



Inhibition of Human Neuroblastoma Cell Line SK-N-MC *In Vivo* and *In Vitro* By a Novel Nutrient Mixture

M.W.Roomi, V. Ivanov, A. Niedzwiecki, M. Rath

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Abstract

Background:

Neuroblastoma, a peripheral nervous system cancer that can be highly invasive and metastatic, accounts for 8-10% of all solid childhood tumors in children under the age of 15 years. Currently there is no effective treatment for this disease. Enzymes called matrix metalloproteinases (MMPs) which degrade a wide range of connective tissue components surrounding cells are important in facilitating cancer cells invasion and metastasis to other organs. A nutrient mixture (NM) containing lysine, proline, ascorbic acid and green tea extract has shown significant antitumor effects, including inhibition of MMP secretion, on many cancer cell lines. In this work we used the nude mouse xenograft model to investigate anti-cancer efficacy of NM *in vivo*. In addition, we tested the effect of NM *in vitro* for its effects on cancer cells viability, MMP secretion, and invasion.

Methods:

Neuroblastoma cell growth was evaluated by MTT assay and their invasion through collagen matrix by a Matrigel assay. MMP secretion under normal and PMA stimulation (induces MMP9) was evaluated by gelatinase zymography at 0, 10, 50, 100, 500 and 1000 mg/ml NM concentrations. *In vivo* effects of NM on tumor growth were tested in athymic nude mice 5-6 weeks of age, which were inoculated with 3×10^6 neuroblastoma cells (SK-N-MC, ATCC) subcutaneously and randomly divided into two groups; group A was fed a regular diet and group B a regular diet supplemented with 0.5% NM. The tumor growth evaluation and histology was conducted after four weeks.

Results:

NM inhibited the growth of tumors in nude mice by 30%. *In vitro*, NM decreased viability of neuroblastoma cancer cells by 25% and 35% at 500 and 1000 mg/ml. Zymography showed a faint band corresponding to MMP-2 secretion, and PMA-induced MMP-9 secretion. NM inhibited the secretion of both MMP-2 and MMP-9 with their

total block at 100 mg/ml concentration. Interestingly, SK-N-MC cells were not invasive through Matrigel.

Conclusions:

These results suggest that NM has therapeutic potential in treatment of neuroblastoma.

Comment: This study shows that specific nutrients combined in a synergy have a potential to curb neuroblastoma tumor growth in vivo by about 30%. In addition, by inhibiting secretion of MMPs, the enzymes essential for degradation of collagen and connective tissue surrounding all cancer cells, they can curb cancer spread in the body. Prevention of ECM degradation by inhibition of MMP activity has been shown to be a promising therapeutic target in cancer. Our unique nutrient mixture (NM) containing lysine, proline, ascorbic acid and green tea extract inhibited the secretion of both MMP-2 and MMP-9 with their total blockage at NM concentration of 100 mg/ml. These results are significant as they demonstrate therapeutic potential in treatment of neuroblastoma by safe and effective micronutrient synergy.

