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Genotoxic properties of representatives of alkylindazoles and aminoalkyl-indoles which are consumed as synthetic cannabinoids

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ABSTRACT

Synthetic cannabinoids (SCs) cause similar effects as cannabis and are sold in herbal mixtures. Recent investigations indicate that some of these drugs possess genotoxic properties. Therefore, we tested representatives of two groups, namely, aminoalkylindoles (AM-2201 and UR-144) and 1-alkylindazoles (5F-AKB-48 and AM-2201-IC) in single cell gel electrophoresis and micronucleus (MN) assays with human lymphocytes and in Salmonella/microsome assays. All drugs except AM-2201 caused DNA-migration, the LOELs were between 50 and 75 μM. Furthermore, all SCs caused inhibition of cell division and significant induction of MN which reflect structural and numerical chromosomal aberrations. The LOEL values were 50 μM for UR-144 and 5-AKB-48 and 75 μM for the other drugs. Also the levels of nucleoplasmatic bridges which are formed from dicentric chromosomes were elevated under identical conditions while the frequencies of nuclear buds were not affected. These findings show that representatives of both groups cause chromosomal damage while the negative results in Salmonella assays (in strains TA98, TA100, TA1535, TA1537 and TA102) in absence and presence of metabolic activation indicate that they do not induce gene mutations. Taken together, these findings indicate that SCs may cause adverse health effects in users as a consequence of damage of the genetic material.

1. Introduction

In 2004, the first "Spice" compounds were sold in herbal mixtures as a substitute of marijuana. These chemicals are marketed *via* the Internet and in hemp shops (UNODC, 2011, 2013) and cause psychoactive effects which are similar to those of tetrahydrocannabinol (THC) (Seely et al., 2012). So far, about 140 chemicals have been synthesized which bind to cannabinoid receptors (Auwarter et al., 2009; Uchiyama et al., 2009). The chemical structures of cannabinoid mimetic compounds differ strongly (Presley et al., 2013) and only few data which concern their toxicological properties are available at present. Recently, we investigated the acute cytotoxicity, immunological effects, estrogenic properties and genotoxic activities of representatives of different groups of these chemicals in *in vitro* assays with human derived cells and found that some of them cause damage of the genetic material (Koller et al., 2013, 2014).

Since it is possible that these drugs cause adverse health effects in users as a consequence of their impact on DNA-stability we conducted further genotoxicity experiments with four representatives (AM-2201, AM-2201-IC, 5F-AKB-48 and UR-144) of two chemical groups of synthetic cannabinoids (SC) which have not been studied so far. The results of these investigations are described in the present paper. AM-2201 (1-(5-fluoropentyl)-1H-indol-3-yl-(naphthalen-1-yl) methanone) is among the most frequently detected SCs in the US in herbal mixtures and powder products (Seely et al., 2013). The name of this drug is derived from the initials of the producer Alexandros Makriyannis. The compound belongs to the aminoalkylindoles and several structurally related compounds (e.g. AB-001 and AM-1248) are currently marketed. Another representative of this group is UR-144 (1-(pent-4-en-1-yl)-1H-indol-3-yl-(2,2,3,3-tetramethylcyclopropyl)-methanone) which was detected for the first time in herbal incense products in Korea in 2012 (Choi

Abbreviations: 2-AA, 2-aminoanthracene; BN-MN, binucleated cells with micronucleus; CBMN assay, cytokinesis-block micronucleus assay; MN, micronucleus; Nbuds, nuclear buds; CBPI, cytokinesis-block proliferation index; CT, cytostasis; LOEL, lowest observed effect level; 2-NF, 2-nitrofluorene; NOEL, no observed effect level; 4-NQO, 4-nitroquinoline-*N*-oxide; 9-AAC, 9-aminoacridine; N4-ACT, N4-aminocytidine; NPB, nucleoplasmatic bridge; PHA, phytohemagglutinine; SCGE, single cell gel electrophoresis; THC, tetrahydrocannabinol.

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et al., 2013). The drug was first synthesized in the Abbott Laboratories in 2006 (Frost et al., 2010), and its structure is similar to that of JWH-018, the first SC which was identified in herbal mixtures (Uchiyama et al., 2013; Zuba et al., 2011). This compound caused positive effects in single cell gel electrophoresis assays (SCGE) with human derived cells (Koller et al., 2013). UR-144 was detected in many European countries, Japan and in the US (Choi et al., 2013) (Kavanagh et al., 2013; Uchiyama et al., 2013). Furthermore, we investigated in the present study two alkylindazoles. 5F-AKB-48 is a fluorophenyl-derivative of AKB-48 (N-(1-adamantyl)-1-(5-fluoropentyl)-1H-indazole-3-carboxamide), which was named after a Japanese girl group (Uchiyama et al., 2012), and AM-2201-IC (1-(5-fluorpentyl)-N-(naphtalen-1-yl)-1H-indazole-3-carboxamide).

The different drugs were tested in SCGE assays (comet assays) with human lymphocytes, which enable the detection of single and double strand breaks and apurinic sites (Tice et al., 2000). In order to find out whether the effects which were seen with some of the drugs in this assay lead to chromosomal damage, we tested them also in micronucleus assays (MN) with human lymphocytes. It is known that MN are formed as a consequence of structural and numerical chromosomal damage (Norppa and Falck, 2003). Furthermore, we evaluated in the same experiments also other nuclear anomalies, which reflect DNA-alterations, namely, nuclear buds (Nbuds) which result from gene-amplifications and nuclear bridges (NPBs) which are formed as a consequence of dicentric chromosomes (Fenech et al., 2003). In order to find out whether the different drugs induce gene mutations, *Salmonella*/microsome assays were conducted with strains TA98 and TA100 in presence and absence of metabolic activation mix (S9-mix).

