



In Vitro Inhibition of MMPS, Invasion and Growth of Human Fanca and Fancc Lymphoblasts by a Unique Nutrient Mixture

M.W.Roomi, V. Ivanov, A. Niedzwiecki, M. Rath
Dr. Rath Research Institute, Oncology, 1260 Memorex Drive, Santa Clara, CA
95050

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Abstract

Introduction:

Fanconi anemia (FA) is a rare genetic disorder characterized by progressive anemia, birth defects, chromosome fragility and high propensity to development of cancer. Aplastic anemia and head and neck squamous cell carcinomas are the major causes of mortality and morbidity in FA patients. Matrix metalloproteinases (MMPs) have received much attention in recent years for their role in various malignancies, and have been implicated in tumor invasion and metastasis. Biological agents that prevent extracellular matrix (ECM) degradation by MMPs have been shown to be promising therapeutic approaches to cancer. A nutrient mixture (NM) containing ascorbic acid, lysine, proline and green tea extract showed significant anticancer activity against a number of cancer cell lines.

Objective:

We investigated the effect of NM on human FANCA and FANCC lymphoblasts for viability, MMP secretion and invasion.

Methods:

Human FANCA lymphoblasts GM13022 and HCS536 were cultured in RPMI supplemented with 15% FBS and antibiotics. The cells were then challenged with NM at 0, 10, 50, 100, 500 and 1000 µg/ml concentration in triplicate at each concentration. Cell proliferation was assessed by counting cells stained with Trypan blue, invasion was evaluated through Matrigel and MMP activity by gelatinase zymography. Cells were also treated with PMA to induce MMP-9 activity.

Results:

NM exhibited 20% inhibition of HCS536 lymphoblast growth compared to the control at 10 µg/ml, and 40% at 50-1000 µg/ml concentrations. However, NM was not toxic to GM13022 lymphoblast even at the highest concentration. Invasion through Matrigel was inhibited in HCS536 at 100 and 500 µg/ml by 27% and 93%. In GM13022, NM had little effect at 50 and 100 µg/ml but at 500 µg/ml

NM completely blocked invasion. GM13022 lymphoblasts exhibited only MMP-9 secretion, which was enhanced by PMA. NM inhibited MMP-9 secretion at 500 µg/ml less than at 1000 µg/ml concentration. Interesting HCS536 lymphoblasts did not demonstrate MMP activity even with PMA stimulation.

Conclusions:

The nutrient mixture inhibited MMP secretion and Matrigel invasion in FANCA, and invasion and proliferation in FANCC lymphoblasts, suggesting NM has a potential therapeutic use in the treatment strategy in FA neoplasia.

Comment:

Fanconi anemia (FA) is a rare genetic disorder with high propensity to development of cancer. Biological agents that prevent extracellular matrix degradation by MMPs have been shown to be promising therapeutic approaches to cancer. A micronutrient mixture (NM) containing ascorbic acid, lysine, proline and green tea extract has shown significant anticancer activity against a number of cancer cell lines. We investigated the effect of NM on human FANCA and FANCC lymphoblasts for viability, MMP secretion and invasion. NM inhibited MMP secretion and Matrigel invasion in FANCA, and invasion and proliferation in FANCC lymphoblasts, suggesting NM has a potential therapeutic use in the treatment strategy in FA neoplasia.

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Dr. Rath Research Institute, Oncology, Santa Clara, CA

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Objective:
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Methods:
1. Human FANCA lymphoblasts GM13022 and FANCC lymphoblasts HCS536 were cultured in RPMI supplemented with 15% FBS and antibiotics.
2. The cells were then challenged with NM at 0, 10, 50, 100, 500, and 1000 µg/ml concentration in triplicate at each concentration.
3. Cell proliferation was assessed by counting cells stained with Trypan blue, invasion was evaluated through Matrigel and MMP activity by gelatinose zymography. Cells were also treated with PMA to induce MMP-9 activity.

Results:
1. The nutrient mixture (NM) exhibited 20% inhibition of HCS536 lymphoblast growth compared to the control at 10 µg/ml, and 40% at 50-1000 µg/ml concentration (Figure 1). However, NM was not toxic to GM13022 lymphoblasts, even at the highest concentration (Figure 2).
2. GM13022 lymphoblasts exhibited only MMP-9 secretion, which was enhanced by PMA (Figure 3). NM inhibited MMP-9 secretion at 500 µg/ml less than at 1000 µg/ml concentration. HCS536 lymphoblasts did not demonstrate MMP activity even with PMA stimulation.
3. Invasion through Matrigel was inhibited in HCS536 at 100 and 500 µg/ml by 27% and 93% (Figure 4). In GM13022, NM had little effect at 50 and 100 µg/ml, but at 500 µg/ml NM completely blocked invasion (Figure 5).

Composition of Nutrient Mixture (NM):

Nutrient	Per Stock Solution
Ascorbic C, as ascorbic acid and in Na ₂ C ₂ O ₄ and ascorbate 100 mg	100 mg
L-lysine	1000 mg
L-proline	100 mg
L-leucine	500 mg
16-Amyl Cyclamate	200 mg
Standardized Green Tea Extract 80% polyphenols	1000 mg
Selenium	50 µg
Copper	2 mg
Manganese	1 mg

Figure 1 - Effect of NM on HCS536 Lymphoblast Growth
Significance of p = 0.01

Concentration (µg/ml)	Cell Proliferation (% of Control)
Control	100
NM 10	~75
NM 50	~55
NM 100	~45
NM 500	~55

Figure 2 - Effect of NM on GM13022 Lymphoblast Growth

Concentration (µg/ml)	Cell Proliferation (% of Control)
Control	100
NM 10	~100
NM 50	~100
NM 100	~100
NM 500	~100

Figure 3 - Effect of NM on GM13022 Lymphoblast MMP Secretion
3A - Untreated cells 3B - PMA treated cells

Figure 4 - Effect of NM on HCS536 Lymphoblast Matrigel Invasion
Significance of p = 0.0001

Concentration (µg/ml)	Inhibition of Invasion (%)
Control	0
NM 50	~10
NM 100	~27
NM 500	~93

Figure 5 - Effect of NM on GM13022 Lymphoblast Matrigel Invasion
Significance of p = 0.0001

Concentration (µg/ml)	Inhibition of Invasion (%)
Control	0
NM 50	~5
NM 100	~5
NM 500	~100

Conclusion:
The nutrient mixture inhibited MMP secretion and Matrigel invasion in FANCA, and invasion and proliferation in FANCC lymphoblasts, suggesting NM has a potential therapeutic use in the treatment strategy in FA neoplasia.

Legend: 1 - Markers, 2 - Control, 3-7 NM 10, 50, 100, 500, 1000 µg/ml