

Aplastic anaemia following exposure to 3,4-methylenedioxymethamphetamine ('Ecstasy')

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Summary. We report two cases of aplastic anaemia following exposure to 'Ecstasy' (MDMA, 3,4-methylenedioxymethamphetamine). In both cases the aplastic anaemia resolved spontaneously 7-9 weeks after presentation. Long-term bone marrow culture study of one patient demonstrated complete normalization of haemopoiesis at time of haematological recovery, suggesting either that damage to the haemopoietic stem cell had been only transient, or that a more mature, committed progenitor

cell was the target. Because MDMA may have been a factor in the aetiology of the bone marrow suppression in these two cases, we recommend close haematological monitoring of young adults presenting with toxicity from MDMA, and a detailed history of exposure to recreational drugs in all new patients presenting with aplastic anaemia.

Keywords: aplastic anaemia, 'Ecstasy', 3,4-methylenedioxymethamphetamine, long-term marrow culture.

MDMA (3,4-methylenedioxymethamphetamine, or 'Ecstasy') is a Class I controlled drug. It was originally patented as an appetite suppressant and later investigated as a mood-modifying drug. Over the last 5-6 years there have been an increasing number of reports of acute severe toxicity and deaths following the recreational misuse of this drug. Such toxicity includes hyperthermia, cardiac arrhythmias, acute renal failure, rhabdomyolysis, disseminated intravascular coagulation, convulsions and hepatotoxicity (Dowling *et al.* 1987; Brown & Osterloh, 1987; Chadwick *et al.* 1991; Henry *et al.* 1992; Fahal *et al.* 1992; Barrett & Taylor, 1993).

We report two cases of aplastic anaemia (AA), one presenting 2 months following ingestion of 'Ecstasy', and the other case occurring after a 12-month period of regular recreational use of 'Ecstasy', LSD and cocaine. Spontaneous recovery of the AA occurred in both patients within 7-9 weeks of presentation. In the absence of a recognized cause of the AA, we suggest that MDMA may have been a common factor in the aetiology of the marrow aplasia in these patients.

CASE REPORT 1

A 21-year-old male student (patient 1) presented in February 1992 with a 1 month history of a flu-like illness with headache, cough, malaise and myalgia. This was followed 2 weeks later by spontaneous bruising, epistaxis and gum bleeding. He admitted to intermittent ingestion of 'Ecstasy', LSD and cocaine at 2-3-monthly intervals during the previous 12 months. His last ingestion of 'Ecstasy' occurred 1 week prior to presentation. Clinical examination revealed scattered petechiae and ecchymoses but no other abnormalities. Blood count showed a haemoglobin concentration (Hb) of 13.0 g/dl, white cell count (WBC) $5.4 \times 10^9/l$, and a platelet count $3 \times 10^9/l$. 2 weeks later his Hb had fallen to 9.0 g/dl with a reticulocyte count of < 0.1%, WBC was $2.1 \times 10^9/l$, neutrophil count $0.4 \times 10^9/l$, and platelet count $8 \times 10^9/l$ despite platelet transfusions. Bone marrow aspirate and trephine biopsy were hypocellular with absent megakaryocytes and reduced erythropoiesis and granulopoiesis, and there was no increase in marrow reticulin. These findings were consistent with a diagnosis of severe aplastic anaemia. The marrow aspirate also demonstrated vacuolation of residual myeloid and early erythroid precursors. Ham's test and urinary haemosiderin were negative, and coagulation screen, serum B12 and red cell folate were normal. No abnormalities of serum

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electrolytes, urea, creatinine and liver function tests were detected, and there was no serological evidence of a recent viral infection (hepatitis A, B and C, EBV, CMV, HIV and parvovirus). He required regular platelet transfusions for 3 weeks, but then a spontaneous, sustained improvement in his blood count occurred (see Fig 1, patient 1). 2 months later his blood count was normal (Hb 14.1 g/dl, WBC $6.3 \times 10^9/l$, neutrophils $4.0 \times 10^9/l$ and platelets $205 \times 10^9/l$), although his MCV was mildly increased at 99 fl. His blood count remains normal, apart from an MCV of 98 fl, 20 months after initial presentation.

