Labor Berlin
Laboratoriumsmedizin & Toxikologie

Cannabinomimetics – A new challenge in routine clinical toxicology

A case report of an intoxication with JWH-018

Thomas Grobosch and Torsten Binscheck

Labor Berlin - Charité Vivantes GmbH, Laboratory Medicine and Toxicology, Oranienburger Str. 285, 13437 Berlin, Germany.

Background

John W. Huffman (JWH), an organic chemist at Clemson University, synthesized analogues and metabolites of Δ9-tetrahydrocannabinol (THC) [1] (**fig.1**). These synthetic cannabionoids (SCs) act as a full agonist at both the CB_1/CB_2 cannabinoid receptors, but with higher potency than THC. SCs are marketed as herbal incense mixtures or "legal highs" under brand names such as "Spice" "and "B 52" etc. with concentration of 150 mg JWH-018/g [2] (**fig. 2**).

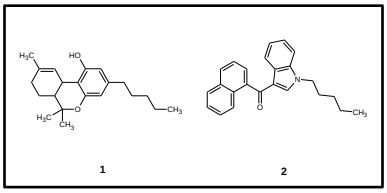

Fig. 1: *Chemical structures of THC (**1**) and JWH-018 (**2**)*

Fig. 2: „Legal highs" products containing SCs

Due to their reported cannabis-like effects, many European countries have banned these substances. A variety of acute effects such as anxiety, sedation, depression, excitability, agitation, chest pain, tremors, sweating, tachycardia followed by bradycardia and severe acute psychosis were reported [3].

JWH-018 (1-Naphthyl(1-pentyl-1H-indol-3-yl)methanone) is hydroxylated at the indole ring and pentyl side chain (carboxylation, hydroxylation), and the hydroxylated metabolites are extensively conjugated with glucuronide [4].

Case history

A 24-yr-old woman (40 kg, 168 cm) with a history of anorexia nervosa/bulimarexia was found unresponsive, breathless and asystolic in bed by her father. After 30 min reanimation attempts of the emergency doctor the patient was defibrillated (6-times) and then transported to hospital. On admission, the EGC showed a prolonged QT interval (QTc=670 ms; normal:< 460 ms) and developed a significant metabolic acidosis with severe hypokalaemia (2.2 mmol/L; normal range: 3.6 to 4.5 mmol/L). With suspicion of an intoxication with diuretics and/or laxatives a plasma and urine sample was sent to our laboratory for toxicological analysis.

Analytical Methods

Systematic toxicological analysis (STA):

Immunoassay (Olympus AU 640/CEDIA Thermo Fisher), HPLC-DAD / TOX.I.S., HS-GC

Qualitative Screening (XLC-QQTOF): Symbiosis Pico /Triple TOF 5600

Sample volume: 100 µL plasma/serum

On-line extraction and Chromatography:

SPE sorbent: OASIS® WCX 10x1 mm (Waters) / sample
SPE solvents: NH_4Ac solution, MeOH
Analytical column: Luna PFP(2), 150 x 2 mm, 5 µm (Phenomenex)
Mobile phase A/B: Formic acid aq/MeOH

QqTOF:

Instrument: Triple TOF 5600 (AB SCIEX)
Source/Ionization mode: TurboIonSpray / (+ESI)
Resolution : 30,000 at 300 m/z
Acquisition mode: MSTOF/MSMSTOF (m/z: 101/50 – 950) +IDA/DBS

Data management:

Software: Analyst TF 1.5.1, Peak View 1.1 (AB SCIEX)
SmileMS - Version 1.1. (GeneBio, Geneva, Switzerland)

Cannabinomimetic (LC-MS/MS): Symbiosis Pico (Spark Holland)/QTRAP 3200 (AB SCIEX)

Sample volume: 50 µL
+ 25 µL enzyme solution (*hydrolysis:* 1 h at 50°C)
+ 100 µL IS-solution (automatically added)

Chromatography:

Analytical column: Luna Phenyl-Hexyl, 50 × 2 mm, 5 µm (Phenomenex)
Mobile phase A/B: HAc with NH_4Ac and HAc with NH_4Ac:methanol
Flow rate: 0.3 ml/min
Injection volume : 50 µL

Mass spectrometry:

The MS apparatus (QTRAP 3200) operated in the positive-ion detection mode (+ESI); LOQ was 1 µg/L.

Following mass transitions were used (m/z: Q1-> Q3/1, Q3,2, Q3/3):

JWH-018: 342.1 - > 155.2 / 127.2 / 214.3
JWH-018,-N-(5-hydroxypentyl)-: 358.1 - > 155.2 / 127.2 / 230.3
JWH-018,-N-pentanoic acid: 372.1 - > 155.2 / 127.2 / 244.2

JWH-018, 4-hydroxyindole-D9: 367.2 - > 155.2 (IS)

Results

Urine: Diuretics, laxatives, digitoxin, digoxin, aconitine and taxines were screened all negative. Drug screening (amphetamines, benzodiazepines, barbiturates, cocaine, methadone, opiates, and THC) using TOX.I.S. and Olympus AU 640 (CEDIA/DRI) was only positive for benzodiazepines in the immunoassay. Qualitative XLC-QQTOF analysis identified midazolam (LC-MS/MS: 45 µg/L), fentanyl (LC-MS/MS: 2.8 µg/L) and the N-(5-hydroxypentyl)-metabolite of JWH-018 (LC-MS/MS: 110 µg/L; **fig. 3**). Creatinine was 0.48 g/L; pH was 7.8.

