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BRIEF REPORT
Rapamycin (Sirolimus) in Tuberous Sclerosis Associated Pediatric Central Nervous System Tumors

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Tuberous sclerosis complex (TSC) is associated with hamartomatous growths including subependymal giant cell astrocytomas (SEGAs). Since chemo-radiation therapies offer scant benefit, oncologists had traditionally been little involved in managing SEGAs. Recent evidence demonstrating rapamycin efficacy in adults and children with TSC-associated tumors foresee a practice change. We summarize our institutional experience and literature review that highlight potential benefits and hazards of rapamycin therapy, for TSC patients with SEGA, and other syndromal brain tumors. Pediatr Blood Cancer 2010;54:476–479. © 2009 Wiley-Liss, Inc.

Key words: mTOR; rapamycin; sirolimus; subependymal giant cell astrocytoma; tuberous sclerosis complex

INTRODUCTION

Tuberous sclerosis complex (TSC) is an autosomal dominant disorder caused by inactivating mutations in the tumor suppressor genes hamartin (TSC1) or tuberin (TSC2), associated with potential hamartomatous tumors. Approximately, 10% of TSC patients develop low-grade CNS lesions known as subependymal giant cell astrocytoma (SEGA) [1]. SEGAs are challenging tumors; slow-growing with often no symptoms until obstructive hydrocephalus develops; watchful monitoring and early surgical intervention have been traditional mainstays of therapy. There is sparse evidence of spontaneous regression or growth stabilization; radiotherapy or chemotherapy typically would not halt progression [2]. Up to now, oncologists were rarely involved in managing TSC. Recent evidence suggesting rapamycin efficacy in patients with TSC-associated tumors may predict changing practice. We present three pediatric patients with TSC and large SEGA treated with rapamycin, and discuss potential implications of these findings.

CASES

Case 1

A 9-year-old diagnosed with nonfamilial TSC at 3 months was referred after a routine MRI disclosed an increasing SEGA. His history included stable renal angiomyolipomas and well-controlled complex partial seizures; he was in grade-appropriate schooling with educational assistance. A right-sided SEGA detected at age 2 on routine MRI had remained small at age 4. Rapamycin was started after his MRI at age 9 showed tumor progression, associated with ventriculomegaly without symptoms. After loading with 5 mg TID, he started 5 mg daily, titrated to 7 mg daily while on carbamazepine (known to induce rapamycin metabolism), with trough levels between 10 and 15 ng/ml. Follow-up MRI demonstrated a 65% SEGA decrease 3 months after rapamycin initiation from $35 \times 24 \times 34$ mm before therapy (Fig. 1A) to $24 \times 16 \times 26$ mm (Fig. 1B) with near-resolution of hydrocephalus; unchanged at 6 and 9 months follow-up. He had intermittent oral ulcers and myalgias, transient hypercholesterolemia, and gingival hypertrophy that responded to dental brace readjustment. His facial angiofibromas decreased significantly on rapamycin. It was elected to stop rapamycin after 1 year. Unfortunately, regrowth was noted 3 months after rapamycin discontinuation (Fig. 1C) and rapamycin was restarted. His 3-month follow-up MRI again demonstrated reduced SEGA size.

Case 2

A 13-year-old with TSC was referred after an increasing SEGA was noted. A twin without familial TSC, TSC was diagnosed at age 7 after dizziness and a falls prompted a head CT scan that demonstrated tubers. Renal angiomyolipomas and cardiac rhabdomyomas were subsequently shown. A SEGA at the right foramen of Monro was noted at age 9. Routine neuroimaging at age 13 detected significant SEGA growth and symptom review found mild intermittent headaches. After a 15 mg loading dose, he started 6 mg rapamycin daily, with maximum trough of 14 ng/ml. Headaches improved within 1 month, with occasional mouth sores and mild transient hypercholesterolemia. Three-month follow-up MRI showed a 60% SEGA decrease.

