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(−)-Anonaine Induces DNA Damage and Inhibits Growth and Migration of Human Lung Carcinoma H1299 Cells

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ABSTRACT: The anticancer effects of (−)-anonaine were investigated in this current study. (−)-Anonaine at concentration ranges of 50−200 μM exhibited significant inhibition to cell growth and migration activities on human lung cancer H1299 cells at 24 h, albeit cell cycle analyses showed that (−)-anonaine at the above concentration ranges did not cause any significant changes in cell-cycle distributions. Significant nuclear damages of H1299 cells were observed with 10−200 μM (−)-anonaine treatment in a comet assay, whereas higher concentrations (6 and 30 mM) of (−)-anonaine concentrations were required to cause DNA damages in an *in vitro* plasmid cleavage assay. In summary, our results demonstrated that (−)-anonaine exhibited dose-dependent antiproliferatory, antimigratory, and DNA-damaging effects on H1299 cells. We inferred that (−)-anonaine can cause cell-cycle arrest and DNA damage to hamper the physiological behavior of cancer cells at 72 h, and therefore, it can be useful as one of the potential herbal supplements for chemoprevention of human lung cancer.

KEYWORDS: (−)-Anonaine, lung cancer, antiproliferation, DNA damage, comet assay, cell migration

INTRODUCTION

Lung cancer is one of the leading deaths in both males and females globally.[1] Non-small cell lung cancer (NSCLC) is lung cancer of any epithelial type (including adenocarcinoma, squamous cell carcinoma, and large cell carcinoma) other than small cell lung cancer (SCLC). NSCLCs account for approximate 85% of all lung cancer cases and are relatively insensitive to chemotherapy clinically, as compared to SCLCs.[2] Although various strategies for treating lung cancers, such as chemotherapy, radiotherapy, and radiosurgery, have been reported,[3] development of novel and effective drugs against lung cancers still remains a challenge.

Michelia alba DC (Magnoliaceae) is a flowering plant native to tropical and subtropical southeast Asia, whose various constituents have been widely used for medicinal purposes. While extracts from its white yellowish fragrant flowers are a potent smooth-muscle relaxant and used as an abortive agent, the bark of *M. alba* DC is commonly used to treat malaria, syphilis, and gonorrhea in oriental medicine.[4] (−)-Anonaine (Figure 1), an aporphine isoquinoline alkaloid isolated from leaves of *M. alba* DC, has been shown to exhibit various pharmacological effects that include antidepressant,[5] antiperoxidative,[6] antifungal, and antibacterial activities.[7] However, the antitumor activities of (−)-anonaine are poorly understood and remain to be elucidated.

In our current work, we investigated the anticancer potential of (−)-anonaine by measuring its cytotoxic, DNA-damaging, and antimigratory effects on human lung carcinoma H1299 cells. Effects of (−)-anonaine on cell-cycle progression were also examined. Our data suggested that (−)-anonaine could be beneficial in inhibiting both growth and migration of lung cancers, and its underlying mechanisms of such anticancer activities merit further investigation. This is the first study to demonstrate (−)-anonaine biofunctions on H1299 human lung cancer cells.

MATERIALS AND METHODS

Purification of (−)-Anonaine. Stems of *M. alba* DC were collected from Fooyin University, Kaohsiung County, Taiwan, in March 2005. A voucher specimen (Michelia 2) was characterized by Dr. Yen-Ray Hsui of the Division of Silviculture, Taiwan Forestry Research Institute, Taipei, Taiwan, and deposited in the School of Medical and Health Sciences, Fooyin University, Kaohsiung County, Taiwan. The air-dried stems of *M. alba* DC (3.0 kg) were extracted with methanol (MeOH) (50 L × 6) at room temperature, and a MeOH extract (156.7

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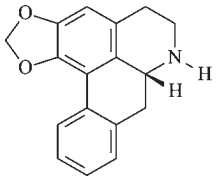

Figure 1. Structure of (−)-anonaine.

