

A case of fatal intoxication due to the new designer drug 25B-NBOMe

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Abstract *N*-Benzyl-substituted phenethylamines (NBOMes) have emerged as novel hallucinogenic designer drugs with potent serotonin-receptor activation. We present the first scientific case report of fatal intoxication with 2-(4-bromo-2,5-dimethoxyphenyl)-*N*-(2-methoxybenzyl)ethanamine (25B-NBOMe). The plasma concentration of 25B-NBOMe upon admission was low, 3.15 ng/ml, but it exceeded concentrations reported previously in NBOMe intoxications cases. Our case documents complete clinical and pathophysiological findings that developed shortly after drug ingestion and highlights the danger of 25B-NBOMe use at small doses, based on autopsy and toxicological analyses. The patient presented symptoms consistent with serotonin syndrome.

Keywords 25B-NBOMe · 2-(4-Bromo-2,5-dimethoxyphenyl)-*N*-(2-methoxybenzyl)ethanamine · 25C-NBOMe · New designer drug · Serotonin syndrome · Rhabdomyolysis

Introduction

N-Benzyl-substituted phenethylamines (NBOMes) have emerged as novel designer drugs due to their availability online at low costs, and a lack of information on their toxicities at small doses [1,2]. Six NBOMe derivatives were shown to cause potent hallucinogenic and psychostimulatory effects through activation of 5-hydroxytryptamine (serotonin) HT_{2A} receptor and α -adrenergic receptor, respectively [2–4]. The use of 2-(4-bromo-2,5-dimethoxyphenyl)-*N*-(2-methoxybenzyl)ethanamine (25B-NBOMe), the most potent hallucinogenic derivative among them, has recently become prevalent worldwide [2,5] and is regulated by law in USA and Japan. 25B-NBOMe can be taken as a liquid, powder, tablet, or via preloaded blotter paper [2]. Two autopsy reports document traumatic deaths resulting from 25I-NBOMe-induced hallucination [6,7]. However, no reports of deaths due to 25B-NBOMe have been reported except in an abstract [8] or as general information on the Internet [2]. A report on seven cases of NBOMe intoxication demonstrated symptoms similar to serotonin syndrome [9], including a triad of mental-status changes, autonomic hyperactivity, and neuromuscular abnormalities [10].

Case history

A male drug dealer (approximately aged 20 years) ingested blotter paper laced with NBOMe (commercial name “Blue Magic Master”). He began speaking inarticulately and squealing, and became violent and convulsive at approximately 7:00 a.m. after night-driving with his friends. He calmed down after self-administration of hypnotics etizolam and flunitrazepam, but became violent again at

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approximately 7:50 a.m. When the ambulance service arrived (8:02 a.m.), he showed the symptoms of stupor, systolic hypotension (90 mmHg), tachycardia (156/min), tachypnea (48/min), hyperthermia (41.5 °C), mydriasis, and sluggish light reflex.

He was comatose on admission (8:53 a.m.) and found to have slight hypertension (139/44 mmHg), tachycardia, and hyperthermia. His hypotension progressed over time and required catecholamines. He was treated with activated carbon, a saline purgative and cold fluids, and underwent body cooling. Laboratory examination revealed thrombocytopenia, hemorrhagic diathesis, rhabdomyolysis, acidosis, and renal, hepatic and multiple other organ failure. Fresh frozen plasma, platelet concentrates, and concentrated red cells were infused repeatedly. Myoclonus appeared during the night, and diazepam and fosphenytoin were administered. Glycerol was infused to attenuate brain edema due to traumatic subarachnoid hemorrhage.

On the second day of admission, the rhabdomyolysis, systemic edema, hypotension, acidosis, and multiple organ failure progressed. Renal failure progressed to anuria, which required continuous renal replacement therapy. Catecholamines, the muscle relaxant vecuronium, and an antidiuretic hormone were infused. He was diagnosed with serotonin syndrome based on the psychological symptoms (mental derangement, coma, and agitation), autonomic symptoms (hyperthermia, sweating, tachycardia, tachypnea, diarrhea, and hypotension), and neurological symptoms (myoclonus, tendon hyper-reflexia, and mydriasis) [10]. He died approximately three and half days after ingestion of the blotter paper.

