Inhibitory effect of a mixture containing ascorbic acid, lysine, proline and green tea extract on critical parameters in angiogenesis

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Abstract. Degradation of extracellular matrix (ECM) is a hallmark of tumor invasion, metastasis and angiogenesis. Based on the Rath multitargeted approach to cancer using natural substances to control ECM stability and enhancing its strength, we developed a novel formulation (NM) of lysine, proline, ascorbic acid and green tea extract that has shown significant anti-cancer activity against a number of cancer cell lines. The aim of the present study was to determine whether NM exhibits anti-angiogenic and anti-metastatic effects using *in vitro* and *in vivo* experimental models. Angiogenesis was measured using a chorioallantoic membrane (CAM) assay in chick embryos and bFGF-induced vessel growth in C57BL/6J female mice. To determine the *in vivo* effect of NM on the tumor xenograft growth, male nude mice were inoculated with $3x10^6$ MNNG-HOS cells. Control mice were fed a mouse chow diet, while the test group was fed a mouse chow diet supplemented with 0.5% NM for 4 weeks. *In vitro* studies on cell proliferation (MTT assay), MMP expression (zymography) and Matrigel invasion were conducted on human osteosarcoma U2OS, maintained in McCoy medium, supplemented with 10% FBS, penicillin and streptomycin in 24-well tissue culture plates and tested with NM at 0, 10, 50, 100, 500, and 1000 µg/ml in triplicate at each dose. NM at 250 µg/ml caused a significant ($p<0.05$) reduction in bFGF-induced angiogenesis in CAM. NM inhibited tumor growth of osteosarcoma MNNG-HOS cell xenografts in nude mice by 53%; furthermore, tumors in NM-treated mice were less vascular and expressed lower levels of VEGF and MMP-9 immunohistochemically than tumors in the control group. In addition, NM exhibited a dose-dependent inhibition of osteosarcoma U2OS cell proliferation (up to 60% at 1000 µg/ml), MMP-2 and -9 expression (with virtual total inhibition at 500 µg/ml NM), and invasion through Matrigel (with total inhibition at 100 µg/ml NM). Moreover, NM decreased U2OS cell expression of VEGF, angiopoietin-2, bFGF, PDGF and TGFß-1. These results together with our earlier findings suggest that NM is a relatively non-toxic formulation, which inhibits growth, invasion, metastasis, and angiogenesis of tumor cells.

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Introduction

Angiogenesis is the formation of new capillaries from pre-existing blood vessels. It is considered to be a fundamental process in physiological and pathological conditions, and essential for solid tumor growth, invasion and metastasis. Angiogenesis not only allows the tumor to increase in size, but also provides a route for metastasis to distal sites in the body. The degree of vascularization in a tumor has been correlated with metastatic potential and prognosis of the disease.

Efforts have been made to identify anti-angiogenic agents as potential cancer treatments. An increasing number of clinical trials are testing the therapeutic efficacy and tolerance of angiogenic agents, targeting matrix metalloproteinases (MMPs), angiogenic growth factors, and their receptors (1).

Earlier work by Rath *et al* defined common pathomechanisms for all cancers, the destruction of ECM as a precondition for cancer cell invasion, growth and metastasis, and suggested intervention through natural inhibitors of plasmin-induced proteolysis, such as lysine and its analogues (2). Degradation of the ECM by migrating endothelial cells and their subsequent invasion of the underlying stroma of neighboring tissues, where they organize into new capillary structures, are also critical in angiogenesis.

Regulation of angiogenesis is achieved through a balance of pro- and anti-angiogenic stimuli. The two major pro-angiogenic factors are MMPs that degrade ECM, and vascular endothelial growth factor (VEGF), a stimulatory factor for cell migration. The prevention of ECM degradation through the inhibition of MMP activity, particularly MMP-2 (gelatinase A) and MMP-9 (gelatinase B), has been shown to be a promising therapeutic approach to blocking the invasion process that occurs during angiogenesis and tumor progression. VEGF, which is specific and critical for blood vessel formation, is one of the most powerful stimulators of angiogenesis.

Blood vessels local to the tumor respond to the malignant cell elaboration of VEGF and fibroblast growth factor (FGF), inducing local blood vessels to sprout branches to feed the metastases. This causes small micrometastases to grow beyond the 2-mm size, which is functionally dormant and becomes a threat to the patient when rapid growth causes local damage (3). VEGF is secreted by tumor cells and promotes the proliferation of endothelial cells by binding to cell surface receptors, and migration toward the tumor. Since endothelial cells can communicate directly with tumor cells by producing growth-promoting factors, the inter-relationship between endothelial and tumor cells and the imbalance between angiogenic factors and angiogenic inhibitors can promote tumor vascularization. The identification of novel angiogenic inhibitors that target both proliferating endothelial and tumor cells and MMP inhibitors may therefore lead to the therapeutic regulation of tumor growth.

Our previous work confirmed the direction described by Rath and Pauling (2) and resulted in identifying a novel formulation of lysine, proline, ascorbic acid and EGCG-enriched green tea extract (NM), which has shown significant anti-cancer activity against a large number of cancer cell lines, blocking cancer cell growth, tissue invasion and MMP activity both *in vitro* and *in vivo* (4-6). The aim of this study is to determine the effectiveness of this novel nutrient formulation as an inhibitor of angiogenesis using both *in vitro* and *in vivo* models.

