The dichloromethane fraction of *Stemona tuberosa* Lour inhibits tumor cell growth and induces apoptosis of human medullary thyroid carcinoma cells

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Abstract: Medullary thyroid carcinoma (MTC), a neuroendocrine tumor arising from the thyroid gland, is known to be poorly responsive to conventional chemotherapy. The root of *Stemona tuberosa* Lour, also called Bai Bu, is a commonly used traditional Chinese antitussive medicine. The present study investigated this medicinal herb for the first time with respect to its anticancer activity in human medullary thyroid carcinoma cells. Four extracts of *Stemona tuberosa* Lour, including the n-hexane fraction, (ST-1), dichloromethane (DCM) fraction, (ST-2), ethyl acetate (EtOAc) fraction, (ST-3), and methanol fraction, (ST-4) were examined for antiproliferative effects in two MTC cell lines. We observed that only the DCM fraction ST-2 inhibited cell growth and viability in a dose-dependent manner. Furthermore, we found that ST-2 also induced the apoptosis of MTC-SK cells. Caspase-3/7 was activated, while caspase-9 was not, implying that at least a caspase-dependent apoptotic pathway was involved in this process. In addition, the multicellular spheroids of MTC-SK were destroyed and the cell morphology was changed by ST-2. Our results show the strong apoptotic effects of the DCM fraction of *Stemona tuberosa* Lour on human medullary thyroid carcinomas, so suggesting a new candidate for chemotherapy of the so far chemo-resistant medullary thyroid carcinoma.

Keywords: apoptosis, chemoresistance, medullary thyroid carcinoma, plant-derived compounds, *Stemona tuberosa* Lour

Introduction

Human medullary thyroid carcinoma (MTC), a malignant neoplasm derived from parafollicular cells, represents about 5%–10% of thyroid tumors (Vitale et al 2001). It may occur sporadically, in a familial form without associated endocrinopathies, or combined with other endocrinopathies as multiple endocrine neoplasia type 2A or 2B with autosomal dominant inheritance (Eng et al 1996). The clinical course of patients with MTC is variable, ranging from indolent to extremely aggressive, and it is related to the stage of the disease. At present, the primary treatment of MTC is the surgical removal of all neoplastic tissue. Non-surgical approaches to MTC treatment have met with inconsistent results. Chemotherapy generally is used in patients with rapidly progressive, measurable, metastatic disease. To date, none of the regimens reported has been found to be particularly effective, and the response rate has been generally low (Orlandi et al 2001).

Oriental medicinal herbs with anticancer activity are currently in the spotlight as a complementary or alternative medicine. The root of *Stemona tuberosa* Lour is a traditional Chinese medicinal plant known for its antitussive and anti-ectoparasitic activity (Lee and

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Chiang 1994; Chung et al 2003). Alkaloids, stilbenoides, and tocopherols have been identified as main constituents of the plant (Li et al 2007; Schinner et al 2007). Recently, *S. tuberosa* extracts have been attracting new interest for their multi-biological functions including anti-tuberculotic, antifungal, demulcent and anticancer activity (Rinner et al 2004; Greger 2006). In our previous work we report for the first time that *S. stemona* whole extract, provided by Prof. H. Greger, Institute of Botany, University of Vienna, Austria (Greger 2006; Li 2007), had apoptotic effects on a human medullary thyroid carcinoma cell line, GSJO (Rinner et al 2004). Nevertheless, the anticancer activity of this plant, including cell growth inhibition, changes in aggregation, and in the pathways of apoptosis, required further investigations.

In this study we aimed to test the chemosensitivity and potential mechanisms of action of *Stemona tuberosa* Lour in chemo-resistant tumor cells. From *S. tuberosa* n-hexane, dichloromethane, ethyl acetate and methanol extracts were successively obtained and tested for their anticancer activity in two human medullary thyroid carcinoma cell lines and one human fibroblast cell line.

Materials and methods

Plant extraction

The roots of *Stemona tuberosa* Lour were provided by Prof. Tran Hung, Faculty of Pharmacy, University of Medicine and Pharmacy at Ho Chi Minh City, Vietnam. A voucher specimen (IBK-SST-Sttu 1) is deposited at the Institute of Pharmacy/Pharmacognosy at the University of Innsbruck. The ground plant material was consecutively extracted in a Soxhlet apparatus by n-hexane, (ST-1), DCM, (ST-2), EtOAc, (ST-3), and methanol, (ST-4). The solvents were evaporated under reduced pressure. Aliquots of 10 mg were dissolved in 1 ml of dimethyl sulphoxide (DMSO, Sigma, Vienna, Austria) and stored at –20 °C.

Reagents

Acetic acid (p.A. quality), acetonitrile (gradient grade), EtOAc, n-hexane, DCM and methanol (all p.A. quality) were purchased from Merck (Darmstadt, Germany). Water was produced by reverse osmosis followed by distillation, nitrogen (99.995%) for mass spectrometry experiments was produced by a nitrogen generator (Peak Scientific Instruments Ltd., Fountain Crescent, UK).

HPLC-MS instrumentation

HPLC was performed using a HP 1100 Liquid Chromatograph (Agilent Technologies, Waldbronn, Germany) equipped with a G1311A quaternary pump, a G1315B diode array detector (DAD), a G1313A automatic injector and auto sampler, a G1316A column oven, a G1322A vacuum degasser. The system was controlled by ChemStation software (version 9.01).

The HPLC was coupled to an Esquire 3000^{plus} ion-trap mass spectrometer (Bruker Daltonics, Bremen, Germany) using an Electrospray ionization source, the system was controlled by Bruker Daltonics Esquire software 5.0.

