#2260 Modulation of P-glycoprotein expression by a novel nutrient mixture in multidrug resistant human uterine sarcoma cell line MES-SA/Dx_5 but not in drug sensitive MES-SA cell line

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:

We have characterized a nutrient mixture containing lysine, proline, ascorbic acid and green tea extract as a novel antineoplastic agent with a broad spectrum of antitumor activity against a number of cancer cell lines.

2. Objective:

We investigated the effect of NM on modulation of P-glycoprotein (Pgp) in the drug-resistant human uterine sarcoma cell line MES-SA/Dx_5 and compared it with the effect on drug-sensitive cell line MES-SA. In addition we also studied the effect of NM on MMP expression and Rhodamine-123 accumulation and efflux.

3. Materials and Methods:

Human drug insensitive uterine sarcoma cell line MES-SA/Dx_5 and drug sensitive cell line MES-SA (ATCC) were grown in RPMI 1640 medium, supplemented with fetal bovine serum and antibiotics. At near confluence, the cells were tested with NM at 0, 50, 100, 250, 500 and 1000 µg/ml, in triplicate at each dose. Cell proliferation was evaluated by MTT assay, MMPs by gelatinase zymography, and Pgp expression by Western blot and immunodetection using FITC-conjugated antibody and rhodamine-123 (Rh-123) accumulation and efflux assays.

Composition of the Nutrient Mixture

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. In MES-SA cell line, NM showed dose-response toxicity of 40% at 50 and 70% at 1000 µg/ml, whereas NM exhibited antiproliferative effect on MES-SA/Dx_5 by 20% at 50 and 100 µg/ml and by 40% at 250, 500 and 1000 µg/ml, as shown in Figures 1A-B.

Figure 1A - Effect of NM on growth of MES-SA cells: MTT 24h

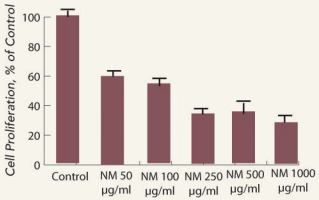
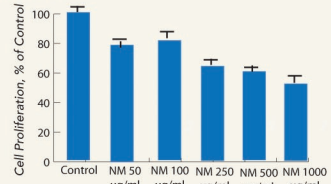


Figure 1B - Effect of NM on growth of MES-SA/Dx_5 cells: MTT 24h



2. In both cell lines, zymography demonstrated a band corresponding to MMP-2 in normal cells and MMP-9 with PMA treatment. Both MMPs showed dose-response inhibition by NM. See Figures 2A-B for MES-SA/Dx_5 MMP expression; MES-SA MMP expression not shown.

Figure 2A- Effect of NM on expression of MMP-2 and -9 by untreated MES-SA/Dx_5 cells

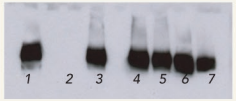
Legend: 1 -Markers, 2- Control, 3-7 NM 50, 100, 250, 500, 1000 µg/ml

Figure 2B- Effect of NM on expression of MMP-2 and -9 by PMA (100 ng/ml) -treated MES-SA/Dx_5 cells

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

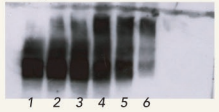
3. NM treatment also showed diminished dose-dependent Pgp expression by MES-SA/Dx_5 cell line by Western blot and by immunodetection, whereas MES-SA did not exhibit Pgp by Western blot or by immunostaining, as shown in Figures 3A-D.

Figure 3A - Pgp expression of MES-SA and MES-SA/Dx_5 cells: Western Blot



Legend: 1 - Pgp standard from Santa Cruz 2- MES-SA 10 µg/ml 3-Pgp standard from Santa Cruz, 4-7 MES-SA/Dx_5 10, 7.5, 5, 2.5

Figure 3B - Effect of NM on Pgp expression of MES-SA/Dx_5 cells: Westerm Blot



Legend: 1 -6 NM 0, 50, 100, 250, 500, 1000 µg/ml

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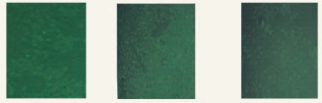
Figure 3C- Effect of NM on Pgp expression of MES-SA cells: Immunostaining



Control NM 100 µg/ml NM 500 µg/ml

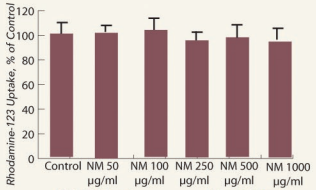
Figure 3D- Effect of NM on Pgp expression of MES-SA/Dx_5 cells: Immunostaining

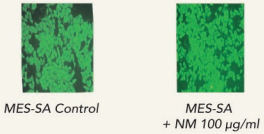
Control NM 50 µg/ml NM 100 µg/ml

NM 250 µg/ml NM 500 µg/ml NM 1000 µg/ml

4. NM enhanced the accumulation (Figures 4A-B) and efflux (Figures 4C-D) of Pgp substrate Rh-123 in MES-SA/Dx_5 uterine sarcoma cell line but not in the drug-sensitive cell line MES-SA.

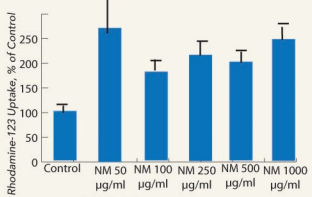
Figure 4A - Effect of NM on rhodamine-123 uptake in MES-SA cells

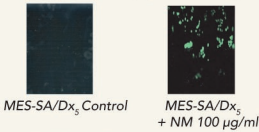




MES-SA Control MES-SA + NM 100 µg/ml

Figure 4B - Effect of NM on rhodamine-123 uptake in MES-SA/Dx_5 cells





MES-SA/Dx_5 Control MES-SA/Dx_5 + NM 100 µg/ml

Figure 4C - Effect of NM on rhodamine-123 residue in MES-SA cells

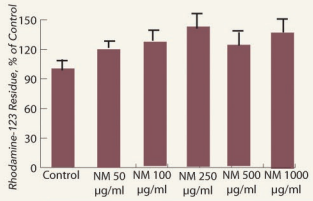
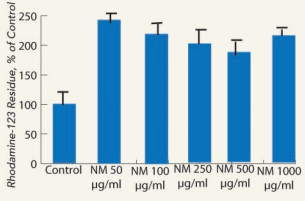


Figure 4D - Effect of NM on rhodamine-123 residue in MES-SA/Dx_5 cells



5. Conclusion: In summary, this study demonstrated that Pgp is modulated by NM, which may be an attractive potential agent for therapeutic use in cancer treatment.