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Clomiphene - targeting of the unchanged drug results in unusual prolonged detection windows in urine

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Abstract

The prohibited selective estrogen receptor modulator (SERM) clomiphene is frequently detected in sports drug testing. Here, an increasing number of positive findings at low urinary concentration (<1 ng/mL) was observed, demonstrating exclusively the presence of clomiphene parent compound, while other metabolites were not detected. To investigate the urinary detection window for clomiphene as target analyte 8 urine specimens of female patients, therapeutically treated with clomiphene were analyzed. The volunteers provided urine samples after a period of 3-11 month after administration. Unchanged clomiphene was detectable up to 11 month and hydroxy-clomiphene up to 5 month after administration.

Introduction

At the Cologne doping control lab the prohibited selective estrogen receptor modulator (SERM) clomiphene was frequently detected. Here, a conspicuous high number of clomiphene findings at low urinary concentrations (< 1 ng/mL) was observed, demonstrating exclusively the presence of clomiphene parent compound, while other metabolites were not detected. Comparable findings have already been reported by Ahi *et al*., showing only the presence of the parent drug clomiphene [1]. Furthermore, recent studies have demonstrated long urinary detection windows of more than 3 months for clomiphene and its hydroxy-metabolites [2]. To collect more data on urinary detection windows of clomiphene parent compound urine specimens of female patients (n=8), therapeutically treated with clomiphene were investigated. Samples of female patients were provided after a period of 3-11 months after administration.

Experimental

Sample preparation

Briefly, after enzymatic hydrolysis (1h, 50°C, pH=7.0) with β-glucuronidase from E. Coli analytes were extracted with TBME at a pH of 9.6. The separated organic layer was evaporated and the dry residue was reconstituted in 60 µL of a mixture of 5 mM ammonium acetate buffer containing 0.1 % glacial acid and methanol (1:1, v:v).

Post-administration urine specimens

Eight female volunteers that have been treated with clomiphene provided urine samples 3-11 months after the last administration. An informed consent of all volunteers was received.

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patient	sex	age (y)	height (cm)	weight (kg)	dosis (mg)	urine sampling (months after administration)
1	female	27	165	50	5 days x 50 (4 times)	11
2	female	30	160	63	2 days x 50/3 days x 25	8
3	female	28	157	71	5 days x 50	3
4	female	28	156	46	5 days x 25	3
5	female	35	161	50	5 days x 50 (3 times)	5
6	female	39	164	99	5 days x 50	6
7	female	37	160	51	5 days x 50	3
8	female	27	174	127	5 days x 50	6

Table 1: Data on the investigated specimens

LC-MS/MS

The LC was equipped with an Agilent (Darmstadt, Germany) Zorbax SB-C8 column (150 x 4.6 mm; 3.5 µm). A reverse phase gradient was employed using eluent A: ammonium acetate buffer (5 mmol/L, 0.1 % glacial acid; pH 3.5) and B: acetonitrile starting at 1 % B and changing to 100 % B within 12 min. After isocratic elution at 100 % B for 1 min the system was re-equlibrated for 2.5 min. The flow rate was set to 0.8 mL/min.

For tandem mass spectrometry a 6500 QTrap hybrid triple quadrupole/linear ion trap mass spectrometer was used controlled by Analyst Software 1.6.1 (Sciex, Darmstadt, Germany). Electrospray positive ionization was used with ionspray voltage +5500 V and an ion source temperature of 450°C, nitrogen was used as collision gas (3.0 x 10^{-3} Pa) delivered from a nitrogen generator (CMC Instruments, Eschborn, Germany). In multiple reaction monitoring (MRM) mode diagnostic ion transitions of clomiphene (*m/z* 406-100/72/58) and its metabolites hydroxy-clomiphene (*m/z* 422-100/72/58) and hydroxy-methoxyclomiphene (*m/z* 438-72) were recorded.