HPLC–DAD-MS conditions

Separations were done on a Phenomenex Synergi Polar RP column (150 × 4.6 mm, 4 µm particle size, Phenomenex. Torrence, USA) with a solvent gradient of water (A) and acetonitril (C), both with 0.1% acetic acid. The Phenomenex SecurityGuard system equipped with a Synergi Polar RP cartridge (4 mm × 2 mm) was used as guard column. Gradient: t = 0 min 65% A; t = 10 min 50% A; t = 20 min 2% A. Between runs the column was equilibrated with 65% A for 10 min. The system was operated at a flow rate of 1 ml/min at 25 °C. The injection volume was 10 µl. DAD derived chromatograms were recorded at 210, 270, 295 and 300 nm. A static 1:5 splitter was used to hyphenate the HPLC device to an Esquire 3000^{plus} ion-trap mass spectrometer (Bruker Daltonics, Bremen, Germany). Mass spectrometry experiments were performed in alternating ESI-mode with a spray voltage of 4500 V; N2 at 3.5 bar as sheath gas and N2 at 10 l/min, 350 °C as dry gas. Mass spectra were recorded over the range 100–1500 m/z. The Bruker ion-trap mass spectrometer was operated under an ion current control (ICC) of approximately 50,000 with a max accumulation time of 20 ms. For HPLC-DAD-MS analysis aliquots of 10 mg ST-1 – ST-4 were dissolved in 1 ml methanol and filtered through a 0.45 µm filter (PET, Machery-Nagel, Düren, Germany).

Cell lines and cell culture

The human medullary thyroid carcinoma cell lines MTC-SK (Pfragner et al 1990), SINJ (Pfragner et al 1993) and the normal human skin fibroblast cell line, HF-SAR were established in our laboratory. The MTC-cells were maintained in Ham's F-12: M199 medium (1:1; Biowhittaker, Verviers, Belgium) containing 10% fetal bovine serum (PAA Laboratories, Pasching, Austria) at 37 °C, in an atmosphere of 5% CO_2. HF-SAR fibroblasts were cultured in MEM-E medium, supplemented with 10% fetal bovine serum, at 37 °C, 5% CO_2. All cell lines were mycoplasma – free.

Cell counting

MTC-SK and SINJ cells were seeded at a density of 2×10^5 cells/ml into 24-well plates (Sarstedt, Wiener Neudorf, Austria) and incubated for 24 and 48 h in completed medium with DMSO (control) or supplemented with 50 μg/ml of each extract. Cells were counted after pipetting the cell clusters into single cells, and cell numbers were determined with an automated cell counter (Casy-1Cell Counter and Analyzer, Schärfe System, Reutlingen, Germany). Each sample was measured in triplicate. The mean value and S.D. were calculated automatically by the cell counter.

WST-1 cell viability assay

Cell proliferation and viability were quantified using the WST-1 cell proliferation reagent (4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1, 3-benzene disulphonate) (Roche Diagnostics, Vienna, Austria) according to the manufacturer's protocol. This method is based on the ability of viable cells to metabolize tetrazolium salt WST-1 to formazan by mitochondrial dehydrogenases. MTC-SK and SINJ suspension cells were seeded at a density of 2×10^5 cells/ml in 24-well plates (Sarstedt, Wiener Neudorf, Austria). After 24, 48 or 72 h treatment with different concentrations of solvent extracts or DMSO, cells were pipetted carefully into single cells and transferred into 96-well plates; then 10 μl WST-1 labeling mixture per well was added. After incubation at 37 °C for 2 hours, the samples were quantified spectrophotometrically by measuring the absorbance of the formazan product at 450 nm with an ELISA plate reader. For HF-SAR adherent cells, cells were seeded directly into 96-well plates at a density of 1×10^5 cells/ml. After the adherence to plates, cells were treated with extracts for 24, 48 and 72 h and measured as above. Each sample was tested for 6 parallels; means and S.D. were calculated.

TUNEL assay

Cells were detected using the *In Situ Cell Death Detection Kit, Fluorescein* (Roche, Lewes, UK cat # 11684795910) according to manufacturer's instructions. Briefly, cells were treated with ST-2 for 24 h, washed with PBSA, and harvested. The cells were fixed with freshly prepared 4% paraformaldehyde for 60 min at room temperature and treated with 0.1% Triton X-100 solution for 2 min on ice. Intracellular DNA fragments were then labeled by exposing the cells to TUNEL reaction mixture for 1 h at 37 °C, in a humidified atmosphere and protected from light. The cells were washed with PBSA twice, then transferred to slides and analyzed under a fluorescence microscope (Nikon eclipse TE300, Tokyo, Japan).

Caspase 3/7 activity, caspase 9 activity, and ATP luminescent cell viability assay

Caspase 3/7, caspase 9 and cell viability activity were measured in one sample by using three homogenous, luminescent assay kits (Promega, Madison, WI, USA, cat # G8091, cat # G8211, and cat # G7570, respectively) according to the manufacturer's instructions. Briefly, 1 ml MTC-SK cells were seeded into 24-well plates at a density of 2×10^5 cells/ml and incubated in completed Ham's F-12: M199 medium with 25 μg/ml, 50 μg/ml, or 100 μg/ml ST-2, 5 mM CPT positive control, or 5 μl DMSO negative control for 4 h at 37 °C. After pipetting thoroughly into single cells, each sample was transferred into three 96-well white-walled plates (Nunc, Roskilde, Denmark) and measured separately for its caspase 3/7, caspase 9 and cell viability activity. The luminescence of each sample was measured with a plate-reading luminometer (Mediators PhL, Mediators Diagnostika, Vienna, Austria) as directed by the manufacturer of the instrument. Each treatment was measured for 3 parallel samples, and mean value and S.D. were calculated. Next, we investigated whether an inhibition of caspases inhibited apoptosis in MTC-SK cells. MTC-SK cells were seeded into microplates. One group was treated with 25 μg/ml, 50 μg/ml or 100 μg/ml ST-2, or 5 μl DMSO negative control, as described above. Another group was co-treated with 50 μM of the specific caspase-3 inhibitor Ac-DEVD-CHO (Promega, Madison , WI, USA, cat # G5961). After 36 h and 72 h the cell numbers were counted by Casy-1 Cell Counter and Analyzer. Cell proliferation and viability were determined by the WSt-1 cell proliferation assay.