CASE REPORT 2

An 18-year-old female student (patient 2) was admitted in April 1992 with an 11 d history of general malaise. Clinical examination was normal apart from pallor, tachycardia and a fever of 40°C. She had ingested 'Ecstasy' once in February and twice in March 1992, but there was no exposure to any other drugs or chemicals. Blood count showed Hb 6.8 g/dl, reticulocytes <1%, WBC $1.2 \times 10^9/l$, neutrophils $0.5 \times 10^9/l$, platelets $20 \times 10^9/l$ and MCV 90 fl. Bone marrow aspirate was moderately hypocellular with reduced erythropoiesis, granulopoiesis and megakaryocytes, consistent with non-severe aplastic anaemia. There was also vacuolation particularly in the myeloid precursors which was also present, but less marked, in the erythroid precursors. Bone marrow cytogenetic analysis was normal. There was no increase in spontaneous or diepoxybutane-induced chromatid breakages of cultured peripheral blood lymphocytes, thus excluding a diagnosis of Fanconi anaemia. Ham's test, viral screen (hepatitis A, B and C, EBV, CMV and HIV) and autoantibody screen were all negative. Liver function tests and serum urea and creatinine were normal. During the next 10 d the neutrophil count fell to $0.3 \times 10^9/l$ and platelet count to $15 \times 10^9/l$. Whilst being assessed for possible bone marrow transplant, her blood count then began to improve spontaneously (see Fig 1, patient 2). 2 months after initial presentation, the blood count was normal with Hb 12.1 g/dl, WBC $4.1 \times 10^9/l$ and platelet count $344 \times 10^9/l$.

METHODS

Bone marrow specimens. Normal bone marrow cells were obtained from posterior iliac crest marrow aspirates from haematologically normal donors after informed consent. Marrow specimens from aplastic anaemia patients were part of aspirates taken for diagnostic purposes. Aspirates were diluted 1:1 Iscove's Modification of Dulbecco's Medium (IMDM, Gibco) supplemented with 100 IU/ml penicillin-streptomycin and 10 U/ml preservative-free heparin, centrifuged on Ficoll-Hypaque, and the bone marrow mononuclear cells (BMMC) obtained were washed twice in the above supplemented medium.

Committed bone marrow progenitor assay. Normal and AA BMMC (10^5) were cultured in 1 ml IMDM supplemented with 30% fetal calf serum (FCS), 1% deionized bovine serum albumin (BSA, Sigma), 10^{-4} M mercaptoethanol and 0.9%

methylcellulose (Terry Fox Laboratory, Vancouver), in 35 mm petri dishes. Growth factor stimulus was 100 ng/ml GM-CSF (5.6×10^6 U/mg, mammalian CHO derived recombinant glycosylated, Sandoz Pharma) and 100 ng/ml IL-3 (8.8×10^6 U/mg, *E. coli* derived recombinant human aglycosylated, Sandoz Pharma). Cultures were set up in duplicate and incubated at 37°C in 5% CO₂/95% air. EPO (2 U/ml, Eprex, Cilag Ltd) was added to all cultures on day 3. On day 14, CFU-GM, BFU-E and CFU-Mix were scored. All reagents were pretested for their ability to support optimal growth.

Long-term bone marrow culture (LTBMC). A modification of previously described techniques was used for the establishment and maintenance of LTBMC (Dexter *et al.*, 1977; Gartner & Kaplan, 1980). Normal and AA BMMC (10^7) were cultured in 25 cm² flasks in 10 ml IMDM (Gibco) supplemented with 10% FCS (ICN Flow), 10% horse serum (ICN Flow) and 10^{-6} M hydrocortisone sodium succinate. Cultures were incubated at 33°C in 5% CO₂/95% air. At weekly intervals, cultures were fed by demi-depopulation of the non-adherent cells and replacement of 5 ml fresh supplemented IMDM. Non-adherent cells were counted, resuspended in IMDM and assayed for committed progenitor cells as above. LTBMC were terminated at week 5.

Statistical analysis. The difference in (a) progenitor cell number between normal and AA bone marrow and patient 1 in clonogenic cultures, and (b) the differences between: CFU-GM in patient 1, normal controls and AA controls at initiation of LTBMC and those harvested at each weekly feeding of the LTBMC, were analysed using the Student's *t*-test after logarithmic transformation of the data.

