ORIGINAL ARTICLE

The Efficacy and Safety of Rapamycin in Children with Tuberous Sclerosis: A Cross-sectional Study

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Abstract

Objectives

Mutations in TSC1 or TSC2 genes have been proposed as the main causative factors responsible for developing Tuberous Sclerosis Complex (TSC). Given the effect of these two genes on the mTOR pathway, rapamycin has emerged as a novel therapeutic agent. The present study evaluated the effectiveness and safety of rapamycin on the multiple manifestations of TSC.

Materials & Methods

Twenty-three eligible children were enrolled in the present cross-sectional study. They were prescribed rapamycin 1mg tablet twice daily for the first two weeks of treatment and then once daily for at least one year. Periodic evaluations through follow-up visits were performed. Besides, growth and developmental statuses were evaluated. All data, including the number and size of brain tuberomas, size of renal angiomyolipomas, and skin lesions, were gathered and recorded, and then analyzed.

Results

During the study period, the mean number of epileptic episodes significantly reduced ($p<0.0001$), and nine cases were seizure-free at the final visit. The mean number of brain tuberomas decreased from 19.3±11.0 at the initial visit to 11.1±5.6 and 8.2±3.2 in the subsequent visits ($p<0.001$). The mean size of brain tuberomas similarly decreased from 17.9±18.5 cm at enrollment to 13.7±5.1 cm and 6.9±5.1 cm in the second and third visits, respectively ($p=0.029$). The mean size of renal angiomyolipomas significantly

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decreased ($p<0.001$). A significant trend toward a decrease in the number of skin lesions was observed ($p<0.0001$). No relationship was observed between the effects of rapamycin and the patient's age or sex ($p>0.05$). Changes in patients' growth and developmental features were not statistically significant through subsequent visits ($p=0.507$).

Conclusion

This study revealed the effectiveness and safety of rapamycin on TSC among our patients.

Keywords: Rapamycin; Tuberous Sclerosis; Efficacy; Safety
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Introduction

Tuberous Sclerosis Complex (TSC) is a rare autosomal dominant disorder estimated to affect about one million people globally (1, 2). The disease shows highly variable manifestations because every organ is involved in the human body. Epilepsy, intellectual disability, autistic spectrum disorder, neuropsychiatric problems, and skin, heart, lung, and kidney lesions are some of the most common findings in TSC (3, 4). Inactivating mutations in TSC1 or TSC2 have been identified as the main causative factors responsible for developing the disease (5-7). These two mutations lead to the formation of abnormal parenchymal proteins and an increase of mammalian Target of Rapamycin (mTOR) pathway activity (7).

In recent years, rapamycin has emerged as a novel and exciting therapeutic option in patients with TSC. Multiple clinical and experimental trials have assessed its safety and efficacy in TSC patients (8-10). The effectiveness of rapamycin has been shown in treating renal angiomyolipomas and epileptic episodes (7). The efficacy and safety of 1 mg/m^2 of rapamycin for the treatment of TSC were demonstrated by Zou et al. They showed a 25% of seizure-free and 73.1% of effective rate among fifty-two children receiving rapamycin for twenty-four weeks (11). Another investigation by Canpolat et al. indicated a significant improvement in facial adenoma sebaceum or frequency of convulsions in seven cases treated with six months of rapamycin (12). Given all these encouraging findings, the authors sought to evaluate the efficacy of rapamycin in controlling epileptic episodes among Iranian candidates. Due to the low disease prevalence, this study was conducted with a small sample size. To the best of our knowledge, no previous studies have been conducted to evaluate the efficacy and safety of rapamycin in TSC-affected children (<18 years old) in Iran, and this study is the first one. Hence, the authors evaluated the effectiveness of rapamycin on the size of Subependymal Giant Cell Astrocytomas (SEGAs), Cortical Tubers, Renal Angiomyolipomas, Seizures frequencies, as

well as the development and learning skills among TSC patients.

