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RESEARCH ARTICLE

MDMA toxicity: no evidence for a major influence of metabolic genotype at CYP2D6

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Abstract

3,4 Methylenedioxymethamphetamine (MDMA or ecstasy) has become a major drug of abuse over the last decade. It produces a mixture of systemic and neuropsychological effects. Animal studies show a range of short- and long-term toxic effects, both systemically and neurochemically. In humans, toxicity and death due to the drug have been attributed to a variety of causes, with 'idiosyncratic', or non-dose-related, reactions often cited. It has recently been established that MDMA is metabolized via the cytochrome P450 enzyme, debrisoquine hydroxylase. This enzyme is coded by the gene CYP2D6. This gene contains mutations which effect the function of the enzyme, and individuals homozygous for these mutations are known as poor metabolizers. Between 3 and 10% of the Caucasian population are thus affected, and therefore may be less able to metabolize MDMA. In this paper we examine the hypothesis that individuals selected on the basis of having had an adverse reaction to MDMA will be more likely than the general population to have homozygous mutations at CYP2D6. We obtained retrospectively seven cases of toxicity or death thought to be due to MDMA. DNA was extracted from these patients, and their genotype ascertained. None of this small sample was shown to be homozygous for the mutation at CYP2D6. Three possible explanations are offered for these results. (1) The non-dose-related nature of MDMA toxicity may be due, either alone or in combination, to contaminants in the drug, or ambient environmental/physiological factors. (2) Our genotyping methods may have missed one of the rare additional mutations which effect gene function at CYP2D6. (3) Our study sample may be too small to demonstrate a statistically significant result. A larger study is currently under way.

Introduction

The hallucinogenic amphetamine, 3,4 methylenedioxymethamphetamine (MDMA, E, or ecstasy) was first synthesized and patented by Merck in 1914 as an appetite suppressant. With little or no subsequent commercial interest, the patent expired. There was little mention of MDMA in the literature until the late 1970s, apart from work on toxicology in animals.[1] The first description of its actions in humans described the production of 'an easily controlled altered state of consciousness with emotional and sensual overtones'.[2] It was the latter qualities, and the concept of MDMA being used as an 'affective enhancer', which led to its proposed use in psychotherapy.[3,4] In 1985 MDMA was placed on Schedule 1 of controlled substances by the Drug Enforcement Agency in the USA. This

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was because of increased recreational use and a report[5] that one of its analogues, 3,4 MDA (3,4 methylenedioxyamphetamine) produced toxic effects on brain serotonin in rats. An increase in usage in universities, and the spread of the drug to Europe and beyond, led to frequent medical and media reports of toxicity and death due to the drug.[6–9] A literature search using the key words 'MDMA' and 'toxicity' indicated none in 1985, nine in 1987, 19 in 1991, 15 in 1994 and 20 in 1996.

MDMA has stimulant effects in humans characterized by increased heart rate, increased blood pressure, dry mouth, decreased appetite, sweating and jaw clenching.[10] Blood biochemical indices are usually within the reference range. Neuropsychological effects include a subjective feeling of euphoria. Subjects report that their 'attention is focused on the here and now rather than future or past'.[10] MDMA, in typical doses, rarely produces frank hallucinations despite being classified as an hallucinogenic amphetamine.[11]

Animal studies with MDMA have shown an anticipated spectrum of signs consistent with sympathomimetic stimulation, including piloerection, mydriasis and hyperthermia. In larger doses, seizures are induced.[1] Prolonged administration to dogs has been shown to cause testicular atrophy and prostatic hypertrophy.[12] Neurochemical evidence points to induced deficits in the serotonin pathways, in a dose-related fashion.[5,13–15] Furthermore, these effects appear to last beyond the cessation of MDMA intake.[16] Damage to dopaminergic neurones in animals has been shown to occur with high doses.[17] Toxic systemic effects in humans include cardiac arrythmias and asystole,[7,18] rhabdomyolysis, disseminated intravascular coagulation, hyperthermia, acute renal failure and hepatotoxicity.[6,8,9,18–20] Toxic neuropsychiatric effects vary from short-lived anxiety states to chronic psychoses.[21,22] Major depression, panic disorders and memory disturbances have also been described.[23,24] As noted above, animal studies demonstrate a possible long-term neurotoxic effect on serotoninergic and, at higher doses, dopaminergic pathways with MDMA use. Human studies thus far, using indirect methods of serotonin function (i.e. CSF 5HIAA measurement and neuroendocrine challenge), have been inconclusive.[25,26] The utilization of positron emission tomography (PET) and single photon emission computed tomography (SPECT) for evaluating the status of the serotonin system in patients who have taken MDMA is currently being investigated. It is possible that long-term neurotoxic effects may begin to emerge in the coming years, given the relative novelty of abuse of MDMA.