g) was obtained upon concentration under reduced pressure. The MeOH extract, suspended in H_2O (1 L), was partitioned with chloroform ($CHCl_3$) (2 L × 5) to give fractions soluble in $CHCl_3$ (89.2 g) and H_2O (60.8 g). The $CHCl_3$-soluble fraction was chromatographed over silica gel (800 g, 70−230 mesh) using *n*-hexane, *n*-hexane/EtOAc mixtures, EtOAc/$CHCl_3$ mixtures, $CHCl_3$/MeOH mixtures, and MeOH mixtures as eluents to produce five fractions. Part of fraction 4 (25.63 g) was subjected to silica gel chromatography by eluting with $CHCl_3$/MeOH (100:1), enriched with MeOH to furnish three fractions (4-1−4-3). Fraction 4-1 (11.62 g) was further purified on a silica gel column using $CHCl_3$/MeOH mixtures to obtain (−)-anonaine (68 mg). The structure of (−)-anonaine was identified by spectroscopic analysis (purity >95%). This compound was identified by spectroscopic data analysis [^{1}H and ^{13}C nuclear magnetic resonance (NMR), correlation spectroscopy (COSY), nuclear Overhauser effect spectrometry (NOESY), heteronuclear multiple-bond correlation (HMBC), and heteronuclear multiple-quantum coherence (HMQC)].

Chemicals and Reagents. Dimethyl sulfoxide (DMSO), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and propidium iodide (PI) were purchased from Sigma (St. Louis, MO). RPMI-1640, antibiotics, and other supplements for cell cultures were purchased from Invitrogen (Carlsbad, CA). Fetal bovine serum (FBS) was purchased from Hyclone (Logan, UT). The cell line was purchased from American Type Culture Collection (ATCC) (Manassas, VA). All other reagents and chemicals used were obtained in the purest forms possible from commercial sources.

Cell Cultures. H1299 (human lung adenocarcinoma) and NB4 [human acute promyelocytic leukemia, to provide nuclear extracts for the comet−nuclear extract (comet−NE) assay mentioned below] cells were routinely maintained in complete RPMI-1640 supplemented with 10% fetal bovine serum (FBS), 100 units/mL penicillin, 100 μg/mL streptomycin, and 0.03% glutamine. Cells were kept at 37 °C in a humidified atmosphere containing 5% CO_2.

Cell Growth Assay. (−)-Anonaine was dissolved in DMSO and diluted in culture medium to the indicated concentrations. At the treatment stage, the final DMSO concentration was never higher than 0.05%. Under these conditions, DMSO was not shown to be toxic or DNA-damaging to the cells. Cell growth inhibition was determined by the MTT assay as previously described.[8] In brief, H1299 cells were seeded in 96-well plates at a density of 4×10^3 cells/well and allowed to attach for 4−8 h at 37 °C. The medium was then replaced with 150 μL of complete RPMI-1640 or medium containing (−)-anonaine to desired final concentrations in triplicates (final DMSO concentrations for control and treatments were 0.05%). After the cells were treated for 24 or 48 h, the medium was aspirated and replaced with 100 μL of fresh medium containing 0.5 mg/mL MTT. The plates were further incubated for 2 h at 37 °C before the MTT-containing medium was aspirated and 100 μL of DMSO was added to solubilize the purple formazan precipitates. After the plates were gently shaken for 20 min in the dark to ensure maximal dissolution of formazan crystals, the plates were read at 595 nm on a microtiter plate reader. The absorbance (A) reflected the number of viable cells and was expressed as a percentage of viable cells in the control using the following formula: $[(A_{sample} - A_{blank})/(A_{control} - A_{blank})] \times 100\%$.

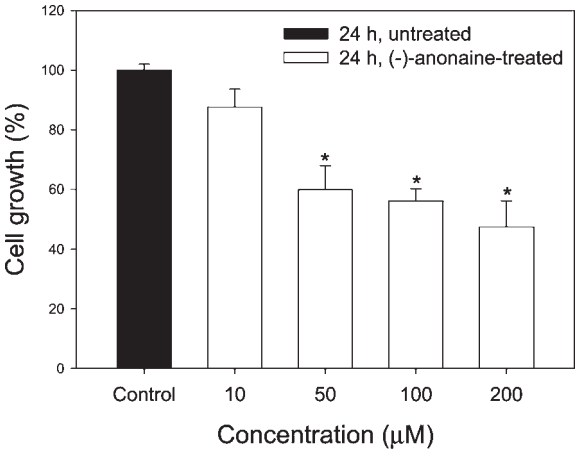

Figure 2. Effects of (−)-anonaine on the proliferation of H1299 cells. H1299 cells were cultured in complete RPMI-1640 in 96-well plates at a density of 4×10^3 cells/well for 4−8 h to allow for complete adherence. Then, cells were either left untreated or treated with (−)-anonaine at doses of 10, 50, 100, and 200 μM for 24 h. The MTT assay was performed, as described in the Materials and Methods, to evaluate cell growth. The values indicate the percentage of total viable cells compared to the untreated control and represent mean ± SD of three independent experiments. Significance was accepted at $p < 0.05$ (*) versus the control.

