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Inhibitory Effects of AVEMAR on Proliferation and Metastasis of Oral Cancer Cells

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ABSTRACT

Oral cancer is keeping its 4th rank on the death causing cancers among Taiwan males, and its metastatic and recurrent rates remain high and a life-threatening issue to the citizens. Fermented wheat germ extract (AVEMAR) is used in clinical cancer nutritional therapy in gastrointestinal cancers but not in oral cancer yet. In this study, the potential of AVEMAR to inhibit tumor proliferation and metastasis of oral cancer was first investigated. Antiproliferative activity of AVEMAR was determined in human oral squamous carcinoma SCC-4 cells by MTT methodology. Wound-healing migration, transwell invasion, and Western blotting assays were carried out to examine the in vitro antimetastatic effects and involved signaling molecules for AVEMAR in oral cancer cells. AVEMAR at 0.2–1.6 mg/ml significantly inhibited the cell viability with IC_{50} values of 1.19 and 0.98 mg/ml for 24-h and 48-h treatment. Furthermore, AVEMAR could induce cell apoptosis and inhibit the migration and invasion of metastatic SCC-4 cells at a similar dose range. Notably, AVEMAR suppressed the expression of matrix metalloproteinase (MMP)-2 and urokinase plasminogen activator (u-PA), but not MMP-1 or MMP-9, in SCC-4 cells. These results strongly support the antiproliferation and in vitro antimetastatic capacity of AVEMAR which may extend its contributions from cancer nutrition supplements to preventive agent for oral cancer.

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Introduction

Oral cancer, which may arise from tongue, floor of the mouth, buccal mucosa, gingival, lips, and oropharynx, is one of the most common cancers and it constitutes a major health problem particularly in Eastern countries (1,2). The International Agency for Research on Cancer (IARC) reported that there were 300,373 newly diagnosed cases and 145,353 deaths of oral cancer in 2012, accounting for 2.1% of all newly diagnosed cancer cases and 1.9% of all cancer deaths, respectively. Most oral cancer incidence and mortality occurred in developing countries. In addition, the estimated 5-yr prevalence of oral cancer is 702,149 worldwide (3). Clinically, treatment of oral cancer has relied primarily on classical modalities encompassing surgery for most of the cases, together with radiation, and chemotherapy, or a concurrent radio-chemotherapy after surgery. Despite significant advances in surgery and therapy over the past decades (4), oral cancer is still characterized by poor prognosis and a low survival rate worldwide (5). In oral cancer patients diagnosed with malignancies at advanced stages, there are usually high occurrences of invasion to lymph nodes and distant metastasis, together with high risks of second malignancy (6).

Cancer metastasis, which means the spreading process of tumor cells from the primary neoplastic location to distant sites and its sequential proliferation in the new sites, is the most common cause of death among cancer patients. Metastasis of cancer cells may involve multiple processes and complicated alterations inside and outside of the cell. For instance, there are dramatically sequential changes in adhesive network among neighbor cells and the extracellular matrixes which may lead to abnormal intercellular interactions. Thus, the degradation of extracellular matrixes and components of the cellular membrane mediated by the proteinases, such as urokinase plasminogen activator (u-PA) and matrix metalloproteinases (MMPs), was a character for cancer metastasis (7). Several of the proteinases, including MMP-1, MMP-2, MMP-9, and u-PA, identified to be responsible for

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degrading the cellular membrane components are known to play a critical role in cancer metastasis (8). Therefore, targeting MMPs and u-PA as a method of migration/invasion inhibition could be a potential strategy in cancer metastasis prevention (9,10).

