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Matrine Enhances the Inhibitory Effect of 5-FU on SW480 Cells *in vitro* and *in vivo*

(Matrin Meningkatkan Kesan Perencatan 5-FU pada Sel SW480 *in vitro* dan *in vivo*)

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ABSTRACT

We investigated the antitumor effects of the combination of matrine-a purified alkaloid extracted from Sophora flavescence-*and 5-fluorouracil (5-FU) on SW480 cells. This combination inhibited the growth of SW480 cells in a synergistic or additive manner by disrupting their progression through the cell cycle. Exposure of SW480 cells to matrine and 5-FU was followed by an increased rate of expression for caspase-3, caspase-9 and poly-ADP ribose polymerase (PARP) and inhibited the subcutaneous transplantation of SW480 tumors into Balb/c nude mice. Histopathological analysis showed that this effect was most pronounced in the spleens of treated animals. Typical cytotoxic effects observed in 5-FU-treated mice included fibrosis and lymphopenia, whereas in mice treated with 5-FU combined with matrine, the spleen ultrastructure remained intact. These findings indicate that matrine may enhance the therapeutic effectiveness of 5-FU in SW480 tumors by enhancing apoptosis and overcome the threat to immunocompetence associated with 5-FU.*

Keywords: Colorectal cancer; combination chemotherapy; cytotoxicity; Sophora flavescence*; 5-fluorouracil*

ABSTRAK

Kami mengkaji kesan antitumor daripada gabungan matrin - alkaloid yang ditulenkan diekstrak daripada Sophora flavescence *dan 5-fluorourasil (5-FU) pada sel SW480. Gabungan ini menghalang pertumbuhan sel SW480 secara sinergi atau tambahan dengan mengganggu perkembangan mereka melalui kitaran sel. Pendedahan sel SW480 kepada matrine dan 5-FU diikuti dengan kadar peningkatan ekspresi untuk caspase-3, caspase-9 dan poli-ADP ribosa polimerase (PARP) dan menghalang pemindahan subkutaneus tumor SW480 ke tikus bogel Balb/c. Analisis histopatologi menunjukkan bahawa ini memberi kesan paling ketara dalam limpa haiwan yang dirawat. Kesan sitotoksik biasa diperhatikan dalam tikus 5-FU-dirawat termasuk fibrosis dan limfofenia, manakala pada tikus yang dirawat dengan 5-FU digabungkan dengan matrin, ultrastruktur limpa kekal utuh. Penemuan ini menunjukkan bahawa matrin boleh meningkatkan keberkesanan terapeutik 5-FU dalam tumor SW480 dengan meningkatkan apoptosis dan mengatasi ancaman kepada kecekapanimmuno yang dikaitkan dengan 5-FU.*

Kata kunci: Gabungan kemoterapi; kanser kolorektal; sitotoksiti; Sophora flavescence*; 5-fluorourasil*

INTRODUCTION

Colorectal cancer (CRC) is a major cause of death from cancer worldwide. In China, CRC ranks fifth among cancer deaths and its occurence is increasing rapidly (Zhao et al. 2010). The 5-year survival rate associated with current treatments hovers around 50%. The chemotherapeutic agent 5-fluorouracil (5-FU) has been the first choice for chemotherapy and the basis of combination chemotherapy for CRC for many years. As a single agent, 5-FU has a considerable curative effect. However, it is highly toxic. Consequently, it is usually used in combination with other chemotherapeutic agents.

Sophora flavescens is a plant that is typically used in traditional Chinese medicine to treat individuals with viral hepatitis, enteritis, cancer, viral myocarditis, gastrointestinal hemorrhage, and skin diseases (colpitis, psoriasis and eczema) (Liu et al. 2006; Luo et al. 2007; Yamazaki 2000). Matrine, a purified alkaloid extracted from the dried roots of *S flavescence*, has demonstrated anti-tumor activity both *in vitro* and *in vivo* (Dai et al. 2009; Jiang et al. 2007). The ability to inhibit tumor growth while enhancing immune function and improving the patient's quality of life has been demonstrated in numerous studies (Chen et al. 2006; Crew & Neugut 2006), including studies in the cervix, stomach, breast and lung, as well as in hepatocellular carcinoma, leukemia and multiple myeloma (Dai et al. 2005; Han et al. 2010; Yu et al. 2009; Zhang et al. 2007). The mechanisms for its antitumor activity appear to be the inhibition of cell proliferation and induction of apoptosis (Ma et al. 2008). We examined the antitumor effect of combining the matrine with 5-FU on human CRC cells both *in vitro* and *in vivo*. The possible roles and mechanism of matrine in the treatment of CRC were also explored.

