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ORIGINAL ARTICLE

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Activity of irofulven (6-hydroxymethylacylfulvene) in the treatment of glioblastoma multiforme-derived xenografts in athymic mice

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Abstract *Purpose*: This study was conducted to define the activity of irofulven in the treatment of a series of xenografts derived from human glioblastoma multiforme growing subcutaneously and intracranially in athymic nude mice. *Methods*: Athymic mice bearing subcutaneous or intracranial tumors were treated with irofulven at a 10% lethal dose with responses compared to tumor-bearing mice treated with drug vehicle. *Results*: Irofulven was active against all tumor lines tested with growth delays ranging from 5.6 to 81.6 days (all values statistically significant, $P \leq 0.001$). Irofulven also produced a statistically significant ($P \leq 0.001$) increase in the median survival of mice bearing D-456 intracranial xenografts with a 162% increase in median survival. *Conclusions*: Irofulven is active in a spectrum of human glioblastoma multiforme-derived xenografts and evaluation in patients with this neoplasm is warranted.

Keywords Glioblastoma multiforme · Irofulven · Nude mice · Xenografts · CNS tumors

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Introduction

Malignant glioma, particularly glioblastoma multiforme (GBM), is one of the most challenging malignancies seen in humans. Despite treatment with surgery, radiotherapy and chemotherapy, virtually all patients with GBM demonstrate early failure at the primary tumor site and die quickly [3]. Surgical intervention and radiotherapy are limited both by neurotoxicity and the distant spread of tumor cells. Systemic treatment with chemotherapy, although improving with several newer agents displaying anti-glioma activity [6, 7, 8], has not yet resulted in a meaningful increase in survival for patients with GBM. Newer strategies employing advances in molecular biology such as gene therapy or anticancer vaccines have not yet produced true benefits for patients. Although this remains a hope for the future, more timely improvements in the outcome for patients with GBM will require advances in systemic chemotherapy.

Irofulven is a novel cytotoxic agent related to mushroom-derived illudin toxins. Specifically, irofulven is a semisynthetic derivative of the natural product illudin S, which reacts with DNA in a unique and unknown way to produce cell death. This agent is active in vitro and in vivo against a broad spectrum of human and murine tumor cell lines, including those derived from adult breast, colon, gastric, lung, ovarian, prostate and pancreatic carcinomas, melanoma, and childhood neuroblastoma and rhabdomyosarcoma [9, 10, 11, 12, 13, 14, 19].

We now report the marked activity of irofulven in the treatment of a series of xenografts derived from GBM growing subcutaneously and against D-456 MG growing intracranially in athymic nude mice.

Materials and methods

Animals

Male and female athymic BALB/c mice (*nu*/*nu* genotype, 6 weeks old or older) were used for all studies as previously described [1].

Xenografts

A panel of four GBM-derived xenografts were used for all in vivo studies and were maintained as previously described [5].

Subcutaneous xenograft transplantation

Tumors were removed from the host under sterile conditions in a laminar flow containment hood. Tumor was segmented and placed into a modified tissue press and passed through a bilayered mesh cytosieve to form a tumor homogenate. This homogenate was then passed through a 19-gauge needle before being placed into a 250-μl Hamilton syringe dispenser. This was used to inoculate the right flank of animals with 50 μl of tumor homogenate as previously described [1].

Intracranial xenograft transplantation

Intracranial (i.c.) tumor transplantation into the right cerebrum was performed as described previously with inoculation volumes of 10 μl [4]. D-456 MG was used for these studies because prior studies with this xenograft have demonstrated highly reproducible survival curves following i.c. inoculation.

Drugs

Irofulven was provided by MGI Pharma (Bloomington, Minn.).

Tumor measurements

Subcutaneous (s.c.) tumors were measured twice weekly with hand-held calipers purchased from the Scientific Products Company (McGraw, Ill.). Tumor volume was calculated according to the following formula: $[(\text{width})^2 \times (\text{length})]/2$.

Xenograft therapy

Irofulven was given to mice via intraperitoneal injection at a dose of 7 mg/kg (21 mg/m^2) in 5% ethanol in 0.9% saline on days 1–5 and 8–12 which represents the dose lethal to 10% of treated animals. For s.c. studies, groups of randomly selected mice were treated when the tumor volume was within the range 100–500 mm^3 and compared to control animals receiving no treatment. For i.c. studies, groups of ten randomly selected animals were treated using the same regimen of irofulven as described above for the s.c studies on the day which represented 50% of the time elapsing between the initial tumor inoculation and the median day of death as previously defined in i.c. tumor-bearing mice receiving no therapy. Control animals were treated with drug vehicle, specifically 5% ethanol in 0.9% saline.

Assessment of response

The response of s.c. xenografts was assessed by delay in tumor growth and by tumor regression. Growth delay, expressed as T–C, was defined as the difference in days between the median time required for tumors in treated (T) and control (C) animals to reach a volume five times greater than that measured at the start of treatment. Tumor regression was defined as a decrease in tumor volume over two successive measurements. Statistical analysis was performed using a personalized SAS statistical analysis program, the Wilcoxon rank order test for growth delay, and Fisher's exact test for tumor regression as described previously [4]. The response of i.c. xenografts was assessed in terms of the percentage increase in median survival. Statistical analysis was performed using the Wilcoxon rank-order test as described previously [4].

Results

Toxicity

Among the 50 treated animals, two deaths were attributable to drug toxicity. The median weight loss nadir was 24% among the surviving animals. No neurologic toxicity, including seizure activity, was noted. Animals dying of i.c. tumors typically died suddenly without any obvious antecedent physical changes.

