Genetic toxicity of cocaine

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Cocaine is a widely abused drug. Recently, it has been shown to induce teratogenesis in both humans and animals. Cocaine-induced teratogenicity has been associated with reactive oxygen species (ROS), which are generated by cytochrome P450 during cocaine biotransformation. Since ROS have been reported to induce genotoxicity, it is of interest to know whether cocaine and/or its metabolites are also genotoxic. In this study, Chinese hamster ovary K1 cells were employed as a model system to investigate the genetic toxicity of cocaine in the presence or absence of rat liver S9 fraction. Cocaine-induced cytotoxicity was potentiated when S9 was present, indicating the cytochrome P450 metabolism plays a role in cocaine-mediated cytotoxicity. Cocaine treatments per se induced a few chromosome aberrations while treatments of cocaine plus S9 caused a significant increase in chromosomesome aberrations. In contrast, cocaine induced micronuclei (MN) formation and hypoxanthine-guanine phosphoribosyltransferase mutation only in the presence of S9. Therefore, cocaine itself is at best a weak clastogen, whereas metabolite(s) of cocaine is/are truly inducer(s) of clastogenesis and mutagenesis. Cocaine treatments alone also induced a significant increase in sister chromatid exchange frequency but the addition of S9 did not affect the results. Free radical scavengers, including superoxide dismutase and catalase, efficiently decreased the frequency of cocaine plus S9-induced MN, implying that ROS are indeed important components in cocaine-induced genotoxicity. The observation that non-toxic doses of cocaine can inhibit intercellular metabolic cooperation suggests that cocaine may also be a tumor promoter. Our data supports that cocaine could possess genotoxicity in addition to its well-known neurotoxicity and teratogenicity.

Introduction

Cocaine is a powerfully addictive drug of abuse. The epidemic of cocaine abuse has been a major public health and social problem worldwide. In the US alone, ~1.7 million Americans (~0.8% of the population aged 12 years and older) abused cocaine at least once per month, according to the results of the National Household Survey on Drug Abuse in 1996 (1). Because of cocaine’s strong addictive potential, numerous studies focusing on its neurotoxicity or behavioral pharmacology have been performed. Recently, cocaine-induced teratogenicity has been a new concern because prenatal cocaine exposure occurs in 6–15% pregnancies in large North American cities (2–5). Cocaine can easily cross the placenta to cause developmental defects. Pregnant women abusing cocaine have been shown to give birth to babies with neurological and ocular abnormalities, decreased body weight and length or premature birth (6–10). The developmental toxicities of cocaine have also been studied in rabbits, mice, chickens, swine and rats. These developmental defects include aortic arch anomalies, central nervous system abnormalities, a variety of external and skeletal anomalies, retarded maturation of respiratory system and behavioral changes (11–16). Upon in vivo exposure to cocaine, reactive oxygen species (ROS) are generated, resulting in lipid peroxidation and glutathione depletion (17–19). It has been reported that certain pesticides and drug-induced ROS are associated with teratogenicity (20,21). Therefore, cocaine-induced ROS may also be involved in the process of teratogenicity, although the underlying mechanism for its teratogenicity is not clear (22,23).

One of the mechanisms of cocaine-induced ROS is through the N-demethylation of cocaine by microsomal oxidative enzymes to produce N-hydroxynorcocaine, which is rapidly converted to norcocaine nitroxide, a free radical metabolite (24–26). In addition, superoxide, driven by the Fenton reaction to generate hydroxyl radicals, is formed during the biotransformation process of cocaine (27). Norcocaine nitroxide and its nitrosonium ion may deplete glutathione, leading to lipid peroxidation and binding of intracellular macromolecules (28). Thus, the ROS induced by cocaine is likely to be generated through its metabolites.