RESULTS

Haematological recovery

Both patients had a rapid recovery of peripheral blood counts within 7 and 9 weeks respectively (see Fig 1). An early rise in the platelet count was quickly followed by the neutrophil count, with a later gradual increase in haemoglobin concentration. In patient 2 there was a rebound overshoot of the platelet count which then fell back into the normal range. Repeat bone marrow aspirate and trephine biopsy was performed in each patient during the phase of haematological recovery and demonstrated that the marrow cellularity had returned to normal, with normal activity and maturation of erythropoiesis and granulopoiesis and normal numbers of megakaryocytes. Vacuolation of marrow precursors was less evident in both cases.

Clonogenic marrow cultures (patient 1)

At diagnosis, multipotent CFU-Mix, bipotent CFU-GM and early erythroid progenitors (BFU-E) from patient 1 were reduced compared with normal controls ($P < 0.0001$) and comparable to results from patients with untreated aplastic anaemia (see Table 1). 6 weeks later, when haematological recovery was evident, CFU-Mix were still undetectable. However, CFU-GM had increased to a value greater than that seen in untreated AA patients and in a control group of AA patients who had been treated with antilymphocyte globulin (ALG). Some recovery of BFU-E in patient 1 was also evident.

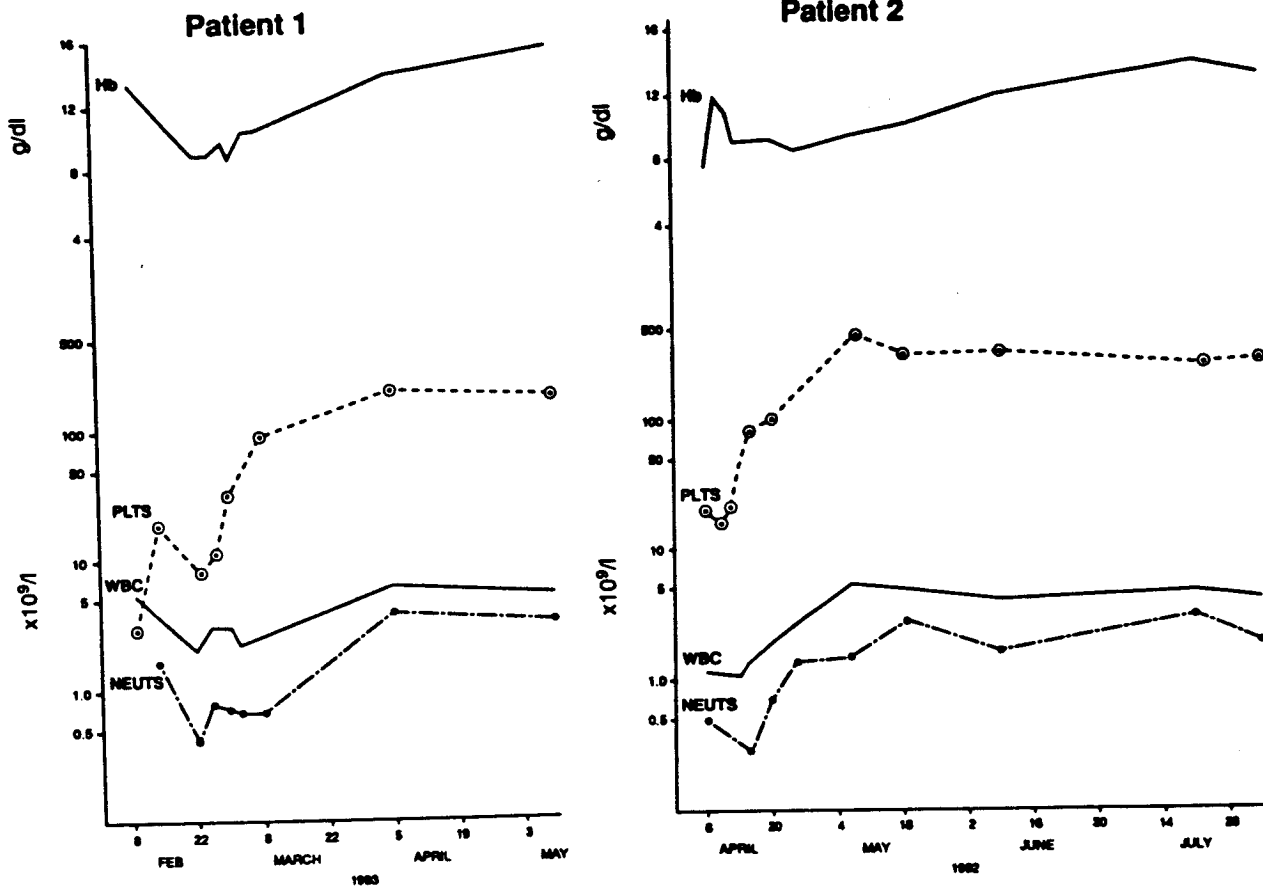


Fig 1. Haematological charts of the two patients, summarizing blood counts from initial presentation to time of haematological recovery. Hb = haemoglobin concentration (g/dl); PLTS = platelet count $\times 10^9/l$; WBC = white cell count $\times 10^9/l$; NEUTS = neutrophil count $\times 10^9/l$. The y axis is a log scale for WBC, NEUTS and PLTS, and an arithmetic scale for Hb.