Fermented wheat germ extract (AVEMAR) is a product of industrial fermentation of wheat germ with standardized contents of two quinones, 2-methoxy benzoquinone and 2,6-dimethoxybenzquinone, and plant flavonoids (11). In literature, it has been reported to be safe and used as a dietary supplement in clinical practice (12–14). One of the highlights is that AVEMAR increased the progression-free survival rates of patients suffering from stage-III melanoma (13). AVEMAR was also used among children with solid tumors, and AVEMAR supplementation had the capacity to reduce the incidence of treatment-related febrile neutropenia (15). For several common cancers, such as breast, colon, lung, and prostate cancer, AVEMAR was reported to be serving as an effective adjuvant agent in cancer therapy (13,15–17). In 2011, Mueller and colleagues screened and compared the cytotoxicity of AVEMAR to a spectrum of 32 human cancer cell lines, including testicular cancer (H12.1, 2102EP, 1411HP, 1777NRpmet), colon cancer (HCT-8, HCT-15, HCT-116, HT-29, DLD-1, SW480, COLO205, COLO320DM), non-small cell lung cancer (NSCLC) (A549, A427, H322, H358), head and neck cancer (FADU, A253), cervical epidermoid carcinoma (A431), mammary adenocarcinoma (MCF-7, BT474), ovarian adenocarcinoma (A2780), gastric cancer (M2), anaplastic thyroid cancer (8505C, SW1736), papillary thyroid cancer (BCPAP), follicular thyroid cancer (FTC133), melanoma (518A2), hepatoma (HepG2), glioblastoma (U87MG), and neuroblastoma (SHSY5Y, SIMA) (18). However, no literature about the effects of AVEMAR on oral cancer cell line(s) was reported.

To sum up, an effective tumor-suppressive agent during the post-operative period beneficial to oral cancer patients, who are at high risk of metastasis and recurrence. AVEMAR was already used in clinical practice and reported to have potential to serve as anticancer agent in addition to dietary supplement for other cancers. Therefore, the purpose of this article is to evaluate the effects of AVEMAR on SCC-4 oral cancer cells and investigate the possible mechanisms.

Materials and methods

Chemicals and AVEMAR

Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), glutamine, and penicillin/streptomycin/neomycin were obtained from Gibco BRL (Grand Island, NY, USA). Antibodies against MMP-1, MMP-2, MMP-9, and u-PA were purchased from Santa Cruz Biotechnology, Inc. (Heidelberg, Germany) and/or Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were of the highest grade commercially available, and supplied either by Merck (Darmstadt, Germany) or Sigma. AVEMAR was obtained by authors, Dr. Yang and Dr. Lu, and imported by You-Lih Pharmaceutical Co. Ltd. in Taiwan from Biropharma USA Inc., New York, USA. AVEMAR was stored as dried powder at 4°C in packets until use. For experimentation, AVEMAR was freshly prepared in sterile water to a final concentration of 300 mg/ml. Later, the solution of AVEMAR was centrifuged with 150 *g* to remove the insoluble materials and then subject to filtration with 0.22-μM filter.

Cell culture and assessment of cell morphology and viability

The human oral squamous cell carcinoma SCC-4 (ATCC, CRL-1624) cells were grown in DMEM supplemented with 10% heat-inactivated FBS, 2 mM glutamine, and 1% penicillin/streptomycin/neomycin in a humidified incubator (5% CO_2 in air at 37°C). Cell viability (2×10^5 cells/12-well plate of SCC-4 cells) with or without treatment of AVEMAR was directly observed by phase-contrast microscopy using trypan blue exclusion method and quantitated with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as previously published (19–21). For all experiments, SCC-4 cells were incubated with AVEMAR (0, 0.05, 0.1, 0.2, 0.4, 0.8, and 1.6 mg/ml) for 24 or 48 h as indicated.

Cell apoptosis assay

Apoptosis of SCC-4 cells was assessed using Annexin V, a protein that binds to phosphatidylserine residues which are exposed on the cell surface of apoptotic cells, as previously described (22,23). Briefly, cells were treated with 0, 0.05, 0.1, 0.2, 0.4, 0.8, and 1.6 mg/ml of AVEMAR for 48 h. After treatment, cells were washed twice with phosphate-buffered saline (PBS) (pH 7.4), and resuspended in staining buffer containing 1 μg/ml of propidium iodide and 0.025 μg/ml of Annexin V-fluorescein isothiocyanate (V-FITC). Double labeling was performed at room temperature for 10 min in the dark before flow cytometric analysis. Cells were immediately analyzed using FACScan and the CellQuest program (Becton Dickinson).