Besides their teratogenic effects, ROS have also been shown to be genotoxic. The ROS-induced genotoxicities include DNA damage (29,30), gene mutation (30–32), micronuclei (MN) formation (33–37) and chromosome aberrations (38–41). However, there are relatively few studies on cocaine-induced genotoxicity, in contrast to its teratogenicity. We have hypothesized previously that abused drugs, including cocaine, could induce genotoxicities if carefully scrutinized (42). Although the carcinogenicity of cocaine remains to be determined, cocaine has been predicted as a carcinogen by CASE, an expert structure–activity relational system which has been shown to be highly predictive for suspect carcinogens (43). In fact, Pott’s puffy tumor, a rare complication of frontal sinusitis, has been shown to be associated with intranasal cocaine abuse (44). A recent histopathologic and molecular approach performed on smokers of marijuana and/or cocaine also showed that smoking of marijuana and/or cocaine, like tobacco
smoking, increased the risk for the subsequent development of lung cancer (45). Since cocaine can generate ROS through its metabolites and may be carcinogenic, we therefore investigated the genotoxic activity of cocaine to substantiate the role of cocaine in the scope of genetic toxicology.

Materials and methods

Chemicals and media

McCoy’s 5A medium and minimal essential medium were purchased from Gibco BRL (Grand Island, NY). Cocaine–HCl was prepared from a batch of pharmaceutical-grade drug, which met the requirements of British Pharmacopoeia 1993, in the Research Division, National Narcotics Bureau, Department of Health, Taipei, Taiwan. The purity of cocaine–HCl, determined by gas chromatography–mass spectrometry, was 100% (as cocaine base) with negligible amounts of impurities. Since the hydrochloride salt of cocaine could readily dissolve in water, distilled water was used as the solvent for all experiments. Rat liver homogenate fraction (S-9 fraction) was purchased from ICN Pharmaceuticals (Aurora, OH). 5-Bromo-2’deoxyuridine (BrdU), colcemid, 12-O-tetradecanoylphorbol-13-acetate (TPA) and other chemicals were purchased from Sigma (St Louis, MO).

Cell cultures

The Chinese hamster ovary K1 (CHO-K1) cell line was purchased from ATCC and the cells were grown in McCoy’s 5A medium with 10% fetal bovine serum (HyClone, Logan, UT). Chinese hamster lung fibroblast V79 cell line (46), a gift of Dr J.E.Trosko (Michigan State University), was cultured in minimal essential medium with 10% fetal bovine serum (HyClone, Logan, UT). Chinese hamster ovary K1 (CHO-K1) cell line was purchased from Sigma (St Louis, MO).

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Assay for chromosome aberrations

In short, 33 DNA in metaphase preparations and analysis of chromosome aberrations were performed as described previously with modifications (47,48). In a short, 33 DNA in metaphase preparations and analysis of chromosome aberrations were performed as described previously with modifications (47,48).

Cytotoxicity assay

One-hundred CHO-K1 cells were seeded in each 60 mm dish for 4 h. Then, the cells were treated with various doses of cocaine in the presence or absence of S9 fraction for 2 h. S9 was added at a concentration of 10 μmol/L with glucose 6-phosphate (0.15 mmol/L) and NADP (0.315 mmol/L). After treatments of cocaine with or without S9, the cells were washed twice with phosphate-buffered saline (PBS) and replaced with fresh media. The cells were further cultured for a week, and the colonies were stained with 10% Giemsa solution. The relative cloning efficiency was calculated as a percentage of the untreated cells.

Microinucleus test

For MN analysis, the method of Wang and Huang was adopted (37). Briefly, 106 CHO-K1 cells were seeded in each 60 mm petri dish and cultured overnight. Then, the cells were treated with or without S9 fraction for 2 h. Subsequently the cells were washed twice with PBS and incubated for 24 h with cytochalasin B (1 μg/mL). The cells were soaked in 0.5% KCl for 3 min, followed by incubation for 5 min with Carnoy’s solution (methanol:acetic acid, 20:1), and stained with 10% Giemsa solution. The frequency of MN formation was expressed as a percentage of binucleated cells with MN. To observe the effect of free radical scavengers on cocaine-induced MN, catalase (Sigma; 100 μg/ml) or superoxide dismutase (SOD; Serva; 10 μg/ml) was co-incubated with cocaine-HCl for 2 h in the presence of S9 fraction.