It is known that there is an interindividual variation in the propensity to the adverse effects of MDMA noted above. Some individuals have died after a single tablet of MDMA, while others have suffered little more than a few hours of sympathetic overdrive after two or three times the dose. Various explanations have been proposed. Drug impurity, the veracity of the subject's report, ambient environment (e.g. lack of water with sustained physical activity) or concomitant physical ailments may all have a role to play in individual cases.[18]

Pharmacogenetics of the metabolism of MDMA

It has recently been established that MDMA is metabolized to DHMA (dihydroxy-methamphetamine), a catecholamine, via the hepatic cytochrome P_{450} enzyme, debrisoquine hydroxylase. This enzyme is coded by the gene CYP2D6 on chromosome 22.[27] There is a common genetically determined variation in the population with regard to debrisoquine metabolism,[28] caused by mutations within the CYP2D6 gene which effect its function. An individual homozygous for these mutations has no functioning enzyme and is known as a poor metabolizer. The mutations are inherited in an autosomal recessive fashion. Between 3 and 10% of the Caucasian population are homozygous for mutations.[29] A paper by Colado *et al.*[30] examined the hypothesis first set out by Tucker *et al.*[27] They used the Dark Agouti rat model[31] to determine if low activity of the debrisoquine hydroxylase enzyme might result in a lower rate of MDMA metabolism, with resulting higher plasma levels of MDMA and therefore a greater risk of toxicity. They found that the female Dark Agouti rat, which metabolizes debrisoquine more slowly than the male, was indeed more susceptible to the hyperthermic effects of MDMA. Colado *et al.*[30] suggest that humans of the poor metabolizer phenotype will be more susceptible to the acute toxic effects of MDMA.

In this paper, we test the hypothesis that individuals selected on the basis of having had an

adverse reaction to MDMA will be more likely than the general population to have homozygous mutations at CYP2D6, and by implication a poor metabolizer phenotype.

Methods

We obtained blood samples from seven cases referred by medical colleagues. The cases were referred on the basis of a history of MDMA intake, and side effects severe enough to necessitate hospital attendance or admission. The spectrum of symptoms therefore varied widely from mild intoxication to death. All our patients had routine toxicology performed. This did not include a specific test for MDMA, although it can be detected under the broad heading of 'amphetamines'; MDMA becomes undetectable in routine screening after approximately 24–36 hours. A negative screen, therefore, is informative only in that it helps to rule out other drugs which may be producing the clinical picture. There is currently no local Irish study which has looked at the purity, or lack thereof, of MDMA for sale on the streets. With the consent of the patient/family and treating doctors we obtained a blood sample, and this was analysed as described below. One hundred and sixty normal population controls were obtained from blood donors. These individuals were not screened for psychiatric disorder or history of drug abuse.

Methods of genotyping

DNA was extracted from anticoagulated blood using standard procedures. Following a method obtained from Ann Daly (personal communication) genomic DNA (0.5 μg) was amplified using the polymerase chain reaction and primers 5-TGCCGCCTTCGCCAACCACT-3′ and 5′-GGCTGGGTCCCAGGTCATAC-3′. The reaction was 30 cycles of 60 s at 95°C, 90 s at 63°C, and 180 s at 70°C in a total volume of 50 μl containing 10 mM TRIS, pH 8.3, 1.5 mM $MgCl_2$, 50 mM KCl, 0.1% (v/v) Triton X-100, 4% DMSO, 200 μM of dNTPs, 0.25 μM primers and 1.5 units of Taq polymerase; 20 μl of product was digested with 15 units of *Bsa*Al and 15 units of *Bst*Nl for 3 hours at 50°C and the fragments separated on a 8% polyacrylamide gel, and stained with ethidium bromide. The B allele is represented by bands at 380 bp, 180 bp, 130 bp and 100 bp; the A allele by bands at 280 bp, 160 bp, 130 bp and 100 bp; and the wild-type by bands at 280 bp, 180 bp, 130 bp and 100 bp.

