Matrine inhibits the proliferation, invasion and migration of castration-resistant prostate cancer cells through regulation of the NF-κB signaling pathway

QI LI1,4*, YIMING LAI2*, CHENGBIN WANG1, GUIBIN XU5, ZHENG HE3, XIAOHONG SHANG4, YI SUN1, FAN ZHANG1, LEYUAN LIU5,6 and HAI HUANG2,5

1Department of Clinical Laboratory, The PLA General Hospital, Beijing 100853; 2Department of Urology, The Sun Yat-sen Memorial Hospital, Sun Yat-sen University, Guangzhou 510120; 3Beijing Center for Physical and Chemical Analysis, Beijing 100094; 4Department of Clinical Laboratory, Xiyuan Hospital, China Academy of Chinese Medical Sciences, Beijing 100091, P.R. China; 5Center for Cancer and Stem Cell Biology, Institute of Biosciences and Technology, Texas A&M Health Science Center, Houston, TX 77030; 6Department of Molecular and Cellular Medicine, College of Medicine, Texas A&M Health Science Center, College Station, TX 77843, USA

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Abstract. Matrine is a naturally occurring alkaloid extracted from the Chinese herb Sophora flavescens. It has been demonstrated to exhibit antiproliferative properties, promote apoptosis and inhibit cell invasion in a number of cancer cell lines. It has also been shown to improve the efficacy of chemotherapy when it is combined with other chemotherapy drugs. However, the therapeutic efficacy of matrine for prostate cancer remains poorly understood. In the present study, we showed that matrine inhibited the proliferation, migration and invasion of both DU145 and PC-3 cells in a dose- and time-dependent manner. It also reduced the cell population at S phase and increased the cell population at sub-G1 phase. The increases in both the apoptotic cell population and cell population at S and sub-G1 phases consistently indicated a pro-apoptotic effect of matrine. Decreases in levels of P65, p-P65, IKKα/β, p-IKKα/β, IκBα and p-IκBa as detected by immunoblot analysis in the matrine-treated DU145 and PC-3 cells suggested an involvement of the NF-κB signaling pathway. Therefore, it is a novel promising addition to the current arsenal of chemotherapy drugs for the treatment of androgen-independent prostate cancer.

Introduction

Prostate cancer is the most common cancer and the second leading cause of cancer-related mortality among men in the US (1). Rates of prostate cancer are high in Western countries and low in Asian countries. Lifespan expansion, environmental changes and improvement of diagnostic techniques have contributed to the recent increases in prostate cancer incidence in China. Prostate cancer has now become the number one cancer of the urogenital system in China (2).

For patients with early-stage prostate cancer, androgens are the major regulators of cellular proliferation, and the growth of tumors is androgen-dependent. The treatment for patients who are not suitable for surgical intervention is by androgen deprivation (3). Long-term treatment with androgen deprivation was reported to have adverse effects. Nevertheless, there is no curative treatment for 70-80% of androgen-independent prostate cancer patients (4). Therefore, developing novel therapeutic modalities for the treatment of androgen-independent prostate cancer has now become the focus of research.

Sophora flavescens Aiton is a type of leguminous plant growing in China, Japan and some European countries. Its dry root is widely used in Traditional Chinese Medicine for the treatment of viral hepatitis (5-7), cardiac (8,9) and skin diseases (10). Matrine, an alkaloid extracted from the dry root of Sophora flavescens Aiton with low toxicity, has a molecular formula of C15H24N2O (10-12). It has been shown that matrine can inhibit proliferation and induce apoptosis of cancer cells developed from breast (13,14), pancreatic (15), liver (16-18), lung (19), gastric (20) and colon cancer (21,22), acute myeloid leukemia (23), multiple myeloma (24), retinoblastoma (25,26), esophageal cancer (27), nasopharyngeal carcinoma (28), osteosarcoma (29), harboring melanoma (30), rhabdomyosarcoma (31) and cervical cancer (32). In addition, matrine was shown to enhance the immune functions of patients and thus improve the quality of life of patients (33). Although matrine has been used clinically to treat a number of types of cancers.
in recent years, the therapeutic efficacy of matrine for prostate cancer remains poorly understood.