Assay for chromosome aberrations

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Effect of cocaine on the cytotoxicity with or without S9 fraction

Cocaine treatments alone caused a dose-dependent decrease in 6-TG-resistant V79 cells in each 60mm dish. Four hours after plating, appropriate doses of cocaine were added for 3 days. 6-TG was added to a final concentration of 10 μmol/L 1 h after addition of cocaine. Fresh medium containing only 6-TG was replaced after 3 days. The cells were further cultured for 3–4 days and stained with 10% Giemsa solution. TPA (1 ng/mL) was used as the positive control.

Results

Effect of cocaine on the cytotoxicity with or without S9 fraction in CHO-K1 cells

To determine the cytotoxicity of cocaine, colony formation assay was used in the presence or absence of rat liver extract S9 fraction. CHO-K1 cells were treated with cocaine with or without S9 for 2 h, and the results are shown in Figure 1. Cocaine treatments alone caused a dose-dependent decrease in survival ratio and co-incubation with S9 fraction potentiated cocaine-induced cytotoxicity. Consistent results were also obtained in the following cytotoxicity experiments concurrently performed with genotoxicity assessments.

Fig. 1. Cytotoxicity of cocaine in CHO-K1 cells. Cells were treated with various doses of cocaine for 2 h in the presence or absence of S9. The cytotoxicity was determined by colony formation assay and calculated as a percentage of the control.

Assay for inhibition of intercellular metabolic cooperation

The assay was performed as described previously (50). CHO-K1 cells (3×103) were seeded in each 60 mm dish overnight. Cells were treated with various doses of cocaine for 2 h and then washed with PBS. BrdU (20 μM) was subsequently added to the cultures in the dark for 22 h. Colcemid (0.2 μg/mL) was added and, after 2 h, mitotic cells were collected by gentle flushing with PBS. Following centrifugation, cells were treated with 5 ml of 0.5% potassium chloride for 5 min, and fixed with methanol:acetic acid (3:1). Then, the cells were dropped onto slides and dried in the air. The samples were incubated with H33258 (20 μg/mL) in Sorenson buffer (prepared by pouring 0.045 g/ml KH2PO4 to 0.047 g/ml Na2HPO4 until the pH reached 6.8), irradiated with black UV light (356 nm, model xx-15, purchased from San Gabriel, CA) at 57°C for 20 min, and stained with 10% Giemsa solution for 10 min. The slide preparations were observed under a light microscope. The results were expressed as SCE events per cell. Thirty mitotic cells were counted for each independent experiment. Each treatment was performed in triplicate.

Genotoxicity of cocaine

Fig. 2. Effect of cocaine on the induction of MN in CHO-K1 cells. Cells were treated with cocaine for 2 h in the presence or absence of S9. The assays were performed as described in Materials and methods. Parallel cytotoxicity was determined with colony formation assay. The data were expressed as percentages of binucleated cells with MN. The results presented are the averages of three independent experiments (±SEM).

\*\(P < 0.05\); \**\(P < 0.01\); performed with Student’s \(t\)-test.

**MN induction**

The potential of cocaine to induce MN is shown in Figure 2. Treatments of CHO-K1 cells with cocaine in the presence of S9 fraction caused dose-dependent MN induction while those in the absence of S9 fraction failed to induce MN.

**Chromosome aberrations (CAs)**

Several types of CAs were observed when CHO-K1 cells were treated with cocaine in the presence or absence of S9 (Table I). The parallel cytotoxicity assay was performed and the results were similar to those presented in Figure 1 (data not shown). Cocaine alone induced a slight increase of dicentric chromosomes and chromatid-type gaps. A few aberrant metaphases could also be observed under the conditions used. When cocaine was co-treated with S9, both chromatid- and chromosome-type exchanges were significantly increased. The aberrant metaphases were increased in a dose-dependent manner. Hence, cocaine-induced CAs were significantly increased in the presence of S9 fraction.