Materials & Methods

Study Population

A cross-sectional study was conducted at two teaching hospitals affiliated with the Tehran University of Medical Sciences (TUMS), Tehran, Iran, between 2015 and 2018. All consecutive children diagnosed with TSC based on the 2012 International Tuberous Sclerosis Complex Consensus Conference diagnostic criteria (13) and followed in the hospitals were considered for initial enrollment. History taking and physical examination were done to select appropriate candidates for receiving rapamycin. All patients with no indication of tuberomas surgery, no history of cytopenia (White Blood Cell <4000 Cells/ mm3), or active infections were finally enrolled in the study. All participants or their parents signed informed consent before entering the study and accepted well-timed attendance for follow-up visits.

Prior to the beginning of the treatment regimen, the following laboratory and imaging investigations were done for all participants:

- A Complete Blood Count (CBC) test
- Neurologic assessment, including brain magnetic resonance imaging (MRI) with and without venous contrast, Electroencephalography (EEG), and Auditory Brainstem Response (ABR) test
- Cardiac functions assessment with echocardiography and electrocardiography
- Visual assessment with ophthalmoscopy and fundoscopic examinations
- Growth and development assessment by a pediatric neurologist expert
- Abdominal and renal ultrasound examination for assessment of any tuberomas

All findings related to the above examinations were gathered and recorded, constructing a baseline database for the future valid and reliable comparisons.

Study Protocol

A rapamycin Tablet (1 mg) was prescribed for all participants twice a day for the first two weeks and then was continued once a day for at least a year (11, 14). All previously administered medications were assessed (by a physician) and continued if no drug interactions with rapamycin were available. The follow-up protocols were scheduled as follows:

- An office visit every two weeks in the first three months of the treatment period
- Monthly visits in months 4-6
- Then office visits every six months

A CBC test was requested in each visit to assess the possible development of cytopenia or bone marrow toxicities related to the drug. In each visit, skin examination for the size and shape of dermatologic lesions (in the face, back, and ash leaf) was regularly performed. The numbers and severity of the lesion (Low: less than one lesion, Moderate: 5-10 lesions, Severe: More than ten lesions) in all three anatomic segments were determined and recorded. For evaluation of any previous renal or abdominal lesions, abdominal ultrasounds were requested every three months. A determined radiologist interpreted all brain ultrasound and brain MRI findings from the radiology department of Imam Khomeini Hospital. The sizes of lesions were recorded and compared with previous findings. In each follow-up visit, the frequency of epileptic episodes and their respective features were checked for patients with seizures. The neurodevelopmental statuses of participants were

evaluated by a pediatric neurologist (using Denver Developmental Screening Test; as a validated and reliable test) (15) at each scheduled visit.

The TUMS institutional review board approved the study according to the Helsinki declaration (No: IR.TUMS.IKHC.1396.3726).

Sample size

Based on former investigations by Franz et al. in 2013 (16) and using the following formula, the proposed sample size was calculated at twenty-two subjects. With twenty-six cases as the proposed sample size, the study had a power of 80% and an alpha error of 0.05.

$$n = \left(\frac{Z_{1-\alpha/2} \times \delta}{d} \right)^2$$

$$n = \frac{2 \times (z_{1-\alpha/2} + z_{1-\beta})^2 \delta^2}{d^{*2}} = \frac{16 \times \delta^2}{d^{*2}}$$

α, Z1-α/2=1.96, d=0.05, 1-β (95%) =1.64 = 0.05

Statistical Analysis

Statistical analysis was done using SPSS Statistics 25.0 (SPSS Inc, Chicago, IL). Continuous variables were presented as mean ± SD, and categorical variables were expressed as percentages. Continuous variables were compared using the standard t-test. Categorical variables were compared using the chi-square test or Mann-Whitney U test regarding the presence or absence of normal distribution. A P-value of less than 0.05 was considered a significant value.

Results

Overall, twenty-six patients who met the inclusion criteria were enrolled. Three patients were excluded from the final analysis; one case passed away (due to sepsis), and two subjects developed complications (secondary amenorrhea and lower limb edema). The mean age of participants at the time of enrollment was 9.7±5.1 years (minimum: four months; maximum: 17 years) and 13 participants (56.5%) were male. No relationship between the effects of rapamycin and the patient's age or sex was observed (p>0.05). No notable side effects were reported in the participants receiving rapamycin.