Results

HPLC-DAD-MS Analysis of the *Stemona* extracts (ST-1 – ST-4)

The extracts ST-1 – ST-4 were analyzed by HPLC-DAD-MS, the resulting HPLC-DAD chromatograms are shown in Figure 1. Particularly two classes of compounds seem to be of interest considering their published activities, namely pyrrolo- or pyridoazepine alkaloids and stilbenoids (Pacher et al 2002; Greger 2006). Whereas alkaloids as croomine and tuberostemonine derivatives were present (not visible in the UV traces) in all four extracts (*Rt* 4–8 min), no stilbenoids could be detected by UV or MS. In ST-1 and ST-2 fatty acids and phytosterols were visible in the MS chromatograms as side compounds. However, none of the prominent HPLC-peaks was elucidated in this preliminary analysis. The phytochemical investigation of the most active extract ST-2 is an ongoing project.

Effects of *Stemona tuberosa* Lour on cell growth of MTC-SK cells

S. tuberosa n-hexane, DCM, EtOAc and methanol fractions were evaluated by cell counting for their ability to inhibit growth and proliferation in the MTC-SK cell line. MTC-SK cells were seeded at a density of 2.26×10^5 cells/ml and incubated for 48 h in completed medium with DMSO (control) or supplemented with 100 μg/ml of each fraction, then the cells were counted with an automated cell counter. After 48 h treatment with ST-2, the numbers of total viable MTC-SK cells were 2.31×10^5 cells/ml, which were significantly lower than those treated by DMSO or other fractions, which were 3.86×10^5 cells/ml by DMSO, 3.7×10^5 cells/ ml by ST-1, 3.68×10^5 cells/ml by ST-3, 4.03×10^5 cells/ ml by ST-4 (t-test $p < 0.01$) (Figure 2A). The data obtained showed that only the dichloromethane fraction, ST-2, induced a significant inhibition of growth and proliferation at the concentration of 100 μg/ml in the MTC-SK cells. On the contrary, the n-hexane, EtOAc, and MeOH fractions had no antiproliferative activity at levels as high as 100 μg/ml (Figure 2A). Moreover, treatment with ST-2 showed growth suppression in a dose-dependent manner. This experiment is shown in Figure 2B. The cell numbers obtained by cell counting after ST-2 treatment were 4.76×10^5 cells/ml at 0 μg/ml, 4.07×10^5 cells/ml at 25 μg/ml, 1.8×10^5 cells/ml at 50 μg/ml and 1.74×10^5 cells/ml at 100 μg/ml.

Effects of *Stemona tuberosa* Lour on cell viability of MTC-SK, SINJ and HF-SAR cells

To further confirm the changes in viability, we tested the mitochondrial dehydrogenases activity by using reagent WST-1. The results showed that cell viability significantly decreased in MTC-SK cells with increasing exposure concentration of the ST-2 but no significant effect was found with other ST fractions (Figure 3A).

To study generalized applicability of *Stemona tuberosa* Lour, we tested its effects on another human medullary thyroid carcinoma cell line, SINJ, and on normal non-cancer human fibroblast cells, HF-SAR. Similar to MTC-SK cells, the SINJ medullary thyroid carcinoma cells were also suppressed by the ST-2 fraction in a dose-dependent manner. The HF-SAR fibroblast cell line was not influenced by *S. tuberosa* fractions (Figures 3B and 3C). This result revealed that there were sensitivity differences between cancer cells