In the present study, we investigated the impact of matrine on the proliferation, migration, invasion, cell cycle and apoptosis of androgen-independent human prostate cancer cell lines DU145 and PC-3, and explored the mechanisms underlying the antitumor activity of matrine on these androgen-independent prostate cancer cells. Our aim was to develop new strategies for the treatment of androgen-independent prostate cancer.

**Materials and methods**

**Cell lines and cell culture.** Matrine (chemical formula, C15H24N2O; molecular weight, 248.36) was purchased from Sun Yat-sen University (Guangzhou, China). Human prostate cancer cell lines DU145 and PC-3 were purchased from the Center for Experiment Animals of Sun Yat-sen University (Guangzhou, China), and cultured at 37°C in RPMI-1640 medium (Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin (Invitrogen, Carlsbad, CA, USA) in a humidified CO2 incubator.

**Cell proliferation assay.** The cell proliferation rate was assessed using the MTS assay (Promega, Biosciences, USA) according to the manufacturer's protocols. Briefly, 10,000 cells were seeded in a well into 96-well plates (Corning, New York, NY, USA) containing 100 µl culture medium plus different concentrations of matrine for 48 h, harvested, fixed in 70% ethanol and stained with 1% crystal violet. The lower portion of the filter were fixed, stained and counted.

**Cell migration assay.** Cells suspended in serum-free RPMI-1640 medium were seeded into the upper chamber, and fetal bovine serum (10%) was added to the bottom chamber. After an incubation for 48 h at 37°C in the presence of 5% CO2, the cells on the upper side were removed with a cotton swab, and the cells on the bottom side of the filter were fixed, stained and counted.

**Cell invasion assay.** In vitro invasion assays were performed with a BD Bio-Coat Matrigel invasion assay system according to the manufacturer's protocol. Cells were seeded 24 h after treatment with different concentrations of matrine for 48 h. Cells suspended in serum-free DMEM-F12 medium (11330050B; Invitrogen, Life Technologies) were seeded into the upper chamber, and fetal bovine serum (10%) was added to the bottom chamber. After an incubation for 48 h at 37°C in the presence of 5% CO2, the cells on the upper side were removed with a cotton swab, and the cells on the bottom side of the filter were fixed, stained and counted.

**Cell migration assay.** Cells suspended in serum-free RPMI-1640 medium were seeded into the upper chamber of a Transwell® well (BD, USA) for 24 h after treatment with different concentrations of matrine for 48 h. The lower chamber of each well was filled with 600 µl of RPMI-1640 medium with 10% fetal bovine serum and incubated for 48 h at 37°C in the presence of 5% CO2. Cells were fixed and stained, non-migratory cells in the upper chamber were removed, and migrated cells were counted in 10 random high-power fields.

**Analysis of cell cycle.** The cell cycle was evaluated using a KeyGen kit from BD. At first, cells were treated with different concentrations of matrine for 48 h, harvested, fixed in 70% pre-chilled ethanol (-20°C) and were set at 4°C overnight. Cells were then re-suspended in propidium iodide (PI) buffer (50 g/ml PI and 100 µg/ml RNase) and incubated at room temperature for 30 min in the dark. Cells were then washed twice (3 min each wash) with 1X PBS and subjected to flow cytometry (BD Calibur, USA). The excitation wavelength was 488 nm and the emitted red fluorescence was collected through a 630 nm long-pass filter. DNA analysis was performed with ModFit software (BD).

**Detection of apoptotic cells.** Apoptosis was evaluated using the Annexin V/FITC apoptosis detection kit from BD. At first, cells were treated with different concentrations of matrine for 48 h and harvested by twice centrifugation at 1,000 rpm (5 min each spin). Cells were then washed twice (3 min each wash) in binding buffer, 1x106 cells were resuspended in 1 ml of binding buffer containing 1.25 µl of Annexin V-FITC (BD Pharmingen, San Diego, CA, USA) and 10 μl of PI, and incubated for 15 min at room temperature in the dark. Finally, cell cycle analysis was performed by flow cytometry. Scatter plots were performed against the intensities of the FITC fluorescence and PI fluorescence. The scatter plot was divided into four quadrants: the left lower quadrant [Annexin V-FITC (-) and PI (-)] representing viable cells, the left upper quadrant [Annexin V-FITC (-) and PI (+)] necrotic cells, right lower quadrant [Annexin V-FITC (+) and PI (-)] early apoptotic cells, and right upper quadrant [Annexin V-FITC (+) and PI (+)] late apoptotic cells.