**hgprt mutation**

The mutagenic effect of cocaine at the \(hgprt\) locus is shown in Table II. Cocaine treatments per se did not induce \(hgprt\) gene mutation, but the mutation frequencies increased at dose ranges of 0.2–0.8 mg/ml of cocaine in the presence of S9. A 7.25-fold increase in mutation frequency was observed when 0.8 mg/ml of cocaine was applied in the presence of S9 (\(P < 0.05\)). However, the mutation frequency of cocaine plus S9 fell rapidly when the doses of cocaine went higher than 1 mg/ml (data not shown). Therefore, cocaine is mutagenic only when S9 is present, and the mutagenic effect was observed only within a narrow dose range.

**Sister-chromatid exchange**

CHO-K1 cells, treated with various doses of cocaine \(± S9\) for 2 h. Cells were washed with PBS twice and further incubated for 13 h. Colcemid (0.2 µg/ml) was added to the culture for another 2 h and cells were harvested, washed with PBS and centrifuged. Five milliliters of 0.5% KCl were added to the pellet for 6 min. The cell suspension was centrifuged, pelleted again and fixed with a solution (methanol: acetic acid, 3:1). Cells were dropped onto slides and dried in the air. 10% Giemsa solution was applied to stain cells on slides. Each value represents aberrant chromosome numbers per 100 metaphases counted.

### Table I. Effects of cocaine with or without S9 on chromosome aberrations in CHO-K1 cells

<table>
<thead>
<tr>
<th>Cocaine (mg/ml)</th>
<th>Aberrant metaphases</th>
<th>Chromatid-type S9</th>
<th>Chromosome-type S9</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gap Break Exchange</td>
<td>Gap Break Ring/ Dichromat</td>
<td></td>
</tr>
<tr>
<td>-S9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2.5</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>+S9a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.5</td>
<td>3</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>1.5</td>
<td>14</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>19</td>
<td>7</td>
<td>23</td>
</tr>
<tr>
<td>2.5</td>
<td>15</td>
<td>4</td>
<td>18</td>
</tr>
<tr>
<td>3</td>
<td>32</td>
<td>19</td>
<td>41</td>
</tr>
</tbody>
</table>

**Experiment 2**

| -S9             |                     |                   |                    |
| 0               | 0                   | 1                 | 0                  |
| 0.5             | 0                   | 1                 | 0                  |
| 1               | 0                   | 1                 | 0                  |
| 2               | 2                   | 0                 | 0                  |
| 2.5             | 4                   | 2                 | 0                  |
| 3               | 2                   | 1                 | 2                 |
| +S9b            |                     |                   |                    |
| 0               | 1                   | 0                 | 0                  |
| 0.5             | 2                   | 4                 | 0                  |
| 1               | 3                   | 3                 | 4                  |
| 1.5             | 8                   | 2                 | 9                  |
| 2               | 15                  | 7                 | 10                 |
| 2.5             | 11                  | 2                 | 10                 |
| 3               | 30                  | 9                 | 4                  |

CHO-K1 cells were treated with various doses of cocaine \(± S9\) for 2 h. Cells were washed with PBS twice and further incubated for 13 h. Colcemid (0.2 µg/ml) was added to the culture for another 2 h and cells were harvested, washed with PBS and centrifuged. Five milliliters of 0.5% KCl were added to the pellet for 6 min. The cell suspension was centrifuged, pelleted again and fixed with a solution (methanol: acetic acid, 3:1). Cells were dropped onto slides and dried in the air. 10% Giemsa solution was applied to stain cells on slides. Each value represents aberrant chromosome numbers per 100 metaphases counted.

aNumber of metaphases with aberrant chromosomes except gaps.

bStatistic significance between cocaine with S9 treatments and cocaine without S9 treatments, and statistic significance in dose dependency, according to SAS GENMOD Procedure; \(P < 0.0001\) in both cases (from pooled data of the two independent experiments).