Results

The frequency of the A and B mutations in our control population was 0.01 and 0.16, respectively. Five individuals were homozygous for the B mutation (frequency of 0.032) and none were homozygous for the A mutation. It is assumed that those individuals homozygous for one or other mutation are of the poor metabolizer phenotype. Case summaries and genotypes of the 7 MDMA individuals are shown in Table 1.

Discussion

Prospective human investigations on the metabolism and toxicity of a controlled, and occasionally lethal, drug such as MDMA, are plainly difficult to perform, both ethically and practically.

In this study, we were limited to a retrospective study of patients suffering toxic reactions to MDMA. In addition, there were no facilities available for the confirmation of the presence of MDMA in the blood. As with any retrospective study of an illicit drug, we are dependent upon accuracy and reliability of the subject's history.

We sought to test the hypothesis that individuals selected on the basis of severe adverse effects to MDMA might be more likely than the general population to have mutations at the CYP2D6 gene and by implication be of the poor metabolizer phenotype. We obtained blood or tissue from seven subjects with a history of MDMA intake and adverse effects as described in the case summaries. DNA was extracted as described above, and the mutations at the CYP2D6 gene assessed using molecular biology techniques. If this hypothesis was proved true, adverse effects may be anticipated from prior knowledge of the individual's genotype. However, none of our subjects was found to be homozygous for the mutation at CYP2D6, and therefore unlikely to be of the poor metabolizer phenotype. We suggest three possible explanations for our findings, as follows:

Contamination and ambient environment. The non-dose-related, or idiosyncratic, nature of MDMA toxicity may be due to contaminants in the tablet, a factor for which it is not possible to control, as noted above. Dehydration, water

Table 1. *Case summaries and genotype results*

Circumstances and amount	Clinical course	Investigations	Outcome	A mutation	B mutation
Case 1: 17-year-old female 2 MDMA tablets at party at 10pm Nausea and vomiting overnight Collapsed 12 hours post ingestion History of MDMA use previously	Cyanosed. Respiratory arrest Fixed dilated pupils. GCS 3/15 (P)120/min(R)0/min(BP)90/70(T)34.2 Dehydrated. Intubated and ventilated	Toxicology: negative C.K.1800(34–170)	Brain death diagnosed 72 hours after admission	wt/wt	wt/wt
Case 2: 22-year-old male 3 MDMA tablets taken Attended A&E 5 hours later C/O sweating and palpitations Regular MDMA user	Agitated (P)110/min(R)22/min(BP)120/90(T)36.8	Toxicology: negative	Asymptomatic on D/C	wt/wt	B/wt
Case 3: 19-year-old male MDMA tablet taken 7/8 pints of beer drunk Attended A&E 4 hours later Regular MDMA user	Panicky, sweating and tremulous (P)100/min(R)22/min(BP)120/90(T)36.8	Toxicology: positive for alcohol	Settled with 10 mg diazepam p.o. Discharged from A/E	wt/wt	wt/wt
Case 4: 17-year-old male ? quantity of MDMA taken Collapsed at party, following seizure	Unconscious GCS 3/15 (P)60/min(R)30/min(BP)150/100(T)37.5°C Further seizures intubated/ventilated	CT brain cerebral oedema Toxicology: negative	Brain death diagnosed 36 hours after admission	wt/wt	wt/wt
Case 5: 18-year-old female 2 MDMA tablets taken Attended A&E 4 hours later Regular MDMA user	Nauseated, dizzy & c/o palpitations (P)150/min(R)24/min(BP)140/100 (T)38. Dehydrated	Toxicology: negative Raised LFTs	Treated symptomatically Discharged well after 4 days	wt/wt	wt/wt
Case 6: 19-year-old female 1 MDMA tablet taken. Seizure. History of MDMA use.	Drowsy, agitated and convulsing Intubated, Apyrexial	Toxicology: negative	Full recovery	wt/wt	wt/wt
Case 7: 16-year-old female 1/2 MA tablets taken Collapse ?History of MDMA use	Respiratory arrest. Unconscious. GCS 3/15	Toxicology: negative Hyponatremia-(Na)125	Brain death diagnosed	wt/wt	wt/wt

intoxication, physical overactivity and coincidental medical problems have all individually or jointly been implicated in producing the overall clinical picture.

