#2261 Antitumor and anti-inflammatory effect of a novel nutrient mixture on human lymphoma cell line U-937

M.W. Roomi, N.W. Roomi, M. Rath and A. Niedzwiecki
Dr. Rath Research Institute, Oncology Division, Santa Clara, CA 95050

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1. Introduction:

Phytochemicals and dietary antioxidants are known to decrease the risk of inflammation and prevent cancer development. We have developed strategies to inhibit cancer progression and its spread by natural products. A nutrient mixture (NM) containing ascorbic acid, lysine, proline and green tea extract has exhibited anticancer activity in vitro and in vivo in a number of cancer cell lines.

2. Objective:

We investigated the effect of NM on human lymphoma cell line U-937 in vitro by measuring: cell proliferation, MMP expression, invasion, apoptosis, and Cox-2 and Cox-1 protein expression.

3. Materials and Methods:

Human lymphoma cell line U-937 (ATCC) was cultured in RPMI medium supplemented with fetal bovine serum and antibiotics. U-937 cells were seeded on 24-well tissue culture plates with 40nM phorbol 12-myristate 13-acetate (PMA). After 24 hrs, the cells were treated with NM at 0, 50, 100, 250, 500 and 1000 µg/ml, in triplicate at each dose. Cell proliferation was evaluated by MTT assay, MMP expression by gelatinase zymography, invasion through Matrigel, apoptosis by using live green caspase detection kit (Molecular Probe), and COX-2 and COX-1 expression by Western blot.

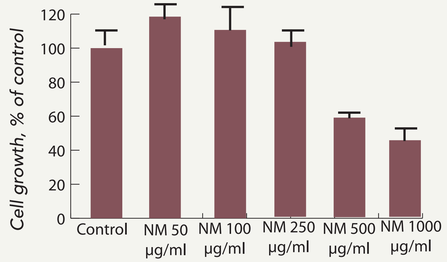
Composition of the Nutrient Mixture (NM)

Nutrient	Proportion
Vitamin C (as ascorbic acid and as Mg, Ca and palmitate ascorbate)	710 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 µg
Copper	2 mg
Manganese	1 mg

4. Results:

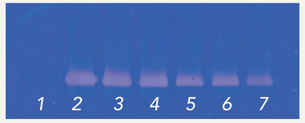
1. NM was not toxic to U-937 cells at a concentration of 250 µg/ml and exhibited an antiproliferative effect at 500 µg/ml concentration, as shown in Figure 1.

Figure 1 - Effect of NM on growth of U937 cells



2. Zymography demonstrated only one band corresponding to MMP-9, which was inhibited by NM in a dose-dependent manner, as shown in Figure 2.

Figure 2- Effect of NM on expression of MMP-2 and -9 by PMA (100 ng/ml) -treated U937 cells



Legend: 1 -Markers, 2- Control, 3-7 NM 50, 100, 250, 500, 1000 µg/ml+ PMA

3. Matrigel invasion was significantly reduced (by 95%) at 250 µg/ml NM and completely blocked at 500 µg/ml NM. See Figures 3 and 4.

Figure 3- Effect of NM on Matrigel invasion of U937 cells

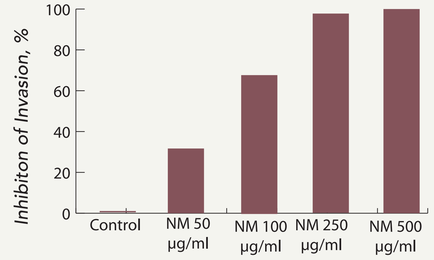
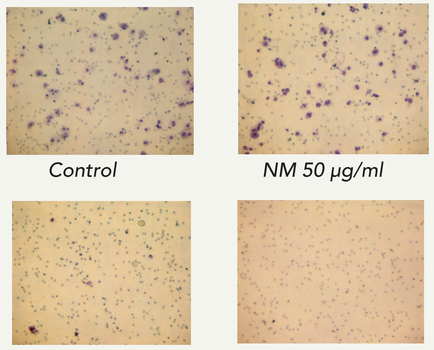


Figure 4- Photomicrographs of U937 Matrigel invasion



4. NM induced slight apoptosis at 100 µg/ml and moderate at 500 and 1000 µg/ml concentration. See Figures 5 and 6.

Figure 5- Effect of NM on apoptosis of U937 cells: quantitative analysis

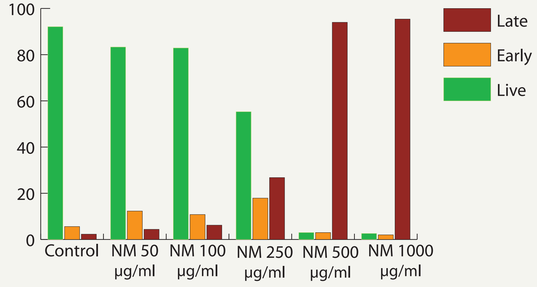
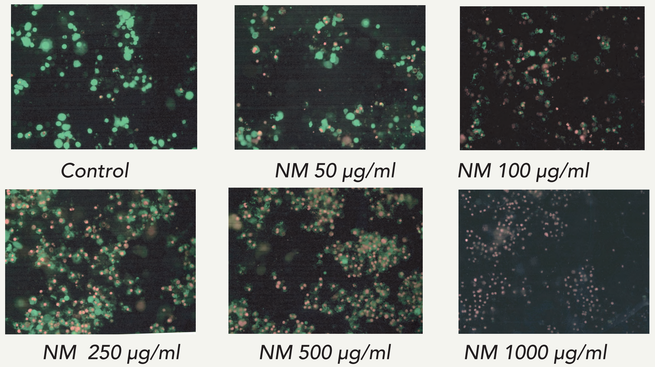
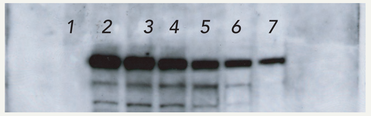


Figure 6- Photomicrographs of apoptosis U937



5. NM inhibited Cox-2 expression in a dose-dependent fashion and had no effect on Cox-1 expression. See Figure 7 for Cox-2; Cox-1 figure not shown.

Figure 7- Effect of NM on Cox-2 expression of U937 cells



Legend: 1 -Markers, 2- Control, 3-7 NM 50, 100, 250, 500, 1000 µg/ml

5. Conclusion:

Our results suggest that NM is an excellent candidate for therapeutic use in the treatment of hematological malignancies.