Wound-healing migration assay

To determine the effects of AVEMAR on cell migration, an in vitro wound-healing assay was performed. For the

cell migration assay, SCC-4 cells (3×10^5 cells/well) were seeded into 12-well culture plates and grown in medium containing 10% FBS to a nearly confluent cell monolayer. At confluence, monolayers were wounded using a 200-μl micropipette tip, washed twice with PBS, and incubated with 0, 0.05, 0.1, 0.2, 0.4, 0.8, and 1.6 mg/ml of AVEMAR in serum-free medium for 48 h. Next, cells were washed twice with PBS, fixed in 100% methanol, and stained with Giemsa stain solution. The cultures were photographed at $\times$200 magnification to monitor the migration of cells into the wounded area and the closure of wounded area was calculated. Percentage inhibition of migrating cells was quantified, with untreated (control) cells representing 100%.

Cell invasion assay

Invasion assays were performed using BD Matrigel^{TM} invasion chambers (Bedford, MA, USA). Matrigel is a solubilized basement membrane preparation extracted from the Engelbreth-Holm-Swarm mouse sarcoma, a tumor rich in extracellular matrix proteins. Briefly, for the invasion assay, 10 μl Matrigel (0.5 mg/ml) was applied to 8-μm polycarbonate membrane filters, and the bottom chamber of the apparatus contained standard medium (750 μl) with 10% FBS. Top chambers were seeded with 1×10^5 cells in 500 μl serum-free medium, and the cells were incubated with the indicated concentrations of AVEMAR. Then cells were allowed to freely migrate for 48 h at 37°C in incubator. After the incubation, the nonmigrated cells on the top surface of the membrane were removed with a cotton swab. The migrated cells on the bottom side of the membrane were fixed in cold 75% methanol for 15 min and washed thrice with PBS. Further, cells were stained with Giemsa stain solution and then destained with PBS. Images were obtained using a light microscope at $\times$200 magnification, and invading cells were quantified by manual counting. Percentage inhibition of invading cells was quantified, with untreated (control) cells representing 100%.

Western blotting analysis

SCC-4 cells (5×10^5 cells/10-mm plate) were incubated with indicated concentrations of AVEMAR for 48 h and harvested for Western blotting analysis to reveal the altered expression levels of investigated proteins as previously published (20,21). Briefly, the cells collected after treatments were then suspended in 100 μl lysis buffer (50 mM Tris, 150 mM NaCl, 0.1% SDS, 0.5% sodium deoxycholate, 1% Triton X-100, 1$\times$ protease cocktail) (Sigma Aldrich, St. Louis, MO), and suspensions were kept on ice for 20 min, and then centrifuged at 13,000 g for 30 min at 4°C. Total protein content was determined by Bradford protein assay reagent (Bio-Rad, Hercules, CA, USA) using bovine serum albumin (BSA) as a standard. Protein extracts were reconstituted in sample buffer (62 mM Tris-HCl, 2% SDS, 10% glycerol, and 5% β-mercaptoethanol) and the mixture was boiled at 97°C for 5 min. Equal amounts (50 μg) of denatured protein samples were loaded into each lane, separated by 8–15% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred onto polyvinylidene difluoride (PVDF) membranes overnight. Membranes were blocked with 5% nonfat dried milk in PBS, which contains 1% Tween-20 for 1 h at room temperature followed by incubation with primary antibodies (MMP-1, MMP-2, MMP-9, and u-PA) for 2 h. Monoclonal antibodies to MMP-1 (sc-21731), MMP-2 (sc-10736), MMP-9 (sc-10737), and uPA (sc-14019) were purchased from Santa Cruz (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and diluted to 1/100 during use. The blots were then incubated with horseradish peroxidase-conjugated anti-mouse or anti-rabbit antibody (1:5000) at room temperature overnight. Signals were detected via enhanced chemiluminescence by using Immobilon Western HRP Substrate (Millipore, Billerica, USA).

