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Original Article

Genotoxic Effects of *N*-nitrosoketamine and Ketamine as Assessed by In Vitro Micronucleus Test in Chinese Hamster Lung Fibroblast Cell Line

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Abstract

Objectives: Ketamine hydrochloride (KT) is a secondary amine that has been safely used as an injectable anesthetic and analgesic to avoid the production of nitroso compounds in the stomach. However, ketamine in the tablet form has recently become an abused, recreational drug. The aim of this study was to investigate the genotoxic effects of *N*-nitrosoketamine (NKT) and KT on the basis of an in vitro micronucleus (MN) test using a Chinese hamster lung fibroblast cell line (CHL/IU).

Methods: NKT was synthesized from KT in our laboratory. In the MN tests, CHL/IU cells were continuously treated with either NKT or KT for 24, 48, or 72 hours without the S9 mix. The cells were also treated with NKT or KT with or without the S9 mix for 6 hours, followed by a recovery period of 18, 42, or 66 hours (short-term treatment). The results were considered to be statistically significant when the p-values of both Fisher's exact test and the trend test were less than 0.05.

Results: After the short-term treatment with either NKT or KT with and without the S9 mix, the frequency of micronuclei significantly increased. However, the frequency of micronuclei did not significantly increase after the continuous treatment with either NKT or KT. Both NKT and KT were determined to be genotoxic in the short-term treatment with or without the S9 mix, but they were determined to be nongenotoxic in continuous treatment.

Conclusion: Our findings suggest that NKT has a stronger genotoxic effect than KT.

Key words: *N*-nitrosoketamine, ketamine, genotoxicity, CHL/IU, micronucleus test

Introduction

Secondary and tertiary amines react with nitrous acid to form *N*-nitroso compounds in vitro and in the stomach in vivo (1–3). Numerous animal studies have demonstrated the carcinogenicity of nitroso compounds (4–7). Ketamine ((±)-2-methylamino-2-(2-chlorophenyl)cyclohexanone) hydrochloride (KT), used as an intravenous or intramuscular anesthetic, is a secondary amine. However, KT in the tablet form has recently become an abused, recreational drug (8–12). We are concerned about the gastric production of *N*-nitrosoketamine (NKT) and its genotoxicity in persons who abuse KT in the tablet form. However, there have been so far no reports on the genotoxicity of NKT. The micronucleus (MN) test has been used as a short-term screening assay for detecting mutagens and carcinogens (13), and the MN test using a Chinese hamster lung fibroblast cell line (CHL/IU) (14) is the most widely used assay system in Japan. The aim of this study was to investigate the genotoxic effects of NKT and KT by the in vitro MN test.

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Materials and Methods

Chemicals

KT was purchased from Sigma-Aldrich (St. Louis, MO, USA). Eagle's minimum essential medium (MEM), Dulbecco's phosphate-buffered saline (PBS), dimethylsulfoxide, and glucose-6-phosphate (G-6-P) were also purchased from Sigma-Aldrich. Acetic acid, sodium nitrite ($NaNO_2$), chloroform ($CHCl_3$), sodium sulphate (Na_2SO_4), potassium chloride (KCl), magnesium chloride ($MgCl_2$), and benzo(a)pyrene (BaP) were purchased from Wako Pure Chemical Industries Ltd., (Osaka, Japan). Ethylenediaminetetraacetic acid disodium salt (EDTA)

was purchase from Dojindo Laboratories (Kumamoto, Japan). Trypsin, calf serum, and hydroxyethylpiperazinylethanesulfonic acid (HEPES) buffer solution were purchased from Invitrogen, (Carlsbad, CA, USA). Nicotineamide adenine dinucleotide phosphate disodium salt (NADP) was purchased from Roche Diagnostics (Indianapolis, IN, USA). Giemsa solution was purchased from Merck (Darmstadt, Germany). Mitomycin C (MMC) was purchased from Kyowa Hakko Kogyo Co., Ltd. (Tokyo, Japan). All chemical agents used were of the highest analytical grade commercially available.

Preparation of N-nitrosoketamine

NKT was prepared from KT in our laboratory by the following method. One gram of $NaNO_2$ was added to 1 g of KT dissolved in 12 ml of 20% acetic acid to form the *N*-nitroso compound. After stirring for 10 minutes, the mixture was poured into ice-cold water, and then the *N*-nitroso compound was extracted using $CHCl_3$. The organic phase was dehydrated by adding Na_2SO_4 and then it was evaporated to dryness. The liquid residue was crystallized at 10°C after being distilled in vacuo. The yield was 92%; the melting point was from 79°C to 80°C; the mass spectrum (EI) was m/z 152, 125, 58, 179, 236(M-NO)$^+$ and the mass spectrum (CI) was m/z 267(M+1)$^+$; ^{1}H-NMR chemical shifts (90 MHz, $CDCl_3$) were 1.89 ppm (m, 4H, -C_2H_4-), 2.63 ppm (m, 2H), 2.96 ppm (s, 3H, N-CH_3), 3.00 ppm (m, 2H), and 7.26 ppm (m, 4H, C_6H_4). NKT decomposed at higher than 220°C.