Table I.

	No. of colonies/ 10^5 BMMC plated		
	CFU-Mix	CFU-GM	BFU-E
Patient 1 at presentation	0	17	2
Patient 1 on recovery	0	63	7
Untreated AA patients* (n = 5)	1 ± 1 (0-3)	14 ± 5 (2-30)	5 ± 3 (0-16)
Treated AA patients† (n = 3)	1 ± 1 (0-2)	33 ± 13 (8-51)	7 ± 5 (0-16)
Normal controls (n = 10)	6 ± 1 (1-14)	168 ± 15 (98-226)	88 ± 12 (40-142)

* Untreated AA patients comprised four with severe AA and one with non-severe AA.

† Treated AA patients had received antilymphocyte globulin. There was one complete responder (transfusion independent), one partial responder and one non-responder. Values shown for untreated and treated AA patients and normal controls are mean \pm 1 SE, with the range in parentheses. Results for both groups of AA patients were significantly reduced compared with normal controls ($P < 0.001$ for all assays, except CFU-GM in untreated AA patients, $P = 0.01$, and CFU-Mix in treated AA patients, $P = 0.03$). Cultures from patient 1 were set up in duplicate at diagnosis and were significantly reduced compared with normal controls, $P < 0.0001$, and repeated 6 weeks later during haematological recovery with an increase in colony numbers (see Results).

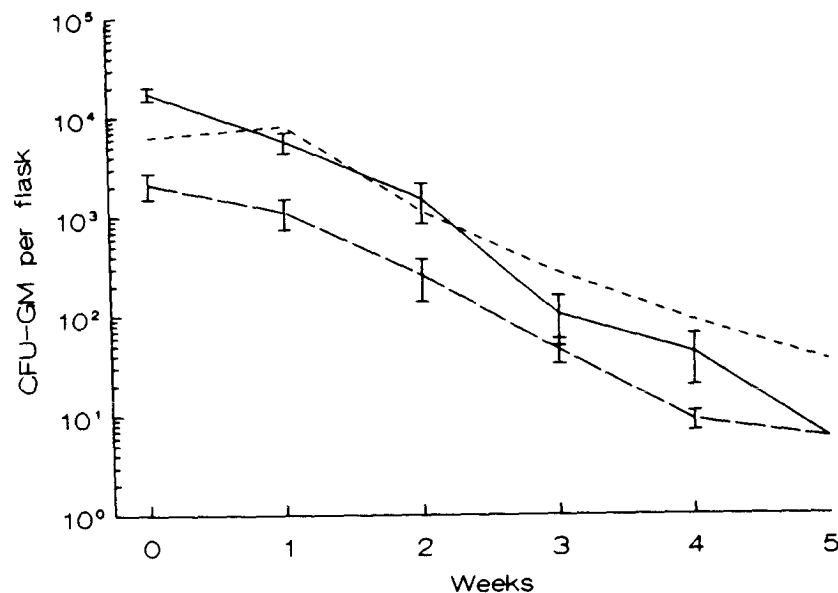


Fig 2. Growth of bone marrow from patient 1 (---) in long-term bone marrow culture (LTBMC). Also shown for comparison are results from a group of eight AA patients (—) and five normal controls (---). For weeks 0, 1 and 2, $P = 0.002$, 0.04 and 0.03 respectively. Weeks 3 and 4 were not statistically significantly different compared with aplastic anaemia controls, and for week 5, $P = 0.01$. Total CFU-GM were assayed from the non-adherent layer of the cultures. No correction has been made for weekly feeding of cultures. Results are expressed as the mean ± 1 SE.