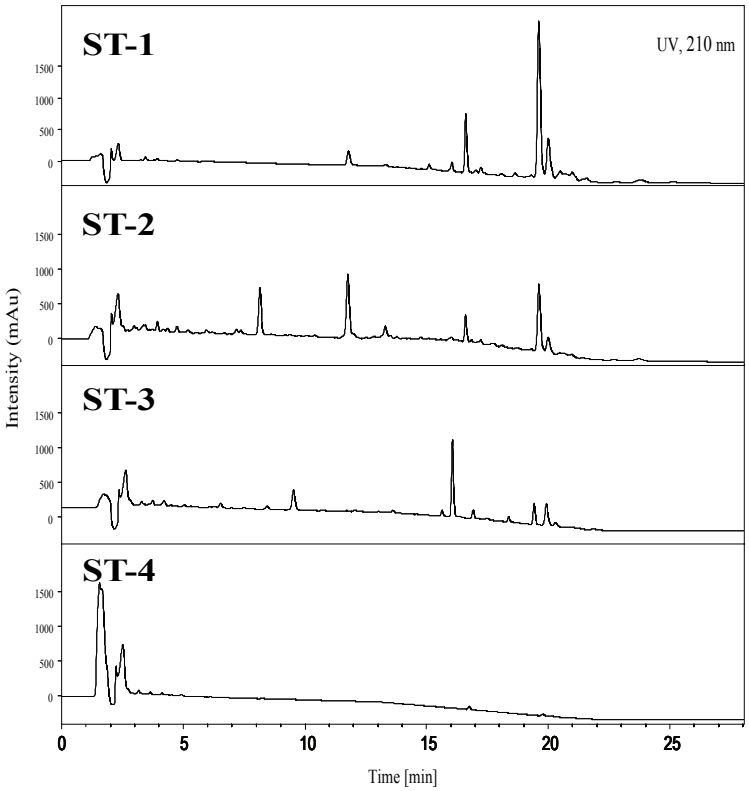

Figure1 HPLC-DAD chromatograms of S-T 1 – ST-4. HPLC stationary phase: Phenomenex Synergi Polar RP column (150 × 4.6 mm, 4 μm); mobile phase: water (**A**) and acetonitrile (**B**) (both with 0.1% acetic acid), gradient: 65% A to 50% A in 10 min, from 50% to 2% in 10 min; flow rate: 1ml/min; temperature: 25 °C; injection volume: 10 μl.

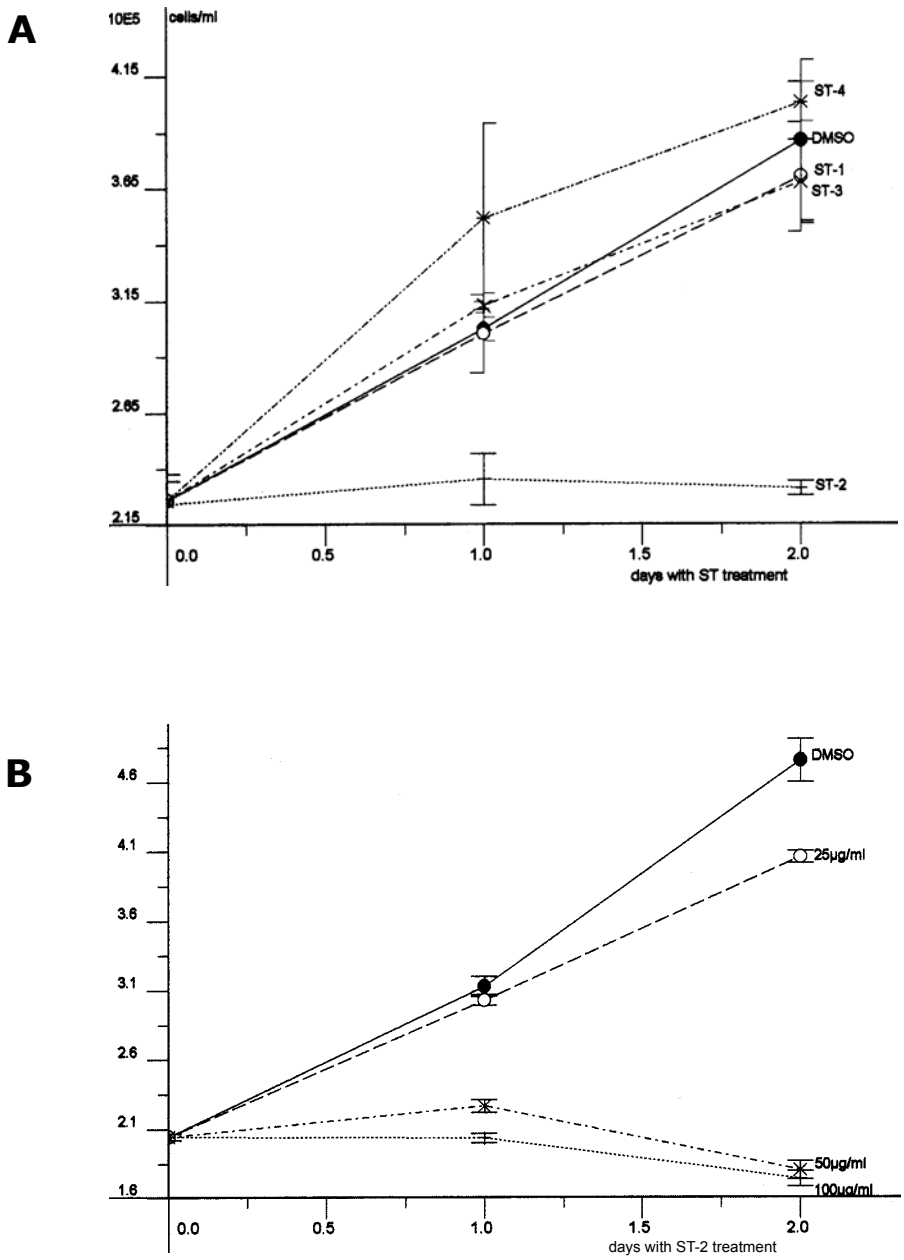

Figure 2 Growth inhibition of human medullary thyroid carcinoma MTC-SK cells by ST-2. (**A**) MTC-SK cells were grown and treated with indicated *Stemona tuberosa* Lour fractions at the concentration of 100 µg/ml, then the number of viable cells was counted and calculated electronically by CASY-1 Cell Counter and Analyzer after 0, 24, 48 h. (**B**) MTC-SK cells were grown and treated with indicated concentration of ST-2 for 48 h. The total living cell numbers were analyzed by Cell Counter and Analyzer after 0, 24, 48 h. All the values represent the means ± S.D. (n = 3).

and normal cells, implying that plant object is a promising chemotherapeutic drug.

