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REVIEW

Gonadal impact of target of rapamycin inhibitors (sirolimus and everolimus) in male patients: an overview

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Summary

The immunosuppressive agents target of rapamycin inhibitors (TOR-I) (sirolimus, and everolimus) have been widely used in kidney transplantation for >10 years. Up to 40% of men receiving a kidney transplant are younger than 50, and fertility as well as erectile function are major concerns. In this review, we provide a synopsis of past studies focusing on gonadal function in men treated with TOR-I, mainly sirolimus, to establish what impact they have on male gonads, and which pathophysiological pathways are involved. A PubMed search for the years 1990–2006 selected articles that focused on the gonadal impact of TOR-I. Primary outcome measures were testosterone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) levels. Secondary outcome measures were sexual function, fertility status and sperm parameters. Treatment with TOR-I results in a decrease in testosterone level, and an opposite increase in LH. Moreover, spermatogenesis seems to be disrupted by TOR-I and FSH levels are increased. Sirolimus and everolimus inhibit the activity of mammalian targets of rapamycin, a serine/threonine kinase involved in numerous cell-growth processes. Molecular mechanisms of action of TOR-I on the testis involve inhibition of a stem cell factor/c-kit-dependant process in spermatogonia. Preliminary results appear to show that TOR-I treatment has deleterious actions on the testis and impairs gonadal function after renal transplantation, but the impact of these effects are unknown.

Introduction

During the last decades, the prevalence of people with a renal transplant in industrialized countries has been progressing over the years. In Europe, the adjusted incidence rate of renal replacement treatment increased from 79 per million population (pmp) in 1990–1991 to 117 pmp in 1998–1999, i.e. 4.8% increase each year [1]. In the UK, The prevalence of renal replacement treatment increased from 157 pmp in 1982 to 626 pmp in 2002 [2]. Nowadays, 30–40% of men receiving kidney transplantation are younger than 50, and fertility as well as erectile function are of major concern. In men on hemodialysis, prevalence of sexual dysfunction and infertility is high and androgen deficiency is frequent: it is estimated that about two-thirds of men on hemodialysis have serum testosterone levels in the hypogonadal range. Sex hormonal disturbances may have an impact on cardiovascular and musculoskeletal systems. Thus, this topic should get better awareness among physicians. The cause of androgen deficiency in men on hemodialysis is multifactorial; defects exist at all levels of the hypothalamic-pituitary-testicular axis, and testosterone may be cleared by hemodialysis [3].

Kidney transplantation is a way to restore hormonal profile, fertility and to recover libido and potency even if its efficiency to restore hormonal profile and erectile function remain controversial [4–7].

Glucocorticoids decrease testosterone synthesis via gonadal steroid receptors and central inhibition. At least in animals, cyclosporine and tacrolimus exhibit a direct

toxic effect on Leydig cells and on the hypothalamic-pituitary axis. However, a recent cross-sectional study of 37 male kidney transplant patients treated with cyclosporine ($n = 21$) and tacrolimus ($n = 16$) concluded that calcineurin inhibitors have favorable effects on sexual hormone levels [follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone] and did not observe any difference in baseline hormone levels between cyclosporine- and tacrolimus-treated patients [8].

Strategies to prolong kidney-allograft survival have become the new challenge in kidney transplantation, and the development of new immunosuppressive regimens designed to reduce the risk of long-term graft loss, by minimizing calcineurin inhibitor (CNI)-related nephrotoxicity, is being strongly promoted [9].

Proliferation-signal inhibitors, the so-called mammalian targets of rapamycin inhibitors (mTOR-I), are a promising, new family of immunosuppressive drugs without nephrotoxicity, that have the capability to prevent acute allograft rejection and possibly chronic allograft nephropathy [10]. They target the proliferation of endothelial and smooth muscle cells by inhibiting both DNA and protein synthesis resulting in arrest of the cell cycle [11–13]. Discovered in early 1970s [14], sirolimus is the leader of mTOR-I family, its antiproliferative properties were demonstrated in 1989 [14–17], and the results of the first trials in transplant recipients occurred in 1996 [18,19].

More recently (after 2004), several studies have emphasized a potential impact of sirolimus on male gonadal function [20–24]. The aims of our review are [1] to highlight the effect of sirolimus and everolimus on male gonads and [2] to determine which pathophysiological pathways are involved.