Materials and methods

Effect of NM on surrogate models for angiogenesis

CAM study. The chick CAM angiogenesis assay was essentially performed as described by Brooks *et al* (7). Briefly, the CAMs of 10-day-old chick embryos were separated from the shell membrane. Filter discs previously coated with cortisone acetate were saturated with 15 µl of recombinant bFGF at a concentration of 1.0 µg/ml. The embryos were allowed to incubate for a total of 24 h. The embryos were next treated with a single I.V. injection of NM (250 or 500 µg/embryo) in a total volume of 100 µl. At the end of a 3-day incubation period, the embryos were sacrificed and CAMs were resected and washed. The number of branching angiogenic blood vessels was counted within the confines of the filter discs for each CAM of each experimental condition.

In vivo mouse Matrigel plug assay. The mouse Matrigel plug assay was performed as described by Passaniti *et al* (8). NM (5 mg/ml) and bFGF (400 ng/ml) in PBS were mixed with Matrigel in proportions not exceeding 1% total volume of Matrigel. A mixture of 0.5 ml Matrigel with bFGF and NM was injected s.c into four C57BL/6J female mice, and the mixture of 0.5 ml Matrigel with bFGF and vehicle was injected s.c. into another group of four C57BL/6J female mice. After 7 days, mice were sacrificed, the skin was excised, fixed and stained with H&E and the Masson-Trichrome method, and representative photographs were taken.

In vivo study with human osteosarcoma MNNG-HOS cells

Cancer cell line and culture. Human osteosarcoma cells MNNG-HOS obtained from ATCC (American Type Culture Collection; Rockville, MD) were maintained in MEM culture supplemented with 10% fetal bovine serum, 100 U/ml penicillin and 100 µg/ml streptomycin. The media and sera were obtained from ATCC, and antibiotics (penicillin and streptomycin) were from Gibco BRL (Long Island, NY). At near confluence, the cultured cells were detached by trypsinizing, washed with PBS, and diluted and emulsified to a concentration of $3x10^6$ cells in 0.2 ml PBS and 0.1 ml Matrigel (BD Bioscience, Bedford, MA) for inoculation.

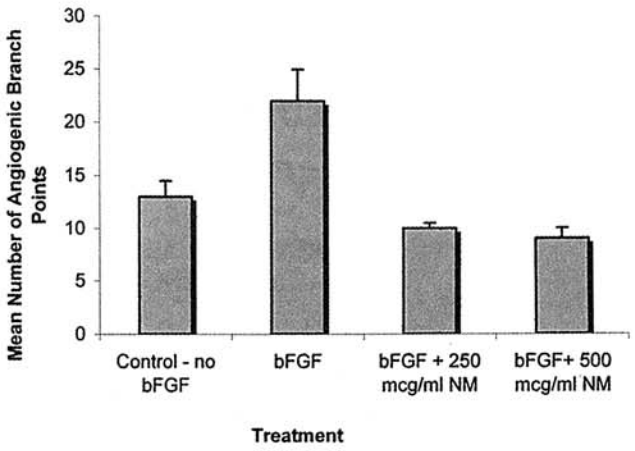

Figure 1. Effect of NM on bFGF-induced angiogenesis in chick CAM assay.

Growth of MNNG-HOS xenografts in nude mice. Male athymic nude mice (NCr-nu/nu), approximately 6 weeks of age on arrival, were purchased from Simonsen Laboratories (Gilroy, CA) and maintained in microinsulator cages under pathogen-free conditions on a 12-h light/12-h dark schedule for 1 week. All animals were cared for in accordance with institutional guidelines for the care and use of experimental animals. After 1 week of housing, the mice were inoculated with $3x10^6$ human osteosarcoma MNNG-HOS cells in 0.2 ml of PBS and 0.1 ml of Matrigel. After implantation, the 12 mice were randomly divided into two groups of 6 mice. From day 1, mice from group A were fed Purina mouse chow diet, and those in group B were fed Purina mouse chow diet supplemented with 0.5% NM. After 4 weeks, the mice were sacrificed, and tumors were excised, measured, weighed, fixed in 10% (v/v) buffered formalin and processed for histology.

Cytochemistry and immunohistochemistry of tumor tissue. Tissue samples were fixed in 10% buffered formalin. All tissues were embedded in paraffin and cut at 4-5 microns. Sections were deparaffinized through xylene and graduated alcohol series to water, and incubated for 5 min in aqueous 3% hydrogen peroxide to block endogenous peroxidase. Histological sections were stained with hematoxylin and eosin (H&E) for evaluation using a standard light microscope.

