Cytoskeleton Interruption in Human Hepatoma HepG2 Cells Induced by Ketamine Occurs Possibly through Suppression of Calcium Mobilization and Mitochondrial Function

Huai-Chia Chang, Ta-Liang Chen, and Ruei-Ming Chen

Graduate Institute of Medical Sciences, College of Medicine, Taipei Medical University, Taipei, Taiwan (H.-C.C., R.-M.C.); Core Laboratories and Department of Anesthesiology, Wan-Fang Hospital, Taipei Medical University, Taipei, Taiwan (H.-C.C., R.-M.C.); and Department of Anesthesiology, Taipei Medical University Hospital, Taipei, Taiwan (T.-L.C.)

Received July 11, 2008; accepted October 6, 2008

ABSTRACT:

Ketamine is an intravenous anesthetic agent often used for inducing and maintaining anesthesia. Cytoskeletons contribute to the regulation of hepatocyte activity of drug biotransformation. In this study, we attempted to evaluate the effects of ketamine on F-actin and microtubular cytoskeletons in human hepatoma HepG2 cells and its possible molecular mechanisms. Exposure of HepG2 cells to ketamine at ≤100 μM, which corresponds to clinically relevant concentrations for 1, 6, and 24 h, did not affect cell viability. Meanwhile, administration of therapeutic concentrations of ketamine obviously interrupted F-actin and microtubular cytoskeletons. In parallel, levels of intracellular calcium concentration-and time-dependently decreased after ketamine administration. Analysis by confocal microscopy further revealed that ketamine-suppressed calcium mobilization from an extracellular buffer into HepG2 cells.

Exposure to ketamine decreased cellular ATP levels. The mitochondrial membrane potential and complex I NADH dehydrogenase activity were both reduced after ketamine administration. Ketamine did not change the production of actin or microtubulin mRNA in HepG2 cells. Consequently, ketamine-caused cytoskeletal interruption led to suppression of CYP3A4 expression and its metabolizing activity. Therefore, this study shows that therapeutic concentrations of ketamine can disrupt F-actin and microtubular cytoskeletons possibly through suppression of intracellular calcium mobilization and cellular ATP synthesis due to down-regulation of the mitochondrial membrane potential and complex I enzyme activity. Such disruption of the cytoskeleton may lead to reductions in CYP3A4 activity in HepG2 cells.

Ketamine, an intravenous anesthetic agent, is often applied to induce and maintain anesthesia during surgical procedures (White et al., 1982). In a clinical environment, ketamine has more stable hemodynamics than barbiturates or inhaled anesthetic agents, so it is usually used in critically ill patients to induce anesthesia (Bourgin et al., 2003). Application of ketamine can be associated with increases in cardiac output, arterial blood pressure, and heart rate through modulation of central and sympathetic nervous system outputs or a reduction of endothelial nitric oxide production (Cook et al., 1991; Chen et al., 2005). In the cellular host defense system, ketamine has been reported to attenuate the activities of neutrophils, leukocytes, and macrophages (Weigand et al., 2000; Chang et al., 2005; Wu et al., 2008). Previous studies also showed that ketamine can interact with and affect the functions of other tissues, including the liver and neurons (Chan et al., 2005; Takadera et al., 2006).

Cytoskeletons are key organelles for maintaining cell morphologies, polarity, movement, and other activities (Canton and Litchfield, 2006). Disturbances in cytoskeletal remodeling result in mitochondrial dysfunction, release of reactive oxygen species, and cell dysfunction (Gourlay and Ayscough, 2005; Adrain et al., 2006; Cherng et al., 2008). Actin and microtubulin are two major monomers contributing to the polymerization of F-actin and microtubular cytoskeletons, respectively (Hill, 1981). Cytoskeletal remodeling requires calcium and energy (Molitoris et al., 1996; Fache et al., 2005). Previous studies showed that calcium mobilization can trigger cytoskeletal remodeling and regulate cell activities (Adamiková et al., 2004; Fache et al., 2005). The ischemia-reperfusion process leads to mitochondrial dysfunction and ATP depletion, and such decreases in cellular ATP levels induce cytoskeletal disruption, which can damage endothelial cells (Nishimura et al., 1998). Mitochondria are important energy-producing organelles and participate in ATP biosynthesis through the respiratory chain reaction (Papucci et al., 2003). Maintenance of the mitochondrial membrane potential is critical for mitochondrial functions and ATP synthesis. Our previous study further showed that ketamine decreases the mitochondrial membrane potential and cellular ATP synthesis, which then suppress phagocytosis and the oxidative ability of macrophages (Chang et al., 2005).

