the second control visit (after 7 months from the beginning of detoxification treatment) a second hair sample of 4 cm in length was taken. The samples were taken to the posterior vertex. Each hair sample was analysed divided into two segments (proximal and distal) for the determination of butalbital and propyphenazone.

At the beginning of detoxification and at the two control visits, also blood and urine samples for the determination of butalbital were drawn.

Both proximal and distal head hair segments were decontaminated by washing twice with dichloromethane and pulverised by means of a bead mill homogenizer Precellys 24 (Bertin Technologies, Montigny-Le-Bretonneux, France) by one cycles of 30 s at 6000 rpm. and 50 mg aliquots were stirred overnight at 45 °C, after the addition of 2 ml of methanol RPE (Carlo Erba, Milan, Italy), and 50 µl of a 50 ng/µl methanol solution of internal standard (IS) Barbitol (Lipomed AG, Arlesheim, Switzerland). After incubation, supernatants were purified by using of Phree-TM™ 124 Phospholipid Removal (Tabbed 1 ml Tube, Phenomenex™, Torrance, CA, USA) in accordance with in-house method. Finally, the filtrate was evaporated to dryness under nitrogen stream and the residue reconstituted in 100 µl of methanol. Aliquots were analyzed by gas chromatography mass spectrometry.

2.3 Instrumentation and analytical conditions

All measurements were performed by gas chromatography-mass spectrometry on a Saturn 2200 Ion trap quadrupole (Varian, Walnut Creek, CA, USA) equipped with a DB-5MS capillary column (30 m × 0.25 mm i.d., 0.25-µm film thickness, Agilent,

Santa Clara, CA, USA). Helium (pure 99,9990%, Sapio, Monza, Milan) was used as the carrier gas with a flow rate of 1 ml/min.

One microliter of the methanol hair/urine/blood extract was injected for full scan electron impact mass spectrometry analysis of butalbital and propyphenazone, elaborated in SMS format. The capillary inlet system was operated in splitless mode. Instrumental conditions were as follows: injection port, 250 °C; gas chromatography temperature program, 100 °C for 2 minutes, ramp 10 °C/minute, ramp 10 °C/min to 290 °C, hold 10 minutes; transfer line, 290 °C; source, 250 °C; manifold, 40 °C. The ions transitions (m/z) were as follows: 168, 181, 225, 124 for butalbital, 215, 230 for propyphenazone and 156 for barbital (IS).

2.4 Calibration and validation parameters

The following validation parameters were evaluated for the gas chromatography mass spectrometry analysis: specificity, linearity, limit of quantification, limit of detection, bias and precision according to literature suggestions [7].

Samples for the calibration curves and quality control samples were prepared by adding appropriate volumes of working standard solutions mixture (Butalbital standard obtained from Lipomed AG, Arlesheim, Switzerland, and Propyphenazone standard obtained from Sigma-Aldrich, St. Louis, MO) to 50 mg drug-free hair. To evaluate specificity, ten different hair samples were collected by drug free volunteers and were analysed to check for peaks that might interfere with the detection of the analytes. Specificity was found to be satisfactory as the chromatograms were free of coeluting peaks: the signal-to-noise (S/N) ratio for interfering peaks was verified to be less than 10. Calibration curves were constructed by plotting the peak area ratios of the selected ion species (for the analytes and IS) versus analyte concentration, in the concentration range 1-100 ng/mg for hair. A linear response over the whole ranges of concentrations with a coefficient of determination (r2) greater than 0.990 both for butalbital and propyphenazone was observed, respectively, 0993 and 0996.

Bias and precision were calculated by analysing three separate spiked samples of hair at three different concentration pools (low concentration at 1 ng/mg; medium concentration at 50 ng/mg; high concentration at 100 ng/mg). The bias has been calculated for each concentration using the following formula: $[(\text{grand mean of calculated concentration}_x - \text{nominal concentration}_x) / \text{nominal concentration}_x] \times 100$ [7]. At each concentration for both analytes, the bias showed satisfactory results ($\pm 20\%$). Regard to precision studies, within run precision and between run precision coefficient of variation values were determined by analysing 5 replicates at three concentration (low, medium and high) and were found to be less than 20%. Detection limits of the employed method were 0.3 ng/mg for butalbital and propyphenazone, respectively. Quantitation limits were 1 ng/mg for both analytes.