Immunohistochemical studies were performed on formalin-fixed, paraffin-embedded sections. Standard immunohisto-chemical staining procedures were used for staining antibodies. After deparaffinization and appropriate epitope retrieval, the sections were incubated with primary antibody. Detection

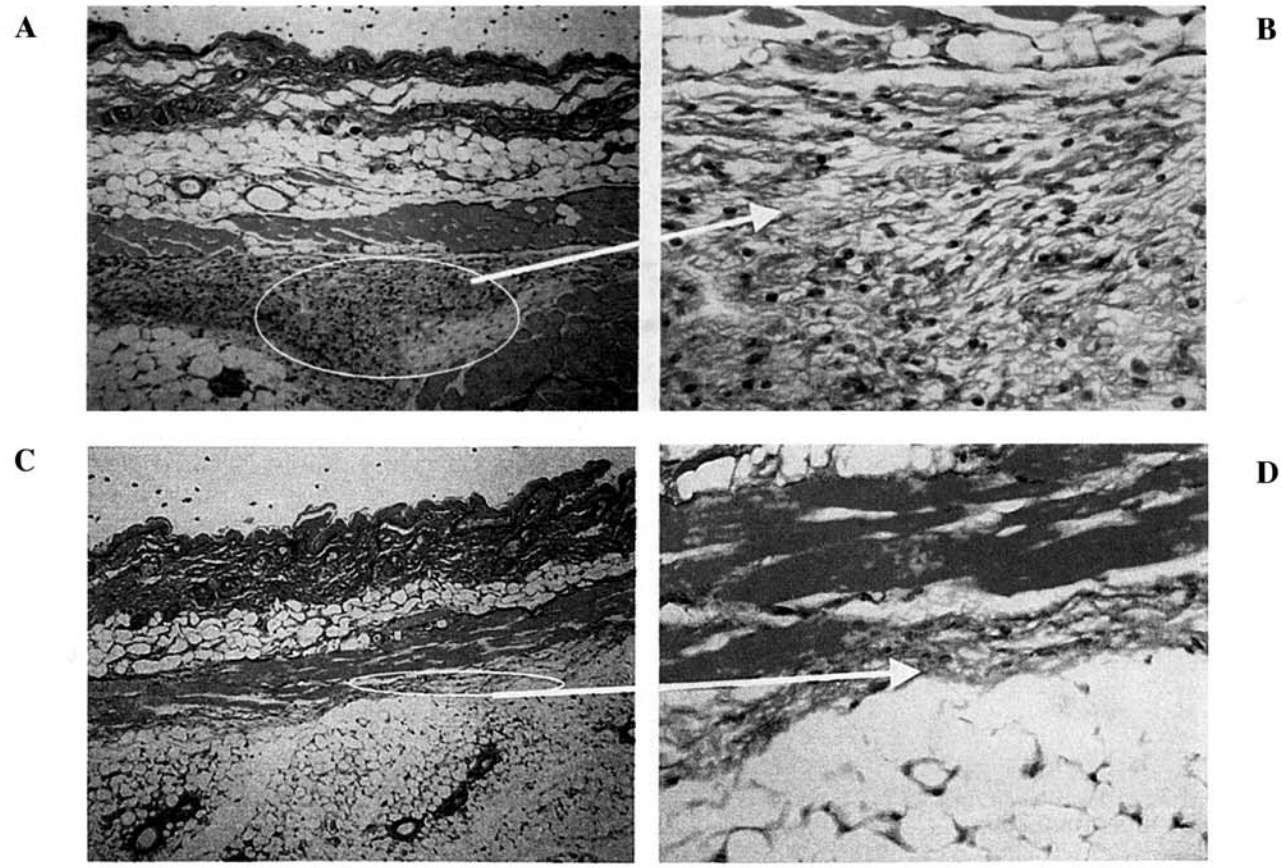

Figure 2. Effect of NM on bFGF-induced vessel growth in C57BL/6J female mice. (A) Control group tissue (original magnification, x200); (B) control group tissue (x400); (C) supplemented 0.5% NM (x200); and (D) supplemented 0.5% NM (x400).

was by biotinylated goat anti-mouse antibodies followed by streptavidin conjugated to horseradish peroxidase with the use of diaminobenzidine as the chromogen. Polyclonal rabbit anti-human antibodies used for MMP-9 and VEGF were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA) and Sigma.

In vitro studies with human osteosarcoma U20S cells

Cell culture. Human osteosarcoma cells U-2OS (American Type Culture Collection) were grown in McCoy medium supplemented with 10% fetal bovine serum, penicillin (100 U/ml) and streptomycin (100 mg/ml). Cells were incubated with 1 ml of media at 37˚C in a tissue culture incubator equilibrated with 95% air and 5% CO_2. At near confluence, the cells were treated with the nutrient mixture (NM) dissolved in media and tested at 0, 10, 50, 100, 500, and 1000 µg/ml in triplicate at each dose. A group of cells was also treated with PMA 200 ng/ml. The plates were then returned to the incubator, and cell proliferation was evaluated after 24 h following incubation with test reagents.

MTT assay. Nutrient effects on osteosarcoma U2OS cell proliferation were evaluated by MTT assay. The MTT assay, a colorimetric assay, is based on the ability of viable cells to reduce a soluble yellow tetrazolium salt [3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyl tetrazolium bromide] (MTT) to a blue formazan crystal by mitochondrial succinate dehydrogenase activity of viable cells. After the addition of MTT (0.5 mg/ml), the plates were covered and returned to the 37˚C incubator for 2 h, the optimal time for formazan product formation. Following incubation, the supernatant was carefully removed from wells, the formazan product was dissolved in 1 ml DMSO, and absorbance was measured at 570 nm in a Bio Spec 1601 Shimadzu spectrometer. The OD_{570} of the DMSO solution in each well was considered to be proportional to the number of cells. The OD_{570} of the control (treatment without supplement) was considered to be 100%.