Statistical analyses

Two independent researchers repeated each protocol for at least three times at different dates. Results are presented as mean $\pm$ SD. All the data were analyzed using analysis of variance, followed by Dunnett's test for pairwise comparison. Statistical significance was defined as $P < 0.05$ for all tests.

Results

Effect of AVEMAR on SCC-4 cell viability

To investigate the inhibitory effects of AVEMAR on SCC-4 cells cell viability, SCC-4 cells were exposed to 0–1.6 mg/ml of AVEMAR for 24 or 48 h (Fig. 1). The results produced from MTT assay showed that 0.05–0.1 mg/ml of AVEMAR treatment for 24 h did not affect the number of SCC-4 cells. However, 0.2, 0.4, 0.8, and 1.6 mg/ml of AVEMAR could significantly ($P < 0.05$) reduce the cell viability of SCC-4 cells by 6.2%, 15.7%, 37.0%, and 65.7%, respectively. Similarly, up to the concentration of 0.1 mg/ml of AVEMAR for 48 h were noncytotoxic to SCC-4 cells, whereas 0.2, 0.4, 0.8, and 1.6 mg/ml of AVEMAR could significantly ($P < 0.05$) reduce the cell viability of SCC-4 cells by 15.5%, 29.5%, 50.8%, and 68.7%, respectively. Overall, the data

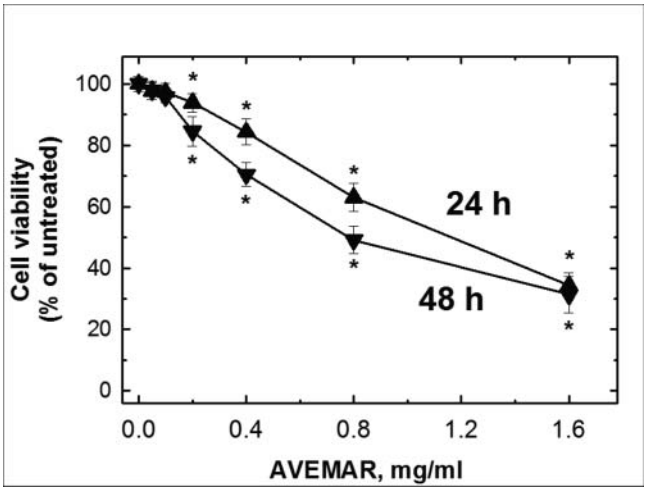

Figure 1. Cytotoxic effects of AVEMAR on human oral cancer SCC-4 cells. SCC-4 cells were cultured in the presence of 0, 0.05, 0.1, 0.2, 0.4, 0.8, and 1.6 mg/ml of AVEMAR for 24 (▲) and 48 (▼) h. The viable cells were then assessed by MTT assay. *P* values were below 0.05, which was the threshold for being significant, over 0.2 mg/ml of AVEMAR after both 24 and 48 h. * indicated significant difference from the untreated control group.

