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RECENT ADVANCES
IN DOPING ANALYSIS
(6)

W. Schänzer
H. Geyer
A. Gotzmann
U. Mareck-Engelke
(Editors)

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N. HALATCHEVA, A. TSOUTSOULOVA-DRAGANOVA, D. CAROVA:
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Dr. N.Halatcheva, Dr.A.Tsoutsoulova-Draganova, D.Carova

ANALYSES OF URINE SAMPLES OF THE FIRST BULGARIAN "METHADONE BABY"

Bulgarian Doping Laboratory, Sofia, N.Gabrovsky str. 1

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**

| <i>Date and Time of collection</i> | <i>Conventional signs</i> |
|---|---------------------------|
| <u>I. Baby - born 28.10.1997, 9.⁴⁵ am</u> | |
| 1. day urine of 28.10.97 | B 1 |
| 2. night urine of 28/29 10.97 | B 2 |
| 3. 30.10.1997- from 15 ³⁰ to 18 ⁰⁰ | B 3 |
| 4. 30.10.1997 - from 18 ⁰⁰ to 24 ⁰⁰ | B 4 |
| 5. 02.11.1997 - from 12 ⁰⁰ to 14 ⁰⁰ | B 5 |
| 6. 02.11.1997 - from 14 ⁰⁰ to 15 ¹⁵ | B 6 |

| <i>Date and Time of collection</i> | <i>Conventional signs</i> |
|--|---------------------------|
| 7. 02.11.1997 - from 15 ¹⁵ to 21 ⁰⁰ | B 7 |
| 8. 02.11.1997 - from 21 ⁰⁰ to 06 ⁰⁰ 03.11.1997 | B 8 |
| 9. 03.11.1997 - from 06 ⁰⁰ to 12 ⁰⁰ | B 9 |
| <u>II. Mother</u> | |
| 1. 28.10.1997 - from 10 ⁰⁰ to 13 ⁰⁰ | M 1 |
| 2. 28.10.1997- from 22 ⁰⁰ to 06 ⁰⁰ | M 2 |

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

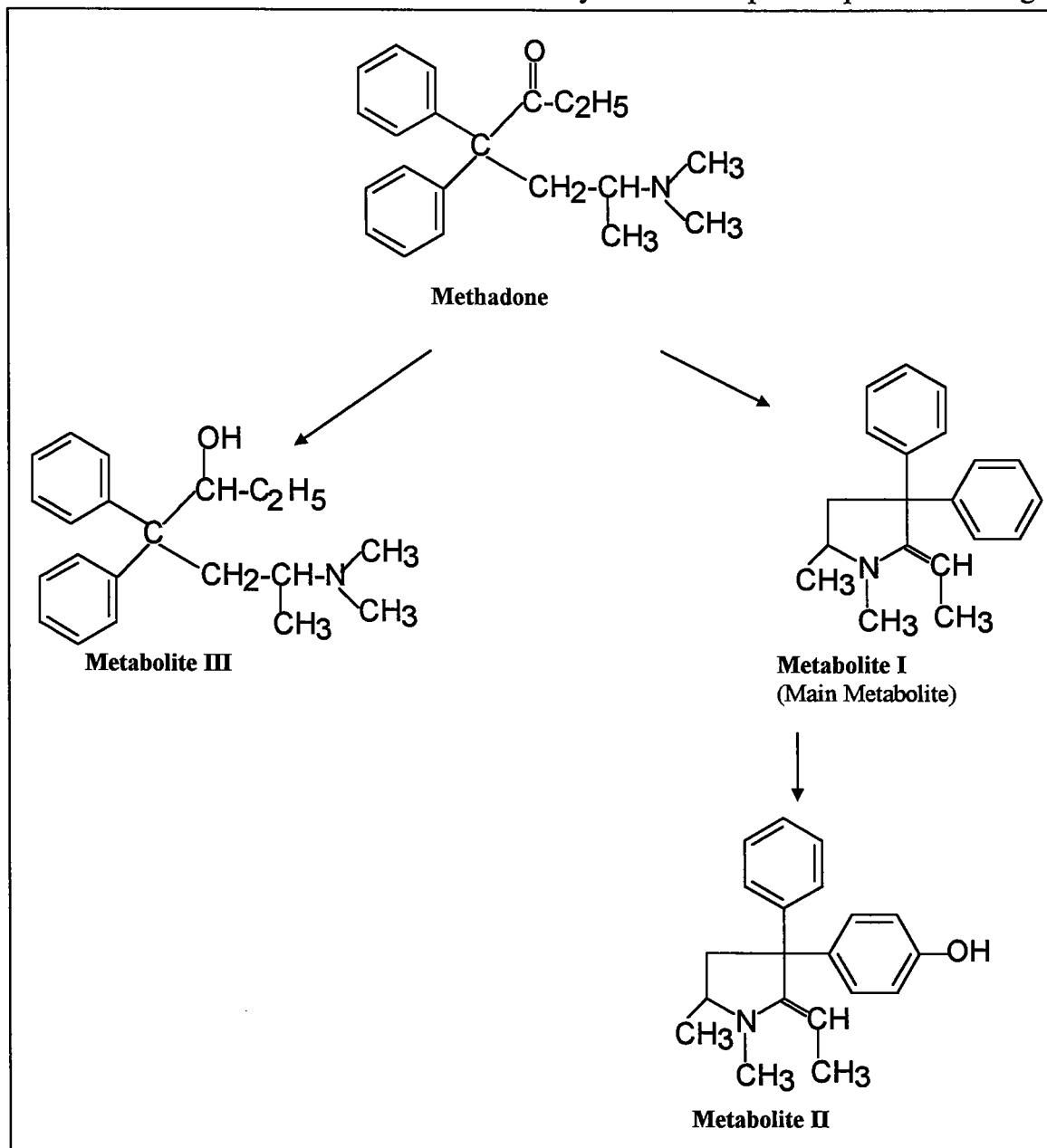


Fig.1 Metabolites of Methadone detected in the urine samples

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.

