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ORIGINAL ARTICLE

Matrine induces apoptosis and autophagy in human lung adenocarcinoma cells via upregulation of Cavin3 and suppression of PI3K/AKT pathway

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Summary

Purpose: *Lung adenocarcinoma is one of the leading causes of mortality and its treatment is limited by the unavailability of effective chemotherapeutic agents. This study was therefore undertaken to evaluate the anticancer effects of Matrine against the human lung adenocarcinoma cells.*

Methods: *CCK-8 assay was used to determine cell viability. Acridine orange (AO)/ ethidium bromide (EB) staining was used for the assessment of apoptosis. Transmission electron microscopy (TEM) analysis was employed for the detection of autophagy. Western blotting was used for the determination of protein expression.*

Results: *The results showed Matrine inhibited the proliferation of the human A549 adenocarcinoma cell line with little effects on the normal MRC5 cells. Investigation of the underlying mechanisms showed that Matrine induced apoptosis in A549 cells. Matrine-induced apoptosis was linked with upregulation of Bax and suppression of Bcl-2. TEM analysis showed that matrine led to development of autophagosomes in A549 cells, suggestive of autophagy. The autophagy induced by Matrine was also accompanied by upregulation of LC3-II and Beclin-1 and suppression of p62. The assessment of the effects of Cavin3 protein showed that Matrine suppressed the Cavin3 in a concentration-dependent manner. Additionally, matrine also blocked the phosphorylation of PI3K and AKT dose-dependently.*

Conclusion: *Taken together, Matrine may be employed for the treatment of lung adenocarcinoma.*

Key words: *lung adenocarcinoma, apoptosis, autophagy, matrine, proliferation*

Introduction

In industrialised countries, lung cancer has been reported to be major cause of cancer related mortality [1]. The 10 year survival rate for non-small cell lung carcinoma (NSCLC) is less than 10% [2]. The most common type of NSCLC is adenocarcinoma [3]. The limited chemotherapy options and constant relapses make it even more difficult to treat [4]. Living organisms produce a vast number of chemical compounds through specialized sets of metabolic reactions constituting the secondary metabolism [5]. The secondary metabolites are usually characteristic of a particular group of organisms. Secondary metabolites, apart from being beneficial to host organisms itself, are seen to possess a variety of health promoting features [6]. Among plant secondary metabolites, alkaloids have shown tremendous pharmacological potential. Many of the plant-derived alkaloids

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have shown the potential to suppress the growth of cancer cells [7]. Matrine is an important alkaloid that has been reported to be present in different plant species, especially the plants of genus *Sophora* [8]. Matrine has been shown to inhibit the proliferation of different types of cancer cells [9]. In 2001 Zhang et al. reported that the growth and differentiation of K-562 cell is suppressed by Matrine [10]. Additionally, matrine has been shown to suppress the growth of murine hepatocellular carcinoma [11]. Nonetheless, so far no study has reported on the anticancer effects of Matrine on human lung lung adenocarcinoma cells. Against this background this study was undertaken to evaluate the anticancer effects of Matrine on human adenocarcinoma together with assessment of apoptosis and autophagy inducing effects of the molecule. It was found that the inhibition of growth of human A549 lung adenocarcinoma cells was via induction of apoptosis and autophagy. These growth inhibitory effects of Matrine were found to be due because of the suppression of PI3K/AKT and upregulation of Cavin3. Taken together, Matrine may prove beneficial in the lung adenocarcinoma treatment

Methods

Growth and proliferation assay

Cell counting kit-8 (CCK-8, MedChemExpress, NJ, USA) was used for estimation of proliferative rates of lung adenocarcinoma cells treated with Matrine (96%, Sigma-Aldrich, St.Louis, Missouri, USA) and proliferation rates were compared with those of normal MRC5 treated cells. In brief, the cells were placed in 96-well plates at of 1×10^6 cells/well and cultured with Matrine for 24h with 0, 2.5, 5, 10, 20, 40, 80 and 180μM, after which CCK-8 was employed to estimate the proliferation rates by the addition of 10μl of CCK-8 solution to each well, at the indicated time intervals. Following 2 h incubation at 37°C, absorbance at 450 nm was read for each sample with a microplate reader.