Long-term bone marrow culture study (patient 1)

Insufficient marrow cells were obtained at diagnosis from patient 1 for LTBMC. 6 weeks later, when the blood count was normal, LTBMC study demonstrated normal stromal layer formation and normal generation of haemopoietic progenitor cells. There was no statistical difference between patient 1 and normal controls in numbers of CFU-GM generated weekly from LTBMC. This contrasts with a major defect in haemopoietic function seen in all the eight control patients with AA whether treated or untreated, where there was a significant reduction in the generation of progenitor cells during the 5 weeks of culture compared with normal controls (see Fig 2).

DISCUSSION

In approximately 30% of cases of AA an identifiable factor or agent, such as a drug or virus, may be implicated in the aetiology of the bone marrow failure (Gordon-Smith, 1979; Young, 1991). In the remaining 70% a recognizable cause cannot be identified. Spontaneous recovery of AA may occur, but it is a rare and unpredictable event (Gordon-Smith & Issaragrisil, 1992). This transient form of AA may sometimes be seen following viral infection due to EB virus, non A-non B hepatitis, or hepatitis A (Smith *et al.*, 1978; Dhingra *et al.*, 1988; Lazarus & Baehner, 1981) or occasionally after drug exposure (Isareli *et al.*, 1985; Keisu *et al.*, 1990; Khelif *et al.*, 1993). Neither of the two patients presented here, however, had evidence of recent exposure to any viruses that are known to cause AA.

There is no *in vitro* assay that will prove whether a particular drug is responsible for AA in an individual.

However, if the drug is taken within 6 months of presentation of the AA there is a stronger case for a temporal association, and this association becomes weaker if exposure occurs later than 6 months (Gordon-Smith, 1979). The latent period from exposure to MDMA and presentation of AA in these patients was 1–2 months and 1–12 months respectively. The relevance of the additional exposure of patient 1 to LSD and cocaine to the subsequent development of AA is not known.

The long-term bone marrow culture (LTBMC) system represents the most physiological *in vitro* system to assess stromal cell mediated haemopoiesis (Dexter *et al.*, 1977; 1990). LTBMC studies in AA have consistently demonstrated a severe haemopoietic defect in all patients, with a normal functioning stromal cell microenvironment in most cases (Gibson & Gordon-Smith, 1990; Marsh *et al.*, 1990). These changes still persist following haematological recovery with immunosuppressive agents such as antilymphocyte globulin (ALG), indicating residual stem cell damage. Restoration of normal haemopoietic function in patients with AA who have been treated with ALG, as assessed by standard LTBMC, has not been documented previously. Furthermore, we are aware of only one patient with spontaneous recovery of AA following a viral infection (non A-non B hepatitis) whose bone marrow has been studied in LTBMC. A full haematological recovery was associated with only partial improvement of haemopoietic function in LTBMC (Marsh *et al.*, 1990). The LTBMC study demonstrating normal generation of haemopoietic progenitors following haematological recovery in patient 1 suggests that damage to the haemopoietic stem cells may have been transient, possibly induced by MDMA, with subsequent

recovery of stem cell function. Alternatively, a more committed progenitor cell could have been the target for damage by MDMA.

The mechanism of MDMA-induced toxicity is not known. Adverse reactions may be idiosyncratic or related to unidentified impurities. Alternatively, genetic differences in metabolism of MDMA may predispose an individual to its toxicity. Cytochrome P450s are important drug-metabolizing enzymes and the debrisoquine polymorphism is an example of a P450 polymorphism (Smith *et al.* 1978; Mayer *et al.* 1990). The human cytochrome P45011D6 (CYP2D6) gene is responsible for the debrisoquine polymorphism and is absent in 8% of caucasians ('poor metabolizers') (Gonzales & Meyer, 1991). Other commonly prescribed drugs have now also been identified as examples of P450 polymorphisms (Lennard, 1990), and recent work has shown that cytochrome P450 in liver and brain is also responsible for the metabolism of MDMA by demethylenation of MDMA to the catechol 3,4-dihydroxymethylamphetamine (DHMA) (Maurer *et al.*, 1993; Tucker *et al.*, 1994). Identification of poor metabolizers of MDMA may predict those individuals who are particularly sensitive to MDMA and hence at more risk of toxicity.

We are not aware of any other reports of bone marrow suppression following ingestion of MDMA. In order to evaluate further this possible association, we suggest that monitoring of the blood count is done routinely in all young adults presenting with evidence of MDMA toxicity. Conversely, for all new patients presenting with AA, particularly young adults, a detailed drug history should include exposure to recreational drugs such as MDMA.

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