Three regulatory compliant test systems show no signs of MDMA-related genotoxicity

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Abstract

3,4 Methylenedioxymethamphetamine (MDMA)-assisted therapy has been recently found to be highly effective for treatment of posttraumatic stress disorder (PTSD). Previous studies have been inconclusive in elucidating potential MDMA genotoxicity. We performed three regulatory compliant studies to investigate the potential of genotoxic effects of MDMA treatment in humans: (1) an *in vitro* bacterial reverse mutation (Ames) assay, (2) an *in vitro* chromosome aberration test in Chinese hamster ovary cells, and (3) an *in vivo* micronucleus study in male Sprague Dawley rats. MDMA was found to not have genotoxic effects in any of the assays at or above clinically relevant concentrations.

Keywords

MDMA, 3,4-Methylenedioxymethamphetamine, Toxicology, PTSD, Genotoxicity

Introduction

3,4 Methylenedioxymethamphetamine (MDMA) or "ecstasy," is an investigational new drug, currently in Phase 3 of human trials. MDMA has been found to have preliminary efficacy in treating post-traumatic stress disorder (PTSD) when delivered in an integrated multimodal intervention through MDMA-assisted therapy, allowing participants to create a stronger bond with their therapist and tap into emotionally upsetting or traumatic experiences (Burge, 2020). MDMA is a sympathomimetic drug that modifies the release, re-uptake, and longevity of dopamine, serotonin, and norepinephrine in the synaptic cleft. Although MDMA does share similar targets to currently approved antidepressants, its mechanism of action, time to onset, and durability of effects and dosing intervals differ greatly (Kalant, 2001; MDMA Investigator's Brochure 12th Edition, 2020). Contrary to currently used psychiatric medications, MDMA is not administered daily for months or years on end, but in three divided dose exposures over the course of a few months (Kalant, 2001). Currently there is mixed reporting on the absence or presence of MDMA genotoxicity in various preclinical test systems (Barenys et al., 2009; Frenzilli et al., 2007; Hariri et al., 2010; Parolini et al., 2014; Yoshioka et al., 2007). In order to settle the lack of consensus in previous literature, we conducted three regulatory compliant studies investigating the genotoxic potential of MDMA in humans: (1) an *in vitro* bacterial reverse mutation (Ames) assay, (2) an *in vitro* chromosome aberration test in Chinese hamster ovary (CHO) cells, and (3) an *in vivo* micronucleus study in male Sprague Dawley rats.

Methods

An *in vitro* chromosome aberration assay (Registre and Proudlock, 2016) was performed in Chinese Hamster Ovary – Wolff Bloom

Litton (CHO-WBL) cells with and without an exogenous metabolic activation system (Aroclor 1254-induced rat liver homogenate with the appropriate buffer and cofactors; S9). The OECD TG473 (OECD, 2016a: 473) and ICH S2(R1) (ICH Expert Working Group, 2011) compliant design utilized 4-hour treatments $\pm$S9 and a 24-hour treatment –S9. MDMA HCl was evaluated at concentrations of 15–240 μg/mL, along with the appropriate positive and negative controls (the highest concentration evaluated represents the 1 mM limit dose for pharmaceuticals). All cultures were harvested at 24 hours (i.e. after a 20-hour recovery for the 4-hour treatments). Structural aberrations were analyzed in 300 total metaphase cells (or $\geqslant$50 aberrant cells) and numerical aberrations (endoreduplication and polyploidy) were evaluated in 400 total cells (half from each duplicate culture).

MDMA HCl was evaluated in the Ames assay (Hamel et al., 2016) in *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537, and in *Escherichia coli* strain WP2 *uvrA*, in triplicate plates using the plate incorporation method. MDMA HCl

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Table 1. In vitro chromosome aberration assay in CHO-WBL cells.