Other rare mutations. Knowledge of an individual's genotype at the A and B mutations at the CYP2D6 gene will predict the poor metabolizer phenotype in 80–90% of cases, depending on the frequency of mutations in the population. There are many additional mutations which also affect gene function, but these are very rare. It is unlikely, therefore, that we have missed these mutations in all or many of our cases. Nonetheless, we intend to genotype all known mutations as part of a larger study which includes individuals with adverse effects as a result of antidepressant and antipsychotic medications.[32]

Small sample size. This small sample of seven patients may be too small to demonstrate a statistically significant result, particularly in the likely presence of contamination and variable ambient environment. We are in the process of collecting clinical samples to enlarge our study population. It is worth noting that, while physiological and environmental factors may change according to the milieu in which MDMA is taken, the metabolic genotype remains chronically stable over time. This provides a degree of predictability of metabolic function, allowing for variability produced by environmental effects. If, with larger patient numbers, pharmacogenotype is shown to have an influence on toxicity, this might suggest the possibility of a 'screening' procedure for those at risk.

We therefore conclude that these preliminary results do not point to a pharmacogenetic aetiology for MDMA toxicity. However, we intend to further clarify this by performing a larger study including all known mutations at, *inter alia*, the CYP2D6 gene.

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References

1. Hardiman HF, Haavik CO, Steevers MH. Relationship of the structures of mescaline and seven analogs to toxicity and behaviour in 5 species of animals. Toxicol Appl Pharmacol 1973;25:299–309.
2. Shulgin AT, Nicholls DE. Characteristics of 3 new psychotomimetics. In: Stillman RC, Willette RE, editors. The pharmacology of hallucinogens. New York: Pergamon, 1978.
3. Greer G. Using MDMA in psychotherapy. Advances 2:57.
4. Greer G, Tolbert R. Subjective reports of the effects of MDMA in a clinical setting. J Psychoact Drugs 1986;18:319–27.
5. Ricuarte GA, Bryan G, Strauss L, *et al.* Hallucinogenic amphetamine selectively destroys brain serotonin terminals. Science 1985;229:986–8.
6. Brown C, Osterloh J. Multiple complications from recreational ingestion of MDMA (Ecstasy). J Am Med Assoc 1987;258:780–1.
7. Dowling GP, McDonough ET, Bost RO. 'Eve' and 'Ecstasy': a report of five deaths associated with the use of MDEA and MDMA. J Am Med Assoc 1987; 257:1615–17.
8. Chadwick IS, Linsey A, Freemont A, *et al.* Ecstasy, 3,4 methylenedioxymethamphetamine (MDMA), a fatality with coagulopathy and hyperthermia. J Roy Soc Med 1991;84:371.
9. Campkin NT, Davies UM. Another death from ecstasy. J Roy Soc Med 1992;85:61.
10. Downing J. The psychological and physiological effects of MDMA on normal volunteers. J Psychoact Drugs 1986;18:335–40.
11. Steele TD, McCann UD, Ricuarte GA. 3, 4-methylenedioxymethamphetamine (MDMA, ecstasy); pharmacology and toxicology in humans and animals. Addiction 1994;89:539–51.
12. Frith CH, Chang CW, Lattin DL, *et al.* Toxicity of 3,4 methylenedioxymethamphetamine in the dog and rat. Fundam Appl Toxicol 1987;9:110–19.
13. Stone DM, Stahl DC, Hanson GR, *et al.* The effects of 3,4 methylenedioxymethamphetamine on monoaminergic systems in the rat brain. Eur J Pharmacol 1986;128:41–8.
14. Stone DM, Hanson GR, Gibbs JW. Differences in the central serotonergic effects of 3,4 methylenedioxymethamphetamine (MDMA) in mice and rats. Neuropharmacology 1987;26:1657–61.
15. Battaglia G, Yeh SY, O'Hearn E, *et al.* 3,4-methylenedioxymethamphetamine and 3,4 methylenedioxyamphetamine destroy serotonin nerve terminals in rat brain. Quantification of neurodegeneration by measurement of [3H]-paroxetine labelled uptake sites. J Pharmacol Exp Ther 1987; 242:911–16.
16. Ricuarte GA, Forno LS, Wilson MA, *et al.* MDMA selectively damages central serotonergic neurones in the primate. J Am Med Assoc 1988;260:51–5.
17. Commins DL, Vosmer G, Virus RM, *et al.* Biochemical and histological evidence that methylenedioxymethamphetamine (MDMA) is toxic to neurones in the rat brain. J Pharmacol Exp Ther 1987;241:338–45.
18. Henry JA, Jeffreys KJ, Dawling S. Toxicity and

