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Potential role of tetrandrine in cancer therapy[1]

CHEN Yu-Jen[2]

Department of Radiation Oncology, Mackay Memorial Hospital, Taipei, Taiwan 104, Department of Martial Arts, Chinese Culture University, Taipei, Taiwan 111, China

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ABSTRACT

Tetrandrine, a bisbenylisoquinoline alkaloid isolated from the dried root of *Stephenia tetrandra* S Moore, exhibits very broad pharmacological actions, including anti-tumor activity. The beneficial effects of tetrandrine on tumor cell cytotoxicity and radiosensitization, multidrug resistance, normal tissue radioprotection, and angiogenesis are most promising and deserve great attention. Tetrandrine has potential either as a tumoricidal agent or as an adjunct to chemotherapy and radiotherapy. To evaluate the potential clinical efficacy of tetrandrine for cancer therapy, more mechanism-based pharmacological, pharmacokinetic, and pharmacodynamic studies are required.

INTRODUCTION

Stephenia tetrandra S Moore is a plant indigenous to main land of China that has long been used as a Chinese herb. In traditional Chinese medicine, it is indicated for the treatment of edema, rheumatic disorders, and inflammatory diseases.

Tetrandrine [(1β)-6,6',7,12-teramethoxy-2,2'-dimethyl-berbaman], a bisbenylisoquinoline alkaloid isolated from the dried root of *Stephenia tetrandra* S Moore, possesses a remarkable pharmacological profile. Several studies have focused on the anti-tumor activity of tetrandrine; however, its potential role in cancer therapy has not been clearly addressed. This article, based on reports in the literature and the work of our own laboratory, reviews the tumor-related pharmacological functions of tetrandrine.

TETRANDRINE AS AN ANTI-NEOPLASTIC AGENT

Tetrandrine has cytotoxic and anti-bacterial activity and fulfills certain structural requirements for anti-tumor activity[1]. Twenty-three different bisbenylisoquinoline alkaloids, including tetrandrine, isotetrandrine, and berbamine, were evaluated for biological activity in tumor cells, bacterial cells, and red blood cells. The effective dose for 50 % growth inhibition (ED_{50}) against HeLa-S3 cervical carcinoma cells was 1 mg/L for tetrandrine and 10 mg/L for isotetrandrine. Tetrandrine and isotetrandrine have the same two-dimensional structure except for the absolute configuration of C-1. This suggests that the stereochemistry at position C-1 influences the anti-tumor activity of bisbenylisoquinolines. The ED_{50} against HeLa cells was 4.4 mg/L for tetrandrine and more than 30 mg/L for tetrandrine dimethiodide, indicating that quaternization reduces the cytotoxicity

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[2] Correspondence to Dr CHEN Yu-Jen, MD, Ph D. *Department of Radiation Oncology, Mackay Memorial Hospital, 92 Chung San North Road, Section 2, Taipei, Taiwan 104, China.*
Phn 886-2-2809-4661. Fax 886-2-2809-6180.
E-mail oncoman@sinamail.com
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of tetrandrine. The *in vivo* therapeutic effect of tetrandrine against Ehrlich ascites carcinoma (EAC) and sarcoma-180 was considerably greater than that of isotetrandrine (ED_{50}/LD_{50} 50/280 for tetrandrine and 100/160 for isotetrandrine against EAC). Hence, this study suggests that, while a definite structure-activity relationship is still unclear, anti-tumor activity is dependent on certain structural features.

Tetrandrine inhibits the proliferation of human esophageal cancer cell lines ECa109 and ECa109-C3 *in vitro*[2]. These tumor cells were treated with various concentrations of tetrandrine (1.0 to 75 mg/L) and the mitotic index, cell number, and DNA synthesis (by [^{3}H]-TdR incorporation assay) were assessed. The ECa 109 cells become rounded and detached from the culture bottle within 24 to 48 h of treatment with tetrandrine (75 mg/L). There was a 37 % inhibition of growth in ECa 109-C3 cells after 5 d exposure to 10 mg/L tetrandrine. The incorporation of [^{3}H]-TdR into ECa109 and ECa-C3 cells was inhibited approximately 20 % to 40 % by tetrandrine at concentrations of 7.5 to 10 mg/L. However, the division of cells which had already synthesized DNA was not affected by tetrandrine. This indicates that tetrandrine may inhibit the proliferation of ECa109-C3 cells through interfering with DNA synthesis.

