Efficiency and Safety of Everolimus for Tuberous Sclerosis Complex - Associated Renal Angiomyolipoma

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Abstract

Introduction and Objectives. Tuberous sclerosis complex (TSC) is a rare autosomal dominant disease with multisystemic involvement. Renal angiomyolipomas (AMLs) affect up to 80% of TSC patients and AML hemorrhage risk increases with size. Everolimus (EVE) is a mTOR inhibitor and is the only approved drug for managing renal AMLs in the adult population. We studied a series of TSC patients who received EVE therapy to evaluate its renal efficiency and safety.

Materials and Methods. 18 patients with TSC and renal AML were enrolled, and they received 10 mg Everolimus daily. AML measurements were performed yearly, by using either computed tomography (CT) or magnetic resonance imaging (MRI). The largest baseline diameter of the biggest renal tumor was compared with the subsequent measurements. Clinical examination was performed, and biological parameters were systematically evaluated.

Results. Of the total of 17 patients who underwent efficiency assessment, 16 (94.1%) responded to EVE by renal tumor shrinkage. A significant AML reduction was observed after 12 months of treatment, from a mean of 74.5 mm to 57.3 mm (23% reduction). The primary endpoint (AML's diameter shrinkage ≥ 25% from baseline) was attained by 8 (47%) patients. In the aspect of adverse events (AE), no treatment interruptions were necessary due to intolerance. The most frequent AE was dyslipidemia, affecting 14 patients (82.3%), followed by anemia in 10 patients (58.8%). Proteinuria was the most common renal AE, which was managed by temporarily lowering the EVE dose. There were no AML-related hemorrhage and no local interventions were necessary.

Conclusions. In our experience, EVE is an efficient and well tolerated treatment for adult TSC patients and associated AML. The most important positive result observed was no new bleeding episodes, which improves both renal and general outcomes.

Key-words: Everolimus, mTOR inhibitor, renal angiomyolipoma, tuberous sclerosis complex

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Introduction and Objectives

Tuberous sclerosis complex or Bourneville's disease is a multisystemic disease that mainly affects the kidneys, brain, skin, lungs, but also other organs. It is a neurocutaneous disorder or a hamartomatosis, with autosomal dominant transmission, caused by mutations of TSC1 gene (encoding hamartin) or TSC2 gene (encoding tuberin) [1]. These proteins create a complex, thus inhibiting the protein synthesis acquired through the mechanistic target of rapamycin cascade (mTOR). Since they have tumor suppressor roles, mutations of either TSC1 or TSC2 are responsible for abnormal cell growth and proliferation. The "two hit" hypothesis states that a second somatic mutation of the other allele of the TSC genes is required for the development of disease (this phenomenon generates loss of heterozygosity – LOH) [2]. The diverse phenotypes of patients suffering of TSC is explained by this phenomenon. The cortex is affected by the formation of cortical tubers which consist of abnormally developed neurons and glial cells and are considered to be the origin of the epileptogenic discharge. In the aspect of neurological dysfunction, epilepsy, intellectual disability, behavioral abnormalities and autism are the main conditions in TSC. Subependymal giant cell astrocytoma (SEGA) causes internal hydrocephalous by obstructing ventricular foramens, while subependymal nodules (SEN) are hamartomas found in the walls of ventricles which may progress into SEGA [3]. The main cause of morbidity and mortality in adult TSC patients is represented by kidney involvement. Renal angiomyolipomas (AML) affect up to 80% of all these patients [4]. AMLs are derived from perivascular epithelioid cells and are frequently associated with cysts [5]. They are known to continuously grow and the bigger they become, the higher the hemorrhage risk is. Most AMLs are easily identified by ultrasound, magnetic resonance imaging (MRI) or computed tomography (CT) due to their fatty content. Sometimes it is difficult to differentiate an AML from a renal cell carcinoma, especially when the fatty component is low. In these situations, histological proof is needed [6]. Spontaneous bleeding is the main AML-related local complication and it is considered a serious risk in tumors exceeding 3-4 cm in diameter. Wünderlich syndrome is a life-threatening complication because of retroperitoneal bleeding, potentially evolving into hemorrhagic shock. Hemorrhage from AMLs affects one quarter to one half of all patients with renal involvement due to TSC [3]. While treatment in acute settings usually consists of arterial embolization of the artery/arteries suppling the tumor or surgical resection (nephrectomy) to control acute bleeding, systemic therapy with mTOR inhibitors is used in patients with AMLs greater than 3 cm, as preventive treatment [7]. Everolimus (EVE) is the sole mTOR inhibitor used for adult patients with renal AML and for SEGA in both adults and pediatric patients with diagnosed TSC. EVE was approved for these indications in our country in November 2014 and the first two Romanian patients started treatment with EVE for TSC- associated AMLs in our department in January 2015.