Hepatocytes play critical roles in the metabolism of endogenous and exogenous substances. Previous studies revealed that interruption of cytoskeleton polymerization may lead to hepatocyte dysfunction or...
even death. In rat hepatocytes, changes in the F-actin cytoskeleton were shown to affect storage-operated calcium channels and organization of the endoplasmic reticulum (Wang et al., 1997). Microtubular cytoskeleton is reported to participate in regulation of cytochrome P450 (P450) 1A, 2B, 2C, or 3A2 expressions possibly through activation of the glucocorticoid receptor or the aryl hydrocarbon receptor (Dvorská et al., 2005). Meanwhile, little is known about the effects of ketamine on cytoskeletal remodeling. Thus, this study was designed to evaluate the effects of ketamine on F-actin and microtubular cytoskeletons of hepatoma HepG2 cells and its possible mechanisms. CYP3A4, the major isoform of P450s in human liver, contributes to ketamine metabolism (Hijazi and Boulieu, 2002). Although CYP3A4 is constitutively expressed, it can also be regulated by xenocochemicals (Martinez-Jimenez et al., 2007). Therefore, the other aim of this study was to further evaluate the effects of ketamine-caused cytoskeleton disruption on CYP3A4 expression.

Materials and Methods

Cell Culture, Drug Treatment, and Cytotoxicity Assay. Human hepatoma HepG2 cells were purchased from American Type Culture Collection (Manassas, VA) and cultured according to a method described previously (Chen et al., 2000). Ketamine was dissolved in phosphate-buffered saline (0.14 M NaCl, 2.6 mM KCl, 8 mM Na2HPO4, and 1.5 mM KH2PO4). Concentration of ketamine (≤100 μM), which correspond to clinical plasma concentrations (Domino et al., 2005; Grant et al., 1983), were chosen as the treated group. Erythromycin N-demethylation activity was determined by using a mouse monoclonal antibody labeled with FITC against mouse immunoglobulin (Cherng et al., 2008). For imaging analysis of HepG2 cells were immunodetected using a method previously described by Chen et al. (2003). CYP3A4 protein was immunodectioned using a goat polyclonal antibody against human CYP3A4 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA). βM protein was immunodectioned using a mouse monoclonal antibody against human βM (Santa Cruz Biotechnology, Inc.) as an internal control. Intensities of the immunoreactive protein bands were determined using a digital imaging system (UVTec, Cambridge, UK).

Reverse Transcription-Polymerase Chain Reaction Assay. mRNA from HepG2 cells exposed to ketamine was prepared for reverse transcription-polymerase chain reaction (RT-PCR) analyses of β-actin, α-actin, microglobulin, CYP3A4, and βM mRNA following the instructions of the ready-to-go RT-PCR beads (GE Healthcare, Little Chalfont, Buckinghamshire, UK) as described previously (Wu et al., 2008). Oligonucleotide sequences for the PCR analyses of β-actin, α-actin, microglobulin, CYP3A4, and βM mRNA were designed and synthesized by Mission Biotech (Taipei, Taiwan). The oligonucleotide sequences of these primers were as follows: 5'-GCTACAGGACCGACCCCTGAA-3' and 3'-TACGATGTTGACGATTGC-5' for β-actin; 5'-ATCTGACCAACACCTTCTACAATGATGCTCCG-3' and 3'-GCTTAACATACTCCACCTCACATCAGC-5' for CYP3A4; 5'-TGGTGCTGCAACTCACC-3' and 3'-CCACACTACATTAAAGTTGATG-5' for CYP3A4; and 5'-GCTTACGTGCTCTGGACCACTTTA-3' and 3'-CTGCGGCTACTCTCTCTCTGTG-5' for βM. According to our preliminary results, the 35 cycles of PCR reactions were chosen as our analyzing condition. The PCR reaction was carried out using 35 cycles of 94°C for 45 s, 60°C for 45 s, and 72°C for 2 min. The PCR products were electrophoretically separated. These DNA bands were photographed and quantified with the aid of a digital imaging system (UVTec).

Statistical Analysis. Statistically significant differences between the control and ketamine-treated groups were determined using a Student’s t test, and differences were considered statistically significant at p < 0.05. Statistical analysis between groups over time was carried out using two-way analysis of variance.