Tetrandrine induces apoptosis of malignant lymphoid and myeloid cells but not of Epstein-Barr virus (EBV) transformed lymphoblastoid cells[3]. Three neoplastic cell lines (BM-13674 EBV-negative Burkitt's lymphoma cell, CEM-C7 T-cell leukemic cell and HL-60 myeloid leukemic cell) and one lymphoblastoid cell line (LCL) were treated with tetrandrine. A dose of 10 mg/L had preferential cytotoxicity against neoplastic cells lines evaluated by trypan blue exclusion and confirmed by a colorimetric MTT assay. After 8 h of treatment with 10 mg/L tetrandrine, oligonucleosomal fragments shown by DNA electrophoresis were noted in all three neoplastic cell lines but not in LCL cells. The induction of apoptosis in CEM-C7 cells by tetrandrine (10 mg/L for 4 h) was much more rapid than that caused by dexamethasone (10 μmol/L for 40 h). Tetrandrine-induced apoptosis in BM-13674 cells does not require *de novo* protein synthesis, as demonatrated by the fact that the protein synthesis inhibitor cycloheximide (at dose of 50 mg/L) had no protective effect against tetrandrine-induced cell death. These results indicate that tetrandrine may have value as an anti-neoplastic agent by virtue of novel mechanisms.

Tetrandrine, berbamine, and coriolus versicolor peptide (CVP) inhibit the proliferation of human myeloid leukemic HL-60 cells *in vitro*[4]. Leukemic HL-60 cells and normal human peripheral blood lymphocytes were treated with tetrandrine (0 to 25 μmol/L) and CVP (0 to >1 g/L). Both tetrandrine and CVP had a concentration-dependent inhibitory effect on the proliferation of HL-60 cells. Tetrandrine, but not CVP, had a concentration-dependent cytotoxic effect on normal lymphocytes. In both morphological assessment and DNA electrophoresis analysis, 24 μmol/L of tetrandrine or berbamine induced characteristic apoptotic changes. However, even high concentrations of CVP (up to 1 g/L) had no such apoptosis-inducing effect. Hence, tetrandrine and berbamine, but not CVP, inhibit the proliferation of human leukemic HL-60 cells by induction of apoptosis.

Tetrandrine induces apoptosis of human monoblastic leukemic U937 cells *in vitro*[5]. U937 cells were cultured in the presence or absence of tetrandrine and evaluated for cell proliferation, clonogenicity, morphological change, DNA fragmentation, and phosphatidylserine (PS) expression. Tetrandrine inhibited cell proliferation in both a concentration- and time-dependent fashion. Cell growth was completely inhibited by tetrandrine at 5 and 10 mg/L. Colony formation was decreased by tetrandrine 2.5 mg/L from (731±26) colonies per 1×10^3 cells in the control agar culture to (202±69) colonies per 1×10^3 cells and was completely suppressed by 5 and 10 mg/L tetrandrine. After 6 h incubation with 5 mg/L of tetrandrine, the treated U937 cells exhibited morphological changes characteristic of apoptosis, ie, membrane blebs, chromatin condensation, and apoptotic bodies. DNA fragmentation on gel electrophoresis was clearly noted after 6 h of treatment with 2.5 mg/L tetrandrine. To quantitate the amount of tetrandrine-induced PS expression, an early event in the apoptotic process, U937 cells were assessed by flow cytometry with propidium iodide and Annexin V-FITC staining. Tetrandrine 2.5 mg/L increased the percentage of PS positive cells up to 71.5 %±5.8 % after 24 h incubation. This study indicates that tetrandrine inhibits proliferation and induces apoptosis of human leukemic U937 cells.

TETRANDRINE AS AN ADJUNCT TO CHEMOTHERAPY

Tetrandrine reverses the resistance to doxoru-

bicin (Dox) in Dox-resistant clones of a Chinese hamster ovary (CHO) cell line[6]. Tetrandrine at non-cytotoxic doses of 1 and 2.5 mg/L, decreased the IC_{50} value of Dox in Dox-resistant CHO cells from 2.78 mg/L to 0.38 mg/L and 0.33 mg/L, respectively. This potentiation phenomenon also occurred when non-cytotoxic doses of tetrandrine (0.5 and 1 mg/L) were used. In both Dox-resistant and -sensitive CHO cells, non-cytotoxic doses of tetrandrine inhibited colony formation. Tetrandrine increased the accumulation of Dox in Dox-resistant but not Dox-sensitive CHO cells. This study indicates that tetrandrine reverses resistance to Dox in Dox-resistant CHO cells and enhances the accumulation of Dox in these cells, which may lead to increased Dox cytotoxicity.