Induction of apoptotic pathway by *S. tuberosa* DCM fraction

Since DNA nucleososomal fragmentation is the hallmark of apoptotic death, TUNEL DNA fragmentation analysis was used to detect broken DNA in the ST-2- treated MTC-SK cells. We observed a dose-dependent green immunofluorescence that was more intensive in MTC-SK cells treated with high concentrations of ST-2. Control cells or MTC-SK treated with low concentrations of ST-2 showed only weak fluorescence signals, suggesting that ST-2 has apoptotic activity (Figure 4A).

We also measured caspase 3/7 activation as an indicator of apoptosis induction since different upstream pathways leading to apoptosis depend on caspase 3/7 induction for final apoptotic execution. Figure 4B shows the effects of different concentrations of ST-2 at the level of caspase 3/7 induction. There was a dose-dependent increase in the caspase 3/7 activity with ST-2 exposure for 4 h. Relative ratio

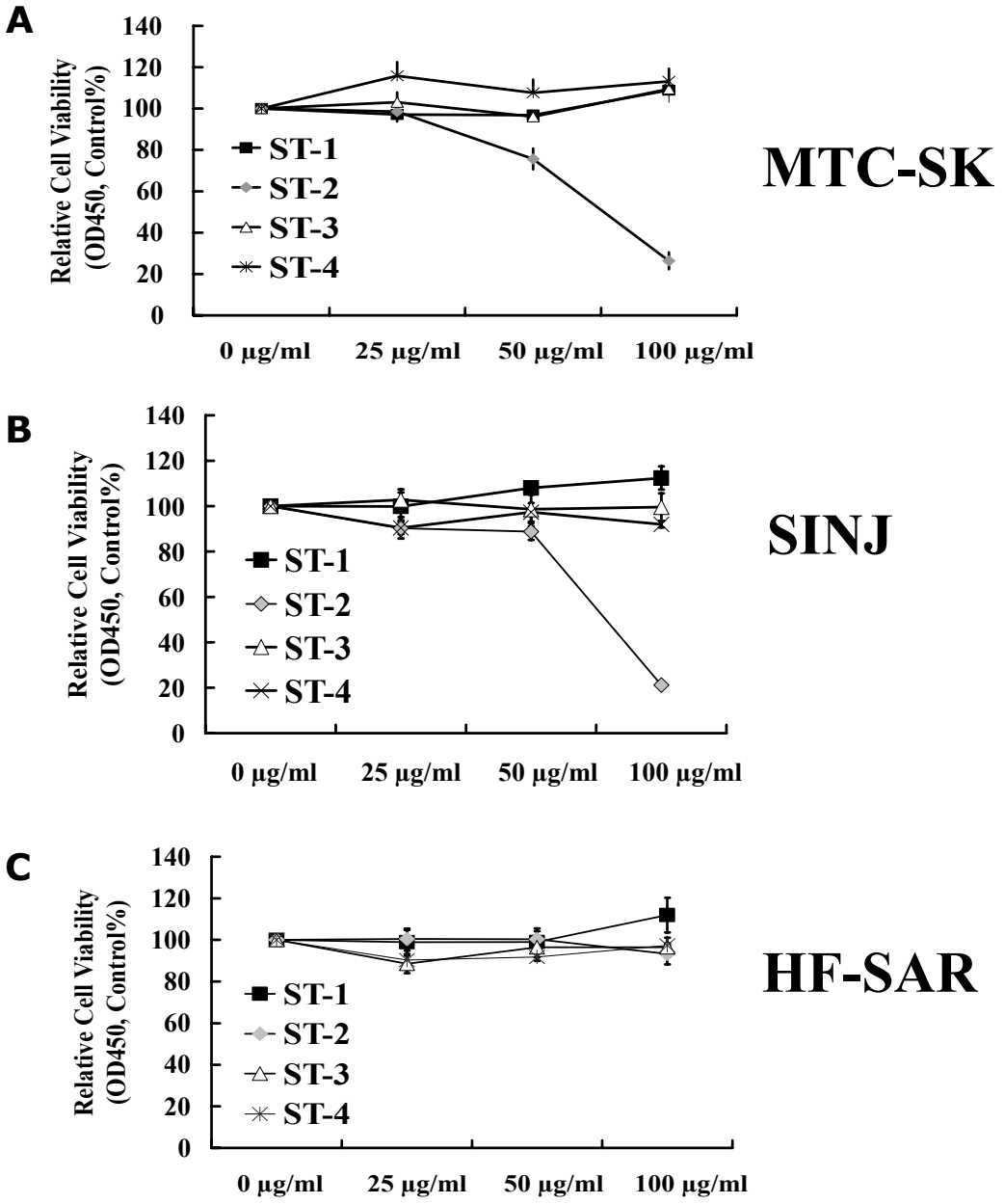

Figure 3 Effects of *S. tuberosa* n-hexane, dichloromethane, ethyl acetate and methanol fractions on MTC-SK, SINJ and HF-SAR cell viability. Cells were treated with indicated concentrations of n-hexane (ST-1), dichloromethane (ST-2), methylacetate (ST-3), or methanol (ST-4) for 48 h, and then cell viability was measured by WST-1 assay. (**A**) MTC-SK cells, (**B**) SINJ cells, (**C**) HF-SAR cells.

of caspase 3/7 activity was 1.42 + 0.08 at 25 μg/ml, 2.18 + 0.03 at 50 μg/ml, and 4.56 + 0.17 at 100 μg/ml.

To confirm whether an intrinsic pathway was involved, the activity of active caspase-9 was treated with ST-2 at concentrations of 25 μg/ml or 50 μg/ml, and decreased at the concentration of 100 μg/ml (Figure 4C). The decrease of caspase 9 may be deduced from the downregulation of the whole number of viable cells, as tested by ATP-luminescent cell viability assay using the same samples (Figure 4D).