Treatment	µg/mL	% Cytotoxicity (RPD)	% Aberrant cells	Trend[a]	% Cells w/>1 Abs	% Endo cells	% Polyploid cells
4-hour −S9 (20-hour recovery)							
Sterile water	1%	0	3.0	NA	0.0	0.0	2.3
MMC	0.5	46	96.2**	NA	46.2	0.0	3.5
MDMA HCl	60	15	3.7	0.3638	0.3	0.3	3.0
	120	8	3.7		0.3	0.3	3.0
	240	13	1.7		0.3	0.3	3.8
4-hour +S9 (20-hour recovery)							
Sterile water		0	3.0	NA	0.0	0.0	2.5
CP	5	47	96.2**	NA	57.7	0.0	1.8
MDMA HCl	60	0	4.0	0.7223	0.0	0.0	3.0
	120	0	2.7		0.3	0.0	2.5
	240	0	4.0		0.0	0.0	2.3
24-hour −S9 (no recovery)							
Sterile water		0	3.7	NA	0.3	0.0	1.8
MMC	0.1	20	68.5**	NA	39.7	0.0	1.8
MDMA HCl	60	0	3.7	0.2498	0.0	0.3	1.5
	120	15	1.7		0.0	0.0	1.8
	240	36	2.7		0.3	0.0	2.3

Abs: aberrations; CP: cyclophosphamide; Endo: endoreduplicated cells; MMC: mitomycin C; NA: not applicable; RPD: relative population doubling.
[a]Cochran-Armitage trend test (% aberrant metaphases; $p \leqslant 0.05$ is significant).
**Significant increase in % aberrant cells ($p \leqslant 0.01$; Fisher's Exact Test, 1-tailed).

was tested as per OECD TG471[14] and ICH S2(R1)[10] at doses from 100 to 5000 µg/plate (the limit dose for this assay), along with the appropriate controls, ±S9.

MDMA HCl was evaluated in the *in vivo* micronucleus assay (Custer et al., 2016), in male Sprague-Dawley rats, as per OECD TG474 (OECD, 2016b) and ICH S2(R1) (ICH Expert Working Group, 2011). Based on a dose range-finding study, doses of 20, 50, and 100 mg/kg/day, as well as the vehicle control, were administered by oral gavage once daily for 2 days (the highest dose level represented the maximum tolerated dose). Animals were housed individually to maximize the tolerability of MDMA. Approximately 24 hours following the last dose, bone marrow was harvested from five animals/group and slides were prepared (archived positive control slides were utilized to verify scorer proficiency). Four thousand polychromatic erythrocytes (PCEs) were scored to determine the frequency of micronucleated (MN) PCEs. Bone marrow cytotoxicity was evaluated by counting 500 total erythrocytes (TE) to determine PCE:TE ratios (normochromatic erythrocytes + PCEs = TE).

Results

All positive and negative control values in all assays were within acceptable ranges, and all criteria for a valid assay were met.

In the *in vitro* chromosome aberration assay, no statistically significant or dose-dependent increases in structural or numerical aberrations were noted in any treatment ($p > 0.05$), and all MDMA-treated cultures were within historical negative control ranges (Table 1). Therefore, MDMA was negative for inducing structural and numerical aberrations in CHO-WBL cells ±S9 under the conditions of the test.

In the Ames assay, no increases in mean revertant frequencies were observed in any tester strain and any dose of MDMA ±S9 (Table 2). Therefore, MDMA HCl was negative for inducing bacterial reverse gene mutations ±S9 under the conditions of this test.