Tetrandrine and dauricine (Dau) reduce Dox resistance in Harringtonine (Har)-resistant human leukemic HL-60 cells[7]. Cell growth, colony formation, cell cycle distribution, and Dox accumulation of Har-resistant HL-60 and/or Har-sensitive HL-60 cells were assessed with various therapeutic combinations. Non-cytotoxic concentrations of tetrandrine (0.5 mg/L) and Dau (5 mg/L) greatly potentiated the growth-inhibitory effect of Dox on Har-resistant HL-60 cells, but only slightly potentiated it in Har-sensitive HL-60 cells. Har-resistant HL-60 cell colony formation was reduced from 60 % by Dox (1 mg/L) to 0.2 % by tetrandrine (0.5 mg/L) plus Dox and to 9.2 % by Dau (5 mg/L) plus Dox. The percentages of Har-resistant HL-60 cells arrested in G_2/M phase were 17 % with Dox, 49 % with tetrandrine plus Dox, and 30 % with Dau plus Dox. Dox accumulation in Har-resistant HL-60 cells treated with tetrandrine increased about 0.2 μg in comparison with Dox alone. Dau had no such effect on Dox accumulation. This investigation suggests that tetrandrine may be effective in overcoming multidrug resistance (MDR).

Tetrandrine and berbamine reverse MDR in adriamycin-resistant human breast cancer MCF-7/Adr and human nasopharyngeal cancer KB_{v200} cells[8]. Cytotoxicity was evaluated *in vitro* by MTT assay and anti-tumor activity by implantation of MCF-7/Adr cells into BALB/c-nu/nu nude mice. Tetrandrine, berbamine, and verapamil had a dose-dependent effect of reversing adriamycin and vincristine resistance in MCF-7/Adr and KB_{V200} cells. Tetrandrine completely reversed vincristine resistance in MCF/Adr cells and adriamycin resistance in KB_{V200} cells at concentrations of 10 μmol/L and 5 μmol/L, respectively. Tetrandrine was more effective in reversing MDR than berbamine or verapamil. All three drugs increased intracellular adriamycin accumulation in MCF/Adr cells. In MCF/Adr-bearing mice, tumor growth was inhibited by 4.4 % by adriamycin, 25.8 % by tetrandrine, and 54.9 % by tetrandrine plus adriamycin. Hence, it appears that tetrandrine has potential as a chemotherapy sensitizer.

Tetrandrine exhibits synergistic anti-cancer effects when combined with Dox or vincristine (Vin) against the human breast cancer cell line MCF-7 and Dox-resistant MCF-7 (MCF-7/Dox) as well as human nasopharyngeal cancer cell lines KB and KB_{v200}[9]. Cell viability was detected by MTT assay and IC_{50} values were calculated by weighted probit analysis. To evaluate the nature of the interaction between tetrandrine and Dox or Vin against the above cell lines, the sum of the fractional inhibitory concentration (SFIC) and an isobologram were assessed. The SFIC values of three different combinations of tetrandrine and Dox ranged from 0.14 to 0.38 for MCF-7 and 0.1 to 0.29 for MCF-7/Dox; those of tetrandrine and Vin combinations ranged from 0.21 to 0.37 for KB and 0.32 to 0.63 for KB_{V200}. The interaction patterns between tetrandrine and Dox or Vin are therefore potentiating because all the estimated SFIC values were less than 1.0. The isobolic curves obtained from tetrandrine plus Dox and tetrandrine plus Vin were both concave. This indicates that the interactions of these 2-drug combinations are synergistic.

Tetrandrine and fangchinoline (FAN) enhance the cytotoxicity of drugs affected by MDR *via* modulation of P-glycoprotein (P-gp)[10]. The human ovarian cancer cell line SK-OV-3 (P-gp-negative) and colorectal cancer cell line HCT15 (P-gp-positive) were used to test the effect of tetrandrine, FAN, and verapamil (VER) on accumulation of the P-gp substrate rhodamine 123 and the cytotoxicity of paclitaxel (TAX) and actinomycin D (AMD). Tetrandrine (3.0 μmol/L), FAN (3.0 μmol/L), and VER (10.0 μmol/L) reduced the dose of TAX needed for 50 % inhibition of cell growth (ED_{50}) on HCT15 cells about 3100-,1900-, and 410-fold, and reduced the ED_{50} of AMD on these cells about 36.0-, 45.9-, and 18.2-fold, respectively. Similar effects, however, were not seen in SK-OV-3 cells. Tetrandrine (3.0 μmol/L), FAN (3.0 μmol/L) and VER (10.0 μmol/L) increased the rate of accumulation rate of rhodamine 123 in HCT15 cells but not in SK-OV-3 cells. The authors concluded that tetrandrine and FAN enhanced the cytotoxicity of drugs affected by MDR via modula-

tion of P-gp.