Gelatinase zymography. MMP expression in conditioned media was determined by gelatinase zymography. Gelatinase zymography was performed in 10% polyacrylamide precast Novex gel (Invitrogen Corporation) in the presence of 0.1% gelatin. Culture media (20 µl) was loaded, and SDS-PAGE was performed with a tris-glycine SDS buffer. After electrophoresis, the gels were washed with 5% Triton X-100 for 30 min. The gels were then incubated for 24 h at 37˚C in the presence of 50 mM Tris-HCl, 5 mM $CaCl_2$ and 5 µM $ZnCl_2$, pH 7.5 and stained with Coomassie Blue R 0.5% for 30 min, then destained. Protein standards were run concurrently, and approximate molecular weights were determined.

Matrigel invasion studies. Invasion studies were conducted using Matrigel™ (Becton-Dickinson) inserts in 24-well plates. Suspended in medium, osteosarcoma U2OS cells were supplemented with nutrients, as specified in the design of the experiment, and seeded onto the insert in the well. Thus, both the medium on the insert and in the well contained the same supplements. The plates with inserts were then incubated in a

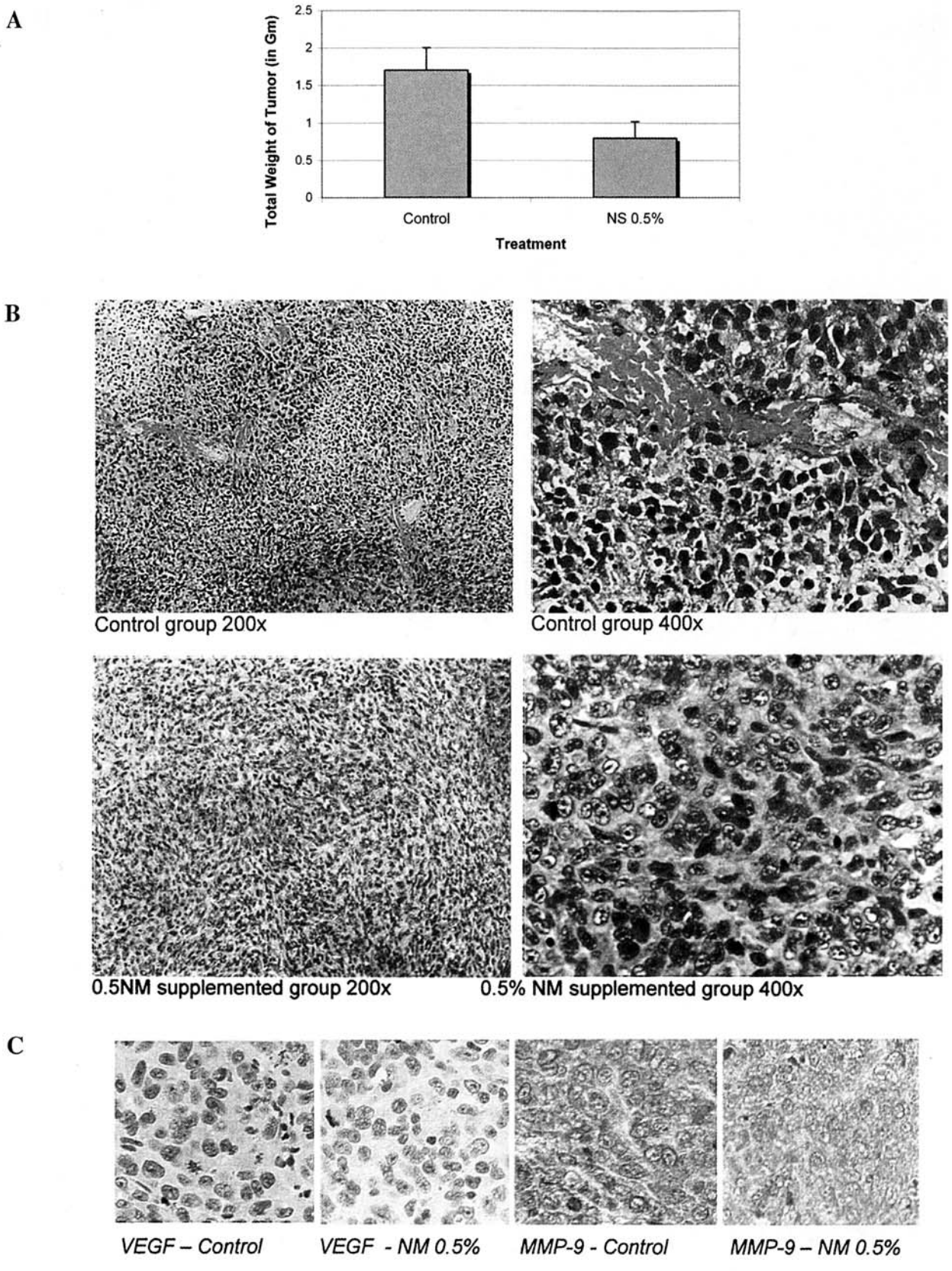

Figure 3. (A) Effect of NM on tumor growth in nude mice with osteosarcoma MNNG-HOS xenografts. (B) Angiogenesis in tumor tissue of nutrient-supplemented and control mice. (C) MNNG osteosarcoma immunohistochemistry.

culture incubator equilibrated with 95% air and 5% CO_2 for 24 h. After incubation, media from the wells were withdrawn. Cells on the upper surface of the inserts were gently scrubbed away with cotton swabs, and cells that had penetrated the Matrigel membrane and migrated onto the lower surface of the Matrigel were stained with H&E and visually counted under the microscope.