The aim of the present study was to investigate the efficiency and safety of EVE treatment in adult patients with diagnosed TSC and renal AML.

Materials and Methods

Study population. This is a single-center prospective study on 18 consecutive patients with TSC - associated renal AMLs performed between January 2015 – March 2019. Patients were previously diagnosed with TSC according to the 2012 International Tuberous Sclerosis Complex Consensus Conference [8]. The inclusion and exclusion criteria [9] are presented in *Table 1*.

Inclusion criteria	Exclusion criteria
Adult patients (age>18 years) with TSC-associated renal AML with high risk of complications (based on factors such as tumor dimension, presence of aneurysm or multiple/bilateral tumors) without need for immediate surgery intervention AML with a diameter >3 cm (based on CT/MRI) Growing AML based on serial imaging studies	Acute AML symptomatology that requires surgical intervention (including AML hemorrhage) Everolimus or sirolimus known hypersensitivity Active infections Pregnant (positive pregnancy test) or breastfeeding women Patients not able or not willing to comply to the study follow up requirements

AML - angiomyolipoma;
TSC - tuberous sclerosis complex;
CT - computed tomography;
MRI - magnetic resonance imaging.

Table 1 - Inclusion and exclusion criteria.

The study was conducted in accordance with the Declaration of Helsinki human rights. All of the patients or their legal representative (when appropriate) signed the informed consent.

Measurements. CT or MRI (same method for the same patient during the whole study duration) were performed at baseline and every year after treatment with EVE was started. Every patient had a baseline measurement of the largest AML which was afterwards compared with the diameter obtained after the initiation of EVE. The biggest baseline diameter was taken into consideration. The evolution of the AML's diameter was used for assessing the efficiency of EVE regarding the prevention of tumor growth. This reduction was evaluated by calculating the percentage of shrinkage in relation with the baseline characteristics. Adverse events were studied (as defined by Common Terminology Criteria for Adverse Events v 3.0) [10] during EVE therapy, by performing clinical and biological evaluation. Biological specimens were evaluated monthly during the first year of treatment and once every two months thereafter. Renal function was assessed by calculating the estimated glomerular filtration rate (eGFR) using the CKD-EPI formula [11] and renal function was defined using the current chronic kidney disease (CKD) nomenclature used by KDIGO [12]. The initial EVE dose was 10 mg daily, with subsequent adjustments according to the patient's tolerance. The serum concentration of EVE was measured periodically, in order to be maintained under the safety trough level of 10 ng/ml, or when a drug, known to interfere with the pathway involved in the metabolization of the mTOR inhibitor was initiated. The primary endpoints of the study were the reduction of the biggest diameter of the largest AML with at least 25% relative to baseline and the absence of new renal lesions. We defined progression of the kidney disease as the enlargement of the studied diameter with 10% or more, the occurrence of new AMLs proved by imaging methods or the appearance of AML-related hemorrhage during the administration of EVE.

Statistical analysis. All statistical analyses were made using *IBM SPSS* software for *Windows*, version 20.0 (*IBM Corporation*, Armonk, NY). Categorical variables were reported as percentages, continuous parametric variables as mean ± SD and those continuous non-parametric as median ± interquartile range.

Results

Baseline characteristics.

Characteristics	Everolimus N=18
Age (years), mean ± SD (min, max)	36.7 ± 11.6 (18-61)
Gender, n	
Female	13
Male	5
eGFR (ml/min/1.73m^2), mean ± SD (min, max)	78.8 ± 35.9 (9.2-126)
stage I CKD, n patients	9
stage II CKD, n patients	5
stage III CKD, n patients	1
stage IV CKD, n patients	2
stage V CKD, n patients	1
Longest diameter of largest renal AML (mm) mean ± SD (min, max)	74.5 ± 28.9 (24-126)
Prior nephrectomy, n patients	5
Prior AML hemorrhage, n patients	7
Duration of EVE treatment, months, mean ± SD (min, max)	32.4 ± 12.9 (5-49)
<12 months	1
12-23 months	4
24-35 months	5
≥ 36 months	9

SD - standard deviation; eGFR - estimated glomerular filtration rate; CKD - chronic kidney disease; AML - angiomyolipoma; EVE - Everolimus.

Table 2 - Baseline characteristics of the studied populations.

In *Table 2* the main baseline characteristics of the study population are presented. Most of the patients were women and many of them were young patients (mean age 36.7 years). Four patients had moderate or advanced chronic kidney disease (CKD) – stage III or higher. Seven patients had a history of bleeding complications due to renal AMLs and radical one-sided nephrectomy was performed prior to enrollment in five patients because of the severity of hemorrhage. The patients' AMLs were generally large and very large, the biggest diameter being 126 mm. Only one patient had a treatment duration of less than one year (and this patient was included only in the safety analysis), while the rest of the population was evaluated for a mean of 32.4 months, some individuals being monitored for over three years.