**Immunoblot analysis.** Protein extracts from cells treated with different concentrations of matrine for 48 h were separated on SDS-PAGE and transferred to polyvinylidene fluoride (PVDF) membranes (Millipore, Billerica, MA, USA). Membranes were blocked and then probed with antibodies against P65 (8242), p-P65 (3033), IKKa (11930), IKKβ (8943), p-IKKa/β (2697), IKBa (4812) and p-IkBα (2859) (1:1,000; Cell Signaling Technology, Beverly, MA, USA) and GAPDH (1:1,000; Kangcheng Biology, Shanghai, China). The blots were then washed with tris-buffered saline/Tween-20 solution and incubated with horseradish peroxidase-conjugated goat anti-rabbit or anti-mouse IgG (1:20,000; Cell Signaling Technology) at room temperature. After washing, the blots were exposed, stained with ECL Plus (Millipore) and visualized on X-ray film. The mean normalized ODs of protein bands relative to the ODs of the GAPDH bands from the same condition were calculated.

**Statistical analysis.** Data are expressed as the mean ± standard deviation (SD) and analyzed using one-way ANOVA. Data concerning cell counts were analyzed by Fisher's exact test (SPSS 17.0; SPSS, Inc., USA). A probability value of <0.05 was considered to indicate a statistically significant result.

**Results**

Matrine suppresses the growth of DU145 and PC-3 cells. After treatment with different concentrations of matrine for different times, both DU145 and PC-3 cells exhibited a dose- and time-dependent growth inhibition (Fig. 1A and B). The growth inhibition rates of both types of cells reached as high as 88% when cells were treated with 6.0 g/l matrine for 72 h (Fig. IC and D). The effectiveness of matrine to inhibit...
cell growth increased with treatment times (Fig. 1E and F). Therefore, matrine effectively suppressed the growth of both androgen-independent prostate cancer cell lines DU145 and PC-3.

**Matrine inhibits the migration and invasion of both DU145 and PC-3 cells.** After treatment with different concentrations of matrine for 48 h, both DU145 and PC-3 cells exhibited significantly reduced abilities of migration and invasion in a dose-dependent manner (P<0.05, ANOVA analysis) (Fig. 2). The inhibitory effects of matrine on the migration of both DU145 and PC-3 cells were near 100% when the cells were treated with 4 g/l matrine for 48 h (Fig. 2A-D). The inhibitory impacts of matrine on the invasion of both DU145 and PC-3 cells were also close to 100% when the cells were treated with 4 g/l matrine for 48 h (Fig. 2E-H). Thus, matrine effectively impaired the migration and invasion of both DU145 and PC-3 cells.

**Matrine decreases the cell population at S phase but increases the apoptotic cell population.** Both DU145 and
PC-3 cells treated with matrine showed significantly lower percentages of cells at the S phase and higher percentages of cells at the sub-G1 phase than the untreated controls (Fig. 3). Matrine displayed no significant impact on the percentage of cells at both the G1 and G2 phases (Fig. 3). Therefore, matrine accelerated the process of DNA synthesis and promoted DNA damage and genome instability. The cell population at the sub-G1 phase consists of apoptotic cells. We further analyzed the impact of matrine on cell apoptosis by flow cytometry. Both DU145 and PC-3 cells exhibited significant increases in the apoptotic cell population upon exposure to matrine (Fig. 4).

Therefore, matrine suppresses DNA replication and enhances apoptosis.

**Matrine inhibits the NF-κB signaling pathway.** To determine the impact of matrine on the expression levels of protein involved in the NF-κB signaling pathway, DU145 and PC-3 cells were treated with different concentrations of matrine for 48 h. The levels of P65, p-P65, IKKα/β, p-IKKα/β, IκBα and p-IκBα in both DU145 and PC-3 cells were significantly reduced upon exposure to matrine (Fig. 5). The results suggest that matrine inhibits the proliferation and enhances the
apoptosis of androgen-independent prostate cancer cells by regulating the NF-κB signaling pathway.

