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Full Length Article

Mechanisms of hepatocellular toxicity associated with new psychoactive synthetic cathinones

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ABSTRACT

Synthetic cathinones are a new class of psychostimulant substances. Rarely, they can cause liver injury but associated mechanisms are not completely elucidated. In order to increase our knowledge about mechanisms of hepatotoxicity, we investigated the effect of five frequently used cathinones on two human cell lines. Bupropion was included as structurally related drug used therapeutically. In HepG2 cells, bupropion, MDPV, mephedrone and naphyrone depleted the cellular ATP content at lower concentrations (0.2–1 mM) than cytotoxicity occurred (0.5–2 mM), suggesting mitochondrial toxicity. In comparison, methedrone and methylone depleted the cellular ATP pool and induced cytotoxicity at similar concentrations (≥2 mM). In HepaRG cells, cytotoxicity and ATP depletion could also be demonstrated, but cytochrome P450 induction did not increase the toxicity of the compounds investigated. The mitochondrial membrane potential was decreased in HepG2 cells by bupropion, MDPV and naphyrone, confirming mitochondrial toxicity. Bupropion, but not the other compounds, uncoupled oxidative phosphorylation. Bupropion, MDPV, mephedrone and naphyrone inhibited complex I and II of the electron transport chain, naphyrone also complex III. All four mitochondrial toxicants were associated with increased mitochondrial ROS and increased lactate production, which was accompanied by a decrease in the cellular total GSH pool for naphyrone and MDPV. In conclusion, bupropion, MDPV, mephedrone and naphyrone are mitochondrial toxicants impairing the function of the electron transport chain and depleting cellular ATP stores. Since liver injury is rare in users of these drugs, affected persons must have susceptibility factors rendering them more sensitive for these drugs.

1. Introduction

In recent years, various synthetic cathinones ("bath salts", research chemicals) with amphetamine-like properties have emerged on the illicit drug market and have become popular alternatives to classic stimulants among drug users (Baumann et al., 2013; Prosser and Nelson, 2012). Cathinone designer drugs are derivatives of cathinone (Fig. 1), a naturally occurring β-keto-amphetamine found in the leaves of the Catha edulis plant, and are chemically and pharmacologically similar to classic illicit stimulants (Baumann et al., 2012; Rickli et al., 2015; Simmler et al., 2013, 2014). Therefore, the health risks posed by synthetic cathinones may be similar to the classic stimulants (Liechti, 2015). A rare, but potentially severe adverse reaction of stimulant use is hepatotoxicity (Andreu et al., 1998; De Carlis et al., 2001; Ellis et al., 1996; Garbino et al., 2001; Jones et al., 1994; Kamijo et al., 2002). Most research concerning stimulant hepatotoxicity has so far focused on 3,4-methylenedioxymethamphetamine (MDMA; "ecstasy"). However, the hepatotoxic mechanism of MDMA is currently not entirely understood and multiple factors including polydrug abuse, hyperthermia, and metabolism appear to be associated with liver injury in MDMA users (Antolino-Lobo et al., 2011b; Carvalho et al., 2012; Dias da Silva et al., 2013a,b). In comparison, data on hepatotoxicity of the newly used synthetic cathinones is currently scarce. A case of acute liver failure after synthetic cathinones use has been described (Fröhlich et al., 2011) and *in vitro* studies showed that mitochondrial dysfunction and oxidative stress contribute to hepatic injury associated with these compounds

Abbreviations: ADP, adenosine-diphosphate; AK, adenylate kinase; BSO, buthionine sulfoximine; CYP, cytochrome P450; DMEM, Dulbecco's Modified Eagle Medium; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); FCCP, trifluoromethoxy carbonylcyanide phenylhydrazone; FCS, fetal calf serum; GR, glutathione reductase; GSH, glutathione (reduced); GSSG, glutathione (oxidized); KPE, potassium phosphate buffer; MDMA, 3,4-methylenedioxymethamphetamine; MDPV, 3,4-methylenedioxypyrovalerone; O_2^-, superoxide; OCR, oxygen consumption rate; PBS, phosphate-buffered saline; PCA, perchloric acid; SRB, sulforhodamine B; TMRM, tetramethylrhodamine methyl ester; tGSH, glutathione (total); β-NADPH, β-nicotinamide adenine dinucleotide 2′-phosphate; $\Delta\psi_m$, mitochondrial membrane potential

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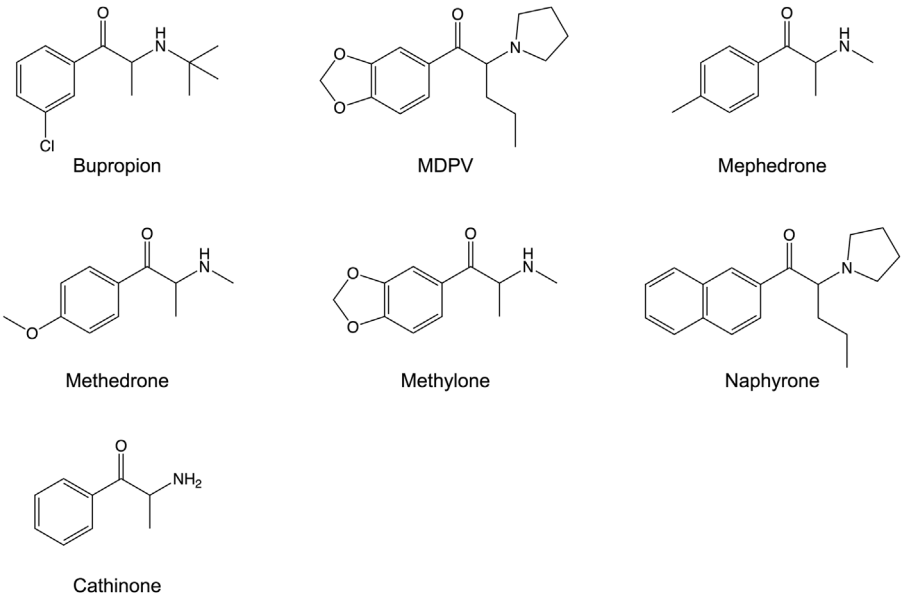

Fig. 1. Structures of the synthetic cathinones used in the study.