Preparation of S9 mix

S9 is the supernatant of male Sprague-Dawley rat liver homogenate, which was centrifuged at 9,000×g and purchased from Kikkoman Co., (Noda, Japan). The homogenate was prepared from 10 g of liver and 30 ml of 0.15 M KCl. Rats were pretreated with phenobarbital (PB) and 5,6-benzoflavone (BF) following the established schedule and then the liver was removed on the fifth day. PB was intraperitoneally (i.p.) injected into the rats at 30 mg/kg body weight on the first day, and 60 mg/kg body weight on the second, third and fourth days, and BF at 80 mg/kg body weight on the third day. The S9 mix contained 200 µl of 20 mM HEPES buffer solution (pH 7.2), 5 µmol of $MgCl_2$, 33 µmol of KCl, 5 µmol of G-6-P, 4 µmol of NADP, and 300 µl of the S9 fraction in a total volume of 1 ml (14, 15).

Cells

CHL/IU cells, obtained from the National Institute of Health Science (Tokyo, Japan), were maintained in MEM supplemented with 10% heat inactivated (56°C for 30 minutes) calf serum.

MN test

The MN test was performed by the method of Matsushima et al. (14) and Li et al. (15) with slight modifications. CHL/IU cells at concentrations from 1.0×10^4 to 1.0×10^5 cells per 5 ml of medium were plated on 60-mm-diameter Petri dishes. After incubating for 48 hours, the CHL/IU cells were exposed to NKT or KT continuously for 24, 48, or 72 hours without S9 mix (24, 48, or 72 hours; continuous treatment). For short-term treatment, the CHL/IU cells were exposed to NKT or KT for 6 hours with or without S9 mix followed by a recovery period of 18, 42, or 66 hours. The CHL/IU cells were washed several times with PBS, exposed to PBS containing 0.1% trypsin and 0.02% EDTA (trypsin-EDTA solution) for 5 minutes at 37°C, collected, and centrifuged at 200×g for 10 minutes. The collected cells were treated with a hypotonic solution (75 mM KCl) for 10 minutes, centrifuged at 200×g for 10 minutes, then suspended in methanol containing 25% acetic acid, centrifuged at 200×g for 10 minutes, fixed three times in methanol containing 2% acetic acid, spread onto clean slides, air dried, and stained with 3.5% Giemsa solution (1/150 M Sörensen's PBS, pH 6.4) for 12 minutes.

All procedures were performed at room temperature. The number of micronucleated cells was counted under a light microscope at 400× magnification. Cells were considered to be micronucleated if they had clear cytoplasmic borders with micronuclei whose diameter was not larger than one-third that of the nucleus. The number of micronucleated cells per 1000 counted cells was recorded.

NKT was dissolved in dimethylsulfoxide, and KT was dissolved in saline for the MN test; these solvents were used as negative controls. The doses of NKT and KT were established at several concentrations ranging from low to high in arithmetic or geometric ratios, while setting the doses of NKT or KT that produced 50% growth ratios of CHL/IU cells as medium doses. MMC was used as a positive control for both the continuous and short-term treatments without S9 mix, and BaP was used as a positive control for short-term treatment with S9 mix.

Statistical procedures

The frequencies of micronuclei were compared with those of the concurrent negative controls using Fisher's exact test. The dose-response relationship was evaluated with the Cochran–Armitage trend test (16, 17). The results were considered to be statistically significant when the p-values of both Fisher's exact test and the trend test were less than 0.05.

Results

The frequencies of micronuclei of cells treated with NKT are shown in Table 1. After the continuous treatment with NKT (Table 1) for 24 hours at a concentration of 31 µg/ml, the frequency of micronuclei induced was 2.2 times as high as that after the continuous treatment with the control solvent, but no increase was observed after treatment for 48 or 72 hours. After treatment with NKT for 24 hours at concentrations of 31, 63, 125, and 225 µg/ml, the increases in the micronuclei frequency were significant as determined using Fisher's exact test but not when determined using the trend test. In addition, after the continuous treatment with NKT for 24, 48, or 72 hours at a concentration of 225 µg/ml, a marked cytotoxicity was observed, and micronucleated cells were not counted after 72 hours because of cytotoxicity. After the short-term treatment, the frequencies of micronuclei of cells treated with NKT with (Fig. 1) and without (Fig. 2) S9 mix significantly increased in a dose-dependent manner. After the short-term treatment with NKT for 6 hours and the recovery periods of 18, 42, and 66