3 Results and discussion

In clinical practice, hair analysis was helpful to monitor objectively the use of analgesic combinations containing butalbital and propyphenazone to document their abuse and the course of the detoxification treatment. In the patient described, by means of the segmental analysis of only two samples of hair taken at the control visits (Table 1), we could estimate the history of the medications taken during about ten months: three previous, one contemporary, and six after detoxification treatment. In the first hair sample collected at the first control visit (after 3 months from the beginning of detoxification treatment), in the distal segment indicating previous use, there were high concentrations of butalbital and propyphenazone, confirming the intense and prolonged abuse of the analgesic combination in the months prior to detoxification. In the proximal segment (comprising the period of detoxification), concurrently with the reduction in the analgesic combination abuse from eight to one suppository per day, the concentrations of butalbital had been reduced to about a third and those of propyphenazone to a fifth of those detected for the two drugs in the distal segment. In the second hair sample collected at the second control visit (after 7 months from the beginning of detoxification treatment), segmental analysis confirmed the positive course of the detoxification treatment and the immediately subsequent period, showing in the distal segment (previous use) concentrations of butalbital and propyphenazone similar to those detected in the proximal segment of the first hair sample. However, the concentrations of butalbital and propyphenazone found in the proximal segment (recent use) were re-increased to the levels prior to detoxification, signalling relapse in the abuse of the analgesic combination in the last three months, as reported by the patient.

Table 1 Results of determinations of butalbital and propyphenazone in head hair samples by GC–MS carried out in a patient with medication-overuse headache. The patient abused an analgesic combination containing caffeine 75 mg, butalbital 150 mg, and propyphenazone 375 mg per suppository. The first hair sample was collected at the first control visit, after 3 months from detoxification; the second hair sample was collected at the second control visit after 7 months from detoxification. Results of butalbital determination in blood and urine samples, collected at the beginning of detoxification and at the two control visits, were presented.

10 Months time Butalbital 150 mg- propyphenazone 375 mg per suppository First hair sample: 6 cm GC-MS Second hair sample: 4 cm GC-MS Blood-Urine GC-months time		Butalbital 150 mg, propyphenazone 375 mg per suppository				First hair sample: 6 cm GC-MS				Second hair sample: 4 cm GC-MS		Blood urine GC-MS	
Month	N. Sup/day	Butalbital ng/mg	Propyphenazone ng/mg	Butalbital mcg/ml (ng/mg)	Propyphenazone (ng/mg)	Butalbital (ng/mg)	Propyphenazone (ng/mg)	Butalbital (mcg/ml)	Propyphenazone (mcg/ml)	Butalbital (mcg/ml)	Propyphenazone (mcg/ml)	Butalbital (mcg/ml)	Propyphenazone (mcg/ml)
-3	8	Distal segment (past use: 3 months prior to detoxification)											
-2	8												

-1	8	17.5	56.0				
+1	Detoxification From 8 to 1	Proximal segment (recent use: 3 months from detoxification to the first hair sample collection)		Distal segment (past use: 2 months from detoxification onwards)		10.02	7.13
-215.45 11.16 26 11.2 +2	<u>1</u>	<u>5.45</u>	<u>11.1</u>	<u>6.26</u>	<u>11.2</u>		
+3	First control visit 4	<i>First hair sample collection</i>		Proximal segment (recent use: 2 months prior to the second hair sample collection)		7.55	5.3
-4620.65 1.5 +58 +68 +4	<u>6</u>			<u>20.6</u>	<u>51.5</u>		
+5	<u>8</u>						
+6	<u>8</u>						
+7	Second control visit_4 During the five days before blood and urine sampling			<i>Second hair sample collection</i>		4.46	3.34