pBR322 Plasmid DNA Cleavage Assay. Conversion of the supercoiled (S) form of plasmid DNA to the open-circular (OC) and/or further linear (L) forms was accessed as an indicator of the DNA strand breaks. The reaction mixtures (10 μL) contained 150 ng of pBR322 plasmid DNA, 0.1 mM $FeSO_4$, varying concentrations of (−)-anonaine (1.2, 6, and 30 mM, respectively), or 0.05% H_2O_2 and were incubated at 37 °C for 30 min. Subsequently, the reaction was stopped by adding 2 μL of 6× gel-loading dye [0.05% bromophenol blue, 40 mM ethylenediaminetetraacetic acid (EDTA), and 50% glycerol (v/v)]. The mixtures were electrophoresed on 1% agarose gel in 1× Tris-acetate-EDTA (TAE) buffer at 50 V for 1−2 h. DNA in the gel was stained with ethidium bromide, visualized, and photographed under ultraviolet light. All studies were repeated 3 times under identical experimental conditions.

Comet−NE Assay. To determine the extent of DNA damage in cells, a modified single-cell gel electrophoresis or the "comet−NE assay" was carried out as previously described.[9] Briefly, NB4 cell lysates were used as the source for the preparation of nuclear extracts as a substitution of adduct-specific enzymes to enhance the sensitivity of the standard comet assay.[10,11] A total of 100 μL of H1299 cells [1×10^6 cells/mL in phosphate-buffered saline (PBS)], pretreated with varying concentrations of (−)-anonaine for 2 h, was mixed with an equal volume of 1.2% low melting point (LMP) agarose prepared in 1× PBS at 37 °C. This mixture was immediately placed on top of a glass slide precoated with 1% normal melting point (NMP) agarose before solidifying at 4 °C. Subsequently, the slides were immersed into freshly made chilled lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris at pH 10, 1% *N*-laurylsarcosine, 1% Triton X-100, and 10% DMSO) and incubated at 4 °C for at least 2 h to allow for complete lysis of cells. The slides were then covered with coverslips and incubated at 37 °C in a sealed humidified chamber for 2 h. After the incubation, the slides were placed in a rinsing chamber, containing 0.3 N NaOH and 1 mM EDTA, for 20 min to allow for salt equilibration and further DNA unwinding before being electrophoresed at 20 V (300 mA) in TBE buffer (1% Tris base, 0.5% boric acid, and 0.093% edetic acid) for 25 min. The slides were rinsed briefly with deionized water and neutralized by slowly immersing them in neutralization buffer (0.4 M Tri-HCl at pH 7.5). Cellular DNA was stained by

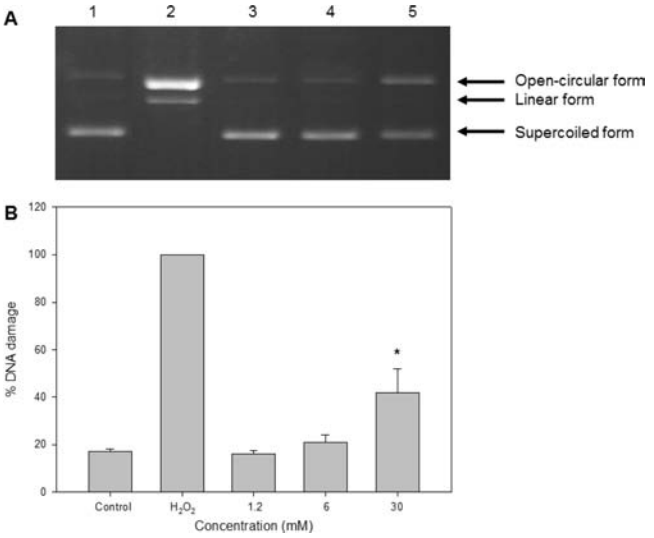

Figure 3. Effects of (−)-anonaine on DNA damage. (A) Equal amounts (150 ng) of pBR322 plasmid DNA were either left untreated (lane 1) or treated with 0.1 mM $FeSO_4$ and 0.05% H_2O_2 (lane 2) or varying concentrations of (−)-anonaine (lanes 3−5) and incubated at 37 °C for 30 min. Subsequently, a $^1/_5$ volume of 6× loading dye was added to stop the reaction, and the mixtures were electrophoresed on a 1% agarose gel at low voltage (50 V) for 1−2 h. After the gel was stained with ethidium bromide, it was visualized and photographed under ultraviolet light. (B) Degree of DNA damage in control and experimental groups was determined by summing band intensities of all three DNA forms and calculating the percent of non-supercoiled forms using the H_2O_2-treatment group as the complete DNA damage control. A representative experimental result was shown here. Significance was accepted at $p < 0.05$ (∗) versus the control.

