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Therapeutic activity of the topoisomerase I inhibitor J-107088 [6-N-(1-hydroxymethyl-2-hydroxyethylamino-12,13-dihydro-13-[(β-D-glucopyranosyl)-5H-indolo[2,3-a]-pyrrolo[3,4-c]-carbazole-5,7(6H)-dione] against pediatric and adult central nervous system tumor xenografts

Abstract Purpose: The in vivo antitumor activity of a novel topoisomerase I inhibitor, J-107088, was tested in athymic nude mice bearing subcutaneous or intracranial pediatric and adult malignant CNS tumor-derived xenografts. Methods: J-107088 was administered to animals on days 1–5 and 8–12 via intraperitoneal injection at a dose of 54 mg/kg (162 mg/m²) per day in 10% dimethyl sulfoxide in 0.9% saline. The xenografts evaluated were derived from a childhood glioblastoma multiforme (D-456 MG), a childhood medulloblastoma (D-341 MED), an adult anaplastic astrocytoma (D-54 MG), an adult glioblastoma multiforme (D-245 MG), and a procarbazine-resistant subtype of D-245 MG [D-245 MG (PR)]. Results: J-107088 produced regressions and significant growth inhibition in all five of the xenograft lines growing subcutaneously. Growth delays ranged from 7.6 days with D-245 MG to 62.1 days with D-456 MG (P < 0.001). J-107088 also produced an 83% increase in survival in mice bearing intracranial D-456 MG (P < 0.001). Conclusion: These results indicate that J-107088 may be active in the treatment of childhood and adult malignant brain tumors and provide the rationale for initiation of clinical trials with this agent.

Keywords J-107088 · Central nervous system tumors · Topoisomerase I inhibitors · Xenografts

Introduction

Malignant tumors of the central nervous system (CNS) are a significant cause of morbidity and mortality among both children and adults. Despite the use of comprehensive multimodality treatment approaches incorporating surgical, radiotherapeutic and chemotherapeutic interventions, treatment results remain modest at best. The majority of children and adults with newly diagnosed glioblastoma multiforme demonstrate rapid and fulminating tumor progression despite aggressive therapy [4]. Virtually all patients with recurrent malignant brain tumors, including glial and neuronal tumors, fail salvage therapy and die quickly. Newer strategies such as gene therapy, cancer vaccines, and biological approaches, including antiangiogenic agents, are being evaluated in a spectrum of phase I trials. However, these approaches remain unproven, offering much promise but no substantive therapeutic benefit to date. Accordingly, the continued search for new, potentially active chemotherapeutic agents is necessary if outcomes are to be improved for patients with brain tumors.

The role of topoisomerase I inhibitors in the treatment of CNS tumors is now emerging. CPT-11 and topotecan have shown modest activity against recurrent malignant glioma in adults, with precise evaluation of antineoplastic activity compromised by the adverse effect of anticonvulsants [8, 10]. Newer topoisomerase I inhibitors are needed, particularly with more favorable pharmacokinetics in patients treated with anticonvulsants.

J-107088 [6-N-(1-hydroxymethyl-2-hydroxyethylamino-12,13-dihydro-13-[(β-D-glucopyranosyl)-5H-indolo[2,3-a]-pyrrolo[3,4-c]-carbazole-5,7(6H)-dione; Fig. 1], is
a novel indolocarbazole which inhibits topoisomerase I and differs from the camptothecins in potentially important ways. Camptothecin and its analogues selectively mediate DNA cleavage at a T \rightarrow G/A site whereas J-107088 mediates cleavage at a C/T \rightarrow G site [17]. Furthermore, the cleavable DNA-topo I complex formed by J-107088 appears to be more stable than that of camptothecin, remaining bound at higher concentrations of NaCl and effectively inhibiting topo I at lower concentrations of added drug [17]. J-107088 does not form any reactive metabolic intermediates and is not a substrate for P450-mediated metabolism in vitro.

We now report the activity of J-107088 in the treatment of a panel of malignant CNS tumor-derived xenografts growing subcutaneously and intracranially in athymic nude mice. J-107088 demonstrated statistically significant antitumor activity against all xenografts tested in both the subcutaneous and intracranial sites.

**Materials and methods**

**Animals**

Male and female athymic BALB/c mice (nu/nu genotype, 6 weeks of age or older) were used in all studies and were maintained as described previously [3].

**Tumor cell lines**

A panel of five xenografts derived from a pediatric high-grade glioma (D-456 MG), adult high-grade gliomas [D-54 MG, D-245 MG, D-245 MG (PR)] and a pediatric medulloblastoma (D-341 MED) were used and maintained as described previously [7, 8].

**Subcutaneous xenograft transplantation**

Tumors were removed from host animals under sterile conditions in a laminar flow contaminant hood. The tumor tissue was placed in a modified tissue press and passed through a bilayered mesh cytosieve. The resultant homogenate was further separated by passage through a 19-gauge needle before being placed into a 250-μl Hamilton syringe dispenser and used to inoculate the right flank of animals with 50 μl of tumor homogenate as described previously [5].

Intracranial xenograft transplantation

Intracranial tumor transplantation into the right cerebrum was performed with inoculation volumes of 5 μl using a 17-gauge needle equipped with a sleeve allowing 4.5 mm penetration as described previously [6].

**Tumor measurements**

Tumors were measured twice weekly with hand-held vernier calipers (Scientific Products, McGraw, III). Tumor volume was approximated according to the following formula: \((\text{width})^2 \times (\text{length})/2\).

**Chemotherapy agent**

J-107088 was synthesized and provided by the Banyu Tsukuba Research Institute (Tokyo, Japan).

**Treatment**

In replicate experiments, J-107088 was administered to animals on days 1–5 and 8–12 via intraperitoneal (i.p.) injection at a dose of 54 mg/kg (162 mg/m²) per day using a 2 mg/ml drug solution in 10% dimethyl sulfoxide in 0.9% saline. Animals were only treated with this regimen once. This represents the dose lethal to 10% of treated animals (LD₁₀). In subcutaneous xenograft studies, randomly selected groups of nine or ten animals began receiving treatment when the tumor volumes were within the range 100–500 mm³. Tumor measurements were subsequently compared with those of control animals receiving drug vehicle. In intracranial xenograft studies, a group of ten randomly selected animals received treatment 12 days after inoculation, a time-point that represents 50% of the time elapsing between initial tumor inoculation and the median day of death as previously defined in intracranial tumor-bearing mice receiving no therapy.

**Assessment of response**

The response of subcutaneous xenografts was assessed according to 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 analyses were performed using a personalized SAS statistical analysis program, the Wilcoxon rank order test for growth delay, and Fischer’s exact test for tumor regression as described previously [6]. The response of the intracranial xenografts was determined by calculating the percentage increase in median survival for treated animals as compared to control animals. Statistical analysis was performed using the Wilcoxon rank order test as described previously [6].

**Results**

**Toxicity**

Among the 101 treated animals, 5 deaths were attributable to drug toxicity. The median nadir weight loss was 4.0% among treated surviving animals. The animals tolerated the treatment well with no neurologic toxicity noted.