**Inhibition of metabolic cooperation**

Treatments of cocaine resulted in dose-dependent inhibition of metabolic cooperation in V79 cells (Figure 4).
Table II. Effect of cocaine with or without S9 on \( hgp rt \) gene mutation assay in CHO-K1 cells

<table>
<thead>
<tr>
<th>Cocaine dose (mg/ml)</th>
<th>Plating efficiency</th>
<th>Total no. of viable cells ((\times 10^4))</th>
<th>Total no. of 6-TG resistant colonies</th>
<th>Mutation frequency ((\times 10^{-6}))</th>
<th>Survival rate (% of control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>–S9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.82 ± 0.04</td>
<td>246</td>
<td>6</td>
<td>2.4 ± 1.2</td>
<td>100</td>
</tr>
<tr>
<td>0.1</td>
<td>0.91 ± 0.03</td>
<td>273</td>
<td>0</td>
<td>0.0 ± 0.1</td>
<td>98 ± 4.1</td>
</tr>
<tr>
<td>0.2</td>
<td>0.93 ± 0.01</td>
<td>279</td>
<td>3</td>
<td>1.1 ± 0.6</td>
<td>99 ± 2.8</td>
</tr>
<tr>
<td>0.4</td>
<td>0.85 ± 0.04</td>
<td>255</td>
<td>9</td>
<td>3.5 ± 2.0</td>
<td>100 ± 6.1</td>
</tr>
<tr>
<td>0.8</td>
<td>0.73 ± 0.05</td>
<td>219</td>
<td>9</td>
<td>4.1 ± 2.3</td>
<td>92 ± 8.3</td>
</tr>
<tr>
<td>1.0</td>
<td>0.85 ± 0.04</td>
<td>255</td>
<td>3</td>
<td>1.2 ± 1.3</td>
<td>90 ± 7.2</td>
</tr>
<tr>
<td>+S9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.72 ± 0.04</td>
<td>216</td>
<td>6</td>
<td>2.8 ± 1.4</td>
<td>98 ± 2.6</td>
</tr>
<tr>
<td>0.1</td>
<td>0.8 ± 0.04</td>
<td>240</td>
<td>3</td>
<td>1.3 ± 1.0</td>
<td>92 ± 3.8</td>
</tr>
<tr>
<td>0.2</td>
<td>0.72 ± 0.06</td>
<td>216</td>
<td>15</td>
<td>6.9 ± 6.9</td>
<td>90 ± 5.2</td>
</tr>
<tr>
<td>0.4</td>
<td>0.73 ± 0.08</td>
<td>219</td>
<td>36</td>
<td>16.4 ± 9.8</td>
<td>89 ± 6.5</td>
</tr>
<tr>
<td>0.8</td>
<td>0.74 ± 0.03</td>
<td>222</td>
<td>45</td>
<td>20.3 ± 2.1*</td>
<td>88 ± 7.7</td>
</tr>
<tr>
<td>1.0</td>
<td>0.84 ± 0.04</td>
<td>252</td>
<td>9</td>
<td>3.6 ± 3.5</td>
<td>86 ± 9.1</td>
</tr>
</tbody>
</table>

*Data pooled from three separate experiments and expressed as the means ± SEM. For each point in each experiment, 10 dishes were used (30 dishes per control or treatment groups). Initial number of cells seeded was \( 1 \times 10^5 \) /dish for mutation and 100 cells /dish for plating efficiency.

Cocaine was added to the cells for 2 h in the presence or absence of S9 fraction. Treated cells were subsequently cultured for 6 days as described in Materials and methods. The 6-TG resistant colonies were stained with 10% Giemsa solution for 10 min.

Survival rate was determined with colony formation assay as described in Materials and methods.

Statistical significance between cocaine with S9 treatments and cocaine without S9 treatments; \( P < 0.0001 \) (according to SAS GENMOD Procedure).

Statistical significance between S9 treatment alone and cocaine plus S9 treatment, performed by Student’s \( t \)-test; \( P < 0.05 \).

Fig. 3. Effect of cocaine on sister-chromatid exchange frequency in CHO-K1 cells. Cells were treated with cocaine for 2 h in the presence or absence of S9, followed by 24 h incubation with 20 \( \mu M \) BrdU. Two hours before cell harvest, colcemid (0.2 \( \mu g/ml \)) was added. Chromosomes were prepared as described in Materials and methods. The cytotoxicity assay was performed concurrently. The results presented are the averages of three independent experiments (±SEM). \(* P < 0.05; **P < 0.01\); performed with Student’s \( t \)-test.

of cocaine needed for such inhibition were relatively non-toxic (at least 70% survival, as determined by colony formation assay).