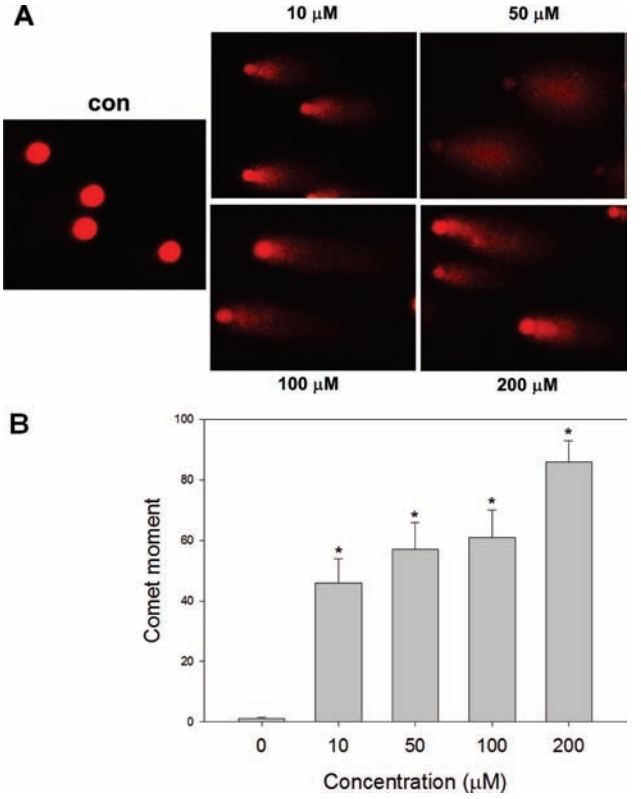

Figure 4. Comet−NE assay to quantify (−)-anonaine-induced DNA damage on H1299 cells. (A) Representative images were taken from PI-stained H1299 cells pretreated with varying concentrations of (−)-anonaine for 2 h. The stained circle spots are nuclei, and the comet tails accompanying nuclei indicate DNA damage. (B) Comet tail moment calculated from 100 cells (mean ± SD) for each experiment was quantified using CometScore software (TriTek Corp.) and normalized against control cells. The values represent the mean ± SD of three separate experiments. (∗) $p < 0.05$ versus the control untreated cells.

adding 40 μL of PI (50 μg/mL) onto the slides and leaving the slides in the dark for 20 min before observation. The slides were examined under a fluorescence microscope (TE2000-U, Nikon, Tokyo, Japan). The images of randomly selected 100 cells per treatment were recorded with a closed-circuit display camera. The damage profile of DNA was accessed by measuring the migration of DNA from the nucleus of each cell with CometScore, version 1.5, software (http://www.tritekcorp.com) for visual scoring and calculating the comet moment, which is defined as the product of the tail length (from the center of the comet head to the end of the tail) and the fraction of total DNA in the tail. The formula for calculating the comet moment is as follows:

$$\sum_{0 \rightarrow n} \frac{[(\text{amount of DNA at distance } X)(\text{distance } X)]}{\text{total DNA}}$$

Wound Healing Assay. The potential of cellular migration was determined by wound healing migration assays, which were performed according to the previously reported method,[12] with minor modification. In brief, 5×10^5 cells were seeded into 12-well culture plates and incubated at 37 °C until reaching complete confluence. The confluent cell monolayer was manually scratched with a sterile pipet tip and thoroughly washed with PBS, followed by adding with either vehicle (medium containing 0.05% DMSO) or various concentrations of (−)-anonaine (ranging from 10 to 200 μM) for the indicated time periods. Subsequently, the recovery of the monolayer was photographed using an inverted phase-contrast microscope (TE2000-U, Nikon, Tokyo, Japan) equipped with NIS-Elements (Nikon) software. Cell migration into the wound areas was calculated as the area covered by migrating cells divided by that of the original scratch and expressed as the fold increase over untreated control cells. The free software TScratch (http://www.cse-lab.ethz.ch/software.html) was used for the calculation.[12]