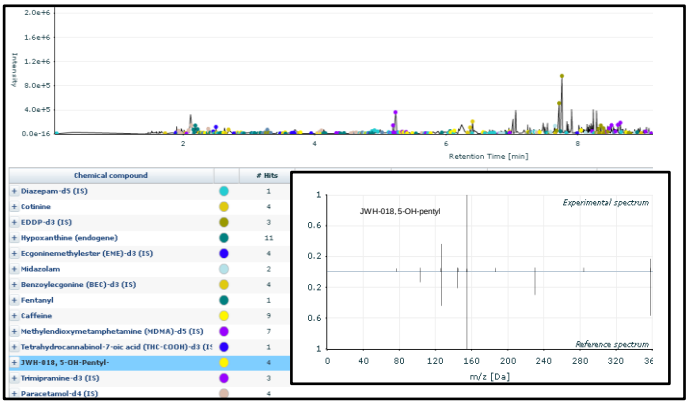

Fig. 3: *Comprehensive report (Smile MS) with experimental and reference spectrum of JWH-018, 5-OH-pentyl*

The quantitative LC-MS/MS analysis of the urine sample yielded following results: JWH-018, 5-OH-pentyl- (11.0 µg/L – **fig. 4**; diluted with water 1:10 -> 110 µg/L) and JWH-018-N-pentanoic acid (4.3 µg/L - **fig. 5**; diluted with water 1:10 -> 43 µg/L).

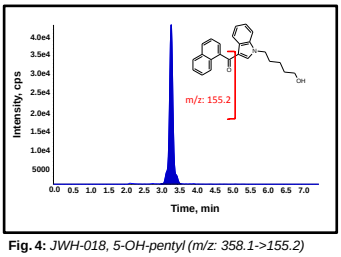

Fig. 4: *JWH-018, 5-OH-pentyl (m/z: 358.1->155.2)*

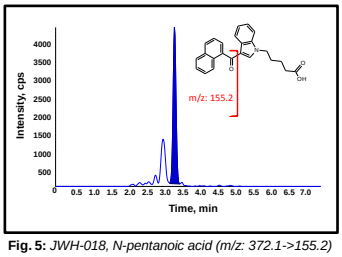

Fig. 5: *JWH-018, N-pentanoic acid (m/z: 372.1->155.2)*

Plasma: STA revealed midazolam (HPLC-DAD: 640 µg/L). XLC-QQTOF analysis screened midazolam, amiodarone (LC-MS: 110 µg/L), fentanyl (LC-MS/MS: 4.7 µg/L) positive; SCs were negative. Quantitative LC-MS/MS analysis of SCs yielded following results: JWH-018 (5.8 µg/L - **fig. 6**, diluted 1:10 -> 58 µg/L), JWH-018, N-pentanoic acid metabolite (4.0 µg/L - **fig. 7**; diluted 1:10 -> 40 µg/L) and JWH-018, 5-OH-pentyl- < 1 µg/L; diluted 1:10 -> < 10 µg/L.

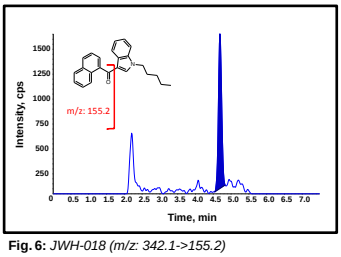

Fig. 6: *JWH-018 (m/z: 342.1->155.2)*

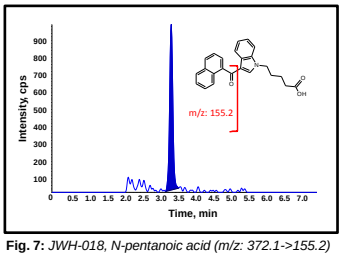

Fig. 7: *JWH-018, N-pentanoic acid (m/z: 372.1->155.2)*

Three days after reanimation, the neuron specific enolase (NSE) in serum was significantly increased (80 µg/L; normal range: < 16 µg/L) - indicating hypoxic brain damage.

Conclusion

Clinicians and users need to be aware of the severe clinical effects following consumption of synthetic cannabinoid preparations marketed as partly legal cannabis alternatives. The cannabinomimetic JWH-018 and similar compounds cannot be detected via standard toxicology tests, therefore MS detection is essential for identification / quantification. It is not clear whether the disturbance of myocardial repolarisation in this case was specifically induced by JWH-018 or might be facilitated by other specific circumstances in this case.

References:

[1] Huffman JW, Zengin G, Wu MJ et al. *Structure-activity relationships for 1-alkyl-3-(1-naphthoyl)indoles at the cannabinoid CB(1) and CB(2) receptors: steric and electronic effects of naphthoyl substituents. New highly selective CB(2) receptor agonists.* Bioorg Med Chem. 2005 Jan 3;13(1):89-112.

[2] Ernst L, Krüger K, Lindigkeit R et al. *Synthetic cannabinoids in "spice-like" herbal blends: First appearance of JWH-307 and recurrence of JWH-018 on the German market.* Forensic Sci Int. 2012 Jun 28.

[3] Young AC, Schwarz E, Medina G et al. *Cardiotoxicity associated with the synthetic cannabinoid, K9 (JWH-018/073) with laboratory confirmation. Am J Emerg Med. 2011 Jul 28.*

[4] ElSohly MA, Gul W, Elsohly KM et al. Liquid chromatography-tandem mass spectrometry analysis of urine specimens for K2 (JWH-018) metabolites. J Anal Toxicol. 2011 Sep;35(7):487-95.