Results

Toxicity of Ketamine to HepG2 Cells. Cell viability was assayed to evaluate the toxicity of ketamine to HepG2 cells (data not shown). Exposure to 25, 50, 75, and 100 μM ketamine for 24 h did not affect the release of γ-glutamyltranspeptidase and lactate dehydrogenase. When the concentration reached 1000 μM, ketamine caused significant 2.5- and 3.2-fold increases in release of γ-glutamyltranspeptidase and lactate dehydrogenase, respectively.

Disruption of F-Actin and Microtubule Cytoskeletons by Ketamine. Confocal microscopy was used to determine the effects of ketamine on F-actin and microtubular cytoskeletons of HepG2 cells. Ketamine was dissolved in phosphate-buffered saline and incubated with 3,3′-dihexyloxacarbocyanine, a positively charged dye, at 37°C in a humidified atmosphere of 5% CO2. The luminescence intensities in cells were analyzed by flow cytometry (BD Biosciences, San Jose, CA).

Assay of Mitochondrial NADH Dehydrogenase Activity. To determine the effects of ketamine on mitochondrial function, mitochondrial dehydrogenase activity was detected using a colorimetric assay following the method of Wu et al. (2005). After drug treatment, HepG2 cells were cultured with new medium containing 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bro-
apeutic concentration of ketamine (100 μM) for 1 h did not affect the microtubular cytoskeleton. After exposure for 6 h, ketamine at 100 μM obviously interrupted polymerization of the microtubular cytoskeleton in HepG2 cells. Remodeling of the microtubular cytoskeleton in HepG2 cells exhibited much greater disturbance after exposure to ketamine for 24 h (Fig. 1C). The fluorescence intensities were quantified and analyzed (Fig. 1D). Exposure of HepG2 cells to a clinically relevant concentration of 100 μM ketamine for 6 and 24 h caused significant decreases of 48 and 77% in the microtubular cytoskeleton, respectively.

Reduction of Bradykinin-Induced Calcium Mobilization by Ketamine. Levels of intracellular calcium in HepG2 cells were quantified to determine the mechanism of ketamine-caused suppression of F-actin and microtubular cytoskeletons (Fig. 2). Exposure of HepG2 cells to 25 μM ketamine did not influence bradykinin-enhanced intracellular calcium levels (Fig. 2A). After administration of 50 μM ketamine for 24 h, bradykinin-caused increases in the levels of intracellular calcium were significantly decreased by 23%. Treatment of HepG2 cells with 75 and 100 μM ketamine for 24 h caused significant decreases of 42 and 61% in intracellular calcium levels (Fig. 2A). Exposure to a therapeutic concentration of ketamine (100 μM) for 1 h significantly decreased the bradykinin-augmented intracellular calcium amount by 19% (Fig. 2B). After administration of 100 μM ketamine for 6 and 24 h, levels of intracellular calcium were reduced by 40 and 63%, respectively (Fig. 2B).

A confocal analysis was used to confirm the effects of ketamine on bradykinin-induced calcium mobilization (Fig. 3). Before bradykinin stimulation, low levels of intracellular calcium were detected in control and ketamine-treated hepatocytes (Fig. 3, A, D, G, and J). When untreated HepG2 cells were exposed to bradykinin for 3 min, the amounts of intracellular calcium were obviously enhanced (Fig. 3B), and the intracellular calcium levels reached a maximum after 5 min (Fig. 3C). In 1-h-treated HepG2 cells, pretreatment with ketamine obviously suppressed bradykinin-induced calcium influx (Fig. 3, E and F). The suppressive effects of ketamine on bradykinin-induced calcium mobilization became much more apparent in 6- and 12-h-treated HepG2 cells (Fig. 3, H, I, K, and L).

Suppression of Cellular ATP Biosynthesis by Ketamine. Cellular ATP levels were quantified to evaluate the roles of mitochondria in ketamine-involved suppression of F-actin and microtubular cytoskeletons (Fig. 4). Exposure of HepG2 cells to 25 μM ketamine for 24 h did not change the levels of cellular ATP (Fig. 4A). After administration of 50 μM ketamine, the amounts of cellular ATP had significantly decreased by 24%. When the concentrations reached 75 and 100 μM, ketamine caused significant (41 and 44%) reductions in cellular ATP synthesis (Fig. 4A). Treatment of HepG2 cells with
Ketamine for 1 h did not influence cellular ATP levels (Fig. 4B). Levels of intracellular ATP were significantly reduced by 31 and 48% after ketamine administration for 6 and 24 h, respectively.