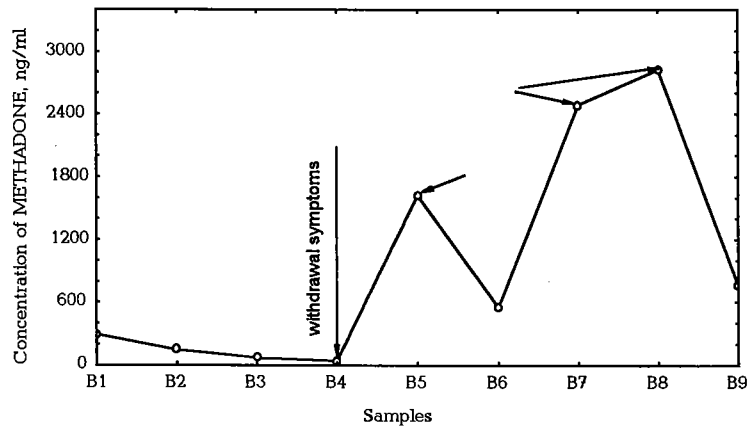


Fig.2 Concentration of Methadone in analysed baby's urine samples with accordance in Time schedule.

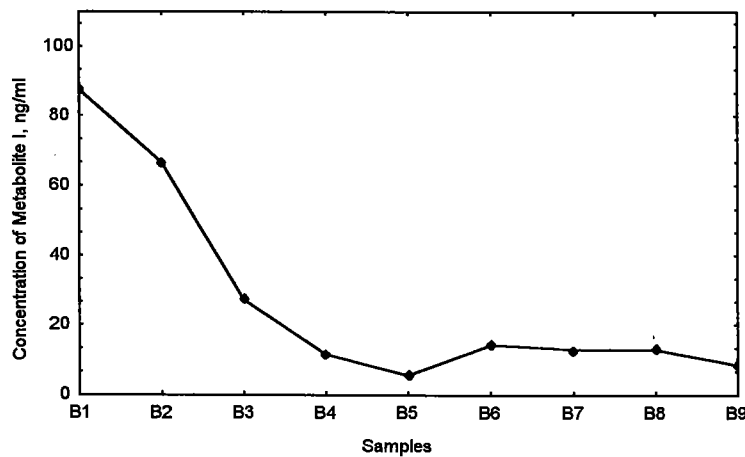


Fig.3 Concentration of Metabolite I in baby's urine samples.

The second day after delivery was a critical day for baby. An initial withdrawal symptom was observed [4]. She did not food 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

| Samples | Methadone, ng/ml | Metabolite I ng/ml |
|---------|------------------|--------------------|
| M 1 | 1030 | 1200 |
| M 2 | 3728 | 2635 |

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.

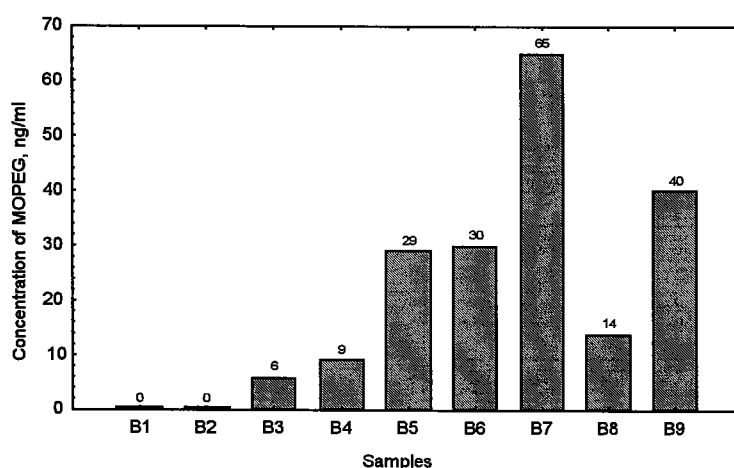


Fig.4 Concentration of MOPEG in baby's samples

Difficulty in the pass of urine for baby is observed upto 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.

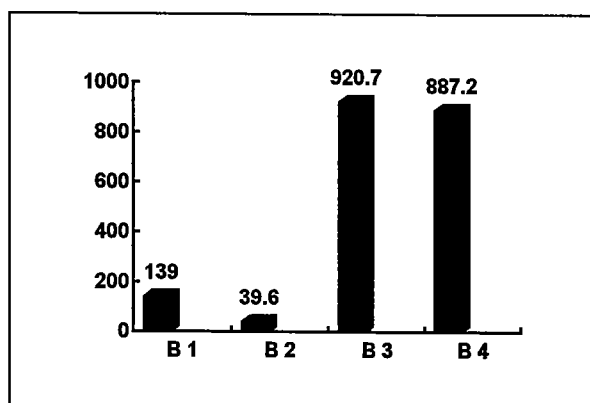


Fig.5 Concentration of Furosemide, ng/ml in baby urine samples

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