Eur J Cancer Clin Oncol, Vol. 22, No. 4, pp. 501–503, 1986.
Printed in Great Britain.

0277-5379/86$3.00+0.00

Sympathetic Nervous System and Glioma Growth

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Abstract—*C-6 glioma cells possess β-adrenergic receptors on the cell surface. Activation of β-adrenergic receptors with β-adrenergic agonists increases intracellular levels of cAMP and leads to differentiation of C-6 glioma cells* in vitro. *The present study shows that growth of C-6 glioma tumor in rats with ablated sympathetic nervous system is augmented as compared to controls. Lack of normal noradrenergic stimulation of C-6 glioma cells may lower intracellular cAMP and allow unrestricted growth of this tumor.*

INTRODUCTION

GLIOMAS and glioblastomas are the most common brain tumors in man. There is no effective treatment for these tumors at present. C-6 glioma tumor, an experimental brain tumor of rats, induced with *N*-methylnitrosourea, has been widely used as a model for human glioma [1]. C-6 glioma produces S-100 protein, an acidic protein present in glial cells, contains glial fibrillary acidic protein, a marker for fibrillary astrocytes and contains 2′3′-cyclic nucleotide-3-phosphohydrolase, a membrane associated enzyme considered to be a marker for myelin and oligodendroglia [2, 3]. Presence of these markers indicates that C-6 glioma is of neural origin. C-6 glioma cells possess β-adrenergic receptors on the cell surface. Stimulation of β-adrenergic receptors with β-adrenergic agonists increases intracellular levels of cyclic adenosine monophosphate (cAMP) significantly [4]. cAMP levels have been shown to be low in C-6 glioma [5] and an increase of intracellular levels of cAMP in glioma cells *in vitro* produces morphological and biochemical maturation of glial tumor cells [6, 7].

We have shown previously that treatment of C-6 glioma bearing rats with the β-adrenergic agonist isoproteranol suppresses growth of this tumor significantly. Addition of the cAMP phosphodiesterase inhibitor papaverine to the treatment schedule suppresses growth of C-6 glioma tumor *in vivo* to a greater extent than does isoproteranol alone [8, 9]. We have also shown that response to this treatment depends on the number of β-adrenergic receptors on the glioma cell surface [9]. We now present data on the influence of sympathetic nervous system ablation on C-6 glioma growth *in vivo*.

METHODS

C-6 glioma cells were obtained from the American Type Culture Collection and maintained in culture in Dulbecco's modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum and *L*-glutamine (10 ml of 200 mM solution per 500 ml of medium) in a humidified atmosphere of 5% CO_2 at 37°C. To induce sympathectomy, newborn Wistar/Furth rats were given i.p. injections of 6-hydroxydopamine (6-OHDA) (Sigma Chemical Company, St. Louis, MO) daily for 10 days in doses of 100 μg/g of body weight. The solution used contained 6-OHDA (10 mg/ml) and ascorbic acid as an anti-oxidant (0.1 mg/ml). Control rats received 0.9% NaCl ascorbic acid solution. Two days after the last injection of 6-OHDA rats were injected in the flank with dispersed viable C-6 glioma cells at a dose of 3×10^3/rat. Tumors were removed 2 or 3 weeks later and weighed. Statistical analyses were done using Student's *t*-test.

RESULTS

6-OH DA treated rats showed bilateral ptosis, evidence of successful sympathectomy.

Tumors removed 3 weeks after cell inoculation were significantly larger in sympathectomized rats than in controls. Mean tumor weight in sympathectomized rats was 500 S.E.M. ± 120mg

Accepted 10 September 1985.
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This work was supported in part by grants from the National Institutes of Health #5R01 NS-18413-02 and by Sarah D. Mayer Memorial Grant for Cancer Research of the American Cancer Society PTD #253.

(n = 13) vs. 240 mg ± S.E.M. 88 (n = 10) in controls (three experiments). The difference is significant at a P value of < 0.05. Growth of the tumors removed 2 weeks after cell inoculation was somewhat but not significantly augmented in the experimental group as compared to controls. Mean tumor weight in sympathectomized rats was 200 S.E.M. ± 59 mg (n = 15) and 180 S.E.M. ± 44 mg in controls (n = 11) (three experiments) (Fig. 1).

