Tumor Inoculation with E.aerogenes Results in Prolonged Survival in a Rat GBM Model

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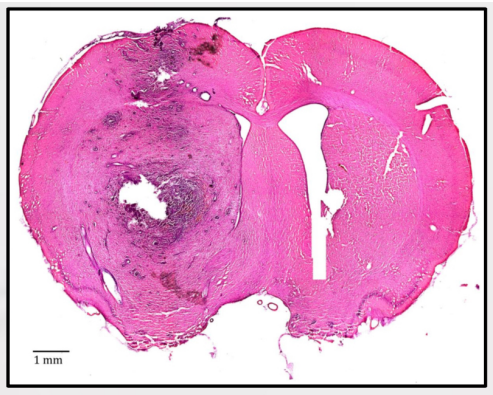
Introduction

Anecdotal reports or case series suggest that incidental wound infections may prolong survival in GBM (1,2). Recently a plea has been made to try intentional infection with live C.novyi as a method of treatment for GBM in patients(3). We explored the effectiveness of implantation of live bacteria in a rat model of GBM

Methods

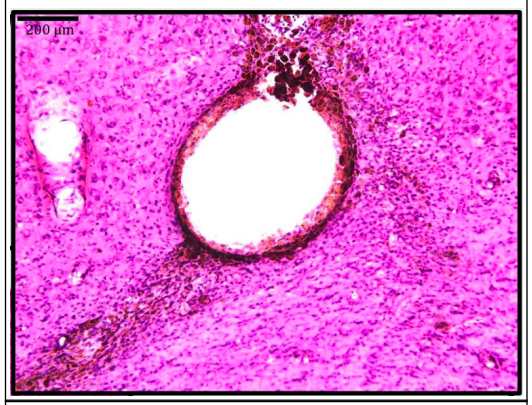
E.aerogenes (ATCC 13048) liquid primary cultures were prepared by single-colony inoculation of Lura Bertani broth, and the bacteria were laden into agarose microbeads. CNS-1 cells (human GBM cell line) were maintained in complete media. In 12 week old Lewis male rats (approx. 300g) 1,250 cells were injected in 10microliter of saline stereotactically into the right caudate. We also had 2 groups without tumor induction, which on day zero received either sterile or bactery laden microbead implantation into the right caudate. Two weeks after the tumorinduction, rats were randomly divided into 3 groups: one group of 5 was treated with stereotactic implantation of 10microliters of bacteria-laden microbeads, one group of 4 with sterile microbeads, and one group of 5 was left untreated. Animals were checked daily.

Tumor Histology



10X micrograph of tissue section 10 days post-implantation of 5 x 103 CNS-1 cells.

Abscess Formation



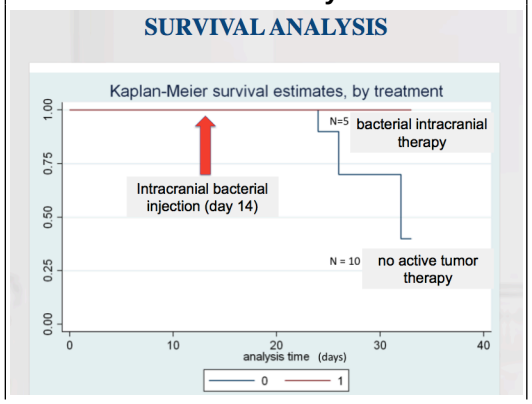
Experimental groups

Group	Day 1	Day 14
A	≤ 5 x 10³ CNS-1 cells (tumor implantation)	∅
B		10 µL sterile microbeads
C		10 µL E. aerogenes microbeads
D	10 µL E. aerogenes microbeads	∅
E	10 µL sterile microbeads	∅
F	sham surgery	∅

Results

By week 4 (after tumor induction) the non-or-sham treated animals started dying, presumably from poor neurological function, at week 5, 6 out of 9 animals in this group had died spontaneously. None of the 5 animals the treatment group (or any of the non-tumor groups) died spontaneously before the end of week 5, at which time the rats were euthanized. Kaplan-Mayer survival curves between treated or un/sham treated GBM animals were significantly different(p=0.039, log-rank test for equality of survivor function)

Survival Analysis



Conclusions

These preliminary data suggest that controlled intracranial E. aerogenes inoculation significantly reduces the risk of tumor-growth induced death in an immunocompetent animal model of GBM. Inoculation with sterile or bacteria laden agarose microbeads alone was tolerated without adverse effects.

Learning Objectives

By the conclusion of this session, participants should be able to 1)recognize the possible promise of bacterial treatment of malignant tumors. 2)be aware that there are models of immunocompetent animals of GBM

References

1. Bowles AP, Perkins E. Longterm remission of malignant brain tumors after intracranial infection: Report of four cases. Neurosurgery. 1999;44(3)636-643.

2. De Bonis P, Albanese A, Lofrese G, et al. Postoperative infection may influence survival in patients with glioblastoma: simply a myth? Neurosurgery. 2011:69(4):864-869.

3. Zwagerman NT, Friedlander RM, Monaco EA. Intratumoral Clostridium Novyi as a potential treatment for solid necrotic brain tumors.Neurosurgery. 2014;75(6):N17-N18.