**Down-Regulation of the Mitochondrial Membrane Potential and Complex I NADH Dehydrogenase Activity by Ketamine.** To determine the mechanism of the ketamine-caused reduction in cellular ATP synthesis, the mitochondrial membrane potential and mitochondrial complex I NADH dehydrogenase activity were assayed (Fig. 5). Exposure of HepG2 cells to a therapeutic concentration of ketamine (100 μM) for 1 h did not affect the mitochondrial membrane potential (Fig. 5A). When administered for 6 and 24 h, ketamine significantly decreased the membrane potential of hepatocyte mitochondria by 25 and 46%, respectively. Treatment of HepG2 cells with a clinically relevant concentration of ketamine for 1 h did not influence the activity of mitochondrial complex I NADH dehydrogenase (Fig. 5B). Meanwhile, after exposure for 6 and 24 h, ketamine significantly decreased mitochondrial complex I activity by 29 and 40%, respectively.

**Effects of Ketamine on α- and β-Actin and Microtubulin mRNA Productions.** Biosyntheses of α- and β-actin and microglobulin mRNA in HepG2 cells were analyzed to evaluate the effects of ketamine on the expressions of these cytoskeletal monomers (Fig. 6). In untreated HepG2 cells, α- and β-actin mRNAs were detected (Fig. 6A, top and middle panels, lane 1). After exposure to a therapeutic concentration of ketamine for 1, 6, and 24 h, the syntheses of α- and β-actin mRNAs were not influenced (Fig. 6A, deleting lanes 2–4).
Levels of β2M mRNA were determined as the internal standards (Fig. 6A, bottom panel). These DNA bands were quantified and analyzed (Fig. 6B). Administration of a clinically relevant concentration of ketamine did not change the production of α- or β-actin mRNA in HepG2 cells. Microglobulin mRNA was detected in control hepatocytes (Fig. 6C, top panel, lane 1). Treatment of HepG2 cells with a clinically relevant concentration of ketamine for 1, 6, and 24 h did not affect synthesis of microglobulin mRNA (Fig. 6C, deleting lanes 2–4). The amounts of β2M mRNA were determined as the internal standards (Fig. 6C, bottom panel). These DNA bands were analyzed and are shown in Fig. 6D. Exposure of HepG2 cells to ketamine did not influence microglobulin mRNA synthesis.

### Inhibition of Erythromycin N-Demethylation and CYP3A4 Protein and mRNA Productions by Ketamine

To evaluate the effects of ketamine-induced suppression of F-actin and microtubular cytoskeletons on the metabolic capacity of HepG2 cells, CYP3A4 expression and activity were analyzed (Fig. 7). In untreated HepG2 cells, the activity of CYP3A4 involved in erythromycin N-demethylation was detected (Fig. 7A). However, after exposure to ketamine for 6 and 24 h, the CYP3A4-involved N-demethylation of erythromycin was significantly decreased by 31 and 54%, respectively. CYP3A4 protein and mRNA were detected in untreated HepG2 cells (Fig. 7, B and D, top panels, lane 1). Administration of a clinically relevant concentration of ketamine for 1 h did not change CYP3A4 protein and mRNA productions (Fig. 7, B and D, top panels, lane 2). Meanwhile, after exposure for 6 and 24 h, ketamine obviously inhibited the syntheses of CYP3A4 protein and mRNA (Fig. 7, B and D, top panels, lanes 3 and 4). Levels of β2M protein and mRNA were determined as the internal standards (Fig. 7, B and D, bottom panels). These protein and DNA bands were quantified and analyzed (Fig. 7, C and E). Treatment of HepG2 cells with ketamine for 6 and 24 h caused significant decreases of 37 and 49% in CYP3A4 protein synthesis (Fig. 7C). Simultaneously, the levels of CYP3A4 mRNA were inhibited by 28 and 49% after ketamine administration for 6 and 24 h, respectively (Fig. 7E).

