Phase I metabolism of the carbazole derivatives EG-018 and MDMB-CHMCZCA – A new class of synthetic cannabinoids circumventing the ‘NpSG’

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Current legal status

The German ‘Act to control the distribution of new psychoactive substances’ (NpSG) became effective on 26th of November 2016. According to the law, synthetic cannabinoids with indole, indazole or benzimidazole core structures are controlled.[1] EG-018, EG-2201 (5-fluoro-pentyl analog of EG-018) and MDMB-CHMCZCA are not covered by the ‘NpSG’ due to their carbazole core structures.

Aims of the study

Phase I metabolism studies were conducted to identify biomarkers for the detection of EG-018 and MDMB-CHMCZCA metabolites in urine samples. Reference spectra of in vitro phase I metabolites generated by pooled human liver microsome assays were used as a positive control as reference standards of metabolites were not commercially available.

Sample preparation

In vitro microsomal phase I metabolism
- Pooled human liver microsomes[2]
- Incubation 1 h at 37 °C with parent substance
- Extraction with ACN

In vivo phase I metabolism
23 urine samples from forensic casework: EG-018 (n=8), MDMB-CHMCZCA (n=15)
- Incubation with β-glucuronidase (1 h, 45 °C)
- Extraction with ACN / 10 M NH₄HCOO

Analytical workflow

In vitro
- Identification of main metabolites
  Instrumentation: LC-MS/MS QTRAP® 5500 (Sliex)
  - Enhanced product ion scan (EPI)
  - Precursor scan (Prec)
  - Multiple reaction monitoring (MRM)
  - LC-QToF-MS impact II® (Brook) (M)
  - Auto MSMS
  - Full scan & Broad band collision induced dissociation (MS/MS) mode

In vivo
- Implementation in routine urine screening
- Phase I biomarker evaluation
- Optimization of the screening method

Results for EG-018

In total, 25 metabolites were generated in pHLM samples incubated with EG-018. Comparing the microsomal metabolic profiles of EG-018 and EG-2201, one identical metabolite (M’01) was detected, formed by mono-hydroxylation and hydrolytic defluorination, respectively.

From a total of 13 in vivo phase I metabolites of EG-018, detected in the urine samples, the ten most abundant metabolites were referred to four different biotransformation steps and confirmed by corresponding signals in the pHLM assay. M’04 a product of N-Desalkylation and hydroxylation was the most abundant in vivo metabolite. M’01, the 5-OH-pentyl metabolite, was also detectable in each urine sample but with a higher variation of its relative intensity than M04 among the investigated collective.

Results for MDMB-CHMCZCA

For MDMB-CHMCZCA, 28 metabolites were identified in the human urine samples and confirmed in vitro by corresponding signals in the pHLM assays. The ten most abundant metabolites were referred to different metabolic steps and evaluated as reliable urinary biomarkers.

Conclusions

M’04, M’01 are suggested as suitable urinary targets to prove EG-018 consumption in urine. For MDMB-CHMCZCA, the metabolites M14 and M07 are characteristic metabolites for urine analysis. Current online monitoring of ‘legal high’ products (see poster 32) indicates that carbazole derivatives, mainly MDMB-CHMCZCA, are sold via the Internet as legal alternatives to the recently banned SCs scheduled under the ‘NpSG’. Therefore, we recommend to update LC-MS/MS screening methods with the respective metabolites.

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Literature

- Gesetz zur Bekämpfung der Verbreitung neuer psychoaktiver Stoffe.

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