Experimental Details

Materials and Methods

All chemicals used in this study were purchased from the Sigma Chemical Company (St Louis, Mo, USA) unless otherwise stated. Matrine and 5-FU were purchased from Yanchi County Pharmaceutical Company, Ltd (China) and the Shanghai Xudong Haipu Pharmaceutical Company, Ltd (China). A 40-μM/mL solution of matrine dissolved it in 0.9% sodium chloride was frozen at –20°C. Antibodies to cleaved caspase-3, cleaved Poly-ADP Ribose Polymerase (PARP), cleaved caspase-9 and β-actin were purchased from Cell Signaling Technology (Danvers, Mass, USA) and Santa Cruz Biotechnology (Santa Cruz, Calif, USA). SW480 was purchased from American Type Culture Collection (ATCC) (CCL228; ATCC, Manassas, Va, USA) and cultured in RPMI 1640 containing 10% fetal bovine serum (FBS), streptomycin 100 μg/mL and penicillin 100 U/mL at 37°C in a 5% CO2 incubator.

General Procedure

Methyl thiazolyl tetrazolium (MTT) assay; Flow cytometric analysis of cell cycle; Western blotting analysis.

Detection Method

In vivo tumor-growth assay. Female BALB/c athymic nude mice aged 4 to 6 weeks old were purchased from the Center of Experimental Animals (Southern Medical University, China). Animal experiments were conducted in accordance with the Animal Research Committee Guidelines of Southern Medical University (SCXK(Yue) 2006-0015, 2006B023). SW480 cells were injected subcutaneously at a concentration of 2×10^6 into the left flank and right inguinal fold of 32 nude mice. The nude mice were then randomized into one of 4 groups: Control group: Animals that received an intraperitoneal (IP) injection of 0.9% sodium chloride; Matrine group: Animals that received matrine 30 mg/kg daily; 5-FU group: Animals that received 5-FU 30 mg/kg twice weekly; and Matrine + 5-FU group: Animals that received matrine 30 mg/kg daily and 5-FU 30 mg/kg twice weekly. Xenografts were measured using a vernier caliper. Both long (a) and short (b) diameters measured at 7-day intervals were used to calculate the xenograft volume (Mizutani et al. 2004): Volume (V) = $a \times b^2/2$ On day 28 after each injection, the mice were sacrificed and the xenografts were harvested to calculate the tumor growth inhibition rate: Inhibition rate (%) = (1 – (mass of xenograft in experimental group ÷ mass of xenograft in control group)) × 100%

Body weights were measured at the start of each experiment and on the last day of each treatment period. Histopathological and morphological analyses of the tumors were performed. Statistical analysis: Data were analyzed using SPSS 13.0 and expressed as the mean±standard deviation (SD). One-way analysis of variance (ANOVA), Welch statistic tests or factorial-designed ANOVA were applied. For multiple comparisons, least significant difference or Games Howell was used, respectively, according to the homogeneity of variances.

Results and Discussion

Inhibitory effects on SW480 cell proliferation was shown in Table 1; Control of SW480 cell progression through the cell cycle was shown in Table 2.

Caspase expression increased after exposure to the combination of matrine and 5-FU (Figure 1) in Western blot analysis. These results indicated that matrine and 5-FU may induce apoptosis in SW480 cells by activating the caspase-3 and caspase-9 pathways.

Changes in tumor volume and weight demonstrated that xenografts had the fastest growth rate in controls. Matrine or 5-FU inhibited tumor growth at rates of 13.71% and 63.07% respectively. Treatment with a combination of matrine and 5-FU at the same doses used in monotherapy had a greater inhibitory effect than treatment with either agent alone (Figure 2(a), 2(b)), with inhibition rates rising dramatically to 80.58%. These agents also appeared to have an effect on body weight, which decreased significantly with 5-FU monotherapy ($p<.05$) then increased significantly with matrine/5-FU combination therapy ($p<.05$) (Figure

TABLE 1. The effect of matrine combined with 5-FU on the inhibition of growth in SW480 cells (n=3)