At each visit, patients were evaluated for the number of brain tuberomas via MRI. The mean number of brain tuberomas was 19.3±11 at the time of enrollment, which decreased to 11.1±5.6 and 8.2±3.2 at the second and third visits; these reductions were statistically significant (P<0.001). A comparison regarding the mean size of brain tuberomas in each follow-up visit showed significant decreases in the second and third visits compared to the time of enrolment (17.9±18.5, 13.7±5.1, and 6.9±5.1mm; p=0.029). Table 1 illustrates a comparative measure of each metric in follow-up visits.

The mean size of renal angiomyolipomas was also evaluated in the follow-up visits. The results showed that the mean size of renal angiomyolipomas decreased from 23.6±33 mm at the enrollment visit to 11.4±7.0 mm and 5.3±3.3 mm in the second and third visits, respectively. This finding was statistically significant (p<0.001). Serum creatinine (Cr) and Blood Urea Nitrogen (BUN) levels were measured concerning renal function. A decrease in serum Cr and an increase in BUN level were observed. However, these findings were not statistically significant (p=0.172). Table 2 demonstrates variations in angiomyolipoma size and renal function tests through each visit.

In participants with seizures, rapamycin's potential effect on controlling the epileptic episodes was

sought. The mean number of episodes at enrollment visit was 4±2 per day; five patients had multiple episodes, and three had no seizure. During follow-up visits, the mean number of epileptic episodes reduced to three and two per day in the second and third visits. At the final visit, nine patients were seizure-free. This reduction in epileptic episodes was statistically significant ($p<0.0001$).

As detailed data on skin lesions are shown in Table 3, a significant trend toward a decrease in lesions ($p<0.0001$) was notable.

Of all participants, 16 were diagnosed with at least one lesion resembling cardiac rhabdomyoma by echocardiography. These lesions had regressed in ten of the 16 participants after one year.

All patients were evaluated regarding statuses of growth and development at each office visit. At enrollment, seven cases (30%) were considered severe learning disabilities, while 13 (56%) were standard. The number of patients with severe learning disabilities in the second, third, and fourth visits was six out of twenty-two (27%), seven out of 17 (41%), and six out of 11 (54%), respectively. Overall, changes in patients' growth and developmental features were not statistically significant ($p=0.507$).

Table 1. Comparison of the Numbers and Sizes of Brain Tuberoma in Each Follow-Up Visit

	Number of Tuberomas	Size of Tuberomas (mm)
1st Visit	19.3±11.0 (2-30)	17.9±18.5 (2-80)
2nd Visit	11.1±5.6 (3-20)	13.7±5.1 (2-70)
3rd Visit	8.2±3.2 (3-15)	6.9±5.1 (0-20)
4th Visit	6.0±3.2 (3-10)	6.0±4.1 (0-15)

Table 2. Comparison of Renal Angiomyolipoma Sizes and Renal Function Test Results in Each Follow-Up Visit

	Angiomyolipoma Size (mm)	Blood Urea Nitrogen (mg/dL)	Serum Creatinine (mg/dL)
1st Visit	23.6±33 (0-180)	26.5±7.5 (11-42)	0.7±0.2 (0.2-1.1)
2nd Visit	11.4±7.0 (0-180)	26.7±11.3 (8-52)	0.7±0.2 (0.4-1.0)
3rd Visit	5.3±3.3 (0-31)	26.4±10.0 (8-49)	0.6±0.1 (0.5-0.8)
4th Visit	3.2±3.2 (0-20)	23.7±11.3 (11-45)	0.7±0.2 (0.5-1.0)

Table 3. Percentage of Patients with Different Severity of Skin Lesions in Each Follow-Up Visit