Results and Discussion

Doping control samples

Between 2015 and 2018 the Cologne doping control lab has reported 35 adverse analytical findings (AAFs) for clomiphene (Fig. 1).

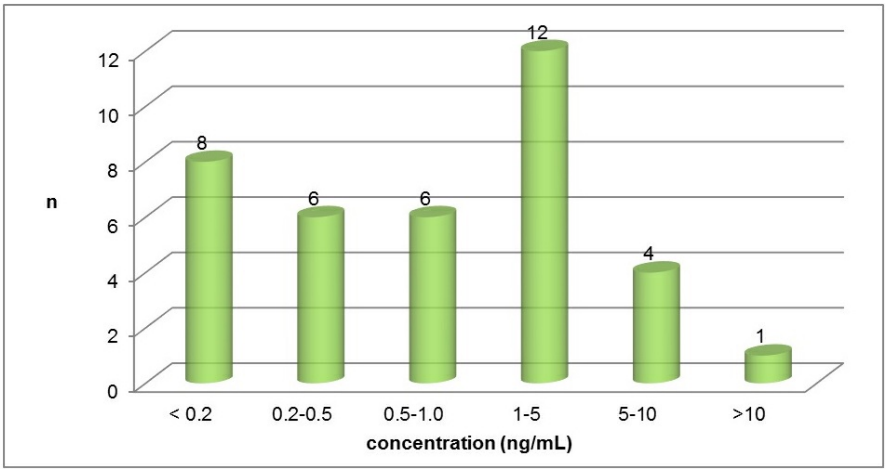

Figure 1: Concentration levels of clomiphene (cis-isomer) in 35 positive clomiphene samples (2015-2018)

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The majority of the AAFs were reported for doping control specimens belonging to combat and strength sport (74%) and for samples from male athletes (86%). The estimated concentrations of clomiphene (cis-isomer) in 57% (n=20) of 35 positive samples were less than 1 ng/mL. In 18 samples (90%) neither hydroxy-clomiphene nor hydroxy-methoxyclomiphene was identified.

Post-administration urine specimens

Clomiphene (cis-isomer) was identified in 7 out of 8 investigated clomiphene post-administration samples at concentration levels ranging from 5 to 100 pg/mL. Even after 11 months, clomiphene was detectable in the urine specimen of patient 1. Patient 1 received the highest absolute dose of clomiphene (4 cycles, 250 mg each). The metabolites hydroxy-clomiphene and hydroxy-methoxyclomiphene were only found at trace amounts in samples of patient 3-5 and 7. After 5 months post-administration no further metabolites could be identified.