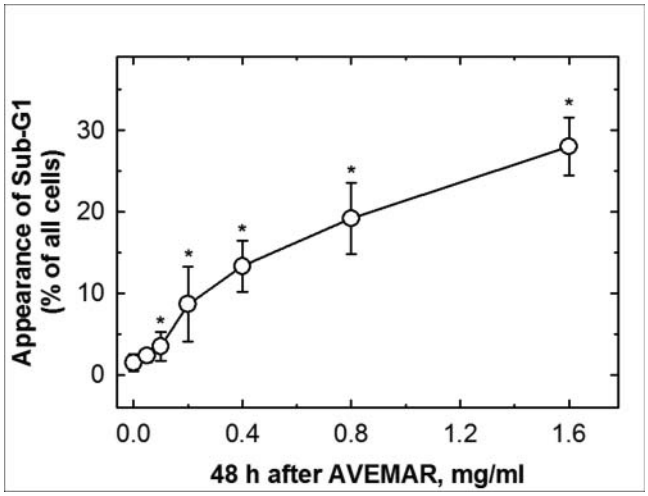

Figure 2. Induction of apoptosis by AVEMAR in human oral cancer SCC-4 cells. SCC-4 cells were cultured in the presence of 0, 0.05, 0.1, 0.2, 0.4, 0.8, and 1.6 mg/ml of AVEMAR for 48 (◯) h. Then the cells were subject to flow cytometry for distribution of cell cycle. The appearance of sub-G1 proportions is shown. Results were presented as mean ± SD of six assays. The *P* values were below 0.05 over 0.1 mg/ml of AVEMAR after 48 h. * indicated significant difference from the untreated control group.

suggested that the IC_{50} value of AVEMAR in inhibition cell viability of SCC-4 cells for 24 and 48 h were 1.19 and 0.98 mg/ml, respectively.

AVEMAR induced cell apoptosis

The induction of cell death together with growth inhibition may both contribute to the reduced cell viability. To investigate the apoptosis induced by AVEMAR, SCC-4 cells were treated with 0, 0.05, 0.1, 0.2, 0.4, 0.8, and 1.6 mg/ml of AVEMAR for 48 h and then stained with Annexin V which was assayed by flow cytometer. Figure 2 shows that AVEMAR induced apoptotic cell death in SCC-4 cells and this effect occurred in a concentration-dependent manner. The percentages of apoptotic cells induced by 0.1, 0.2, 0.4, 0.8, and 1.6 mg/ml of AVEMAR after 48 h were 3.5, 8.7, 13.3, 19.2, and 28.0%, respectively (Fig. 2).

AVEMAR inhibited migration and invasion of SCC-4 cells

To determine the effects of AVEMAR on the migration capacity of SCC-4 cells, confluent monolayers of SCC-4 cells were scraped to remove a section of monolayer and cultured for 24 and 48 h with 0, 0.05, 0.1, 0.2, 0.4, 0.8, and 1.6 mg/ml of AVEMAR. As described in Materials and Methods, the serum-free wound-healing assay is a classical and commonly used method for examining the capacity of cell migration. In Figs.3A and B, the results show that AVEMAR significantly ($P < 0.05$) suppressed the migration capacity of SCC-4 cells after 24 and 48 h, respectively (Fig. 3A and B). In addition, Matrigel transwell assay has been used to determine the invasive activity of SCC-4 cells across the basement membrane, and anti-invasive efficacies of AVEMAR. According to the results, we observed that AVEMAR significantly ($P < 0.05$) decreased the invasion potential of SCC-4 cells in a dose- and time-dependent manner (Fig. 3C and D).

AVEMAR decreased the protein levels of MMP-2 and u-PA in SCC-4 cells

The Western blot assay was performed to monitor the effect of AVEMAR on the levels of MMP-1, MMP-2, MMP-9, and u-PA expression within SCC-4 cells. As shown in Fig. 4A, SCC-4 cells treated with 0, 0.4, 0.8, and 1.6 mg/ml of AVEMAR for 48 h had remarkably altered MMP-2 and u-PA expression (Fig. 4A). In detail, AVEMAR at 0.4, 0.8, and 1.6 mg/ml decreased 14.7, 30, and 51% of MMP-2 and 19, 27.3, and 44.7% of u-PA, respectively (Figs. 4C and E). However, up to the concentration of 1.6 mg/ml, AVEMAR did not seem to change the levels of detectable MMP-1 or MMP-9 expression within SCC-4 cells (Figs. 4B and D).