Apoptosis assay

AO/EB staining followed to examine the effect of Matrine on the viability of lung adenocarcinoma cells and induction of cell apoptosis. The cells were put into 12-well plates at a density of 0.6×10^6 cells/well. Matrine at concentrations of 0, 10, 20 and 40 μM was added to each well and cells were incubated at 37°C for 24 h. Afterwards, the cells were harvested and washed twice with phosphate buffered saline (PBS) followed by fixing with 4% paraformaldehyde. The AO/EB solutions were then separately used to stain the cells. Afterwards, the cells were examined for fluorescence measurements using fluorescent microscope.

Western blotting

Using RIPA lysis and extraction buffer (Thermo Fisher Scientific Waltham, Massachusetts, USA) total proteins were isolated from untreated lung adenocarcinoma cells and cancer cells treated with 10, 20 and 40μM Matrine for 24 h. Bradford method was used to quantify the protein concentrations. About 45 μg of total proteins from each sample were separated electrophoretically on 10% SDS-PAGE. The gel was blotted to nitrocellulose membrane which was given the exposure of primary protein antibodies followed by exposure of secondary antibodies. Enhanced chemiluminescence reagent (ECL) reagent was used for detection of bands corresponding to proteins of interest. The protein expression procedures were normalized with human GADPH protein.

Statistics

The mean and standard deviation (SD) values were calculated from the data obtained from replicas of experimental setups and final representation was made as mean ± SD. Graphpad Prism 7 software was used to perform *t*-test. P value less or equal to 0.05 was taken as an indicator of statistically significant difference.

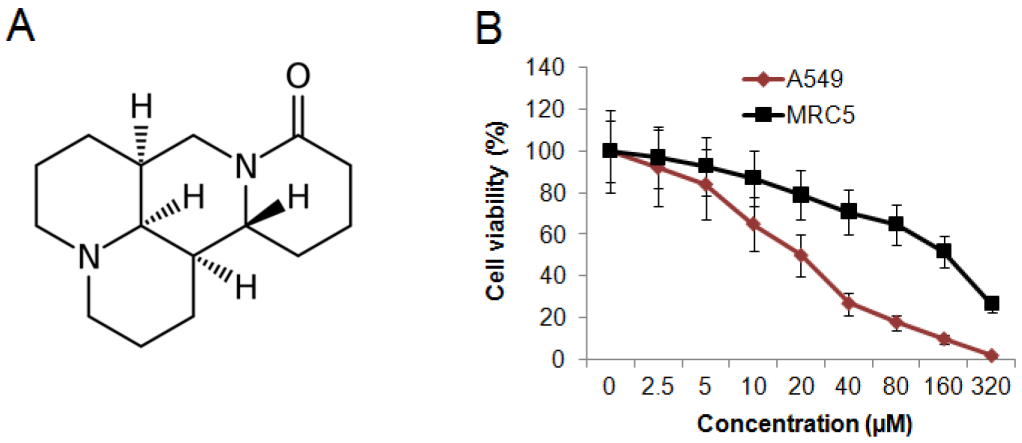

Figure 1. A: Structure of Matrine. **B:** CCK8 assay showing the effect of Matrine on the viability of MRC5 and A549 cells. The experiments were performed in triplicate and expressed as mean ± SD ($p<0.05$).

Results

Inhibition of lung adenocarcinoma proliferation by Matrine

Lung adenocarcinoma growth was suppressed by Matrine acid via apoptotic cell death. To ascertain the effects of Matrine (Figure 1A) on the proliferation of the lung adenocarcinoma A549 cells and the normal MRC5 cells, these cells were treated with 0 to 200 μM concentrations of Matrine for 24 h. Using CCK-8 kit, the cell proliferation rates were determined. It was found that the viability of A549 cancer cells decreased proportionally with increasing doses of Matrine with an IC_{50} of 20 μM (Figure 1B). The effects of Matrine on the normal cells were much lower.

Apoptosis induction in A549 cells by Matrine

Next, several apoptosis assays were performed to investigate if the Matrine-induced growth inhibitory effects were due to induction of apoptosis. Intriguingly, the AO/EB-stained cancer cells were seen with clear nuclear deformation which is indicative of apoptotic cell death (Figure 2). Moreover, the effects were more prominent at higher doses of Matrine. Further support was obtained from western blotting results where it was found that the positively regulating apoptotic proteins, Bax was upregulated and Bcl-2 was downregulated upon Matrine treatment (Figure 3). Taken together, the results infer that the Matrine induces apoptosis in lung adenocarcinoma cells and thus reduces their proliferation rates.