Autopsy findings

The obese man (height of 170 cm and weight of 81 kg) was autopsied 2 days postmortem at the University of Tokyo. There was subcutaneous hemorrhage in the right outer periocular, temporal (18 × 12 cm), and the right buccal (small) regions, as well as bilateral temporal muscle hemorrhage. The brain (1498 g) showed subdural hemorrhage in the right periocular region and subarachnoid hemorrhage, which had spread patchily over the left upper occipital lobe. Pleural (700 ml) and peritoneal (400 ml) effusion, as well as systemic edema, reflected vascular hyper-permeability and fluid infusion. Skeletal muscles appeared whitish and edematous. Histological examination showed rhabdomyolysis with fragmentation in the iliopsoas muscle (Fig. 1a). Immunostaining revealed myoglobin leakage from the muscles (Fig. 1b). The lungs (left: 1209 g, right: 1197 g) showed severe edema and congestion. The heart (total weight: 420 g) showed concentric ventricular hypertrophy, without coronary sclerosis.

Microscopic observation also disclosed focal myocardial fragmentation with hyper-eosinophilia, reflecting hyper-contraction (Fig. 1c). The kidneys showed a cortical “shock kidney” appearance. Immunostaining showed diffuse renal myoglobin deposition (Fig. 1d).

Toxicological analysis

Extraction procedure

25B-NBOMe, 25C-NBOMe, etizolam, flunitrazepam, and 7-aminoflunitrazepam were simultaneously extracted using a liquid–liquid extraction. Plasma (200 µl) was transferred to a glass test tube. As internal standards, 10 µl of 100 ng/ml methoxyphenamine hydrochloride aqueous solution was used for 25B-NBOMe and 25C-NBOMe; 4 µl of 1 µg/ml diazepam-*d*₅ acetonitrile solution was used for etizolam and flunitrazepam; and 4 µl of 1 µg/ml 7-aminoflunitrazepam-*d*₇ acetonitrile solution was used for 7-aminoflunitrazepam. They were added to the plasma, and after briefly mixing by vortex, 1 ml of 5 % ammonium aqueous solution (28 % ammonium solution regarded as 100 %) and 4 ml of ethyl acetate were added to the tube. The mixed solution was shaken for 10 min and then centrifuged for 10 min (1000 g, 4 °C). The organic layer was transferred to another glass test tube and evaporated to dryness under a stream of nitrogen at 40 °C. The dried residue was reconstituted in 100 µl of 10 mM ammonium formate in 0.1 % formic acid aqueous solution/methanol (2:8, v/v), and 4 µl of the supernatant was injected into the liquid chromatography–tandem mass spectrometry (LC–MS–MS) system.

LC–MS–MS conditions

Qualitative and quantitative analyses were performed using a Shimadzu Nexera UHPLC system coupled with a Shimadzu LCMS-8030 triple quadrupole mass spectrometer (Shimadzu, Kyoto, Japan). Chromatographic separation was achieved using a Kinetex C18 column (100 × 2.1 mm i.d., particle size 2.6 µm, Phenomenex, Torrance, CA, USA) maintained at 40 °C. The mobile phase consisted of 10 mM ammonium formate with 0.1 % formic acid in water (A) and methanol (B). The flow rate was held at 0.4 ml/min. The gradient program for 25B-NBOMe and 25C-NBOMe analysis was as follows: 5–65 % B from 0–11.5 min, 65–95 % B from 11.5–13.5 min, and 95 % B until 15 min. At 15.1 min, the concentration of B was returned to 5 % and was held until 17.5 min. For benzodiazepines, the gradient was 5–95 % B from 0–5 min and 95 % B until 6.5 min. At 6.51 min, the concentration of B was returned to 5 % and held until 9 min. The mass spectrometer was operated in the positive mode with an electrospray