2. Materials and methods

2.1. Chemicals

Low melting point agarose (LMPA) and normal melting point agarose (NMPA) were obtained from Gibco (Paisley, UK). Inorganic salts, 2-aminoanthracene (2-AA) dimethysulfoxide (DMSO), propidium iodide, hydrogen peroxide, RPMI 1640, Triton X-100, Trizma base, trypan blue, Histopaque, FCS, 2-nitrofluorene (2-NF), 4-nitroquinoline-*N*-oxide (4-NQO), 9-aminoacridine (9-AAC), N4-aminocytidine (N4-ACT), methyl methanesulfonate (MMS), Mitomycin C, cytochalasin-B, L-glutamine and sodium pyruvate were purchased from Sigma-Aldrich (Steinheim, Germany). PAH was ordered from Remel, Inc. (USA). S9-homogenate was purchased from MP Biomedicals (Illkirch-Graffenstaden, France).

2.2. Test compounds

Four synthetic cannabinoids, namely, 1-(5-fluoropentyl)-1H-indol-3-yl-(naphthalen-1-yl) methanone, (AM-2201, $C_{24}H_{22}FNO$), 1-(pent-4-en-1-yl)-1H-indol-3-yl-(2,2,3,3-tetra-methylcyclopropyl) methanone ($C_{21}H_{27}NO$, UR-144), N-(1-adamantyl)-1-(5-fluoropentyl)-1H-indazole-3-carboxamide, ($C_{23}H_{30}FN_{30}$, 5F-AKB-48) and 1-(5-fluorpentyl)-N-(naphtalen-1-yl)-1H-indazole-3-carboxamide, (AM-2201-IC, $C_{23}H_{22}FN_3O$) were synthesized as crystalline solids (purity 96–99%) at the Institute of Forensic Medicine (University Medical Center Freiburg). Stock solutions were prepared in DMSO and stored at –20 °C.

The doses of the test compounds which were studied in the different experimental models were selected on the basis of international guidelines: In MN experiments, doses were monitored which do not cause substantial inhibition of cell division (OECD, 2012). In SCGE experiments concentrations were tested which do not affect the viability of the cells, as it is known that dead cells may cause misleading (false positive) results (Hartmann et al., 2003; Tice et al., 2000). The concentrations which were tested in Salmonella microsome assays were choosen on the basis of the solubility of the drugs (Fluckiger-Isler and Kamber, 2012; OECD, 1997) (Fig. 1).

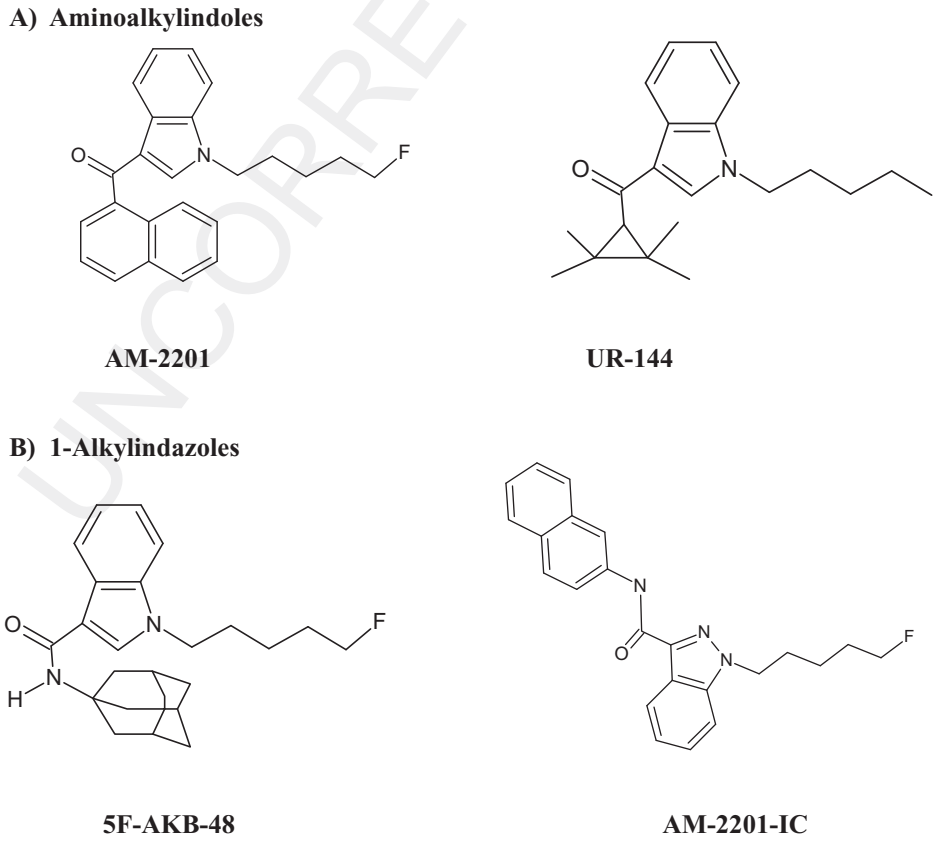

Fig. 1. Chemical structures of the different synthetic cannabinoids.

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