Efficiency of EVE treatment.

Baseline (17 patients) Longest diameter of largest renal AML (mm) mean ± SD (min, max)	74.5 ± 28.9 (24-126)
12 months (17 patients) Longest diameter of largest renal AML (mm) mean ± SD (min, max)	57.3 ± 28. (22-140)
24 months (13 patients) Longest diameter of largest renal AML (mm) mean ± SD (min, max)	51.9 ± 15.5 (20-73)
36 months (8 patients) Longest diameter of largest renal AML (mm) mean ± SD (min, max)	50.2 ± 13.8 (23-63)
Mean % reduction in AML dimension % mean ± SD (min, max)	27.7 ± 13.1(6.66-53.4)
Primary endpoint (≥ 25% AML dimensional reduction) No of patients	8
AML progression No of patients	0
AML hemorrhage during EVE treatment, no patients	0
Nephrectomy due to local complication during EVE treatment, no of patients	0
New CT/MRI- documented AMLs	0

AML - angiomyolipoma; SD - standard deviation; EVE - Everolimus; CT - computed tomography; MRI - magnetic resonance imaging.

The main results are presented in *Table 3*. Only the biggest tumor dimension was taken into account. The primary endpoint was a reduction of at least 25% of the nadir length of the largest AML diameter and it was attained in 8 patients (47% of total). Even though the rest of the individuals did not reach this endpoint, every patient presented tumor shrinkage under EVE treatment. A mean percentual reduction of 27.7 ± 13.1 in the largest diameter of AML was noticed. This dimensional reduction trend was maintained during the whole period of treatment, patients reaching their third year of EVE therapy still benefiting from tumor shrinkage. The mean AML diameter decreased from a baseline of 74.5 mm to 57.3 mm after one year (23% mean decrease), 51.9 mm after two years (30.3% mean decrease) and 50.2 (32.6% mean reduction) after three years. Three of the eight patients who reached the endpoint had a tumor shrinkage between 25-33%, while 5 of them had an AML dimensional reduction between 33%-53.4%. No individuals suffered hemorrhage from AMLs and no patients needed local intervention for the management of complications. Also, no new renal lesions were described in the studied population.

Everolimus safety. In our study no grade 3 or 4 AE were reported, and no treatment withdrawal was necessary.

Adverse events	**Number**
Dyslipidemia	14
Anemia	10
Stomatitis	8
Proteinuria	7
Gingivitis	6
Increased liver enzymes	6
Hypophosphatemia	5
Skin rash	4
Leucopenia	4
Acnea	3
Edema	3
Pruritus	2
Diarrhea	2
Anorexia	2
Irregular menses	2
Ovarian cysts	2
Upper respiratory tract infections	2
Constipation	1
Amenorrhea	1
Urinary infection	1
Other infections	1
Thrombocytopenia	1
Hyperglycemia	1
Diabetes mellitus	1

Table 4 - Adverse events frequency

The most frequent AE *(Table 4)* was dyslipidemia, noted in 14 individuals. Combined hyperlipidemia was more common than isolated hypercholesterolemia. Patients received statins and/or fibrate and the biological response was satisfactory, with the correction or improvement of this AE. Anemia was the second most observed AE, but no blood transfusions were needed, the lowest level of hemoglobin being 8.3 g/dl in one patient. Stomatitis affected 8 patients and gingivitis 6 patients, which were managed by local treatment, associated in some cases with temporary EVE dose reductions. Proteinuria worsened in seven patients. It is important to note that, even though in 4 patients the chronic kidney dysfunction progressed to the next CKD stage, we considered this to have happened due to natural evolution of the disease, rather than a supposedly negative effect of EVE on the renal function. Considering the same aspect, no patients developed AKI during therapy.

Discussions

TSC is a rare autosomal-dominant disease with an incidence of roughly 1 in 6000 live births [13]. Angiomyolipomas affect 80-90% of these patients, bleeding being the most frequent and severe local complication [14]. The rationale for using mTOR inhibitors as systemic treatment in TSC derives from the increased mTOR activity due to TSC1 or TSC2 gene mutations. This study assessed the effects of the mTOR inhibitor EVE regarding dimensional reduction of renal AML in TSC patients. Our study included more female patients than male ones, following the gender pattern described in other studies. This is thought to happen because of the hormonal factors, hamartomas having estrogen and progesterone receptors, generating a higher growth speed in women and higher AML bleeding risk [15].