**Prevention of cocaine-induced MN by ROS scavengers**

To determine whether cocaine-induced genotoxicity was mediated by ROS, enzymes capable of removing free radicals were co-treated with cocaine in the presence of S9, and MN were scored. Both SOD and catalase effectively reduced the cocaine-induced MN (Figure 5).

**Discussion**

In this study, we have shown that short-term exposure of CHO-K1 cells to cocaine and its metabolites is genotoxic. Cocaine treatments alone induced a few CAs while treatments of cocaine plus S9 fraction caused a significant increase in CAs (Table I). Although the CAs induced by cocaine plus S9 seemed to stall at 2–2.5 mg/ml of cocaine treatments, the increase in CAs was nevertheless dose-dependent through calculation with the trend test (SAS GENMOD procedure; Table I). In contrast, cocaine induced MN formation and \( hgp rt \) mutation only in the presence of S9 (Figure 2; Table II), indicating metabolism plays an important role in cocaine-induced genotoxicity. Therefore, we conclude that cocaine itself is at best a weak clastogen, whereas metabolite(s) of cocaine is/are truly inducer(s) of clastogenesis and mutagenesis. However, we also observed that cocaine treatments per se induced a significant increase in SCE frequency, but the addition of S9 did not influence the results (Figure 3). These results imply that SCE induced by cocaine per se may be a separate event from other genotoxicities induced by the metabolite(s) of cocaine. Albeit SCE is a sensitive indicator toward carcinogens and has been implicated with misreplication, its molecular mechanism is still obscure. Since cocaine is known to generate ROS only when it is metabolized (24–26), cocaine-induced SCE may be ROS-independent.

It would then be interesting to learn whether the genotoxicity of cocaine through metabolism is associated with ROS. Kloss
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Fig. 4. Inhibitory effect of cocaine on intercellular metabolic cooperation in V79 cells. 6-TG-sensitive cells (4 \times 10^5) were co-cultured with 100 6-TG-resistant cells in each 60 mm dish. Various doses of cocaine were added for 3 days. One hour after cocaine addition, the cells were treated with 10 µg/ml of 6-TG. After 3 days, the culture was replaced with fresh medium containing 10 µg/ml of 6-TG. The cells were further cultured for 3–4 days. Parallel cytotoxicity test was performed by seeding 100 cells in each dish. Colonies were stained with 10% Giemsa solution. Inhibition of metabolic cooperation was expressed as recovered colony number normalized by survival rate. The results presented are the averages of three independent experiments (±SEM). ***P < 0.001, according to Student’s t-test.

et al. (26) have shown that superoxide is produced when cocaine is metabolized by cytochrome P450. Superoxide is believed to reduce ferric iron to ferrous iron, which then reacts with hydrogen peroxide to form DNA-reactive hydroxyl radical (27). Since hydrogen peroxide is readily generated when superoxide accepts one electron in the presence of a water molecule (52,53), the metabolites of cocaine could induce the production of hydroxyl radicals. Thus, the hydroxyl radicals are generated in the process of cocaine metabolism. Indeed, addition of free radical scavengers, including SOD and catalase, could effectively decrease MN in the CHO-K1 cells treated with cocaine plus S9 (Figure 5). Besides, the minimal concentration of SOD (~30 U) required for inhibition of cocaine-induced MN is far smaller than that of catalase (~15 000 U). The results indicate that inhibition of superoxide generation should be more effective than that of hydrogen peroxide generation in preventing cells from cocaine-caused MN formation. Furthermore, it has been shown that in the CHO-K1 cells, the activity of SOD is relatively low in comparison with that of catalase (54,55). Therefore, the rate-limiting step for removal of hydroxyl radicals is probably occurring at the reaction of reduction of superoxide to hydrogen peroxide, supporting the observation that SOD is more efficient for preventing cocaine-induced MN than catalase.