Cell Cycle Analysis by Flow Cytometry. To determine the phase distribution of DNA content in H1299 cells, PI staining was performed as previously described.[9] Briefly, cells were treated with vehicle (medium containing 0.05% DMSO, as controls) or (−)-anonaine at desired final concentrations for 24, 48, and 72 h. After treatments, the floating and trypsin-detached H1299 cells were collected, washed twice with ice-cold PBS, and then fixed in 70% cold ethanol. The cells were then stained in PBS containing 10 μg/mL of PI, 10 μg/mL RNase A, and 0.05% Triton X-100 for 15 min at room temperature in the dark. The DNA content of H1299 cells was analyzed with a FACScan flow cytometer (Becton-Dickinson, Mansfield, MA). At least 10 000 events were analyzed for each test sample. Data analyses were performed with Cell-Quest and Modfit software (Becton-Dickinson, Mansfield, MA).

Statistical Analysis. All data values are presented as the mean values [± standard deviation (SD)] of at least three independent experiments. Where appropriate, data were analyzed by one-way analysis of variance (ANOVA) followed by the Dunnett's test. Statistical significance was assumed with a p value of less than 0.05.

RESULTS AND DISCUSSION

Antiproliferative Effects of (−)-Anonaine on Human Lung Carcinoma H1299 Cells. The frequency of p53 mutation in