Discussion

Although matrine has been clinically used in recent years to treat a number of types of cancer, the therapeutic efficacy of matrine in androgen-independent prostate cancer and the exact mechanisms remain poorly understood. In the present study, we evaluated the antitumor effect of matrine on androgen-independent prostate cancer cells DU145 and PC-3. We showed in the present study that matrine inhibited the proliferation, migration and invasion of both DU145 and PC-3 cells, in a dose- and time-dependent manner. Matrine also inhibited DNA synthesis and reduced the cell population at the S phase but enhanced DNA damage and cell apoptosis. Therefore, it may be effective to use matrine to treat androgen-resistant prostate cancer.

The antitumor effects of matrine have been previously demonstrated in a number of types of cancers. Matrine was previously reported to inhibit the proliferation of tumor cells by causing cell cycle arrest at the G0/G1 or S phase (13,16,21,24,27,30,34). It was also suggested that matrine enhanced the anticancer activity by inducing apoptosis of tumor cells (13-22,24-31,35). Our data showed that matrine mainly functioned by inhibiting DNA synthesis and enhancing apoptosis, but did not markedly change the cell population at the G1 and G2 phases. It appears that matrine promotes the DNA replication-active cells to commit apoptosis.

NF-κB has been emphasized as a pleiotropic transcription factor in the regulation of diverse biological processes, including the inflammatory response, cell apoptosis and development, as well as tumorigenesis (36). Our previous studies based on traditional gene ontology (GO) and KEGG pathways showed that the highest differential expression in the matrine-treated androgen-independent prostate cancer cells DU145 and PC-3 was IκBα (unpublished data), suggesting that matrine likely interferes with the NF-κB signaling pathway. Thus, we investigated whether the suppression of the cancer cell proliferation and invasion by matrine is through the
Figure 4. Matrine promotes the apoptosis of prostate cancer cells. (A and B) Representative dot plots of apoptotic cells detected in the DU145 (A) and PC-3 cells (B) treated with increasing concentrations of matrine by flow cytometry. (C-H) Plots of the percentages of early (C and D), late (E and F) and total apoptotic cells (G and H) in the DU145 (C, E and G) and PC-3 cells (D, F and H) treated with increasing concentrations of matrine.

Figure 5. Matrine suppresses the NF-κB signaling pathway in prostate cancer cells. (A and B) Representative immune-blot results showing the intensities of P65, p-P65, IKKα/β, p-IKKα/β, IκBα and p-IκBα in DU145 (A) or PC-3 cells (B) treated with matrine at the concentrations as indicated. (C and D) Plots of the relative intensities of proteins shown in panel A and B as representatives against increasing concentrations of matrine in DU145 (C) and PC-3 cells (D). Levels of proteins are the ratios to GAPDH levels.
NF-κB signaling pathway. We found that matrine significantly decreased levels of proteins involving the NF-κB signaling pathway. Therefore, matrine may be a promising reagent for treating androgen-independent prostate cancer by inducing NF-κB signaling pathway-mediated apoptosis.