Case 3

A 10-year-old with TSC underwent evaluation for headaches and MRI revealed a large SEGA in the left foramen of Monro with significant hydrocephalus. She had a history of developmental delay and well-controlled epilepsy on phenytoin. Routine neuroimaging at age 3 and 7 demonstrated subcentimeter SEGAs with mild stable ventriculomegaly. At diagnosis of her large SEGA, chronic papilledema was noted; there were no symptoms apart from progressive headaches and propensity for car-sickness. Rapamycin was started, titrated to 9 mg daily. Three-month follow-up MRI showed 50% SEGA decrease and ventriculomegaly improved. Initial mouth sores self-resolved; maximum trough was 8.4 ng/ml. Three months after rapamycin initiation, papilledema and

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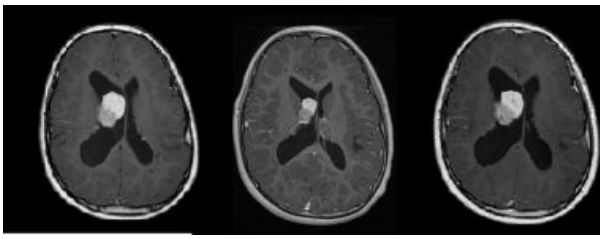

Fig. 1. **A**: Baseline pre-rapamycin (axial T1 MRI); **B**: Response after 3 months of rapamycin (axial T1 MRI); **C**: Regrowth after stopping rapamycin for 3 months (axial T1 MRI).

headaches had resolved, and adenoma sebaceum lesions had significantly improved.

DISCUSSION

mTOR and TSC

Mammalian target of rapamycin (mTOR) is an evolutionarily conserved cytoplasmic serine/threonine kinase involved in cell growth and metabolism [3]. Survival and growth promotion through mTOR signaling is mediated by the mTOR complex (mTORC), which phosphorylates and upregulates ribosomal S6 kinases, inducing cell growth and protein translation, and downregulates 4E binding proteins which inhibit protein translation [1]. TSC1/TSC2 regulate mTOR activity by inhibiting Rheb, a key mTOR activator through the AKT pathway. Upon recognizing inactivating TSC1/TSC2 mutations that led to constitutive mTOR activity and tumorigenesis, thereby resulting in TSC [2], many postulated that mTOR inhibitors would be ideal contributors to TSC therapy. Rapamycin (sirolimus; Rapamune®) was initially identified as an antimicrobial agent, but became best established as transplant immunosuppressive therapy [4]. Rapamycin can complex with FK506 binding protein-12 (FKBP12) and inhibit mTOR's phosphorylation activity, leading to cell size reduction or apoptosis [5]. Rapamycin also inhibits tumor angiogenesis [4]. Rapamycin has thus been a study target in multiple tumors, including TSC-associated ones, and in diverse disorders, from coronary artery disease to various common cancers [3–5].

Indications for Rapamycin in TSC

Early clinical studies support rapamycin efficacy in TSC tumors of the CNS, kidneys, and lungs [3,6,7]. Our cases, together with cases summarized (Table I), suggest dramatic response of TSC-associated CNS tumors to rapamycin, pointing to a potentially alternative to surgery [2,8]. As in other tumors, SEGA tissue showed that biallelic TSC1/TSC2 loss results in mTOR activation [9]. In addition, rapamycin showed promising efficacy in preventing seizures, and prolonging survival along with potentially improving learning/behavioral deficits in mouse TSC models [10,11]. Other rodent models' studies have, in contrast, suggested memory impairment [12]. Unfortunately, our patients did not undergo serial neuropsychologic testing during treatment. Potential neuropsychologic consequences require further delineation, although there are no definitive clinical concerns in human trials to date [7]. Rapamycin's activity outside the CNS has also been suggested. In adult, TSC-associated renal angiomyolipomas and sporadic lymphangiomatosis, rapamycin resulted in significant shrinkage [6,7].

Rapamycin levels for antitumor effects are currently based on levels used for immunosuppression, aiming for 24 hr trough levels between 10 and 15 ng/ml. However, antitumor activity may occur at lower levels, as in Case 3, where response occurred with maximum trough of 8.4 ng/ml. Avoidance of adverse effects and consideration of drug interactions require ongoing attention [2].