Table 1 Frequencies of micronuclei of cells treated with NKT

Treatment timeb (hours)	S9 mix	Concentration (μg/ml)	Micronucleated cells (‰)	Fisher's p-value	Trend p-value
24	–	0	11.0		p=0.168
	–	16	13.0	0.6636	
	–	31	24.5	0.0017	
	–	63	18.5	0.0383	
	–	125	16.5	0.0383	
	–	225	20.0 (Tox)	0.0289	
	–	MMC (0.02)	38.5	p<0.0001	
48	–	0	14.0		p=0.679
	–	16	11.5	0.5734	
	–	31	11.5	0.5734	
	–	63	9.5	0.2403	
	–	125	9.5	0.2403	
	–	225	12.0 (Tox)	0.6758	
	–	MMC (0.02)	164.5	p<0.0001	
72	–	0	11.0		p=0.734
	–	16	14.0	0.6758	
	–	31	13.5	0.5658	
	–	63	15.0	0.3287	
	–	125	13.0	0.6636	
	–	225	NT		
	–	MMC (0.01)	90.0	p<0.0001	
6+18R*	–	0	9.0		p<0.001
	–	100	10.0	0.7760	
	–	150	11.0	0.4851	
	–	200	11.5	0.4052	
	–	225	23.0	p<0.0001	
	–	250	41.5	p<0.0001	
	–	MMC (0.04)	45.5	p<0.0001	
6+18R*	+	0	8.5		p<0.001
	+	100	23.0	p<0.0001	
	+	150	37.0	p<0.0001	
	+	200	54.0	p<0.0001	
	+	225	39.0	p<0.0001	
	+	250	34.0	p<0.0001	
	+	BaP (10)	43.5	p<0.0001	
6+42R*	–	0	8.3		p<0.001
	–	100	12.5	0.1247	
	–	150	10.5	0.3875	
	–	200	11.5	0.2545	
	–	225	16.5	0.0057	
	–	250	68.0	p<0.0001	
	–	MMC (0.04)	45.0	p<0.0001	
6+42R*	+	0	9.3		p<0.001
	+	50	13.0	0.1823	
	+	100	60.0	p<0.0001	
	+	150	70.0	p<0.0001	
	+	225	122.0	p<0.0001	
	+	250	114.5	p<0.0001	
	+	BaP (10)	33.0	p<0.0001	
6+66R*	–	0	9.3		p=0.024
	–	140	12.0	0.3404	
	–	160	9.0	1.0000	
	–	180	10.5	0.6753	
	–	200	10.8	0.5745	
	–	225	15.5	0.0384	
	–	250	17.0	0.0114	
	–	MMC (0.04)	38.0	p<0.0001	
6+66R*	+	0	9.0		p<0.001
	+	100	47.0	p<0.0001	
	+	150	98.0	p<0.0001	
	+	200	98.0	p<0.0001	
	+	225	103.0	p<0.0001	
	+	250	71.5	p<0.0001	
	+	BaP (10)	51.0	p<0.0001	

R*: Treatment with NKT for 6 hours followed by recovery period of 18, 42 or 66 hours.
Tox: Cytotoxicity was observed but micronucleated cells were available for counting.
NT: Cytotoxicity was observed and micronucleated cells were not available for counting.

