#5573 - Chemopreventive Effect of a Novel Nutrient Mixture on Lung Tumorigenesis Induced by Urethane in Male A/J Mice.

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Introduction:

Incidence of lung cancer, the second leading cause of cancer death in both men and women in the USA, is decreasing slightly in men, but rising sharply in women. Perhaps the greatest risk factor for developing lung cancer is exposure to inhalation carcinogens, most commonly those found in tobacco smoke. Other risk factors include asbestos and radon exposure. Despite improvements in therapy, the cure rate for lung cancer remains low. In general, surgery, radiation and chemotherapy are used in the management of lung cancer. But these treatments have adverse and toxic effects. We have developed strategies to inhibit cancer development and its spread using naturally occurring nutrients such as lysine, proline, ascorbic acid and green tea extract (NM). Such a unique formulation was shown to exhibit synergistic anticancer activity *in vivo* and *in vitro* in a number of cancer cell lines, inhibiting cancer cell growth, MMP secretion, invasion, metastasis and angiogenesis.

Objective:

The present study examines the *in vivo* effect of NM on the development of urethane-induced lung tumors in male A/J mice

Methods:

1. A/J male mice (n=25), six weeks old, were either given a single intraperitoneal injection with urethane (1 mg/g body weight) in saline (n=20) or an injection of vehicle only (n=5).
2. After two weeks the urethane-injected mice were divided into two groups. The Control group was fed a regular diet, while the NM group was fed the same diet supplemented with 0.5% NM. The Negative Control group was fed the regular diet.
3. After 20 weeks, mice were sacrificed, the lungs were excised and weighed, and tumors were counted and processed for histology.

Composition of the Nutrient Mixture (NM)

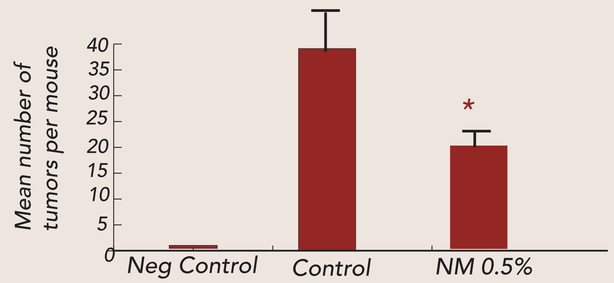
Nutrient	Proportion
Vitamin C (as ascorbic acid and as Mg, Ca and palmitate ascorbate)	710 mg
L-Lysine	1000 mg
L-Proline	750 mg
L-Arginine	500 mg
N-Acetyl Cysteine	200 mg
Standardized Green Tea Extract (80% polyphenol)	1000 mg
Selenium	30 µg
Copper	2 mg
Manganese	1 mg

Results:

1. Mice in both urethane-injected groups developed tumors; however the mice supplemented with 0.5% NM demonstrated significantly reduced mean number of tumors (by 49%, $p<0.0001$) than the control group of mice, as shown in Figures 1and 2. Mice injected with the vehicle alone did not develop tumors.

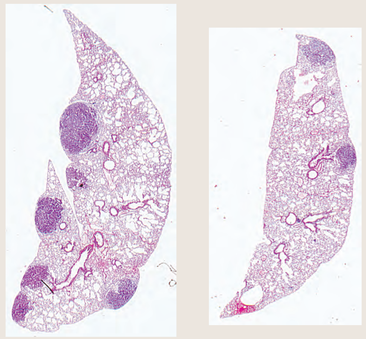
Figure 1 - Representative lung specimens of urethane-treated AJ mice

Figure 2 - Effect of supplementation with NM 0.5% on number of tumors per mouse



2. Pulmonary lesions were morphologically similar for both groups, but lesions were smaller in the NM-supplemented group. Histological examination revealed adenomas were characterized by well-differentiated cuboidal cells with irregular round nuclei and clear to lightly eosinophilic cytoplasm forming glandular or papillar structures. See Figures 3 and 4.

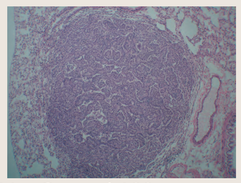
Figure 3 - Representative lung crossections of AJ mice



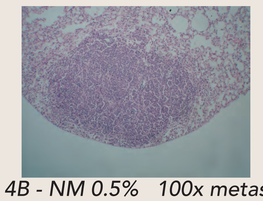
3A - Control

3B - NM 0.5%

Figure 4 - Representative lung histology from AJ mice



4A - Control 100x metastatic lesion



4B - NM 0.5% 100x metastatic lesion

3 Mean lung weights of animals from the NM-supplemented group were significantly lower (by 17%, $p=0.0025$) than lung weights from the Control group, as shown in Figure 5. Data from the Control, NM-supplemented and Negative Control groups were pooled. In analyzing the relationship between the lung weight and tumor number, a significant positive correlation was obtained (correlation coefficient $r= 0.7891$, $p<0.0001$), as shown in Figure 6. There was no significant difference in the body weights of the control and NM-supplemented mice.

Figure 5 - Effect of NM on mean lung weight of AJ mice

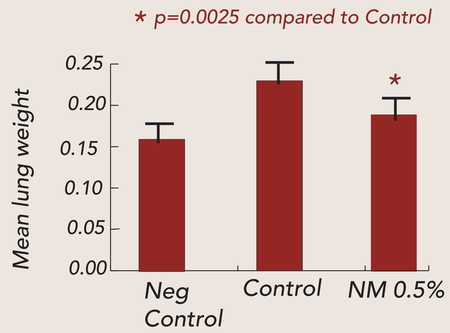
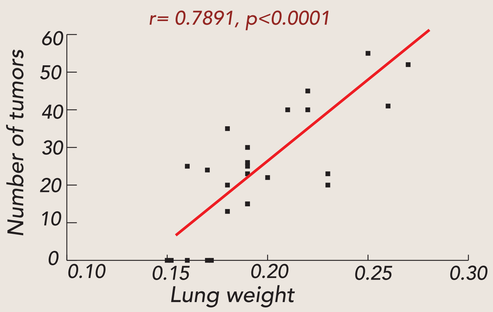


Figure 6 - Correlation bewteen lung weight and tumor multiplicity in AJ mice



4. In contrast to mean lung weights, mean weights of livers, kidneys, hearts and spleens of AJ mice from the various groups did not significantly differ, as seen in Table 1.

Table 1 - Organ weights (in g) of groups of AJ mice

Organs	Negative Control	Control (urethane)	NM 0.5%
Liver	1.02 ± 0.16	1.25 ± 0.15	1.10± 0.11
Kidney	0.35 ± 0.07	0.38 ± 0.05	0.36 ± 0.04
Heart	0.10 ± 0.01	0.11 ± 0.01	0.10 ± 0.004
Spleen	0.09 ± 0.03	0.08 ± 0.02	0.07 ± 0.02

Conclusions:

These results suggest that NM has inhibitory potential on the development of mouse lung tumors induced by urethane.