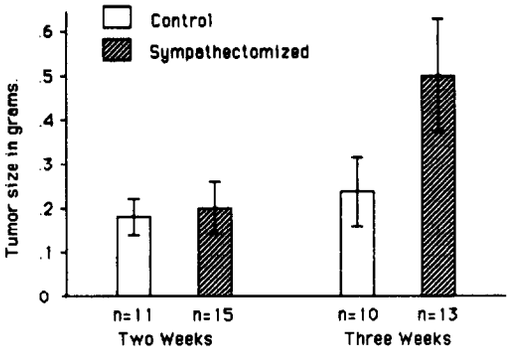

Fig. 1. Effect of sympathectomy on the growth of C-6 glioma in rats. C-6 glioma tumor size 2 weeks *or* 3 weeks *after cell inoculation. Vertical bars show* S.E.M. n = *number of animals.*

DISCUSSION

We have shown that ablation of the sympathetic nervous system augments growth of C-6 glioma *in vivo*. We have shown previously that treatment of C-6 glioma bearing rats with β-adrenergic agonists suppresses growth of this tumor [8, 9].

C-6 glioma cells possess receptors for several neurotransmitters, the most extensively studied at present being the β-adrenergic receptor. *In vitro* stimulation of the β-adrenergic receptor by the β-adrenergic agonists norepinephrine or isoproterenol activates adenylate cyclase and induces an up to 200-fold increase in the intracellular content of cAMP in C-6 glioma cells [4].

Cyclic 3′, 5′-adenosine monophosphate is intimately involved in regulation of cell proliferation and differentiation. Intracellular cyclic AMP varies at different stages of the cell cycle, being minimal during mitosis and maximal in the G_0 and early G_1 phases [10]. In most cases, cAMP drives cells towards the G_0 and G_1 stages of the cell cycle and towards a full expression of their differentiated functions.

Malignant cells have been shown to be deficient in the regulatory mechanisms which allow cells to remain in G_0 and G_1 and to have a low content of cAMP [11]. Malignant transformation can be linked to a defect in the cells' ability to respond to cAMP or in its capacity to raise intracellular cAMP levels in response to growth regulatory signals [12]. With many tumor lines *in vitro*, addition of analogues of cAMP or of agents which elevate intracellular cAMP levels causes differentiation [13–16]. There is also some evidence that treatment of tumor-bearing animals with cAMP, cAMP analogues, or cAMP phosphodiesterase inhibitors slows tumor growth. This has been shown for rats bearing Walker carcinosarcoma [17] and for mice bearing GC3HED, L51784, and L1210 tumors [18].

Exposure of glioma cells to NE or cAMP analogues affects the cells in several ways. Glycogenolysis, lactate dehydrogenase, ornithine decarboxylase, cAMP, and the glial specific enzyme CNP, are all increased [19, 20]. The increase in CNP indicates that the stimulatory effect of NE on cAMP concentration in glioma cells induces functions specific for differentiated glial cells. Stimulation of the β-adrenergic receptor on C-6 glioma cells also causes marked changes in cell morphology, the cells becoming more differentiated. Cells treated in culture with NE assume a spindlelike morphology, the cytoplasm retracts to form a compact cell body with multiple processes which occasionally show beadings and bifurcations [5]. Therefore, C-6 glioma, in the presence of NE, acquires a morphology similar to that of mature astrocytes in brain tissue. Comparable morphological changes can be induced with db-cAMP and theophylline.

Similar considerations may apply to human glioma. It has been shown that the human glioma tumor line 1181 N 1 contains all of the components of the cAMP regulatory system and that cAMP increases rapidly when these cells are exposed to NE or epinephrine [6]. In our experiments lack of sympathetic innervation, and secondary to it, lack of normal noradrenergic stimulation of the tumor cells, may lower the intracellular cAMP and allow unrestricted growth of glioma tumor. An understanding of the interaction between the sympathetic nervous system and glioma growth may have therapeutic implications for this tumor in the future.

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