Table 2 Frequencies of micronuclei of cells treated with KT

Treatment time (hours)	S9 mix	Concentration (μg/ml)	Micronucleated cells (‰)	Fisher's p-value	Trend p-value
24	–	0	8.5		p=0.980
	–	50	9.5	0.8674	
	–	100	11.5	0.4273	
	–	200	9.5	0.8674	
	–	300	12.5	0.2775	
	–	400	10.0	0.7417	
	–	500	8.5	1.0000	
	–	MMC (0.02)	24.5	p<0.0001	
48	–	0	10.0		p=0.578
	–	50	11.0	0.8770	
	–	100	10.5	1.0000	
	–	200	8.5	0.7417	
	–	300	10.0	1.0000	
	–	400	8.0	0.6161	
	–	500	10.0	1.0000	
	–	MMC (0.02)	75.5	p<0.0001	
72	–	0	9.0		p=0.709
	–	50	11.0	0.6341	
	–	100	9.0	1.0000	
	–	200	8.5	1.0000	
	–	300	8.0	0.8636	
	–	400	8.5	1.0000	
	–	500	9.5	1.0000	
	–	MMC (0.01)	36.0	p<0.0001	
6+18R*	–	0	10.0		p<0.001
	–	800	10.0	1.0000	
	–	1000	10.5	0.8916	
	–	1200	9.5	1.0000	
	–	1400	15.5	0.0762	
	–	1600	24.0	p<0.0001	
	–	MMC (0.04)	42.5	p<0.0001	
6+18R*	+	0	9.8		p<0.001
	+	800	20.5	0.0012	
	+	1600	27.3	p<0.0001	
	+	1800	33.0	p<0.0001	
	+	2000	47.0	p<0.0001	
	+	BaP (10)	39.0	p<0.0001	
6+42R*	–	0	10.5		p<0.001
	–	800	9.5	0.7859	
	–	1000	9.5	0.7859	
	–	1200	9.0	0.6802	
	–	1400	8.0	0.4027	
	–	1600	34.5	p<0.0001	
	–	MMC (0.04)	39.0	p<0.0001	
6+42R*	+	0	9.3		p<0.001
	+	800	13.5	0.1437	
	+	1600	31.0	p<0.0001	
	+	1800	28.0	p<0.0001	
	+	2000	47.0	p<0.0001	
	+	BaP (10)	52.0	p<0.0001	
6+66R*	–	0	9.0		p=0.037
	–	800	13.5	0.1389	
	–	1000	8.0	0.7689	
	–	1200	8.0	0.7689	
	–	1400	10.0	0.7760	
	–	1600	19.5	0.0012	
	–	MMC (0.04)	35.5	p<0.0001	
6+66R*	+	0	10.0		p<0.001
	+	800	21.5	0.0006	
	+	1600	21.5	0.0006	
	+	1800	25.0 (Tox)	p<0.0001	
	+	2000	27.5 (Tox)	p<0.0001	
	+	BaP (10)	40	p<0.0001	

R*: Treatment with KT for 6 hours followed by recovery period of 18, 42 or 66 hours.
Tox: Cytotoxicity was observed but micronucleated cells were available for counting.

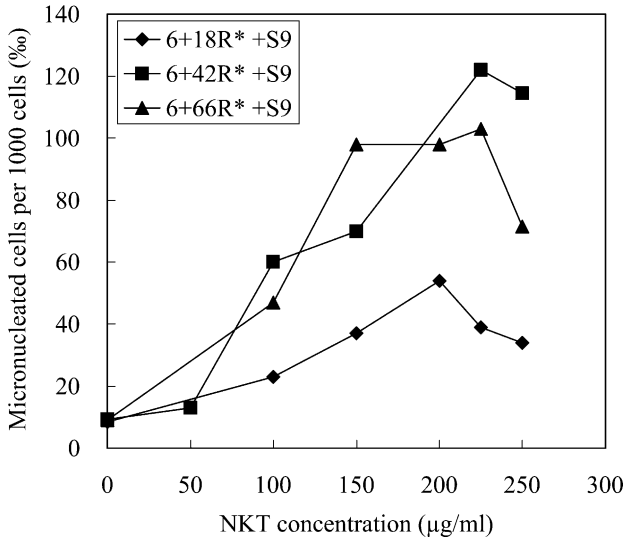

Fig. 1 Frequencies of micronuclei of cells treated with NKT in short-term treatment with S9 mix. R*: Treatment with NKT for 6 hours followed by recovery period of 18, 42 or 66 hours.

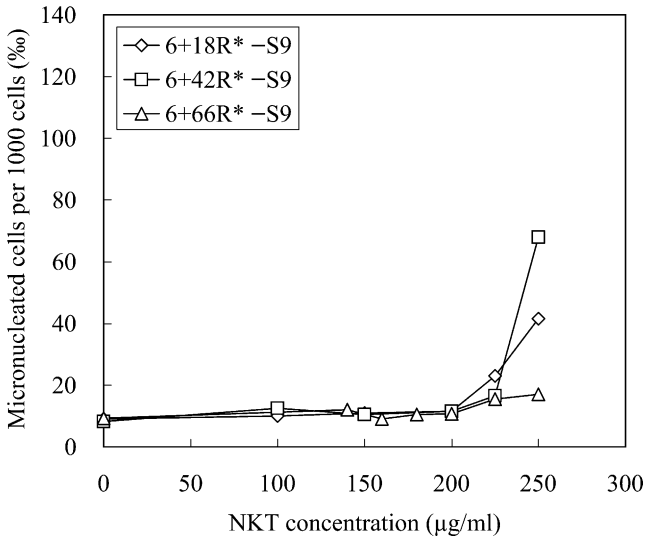

Fig. 2 Frequencies of micronuclei of cells treated with NKT in short-term treatment without S9 mix. R*: Treatment with NKT for 6 hours followed by recovery period of 18, 42 or 66 hours.