### Discussion

This study shows that therapeutic concentrations of ketamine (≤100 μM) can induce dysfunction of HepG2 cells. Administration of neither 25, 50, nor 100 μM ketamine was cytotoxic to HepG2 cells. Concentrations of ketamine used in this study are within the range of clinical relevance (Domino et al., 1982; Grant et al., 1983). Under such clinically relevant concentrations, ketamine suppressed polymerization of F-actin and microtubular cytoskeletons. Levels of intracellular calcium and cellular ATP significantly decreased after ketamine administration. Cytoskeletons and mitochondria are two key organelles that are involved in maintaining cell activities (Canton et al., 2006). In parallel with the suppression of cytoskeletal remodeling, exposure of HepG2 cells to a therapeutic concentration of ketamine (100 μM) inhibited CYP3A4 expression and its enzyme activity. In hepatocytes, CYP3A4 is a major enzyme that participates in the metabolism of xenobiotics (Martinez-Jimenez et al., 2007). Our previous studies showed that a therapeutic concentration of ketamine can suppress macrophage activities and reduce endothelial nitric-oxide synthesis (Chang et al., 2005; Chen et al., 2005). In this study, we further demonstrated that clinically relevant concentrations of ketamine can lead to dysfunction of HepG2 cells due to suppression of cytoskeletal remodeling, calcium mobilization, ATP synthesis, and enzyme activity rather than the death mechanism.

Ketamine can suppress polymerization of F-actin and microtubular cytoskeletons. Treatment of HepG2 cells with a therapeutic concentration of ketamine (100 μM) significantly decreased polymerization of F-actin and microtubular cytoskeletons. Actin and microtubulin are two monomers that comprise F-actin and microtubular cytoskeletons, respectively (Ready et al., 1990; Thai et al., 2006). Treatment of HepG2 cells with ketamine did not affect the biosyntheses of α- and β-actins or microtubulin mRNA. Thus, it is possible that ketamine suppresses F-actin and microtubular cytoskeletons by means other than through inhibition of α- and β-actins or microtubulin expression. A previous study showed that levels of calcium can trigger cytoskeletal remodeling and regulate cell motility (Adamiková et al., 2004; Fache et al., 2005). The present results revealed that ketamine alleviated the bradykinin-induced enhancement of intracellular calcium. In addition, cytoskeletal remodeling is energy-dependent (Molitoris et al., 1996). Our results showed that ketamine caused significant decreases in cellular ATP levels in HepG2 cells. Therefore, possible reasons explaining the ketamine-caused suppression of remodeling of F-actin and microtubular cytoskeletons are decreases in cellular calcium and ATP levels rather than inhibition of monomer syntheses.

Ketamine can decrease bradykinin-induced augmentation of cellular calcium levels in HepG2 cells. Exposure of HepG2 cells to ketamine for 6 and 24 h significantly decreased the bradykinin-enhanced calcium mobilization. Bradykinin increases calcium influx through stimulation of the bradykinin-B2 receptor (Pallone et al., 1998). This endogenous bradykinin-B2 receptor is found in hepatocytes (Yayama et al., 2007). Thus, we suppose that ketamine might interact with bradykinin-B2 receptor during 6- and 24-h-treated peri-
ods. To prove this theory, our other study focuses on cloning B2 receptor cDNA from HepG2 cells. Subsequently, we will evaluate the effects of ketamine on calcium mobilization and cytoskeleton remodeling in B2 receptor-overexpressed HepG2 cells. The suppressive effects of ketamine on intracellular calcium have been reported in a variety of cells and are known to sequentially affect cell activities. In resistant mesenteric arteries, ketamine can decrease voltage-gated calcium influx and norepinephrine-induced calcium release and causes a contractile response in smooth muscle cells (Akata et al., 2001). A decrease in intracellular levels of calcium can be associated with reduced nitric-oxide synthesis in endothelial cells (Fleming and Busse, 1999). Therefore, ketamine can suppress intracellular calcium concentrations in HepG2 cells, which sequentially causes dysfunction of cytoskeletal remodeling and even hepatocyte activities.

Ketamine at a therapeutic concentration decreased cellular ATP synthesis via suppressing the mitochondrial membrane potential and complex I NADH dehydrogenase activity. Treatment of HepG2 cells with ketamine reduced the mitochondrial membrane potential. Maintenance of the mitochondrial membrane potential is critical for ATP synthesis (Papucci et al., 2003). In addition, complex I NADH dehydrogenase activity participates in the respiratory chain reaction (Wu et al., 2005). In parallel with reductions in the membrane potential and complex I activity in hepatocytes mitochondria, ketamine decreased cellular ATP levels. Thus, the ketamine-caused suppression of cellular ATP synthesis is due to a reduction in the mitochondrial membrane potential and NADH dehydrogenase activation. Our previous study also showed that ketamine can inhibit mitochondrial ATP synthesis, which thus suppresses macrophage activities (Chang et al., 2005). ATP is also needed for polymerization of F-actin and microtubular cytoskeletons (Nishimura et al., 1998). In mouse endothelial cells, an ATP defect induces alterations of the F-actin cytoskeleton and leads to cell damage and microvascular dysfunction (Suurna et al., 2006). Therefore, a therapeutic concentration of ketamine can decrease cellular ATP levels, and such inhibition may induce interruption of F-actin and microtubular cytoskeletons and decrease hepatocyte activities.