Adverse clinical observations, as well as reduced bodyweight and food consumption, were observed in the *in vivo* micronucleus assay at all MDMA doses evaluated. Clinical observations including stereotypy, piloerection, decreased activity, hunched posture, salivation, hypersensitivity to touch, rapid breathing, and body weight loss were increasingly severe with higher dose levels. These adverse effects were dose limiting, and the assay was evaluated at three dose levels with the high dose group covering the maximum tolerated dose, which was 100 mg/kg/day. However, no statistically significant or dose-dependent increases in %MN-PCEs were observed at any dose of MDMA, and all MDMA-treated group means were within historical negative control ranges. In addition, no bone marrow cytotoxicity (decreases in PCE:TE ratios) was observed at any dose level (Supplemental Table 1). Therefore, MDMA was negative for clastogenic and aneugenic activity under the conditions of this assay.

Discussions

MDMA did not show any signs of genotoxicity across the three robust test systems analyzed. These results are consistent with past articles from Hariri (Hariri et al., 2010) and Yoshioka (Yoshioka et al., 2007). We highlight that these studies are the recommended studies to rule out genotoxicity potential by regulators internationally (ICH Expert Working Group, 2011). Additionally, all studies conducted here were carried out with test

Table 2. Ames assay results – average revertant colonies per plate (SD).

Treatment	Dose level (μg/plate)	TA98	TA100	TA1535	TA1537	WP2uvrA
−S9						
Sterile water	100 μL	14 (4)	98 (22)	9 (3)	6 (2)	42 (5)
ICR	0.5				201* (30)	
2NF	2.5	468* (125)				
SA	1		317* (23)	416* (39)		
NQNO	2					576* (55)
MDMA HCl	100	12 (2)	77 (4)	12 (4)	7 (5)	41 (10)
	250	14 (9)	82 (11)	9 (3)	6 (4)	37 (2)
	500	12 (3)	92 (4)	11 (2)	6 (2)	44 (5)
	1000	15 (3)	94 (16)	7 (4)	8 (3)	38 (9)
	2500	21 (6)	80 (8)	9 (3)	6 (1)	35 (15)
	5000	10 (2)	70 (7)	7 (3)	9 (5)	33 (9)
+S9						
Sterile water	100 μL	16 (4)	84 (11)	5 (2)	4 (3)	37 (4)
2AA	2.5	1499* (187)	963* (112)	201* (12)	73* (19)	
2AA	10					151* (33)
MDMA HCl	100	18 (4)	93 (11)	10 (5)	4 (1)	38 (8)
	250	14 (3)	83 (13)	6 (3)	6 (2)	35 (5)
	500	14 (3)	90 (14)	9 (0)	6 (1)	43 (5)
	1000	17 (6)	85 (6)	8 (1)	6 (3)	40 (3)
	2500	13 (4)	87 (7)	12 (2)	8 (4)	39 (4)
	5000	17 (2)	89 (4)	8 (2)	4 (4)	35 (8)

Data reported as mean (standard deviation). All plates had normal background lawns.
2AA: 2-aminoanthracene; 2NF: 2-nitrofluorene; ICR: ICR-191; NQNO: 4-nitroquinoline-N-oxide; SA: sodium azide.
*Positive response or increase: ≥2-fold (TA100) or ≥3-fold (TA98, TA1535, TA1537 and WP2 uvrA) negative control values.

system exposures above the relevant clinical C_{max} for MDMA (of 195–252 ng/mL) (MDMA Investigator's Brochure 12th Edition, 2020; Vizeli and Liechti, 2017). We note that results from Barenys et al. (2009), did show signs of DNA damage in sperm and testes of male rats. However we caution that the experiments from Barenys and colleagues were performed with 36 drug exposure days in rapid succession prior to collection of tissue for the comet test, resulting in many fold higher dosages than ever expected or allowed clinically. Additionally, the Barenys study administered MDMA subcutaneously which would be resultant in increased exposure compared to the oral route (as MDMA has a relative oral bioavailability of 37–68% (MDMA Investigator's Brochure 12th Edition, 2020)). Overall, these results indicate the apparent lack of genotoxicity for MDMA. We hope that these results can guide the safe use of MDMA in humans.

Declaration of conflicting interests

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Supplemental material

Supplemental material for this article is available online.

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