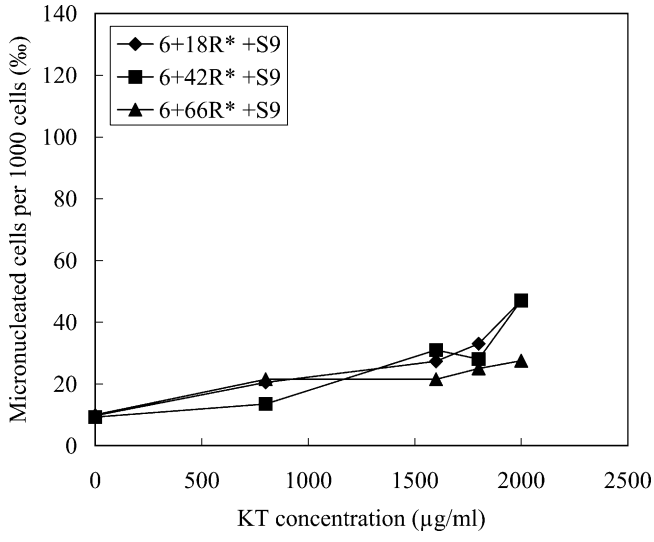

Fig. 3 Frequencies of micronuclei of cells treated with KT in short-term treatment with S9 mix. R*: Treatment with NKT for 6 hours followed by recovery period of 18, 42 or 66 hours.

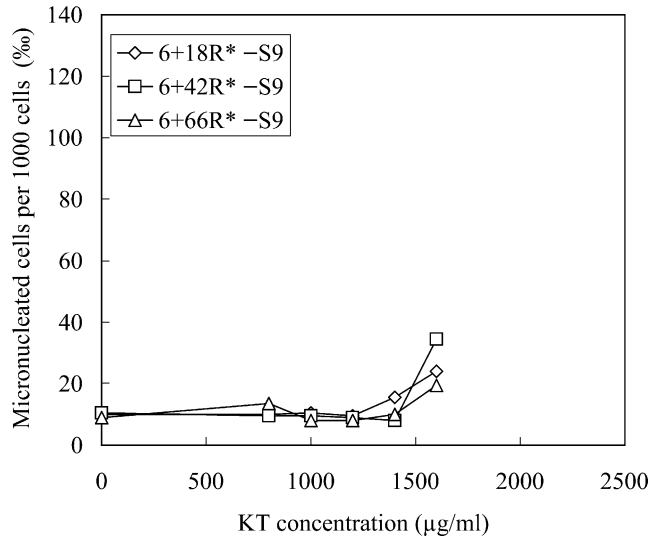

Fig. 4 Frequencies of micronuclei of cells treated with KT in short-term treatment without S9 mix. R*: Treatment with NKT for 6 hours followed by recovery period of 18, 42 or 66 hours.

hours, the highest frequencies of the micronuclei were 54.0‰, 122.0‰, and 103.0‰ in the presence of S9 mix, and 41.5‰, 68.0‰ and 17.0‰ in the absence of S9 mix respectively. Dose-dependency was observed in all treatments, both with and without S9 mix.

The frequencies of micronuclei of cells treated with KT are shown in Table 2. After the continuous treatment with KT for 24, 48, or 72 hours (Table 2), no significant increases in the frequency of micronuclei were observed. After the short-term treatment with KT for 6 hours, the frequencies of micronuclei of cells treated with KT with (Fig. 3) and without (Fig. 4) S9 mix increased significantly in a dose-dependent manner. The highest frequencies of micronuclei after treatment with KT for 6 hours and the recovery periods of 18, 42, and 66 hours were 47.0‰, 47.0‰, and 27.5‰ in the presence of S9 mix, and 24.0‰, 34.5‰, and 19.5‰ in the absence of S9 mix, respectively. A dose-dependent response was observed in the presence of S9 mix. The short-term treatment with either NKT or KT increased the frequency of micronuclei of cells treated with and without S9 mix. However, the continuous treatment with either NKT or KT did not significantly increase micronuclei frequency.

Discussion

In the present study, we investigated the genotoxicity of KT and NKT with the in vitro MN test using CHL/IU cells, which has a good correlation with mutagenicity determined by the Ames test and carcinogenicity (15). Genotoxicity determined by the MN test using CHL/IU cells was generally determined to be positive when the frequency of micronuclei was higher than 40‰ and negative when lower than under 20‰

(14). Scott et al. (18) have reported the high concentrations of test chemicals used to induce chromosome aberrations (including micronuclei) indirectly through either nonphysiological osmolarity, pH or an ion unbalance. Our tests were performed using concentrations of less than 10 mM (KT 2740 μg/ml, NKT 2660 μg/ml).

