

Tacrolimus Treatment in Myasthenia Gravis

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Abstract: Tacrolimus (FK506) is a new macrolide immunosuppressant isolated from *Streptomyces tsukubaensis* that acts by a variety of different mechanisms, including inhibition of calcineurin. Although the mechanism of action of tacrolimus is similar to that of ciclosporin A, tacrolimus is 10 to 100 times more potent. Tacrolimus also has an effect on glucocorticoid receptor-mediated gene expression. The therapeutic efficacy of tacrolimus in primary immunosuppression has been evaluated in numerous clinical trials in patients receiving hepatic, renal, heart, lung, pancreas, intestinal, or bone marrow transplantation. Because tacrolimus displays a variety of different mechanisms of action and shows a synergism with corticosteroids, it is presumed that tacrolimus provides a new and useful therapeutic approach for autoimmune diseases, including myasthenia gravis.

1. INTRODUCTION

Myasthenia Gravis (MG) is an autoimmune disorder of neuromuscular transmission primarily caused by auto-antibodies specific to the human nicotinic acetylcholine receptor (AChR), and is characterized by fatigability and weakness of the striated muscles [1, 2]. The prevalence of MG in Japan has been estimated to be 51 per 1 million persons [3]. Autoimmune MG is probably the best understood of the human autoimmune diseases, and our knowledge of its pathogenesis and treatment has increased [4, 5]. The thymus gland is thought to play an important role in the pathogenesis of MG. A large number of young-onset MG patients have been found to have a hyperplastic thymic medulla containing AChR-antibody-producing cells, sensitized T cells, and antigen-presenting muscle-like myoid cells [2]. These findings suggest that the primary sensitization against muscle antigen in MG takes place in the thymus.

Anti-cholinesterase inhibitors provide symptomatic improvement for a time in most patients with MG and they are often used as the initial therapy. This group of drugs hinders the hydrolysis of ACh at the neuromuscular junction, and has no alteration of the immunologic activity involved in generation of the disease symptoms. In many cases, treatment with anti-cholinesterase inhibitors is not sufficient as a sole therapy. Thymectomy and/or immuno-suppressant medication, in particular corticosteroids (CSs), are commonly employed to improve MG symptoms and to prevent destruction of the neuro-muscular junction by AChR-antibodies, although oral anti-cholinesterase inhibitor treatment is useful as adjunctive symptomatic therapy. However, a subset of MG patients cannot achieve remission even after thymectomy and administration of CSs, or cannot take enough doses of CSs because of their adverse side effects.

Currently, MG is treated with several immuno-modulating or immunosuppressive agents, including azathioprine, ciclosporin, and cyclophosphamide [6]. Tacrolimus (FK506) is a new and unique macrolide immunosuppressant isolated from

Streptomyces tsukubaensis [7]. Fig. (1) shows the chemical structure of tacrolimus. Tacrolimus acts through inhibition of the calcium-calcineurin pathway, interfering with T lymphocyte activation and transcription of inflammatory cytokine genes, including interleukin-2 (IL-2) (Fig. 2). Although the mechanism of action of tacrolimus is similar to that of ciclosporin A [8], tacrolimus is 10 to 100 times more potent. Actually, tacrolimus has already been used successfully for transplantation [9-13], rheumatoid arthritis [14, 15], polymyositis [16], and systemic lupus erythematosus [17, 18]. Additionally, *in vitro* studies have indicated that tacrolimus may enhance CSs effects [19, 20]. Thus, low doses of tacrolimus are expected to be effective in the treatment of autoimmune disorders, especially in steroid-dependent patients. I summarize herein the current understanding of the efficacy of tacrolimus in treating MG.

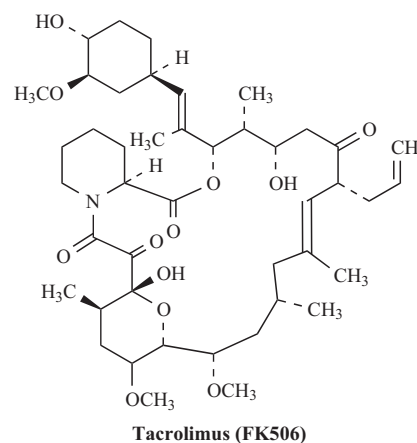


Fig. (1). The chemical structure of tacrolimus (FK506).

2. PHARMACODYNAMIC PROPERTIES

The immunosuppressive effects of tacrolimus are considered to result primarily from an interference with T-cell function. Fig. (2) shows the mechanism of tacrolimus on T cells.

Tacrolimus is a macrolide immunosuppressant that acts by a variety of different mechanisms, including inhibition of calcineurin. Tacrolimus forms complexes with immuno-

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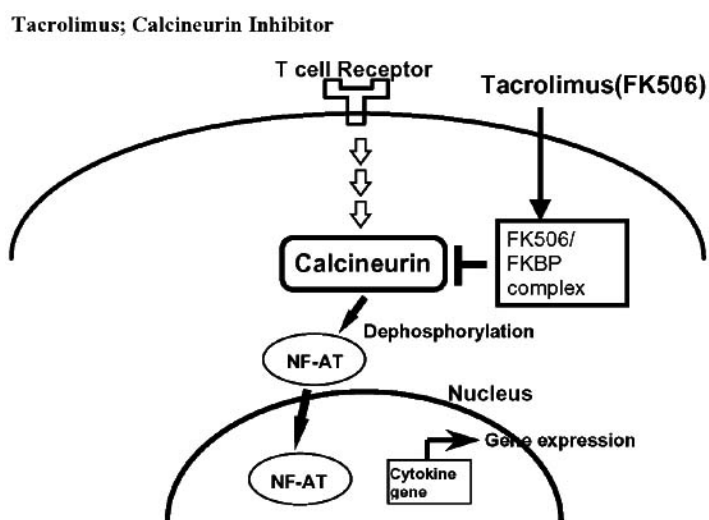