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References

1. Siegel R, Ma J, Zou Z and Jemal A: Cancer statistics, 2014. CA Cancer J Clin 64: 9-29, 2014.
2. Sim HG and Cheng CW: Changing demography of prostate cancer in Asia. Eur J Cancer 41: 834-845, 2005.
3. Quon H and Loblaw DA: Androgen deprivation therapy for prostate cancer-review of indications in 2010. Curr Oncol 17 (Suppl 2): S38-544, 2010.
4. Gioselli D: Signal transduction in prostate cancer progression. Clin Sci 108: 293-308, 2005.
5. Long Y, Lin XT, Zeng KL and Zhang L: Efficacy of intramuscular matrine in the treatment of chronic hepatitis B. Hepatobiliary Pancreat Dis Int 3: 69-72, 2004.
6. Liu J, Zhu M, Shi R and Yang M: Radix Sophorae flavescentis for chronic hepatitis B: A systematic review of randomized trials. Am J Chin Med 31: 337-354, 2003.
7. Ma ZJ, Li Q, Wang JB, Zhao YL, Zhong YW, Bai YF, Wang RL, Li JY, Yang HY, Zeng LN, et al: Combining oxymatrine or matrine with lamivudine increased its antireplication effect against the hepatitis B virus in vitro. Evid Based Complement Alternat Med 2013: 186573, 2013.
8. Liu X, Wang X, Guo Y, Deng N, Zheng P, Xu Q, Wu Y and Dai G: Regulation of endothelial nitric oxide synthase and asymmetric dimethylarginine by matrine attenuates isoproterenol-induced acute myocardial injury in rats. J Pharm Pharmacol 64: 1107-1118, 2012.
9. Li Y, Wang B, Zhou C and Bi Y: Matrine induces apoptosis in angiotensin II-stimulated hyperplasia of cardiac fibroblasts: Effects on Bcl-2/Bax expression and caspase-3 activation. Basic Clin Pharmacol Toxicol 101: 1-8, 2007.
10. Liu JY, Hu JH, Zhou GG, Li FQ, Wang J and Sun HJ: Effect of matrine on the expression of substance P receptor and inflammatory cytokines production in human skin keratinocytes and fibroblasts. Int Immunopharmacol 7: 816-823, 2007.
11. Lai JP, He XX, Jiang Y and Chen F: Preparative separation and determination of matrine from the Chinese medicinal plant Sophora flavescent Ait by molecularly imprinted solid-phase extraction. Anal Bioanal Chem 375: 264-269, 2003.
12. Sun J, Mao J, Liu X, Wang Y, Sun Y and He Z: Separation and mechanism elucidation for six structure-like matrine-type alkaloids by micellar liquid chromatography. J Sep Sci 32: 2043-2050, 2009.
13. Yu P, Liu Q, Liu K, Yagasaki K, Wu E and Zhang G: Matrine suppresses breast cancer cell proliferation and invasion via VEGF-Akt-NF-kappaB signaling. Cytotechnology 59: 219-229, 2009.
14. Shao H, Yang B, Hu R and Wang Y: Matrine effectively inhibits the proliferation of breast cancer cells through a mechanism related to the NF-kB signaling pathway. Oncol Lett 5: 517-520, 2013.
15. Liu T, Song Y, Chen H, Pan S and Sun X: Matrine inhibits proliferation and induces apoptosis of pancreatic cancer cells in vitro and in vivo. Biol Pharm Bull 33: 1740-1745, 2010.
16. Zhang QJ, Li YM, Liu T, He WT, Chen YT, Chen XH, Li X, Zhou WC, Yi JF and Ren ZJ: Antitumor effect of matrine in human hepatoma G2 cells by inducing apoptosis and autophagy. World J Gastroenterol 16: 4290-4296, 2010.
17. Gong X, Gao Y, Guo G, Vondran FW, Schwartlander R, Efimova E, Pless G, Saueru IM and Neuhaus P: Effect of matrine on primary human hepatocytes in vitro. Cytotechnology 67: 255-265, 2015.
18. Wang L, Gao C, Yao S and Xie B: Blocking autophagic flux enhances matrine-induced apoptosis in human hepatoma cells. Int J Mol Sci 14: 2327-2339, 2013.
19. Zhang Y, Zhang H, Yu P, Liu Q, Liu K, Duan H, Luan G, Yagasaki K and Zhang G: Effects of matrine against the growth of human lung cancer and hepatoma cells as well as lung cancer cell migration. Cytotechnology 59: 191-200, 2007.