The continuous treatment differs from the short-term treatment in both exposure time and the changing of the culture medium. CHL/IU cells were exposed to NKT or KT, for 24, 48 or 72 hours without changing the culture medium in the continuous treatment. On the other hand, CHL/IU cells were exposed to NKT or KT with S9 mix for 6 hours and then were cultured for 18, 42 or 66 hours with the culture medium in the short-term treatment. Cytotoxicity in the short-term treatment with S9 mix is generally lower than that in the continuous treatment without S9 mix, because of the short time exposure to the chemicals. Therefore, the maximum concentrations of KT and NKT in the short-term treatment were higher than those in the continuous treatment.

Chemicals are generally metabolized in the liver after absorption. S9 mix was added during the short-term treatment to investigate the effects of metabolites. S9 used was prepared from a rat liver pretreated with phenobarbital and benzoflavone. Phenobarbital is known to induce CYP2B whereas benzoflavone mainly induces CYP1A1. S9 contains metabolic enzymes for many carcinogens and drugs (14, 19, 20). The frequencies of micronuclei of cells treated with KT in the short-term treatment without S9 mix did not increase at a low KT concentration, but they significantly increased at the maximum KT concentration. These results suggest a weak genotoxicity in accordance with a previous report (21). On the other hand, Yanagihara et al. reported that KT is metabolized to norketamine mainly by CYP2B6 in the short-term treatment with KT (22). The frequency of micronuclei increased more significantly at a low KT concentration with S9 mix than at that without S9 mix. Therefore, KT metabolites enhanced the induction of micronuclei.

In the short-term treatment with NKT without S9 mix, the frequency of micronuclei increased significantly at high concentrations, but it did not increase at low concentrations. In the short-term treatment with NKT with S9 mix, the frequency of micronuclei increased more significantly at a low dose than in that without S9 mix. Therefore, the NKT metabolites enhanced the induction of micronuclei. Shimizu et al. demonstrated that *N*-nitrosoephedrine (NEP) and *N*-nitrosomethamphetamine (NMA) show a strong mutagenicity as determined by the Ames test when S9 mix was added (23). The mechanism underlying the enhancement of micronuclei induced by NKT has not yet been clarified. However, the O^6-guanine methylation of DNA has been suggested to not only correlate with mutagenicity but also with carcinogenicity (24).

The maximum plasma KT concentration was approximately 40 ng/ml in an experiment of healthy volunteers who ingested tablets containing 50 mg of KT, and the time course of plasma KT concentration was similar to that in healthy volunteers administered 50 mg of KT in the syrup form (25). The normal concentrations in the tablet form, which has recently been abused for recreational usage, tend to range from 100 to 200 mg (8, 26). Therefore, plasma KT or NKT concentration does not increase the frequency of micronuclei induced by KT at 2000 μg/ml or NKT at 250 μg/ml when a drug abuser ingests 200 mg of KT. Commercial medical tablets are designed to disintegrate and dissolve in the stomach or in the intestine within the regular use for bioavailability. Drugs absorbed from the intestine are then carried to the liver and metabolized. If such tablet-form abused drugs do not disintegrate and thus remain in the stomach, then KT concentration may increase at some sites in the stomach for a long time, stomach cells are then locally exposed to nonmetabolized KT or synthesized NKT in the stomach at high concentrations for a long time. The MN test was performed to determine the limit of concentration for CHL/IU cells using KT or NKT in both continuous treatment and short-term treatment without S9 mix, to evaluate genotoxicity at high concentrations in long-term usage and frequent exposure to KT or NKT.

KT has safely been used as an anesthetic and analgesic through intravenous or intramuscular injection. However, KT has also been abused as a recreational drug administered via intravenous injection (10, 27), intramuscular injection (10, 11, 26), nasal inhalation of powder (8, 26), and the ingestion of tablets (8, 28–30). The reported symptoms in KT abusers include hallucinations (8–12), drug dependency (10–12), flashbacks (9, 10), tolerance (10–12), and anxiety (9). Tablets seized by the police tend to contain KT alone or are adulterated with 3,4-methylenedioxymethamphetamine, methamphetamine (MA), and ephedrine (EP) (30–32). All these chemicals are secondary amines that react with nitrous acid to form *N*-nitroso compounds both in vitro and in the stomach (1–3). KT is a secondary amine that shows a weak genotoxicity in the in vitro sister chromatid exchange test (21). However, the mutagenicity of NKT has not yet been reported. Four patients were reported to die from severe toxic hepatitis and hundreds of patients who had taken *N*-nitrosofenfluramine have received medical care for acute severe hepatitis (33) from 2001 to 2002 in Japan. Many *N*-nitroso compounds induce severe acute hepatitis, therefore, we also suspect that hepatitis may thus be induced by NKT produced in the stomach of KT abusers. Drugs and their metabolites are usually identified by gas chromatography-mass spectrometry (GC-MS). Most *N*-nitroso derivatives of drugs have not yet been studied and or published in the spectrum library. A mass spectrum of NKT would be very useful for identifying NKT in urine, stomach residues or blood, investigating the cause of disease, and establishing the optimal medical treatment. Moreover, KT is going to be regulated as a narcotic in 2006 in Japan, and the identification of NKT in urine as proof that KT tablets have been consumed can be a measure in regulating drug abuse by police. In addition, NKT analysis by GC-MS must be performed at lower than 210°C, because NKT, as well as NMA, decomposes resulting in changes in mass fragments or intensities at higher than 220°C (34).