Potential Limitations to Rapamycin Effect and/or Use

Side effects include oral ulcers, acneiform rash, arthralgias, and diarrhea, thrombocytopenia, hyperlipidemia, and lipoproteinemia [2]. Side effects appear generally self-limited, but may require temporary dose reduction or cessation [7]. There are, however, sequelae of impaired wound healing and immunosuppression, including opportunistic infections and lymphoproliferative disease in transplant populations [2]. Notably, anticancer properties of rapamycin appear dominant to its immunosuppressant effects [4], and rapamycin is thought to hold a more favorable profile than other immunosuppressants including reduced post-transplant malignancies [13]. Continuous mTOR inactivation may also affect negative feedback loops involving upstream AKT/PI3K signaling; as activated PI3K often associates with more aggressive tumors, this warrants monitoring for malignant transformation with mTOR inhibition [14]. In vivo rapamycin resistance has been suggested from animal models [5]. This argues for potential need for combination therapies to maximize efficacy.

Indications for Rapamycin in Other CNS Tumors

Increased mTOR pathway activity has been demonstrated in tumors in the setting of neurofibromatosis type-1 (NF1). Increased ribosomal S6 activity was shown in mutant Nf1 mouse optic gliomas and human NF1-associated pilocytic astrocytomas, with abnormal astrocytes' increased growth arrested upon mTOR inhibition [15]. Rapamycin is currently assessed in NF1-associated plexiform neurofibromas in children (http://www.cancer.gov/CLINICALTRIALS. Accessed July 1, 2009).

Most rapamycin trials in CNS tumors have involved high-grade glioma (HGG) patients. A phase I study of neoadjuvant rapamycin in recurrent PTEN-deficient glioblastoma patients found reduced tumor proliferation in 7 of 14 patients [14]. Other adult studies have combined rapamycin with molecularly targeted agents such as EGFR inhibitors [16] or erlotinib (http://www.cancer.gov/CLINICALTRIALS. Accessed July 1, 2009).

Other mTOR Inhibitors

Rapamycin results have triggered interest in other small-molecule therapies in oncology, including derivative mTOR inhibitors, everolimus (RAD001; Certican®) and temsirolimus (CCI-779; Torisel™). These analogs inhibit mTORC2 and thereby impair AKT signaling and cell survival [17].

A phase I pediatric study of everolimus has demonstrated acceptable safety [17]. A phase III study on SEGAs in TSC is pending (Protocol IDs CRAD001M2301, NCT00789828), as is a phase II pediatric chemotherapy-resistant low-grade glioma study. Adult CNS tumor trials underway include: phase I/II chemo-radiation trial in new glioblastoma; and phase II trials in recurrent

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TABLE I. Case Reports of Rapamycin in CNS Tumors