VEGF, IL-6, IL-8, FGF, TGFβ and angiopoietin assay. Conditioned media were collected after confluent cell culture

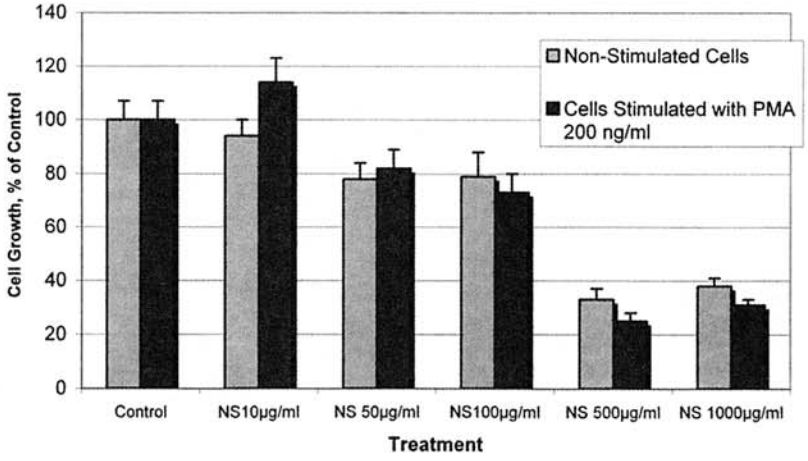

Figure 4. Effect of NM on osteosarcoma U2OS cell growth.

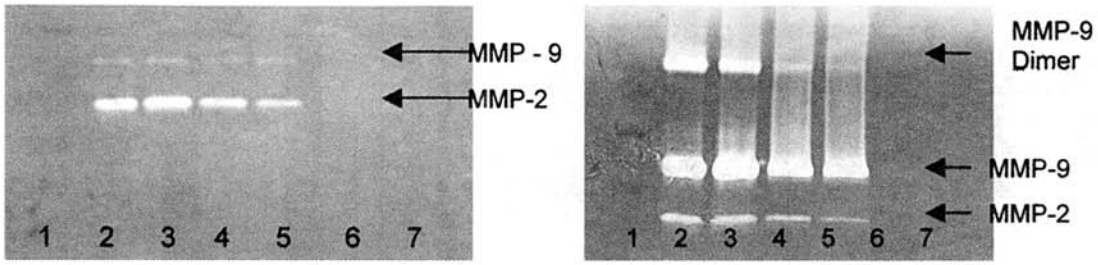

Figure 5. Effect of the nutrient mixture on MMP-2 and MMP-9 expression by human osteosarcoma U2OS cells. 1, Markers; 2, control; and 3-7, nutrient mixture 10, 50, 100, 500 and 1000 µg/ml, respectively. Left, Unstimulated U2OS cells; right, PMA (200 ng/ml)-stimulated U2OS cells.

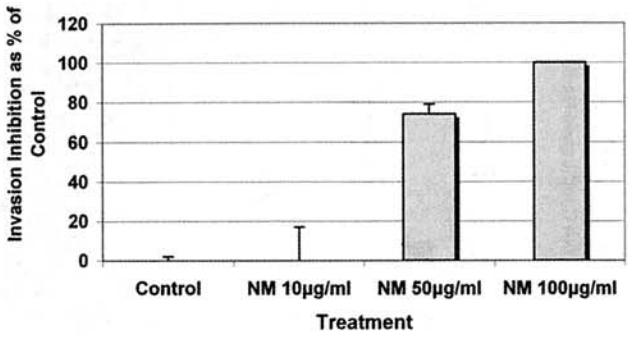

Figure 6. Effect of NM on Matrigel invasion and migration by osteosarcoma cells U2OS.

incubation for 24 h in serum-free medium with the indicated supplements. Triplicate samples were pooled, and the level of respective proteins was measured in duplicate using an immunoassay kit (BioSource International and Quantikine, R&D) according to the manufacturer's protocol. Values are expressed as the mean percentage of unstimulated control.

Composition of the nutrient formulation (NM). Stock solution of the nutrient mixture (total weight 4.2 Gm) is composed of the following: vitamin C (as ascorbic acid and Mg, Ca, and palmitate ascorbate) 700 mg; L-lysine 1000 mg; L-proline 750 mg; L-arginine 500 mg; N-acetyl cysteine 200 mg; standardized green tea extract (80% polyphenol) 1000 mg; selenium 30 mg; copper 2 mg; and manganese 1 mg.

Statistical analysis. The results were expressed as means ± SD for the groups, and data were analyzed by an independent sample *t*-test.