Time (h)	5-FU (mg/mL)	Growth inhibition rate (%) 5-FU	5-FU+Matrine	*F*	*P*
24	3.125	10.66±1.92	24.18±7.63*	8.667	.041
	6.25	21.22±2.24	36.20±6.11*	15.865	.016
	12.5	23.71±3.40	43.57±5.36*	29.429	.006
48	3.125	21.25±3.28	59.30±3.44*	360.018	< .001
	6.25	31.12±0.22	70.76±3.30*	431.173	< .001
	12.5	57.36±5.39	91.89±3.06*	92.856	.001
72	3.125	34.12±4.18	53.26±3.49*	37.132	.004
	6.25	49.67±6.98	81.13±0.97*	23.745	.008
	12.5	65.39±6.16	89.58±1.27*	44.326	.003

*$p < .05$ compared with 5-FU alone

TABLE 2. Changes in distribution of SW480 cells throughout the cell cycle (n=3)

Group	Cell cycle phase (%)		
	G0/G1	S	G2/M
Control	45.03±1.89	41.10±4.29	13.90±2.36
Matrine	57.47±1.66*	30.87±3.04*	11.70±1.47
5-FU	60.11±1.53*	29.63±1.46*	10.26±0.66*
Matrine+5-FU	72.70±1.05*	16.67±0.47*	10.63±1.52*
F	157.864	40.136	3.072
P	< .001	< .001	0. 091

*p< .05 vs control group

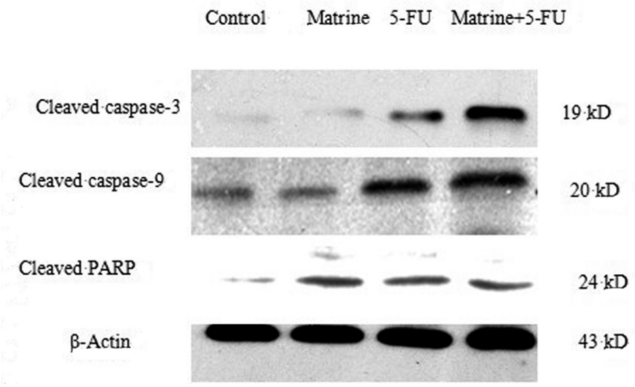

FIGURE 1. Activation of the caspase pathway in Western blot

2(c)). Histopathological analysis showed large areas of necrosis and apoptosis of tumors in mice treated with combination therapy. The 5-FU group demonstrated a diffuse distribution of necrosis and relatively few apoptotic cells compared with the combination matrine/5-FU group, which demonstrated an increased number of apoptotic cells in some areas of the tumor (Figure 2(d)) compared with the 5-FU monotherapy group.

It has been reported that matrine shows antitumor activity in many types of cancer. It has also been shown that the combination of matrine and cisplatin is superior to cisplatin alone in patients with non–small-cell lung cancer accompanied by subclinical pleural metastasis (Yang et al. 2012). It has been suggested that matrine inhibits tumor growth by modulating genes and proteins that participate in apoptosis or facilitate proliferation, such as N-ras, p53 and the caspases (Jiang et al. 2007; Zhang et al. 2007). In a recent study, it was reported that matrine inhibits the growth of gastric carcinoma by up-regulating Beclin 1 and inducing autophagy (Zhang et al. 2011). Matrine can stop K-562 cells from entering the S-phase of the cell cycle by inhibiting the expression of myc (Zhang et al. 2001). In this study, we demonstrated that low doses of matrine can enhance the inhibitory effects of 5-FU in SW480 cells by contributing in a synergistic or additive manner to blocking progression through specific phases of the cell cycle.

Apoptosis is a key target for new anti-cancer therapies. This form of cell death is coordinated by a network of genes and gene products involved in both caspase-dependent and caspase-independent pathways (Broker et al. 2005). The caspases constitute a unique class of aspartate-specific proteases that serve as central components of the apoptotic response. There are 2 types of caspases: Initiator caspases (caspases 2, 8, 9 and 10) and effector caspases (caspases 3, 7 and 6) Lopez-Hernandez et al. 2003). We found that matrine and 5-FU may induce apoptosis in SW480 cells by activating caspase-3 and caspase-9. We found that the combination of matrine and 5-FU had a considerably greater inhibitory effect on the growth of SW480 tumors than monotherapy with either agent. Histological analysis showed that this combination of agents resulted in more apoptotic tumor cells than other groups. Some researchers associate the antitumor activity of matrine with its immunomodulatory properties, specifically with its ability to activate both cellular and humoral immune responses (Lin et al. 2011). In the present study, we found that mice that received 5-FU alone were more likely to exhibit diarrhea, stiff movements and weakness compared with those who received combination therapy. By contrast, the rate of weight loss decreased and the quality of life improved in the animals that received matrine. An injection of Sophora can reduce the toxicity and adverse effects of chemotherapy (Zhu et al. 2009). Furthermore, matrine may be able to inhibit the production of tumor necrosis factor-α and interleukin-6 in murine macrophages and prevent cachexia-related symptoms associated with