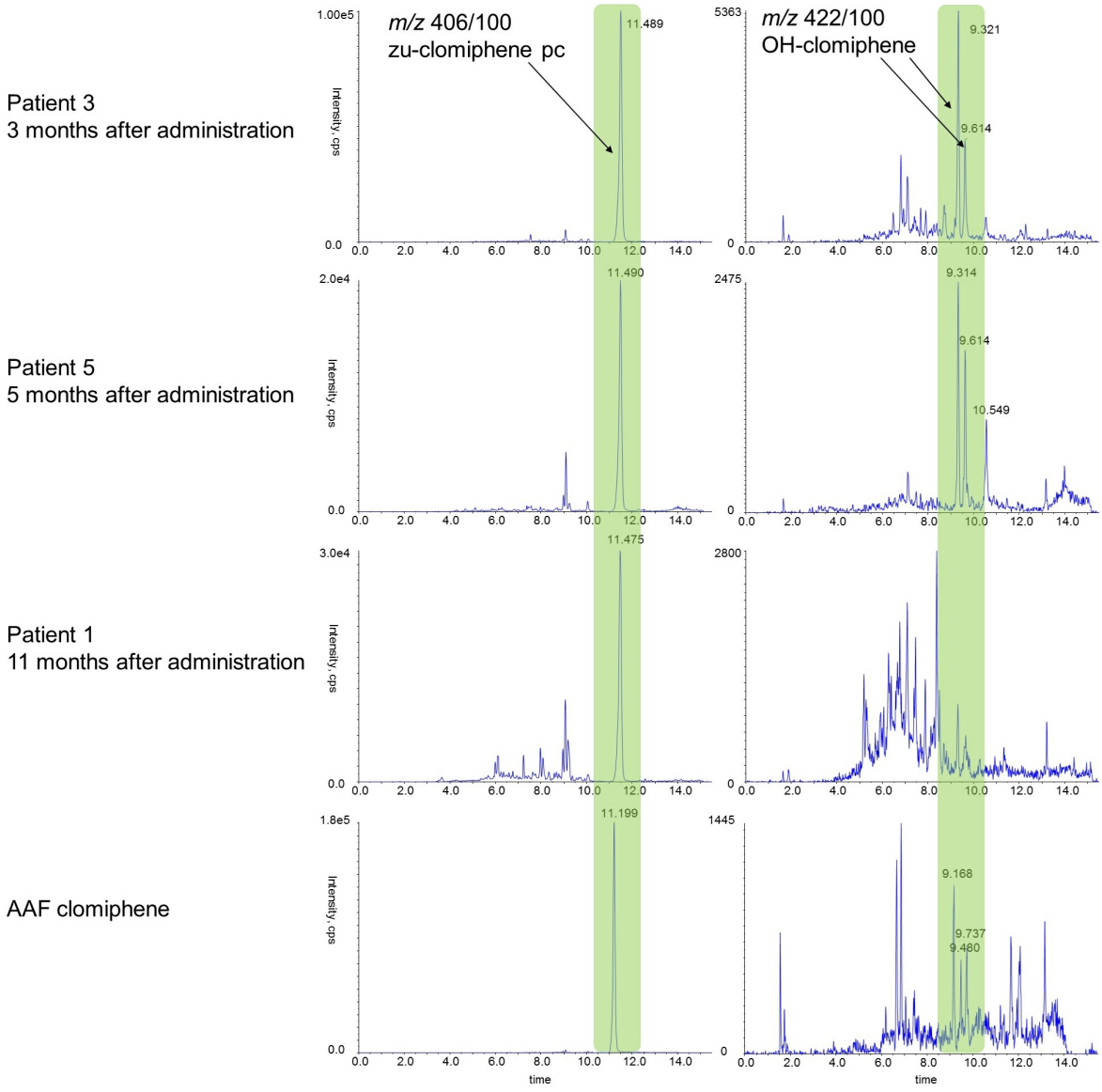

Figure 2: Chromatograms of post-administration urine specimens (3, 5, 11 months after last administration) and a typical low concentrated AAF (clomiphene (cis) ca. 0.2 ng/mL)

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Conclusions

After a typical therapeutic dose, clomiphene (cis-isomer) was identified up to 11 months after application providing unusual long detection windows for the analyte. Furthermore, additional metabolites (hydroxy-clomiphene and hydroxy-methoxyclomiphene) were only detectable in trace amounts up to 5 months after application. In accordance with this result the majority of low concentrated clomiphene AAFs (<1 ng/mL) demonstrated only the presence of clomiphene (cis-isomer), supposably indicating a clomiphene application months ago. However, for an effective clomiphene screening in sports drug testing, both the parent drug and hydroxy-clomiphene should be targeted.

References

1. Ahi S, Beotra A, Upadhyay A, Bhardwaj A, Jain S. (2014) Excretion study of Clomiphene and its correlation with unusual findings in the routine doping control samples. *Recent Advances in Doping Analysis* (22). p75-78
2. Geoffrey D. Miller, Chad Moore, Vinod Nair, Brian Hill, Stuart Willick, Daniel Eichner. Endocrine effects and urinary detection following clomiphene administration in males. Lecture at the USADA meeting 2017, Florida, US.

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