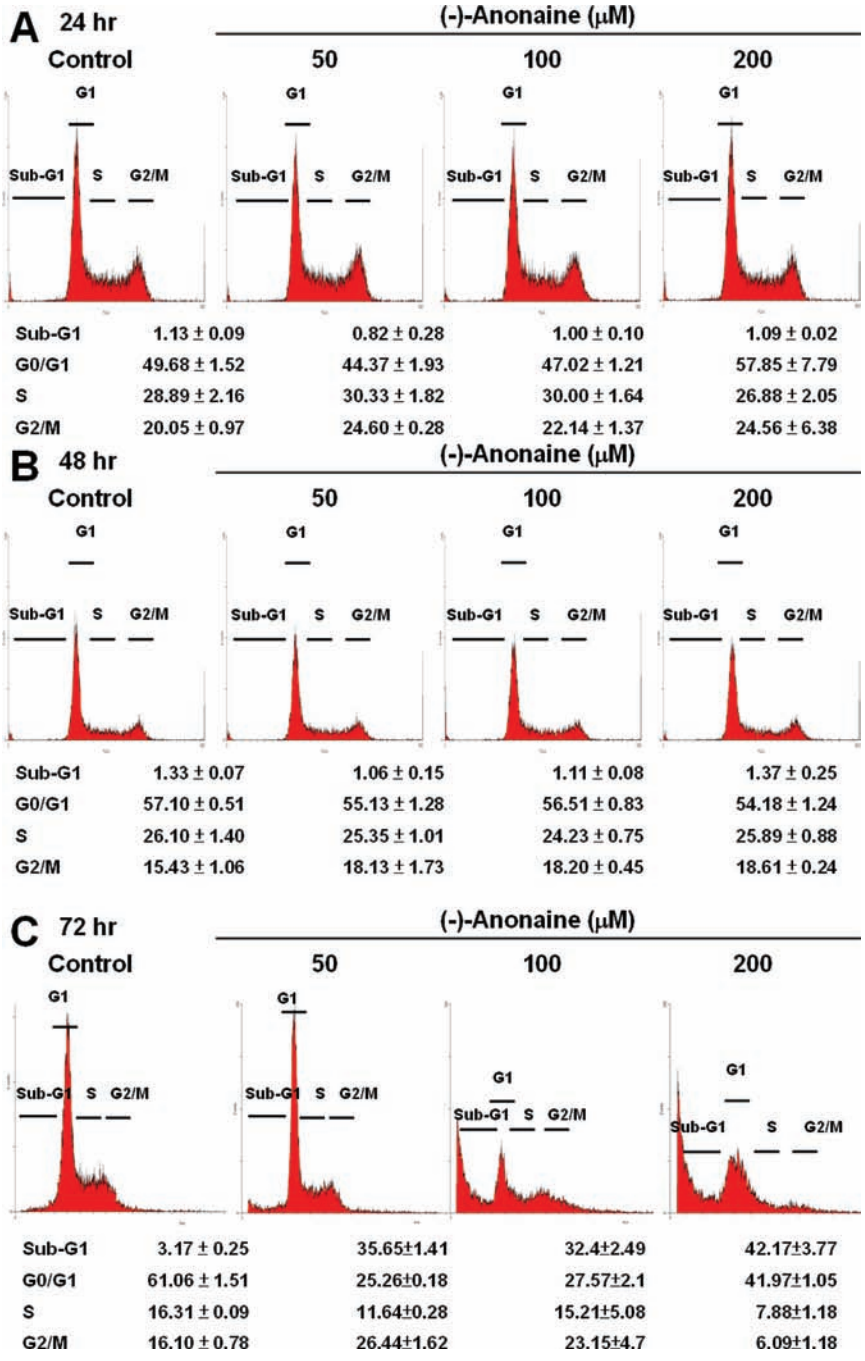

Figure 5. Effects of (−)-anonaine on cell-cycle distribution. H1299 cells were treated with 0, 50, 100, and 200 μM (−)-anonaine for (A) 24, (B) 48 h, and (C) 72 h. Cells were then subjected to flow cytometry analysis as described in the Materials and Methods. Values were obtained from three independent experiments, and representative histograms were shown. Data analyses and quantification were performed with Cell-Quest and Modfit software (Becton-Dickinson, Mansfield, MA).

lung cancer cells, including H1299 is high, and more than 50% of lung cancers carry a mutated or null p53 gene. In category, the H1299 cell is a large cell carcinoma in NSCLC. NSCLC includes squamous cell carcinoma, adenocarcinoma, and large cell carcinoma; moreover, the large cell carcinoma often shows agressiveness and intrinsic multidrug resistance.[13] Accordingly, the H1299 cell represents the highly metastatic and multidrug-resistant NSCLC and, therefore, is widely used in a variety of lung cancer studies.[14−16] In this study, we purified (−)-anonaine from the stems of *M. alba* DC and investigated its antiproliferative and other properties on human lung carcinoma cells. To evaluate the growth inhibition of (−)-anonaine, H1299 cells was treated at 10, 50, 100, and 200 μM concentration for 24 h. The MTT assay was then used to determine the cell growth. As shown in Figure 2, the cell growth of (−)-anonaine-treated H1299 cells decreased from 87 ± 6% (10 μM) to 47 ± 9% (200 μM) in a dose-dependent manner. Our data indicated that (−)-anonaine exhibited strong growth inhibitory effects at micromolar concentration ranges on H1299 cells.

(−)-Anonaine Induced DNA Strand Breaks on the pBR322 Plasmid. Typically, conversion of the supercoiled form of

plasmid DNA to either open-circular or further linear form has been used as an index of DNA damage.[17] The DNA damage of (−)-anonaine was evaluated by an *in vitro* DNA cleavage assay using pBR322 plasmid DNA. Figure 3 showed the cleavage effects of (−)-anonaine on pBR322 plasmid DNA, as compared to the oxidative DNA damage caused by treatment with Fe^{2+} and H_2O_2. In the absence of Fe^{2+} and H_2O_2, the pBR322 plasmid DNA was mainly in the supercoiled form (lane 1, control). However, the supercoiled form decreased and was converted to the relaxing open-circular and linear forms after the addition of Fe^{2+} and H_2O_2 (lane 2, H_2O_2), indicating that the integrity of plasmid DNA was compromised by the oxidative stress caused by H_2O_2. At the concentrations of micromolar (or lower) ranges of (−)-anonaine, we essentially did not observe any shift of DNA fragments from the supercoiled forms to others (data not shown), indicating that (−)-anonaine at lower concentrations did not induce DNA-damaging effects as seen with H_2O_2 treatments. Conversely, when (−)-anonaine was added at higher concentration ranges (6 and 30 mM), a dose-dependent decrease in the percent plasmid DNA migrating as the supercoiled forms was observed. Our result suggested that (−)-anonaine influences the stability of DNA by generating DNA strand breaks, resulting in the decrease of DNA integrity. It is also worth noting that (−)-anonaine caused significant DNA-damaging effects when used at a higher millimolar concentration (i.e., 30 mM) in our *in vitro* plasmid DNA cleavage assay, whereas it effectively reduced cell growth (Figure 2) and caused cellular DNA strand breaks (Figure 4) at micromolar concentrations. These results showed that the plasmid DNA and cellular DNA tended to have different sensitivities to (−)-anonaine, suggesting that the nature for cytoplasma may be helpful for DNA damage compared to the water on the plasmid test. We speculated that, in addition to directly causing DNA damages, (−)-anonaine could possibly influence other pathways or interact with factors essential for the maintenance of cell survival, so that it only requires much lower concentrations of (−)-anonaine to be effective *in vivo*. Therefore, it is demonstrated here that (−)-anonaine can generate DNA strand breaks and decrease the stability of DNA molecules.