We found that only the dichloromethane (DCM) fraction of ST-2 inhibited cell growth and induced apoptosis in MTC-SK cells. The caspase-dependent apoptotic pathway was involved in this process. Executor caspases 3/7 were activated, but initiator caspase of the intrinsic pathway caspase 9, was not involved in this process.

Effect of *S. tuberosa* DCM fraction on multicellular aggregation and cell morphology

As we reported previously (Pfragner 1990, 1993), the MTC-SK cells originally grow as a suspension of multicellular

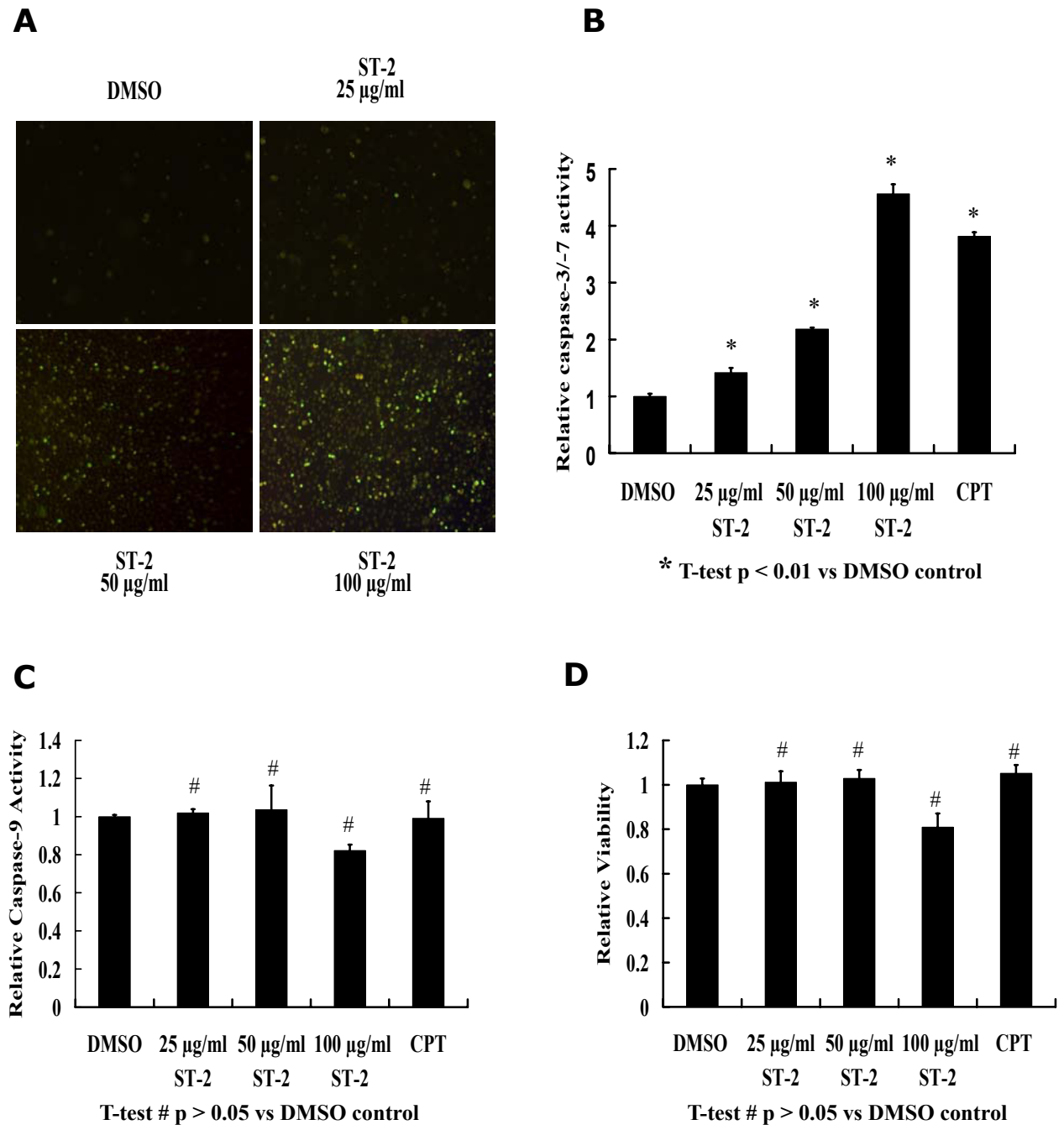

Figure 4 Induction of apoptosis in MTC-SK cells by *S. tuberosa* dichloromethane extracts. (**A**) TUNEL assay. (**B**) caspase-3/-7 activity in the MTC-SK cells with indicated treatment for 4 h. (**C**) caspase-9 activity in the MTC-SK cells with indicated treatment for 4 h. (**D**) cell viability in the MTC-SK cells with indicated treatment for 4 h.

aggregates. It is believed that the ability of cell populations to survive in the aggregated form is a potential index for tumorigenicity, which might also be a reason for drug and radiation resistance in cancer (Olive and Durand 1994). In this study, as shown in Figure 5A, the multicellular tumor spheroids of MTC-SK cells dissociated after treatment with 100 μg/ml ST-2 for 24 h. We also saw alteration of cell aggregates, significant treatment-induced morphological changes indicative of cell death and growth inhibition in ST-2 treated MTC-SK cells (Figure 5A). In addition, after a 24 h ST-2 treatment, the cell counts and cell size in MTC-SK decreased in comparison to the control, and the percentage of dead cells and cell debris increased (Figure 5B). Co-treatment of MTC-SK cells with ST-2 (25 μg/ml, 50 μg/ml, 100 μg/ml) and the specific caspase-3 inhibitor Ac-DEVD-CHO (50 μM) promoted cell survival and proliferation. While ST-2 treatment alone induced apoptosis, the inhibitor reduced this effect significantly (data not shown).