Discussion

AVEMAR, a product from fermented wheat germ extract, has been approved as a dietary supplement for

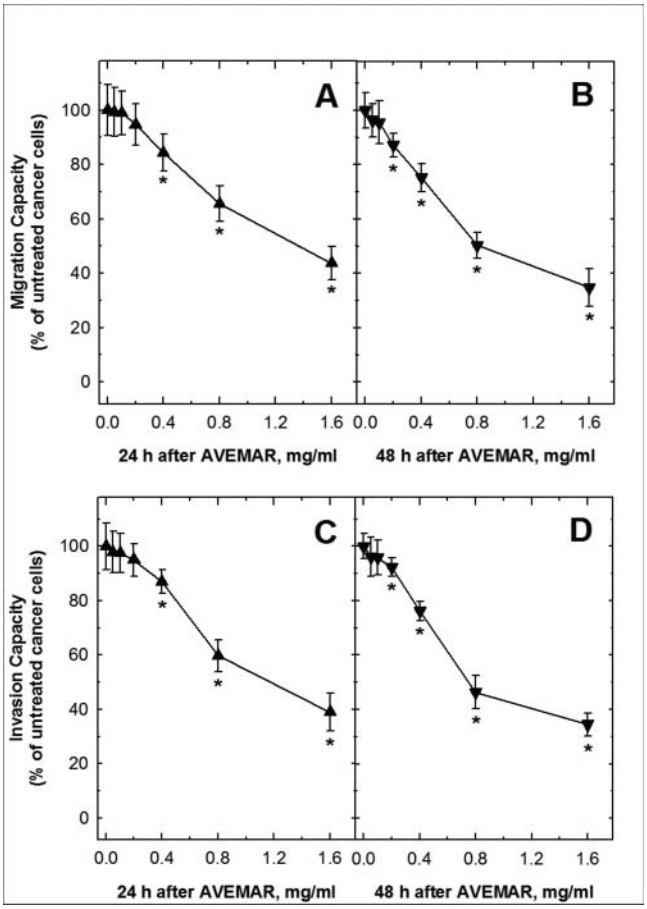

Figure 3. Inhibitory effects of AVEMAR on the migration and invasion capacities of human oral cancer SCC-4 cells. (A and B) Cells in a wounded culture plate were treated with 0, 0.05, 0.1, 0.2, 0.4, 0.8, and 1.6 mg/ml of AVEMAR, and their migration capacities were observed under a phase-contrast microscope (×100 magnification) at 0, 24 (A), and 48 h (B), and the closure of wounded areas was observed. The migration capacities of the untreated freely migrated group were set as 100% for 24 (A) and 48 h (B), separately. (C and D) Cells were treated with 0, 0.05, 0.1, 0.2, 0.4, 0.8, and 1.6 mg/ml of AVEMAR, and after 24 (C) and 48 h (D), the cells invading the membrane were photographed at ×200 magnification and quantitated. The percentage inhibition of invading cells was quantified and expressed on the basis that untreated cells (control) represented 100%. Invasive capacities were determined by counting the stained cells in three microscopic fields per sample. Results were presented as mean ± SD of six assays. * indicated significant difference from the untreated control group.

special medical purposes in cancer patients by the National Institute of Food Safety and Nutrition of Hungary. In animal models, AVEMAR exhibited a remarkable inhibitory effect on metastasis formation and resulted in reduced proliferation rate and increased survival time of the skin grafts (24, 25). In addition, AVEMAR can synergistically enhance the effect of 5-fluorouracil (5-FU) and dacarbazine under experimental conditions when applied in combination with these anticancer agents (26). Moreover, oral administration of AVEMAR inhibits tumor metastasis formation and prolongs the survival rate after chemotherapy and surgery among patients of advanced colorectal cancer (16, 27) and high-risk melanoma (13). In the present study, we have provided evidence showing that AVEMAR could significantly suppress viability and induce apoptosis of SCC-4 oral cancer cells (Figs. 1 and 2). This study also revealed that AVEMAR could potentially inhibit their migration and invasion capacities (Fig. 3). According to our literature survey, this is the first scientific report examining the effects and possible mechanisms of AVEMAR on oral cancer metastasis (Fig. 4), and our results confirmed and extended the in vitro antimetastatic contribution of AVEMAR.