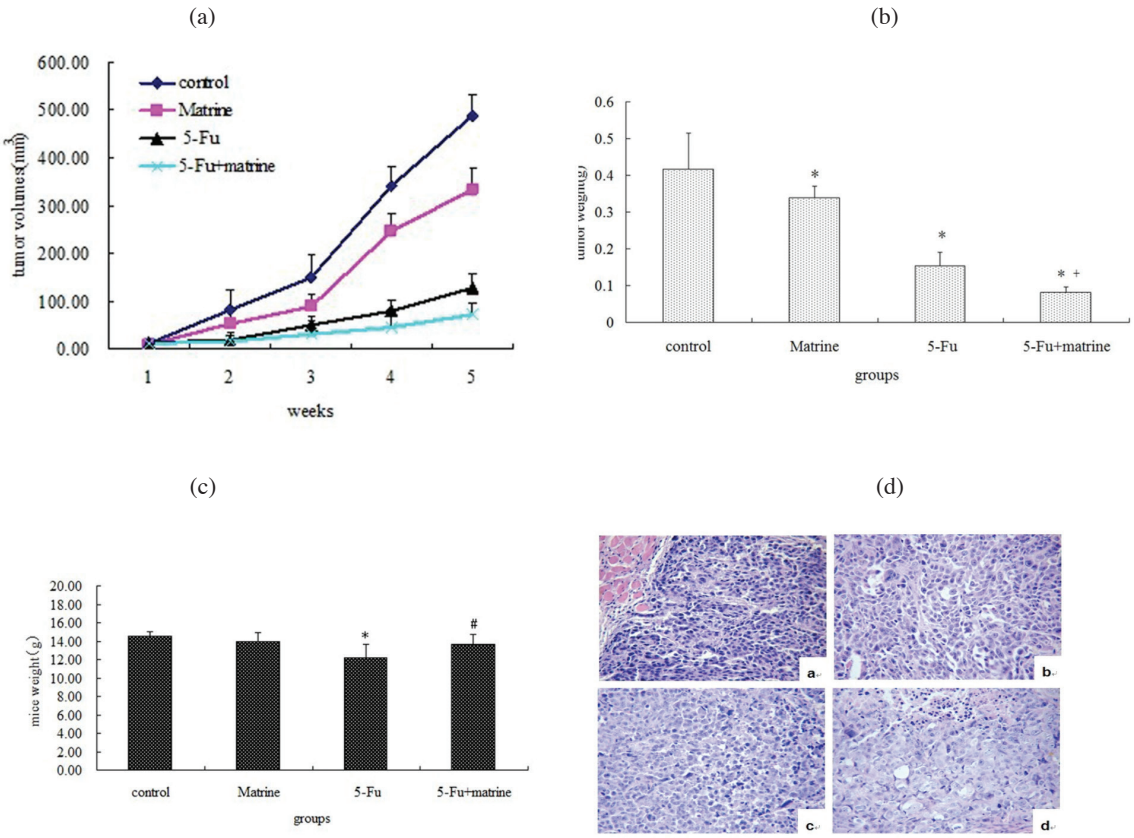

FIGURE 2. Effects on tumor growth from xenografts

colon adenocarcinoma in mice (Zhang et al. 2008). These findings suggest that matrine not only possesses antitumor effects but can also enhance the antitumor activity of chemotherapeutic drugs, including 5-FU and decrease the number and severity of side effects.

CONCLUSION

Matrine may enhance the therapeutic effectiveness of 5-FU in SW480 tumors by enhancing apoptosis and release the side effects associated with 5-FU. Our results also suggested a potential clinical use for matrine/5-FU combination therapy on CRC.

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