Ketamine inhibits CYP3A4 expression and its enzyme activity. Ketamine at a therapeutic concentration decreased microsomal erythromycin N-demethylase activity. Although midazolam and testosterone can also be used as the substrates for CYP3A4 assay, the present study has further determined that ketamine could inhibit CYP3A4 protein and mRNA productions in HepG2 cells. CYP3A4 has been reported to be a major enzyme catalyzing erythromycin N-demethylation (Wang et al., 1997). In the rat liver, ketamine can decrease CYP3A activity (Meneguz et al., 1999). Thus, the present results further demonstrate that ketamine can reduce CYP3A4 enzyme activity, and the suppressive mechanism occurs at least at a pretranslational level. Because CYP3A4 is the principal enzyme responsible for ketamine metabolism in the human liver (Hijazi and Bouliou, 2002), we suggest that the ketamine-induced reduction of CYP3A4 enzyme activity may lead to suppression of hepatocyte clearance of this intravenous anesthetic agent. Previous studies showed that ketamine can modulate P450 gene expression (Meneguz et al., 1999; Chan et al., 2005, 2006). Cytoskeletal disruption has been shown to severely impair basal and inducible expressions of human P450 genes (Dvorák et al., 2005). Our present results show that ketamine can suppress F-actin and microtubular cytoskeletons. Therefore, one of the possible mechanisms explaining the ketamine-caused inhibition of CYP3A4 mRNA production is via suppression of F-actin and microtubular cytoskeletons.

In conclusion, the present study shows that therapeutic concentrations of ketamine (≤100 μM) can suppress polymerization of F-actin and microtubular cytoskeletons in HepG2 cells. Meanwhile, ketamine did not affect actin or microtubulin mRNA production. Instead, ket-
amine alleviated the bradykinin-induced increase in intracellular calcium levels and mobilization. In addition, ketamine decreased the mitochondrial membrane potential, complex I NADH dehydrogenase activity, and cellular ATP levels in HepG2 cells. Simultaneously, ketamine inhibited CYP3A4 mRNA synthesis, and this enzyme is involved in the N-demethylation of erythromycin. Ketamine at clinical concentrations is not cytotoxic to HepG2 cells. Therefore, according to the present data, we suggest that ketamine at clinically relevant concentrations can disturb polymerization of F-actin and microtubular cytoskeletons possibly via suppression of calcium mobilization and

**Fig. 7.** Effects of ketamine on erythromycin N-demethylase activity and CYP3A4 protein and mRNA productions. HepG2 cells were exposed to 100 μM ketamine for 1, 6, and 24 h. A, microsomal erythromycin N-demethylase activity was determined after formation of formaldehyde using the Nash reagent. B and D, top panels, CYP3A4 protein and mRNA were detected using immunoblotting and RT-PCR analyses. B and D, bottom panels, amounts of β2M protein and mRNA were determined as the internal standards. C and E, these protein and DNA bands were quantified and analyzed. Each value represents the mean ± S.D. of six individual experiments. *, values significantly differ from the respective control, p < 0.05. M, DNA 100-base pair marker.
mitochondrial ATP synthesis rather than inhibition of their monomer expressions. The ketamine-caused suppression of cytoskeletal remodeling may lead to inhibition of CYP3A4 expression. There are certain limitations to this study. Because this in vitro study used a human cancer cell line instead of human primary hepatocytes, we cannot draw definite, clinically relevant conclusions about the possible effects of ketamine on hepatocyte functions.

Acknowledgments. We are grateful to Yi-Ling Lin for technical support and data collection for the experiment.

References


Address correspondence to: Dr. Ruei-Ming Chen, Graduate Institute of Medical Sciences, College of Medicine, Taipei Medical University, 250 Wu-Xing St., Taipei 110, Taiwan. E-mail address: ruchen@tmu.edu.tw