Discussion

Oriental medicinal herbs are still an important source of promising lead compounds for the generation of antineoplastic drugs. In this study, as a first step to provide scientific evidence for anticancer activity of *Stemona tuberosa* Lour, the ability of dichloromethane fraction of *Stemona tuberosa* Lour to inhibit cell growth through a mechanism of action which involving induced apoptosis in human medullary thyroid carcinoma cells was demonstrated. We observed that among tested fractions, only the DCM fraction inhibited the growth of MTC-SK and SINJ cells in a dose-dependent manner. *S. tuberosa* DCM showed cytotoxicity against MTC-SK and SINJ cells with a mean LC50 value of 50 μg/ml, which is remarkably low. Moreover, ST-2 did not inhibit normal human cells at the same concentration. The different chemosensitivity to ST-2 DCM reaction between medullary thyroid carcinoma cells and normal cells suggested ST-2 as a promising candidate for chemotherapy.

There is accumulating evidence that naturally occurring compounds and many chemotherapeutic agents with antitumor effects can trigger the apoptosis of cancer cells. In agreement with this line of thought, we provide evidence that ST-2 induced cell death with the classic features of apoptosis. To further characterize the potential apoptotic signaling pathways activated by ST-2, a series of luminescent-based assays were performed. We demonstrated that the executor caspase 3/7 was involved in the apoptosis-inducing mechanism. As reported (Strasser et al 2000), in the intrinsic mitochondrial pathway, apoptosis is triggered by pro-apoptotic members of the bcl-2 family, leading predominantly to activation of the initiator caspase 9. This pathway plays a major role in drug-induced apoptosis (Schmitt et al 2000) whereas we found here that caspase 9 was not activated by ST-2. Together with our previous study (Rinner et al 2004) in which the expression of the antiapoptotic protein bcl-2 was not found to have changed by total ST-extracts, we assume

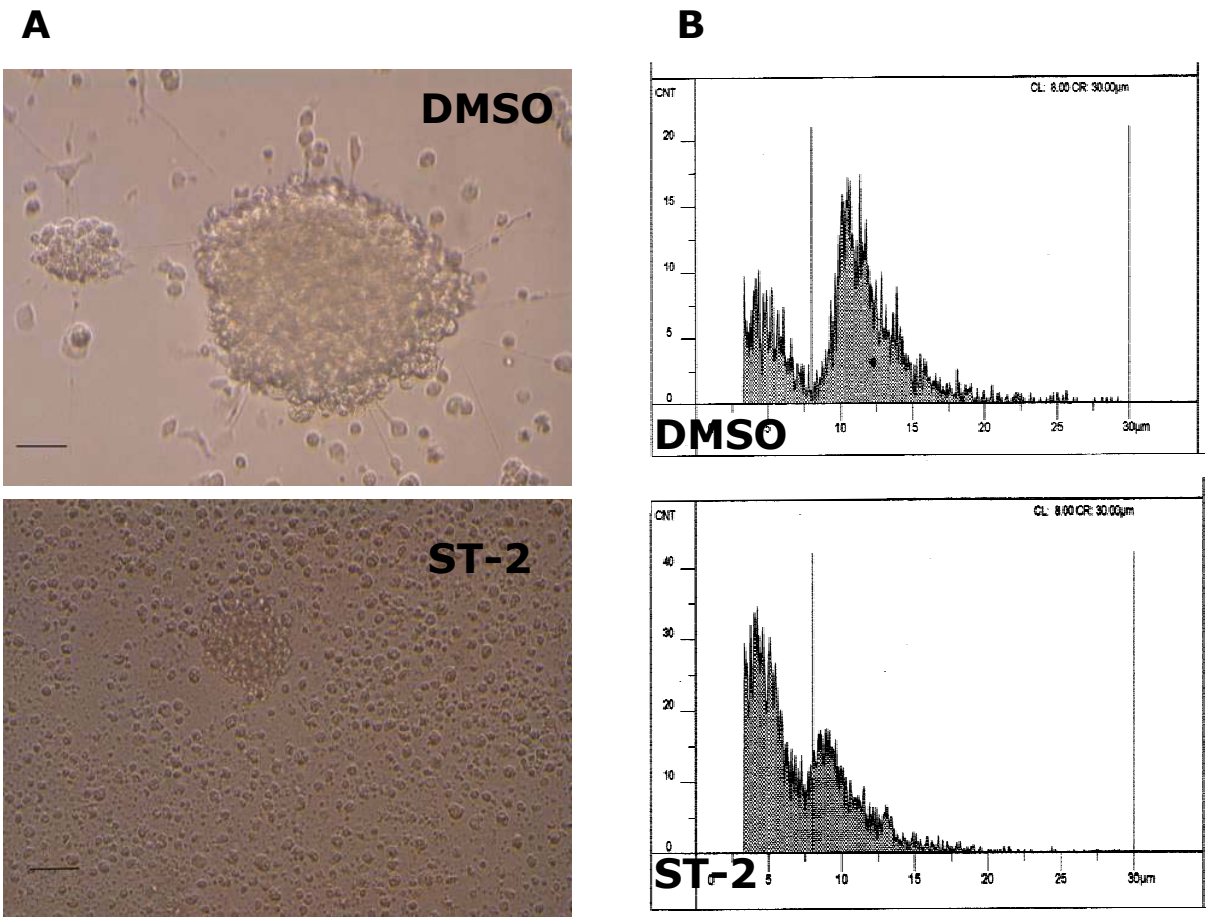

Figure 5 ST-2 influenced the MTC-SK aggregation and morphology. (**A**) Cellular morphology. *Upper*, control MTC-SK cells, growing as a suspension of multicellular aggregates. Bar = 50 μ. *Lower*, treatment with ST-2 for 24 h. The multicellular aggregates disscociated. Bar = 50 μ. (**B**) Cell counts. *Upper*, control MTC-SK cells with a low percentage of dead cells and cell debris (left peak) and a mean diameter of 12.29 μm; *Lower*, cell counts after treatment with 100 μg/ml ST-2 for 24 h, with a high percentage of dead cells and cell debris (left peak) and a mean diameter of 10.83 μ.