		Face Lesions n%	Back Lesions n%	Ash Leaf Lesions n%
1st Visit (n=22)	No Lesion	4(18.1)	5 (22.7)	0
	Low	2 (9.0)	7(32.0)	4(18.1)
	Moderate	5 (22.7)	6(27.3)	8(36.4)
	High	11 (50.0)	4(18.1)	10(45.5)
2nd Visit (n=22)	No Lesion	8 (36.4)	8(36.4)	7(32.0)
	Low	8(36.4)	11(50.0)	8(36.4)
	Moderate	5(22.7)	3(13.6)	7(32.0)
	High	1 (4.5)	0	0
3rd Visit (n=17)	No Lesion	10(58.8)	12(54.5)	7(41.2)
	Low	5(29.4)	4(18.2)	8(47.18)
	Moderate	2(11.8)	0	2(11.8)
	High	0	0	0
4th Visit (n=11)	No Lesion	8(72.7)	8(72.7)	6(54.5)
	Low	3(27.2)	3(27.2)	5(45.5)
	Moderate	0	0	0
	High	0	0	0

Discussion

TSC is a rare hereditary disease both globally and locally in Iran, while so far, there have been multiple studies evaluating the effect of various mTOR inhibitors on TSC manifestations. To our knowledge, such a study has not been conducted in Iran, and this investigation was the first prospective study assessing the effectiveness of mTOR inhibitors among Iranian patients. Besides, due to the general rarity of the disease, the authors could only recruit a few patients, and the final analysis was limited to a small sample size of only twenty-three patients.

According to the results, prescribing rapamycin (a form of mTOR inhibitor) positively reduced the numbers and sizes of brain tuberomas, renal angiomyolipomas, and skin lesions in all three anatomic categories (face, back, and café au lait lesions). Patients receiving rapamycin positively responded to the drug regarding control of epileptic episodes, with nine initially enrolled patients becoming seizure-free by the final scheduled office visit. All these findings were statistically significant and supported the findings from previous studies that mTOR inhibitors can be a solid and practical treatment choice in patients with TSC.

The primary mechanism behind rapamycin's effect on epileptic episodes is not well-known. Proposedly,this mechanism may not differ from traditional antiepileptic drugs (AEDs) with a mix of targeting ion channels, synaptic receptor function, and a direct effect on neurotransmitter release (16-19). The present study relied on parents' reports regarding the frequency of seizures and EEG

findings, consistent with other findings indicating an improvement in seizure control among patients with TSC. A clinical trial study by Krueger et al. (20) reported an increase of about 30% in the number of patients who became seizure-free by the end of the study's follow-up period.

SEGAs, accounting for a significant portion of mortality and morbidity, are essential in TSC patients (7, 21). EXIST-1 trial examined the effects of mTOR inhibitors in the treatment of SEGAs (16). This study randomly assigned patients to oral Everolimus or placebo groups. Their results showed that 35% of patients in the Everolimus arm had at least a 50% significant reduction in the volume of SEGAs after six months. The other studies later confirmed these findings, most notably those of Rosado and Kim (22, 23). Based on their results, it was shown that mTOR inhibitors could be a reasonable therapeutic option in patients with multiple SEGAs who were not suitable candidates for surgical operations.

Renal angiomyolipomas have been presented in 70-90% of TSC patients (24). The therapeutic effect of rapamycin on the treatment of angiomyolipomas was first shown by Wienecke et al. (25). They indicated a progressive reduction in tumor size by follow-up MRI imaging for a 19-year-old male TSC patient who received rapamycin. The most notable study that has evaluated the effect of mTOR inhibitors on renal angiomyolipomas is the EXIST-2 trial (10). It revealed a response rate of 42% in regression of angiomyolipomas among patients treated with Everolimus compared to those who received a placebo. As angiomyolipomas are the leading cause of mortality in TSC patients (26), the international guidelines (27) proposed mTOR inhibitors as the first line of therapy for all TSC patients with renal angiomyolipomas.