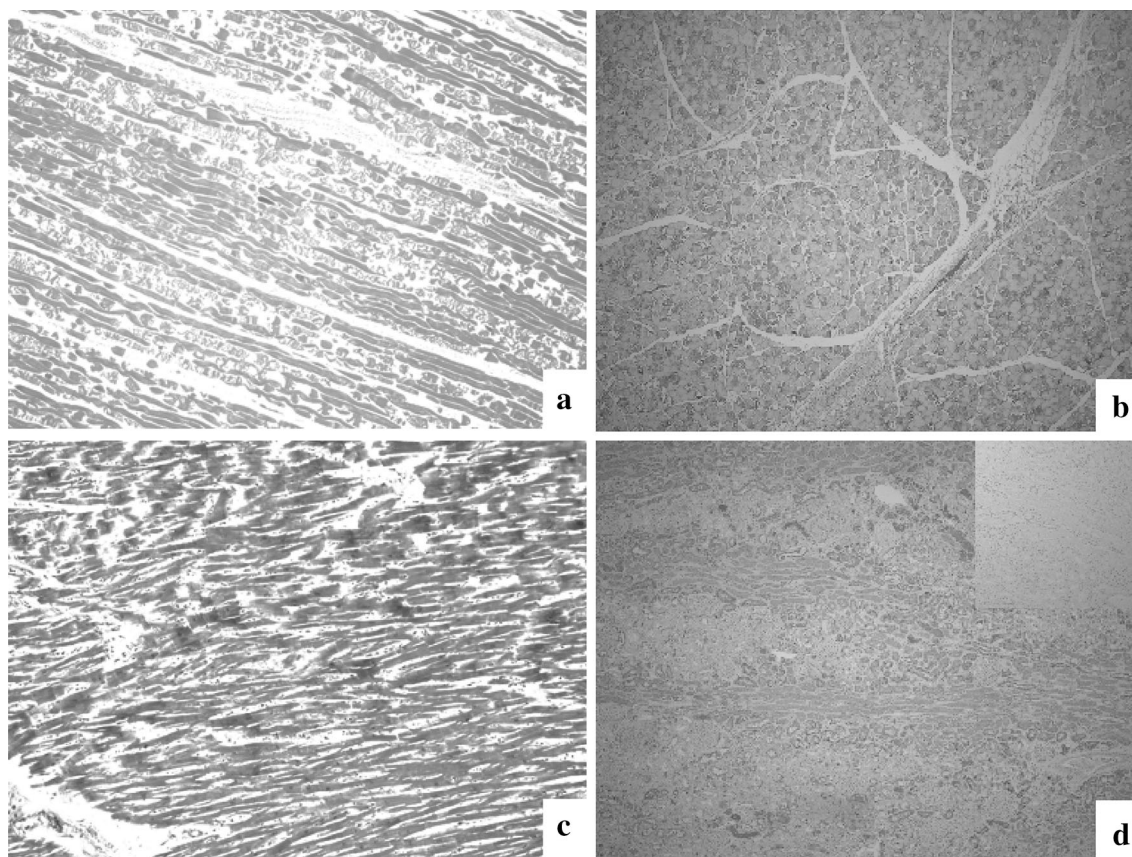


Fig. 1 Histological findings. **a** The iliopsoas muscle with rhabdomyolysis (hematoxylin–eosin staining). **b** Myoglobin leakage (immunostaining with anti-myoglobin antibody) from the iliopsoas muscle. **c** Myocardial fragmentation with hyper-eosinophilia (Elastic-

Masson staining). **d** Myoglobin deposition in the kidney (immunostaining). The *right-upper inset* shows a kidney section without incubation with anti-myoglobin antibodies

ionization interface. Analytes were detected using the multiple reaction monitoring (MRM) mode. In the MRM transitions, two product ions (m/z) were monitored for each compound: 25B-NBOMe 380 > 121 (23) and 380 > 91 (49), 25C-NBOMe 336 > 121 (22) and 336 > 91 (48), methoxyphenamine 180 > 121 (22) and 180 > 149 (16), etizolam 343 > 314 (27) and 343 > 138 (40), flunitrazepam 314 > 268 (30) and 314 > 239 (35), diazepam- d_5 290 > 154 (30) and 290 > 198 (35), 7-aminoflunitrazepam 284 > 135 (30) and 284 > 226 (35), and 7-aminoflunitrazepam- d_7 291 > 138 (30) and 291 > 230 (35). The former in the pair of product ions was used as a quantifier and the latter was used as a qualifier. The values in parentheses after each transition represent the collision energy (V).