Results

Chicken CAM angiogenesis assay. The nutrient mixture caused a significant ($p<0.50$) reduction (from 22 to 10 blood vessel branch points within the confined region of the filter disc) in bFGF-induced angiogenesis compared to no treatment (bFGF only), as shown in Fig. 1. The number of blood vessel branch points is relative to the number of newly sprouting angiogenic vessels.

In vivo bFGF-induced vessel growth. To investigate the anti-angiogenic potential of NM, an extract of basement membrane proteins (Matrigel) impregnated with bFGF, an inducer of neovascularization, was injected subcutaneously (s.c.) into C57BL/6J female mice. Passaniti *et al* found that s.c. injection of Matrigel supplemented with angiogenic factors into C57BL/6J mice reconstituted into a gel supported an intense vascular response (8). The test group of mice received NM in the injection mixture, and control mice received only the vehicle. After 7 days, red blood cells were abundant within the lumen of numerous vessels in control mice (Fig. 2A and B). In contrast, NM strongly suppressed the bFGF-stimulated angiogenesis in supplemented mice (Fig. 2C and D).

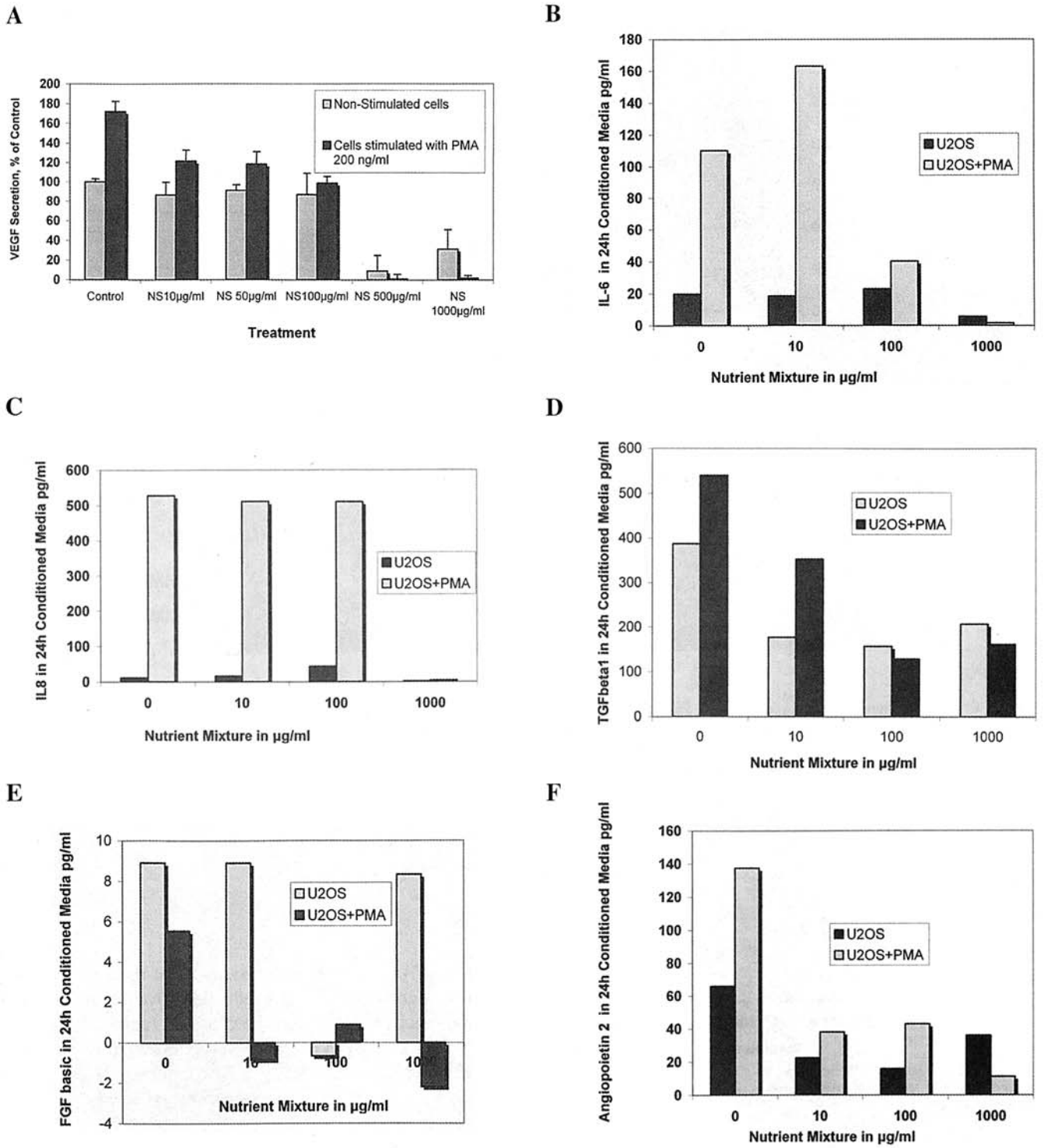

Figure 7. (A) Effect of the nutrient mixture (NM) on VEGF expression of osteosarcoma U2OS. (B) Effect of NM on IL-6 secretion by osteosarcoma U2OS cells. (C) Effect of the nutrient mixture on IL-8 secretion by osteosarcoma U2OS. (D) Effect of the nutrient mixture on TGF ß1 secretion by U2OS osteosarcoma cells. (E) Effect of nutrient mixture on FGF secretion by U2OS osteosarcoma cells. (F) Effect of nutrient mixture on angiopoietin-2 secretion by U2OS osteosarcoma cells.

