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In: W. Schänzer, H. Geyer, A. Gotzmann, U. Mareck-Engelke (eds.) Recent advances in
doping analysis (6). Sport und Buch Strauß, Köln, (1999) 469-474
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ANALYSES OF URINE SAMPLES OF THE FIRST BULGARIAN "METHADONE BABY"

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Introduction

The first Bulgarian baby from parents participating in Methadone maintenance programme (MMP) was born in 1997 year. The quantity determination of Methadone and metabolites were carried out in urine samples of baby and mother collected right after childbirth and during the first 6 day.

The participation of Doping Laboratory in MMP has the consequent aspects:

- Social and public significance - integration of efforts of government and social organisation to solution of this acute public problem - narcomania;
- Practical and research - taken part in observation of a complicated medical incidence; opportunity of investigation and improvement of quantity determination methods.

Experimental

A) Samples collection - The urine samples were taken following the Time Schedule

<table>
<thead>
<tr>
<th>Date and Time of collection</th>
<th>Conventional signs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>I. Baby - born 28.10.1997, 9.45 am</strong></td>
<td></td>
</tr>
<tr>
<td>1. day urine of 28.10.97</td>
<td>B 1</td>
</tr>
<tr>
<td>2. night urine of 28/29 10.97</td>
<td>B 2</td>
</tr>
<tr>
<td>3. 30.10.1997 - from 15(^ {30}) to 18(^ {00})</td>
<td>B 3</td>
</tr>
<tr>
<td>4. 30.10.1997 - from 18(^ {00}) to 24(^ {00})</td>
<td>B 4</td>
</tr>
<tr>
<td>5. 02.11.1997 - from 12(^ {00}) to 14(^ {00})</td>
<td>B 5</td>
</tr>
<tr>
<td>6. 02.11.1997 - from 14(^ {00}) to 15(^ {15})</td>
<td>B 6</td>
</tr>
<tr>
<td>Date and Time of collection</td>
<td>Conventional signs</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>------------------</td>
</tr>
<tr>
<td>7.02.1997 - from 15.15 to 21.00</td>
<td>B 7</td>
</tr>
<tr>
<td>8.02.1997 - from 21.00 to 06.00 03.11.1997</td>
<td>B 8</td>
</tr>
<tr>
<td>9.03.1997 - from 06.00 to 12.00</td>
<td>B 9</td>
</tr>
</tbody>
</table>

**II. Mother**

<table>
<thead>
<tr>
<th>Date and Time of collection</th>
<th>Conventional signs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 28.10.1997 - from 10.00 to 13.00</td>
<td>M 1</td>
</tr>
<tr>
<td>2. 28.10.1997- from 22.00 to 06.00</td>
<td>M 2</td>
</tr>
</tbody>
</table>

**B) Isolation and derivatisation of Methadone and Metabolites** - The procedure II of conjugated stimulants and narcotics is used for isolation of these substances (hydrolysis - Helix Pomatia, extraction with diethyl ether at pH=9.6). Selective derivatisation with N-methyl-N-trimethylsilyl-trifluoroacetamide (MSTFA) and N-methyl-bis(trifluoroacetamide) - (MBTFA).

**C) GC/MS quantitation** - Quantitation of Methadone and Metabolites were performed with GC/MS system (Hewlett Packard GC 5890/ MSD 5970) with electron impact set at 70 eV, column - (HP-5, 16.5 m, ID 0.25 mm, film thickness 0.25 μm). The carrier gas - helium 1 ml/min, split 1:10. Temperature programme was as follows:

- initial temperature 140 °C
- program rate 20 °C/min
- final temperature 310 °C
- temperature of injector 280 °C
- transfer line temperature 280 °C

Selected ion monitoring (SIM) was used with the following ions:

1. m/z 72, 91, 165, 178, 223, 294 for Methadone - underivatized M⁺ = 309
2. m/z 72, 115, 296, 381 for Methadone (O-TMS) M⁺ = 381
3. m/z 77, 91, 165, 276, 304, 373 for the main Metabolite I⁺(N-TFA) M⁺ = 373
4. m/z 278, 367, 382 for Metabolite II⁺ (O-TMS) M⁺ = 382
5. m/z 277, 350, 365 for Metabolite III⁺ (O-TMS) M⁺ = 365

* - See Fig.1

The concentration of Methadone and metabolites in urine samples were calculated by peak areas of the detected signals relative to the internal standard - Propoxyphene - m/z 58 that has a very similar chemical structure as Methadone. The calibration of the GC/MS instrument was
made in the range from 30 ng to 5 μg per ml urine using a working solution of Methadone in methanol with concentration 30μg/ml.

**Results**

It is known that about 10 % of the administrated Methadone are excreted as unchanged [1-4]. The detected metabolites of Methadone in the analysed urine samples are presented on Fig 1.

Fig.1 Metabolites of Methadone detected in the urine samples
The unchanged Methadone and Metabolite I (the main) were detected in the urine samples of the baby, but in mother's urine samples were found all of metabolites showed on Fig. 1.

![Graph of Methadone Concentration](image1)

**Fig. 2** Concentration of Methadone in analysed baby's urine samples with accordance in Time schedule.

![Graph of Metabolite I Concentration](image2)

**Fig. 3** Concentration of Metabolite I in baby's urine samples.

The second day after delivery was a critical day for baby. An initial withdrawal symptom was observed [4]. She did not feed with the mother's milk after the childbirth. The "level of methadone" of baby was decreased up to 37 ng/ml (Fig. 2) and the doctor's team was applied a treatment with Phenobarbital. The feed of the infant with mother's milk was begun at 4-th day after delivery with doses of 5 ml milk, 8 times per day. The concentration of Methadone in child urines (Samples from B5 to B9) was increased. During next weeks the mother's milk was exchanged with artificial little by little.
Table 1
Concentration of Methadone and Metabolite I in the urine samples of mother

<table>
<thead>
<tr>
<th>Samples</th>
<th>Methadone, ng/ml</th>
<th>Metabolite I ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>M 1</td>
<td>1030</td>
<td>1200</td>
</tr>
<tr>
<td>M 2</td>
<td>3728</td>
<td>2635</td>
</tr>
</tbody>
</table>

During the methadone treatment (or any long-term opiate use) the activity of the neurones that respond to noradrenaline is reduced. If the opiates are removed there is apparently a surge of activity in the neurones resulting in a rise in the level of noradrenaline [5]. The 3-methoxy-4-hydroxyphenylglycol (MOPEG) was detected in urine samples of baby. It is one of the urinary excreted metabolites of noradrenaline. The quantity estimation was made and results are shown of Fig. 4.

![Graph showing concentration of MOPEG in baby's samples]

**Fig.4 Concentration of MOPEG in baby's samples**

Difficulty in the pass of urine for baby is observed up to 48-th hour of the birth, for this reason treatment with Furosemide was applied. The sample from B 1 to B 4 were analysed by procedure of diuretics and quantification of furosemide was made. On Fig.5 can be seen the changes of it concentration.
Fig. 5 Concentration of Furosemide, ng/ml in baby urine samples

Acknowledgements

The authors acknowledge Dr. Nicolov (National centre for addictions - Methadone maintenance programme) and Dr. A. Conakchiev, Dr. Dobreva, Dr. Racheva (Hospital "Sheinovo") for providing urine samples from mother and child.

References