TETRANDRINE AS AN ADJUNCT TO RADIATION THERAPY

Tetrandrine enhances the sensitivity to radiation in radiation-resistant human glioblastoma U138MG cells *in vitro*[11]. Human glioblastoma U138MG cells were treated with tetrandrine (0, 2.5, 5.0, or 7.5 mg/L) for 4 h, after which ionizing radiation (0, 2, 4, 6, or 8 Gy) was delivered by a linear accelerator producing a 6 MeV electron beam. Cell survival, cell cycle, cell morphology, and DNA electrophoresis were assessed. Pretreatment with tetrandrine resulted in markedly decreased survival of U138 MG cells compared with radiation alone. Radiation (4 Gy) caused major pertubation of the cell cycle pattern, but this pertubation was prevented by treatment with 5 mg/L tetrandrine before radiation. Tetrandrine alone and tetrandrine plus radiation (4 Gy), but not radiation alone, induced cell detachment, rounding, and characteristic apoptotic bodies after 1 to 2 h. DNA fragmentation was also noted in cells treated with tetrandrine alone as well as with tetrandrine plus radiation. This study suggests that tetrandrine has potential as an adjunct to radiotherapy for glioblastoma.

Radiation-induced damage and inflammation of normal human mononuclear cells (MNC) can be prevented by pretreatment with tetrandrine[12]. Normal human MNC were isolated from the peripheral blood of healthy volunteers and subjected to radiation with and without tetrandrine pretreatment. The trypan blue exclusion test for cell viability, nitroblue tetrazolium reduction test for superoxide production, yeast ingestion assay for phagocytosis, Wright's stain for morphological assessment, and Griess reaction for nitric oxide release were performed. Cell survival increased from 58.3 % in the irradiated (10 Gy) group to 78.0 % in the tetrandrine (2 mg/L)-pretreated group, and similarly, the percentage of necrotic cells declined from 20.7 % to 10.7 % with tetrandrine pretreatment. The drug inhibited some aspects of the inflammatory response induced by ionizing irradiation, including superoxide release and phagocytic activity, but did not significantly influence the production of nitric oxide. Apoptotic changes were not seen in this study. These results indicate that tetrandrine possesses protective activity against ionizing irradiation and suppresses irradiation-induced inflammation of normal human MNC.

The topical administration of tetrandrine or extracts of *Centella asiatica* (Madecassol ointment) reduce acute radiation dermatitis in rats[13]. The gluteal skin of Sprague-Dawley rat was irradiated with different doses of radiation (20, 40, and 80 Gy) with a 6 MeV electron beam produced from a linear accelerator. Tetrandrine gel (1.6 mg/cm^2) and vaseline (as a control) were applied topically to the irradiated skin every day after irradiation and the acute skin reaction was evaluated by a modified dermatitis score system every other day. The animals were sacrificed on d 30 and the irradiated skin was evaluated histologically. The acute skin reaction in tetrandrine- and Madicassol-treated rats appeared earlier but was significantly less severe than in the control group. The peak skin reaction in the tetrandrine group was less serious than that of the control group. At a high radiation dose (80 Gy), the healing effect of tetrandrine was better than Madicassol or vaseline. The histological findings demonstrated that tetrandrine and Madicassol both reduced inflammation in irradiated skin even after high dose irradiation (80 Gy).

TETRANDRINE AS AN INHIBITOR OF ANGIOGENESIS

Tetrandrine inhibits angiogenesis in adjuvant-induced chronic inflammation and tube formation of vascular endothelial cells[14]. Air pouch granulomata in male ddY mice were induced by injection of Freud's complete adjuvant emulsion into the subcutaneous air pouch. Mice were sacrificed by injection of carmine solution via the tail vein 5 d later. The granulomatous tissues were excised and assessed for granuloma weight, carmine content, inflammatory cell count, and pouch fluid weight. The inhibitory pattern of tetrandrine (7.5-30 mg$\cdot$kg$^{-1}\cdot$d^{-1}, ip) on these parameters was similar to that of hydrocortisone (3.8-15 mg$\cdot$kg$^{-1}\cdot$d^{-1}, ip). To investigate the inhibitory modes of action of tetrandrine in the angiogenesis process, the tube formation of rat vascular endothelial cells was assessed. The 50 % inhibitory concentrations of tetrandrine and hydrocortisone on 2 % fetal bovine serum-stimulated tube formation were 1.72 μmol/L and more than 100 μmol/L, respectively. Tetrandrine (10 - 30 μmol/L) inhibited tube formation stimulated by interleukin (IL)-1α and platelet-derived growth factor (PDGF)-BB in a concentration-dependent and non-competitive manner. Tetrandrine thus may reduce endothelial cell tube formation, part of the angiogenetic process, by inhibition of the post-receptor pathway of IL-1α and PDGF-BB in chronic inflammation.

CONCLUSION

Many studies support the contention that tetrandrine has pharmacological potential in cancer therapy. The beneficial effects of tetrandrine include induction of apoptosis in tumor cells, reversal of MDR, sensitization of tumor cells to radiation, reduction of radiation injury in normal MNC and skin, and inhibition of angiogenesis. Although the mechanisms are not clearly addressed in the reviewed publications, the results indicate that further evaluation of tetrandrine should be pursued. In particular, there should be investigation on the mechanisms by which tetrandrine induces its beneficial as well as preclinical studies that may pave the way for clinical trials.

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