Our results showed a good effect regarding tumor shrinkage, with a mean of 27.7 ± 13.1 % reduction of the biggest diameter after the first year of treatment. The main result of EVE therapy was the absence of AML hemorrhage and no need for local interventions. Another important aspect of the treatment with EVE is that the composition of the tumors changed in time, their vascular component diminished, while the fatty parts developed. These imagistic findings are important, because the risk of bleeding decreases with this pattern of evolution [16]. In case of local emergencies, embolization or even ablative procedures (partial/total nephrectomy) are necessary. The poor neurocognitive status in most patients make this kind of interventions more complicated. Also, in nearly half of the cases where embolization is performed, one procedure is not enough and the subsequent reduction in tumor bulk is only moderate. Multiple false aneurysms and parasitic-type arterial supply further limit the efficiency of embolization [7]. Considering these aspects and taking into account the greater bleeding risk of AMLs larger than 3-4 cm, oral therapy with EVE seems an appropriate alternative treatment in preventing tumor growth and diminishing hemorrhage risks. EXIST-2 [17] is a multicenter, randomized, double-blind, parallel group, placebo-controlled phase 3 study that enrolled 118 patients with TSC and at least one AML ≥ 3 cm. 79 patients received 10 mg EVE daily and 39 received placebo. The median duration of the first phase was of 9.7 months with an open-label extension phase of 46.9 months. The AML response rate (defined as over 50% AML volume reduction) increased from 42% (primary data cutoff) to 58% at the end of extension phase, with a median time to response of 2.89 months. 97% of patients had some degree of AML dimensional reduction. Even though the mean response rate in our study is lower than the one described in the EXIST-2 study (32.6% mean dimensional reduction), the same renal efficiency patterns were identified, with a continuous tumor shrinkage as the EVE treatment was maintained. In the same manner as the aforementioned study, the most important renal results were observed during the first 12 months of treatment. The absence of serious adverse events generated no treatment withdrawal in our study. In EXIST-2 study, 16 patients had AML progression, but only five of them (4.5%) discontinued treatment, the other nine continued the treatment due to observed clinical benefit. The EXIST-2 study reported reduction in the best percentage change from baseline in 98 out of 101 evaluable patients (95%), while all the patients in our study presented AML dimensional reduction.

The most frequent AEs in the EXIST-2 study were nasopharyngitis (44.5%), stomatitis (35.7%) and headache (33%) [17]. The main adverse events attributed to EVE reported in literature are stomatitis, amenorrhea, upper respiratory tract infections, hypercholesterolemia, nasopharyngitis, acne-like lesions, irregular menses, sinusitis and pneumonia. Other AE are urinary tract infections, aphthous stomatitis, cough, diarrhea, vomiting, proteinuria, hyperlipidemia etc. Hematological AE noted in the prescription drug information are neutropenia, anemia, leucopenia, lymphopenia and thrombocytopenia. Most frequent complications that reach grades 3-4

are stomatitis, amenorrhea, neutropenia, pyrexia and viral gastroenteritis [18]. Even though current guidelines for TSC management state that long-term safety studies are needed, AEs described with prolonged use of EVE rose no new safety issues. The most adverse events or serious adverse events are generally described during the first year of treatment and are generally easily handled by dose reductions. After 1 year of treatment the frequencies stabilize [18]. Our study followed the trends of EXIST-2. No severe AEs were reported, and no patient needed permanent treatment interruption due to drug toxicity. Most of the AE were mild and their management was possible while maintaining EVE therapy. The most frequent reported adverse event was dyslipidemia, affecting 14 out of the total 18 patients (77.7%). Lipid-lowering drugs were introduced, and biological response was obtained. Anemia was the second most encountered AE, affecting 10 patients (55.5%), but no blood transfusions were needed for management, dose adjustments being enough. Clinically, stomatitis was the most frequent finding in 8 patients (44.4%), along with gingivitis (33.3%). Other observed AEs were increased liver enzymes (33.3%), hypophosphatemia (27.7%), skin rash (22.2%) and leucopenia (22.2%). Renal events were generally mild and were characterized by increased proteinuria in 38.8% of patients, which reduced after dosage adjustments. Severe renal impairment due to EVE was not encountered, no patients suffered AKI and renal disease progression, if present, was more probably due to natural evolution of CKD rather than because of the impact of EVE therapy.

Conclusions

In our experience, systemic treatment with Everolimus for TSC-associated renal AMLs is efficient and well tolerated. No bleeding episodes occurred, and no embolization or surgery were needed. Angiomyolipoma size reduced in all the 17 patients who underwent renal efficiency assessment. AEs were manageable, mostly predictable and their incidence decreased over time, many of them being encountered during the first 12 months of treatment.

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