Effect of NM on growth of osteosarcoma MNNG xenograft in nude mice. Results showed that the nutrient-supplemented nude mice developed significantly smaller tumors (by 53%, p=0.0001) and less vascular ones than did the control group of nude mice (Fig. 3A and B). Immunohistochemical analysis noted decreased VEGF staining, an indicator of angiogenesis, in the NM-treated group compared to the control group. MMP-9 expression was also lower in the NM-treated group compared to the corresponding control group (Fig. 3C).

In vitro osteosarcoma U20S studies

Effect of NM on proliferation. The nutrient mixture exhibited inhibition of proliferation with a maximum of 67% over the control at 500 µg/ml in unstimulated cells and 75% over control in PMA-stimulated cells, as shown in Fig. 4. Results were statistically significant at 500 µg/ml (p=0.006).

Effect of NM on MMP expression using gelatinase zymography. As shown in Fig. 5, zymography demonstrated expression

of MMP-2 and MMP-9 by human osteosarcoma U2OS cells with significantly increased MMP-9 expression in PMA-treated (200 ng/ml) osteosarcoma cells. NM inhibited the expression of both MMPs in a dose-dependent fashion with a virtual total inhibition at 500 μg/ml.

Effect of NM on Matrigel invasion. Invasion of osteosarcoma cells U20S through Matrigel was reduced by 74% at 50 μg/ml and totally inhibited at 100 μg/ml (p=0.003) (Fig. 6).

Effect of NM on VEGF, IL-6, IL-8, FGF, TGFβ and angiopoietin expression. VEGF expression of PMA-treated U2OS cells was increased by 172% over the control. NM significantly (p=0.002) suppressed VEGF expression of both PMA-stimulated (by 172% at 500 μg/ml NM) and untreated osteosarcoma cells (by 91% at 500 μg/ml) (Fig. 7A). PMA increased interleukin-6 (IL-6) expression in the absence of NM by 560%; NM 1000 μg/ml inhibited IL-6 expression by 71% in untreated osteosarcoma cells and 98% in PMA-treated cells (Fig. 7B). Interleukin-8 (IL-8) expression was increased 48-fold by PMA in the absence of NM; NM inhibited IL-8 expression by 89% in untreated osteosarcoma cells and 99% in PMA-treated cells (Fig. 7C). PMA increased TGF-ß expression in the absence of NM by 140%; NM inhibited TGF-ß expression by 47% in untreated osteosarcoma cells and 70% in PMA-treated cells (Fig. 7D). PMA increased FGF expression in the absence of NM by 158%; NM inhibited FGF expression by 71% in untreated osteosarcoma cells and 98% in PMA-treated cells (Fig. 7E). PMA increased angiopoietin-2 expression in the absence of NM by 208%; NM inhibited angiopoietin-2 expression by 45% in untreated osteosarcoma cells and 92% in PMA-treated cells (Fig. 7F).

Discussion

The presence of an adequate blood supply is required for the growth and metastasis of malignant tumors; thus, inhibition of tumor-induced angiogenesis represents a promising approach to cancer therapy. A number of *in vivo* and *in vitro* models have been developed to facilitate the study of angiogenesis. Using various *in vivo* and *in vitro* models, we demonstrated that the nutrient mixture of lysine, proline, ascorbic acid and green tea extract had anti-angiogenic properties.

Results presented in this study further confirm the effectiveness of the Rath approach (2) and provide further evidence of a significant cancer-protecting effect of this specific nutrient mixture in nude mice. Nude mice on a nutrient-supplemented diet with implants of the highly metastatic human osteosarcoma MNNG-HOS cells developed significantly smaller tumors (by 53%, p=0.0001) and less vascular ones than the tumors grown in the control group of nude mice. Furthermore, immunohistochemical analysis of these tumors demonstrated lower VEGF staining, an indicator of angiogenesis, in nutrient-supplemented animals compared to the control group of mice. This was accompanied by lower cytoplasmic staining for MMP-9 in tumor tissues taken from nutrient-supplemented mice than those fed the standard diet, indicating decreased matrix degradation. The results correlated with *in vitro* data obtained with this nutrient mixture in osteosarcoma U2OS cells, showing their significant inhibitory effect on cell proliferation, MMP expression and Matrigel invasion.

Angiogenesis induces a release of various angiogenic factors, among them bFGF. We applied the chick embryo chorioallantoic membrane (CAM) assay to test the effects of NM in this comprehensive *in vitro* system in tissue that incorporates all angiogenic processes in one mode. This assay utilizes a microenvironment in which angiogenesis naturally occurs and provides a good model for evaluation of systemically administered antagonists. In addition, it allows the selection of angiogenic inhibitors that interfere with new blood vessel development without affecting pre-existing vessels. Our results demonstrate that this nutrient mixture caused a significant ($p<0.50$) reduction of blood vessel branch points (from 22 to 10 within the confined region of the filter disc) in bFGF-induced angiogenesis compared to no nutrient treatment (bFGF only). The number of blood vessel branch points relates to the number of newly sprouting angiogenic vessels. These findings were confirmed by our *in vivo* study, which showed that NM, included as a component of diet, strongly suppressed bFGF-induced angiogenesis in C57BL/6J female mice.