The most threatening indicator for the prognosis of oral squamous cell carcinoma is its metastasis to the cervical lymph nodes or distant organs. Statistically, about 50% of oral squamous cell carcinoma patients present with pathological or clinical evidence of nodal metastases, and the 5-yr survival rate are less than 50% for the patients with cervical lymph node metastasis (28). In the present study, we have examined the SCC-4 cell migration using the wound-healing assay and the results showed that AVEMAR significantly inhibited the migration of SCC-4 cell and there was a dose-dependent response within the dose range of 0.05–1.6 mg/ml. To avoid any possible bias that we found the inhibition of AVEMAR on cancer cell in vitro migration due to reduction in cell viability, we have performed the following examination. As per the data of Fig. 2, the percentages of viable cells (with no sub-G1 appearance) at 48 h after AVEMAR treatment were 97.6%, 96.5%, 91.3%, 86.6%, 80.8%, and 72.0% for 0.05, 0.1, 0.2, 0.4, 0.8, and 1.6 mg/ml. At the same treatment, the inhibition efficacy of AVEMAR on in vitro migration was 0.7%, 1.0%, 5.3%, 15.7%, 34.5%, and 56.3% for 0.05, 0.1, 0.2, 0.4, 0.8, and 1.6 mg/ml as shown in Fig. 3B. Obviously, 48-h AVEMAR treatment inhibited in vitro migration capacity and cell viability at the same time, while the effects on inhibition of in vitro migration capacity was more efficient than that on the cell viability. Also, measurement of the inhibition of invasion of cancer cells using Boyden chamber Matrigel invasion assay is a widely accepted in vitro assay for screening compounds that can inhibit metastasis (29). Results from our invasion assay also strongly supported that AVEMAR inhibited SCC-4 cell metastasis dose-dependently within the doses of 0.05–1.6 mg/ml (Fig. 3). In 2002, Comin-Anduix and colleagues found that AVEMAR was capable of inhibiting the cell growth of Jurkat T-cell leukemia cells at 0.2 mg/ml and inducing apoptosis at 48 h. Also, after oral intake of AVEMAR 9 g/day for 9 months, the peak

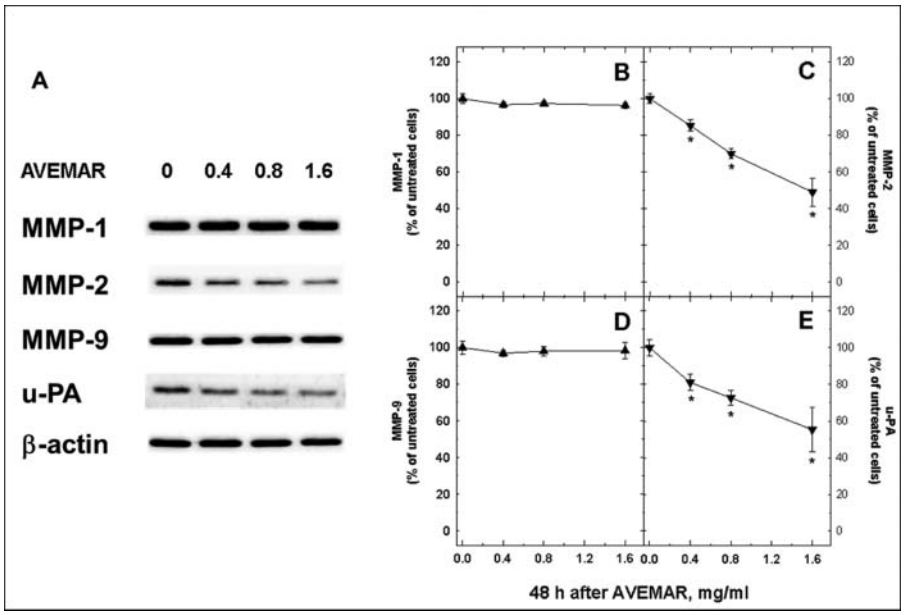

Figure 4. Alterative patterns of AVEMAR on expression levels of MMPs and u-PA in human oral cancer SCC-4 cells. Western blot analysis of the protein levels of MMP-1, MMP-2, MMP-9, and u-PA (A) in SCC-4 cells after exposure to 0–1.6 mg/ml of AVEMAR for 24 h. Proteins (50 μg) from each sample were separated using8–15% SDS-PAGE. The typical housekeeping gene β-actin was used as an internal control for loading adjustment. Relative alterations in protein bands were measured using densitometric analysis with the control being 100% as shown in the gel data. Typical results from six independent experiments and the overall quantitative data are presented in (A) and (B–E), respectively.