(−)-Anonaine Induced to Cellular DNA Damage. Traditionally, the comet assay has been considered as an easy, economical, and speedy method to semi-quantify DNA damages, such as single- and double-stranded breaks,[18,19] and, therefore, is an ideal biomarker for the identification of agents with anticancer potentials. In our study, a modified comet assay (comet−NE) was used to determine whether (−)-anonaine could induce cellular DNA damage of H1299 cancer cells.[10,20] Comet images from controls and (−)-anonaine-treated H1299 cells were analyzed, and comet moment, representing the degree of nuclear DNA damages, was calculated and compared to the untreated controls. As shown in Figure 4A, the nuclei (the stained circles) from untreated controls essentially remain intact with almost no visible comet tail. On the other hand, the nuclei from (−)-anonaine-treated H1299 cells exhibited not only a shrunken morphology but also a prominent comet tail. After normalization against control cells, the comet tail moment of (−)-anonaine-treated cells significantly increased in a dose-dependent manner, with a comet score of 46 ± 8 and 86 ± 7 for the lowest and highest concentrations (10 and 200 μM) of (−)-anonaine used, respectively (Figure 4B). (−)-Anonaine is capable of eliciting DNA-damaging effects, as evidenced by the comet assays on H1299 lung cancer cells.

Cell Cycle Distribution of H1299 Cells Affected by (−)-Anonaine Treatments. As another parameter for assessing

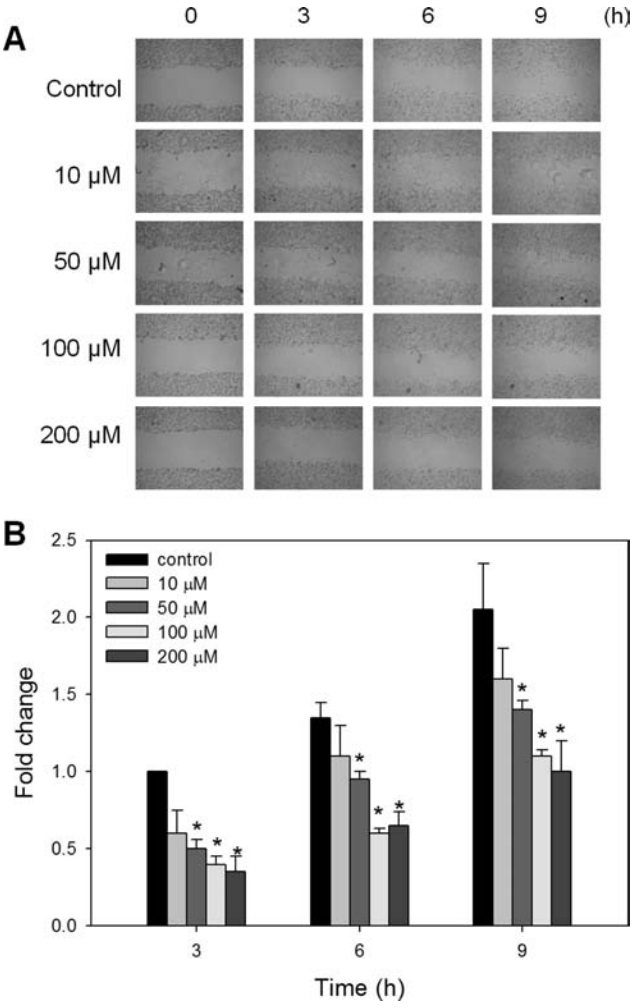

Figure 6. Wound healing assay on (−)-anonaine-treated H1299 cells. H1299 cells were cultured to monolayer confluence on an uncoated 12-well culture dish followed by applying a scratch with a sterile pipet tip. After an extensive wash with PBS, cells were treated with either vehicle (medium containing 0.05% DMSO) or varying concentrations of (−)-anonaine for the indicated time periods. Relative wound closure was calculated as described in the Materials and Methods and is shown as the mean ± SD of three independent experiments. Significance for three different time-point groups was accepted at $p < 0.05$ (∗) versus their corresponding controls.