While skin lesions do not necessarily impact a TSC patient's survival, they have clear cosmetic implications and a relationship with the patient's overall well-being. At first, the positive effect of rapamycin on skin lesions was observed in patients who received the drug for other indications. In EXIST-1 and 2 trials, 42 and 26% of patients in the Everolimus group showed at least partial regression of skin lesions. The efficacy of topical rapamycin in treating skin lesions was reported. This topical agent could certainly reduce systemic adverse effects related to the drug (28-30). In this study, none of the patients had any lesion classified as severe by the time of the third and fourth visits. Moreover, the percentage of patients with no face and back lesions dramatically increased from 18% and 22% to 73% by the fourth visit. Notably, although no patient was free of café au lait lesions at the beginning of the treatment, more than half of the patients (n=6) were free of these lesions by the final visit.

TSC-associated cardiac rhabdomyomas are considered benign (31), and the spontaneous regression of tumors has been reported in more than 50% of cases (32). The authors witnessed a regression rate of around 62% in the participants. Indicatively, some patients probably benefitted from rapamycin treatment. However, more accurate conclusions about the mTOR effect on cardiac rhabdomyomas need case-control studies with larger sample sizes. The study's finding was confirmed by Saffari et al. They showed a rapid decrease in cardiac rhabdomyoma size among 13 patients with mTOR inhibitor treatment (33).

Regarding growth and development status, no statistically significant differences were observed between follow-up visits, which may relate to insufficient patients participating in the third and

fourth visits. Almost all participants were present at the first and second visits; this number fell to 17 and 11 patients in the third and fourth visits.

Previous studies on mTOR inhibitors have shown several side effects, such as stomatitis, fever, diarrhea, nasopharyngitis, pneumonia, and sometimes status epilepticus forms of seizure. The true incidence of these complications has been reported as 20-50% (18, 34), while the authors did not observe any adverse drug effects. Regarding renal function and safety profiles, measuring serum Cr and BUN levels showed no statistically significant alterations during treatment. In line with the study's findings, Franz et al. indicated non-specific adverse reactions among their treatment group (16). However, these findings may notably relate to a small sample size or a relatively short follow-up period.

Finally, the study's results showed that the therapeutic effects of rapamycin were independent of sex and age, making it an excellent option for use in almost every pediatric patient suffering from the disease.

Strength and limitations

The strength of the present study was showing the therapeutic effects of rapamycin on epileptic episodes, declines of the numbers and sizes of brain tubers, renal angiomyolipomas, and skin lesions among Iranian children regardless of age or sex. On the other hand, the study had several limitations; the small sample size was the main one. The study was conducted in two referral university-affiliated hospitals with twenty-five included patients. The authors did not follow the patients after a year. So, multicenter studies with a larger sample size, more safety profiles, and extended follow-up periods are strongly suggested. The other limitation was the need for more patients' motivation to complete the treatment course, which caused different sample sizes in the follow-up visits. Finally, no control or unexposed group was included in the study, which could provide more comparative and informative data. These limitations should be addressed in future studies.

In Conclusion

The results of the present study showed an encouraging effect of rapamycin among TSC patients. The authors noted the positive impacts of rapamycin on the numbers and sizes of brain tuberomas and renal angiomyolipomas. The frequency of epileptic episodes and the severity of skin manifestations significantly decreased. A significant regression of cardiac rhabdomyomas was observed in most affected patients who received rapamycin. Although the beneficial effects of rapamycin on the relief of TSC's manifestations had been previously proved, the study showed that these therapeutic effects were independently related to the patient's sex or age. It may be concluded that rapamycin is a great option that can be used in every patient suffering from TSC disease.

Declarations

Ethics and Consent to participate

The study was approved by the institutional review board of the TUMS and according to the Helsinki declaration. Participants' data were considered confidential.

Availability of data and materials

The corresponding author's datasets related to the study are available on reasonable request.

Author's contributions

Dr. F. D, Dr. M.Z., and Dr. Z.K. carried out the design, coordinated the study, and participated in most experiments. Dr. N.Kh. and Dr. S.D. coordinated and conducted all the experiments and data analysis. Dr. F. D and Dr. M.Z. participated in the research and manuscript preparation. All authors have read and approved the content of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest

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