In addition to these results, NM was also found to inhibit the secretion of pro-angiogenic factors VEGF, IL-6, IL-8, FGF, TGF-ß, and angiopoietin-2 by osteosarcoma U2OS. These factors, which have been identified in various settings of physiologic and pathologic angiogenesis, directly or indirectly affect the endothelium by the activation of surrounding cells to produce other factors with pro-angiogenic activity or the modulation of receptor(s) activities (9).

One of the most promising approaches to cancer is through simultaneous targeting of universal pathomechanisms involved in cancer growth and invasion. Curbing cancer invasiveness can be achieved by the encapsulation of tumors through a decrease in matrix degradation accompanied by optimized ECM structure and its integrity. Degradation of basement membranes by MMPs is key to the invasive potential of cancer cells. Research has shown that highly metastatic cancer cells secrete higher amounts of MMPs than poorly metastatic cells, demonstrating that the invasive and metastatic abilities of these cancer cells *in vitro* and *in vivo* correlates with MMP-9 expression (10). Controlling proteolytic activity of the ECM provides an opportunity to address common mechanisms of metastasis, angiogenesis and tumor growth. This direction, pointed out by Rath and Pauling, suggests targeting plasmin-mediated mechanisms with the use of nutritional components, such as lysine and lysine analogues (2). Lysine interferes with the activation of plasminogen into plasmin by tissue plasminogen activator (tPA) by binding to plasminogen active sites, consequently affecting the plasmin-induced MMP activation cascade (2). Rath and Pauling proposed the use of lysine and its analogues in controlling the spread of cancer and other pathological conditions, resulting in excessive ECM degradation (2). Another study demonstrated a 7-fold reduction in metastasis of transgenic mammary cancer in plasmin-deficient mice (11). Furthermore, expression of urokinase-type plasminogen activator inhibitor type I by human prostate carcinoma cells was shown to inhibit angiogenesis and metastasis to the lung and liver in an athymic mouse model (12). Lysine-mediated effects on

the ECM include increased connective tissue strength and stability. It is well known that the synthesis and structure of collagen fibrils depends upon hydroxylation of proline and lysine residues in collagen fibers, catalyzed by ascorbic acid. Lysine importance in collagen structure and synthesis is attributed to it being the most abundant amino acid in collagen. Both ascorbic acid and lysine are not produced in the human body; therefore sub-optimal levels of these nutrients are possible in various pathological stages and through deficient diets.

The inhibitory effects of individual nutrients composing the nutrient mixture have been reported in both clinical and experimental studies. Ascorbic acid has been reported to have cytotoxic, anti-metastatic and anti-angiogenic actions on malignant cell lines (13-16); in addition, low levels of ascorbic acid have been reported in cancer patients (17-19). Green tea extract is a potent anti-cancer agent that has been reported to have anti-tumorigenic and anti-angiogenic effects against human cancer cell lines (20-25). However, individual nutrients are not as powerful as nutrient synergy. Our previous studies demonstrated that the synergistic anti-cancer effect of ascorbic acid, proline, lysine and EGCG on several cancer cell lines in tissue culture studies was greater than that of individual nutrients (26).

Because of the growing number of agents tested for anti-angiogenic properties, the U.S. National Cancer Institute (NCI) uses a classification system that categorizes anti-angiogenic drugs into five groups: 1) agents that inhibit endothelial cells directly; 2) agents that block activators of angiogenesis; 3) agents that block ECM breakdown; 4) agents that inhibit endothelial-specific integrin signaling; and 5) agents with non-specific mechanisms of action. Many MMP inhibitors are also anti-angiogenic agents. Unfortunately, many compounds have limited efficacy due to problems of delivery and penetration and moderate effects on the tumor cells, accompanied by severe toxicity and damage to healthy tissues. In addition, the activity of these compounds is primarily limited by the development of drug resistance (27).

Tumor cells are rapidly changing because of their genetic instability, heterogeneity and high rate of mutation. We postulate that cancer development and progression can be controlled only through a multi-targeted approach in contrast to the application of selective agents for a highly specific metabolic target. The results of this study and our previous studies indicate that such multifaceted approaches targeting mechanisms common to all types of cancer cells can be achieved through nutrient synergy. We demonstrated the inhibitory effects of this nutrient synergy on cancer cell invasion, proliferation and angiogenesis executed through various mechanisms. We have shown that this nutrient mixture affects MMPs, can induce apoptosis, and modulate the effects of various growth factors.

While clinical studies are necessary to better determine the efficacy of nutrient therapy in both cancer prevention and treatment, the results of these studies suggest that the formulation of green tea extract, lysine, proline, and ascorbic acid is an excellent candidate for adjunctive therapeutic use in the treatment of metastatic cancer by inhibiting MMP expression and invasion, and angiogenesis without cytotoxic effects.

Acknowledgements

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