Age (years)/sex (M/F)/tumor diagnosis/underlying condition/genotype	Indication for rapamycin	Starting daily dose/target daily dose/trough/concurrent therapy	Adverse effects	Response/duration of follow-up		References
				Size pre-rapamycin	Size at last follow-up	
Pediatric cases (0–18 years)						
15; F; SEGA (contralateral resected at 9 months with hemorrhage); complex partial epilepsy; TSC (*TSC2*: point mutation)	Progressive headache, nystagmoid eye movements, with tumor growth	2–7 mg; 10.9 ng/ml; concurrent phenobarbital and lamotrigine	Elevated cholesterol (no therapy change required)	Symptoms improved; decreased size at 5 months MRI; remained seizure-free		Franz et al. [2]
5.5; M; SEGA seizures; sleep and behavioral problems; TS	Intermittent headache and mental status change, with tumor growth	2–5 mg; 9.6 ng/ml; concurrent divalproex sodium, clonidine, quetiapine, and amitriptyline	None	23 × 20 mm, (6 cm^3)	18 × 13 mm, (2.4 cm^3) Unchanged sleep/behavior; decreased size at 3 months MRI; seizures well controlled	Franz et al. [2]
14.5; M; SEGA infantile spasms and seizures as child, off-anticonvulsants; mild cognitive impairment; TSC(*TSC2*: point mutation)	Solid and cystic tumor growth over 9 months	3–6 mg; 10.4 ng/ml	Mild acneiform rash, oral ulcers, and transient hypercholesterolemia (resolved over time)	10.2 × 12.7 mm, (1.1 cm^3)	7.3 × 9.9 mm, (0.41 cm^3) Decreased size (solid and cystic) at 2.5 months MRI	Franz et al. [2]
3; F; low-grade pilocytic astrocytoma (endoscopic biopsy); infantile spasm and partial epilepsy; TSC	Progressively enlarged hypothalamic mass over 16 months, with hydrocephalus	Titrated to 4 mg; 10.2 ng/ml; concurrent topiramate and weaned vigabatrin	Transient hyper-cholesterol-emia (no therapy change required) initial irritability (? hydro-cephalus related)	13.7 × 23.4 mm, (3.6 cm^3)	8.1 × 13.7 mm, (1.7 cm^3) Decreased ventriculomegaly with lesion necrosis at 5-week; resolved ventriculomegaly with decreased lesion and further necrosis at 5/6 months; remained seizure-free tapered off vigabatrin; progressed with speech and rehabilitative therapy	Franz et al. [2]
Adult cases (>18 years)						
21; F; bilateral SEGA; TSC	Intermittent headaches, blurred vision, and imbalance, with tumor growth	0.2 mg/kg/day; 11–13 ng/ml	None	66 × 50 × 43 mm	58 × 42 × 38 mm	Koenig et al. [8]
				Symptoms improved; decreased size at 2.5 months		
21; F; bilateral SEGA; TSC (*TSC2:*10 bp deletion)	Headaches, with tumor growth and mild ventriculo-megaly	6 mg; 7.7 ng/ml	Aphthous ulcers, acnei form rash at ~5 months: not recurrent when resumed; cholesterol elevation (self-resolved)	11 mm (R) 8 mm (L)	7.5 mm (R) 5 mm (L)	Franz et al. [2]
				Symptoms resolved; decreased size at 2.5/5 months; stopped at 5 months (asymptomatic, mild side effects); size re-increased 4 months after stopping; decreased 4 months after resuming and at 20 months		
				13.2 × 10.7 mm, (1.6 cm^3) (R) 6.6 × 10.3 mm, (1 cm^3) (L)	4.3 × 9.5 mm, (0.61 cm^3) (R) 5.6 × 7.3 mm, (0.25 cm^3) (L)	

F, female; L, left; M, male; mo, month; R, right; wk, week.

glioblastoma and refractory low-grade glioma (http://www.cancer.gov/CLINICALTRIALS. Accessed July 1, 2009).

Temsirolimus' use in CNS tumors has included an open-label trial of 65 adults with recurrent glioblastoma multiforme, showing prolonged time to progression; response correlated with S6 kinase activation, suggesting a response mechanism resembling that in TSC [18]. A parallel adult phase II trial in 43 recurrent glioblastoma multiforme patients found good tolerance but no longer-term efficacy [19]. Other trials combining temsirolimus with sorafenib or bevacizumab in recurrent HGG are ongoing (http://www.cancer.gov/CLINICALTRIALS. Accessed July 1, 2009).

FUTURE DIRECTIONS

mTOR inhibitors have sparked a potential re-thinking of the management of TSC patients. Studies need to confirm rapamycin's efficacy in SEGAs, and its impact on outcomes including neuropsychologic function. Currently, it is unclear whether mTor inhibitors are able to obviate or defer the need for surgical resection of symptomatic SEGAs. Studies are required to explore optimal therapy duration and management upon discontinuing therapy, given reports, including Case 1 above, that suggest re-growth of SEGAs upon rapamycin discontinuation. mTOR inhibitors may also serve as initial treatment to facilitate surgery for unresectable SEGAs.

CONCLUSION

Rapamycin offers significant promise in treating SEGAs and other TSC-associated tumors. Results from these three children with TSC treated with rapamycin further support rapamycin's role in potentially leading a changing standard of care in TSC. Further prospective, longer-term clinical investigations are warranted. Still, it is perhaps well time that pediatric neuro-oncologists assume more active roles in the multidisciplinary care for TSC patients.

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