Materials and methods

Literature search strategy

A PubMed search was performed for the years 1990–2006 (not limited to articles written in English) by using the following key words:

1. Immunosuppressive regimen: sirolimus/rapamycin/rapamune/everolimus.
2. Sex hormone status: testosterone/LH/FSH/sex hormone.
3. Erectile dysfunction/fertility/sperm.
4. Kidney transplantation/heart transplantation/liver transplantation.

Methods

Methodological quality of the included studies and their main results were assessed. In cases of redundant papers, we only selected the most relevant (the most recent, the one focusing more on the topic, or the one giving more details about methodology).

We considered all studies in which sirolimus or everolimus were used as an immunosuppressive agent to treat kidney transplant recipients and that focused on their sex-hormone status.

Studies focusing on other solid-organ transplants (e.g. heart, liver) were also considered.

Primary outcome measures were testosterone, FSH and LH levels.

Secondary outcome measures were sexual function, fertility status and sperm parameters.

Impact of antiproliferative agents on the male gonad

Impact of mTOR inhibitors on testosterone, LH and FSH

Table 1 summarizes the results of the main studies on the impact of mTOR-I on sex hormones.

In 2004, a German study first reported the impact of sirolimus on male gonadal function in a series of 132 heart-transplant recipients [21]. A group of 66 male heart-transplant recipients, who were taking sirolimus-based immunosuppression, with either mycophenolate mofetil or tacrolimus, were pair-matched with 66 heart-transplant recipients on a calcineurin-inhibitor-based immunosuppressive protocol. Patients were pair-matched for their age at the time of transplantation, their transplantation date, and serum-creatinine levels. The free androgen index (FAI) and the testosterone/LH ratio were also calculated. This study revealed that heart-transplant recipients treated with sirolimus had significantly lower free testosterone levels and a significant increase in the gonadotrophic hormones, LH and FSH and of SHBG (sex hormone-binding globulin). The duration of sirolimus treatment correlated negatively with the FAI and testosterone/LH ratio [21].

From the same year, the first results from male renal-transplant recipients were published by Fritsche *et al.* [20]. They performed a retrospective case–control study that compared sex-hormone levels in two groups of 28 men who differed in their use or not of sirolimus. The authors tested the influence of potential confusion factors (age, post-transplant follow-up time, creatinine levels, proteinuria, regimens) in a multiple linear regression analysis. Only sirolimus treatment provided a significant result on testosterone changes. However, their population size was small and the many concomitant immunosuppressive agents that were used in both groups (cyclosporine, tacrolimus, mycophenolic acid, and azathioprine prednisolone) rendered it difficult to assess the results [20].

In 2005, another study reported on the influence of immunosuppressive therapy on the restoration of normal

Table 1. Sex-hormone measurements of patients treated with sirolimus.

Authors	Year	Recipient	*n*	Type of study	Groups of patients	Results	*P*
Kaczmarek *et al.* [12]	2004	Heart	132	case control pair matching (age, transplantation date, serum creatinine level)	Sirolimus ($n = 66$) Calcineurin inhibitors ($n = 66$)	↓ testosterone ↑ luteinizing hormone (LH) ↑ follicle-stimulating hormone (FSH)	S S S
Fritsche *et al.* [13]	2004	Kidney	56	Case control Cross sectional	Sirolimus ($n = 28$) Calcineurin inhibitors ($n = 28$)	↓ testosterone ↑ LH ↑ FSH	S S S
Tondolo *et al.* [11]	2005	Kidney	59	Cross sectional	Calcineurin inhibitors ($n = 15$) Sirolimus ($n = 15$) Sirolimus + calcineurin inhibitors ($n = 29$)	↓ testosterone	S
Krämer *et al.* [15]	2005	Kidney	256	Prospective randomized	Combination of everolimus of 1.5 or 3 mg/day with steroids, basiliximab and low-dose cyclosporine	testosterone	NS
Lee *et al.* [10]	2005	Kidney	66	Case control Cross sectional	Sirolimus ($n = 32$) Controls ($n = 34$)	↓ testosterone ↑ LH ↑ FSH	S S S

S, significant; NS, not significant.