that ST-2 does not induce apoptosis of MTC-SK cells through the mitochondrial pathway.

We did not specially study the relationship between growth inhibition and apoptosis induction. But Figure 4 shows the effect of treatment with the DCM fraction on apoptotic mediators caspase 3/7 in MTC-SK. After 4h of treatment an increasing rate of caspase 3/7 activity was detected but this is not completely parallel with the decreasing rates of cell viability. This may be due to the fact that caspase 3 activation is an early-stage event in apoptosis, whereas the decrease in cell viability occurs later than caspase 3/7 activation. When the percentage of viable cells after 48 h treatment in WST-1 assay (Figure 3A) is compared with the percentage of apoptosis (Figure 4B), there is a significant negative correlation between cell viability and early-stage caspase 3 activation ($r = -0.9908$). Moreover, the specific caspase-3 inhibitor Ac-DEVD-CHO blocked the effects of ST-2. Thus, we can assume that in MTC-SK cells, the induction of apoptosis is the main mode of growth inhibition by ST-2.

Almost all conventional cytotoxic anticancer drugs are less effective in killing tumor cells grown as multicellular spheroids than in killing tumor cells grown as monolayer cell cultures. This "multicellular resistance" reflects the relative intrinsic drug-resistant phenotype of most solid tumors growing in vivo and is due to factors such as limited drug penetration or reduced fractions of proliferating cells. As we reported previously (Pfragner et al 1990, 1993), both MTC-SK and SINJ cells grow as muticellular speroids which may account for the chemo-resistance of MTC. In this study, we found that ST-2 altered the phenotype of the MTC cells from originally aggregating cells towards single-cell suspensions. Studies to see whether the disruption of cellular aggregation actually contributes to the growth inhibition by ST-2 are in progress.

In summary, our findings demonstrate the *in vitro* anticancer activity of the dichloromethane fraction of *Stemona tuberosa* Lour in medullary thyroid carcinoma cells, possibly suggesting a new chemotherapeutic agent for the treatment of medullary thyroid carcinoma.

Acknowledgments

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References

Chung HS, Hon PM, Lin G, et al. 2003. Antitussive activity of Stemona alkaloids from Stemona tuberosa. *Planta Med,* 69:914–20.

Eng C, Clayton D, Schuffenecker I, et al. 1996. The relationship between specific RET proto-oncogene mutations and disease phenotype in multiple endocrine neoplasia type 2. International RET Mutation Consortium Analysis. *JAMA,* 276:1575–9.

Greger H. 2006. Structural relationships, distribution and biological activities of Stemona alkaloids. *Planta Med,* 72:99–113.

Lee HL, Chiang YF. 1994. Insecticidal activity of the herbal plant, Stemona tuberosa Lour to mosquito larvae. *Trop Biomed,* 11:87–9.

Li S-L, Jiang R-W, Hon P-M. 2007. Quality evaluation of Radix Stemonae through simulateneous quantification of bioactive alkaloids by high-performance liquid chromatography coupled with diode array and evaporation light scattering detectors. *Biomed Chromatogr,* (in press).

Olive PL, Durand RE. 1994. Drug and radiation resistance in spheroids: cell contact and kinetics. *Cancer Metastasis Rev,* 13:121–38.

Orlandi F, Caraci P, Mussa A, et al. 2001. Treatment of medullary thyroid carcinoma: an update. *Endocr-Relat Cancer,* 8:135–47.

Pacher T, Seger C, Engelmeier D, et al. 2002. Antifungal stilbenoids from *Stemona collinsae. J Nat Prod,* 65:820–7.

Pfragner R, Höfler H, Behmel A, et al. 1990. Establishment and characterization of continuous cell line MTC-SK derived from a human medullary thyroid carcinoma, *Cancer Res,* 50:4160–6.

Pfragner R, Wirnsberger G, Behmel A, et al. 1993. New continuous cell line from human medullary thyroid carcinoma SINJ. Phenotypic analysis and in vivo carcinogenesis. *Int J Oncol,* 2:831–6.

Rinner B, Siegl V, Pürstner P, et al. 2004. Activity of novel plant extracts against medullary thyroid carcinoma cells. *Anticancer Res,* 24:495–500.

Schinner J, Brem B, But PP-H, et al. 2007. Pyrrolo- and pyridoazepine alkaloids as chemical markers in Stemona species. *Phytochemistry,* 68:1417–27.

Schmitt CA, Rosenthal CT, Lowe SW. 2000. Genetic analysis of chemoresistance in primary murine lymphomas. *Nat Med,* 6:1029–35.

Strasser A, O'Connor L, Dixit VM. 2000. Apoptosis signaling. *Annu Rev Biochem,* 69:217–45.

Vitale G, Caraglia M, Ciccarelli A, et al. 2001. Current approaches and perspective in the therapy of medullary thyroid carcinoma. *Cancer*, 91:1797–808.