Results and discussion

Validation of the method

Calibration curves (concentration–peak area) were generated at 0.025–5 ng/ml (8 points) for 25B-NBOMe

($r = 0.9998$, $y = 0.680x + 0.00169$), at 0.015–0.5 ng/ml (6 points) for 25C-NBOMe ($r = 0.9988$, $y = 1.25x + 0.0000790$), at 0.1–20 ng/ml (8 points) for etizolam ($r = 0.9999$, $y = 1.13x - 0.000334$), at 0.1–5 ng/ml (6 points) for flunitrazepam ($r = 0.9999$, $y = 0.859x - 0.000421$), and at 0.5–20 ng/ml (6 points) for 7-aminoflunitrazepam ($r = 1.0000$, $y = 0.723x - 0.00111$). Precision, accuracy, recovery, matrix effect, limit of detection (LOD: signal/noise ≥ 3), and limit of quantification (LOQ: signal/noise ≥ 10) are summarized in Table 1. Precision ($n = 10$), accuracy ($n = 10$), recovery ($n = 4$), and matrix effect ($n = 4$) were determined by replicate analyses at each concentration of analytes (approximately the mid point of the calibration range).

Quantification of 25B-NBOMe, 25C-NBOMe, and hypnotics

Preliminary survey of the blotter paper detected 25B-NBOMe and 25C-NBOMe. LC–MS–MS revealed 3.15 ng/ml of 25B-NBOMe, and 0.43 ng/ml of 25C-NBOMe in plasma at admission (Table 2). The plasma 25B-NBOMe

Table 1 Precision, accuracy, recovery, matrix effect, LOD, and LOQ of analytes

Analyte	Concentration (ng/ml)	Precision (%RSD)	Accuracy (%)	Recovery (%)	Matrix effect (%)	LOD (ng/ml)	LOQ (ng/ml)
25B-NBOMe	2	5.02	100	85.8	96.6	0.01	0.025
25C-NBOMe	0.2	6.38	101	90.5	96.4	0.005	0.015
Etizolam	5	4.96	94.1	95.0	102	0.02	0.1
Flunitrazepam	1	6.55	103	88.2	79.8	0.025	0.1
7-Aminoflunitrazepam	2	5.94	97.4	77.8	67.8	0.1	0.5

LOD limit of detection, LOQ limit of quantification, RSD relative standard deviation

Table 2 Antemortem and postmortem concentrations (ng/ml) of 25B-NBOMe, 25C-NBOMe, and benzodiazepines in plasma samples of the patient

Date/time (post-admission)	25B-NBOMe	25C-NBOMe	Etizolam	Flunitrazepam	7-Aminoflunitrazepam	
1st day	9:05 (0)	3.15	0.433	12.9	1.92	2.70
	15:00 (~6 h)	0.448	0.050	3.53	0.487	1.18
	21:00 (~12 h)	0.160	0.018	2.30	0.292	0.934
2nd day	4:00 (~19 h)	0.115	<0.015	1.18	0.190	0.814
	14:00 (~29 h)	0.065	<0.015	0.616	0.162	0.679
	21:00 (~36 h)	0.049	<0.015	0.496	0.118	0.667
3rd day	4:00 (~43 h)	0.027	<0.015	0.285	<0.1	0.524
	20:00 (~59 h)	0.029	<0.015	0.198	<0.1	0.570
4th day	4:00 (~67 h)	<0.025	<0.015	0.195	<0.1	0.575
	15:00 (~78 h)	<0.025	<0.015	0.170	<0.1	0.523
6th day	Right heart cavity ^a	0.177	0.018	0.154	<0.1	0.534
	Left heart cavity ^a	0.360	0.039	0.145	<0.1	0.574
	Urine ^a	0.051	<0.015	<0.1	<0.1	<0.5

^a Autopsy sample

levels were 0.448 and 0.16 ng/ml, at 6 and 12 h, respectively, post-admission. The concentrations of 25B-NBOMe on the 4th day was below the LOQ (0.025 ng/ml), but the postmortem concentration was increased (0.36 ng/ml in the left heart). In addition, etizolam, flunitrazepam, and 7-aminoflunitrazepam (metabolite), which were suspected of being self-administered, were detected (Table 2). The benzodiazepine concentrations were similar to those at the therapeutic levels [11]. In addition, as benzodiazepines are used to treat serotonin syndrome [10], these drugs are thought not to have contributed to the death.

This is the first scientific report on a case of fatal intoxication due to 25B-NBOMe, in contrast to the two autopsy reports on traumatic deaths resulting from 25I-NBOMe-mediated hallucinations [6,7]. 25B-NBOMe and 25C-NBOMe in the patient were presumably derived from the same dubious product [5]. This case report documents the extremely rare clinical and pathophysiological changes that developed shortly after drug ingestion, together with a full autopsy, and histological and toxicological analyses.