Hair analysis provides the context to interpret the concentrations of butalbital in blood and urine. In fact, at the second control visit (Table 1), butalbital concentrations in blood and urine (respectively, 4.46 mcg/ml and 3.34 mcg/ml) were lower than those detected before (10.02 mcg/ml and 7.13 mcg/ml) and two months after detoxification treatment (7.55 mcg/ml and 5.30 mcg/ml). These findings reflected the most recent decrease in the analgesic combination consumption to four suppositories per day, over the last five days. Relying only on these butalbital concentrations in blood and urine, it would not have been possible to detect the important relapse into abuse of the analgesic combination in the previous months. In the segment comprising the detoxification period, the concentrations of propyphenazone in the keratin matrix had decreased in both hair samples more than those of butalbital, in harmony with a significantly shorter ~~-2 hours~~(1–2 h) elimination half-life of this drug [8] than that of butalbital (about 61 hours) [2]. Notably, the incorporation of the two drugs in the keratin matrix was proportional to the doses taken. In fact, the ratios between the concentrations of butalbital and propyphenazone measured in the segments of hair matching with the abuse of eight suppositories per day (the distal segment of the first hair sample and the proximal segment of the second hair sample) were ~~respectively~~, respectively, 0.32 and 0.39, therefore close to the 0.4 ratio between the doses of butalbital and propyphenazone present in one suppository of the analgesic combination.

There is limited information on the disposition of barbiturates in biological matrices other than blood and urine and in literature we have not found other determinations of butalbital and propyphenazone in the hair. For this reason, we cannot make direct comparisons. However, it is possible to tentatively relate the concentrations of butalbital to those of phenobarbital measured in the hair of two subjects taking toxic doses. These subjects had in their hair 16.2 and 14.7 ng/mg of phenobarbital. Moreover, two volunteers treated with phenobarbital 100 mg/day for five days had concentrations of 3.3 and 0.1 ng/mg [9]. Also in patients in antiepileptic treatment the concentrations of phenobarbital in the hair increased with increasing dosages [10]. Considering that phenobarbital is more potent and has longer duration of action than butalbital, our patient had very high concentrations, indicating, in agreement with her clinical history, a prolonged use of doses much higher than the maximum recommended, that is, 600 mg/day and 700 mg/day ~~respectively~~, respectively, for butalbital [2] and propyphenazone [8].

The toxic effects of butalbital are reported with blood concentrations ~~>~~8 mcg/ml [11]. In our patient, blood concentrations of butalbital were 10.2 mcg/ml prior to detoxification. In the two blood samples collected at the control visits, butalbital concentrations had reduced, but remained superior to 2.1 mcg/ml, the peak level reported after a therapeutic oral dose of 100 mg of butalbital [12]. It has recently been recommended not to prescribe butalbital-containing analgesic combinations to migraine patients as first choice [13]. They should only be prescribed in exceptional cases and the use should be monitored to prevent complications [2,13].

4 Conclusion

Hair analysis may be useful adjunct to conventional (urine, blood) drug testing in toxicology. Specimens can be more easily obtained with less embarrassment, and hair can provide a more accurate history of drug use, abuse or misuse [6]. The keratin matrix is increasingly used to monitor adherence to pharmacological treatments and to document objectively the progress of detoxification and rehabilitation programs of drug addicts [14]. Butalbital is probably the only barbiturate which is abused today. Some analytical methods have been developed for the quantification of butalbital in different matrices such as serum, oral fluid and urine [15–17]. However, to the best of our knowledge, there are no other report of a method for the simultaneous quantification of butalbital and propyphenazone in non-conventional matrix as head hair.

Conflict of interest

The authors declare that they do not have any financial or other relationships that might lead to a conflict of interest.

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Graphical abstract

10 Months time	Butalbital 150 mg, propyphenazone 375 mg per suppository	First hair sample: 6 cm GC-MS	Second hair sample: 4 cm GC-MS		
Month	N, sup/day	Butalbital ng/mg	Propyphenazone ng/mg	Butalbital ng/mg	Propyphenazone ng/mg
-3	8	Distal segment (past use: 3 months prior to detoxification)			
-2	8	Distal segment (past use: 3 months prior to detoxification)			
-1	8	17.5	56.0	Distal segment (past use: 3 months prior to detoxification)	
+1	Detoxification From 8 to 1	Proximal segment (recent use: 3 months from detoxification to the first hair sample collection)		Distal segment (past use: 2 months from detoxification onwards)	
+2	1	5.45	11.1	6.26	11.2
+3	First control visit 4	First hair sample collection		Proximal segment (recent use: 2 months prior to the second hair sample collection)	
+4	6			20.6	51.5
+5	8				
+6	8				
+7	Second control visit 4			Second hair sample collection	

Highlights

- Butalbital is present in analgesic combinations used by headache sufferers.
- Butalbital is probably the only barbiturate which is abused today.
- Developing a GC-MS method for butalbital and propyphenazone determination in hair.
- Monitoring abuse of analgesic combinations with butalbital by hair analysis.

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