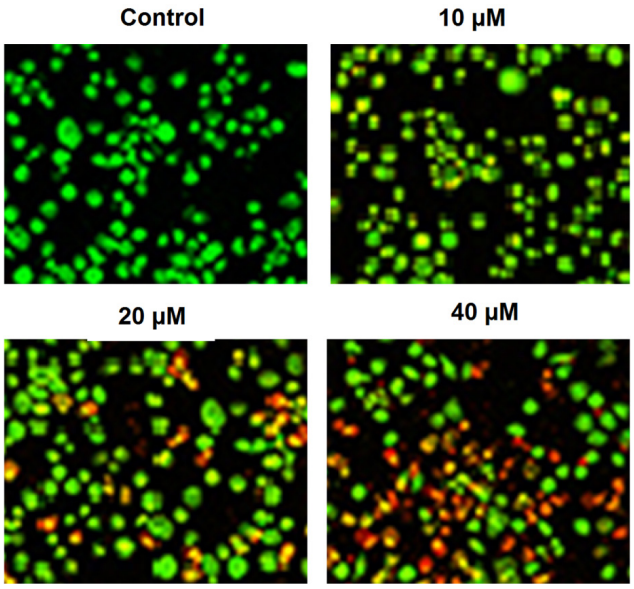

Figure 2. AO/EB staining showing Matrine induction of apoptosis in A549 cells at indicated concentrations. The experiments were performed in triplicate.

Matrine promotes autophagy in lung adenocarcinoma cells

Next, TEM analysis of the A549 cells after treatment with 10 μM Matrine was performed. The results showed that Matrine led to development of autophagosomes in the A549 cells suggestive of autophagy (Figure 4). The autophagy induction was further authenticated by determining the expression of LC3-II, L3-I and p62 expression. The results showed that the expression of LC3-II and Beclin-1 was enhanced and the expression of p62 was decreased (Figure 5).

Matrine modulates the expression of Cavin3 in A549 cells

The impact of Matrine was also assessed on the expression of Cavin3 in A549 cells. The results

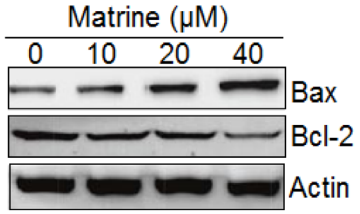

Figure 3. Western blotting showing the effects of Matrine on Bax and Bcl-2 in A549 cells at indicated concentrations. The Figure shows that the expression of Bax increases and of Bcl-2 decreases dose-dependently. The experiments were performed in triplicate.

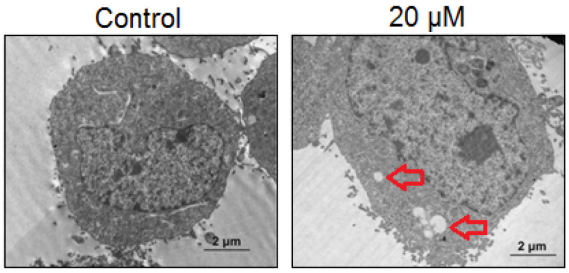

Figure 4. TEM analysis showing induction of autophagy in A549 cells at indicated concentrations of Matrine. Arrows show autophagosomes. The experiments were performed in triplicate.

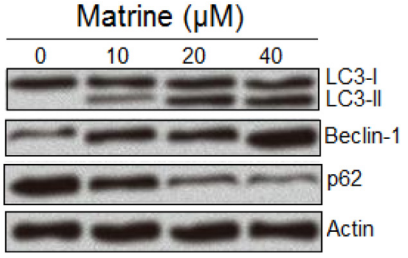

Figure 5. Western blotting showing Matrine increases the expression of LC3-II, Beclin-1 and decreases the expression of p62 in A549 cells at the indicated concentrations. The experiments were performed in triplicate.