degrees of DNA damage, cell cycle distributions of (−)-anonaine-treated H1299 cells were analyzed by flow cytometry. Cells were treated with (−)-anonaine at 0, 50, 100, and 200 μM for 24, 48, and 72 h, respectively, followed by PI staining before being subjected to flow cytometric analysis. It has been reported that (−)-anonaine effectively induced apoptosis in human cervical cancer (HeLa) cells.[10] In cell cycle analysis, the prominent sub-G1 population represents a suggestive indicator for apoptotic cells. Our results presented that treatments with (−)-anonaine for 24 and 48 h did not cause any significant change in the distributions of the sub-G1 phase in H1299 cells (panels A and B of Figure 5) but significant change was induced for the 72 h treatment (Figure 5C). The results showed that, at 72 h, (−)-anonaine treatment resulted in the formation of DNA fragments in the sub-G1 phase. In comparison to the control group, 50, 100, and 200 μM (−)-anonaine induced apoptosis in the sub-G1 phase with 35.65 ± 1.41, 32.4 ± 2.49, and 42.17 ± 3.77% at 72 h,

respectively. In comparison to the findings in Figures 2−4, it was noted that (−)-anonaine may lead to both plasmid and cellular DNA damages as well as growth inhibition. This phenomenon raised the issue that we have to carefully interpret the data from the comet−NE assay (Figure 4). The degree for cellular DNA damage was amplified by the addition of the nuclear extracts; therefore, we cannot expect the original amounts of cellular DNA damage without nuclear extracts, although the cellular DNA damages were present. This amplified signal from comet−NE cannot be cross-compared to other experiments, even though they were performed under the same concentration. Therefore, it was likely that (−)-anonaine elicits its antiproliferating effects on H1299 cells via other mechanism(s) than directly causing cell-cycle arrest. DNA damage is only one of possible mechanisms for antiproliferation effects of (−)-anonaine.[21−24] Further studies are needed to investigate the antilung cancer mechanism from (−)-anonaine. In addition, the possible clinical usage for (−)-anonaine cannot start before confirming the safe response for normal cells. Currently, we cannot exclude the possibility that (−)-anonaine might be a potent carcinogen to induce genomic or gene toxicity.

Evaluation of Antimigratory Effects of (−)-Anonaine on H1299 Cells. In normal physiological conditions, cell migration plays a crucial role for the maintenance of development and homeostasis. The deregulated cellular migration is often responsible for the metastasis and invasion of many malignant tumors. Therefore, we evaluated the inhibition of H1299 cell migration by (−)-anonaine using a wound healing assay. In this experiment, the wound-healing process of H1299 cells was triggered by scratching a cell monolayer, followed by treatments with various concentrations of (−)-anonaine. Subsequently, the closure of wounds was measured at different time points (Figure 6A). The antimigratory capacity of (−)-anonaine was accessed by monitoring how effectively (−)-anonaine can inhibit the cell monolayer to invade the wound. The data illustrated that both lower (50 μM) and higher (100 and 200 μM) concentrations of (−)-anonaine caused a significant inhibition (greater than 25 and 50%, respectively) on the recovery of the scratch in all time points measured (Figure 6B). However, on the basis of our results, we did not provide direct evidence to prove the positive correlation between DNA injury and cell migration, although (−)-anonaine-induced DNA injuries (Figures 3 and 4) to restrain proliferation (Figure 2) and a reduced migratory capacity of H1299 tumor cells were found (Figure 6).

Recent studies reported that the interactions of tumor cells and extracellular matrix (ECM) are critical for accelerating cellular proliferation and migration.[25] In addition, the zinc-dependent endopeptidases and matrix metalloproteinases (MMPs) degrade ECM, allowing tumor cells to invade surrounding tissues, intravasate into the circulation systems, including lymphatic and blood vessels, then travel to distant sites.[26] Our results indicated that (−)-anonaine reduced the migratory capacity of lung cancer cells. Therefore, it will be informative to further investigate if (−)-anonaine would possess the potential to modulate the process of ECM metabolism to subsequently inhibit metastasis and invasion of tumors in future studies. In summary, we suggest that (−)-anonaine could be possible in inhibiting growth and migration against human tumors. Further studies to elucidate the mechanism(s) of anticancer action of (−)-anonaine are warranted.

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