levels of gonadal hormones after renal transplantation. Sex hormone levels were measured after a follow-up period of 56 (+/−55) months post-transplant in a population of 59 male kidney-transplant recipients. They were divided into three groups according to the immunosuppressive regimen used: group I, calcineurin inhibitors ($n = 15$); group II, sirolimus without calcineurin inhibitors ($n = 15$); group III, sirolimus in combination with calcineurin inhibitors ($n = 29$). Even though the number of enrolled patients was limited, differences in testosterone levels were significant between the three groups. The decrease in testosterone was correlated with the use of sirolimus, the lowest testosterone level was observed in group II (sirolimus without CNIs) and the highest level in group I (CNIs without sirolimus) [24]. Recently, Krämer *et al.* studied sex-hormone levels, together with graft function and cardiovascular-risk factors, in a large-scale prospective, randomized trial ($n = 256$) of renal-transplant recipients treated with varying amounts of everolimus (1.5 or 3 mg/day) in combination with low-dose cyclosporine, basiliximab and corticoids. The two groups did not differ significantly concerning cyclosporine, basiliximab and corticoid doses. Gonadal function was evaluated before transplantation and at 6 months after by assessing serum levels of testosterone, FSH and LH. Following transplantation, an increase in testosterone concentration was observed in both everolimus groups. This increase was from 8.3 (+/−5.1) to 11.0 (+/−3.9) nmol/l in the group receiving a low concentration of everolimus (1.5 mg/day), and was from 9.2 (+/−5.6) to 9.9 (+/−3.1) nmol/l in the group receiving a more concentrated everolimus treatment (3 mg/day). Even though these results showed a tendency toward a lower increase in testosterone in those patients receiving the more concentrated everolimus regimen, the difference between the two groups was not significant. We can note that the doses applied in the two groups (2.2 vs. 3 mg/day) were very close and therefore not adequate to emphasize a weak effect of everolimus. Moreover, we do not dispose of data regarding the serum concentrations of everolimus in the two groups.

Nevertheless, the authors concluded that there was a minor inhibitory effect caused by everolimus, as suggested by the slight increase in FSH and LH, and that testosterone levels did not fully recover with increasing dose of everolimus. Erectile dysfunction was only reported in three patients (two from the 3-mg/day everolimus group and one from the 1.5-mg/day group) [22]. However, this study had no control group. In the absence of an mTOR-I-free arm, it is impossible to assess, whether everolimus has an impact on renal function. Therefore, the conclusion that everolimus has any advantage on sirolimus with regard to gonadal side effects is not acceptable.

Impact of mTOR-I on erectile function

We lack information regarding the clinical consequence of testosterone disturbances relative to sirolimus administration, notably that concerning sexual problems.

A recent cross-sectional study performed in Montefiore Medical Centre, NY, gives some initial insight into these problems. The study's population, consisting of 66 renal-transplant recipients, was divided into two groups according to their immunosuppressive treatment: group 1, sirolimus ($n = 32$); group 2, control ($n = 34$). The patients were asked about their sexual performance through a self-administered questionnaire (IIEF, 15 questions), and their sex hormone levels were measured. The authors confirmed that total testosterone was lower and FSH and LH higher in the group of patients treated with sirolimus than in those from the control group, and in multivariate analysis, only the use of sirolimus was significantly correlated with decrease of testosterone (age, race, etiology of renal failure, transplant and dialysis durations, antihypertensive and non-mammalian target of rapamycin-I immunosuppressive treatments not significant). However, even though the IIEF score was slightly lower in the sirolimus group, there was no significant difference in sexual score between the two groups (mean IIEF score: 49/75 in the sirolimus group; 52/75 in the control group). Moreover, free testosterone levels did not differ significantly between the two groups. Therefore, the correlation between testosterone variations and sexual dysfunction needs to be further investigated in a larger population to assess the clinical impact of sirolimus use [23].

Impact of mTOR-I on fertility

Regarding the impact of sirolimus on sperm, we have recently published a case report on a 36-year-old male kidney-transplant recipient treated by an immunosuppressive regimen that contained sirolimus. He presented with dramatic, reversible sperm impairment. This man, who was fertile before transplantation, received initially (for 3 months) 2 mg/day of sirolimus associated with 10 mg/day of prednisone, 200 mg/day of cyclosporine, and then for 33 months 7 mg/day of sirolimus and 10 mg/day of prednisone. Serum sirolimus level during the study period remained within the normal range (10–15 ng/ml). After this time, the patient's wife had failed to become pregnant and a sperm analysis revealed a low sperm count, and a decrease in motility, vitality and percentage of normal sperm. Replacement of sirolimus (sirolimus by tacrolimus) was followed by complete normalization of the sperm parameters. Even if this case does not prove that sirolimus is responsible for such quantitative and qualitative sperm impairment, no other factors were found to explain this result (this patient had testosterone and FSH levels within the normal range, he had conceived a child 2 years before transplantation, and another pregnancy had occurred during the year before transplantation) [25].

