Role of Immunophilins in Recovery of Erectile Function after Cavernous Nerve Injury

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ABSTRACT

Introduction. Immunophilin ligands provide potentially new alternatives for the treatment of erectile dysfunction (ED), which occurs after injury of the cavernous nerves (CNs).
Aim. To review and update current knowledge of the neurotrophic effects and likely mechanism of action of immunophilin proteins with emphasis on the FK506-binding protein (FKBP) subfamily and the role of immunophilin ligands for the treatment of CN injury-induced ED.
Methods. Review of available reports of studies investigating the effects and neurotrophic mechanisms of immunophilin ligands involved in erectile function recovery in rodent models of CN injury.
Main Outcome Measures. Erection parameters and molecular correlations associated with CN injury and functional recovery.
Results. Treatment with prototype immunosuppressive immunophilin ligands FK506 (FK) and rapamycin (Rapa) improve erectile function in animal models of CN injury. Similarly, non-immunosuppressive analogs such as GPI-1046 and FK1706 are effective in recovery of erections after CN injury. Neuronal nitric oxide may influence the erection recovery effects of immunophilin ligands after CN injury. FKBPs 38 and 65 expression changes in the penis and its innervation coincide with the neurotrophic effects of immunophilin ligands. Antioxidative actions of immunophilin ligands contribute to their neurotrophic effects. Immunophilins are localized to nerves coursing in human prostate and penile tissue.
Conclusions. The findings support the hypothesis that immunophilin ligands, working through specific receptor mechanisms that are specific to injured CN, are potentially useful to sustain erectile function in men following radical prostatectomy. **Sezen SF, Lagoda G, and Burnett AL. Role of immunophilins in recovery of erectile function after cavernous nerve injury. J Sex Med 2009;6(suppl 3):340–346.**

Key Words. Erectile Dysfunction; FKBP; Nitric Oxide; Cavernous Nerve; Crush Injury

Introduction

Erectile dysfunction (ED) continues to be a major complication following radical prostatectomy (RP) that impacts the patient's quality of life [1,2]. With the acknowledgment that penile neuropathy is a major pathogenic basis for ED in patients after RP, there has been great interest to promote neuroprotective and nerve regenerative strategies for facilitating functional recovery. In this regard, investigators have sought to evaluate the neurotrophic effects of immunophilin proteins and immunophilin ligands mostly in preclinical cavernous nerve (CN) injury studies for the purposes of developing a potential basis for the treatment of neurogenic ED. In this article, we will review the current literature in this field and briefly present new findings from our laboratory, which contribute toward understanding the mechanisms of immunophilin actions and the beneficial effects of immunophilin ligands in this setting.

Immunophilin Proteins and Ligands

Immunophilins are a large group of cellular proteins that were initially identified as targets for drugs like FK506 (FK), cyclosporine, and

rapamycin (Rapa) [3,4]. Based on the selectivity of this binding, one of the main families of immunophilins are FK506 binding proteins (FKBPs), which act as receptors for FK and Rapa. Several FKBPs have been identified in various cellular and subcellular locations (cytosol, endoplasmic reticulum, mitochondria, and nucleus) [5]. To date, more than 15 FKBP sequences have been identified in the human genome that are expressed in a variety of cell types, and they are designated according to their molecular weights, which range from 12 to 135 kDa [5–7]. All FKBPs have a PPIase (peptidyl-prolyl *cis-trans* isomerase) domain that regulates the folding/unfolding of other proteins. However, the domain compositions differ in different FKBPs (e.g., DNA-binding domains, calmodulin-binding domain, transmembrane motifs, or nuclear localization signal) [3,6]. Reflecting the diversity of their structures, FKBPs are multifunctional proteins that act as chaperone proteins, regulate protein folding, and participate in intracellular protein trafficking. FKBPs are proposed to be the modulator proteins of receptors in a cell. For example, FKBP12 is associated with inositol 3-phosphate and ryanodine receptors present on the endoplasmic reticulum and plays a role in calcium release [8]. Similarly, FKBP12 modulates the activity of transforming growth factor (TGF)-β. On the other hand, FKBPs 52 and 51 are known to be components of unliganded steroid receptor heterocomplexes, an interaction that occurs via binding heat shock protein 90 by tetratricopeptide repeat (TPR) domains of FKBPs [9]. It has been suggested that FKBP52 plays a role in the nuclear translocation of steroid hormone receptors [10]. Moreover, FKBPs 38 and 65 are involved in the regulation of cellular functions such as transcription, apoptosis, and inflammatory responses [11–13].

FK (tacrolimus) and Rapa (sirolimus) are two of the prototype immunophilin ligands that are natural macrolide compounds discovered in 1984 as a product of *Streptomyces tsukubaenis* and in 1971 as a product of *Streptomyces hygroscopius*, respectively. Both drugs have been routinely used to prevent allograft rejection following transplant surgeries because of their potent immunosuppressive effects [14,15]. The immunosuppressive effects of FK and Rapa are mediated by binding to FKBP12, one of the small cytoplasmic proteins of the FKBP family, in immune cells, thereby inhibiting their activation [14]. Moreover, both drugs can bind to other FKBPs with varying affinities, although the effects mediated by other FKBPs are not completely understood [16].

Neurotrophic Effects of Immunophilins

Beyond their expressions in immune cells and role in immunosuppression, immunophilins are found to be enriched in the nervous system. Steiner et al. discovered that the FKBP12 level in the rat brain is 10- to 15-fold higher than its expression in immune tissues, suggesting that these receptor proteins and their ligands exert important roles in the nervous system [17]. These investigators further demonstrated regenerative effects of FK by the enhancement of neurite outgrowth in cultures of the rat PC12 cell line and sensory ganglia treated with this drug [18,19]. Neuroprotective effects of immunophilin ligands were also demonstrated in a number of animal studies. In particular, in animal models of focal cerebral ischemia, stroke, and sciatic nerve injury, treatment with FK provided protection against nerve injury and enhanced functional recovery [20–22].

Effects of Immunophilins after CN Injury

Based on solid evidence from in vitro and in vivo studies of the neurotrophic effects of immunophilin ligands, the potential benefit of these drugs for the recovery of erectile function following RP has been investigated in animal models of CN injury. We first demonstrated in a rat model of partial CN injury that treatment with FK (1 mg/kg/day, ip) improved the erectile response induced by electrical stimulation of CN at 1, 3, and 7 days after injury [23]. When the cross sections of CNs were examined under electron microscopy, we also found that FK treatment significantly increased the number of surviving unmyelinated axons at all time points after injury, clearly demonstrating the neuroprotective effects of the immunophilin ligand treatment. In other studies, it was also found that FK was highly effective in maintaining erectile function in a more severely injured rat model involving focal transection or bilateral crush of the CN [24,25]. We have further tested the effects of Rapa in a rat model of bilateral CN injury. Treatment with Rapa (2 mg/kg/day, sc) produced an approximate 50% increase in erectile responses measured as the maximum intracavernous pressure (ICP) and total ICP at 1 and 7 days after injury (unpublished data). Currently, an ongoing clinical trial (phase IV, randomized, double-blind, placebo-controlled) is evaluating the safety and effectiveness of FK in the prevention of ED in men following bilateral nerve-sparing RP [26].