A NUTRIENT MIXTURE INHIBITS GLIOBLASTOMA XENOGRAFT
U-87 MG GROWTH IN MALE NUDE MICE

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Background: Brain tumors are highly aggressive tumors characterized by secretions of high levels of matrix metalloproteinase-2 and -9, leading to tumor growth, invasion and metastasis by digesting the basement membrane and extracellular matrix components. We previously demonstrated the effectiveness of a nutrient mixture (NM) containing ascorbic acid, lysine, proline, and green tea extract in vitro: on activity of urokinase plasminogen activator, matrix metalloproteinases and TIMPs in various human glioblastoma (LN-18, T-98G and A-172) cell lines and on glioblastoma A-172 cell proliferation and Matrigel invasion. Aim: Our main objective in this study was to investigate the effect of the NM in vivo on human glioblastoma U-87 MG cell line. Materials and Methods: Athymic male nude mice inoculated with 3·10^6 U-87 MG cells subcutaneously and were fed a regular diet or a regular diet supplemented with 0.5% NM. Four weeks later, the mice were sacrificed, the tumors were weighed and measured. The samples were studied histologically. Results: NM inhibited tumor weight and tumor burden by 53% (p = 0.015) and 48% (p = 0.010), respectively. Conclusions: These results suggest the therapeutic potential of NM as an adjuvant in the treatment of glioblastoma.

Key Words: glioblastoma U-87 MG cell line, nutrients, xenograft tumor growth.

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Abbreviations used: MMP — matrix metalloproteinase; NM — nutrient mixture; uPA — urokinase plasminogen activator.

Methods: Athymic male nude mice inoculated with 3·10^6 U-87 MG cells subcutaneously and were fed a regular diet or a regular diet supplemented with 0.5% NM. Four weeks later, the mice were sacrificed, the tumors were weighed and measured. The samples were studied histologically. Results: NM inhibited tumor weight and tumor burden by 53% (p = 0.015) and 48% (p = 0.010), respectively. Conclusions: These results suggest the therapeutic potential of NM as an adjuvant in the treatment of glioblastoma.

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sured using a digital caliper, and the tumor burden was calculated using the following formula: 0.5 × length × width. Mean weight of mice at initiation of study and termination of study did not differ significantly between the groups.

**Histology.** Tissue samples were fixed in 10% buffered formalin. All tissues were embedded in paraffin and cut at 4–5 microns. Sections were deparaffinized through xylene and graduated alcohol series to water and stained with hematoxylin and eosin (H & E) for evaluation using a standard light microscope.

**Statistical analysis.** The results were expressed as means ± SD and analyzed by independent sample "t" test using MedCalc software (Mariakerke, Belgium).

**RESULTS AND DISCUSSION**

NM strongly inhibited the growth of U-87 MG xenografts in male nude mice. Mean tumor weight was inhibited by 53% (p = 0.0147) with NM 0.5% dietary supplementation, as shown in Fig. 1, a and tumor burden was inhibited by 48% (p = 0.0098), as shown in Fig. 1, b. There was no significant difference between mean body weights of groups of mice.

![Fig. 1. Effect of NM on mean tumor weight (a) and tumor burden (b) in nude mice grafted with U-87 MG cells. *p = 0.015 (a) and p = 0.01 (b) as compared to control](http://example.com/fig1)

Histologically, the tumors from both groups were irregularly round, subcutaneous masses, composed of solid sheets and nests of irregularly round epithelioid cells with prominent round to ovoid nuclei and lightly staining bluish cytoplasm with ill-defined cell borders. Tumors from control and NM-supplemented mice were similar morphologically, but the tumors from supplemented mice were significantly smaller in size. Areas of tumor necrosis ranged from 50–80% of tumor mass in the control group compared to 10–60% in the NM group. Areas of tumor necrosis were associated with moderate to severe neutrophilic infiltration (Fig. 2, a–d).

Previous in vitro results from the cellular proliferation study of glioblastoma A-172 support the in vivo findings, as NM showed increased toxicity in cells in a dose-dependent manner, with 50% inhibition of cell growth in cells exposed to 1000 μg/ml NM and total block of invasion through Matrigel at 1000 μg/ml NM [8]. In addition, prior studies of several glioblastoma cell lines, demonstrated potent, significant suppression of invasive parameters by the NM, including uPA and MMP-2 and -9 [7].

Brain cancer is the second leading cause of cancer death in children under 20 years, accounting for one third of all cancer deaths in this age group. Current treatment methods for gliomas are generally ineffective and toxic. Thus, there is a need for development of effective therapeutic agents for these cancers with minimal toxicity. Our studies demonstrated that the mixture of the non-toxic components of NM significantly inhibited the growth and tumor burden of glioblastoma cell line U-87 MG in vivo. These results in addition to our prior in vitro studies with glioblastoma cell lines, which documented significant inhibition of MMP-2 and-9 secretion and invasion by NM, suggest the potential of NM as an adjuvant in treatment of glioblastomas.

![Fig. 2. Histopathology of tumors from control (a, b) and NM-supplemented (c, d) mice, × 200. In b and d tumor necrosis and associated neutrophilic inflammation are present](http://example.com/fig2)

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**REFERENCES**