Subcutaneous xenograft therapy

Irofulven was active against all tumor lines tested (Table 1). Growth delays ranged from 5.6 days in D-54 MG to 81.6 days in D-456 MG; all values were statistically significant ($P \leq 0.001$). Tumor regressions were seen in all xenografts except D-54 MG.

Intracranial therapy

Irofulven produced a statistically significant ($P \leq 0.001$) increase in the median survival of mice bearing D-456 MG xenografts growing i.c. with a 162% increase in median survival (Table 2). All mice displayed gross evidence of i.c. tumor at time of death. No animals died from drug toxicity.

Table 1 Effect of irofulven on growth of s.c. human GBM xenografts in mice (and previously reported data with CPT-11 [8] and topotecan [5]). In replicate studies, irofulven was administered via intraperitoneal injection at a dose of 7 mg/kg in 5% ethanol in 0.9% saline on days 1–5 and 8–12. T–C, growth delay in days, is defined as the difference between the median time required for tumors in treated (T) and control (C) animals to reach five times the volume measured at the initiation of treatment. All values, from replicate studies are statistically significant ($P \leq 0.001$) compared with controls. Regression is defined as a decrease in tumor volume over two successive measurements

Xenograft	Histology	Time (days) to 5× initial tumor size in controls	Irofulven			CPT-11		Topotecan	
			T–C (days)	Regressions	Toxic deaths	T–C (days)	Regressions	T–C (days)	Regressions
D-212 MG	Childhood GBM	53.0	54.6	9/9	1/10	81.1	8/8	38.1	6/7
D-456 MG	Childhood GBM	18.1	81.6	9/9	1/10	90+	9/9	26.2	8/8
D-54 MG	Adult GBM	14.5	5.6	0/10	0/10	90+	9/9	9.9	2/10
D-245 MG	Adult GBM	28.0	34.8	10/10	0/10	25.8	10/10	16.5	5/8

Table 2 Effect of irofulven treatment on survival of mice bearing i.c. D-456 MG xenografts. Irofulven was administered via intraperitoneal injection at a dose of 7 mg/kg in 5% ethanol in 0.9% saline on days 1–5 and 8–12

Xenograft	Histology	Median day of death		Increase in median survival (%)[a]
		Control	Treated	
D-456 MG	Childhood high-grade glioma	32	84*	162

*$P \leq 0.001$ compared with controls
[a]Calculated as the median day of death of ten drug-treated mice minus the median day of death of ten control mice divided by the median day of death of control mice

Discussion

GBM is one of the most aggressive and fulminant malignancies seen in humans [3]. Surgical resection can only remove macroscopic tumor leaving behind a considerable number of tumor cells which invariably spread throughout the brain. Radiotherapy produces a reproducible but modest effect, with the majority of tumors recurring at the primary site despite having received maximal radiation. Systemic intervention therefore is required for a tumor refractory to surgery and radiotherapy and which has diffusely invaded brain at initial diagnosis. Unfortunately, conventional systemic approaches such as chemotherapy offer limited benefit. Nitrosoureas have been considered the gold standard since the initial Brain Tumor Study Group trials [3]. More recently, Temodal was approved by the FDA for recurrent malignant glioma with large multi-institutional trials confirming activity in GBM and anaplastic astrocytoma [20, 21]. However, alkylators such as nitrosoureas and Temodal will alone be inadequate, and a broader spectrum of antineoplastics are needed for a tumor such as GBM which is extraordinarily resistant to chemotherapy. Newer therapeutic strategies such as vaccines or gene therapy may prove helpful in the future but remain speculative and unproven to date. Accordingly, immediate progress will require identification of new chemotherapeutic agents active in GBM and which are not cross-resistant with other currently employed drugs.

Irofulven is a semisynthetic derivative of illudin S, which is a natural product isolated from mushrooms of the genus *Omphalotus* (*O. olerius*) or *Lampteromyces* (*L. japonicus*). The function of illudin production by these organisms remains unknown. Initial studies by the NCI with illudin S against a panel of solid tumors and leukemia revealed minimal activity and considerable toxicity. However, despite trivial antineoplastic activity, the illudins produce cell death in novel ways. Illudin S is a potent inhibitor of DNA synthesis that causes cell cycle in S phase. The action of the agent also requires functional DNA helicase activity for DNA repair processes to occur [11]. These unique properties of the illudins led to structure-activity-based synthetic efforts to create chemical derivatives with better therapeutic margins than illudin S [15, 16, 17].

Irofulven is an analog synthesized by Dr. Trevor McMorris that exhibits marked in vitro and in vivo antitumor activity [17, 18]. Additionally, this analog has been found to display favorable solubility properties that allows formulation in a vehicle suitable for intravenous administration. Irofulven undergoes rapid cellular uptake where it covalently binds to cellular macromolecules. The interaction with DNA results in rapid inhibition of DNA synthesis and produces DNA lesions that are difficult for the cell to repair. Irofulven damage induced in tumor cell lines initiates an apoptotic degradation of DNA that leads to cell death but only a cytostatic response in normal cell lines.

These unique properties and broad antitumor activity led us to evaluate irofulven against a panel of human GBM xenografts in athymic nude mice. Marked activity was seen against all xenografts growing at both s.c. and i.c. sites. These results are similar to the activity produced against these xenografts by temozolomide and CPT-11, agents subsequently confirmed in clinical trials to have activity against GBM [6, 8].

Phase I testing of irofulven has been completed using a series of different schedules [2], revealing hematopoietic dose-limiting toxicity. Accordingly, on the basis of our preclinical studies and the completed phase I trials, we will shortly open a phase II trial of irofulven for patients with newly diagnosed and recurrent GBM using a schedule of 24 mg/m^2 on days 1 and 15 every 4 weeks by a 30-min intravenous infusion through a central venous access.

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