Experiments in rats using sirolimus have shown no effects on fertility in females, and in male rats, there was no significant difference in fertility rate compared with the controls at a dosage of 2 mg/kg (approximately 4–11 times the clinical doses adjusted for body surface area in humans). However, reductions in testicular weight and/or histological lesions (e.g. tubular atrophy and tubular giant cells) were observed in rats following the dosages of 0.65 mg/kg (approximately 1–3 times the clinical doses adjusted for body surface area in humans) [18]. In a monkey study, similar testicular lesions were observed after administration of 0.1 mg/kg (approximately 0.4–1 times the clinical doses adjusted for body-surface area in humans) [18]. Regarding sperm count, reduction was observed in male rats following the administration of sirolimus for 13 weeks at a dosage of 6 mg/kg (approximately 12–32 times the clinical doses adjusted for body-surface area), but the counts showed improvement by 3 months after dosing was discontinued [26].

Therefore, a direct link between sirolimus and spermatogenesis must be suspected, and further studies are strongly encouraged to confirm these preliminary results.

Pathophysiological pathways

The molecular mechanisms by which sirolimus induces a decrease in testosterone level and sperm impairment still remain partly unknown.

Sirolimus binds to FK BP12, and this complex then binds to the mammalian target of sirolimus (mTOR), which is a major regulatory kinase. The sirolimus-FK BP12– mTOR complex acts mainly by two pathways: [10] (i) inhibition of p70 S6 kinase, which is a key molecule in cytokine-induced proliferation and (ii) inhibition of the enzymatic activity of the cyclin-dependant kinase, cdk2-cyclin E complex, which functions as a regulator of the G1/S transition.

Such mechanisms occur in T cells; however, sirolimus also blocks B-cell activation [27] and inhibits growth-factor-stimulated proliferation of parenchymal cells, such as fibroblasts and smooth muscle cells [28].

A recent study demonstrated that c-kit-induced activation of phosphatidylinositol 3-kinase is essential for spermatogonial proliferation and male fertility. Feng *et al.* [29] have described the mechanisms of action of sirolimus in testis (Fig. 1). These authors found that sirolimus plays a central inhibitory role in a stem cell factor (SCF)/c-kit-dependant process in spermatogonia via the phosphoinositide 3-kinase (PI3-K)/(AKT)/p70S6K pathway. c-kit belongs to the platelet-derived growth-factor family receptor of receptor tyrosine kinases and is a major regulator of spermatogenesis. In the testes, c-kit is predominantly expressed in type A spermatogonial cells. The ligand

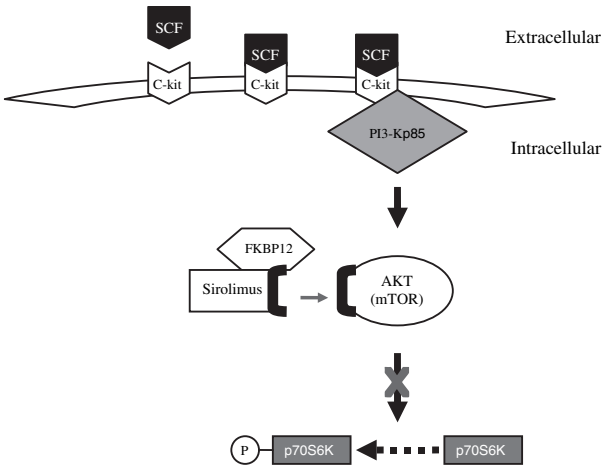

Figure 1 Mechanisms of action of sirolimus.

of c-kit is the SCF. The SCF is expressed by sertoli cells. In response to SCF, c-kit may trigger multiple signaling pathways to regulate the proliferation and/or differentiation of the spermatogonia. SCF induces the binding of the PI3-K p85 subunit to c-kit. p70S6K is phosphorylated on Thr- 389 by SCF/c-kit through the wortmannin and sirolimus-sensitive pathway in this type of cell. By using co-transfection experiments, Feng *et al.* [29] have demonstrated that SCF/c-kit recruits the PI3-K/mTOR pathway to activate p70S6K; the latter interacts with the cell-cycle machinery to induce spermatogonial proliferation. The cell cycle through the G1/S checkpoint is regulated by multiple mitogenic signaling pathways, including Ras-p42/p44 mitogen-activated protein kinase, PI3-K/p70S6K, and the PI3-K-dependent sirolimus-insensitive pathway. Feng *et al.* also performed a bromodesoxyuridin (BrdUrd) experiment, which indicated that the SCF/c-kit-induced spermatogonial cell proliferation was completely abolished by sirolimus. The mechanisms by which sirolimus blocks BrdUrd incorporation induced by SCF is probably caused by the inhibitory function of sirolimus on cyclin D3 expression and phosphorylation of Rb, both of which are necessary to promote the cell-cycle progression that is induced by SCF in spermatogonia. SCF/c-kit induces the sirolimus-sensitive PI3-K/p70S6K/cyclin D3 pathway by regulating the cell-cycle progression and growth in spermatogonial cells.