NMA is produced in the stomach under acidic conditions (35) when MA is used as a stimulant and it can be detected in the urine of MA addicts (34). NEP is easily produced under acidic conditions in the presence of nitrite (23) from ephedrine, which is used as a bronchodilator and to produce MA. Both

NMA and NEP have been shown to have a strong mutagenicity by the Ames test (23). Moreover, NEP induces hepatocellular carcinoma in rats (36) and mice (37).

In the present study, we found that NKT is strongly mutagenic and easily produced from KT under acidic conditions in the presence of nitrite. The correlation of mutagenicity determined by the in vitro MN test using CHL/IU cells with that determined by the Ames test and carcinogenicity has thus been shown (15). Therefore, NKT might be a carcinogenic in experimental animals. Drug abuse is a great social problem worldwide. The genotoxic studies of *N*-nitroso compounds in abused drugs are therefore urgently needed and they are considered to be an important part of the strategy for preventing drug abuse.

References

(1) Lijinsky W. Reaction of drugs with nitrous acid as a source of carcinogenic nitrosamines. Cancer Res. 1974;34:255–258.

(2) Raineri R, Poiley JA, Andrews AW, Pienta RJ, Lijinsky W. Greater effectiveness of hepatocyte and liver S9 preparations from hamsters than rat preparations in activating *N*-nitroso compounds to metabolites mutagenic to Salmonella. J Natl Cancer Inst. 1981;67:1117–1122.

(3) Sen NP, Smith DC, Schwinghamer L. Formation of *N*-nitrosamines from secondary amines and nitrite in human and animal gastric juice. Food Cosmet Toxicol. 1969;7:301–307.

(4) Cardesa A, Pour P, Althoff J, Mohr U. Vascular tumors in female mice after intraperitoneal injection of dimethylnitrosamine. J Natl Cancer Inst. 1973;51:201–208.

(5) Druckrey H, Preussmann R, Ivankovic S, Schmahl D. Organotropic carcinogenic effects of 65 various *N*-nitroso-compounds on BD rats. Z Krebsforsch. 1967;69:103–201.

(6) Magee PN, Barnes JM. The production of malignant primary hepatic tumours in the rat by feeding dimethylnitrosamine. Br J Cancer. 1956;10:114–122.

(7) Toth B, Magee PN, Shubik P. Carcinogenesis study with dimethyl-nitrosamine administered orally to adult and subcutaneously to newborn BALB/C mice. Cancer Res. 1964; 24:1712–1721.

(8) Dalgarno PJ, Shewan D. Illicit use of ketamine in Scotland. J Psychoactive Drugs. 1996;28:191–199.

(9) Siegel RK. Phencyclidine and ketamine intoxication: a study of four populations of recreational users. NIDA Res Monogr. 1978;21:119–147.

(10) Jansen KL. Ketamine—can chronic use impair memory? Int J Addict. 1990;25:133–139.

(11) Kamaya H, Krishna PR. Ketamine addiction. Anesthesiology. 1987;67:861–862.

(12) Hurt PH, Ritchie EC. A case of ketamine dependence. Am J Psychiatry. 1994;151:779.

(13) Heddle JA, Hite M, Kirkhart B, Mavournin K, MacGregor JT, Newell GW, Salamone MF. The induction of micronuclei as a measure of genotoxicity. A report of the U.S. Environmental Protection Agency Gene-Tox Program. Mutat Res. 1983;123:61–118.

(14) Matsushima T, Hayashi M, Matsuoka A, Ishidate M Jr, Miura KF, Shimizu H, Suzuki Y, Morimoto K, Ogura H, Mure K, Koshi K, Sohuni T. Validation study of the in vitro micronucleus test in a Chinese hamster lung cell line (CHL/IU). Mutagenesis. 1999;14:569–580.

(15) Li J, Suzuki Y, Shimizu H, Fukumoto M, Okonogi H, Nagashima T, Ishikawa T. In vitro micronucleus assay of 30 chemicals in CHL cells. Jikeikai Med J. 1993;40:69–83.

(16) Cochran WG. Some methods for strengthening the common 2 tests. Biometrics. 1954;10:417–451.