25B-NBOMe and 25C-NBOMe have been thought to be highly active at extremely small doses [2,4]. The plasma 25B-NBOMe concentration was low (3.15 ng/ml) at admission, but far exceeded concentrations reported in other cases of non-fatal 25B-NBOMe intoxication (0.18 ng/ml) [12]. The symptoms of NBOMe intoxication were thought to emerge 15–60 min after administration, with the plateau lasting for 3–4 h and a total duration of 8–10 h [2]. According to the police investigation, he was estimated to have taken the drug about 2–3 h before admission. Accordingly, 3.15 ng/ml would reflect a fatal blood concentration of 25B-NBOMe. Notably, the symptoms progressed during the 3 days before death, in contrast with the rapid decrease in 25B-NBOMe concentration in the blood. The blood concentration of 25C-NBOMe (0.433 ng/ml) was comparable to that reported in the cases of traumatic death related to 25I-NBOMe intoxication (0.41 ng/ml) [7]. It should be noted that the concentrations of 25B-NBOMe in our case was much lower than the fatal concentrations of methamphetamine (1–18 µg/ml) and 3,4-methylenedioxymethamphetamine (MDMA, 0.4–0.8 µg/ml) [11,13].

Thus, this case, free from any other illicit drug use, diseases or serious injuries, provides the first solid evidence of the toxicity of 25B-NBOMe at an extremely small dose. Public awareness of this information is critical to prevent accidental 25B-NBOMe overdose.

Various serotonergic drugs including selective serotonin reuptake inhibitors, opiates, amphetamine-like illicit drugs, and herbal products have been implicated in serotonin syndrome [10]. Given the high affinities of 25B-NBOMe [14,15] with the 5-HT_{2A} receptor, the potent and persistent activation of the 5-HT_{2A} receptor by 25B-NBOMe would enhance and maintain the pathogenesis of serotonin syndrome, even at low doses. Thus, this case supports the notion that serotonin syndrome primarily contributes to the pathogenesis of NBOMe intoxication [9]. Meanwhile, NBOMes are supposed to deteriorate serotonin syndrome when used concomitantly with other illicit drugs (e.g., marijuana) [6] or other serotonergic drugs [7], but such drugs were not used in this case.

The patient's plasma at admission contained etizolam and flunitrazepam, both of which are supposedly beneficial for patients with serotonin syndrome [9]. 25B-NBOMe was eliminated very rapidly from the blood (the concentrations at 6 and 12 h were 14.2 and 5.1 % of the concentration at admission, respectively) through rapid fluid infusion and detoxification procedures. Despite the hypnotic administration and the rapid 25B-NBOMe elimination, symptoms deteriorated, resulting in death through sustained 5-HT_{2A} activation by 25B-NBOMe. The interaction of 25B-NBOMe with tissue 5-HT_{2A} receptor remains to be addressed in clinical and experimental studies. In an animal model of serotonin syndrome, post-administration of a 5-HT_{2A} antagonist mirtazapine completely inhibited hyperthermia induced by two serotonergic drugs [16].

Concentrations of 25B-NBOMe and 25C-NBOMe were much higher postmortem than those after the 3rd day of admission. From detailed analyses of specimens at autopsies and animal studies, basic drugs such as methamphetamine were proposed to diffuse postmortem into the left cardiac cavity from the lungs via the pulmonary vein, thereby increasing postmortem intra-cardiac concentrations [17]. Postmortem intra-cardiac concentrations of 25B-NBOMe (basic drug) would have increased through redistribution from the lungs [13,17].

Conclusions

This report on fatal 25B-NBOMe intoxication with clinical, histological and toxicological analyses showed that administration of 25B-NBOMe at relatively lower doses is much more dangerous than administration of methamphetamine and MDMA. It is important to collect blood

samples upon hospital admission for toxicological analyses (lethal and toxic concentrations) in cases suspected of NBOMe-related intoxication.

Conflict of interest There are no financial or other relations that could lead to a conflict of interest.

Ethical approval Informed consent was obtained from all individuals included in the study, who supplied several milliliters each of blood as blank matrix for validation experiments.

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