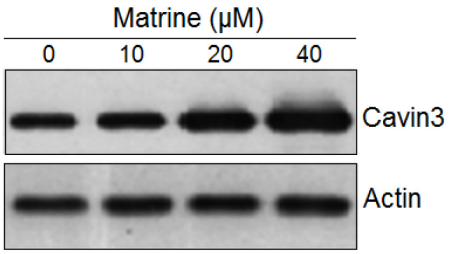

Figure 6. Western blotting showing the effects of Matrine on the expression of Cavin3 in A549 cells at indicated concentrations. The experiments were performed in triplicate.

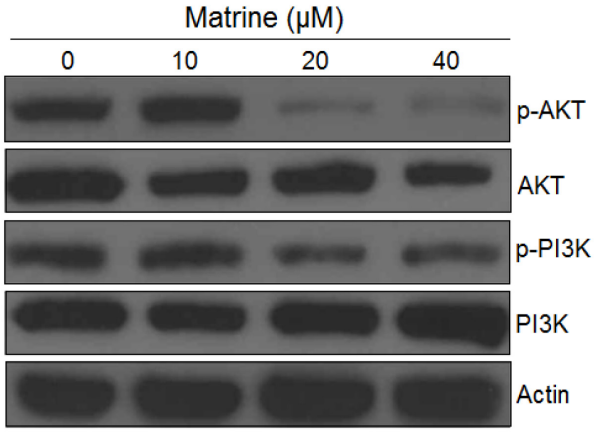

Figure 7. Western blotting showing the effects of Matrine on the expression of PI3K/AKT signalling in A549 cells at indicated concentrations. The Figure depicts the expression of p-PI3K and p-AKT decreased dose-dependently. The experiments were performed in triplicate.

showed that the expression of Cavin3 was significantly upregulated in A549 cells (Figure 6). These effects of Matrine were concentration-dependent.

Matrine blocks the PI3K/KT pathway in A549 cells

The effects of Matrine were also evaluated on the PI3K/AKT pathway. The results showed concentration dependent inhibition of phosphorylation of PI3K and AKT (Figure 7). Nonetheless, there was no apparent effect on the total PI3K and AKT protein levels.

Discussion

Alkaloids constitute a large diverse group of plant secondary metabolites that have low molecular weight and also contain nitrogen [12]. They are generally synthesized from amino acids. Alkaloids have been reported from at least 20% of the plant species wherein these metabolites have defensive role against biotic or abiotic stresses. A wide array of alkaloids have been reported to exhibit enormous pharmacological potential [13]. Constantly, active research is going on to examine the anticancer effects of Matrine against the different human cancers. This study was undertaken to evaluate the anticancer effects of this molecule, a naturally occurring alkaloid of plant origin, against the human lung adenocarcinoma cells. The cell proliferation assay showed significant inhibition of the lung adenocarcinoma cells growth upon Matrine treatment. Previous research has also shown that Matrine and its derivatives halt the proliferation of esophageal cancer cells via induction of apoptosis [14]. The investigation of the underlying mechanism revealed the induction of apoptosis by Matrine in the A549 cells. The Bax/Bcl-2 ratio was also increased which is an important indicator of apoptosis [15]. Apoptosis plays key role in eliminating the defective cells and thus drugs that promote apoptosis are currently being studied extensively [16]. TEM analysis showed that Matrine also induces autophagy in the A549 lung adenocarcinoma cells which was accompanied by alteration of the LC3, Beclin-1 and p62 expression. Several studies have shown that LC3-II expression is increased and that of p62 is significantly decreased during the induction of autophagy [17]. We also observed similar results in our study. This study also examined the effects of Matrine on the cavin3 expression. Cavin3 expression has been shown to be downregulated in several cancer types [18] and herein we found that Matrine upregulated the expression of Cavin3. PI3K/AKT signalling is one of the critical signalling cascades that is dysregulated in cancer cells [19]. Herein we observed that Matrine blocks the PI3K/AKT signalling pathway. Hence more studies are required to establish Matrine as a lead molecule for the development of systemic therapy for lung adenocarcinoma.

Conclusion

Taken together, the results of the present study showed that Matrine inhibited the growth of the human lung adenocarcinoma cells via induction of apoptosis and autophagy. The efficacy of its anticancer properties may be augmented via semi-synthetic chemistry approaches and it may act as a crucial lead molecule for discovery of more efficient drugs against lung adenocarcinoma.

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Conflict of interests

The authors declare no conflict of interests.

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