20. Song S, Zhu S, Zhang Z, Mo Z, Ke Q and Luo Z: A study on the inhibitory effect of matrine on gastric cancer SGC-7901 cells. Afr J Tradit Complement Altern Med 10: 435-438, 2013.
21. Zhang S, Cheng B, Li H, Xu W, Zhai B, Pan S, Wang L, Liu M and Sun X: Matrine inhibits proliferation and induces apoptosis of human colon cancer LoVo cells by inactivating Akt pathway. Mol Biol Rep 41: 2101-2108, 2014.
22. Chang C, Rao DL, Qin XM, Wang H and Xiong L: The inhibitory effect of matrine on the growth of human colorectal cancer HT29 cells: An experimental observation. Zhongguo Zhong Xi Yi Jie He Za Zhi 34: 62-65, 2014 (In Chinese).
23. Zhang S, Zhang Y, Zhuang Y, Wang J, Ye J, Zhang S, Wu J, Yu K and Han Y: Matrine induces apoptosis in human acute myeloid leukemia cells via the mitochondrial pathway and Akt inactivation. PLoS One 7: e46853, 2012.
24. Yu Q, Chen B, Zhang X, Qian W, Ye B and Zhou Y: Arsenic trioxide-enhanced, matrine-induced apoptosis in multiple myeloma cell lines. Planta Med 79: 775-781, 2013.
25. Shao Q, Zhao X and Yao L: Matrine inhibits the growth of retinoblastoma cells (SO-Rb50) by decreasing proliferation and inducing apoptosis in a mitochondrial pathway. Mol Biol Rep 41: 3475-3480, 2014.
26. Zhao B, Li B, Bae S, Shen L, Ren R, Jonas JB, Xu X, Lu Q and Liu Q: Effects of matrine on proliferation and apoptosis of cultured retinoblastoma cells. Graefes Arch Clin Exp Ophthalmol 250: 897-905, 2012.
27. Wang Q, Du H, Geng G, Zhou H, Xu M, Cao H, Zhang B, Song G and Hu T: Matrine inhibits proliferation and induces apoptosis via BID-mediated mitochondrial pathway in esophageal cancer cells. Mol Biol Rep 41: 3009-3020, 2014.
28. Xie M, He G, Wang R, Shi S, Chen J, Ye Y, Xie L, Yi X and Tang A: Matrine-induced apoptosis of human nasopharyngeal carcinoma cells via in vitro vascular endothelial growth factor-A/extracellular signal-regulated kinase2 pathway inactivation. Horm Metab Res 46: 556-560, 2014.
29. Liang CZ, Zhang JK, Shi Z, Liu B, Chen Q and Tao HM: Matrine induces caspase-dependent apoptosis in human osteosarcoma cells in vitro and in vivo through the upregulation of Bax and Fas/Fasl, and downregulation of Bcl-2. Cancer Chemother Pharmacol 69: 317-331, 2012.
30. Jin H, Sun Y, Wang S and Cheng X: Matrine activates PTEN to induce growth inhibition and apoptosis in 1000 BAF harboring melanoma cells. Int J Mol Sci 14: 16040-16057, 2013.
31. Guo L, Xue TY, Xu W and Gao JZ: Matrine promotes G0/G1 arrest and down-regulates cyclin D1 expression in human rhabdomyosarcoma cells. Panminerva Med 55: 291-296, 2013.
32. Zhang L, Wang T, Wen X, Wei Y, Peng X, Li H and Wei L: Effect of matrine on HeLa cell adhesion and migration. Eur J Pharmacol 563: 69-76, 2007.
33. Chen J, Mei Q, Xu YC, Du J, Wei Y and Xu ZM: Effects of matrine injection on T-lymphocyte subsets of patients with malignant tumor after gamma knife radiosurgery. Zhong Xi Yi Jie He Za Zhi 4: 78-79, 2006 (In Chinese).
34. Chang C, Liu SP, Fang CH, He RS, Wang Z, Zhu YQ and Jiang SW: Effects of matrine on the proliferation of HT29 human colon cancer cells and its antitumor mechanism. Oncol Lett 6: 699-704, 2013.
35. Li LQ, Li XL, Wang L, Du WJ, Liang HH, Liu X, Liang DS, Lu YJ, Shan HL, et al: Matrine inhibits breast cancer growth via miR-21/PTEN/Akt pathway in MCF-7 cells. Cell Physiol Biochem 30: 631-641, 2012.
36. DiDonato JA, Mercurio F and Karin M: NF-κB and the link between inflammation and cancer. Immunol Rev 246: 379-400, 2012.