deaths from 3,4 methylenedioxymethamphetamine (ecstasy). Lancet 1992;340:384–7.
19. Screaton GR, Cairns HS, Sarner M, *et al.* Hyperpyrexia and rhabdomyolysis after MDMA (ecstasy) abuse. Lancet 1992;339:677–8.
20. Milroy CM, Clark JC, Forrest AR. Pathology of deaths associated with 'ecstasy' and 'eve' misuse. J Clin Pathol 1996;49:149–53.
21. Greer G, Straussman RJ. Information on ecstasy. Am J Psychiatry 1985;142:1391.
22. Creighton FJ, Black DL, Hyde CE. Ecstasy psychosis and flashbacks. Br J Psychiatry 1991;159:713–15.
23. McCann UD, Ricuarte GA. Lasting neuropsychiatric effects of methylenedioxymethamphetamine (ecstasy) in recreational users. J Clin Psychopharmacol 1991;11:302–5.
24. Pallanti S, Mazzi D. MDMA (ecstasy) and precipitation of panic disorder. Biol Psychiatry 1992;32:91–4.
25. Ricuarte GA, Finnegan KJ, Irwin I, *et al.* Aminergic metabolites in the CSF of humans previously exposed to MDMA-preliminary observations. Ann NY Acad Sci 1990;648:371–82.
26. Peroutka S, Pascoe N, Faull KF. Monoamine metabolites in the CSF of recreational users of 3,4 methylenedioxymethamphetamine (MDMA). Res Commun Subst Abuse 1987;8:125–8.
27. Tucker GT, Lennard MS, Ellis SW, *et al.* The demethylation of methylenedioxymethamphetamine (ecstasy) by debrisoquine hydroxylase (CYP2D6). Biochem Pharmacol 1995;47:1151–6.
28. Mahgoub A, Idle JR, Dring LG, *et al.* Polymorphic hydroxylation of debrisoquine in man. Lancet 1977;ii:584–6.
29. Kimura S, Umeno M, Skoda R, *et al.* The human debrisoquine 4-hydroxylase (CYP2D6) locus: sequence and identification of the polymorphic CYP2D6 gene, a related gene, and a pseudogene. Am J Human Genet 1989;45:889–904.
30. Colado MI, Williams JL, Green AR. The hyperthermic and neurotoxic effects of 'Ecstasy' and 3,4 methylenedioxyamphetamine (MDMA) in the Dark Agouti (DA) rat, a model of the CYP2D6 poor metabolizer phenotype. Br J Pharmacol 1995;115:1281–9.
31. Al-Dabbagh SG, Idle JR, Smith RL. Animal modelling of human polymorphic drug oxidation—the metabolism of debrisoquine and phenacetin in the rat inbred strains. J Pharm Pharmacol 1981;33:161–4.
32. Gill M, Hawi Z, Webb M. Homozygous mutations at cytochrome P4502D6 in an individual with schizophrenia: implications for antipsychotic drugs, side effects and compliance. Irish J Psychol Med 1997;14:38–9.