(Valente et al., 2016a,b).

In the current study, we aimed to investigate in more detail the mechanisms of hepatocellular toxicity of several synthetic cathinones (Fig. 1) with a focus on mitochondrial toxicity. We therefore investigated the toxicity of the cathinone designer drugs 3,4-methylenedioxypyrovalerone (MDPV), 4-methylmethcathinone (4-MMC; mephedrone), 4-methoxymethcathinone (4-MeOMC; methedrone), 3,4-methylenedioxymethcathinone (βk-MDMA; methylone), and naphthylpyrovalerone (naphyrone) in two human hepatocyte cell lines. In addition, we included bupropion, a synthetic cathinone used as an antidepressant and as a smoking cessation aid.

2. Methods

2.1. Test substances

Naphyrone was synthesized as previously described by Meltzer et al. (2006). Methylone, mephedrone, methedrone, and MDPV were purchased from Lipomed (Arlesheim, Switzerland) with HPLC purity of > 98.5%. Bupropion was purchased from Cayman Chemicals (Ann Arbor, MI, USA), with a purity of > 98%. All drugs were obtained as racemic hydrochloride salts. Drug stocks were made in autoclaved Milli-Q water and were freshly prepared for each assay.

2.2. Cell line and culture

The HepG2 cell line was obtained from the American Type Culture Collection (ATCC, Manassas, USA) and cultured in Dulbecco's Modified Eagle Medium (DMEM, 1 g/l glucose) supplemented with 10% heat inactivated fetal calf serum (FCS), 10 mM HEPES buffer, 2 mM GlutaMAX™, 1% MEM non-essential amino acids, and penicillin-streptomycin (10,000 U/ml corresponding to 10 mg/ml). Cell culture medium and supplements were purchased from Invitrogen (Basel, Switzerland).

The HepaRG cell line was obtained from Biopredic International (Saint Grégoire, France) and cultured in William's E medium (no glutamine) supplemented with 10% FCS, 2 mM L-glutamine, 50 µM hydrocortisone hemisuccinate (Sigma Aldrich, Buchs, Switzerland), 0.05% human insulin (9.5–11.5 mg/ml insulin, Sigma Aldrich), and penicillin-streptomycin (10,000 U/ml corresponding to 10 mg/ml). The cells were cultured at 37 °C in a 5% CO_2 humidified atmosphere and passaged using trypLE™ Express reagent (Invitrogen) when they reached 70–80% confluency.

2.3. Cytotoxicity of HepG2 cells

Cytotoxicity was assessed with the ToxiLight BioAssay Kit from Lonza (Basel, Switzerland) and conducted according to the manufacturer's manual. Briefly, 25,000 HepG2 cells per well were seeded in a 96-well plate. The following day, the cells were treated with 100 µl of the test substances dissolved in medium (0.01 mM, 0.1 mM, 1 mM, and 2 mM for each drug and additionally 0.2 mM and 0.5 mM for bupropion and naphyrone). Treatment with 0.5% Triton X-100 was used as a positive control. After 24 h, 50 µl of the ToxiLight assay buffer was added to 20 µl of supernatant and luminescence was measured with a Tecan M200 Pro Infinity plate reader (Männedorf, Switzerland) after 5 min incubation. The luminescence signal was then compared to medium control.

2.4. ATP content in HepG2 cells

The ATP content was assessed with the CellTiter-Glo Luminescent Cell Viability Assay from Promega (Dübendorf, Switzerland) according to the manufacturer's manual. Briefly, 25,000 HepG2 cells per well were seeded in a 96-well plate and cultured overnight. The cells were then treated for 24 h with 100 µl of the test substances dissolved in medium (concentrations as for cytotoxicity). Treatment with 0.5% Triton X-100 was used as a positive control. Thereafter, 50 µl of the supernatant was discarded and 50 µl of CellTiter-Glo reagent was added to each well. The plate was then shaken for 15 min at room temperature to induce cell lysis. Thereafter, the luminescent signal was measured with a Tecan M200 Pro Infinity plate reader and compared with medium control.

2.5. Cytotoxicity and ATP content in HepaRG cells

HepaRG cells were cultured and differentiated as follows. 10,000 cells per well were seeded in a 96-well plate and the growth medium was replaced with fresh medium every 3–4 days for 2 weeks. Thereafter, the medium was replaced with medium containing 2% of DMSO in order to differentiate the cells into cholangiocyte- and hepatocyte-like cells. The medium was again replaced with fresh medium every 3–4 days for 2 weeks and finally replaced with medium containing no DMSO and only 2% FCS. After three days, the medium of some cell preparations was replaced with the same medium containing 20 µM rifampicin (Sigma Aldrich) every 24 h for cytochrome P450 (CYP) induction. The uninduced cell preparations were treated

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