plasma concentration of AVEMAR could reach 0.5–1 mg/ml, which is similar to that in this study (30). In 2015, Shibuya and colleagues found that administration of AVEMAR at 1 g/kg could suppress the tumor growth and delay the tumor formation in specimens from patients, and treating the colorectal carcinoma cells with AVEMAR at 1 mg/ml could inhibit the synthesis of pentose phosphate pathway enzymes and inhibit their growth (31). Thus, the effaceable concentration of AVEMAR in this study is at a physiological achievable level which could be applied in clinical practice. The inhibitory effects of AVEMAR on metastasis in addition to proliferation strongly enhance its value in clinical use for both cancer prevention and therapy.

To further explore the detailed mechanisms, the levels of typical metastasis-associated molecules were assayed. It is well described that MMPs form a group which plays a crucial role in the activation of gelatinase A followed by the degradation of gelatin and collagen extracellular matrix, eventually enhancing tumor migration and invasion. It is noteworthy that in our study AVEMAR significantly inhibited the expression of MMP-2, not MMP-1 or MMP-9, in SCC-4 cells (Fig. 4). In addition, AVEMAR also significantly inhibited the expression of u-PA, which is an upstream activator of MMPs that plays a crucial role during metastases (32,33). We have also performed RT-PCR to investigate whether the inhibition of MMP-2 and u-PA by AVEMAR was a consequence of the alteration at the transcriptional level. The results showed that consistent alterations in mRNA and protein levels were found regarding MMP-2, u-PA (both down-regulated), and MMP-9 (not significantly changed) (data not shown). Since it isreported that the activities of MMPs and u-PA are regulated by their specific endogenous inhibitors, Tissue inhibitor of metalloproteinase (TIMPs) and plasminogen activator inhibitors (PAIs), respectively, whether AVEMAR not only inhibits the activity of MMP-2 and u-PA, but upregulate the activity of their inhibitors TIMP-1, TIMP-2, PAI-1, and PAI-2 in SCC-4 cells will be revealed in the near future.

The effective and powerful components in this extracted compound still need to be certified in preclinical models. Previous study showed that AVEMAR exerts anticancer activity on 32 types of cancer cell lines, including testicular cancer, colon cancer, NSCLC, head and neck cancer, cervical epidermoid carcinoma, mammary adenocarcinoma, ovarian adenocarcinoma, gastric cancer, thyroid cancer, melanoma, hepatoma, glioblastoma, and neuroblastoma cell lines (18). Among the cancer cells examined, the highest efficacy of AVEMAR was found to have an IC_{50} value of 0.042 mg/ml in neuroblastoma cells and 0.3–0.54 mg/ml in the eight colon cancer cell lines examined (18). In this study, the IC_{50} value of AVEMAR was about 0.98–1.19 mg/ml in SCC-4 oral

cancer cells. The IC_{50} value in this study is a little higher in oral cancer than those in neuroblastoma cells or colorectal cancer cells (18). We have proved that the suppression of cell viability could not only be due to the induction of apoptosis, but also due to the inhibition of cell proliferation as well (Figs. 1 and 2). In the future, further investigations focusing on combination of AVEMAR with other anticancer drugs, such as 5-FU and cisplatin, to enhance its in vivo and in vitro antimetastatic and anti-proliferation activities are warranted.

In conclusion, our observations indicate that AVEMAR exerts an inhibitory effect on the essential steps of metastasis, including migration and invasion of SCC-4 cells, in addition to cancer proliferation. To the best of our knowledge, this is the first scientific report examining the inhibitory efficacies and mechanisms of AVEMAR from multiple angles on oral cancer metastasis.

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Declaration of Interest

The authors declare no conflict of interest.

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