Another study found that the disrupted binding of PI3-K to c-kit resulted in defective oogenesis and spermatogenesis. The males were found to be sterile due to a block in the early stages of spermatogenesis [30].

A study of the effect of sirolimus in a cultured pituitary gonadotrophic cell line that expresses the gonadotrophin-releasing hormone (GnRH) receptor and responds to GnRH stimulation (aT3–1) showed that, compared with untreated cells, aT3–1 cells pretreated with sirolimus had 49% less activation following GnRHa stimulation. Sensitivity to sirolimus by aT3–1 cells was explained by an involvement of mTOR in GnRH signaling [31].

Discussion

The role of testosterone in male sexual function is double: testosterone is the main hormonal mediator of a man's libido (central action) [32] and it also has a major role in stabilizing the levels of intracavernosal NO synthase (NOS), the enzyme responsible for triggering the nitric oxide (NO) cascade required to have an erection (peripheral action) [33]. Testosterone circulates in plasma nonspecifically bound to albumin, specifically bound to SHBG and, in a small percentage unbound (free testosterone). The sum of free and non-SHGG-bound testosterone is generally referred to as 'bioavailable' testosterone. So far, it is controversial which testosterone assay is most appropriate in the diagnosis of low testosterone levels, especially in aging males [34]. The Endocrine Society recommends making an initial diagnosis of androgen deficiency and confirming this diagnosis by using morning measurement of total testosterone level. Accurate and reliable assays for free or bioavailable testosterone measurements usually are not available in local laboratories [35].

The latest studies describe the action of mTOR-I and its different biochemical pathways. In summary, upon stimulation with stem cell factor, c-kit recruits the sirolimus-sensitive PI3-K/p70S6K pathway to induce cyclin D3 expression and phosphorylation of Rb, which leads to spermatogonial proliferation. AKT is the main transducer that links c-kit/PI3-K to p70S6K and is also important for spermatogonial proliferation.

All data in transplant recipients who received TOR-I are consistent with sirolimus-related testosterone suppression, In contrast, FSH and LH concentrations were increased. Even if the level of evidence of clinical studies analyzed in this review is lowered by the possibility that the patients studied take other drugs (such as beta-blockers, ACE-I and diuretics) that may influence sex hormone levels, the observed pattern characterizes a hypergonadotrophic hypogonadism, which strongly suggests a direct action of sirolimus on the gonad. However, recent data indicate that the point of action may be double: central (pituitary) and peripheral (gonad).

Despite the large number of patients routinely treated by sirolimus-containing immunosuppressive regimens, there is a lack of clinical data concerning the impact of sirolimus on erectile function and fertility. The early data show a decrease in testosterone levels observed in patients treated by sirolimus, but impairment of sexual function

cannot be ascertained. For everolimus, which is also an mTOR-I, there have been no clear studies to show its clinical impact on patients with transplants. Therefore, it is impossible to assess if everolimus is less toxic to the gonads than sirolimus, and there is a need for a comparison in terms of sex-hormonal status and erectile function between renal-graft recipients treated with everolimus and those treated with sirolimus.

Knowledge of short- and long-term benefits and the side effects of sirolimus and everolimus in their various uses as immunosuppressives still remain to be completed. Hence, there is an urgent need for randomized-control trials examining the evolution in time of sex hormones, sperm count and erectile function (using a validated questionnaire, such as the IIEF score).

There has been considerable variability in the use of immunosuppressive agents by clinicians, both in their combinations and dosage regimens. Various immunosuppressive regimens, and their combinations, have to be better defined to determine if a dose effect exists. There is a concern that newer drugs or combinations, whilst apparently improving early graft outcome, may in fact increase the risk of impotence or infertility. Both transplanted patients and physicians should be aware of this potential deleterious effect, as long as we do not have evidence based on randomized-control studies on this topic.

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