The cytotoxicity of cocaine is also potentiated in the presence of S9 fraction (Figure 1). The result is consistent with previous studies that in hepatocytes where cocaine is actively metabolized by cytochrome P450, cocaine-induced toxicity is effectively reduced by the addition of P450 inhibitors (56,57). We also observed that addition of SOD or reducing agents could decrease the cytotoxicity induced by cocaine plus S9 in the CHO-K1 cells (data not shown), indicating that oxidative stress plays an important role in cocaine-induced cytotoxicity.

Human and animal studies have demonstrated that cocaine gains access to fetal plasma and brain tissue shortly after maternal uptake (58,59). The efficient transfer of cocaine across the placenta and blood-brain barriers can be explained by its low molecular weight and amphiphilic nature. In human volunteers receiving a dose of 0.5 mg/kg cocaine, the detected cocaine levels were between 0.1 and 1.0 µM in the serum (60). However, the levels of cocaine are often considerably higher in active abusers, reaching 0.1 mM (61). In this report, we adopted the method of short-term (2 h) treatments to prevent the degradation of cocaine, which is sensitive to oxygen and UV light, and to avoid overexposure to S9, which is cytotoxic in long-term treatments. The doses of cocaine that were capable of inducing genotoxicities in this study were ~0.4–0.8 mg/ml (~0.9–1.8 mM). Although such concentrations may seem too high even for active abusers, the lungs or nasal cavities of abusers may be locally exposed to high concentrations of cocaine through sniffing or smoking crack/cocaine powder. In fact, Barsky et al. (45) have reported that habitual smokers of marijuana and/or cocaine have an increased frequency of molecular abnormalities in bronchial epithelium that are similar to those identified in tobacco smoking, implying

Fig. 5. Effects of SOD and catalase on cocaine-induced MN in CHO-K1 cells. Cocaine was applied to cells for 2 h in the presence or absence of S9, then washed with PBS and incubated for 24 h with cytochalasin B (1 µg/ml). The cells were soaked in 0.5% KCl for 3 min followed by 6 min incubation with methanol:acetic acid (20:1). Cells were stained by 10% Giemsa solution. The results presented are the averages of three independent experiments (±SD). Significant difference between cocaine/S9-treated and cocaine/S9 plus SOD- or catalase-treated cells is given by *P < 0.05 (Student’s t-test). a, untreated control; b, S9 alone; c, cocaine (1 mg/ml) alone; d, cocaine plus S9; e, cocaine plus SOD (10 µg/ml); f, cocaine plus catalase (200 µg/ml).
that smoke constituents of marijuana, cocaine and/or tobacco could produce precancerous lesions. Although these lesions are not specific for cocaine, it nevertheless suggests that habitual smoking of cocaine could increase the risk for the subsequent development of lung cancer. Because we adopted the strategy of short-term treatments in this study, the extrapolation of the results inevitably had to be compromised by high concentrations and maximum tolerable doses. It would be interesting to know whether long-term treatments with repeated low doses of cocaine can also cause genetic toxicities. It has been predicted that cocaine could be a rodent carcinogen according to a computer system CASE, which is an expert structure–activity relational system (43). In addition, some case reports have associated cocaine abusers with tumor generation (44,62). Damage of genetic materials plays an important role in carcinogenesis (63,64). As demonstrated in this paper, genotoxicity can be induced by cocaine metabolism through ROS generation. Although there should be a threshold for endogenous ROS which are produced as part of normal cellular processes, overexposure to ROS has been associated with a number of degenerative diseases including aging and cancers (65,66). Therefore, long-term cocaine abuse may result in excess exposure to ROS and increases the likelihood of contracting cancers. Furthermore, a relatively low and non-toxic concentration of cocaine (0.1 mg/ml, or ~0.23 mM) was able to inhibit metabolic cooperation in V79 cells. Since inhibition of metabolic cooperation has been recognized as a tumor promoter marker (51), the results also imply the potential of cocaine to promote tumorigenesis.

In summary, we report here that cocaine, especially after its biotransformation, is genotoxic, which may explain why cocaine is teratogenic and possibly carcinogenic. Further scrutiny on the epidemiology of cocaine-associated cancers and the molecular mechanism of cocaine-induced genotoxicity is imperative.

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References

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