(17) Armitage P. Test for linear trends in proportions and frequencies. Biometrics. 1955;11:375–386.

(18) Scott D, Galloway SM, Marshall RR, Ishidate M Jr, Brusick D, Ashby J, Myhr BC. International commission for protection against environmental mutagens and carcinogens. Genotoxicity under extreme culture conditions. A report from ICPEMC Task Group 9. Mutat Res. 1991;257:147–205.

(19) Morel G, Ban M, Bonnet P, Zissu D, Brondeau MT. Effect of beta-naphthoflavone and phenobarbital on the nephrotoxicity of chlorotrifluoroethylene and 1,1-dichloro-2,2-difluoroethylene in the rat. J Appl Toxicol. 2005;25:153–165.

(20) Garcia Franco S, Dominguez G, Pico JC. Alternatives in the induction and preparation of phenobarbital/naphthoflavone-induced S9 and their activation profiles. Mutagenesis. 1999; 14:323–326.

(21) Adhvaryu SG, Trivedi AH, Jani KH, Vyas RC, Dave BJ. Genotoxic effects of ketamine on CHO cells. Arch Toxicol. 1986;59:124–125.

(22) Yanagihara Y, Kariya S, Ohtani M, Uchino K, Aoyama T, Yamamura Y, Iga T. Involvement of CYP2B6 in *N*-demethylation of ketamine in human liver microsomes. Drug Metab Dispos. 2001;29:887–890.

(23) Shimizu H, Takemura N, Ando H, Morita M, Machida K. Mutagenic activity of *N*-nitrosomethamphetamine and *N*-nitrosoephedrine. Cancer Lett. 1983;21:63–68.

(24) Newbold RF, Warren W, Medcalf AS, Amos J. Mutagenicity of carcinogenic methylating agents is associated with a specific DNA modification. Nature. 1980;283:596–599.

(25) Yanagihara Y, Ohtani M, Matsumoto M, Kariya S, Uchino K, Hiraishi T, Ashizawa N, Aoyama T, Yamamura Y, Iga T. Preparation of ketamine tablets for treatment of patients with neuropathic pain. Yakugaku Zasshi. 1999;119:980–987. (Article in Japanese)

(26) Weiner AL, Vieira L, McKay CA, Bayer MJ. Ketamine abusers presenting to the emergency department: a case series. J Emerg Med. 2000;18:447–451.

(27) Felser JM, Orban DJ. Dystonic reaction after ketamine abuse. Ann Emerg Med. 1982;11:673–675.

(28) Dillman J. Ketamine abuse—a new edge. Todays Surg Nurse. 1998;20:37–39.

(29) Jansen KL. Non-medical use of ketamine. BMJ. 1993;306: 601–602.

(30) Shewan D, Dalgarno P. Ecstasy and neurodegeneration. ...such as ketamine. BMJ. 1996;313:424.

(31) Katagi M, Tsutsumi H, Miki A, Nakajima K, Tsuchihashi H. Analyses of clandestine tablets of amphetamines and their related designer drugs encountered in recent Japan. Japanese Journal of Forensic Toxicology. 2002;20:303–319.

(32) Makino Y, Tanaka S, Kurobane S, Nakauchi M, Terasaki T,

Ohta S. Profiling of illegal amphetamine-type stimulant tablets in Japan. J Health Sci. 2003;49:129–137.
(33) Kawaguchi T, Harada M, Arimatsu H, Nagata S, Koga Y, Kuwahara R, Hisamochi A, Hino T, Taniguchi E, Kumemura H, Hanada S, Maeyama M, Koga H, Tomiyasu N, Toyomasu H, Kawaguchi M, Kage M, Kumashiro R, Tanikawa K, Sata M. Severe hepatotoxicity associated with a *N*-nitrosofenfluramine-containing weight-loss supplement: report of three cases. J Gastroenterol Hepatol. 2004;19:349–350.
(34) Ando H, Morita M. Possibility of in vitro and in vivo formations of *N*-nitrosomethamphetamine. Reports of National Research Institute of Police Science. 1983;36:16–20. (Article in Japanese)
(35) Shimizu H, Takemura N, Ando H, Morita M, Machida K. The in vivo formation of mutagenic *N*-nitroso compounds from methamphetamine and ephedrine. Nippon Eiseigaku Zasshi. 1984;39: 573–580. (Article in Japanese)
(36) Eisenbrand G, Preussmann R, Schmahl D. Carcinogenicity of *N*-nitrosoephedrine in rats. Cancer Lett. 1978;5:103–106.
(37) Wogan GN, Paglialunga S, Archer MC, Tannenbaum SR. Carcinogenicity of nitrosation products of ephedrine, sarcosine, folic acid, and creatinine. Cancer Res. 1975;35:1981–1984.