Fig. (2). Tacrolimus (FK506) binds to an intracellular protein, FK binding protein (FKBP). A complex of FK506-FKBP, calcium, calmodulin, and calcineurin is then formed, and the phosphatase activity of calcineurin is inhibited. This effect may prevent the dephosphorylation and translocation of nuclear factor of activated T cells (NFAT), a nuclear component thought to initiate gene transcription for the formation of cytokines. The net result is the inhibition of T-cell activation.

philins called FK-506 binding proteins (FKBP)-12 in the cytoplasm; immunosuppressive activity is primarily mediated by complexes formed with the FKBP-12 isoform. Tacrolimus-FKBP 12 complex binds to and inhibits the activity of calcineurin (a calcium- and calmodulin-dependent protein phosphatase). This inhibition causes a downregulation of the signal transduction pathways in T cells.

Via inhibition of calcineurin, tacrolimus interferes with translocation to the nucleus of various nuclear factors involved in the transcription of cytokines. Affected factors include the cytosolic subunit of nuclear factor of activated T cells and NF κ B. Transcription of early T-cell activation genes is suppressed, affecting the production of interleukin-2 (IL-2), IL-3, IL-4, IL-5, IFN- γ , granulocyte-macrophage colony-stimulating factor (GM-CSF), tumor necrosis factor- α (TNF- α), and proto-oncogenes such as *c-myc* and *c-rel*. Gene transcription is also inhibited by modulation of cyclic adenosine monophosphate (cAMP)-responsive element binding protein activity. Transcription of pro-inflammatory cytokine genes may be inhibited by heat shock protein-mediated interactions between tacrolimus and the glucocorticoid receptor (GR), which enhance translocation of the GR to the nucleus and potentiate binding to response elements. Tacrolimus may similarly interact with progesterone receptors to activate genes promoted by the progesterone response element.

Inhibition of calcineurin by tacrolimus interferes with activation of other enzymes, including phosphatase 1, cAMP-dependent protein kinase, and nitric oxide synthetase. Thus, cellular effects such as cell degranulation and apoptosis in leukocytes are affected.

3. PHARMACOKINETIC PROPERTIES

The pharmacokinetic properties of tacrolimus can vary widely between individuals, and dosage regimens are titrated

according to clinical signs and the concentrations of whole-blood trough drugs like cyclosporin. Generally, the daily tacrolimus dose for MG in Japan is approximately 3.0 mg, and tacrolimus blood trough concentrations remain stable at approximately <10 ng/mL.

Absorption of tacrolimus from the GI tract is variable and incomplete after oral administration, with bioavailability of approximately 25% [21]. Food has a significant effect in reducing the rate and extent of absorption. The drug binds extensively to erythrocytes, and whole-blood concentrations of tacrolimus are approximately 15 to 35 times those measured in plasma. Tacrolimus is almost completely metabolized prior to elimination. Metabolism is *via* 3A4 isoenzymes of the cytochrome P450 (CYP) system, primarily in the liver but also in the intestinal mucosa, and a number of metabolites are formed. The elimination half-life of tacrolimus has been reported to be approximately 12 hours in liver transplant recipients and 19 hours in renal transplant recipients. The primary elimination route for tacrolimus and its metabolites is *via* the biliary tract, and less than 1% of an intravenous dose of tacrolimus is eliminated unchanged in the urine. Tacrolimus is subject to a number of pharmacokinetic and pharmacodynamic drug interactions of potential clinical significance, including those involving other drugs metabolized by the CYP enzyme system.

4. THERAPEUTIC EFFICACY

The therapeutic efficacy of tacrolimus in primary immunosuppression and rescue therapy has been evaluated in numerous clinical trials in patients receiving hepatic, renal, heart, lung, pancreas, intestinal, or bone marrow transplantation. In most clinical trials, tacrolimus-based primary immunosuppression initially has included concomitant administration of CsAs, typically with azathioprine or mycophenolate mofetil, and sometimes with adjunctive antilymphocyte antibody induction therapy.

Tacrolimus is also effective in patients with myasthenia gravis. As a primary prophylaxis, tacrolimus is usually initially administered orally at dosages of approximately 3 mg/day for adult MG patients, with subsequent dosage titration to maintain blood trough levels within a therapeutic range (generally <10 ng/ml for MG but <20 ng/ml for transplantation). The efficacy of tacrolimus has been verified in an open-labeled clinical study of intractable, systemic MG patients conducted in Japan [22].

The main immunosuppressive mechanisms of tacrolimus are to inhibit transcription of IL-2 through the inhibition of a calcineurin-mediated pathway [8, 23], and a recent paper indicates that low-dose tacrolimus is effective only in MG with high interleukin-2 (IL-2) productivity [24], although patients were clinically followed for just 1 month. But our small amount of data regarding IL-2 productivity indicates that low-dose tacrolimus can be effective for patients with even normal or low IL-2 productivity [25]. It is known that tacrolimus enhances corticosteroid (CS) receptor-mediated gene expression *in vitro* [19] and, interestingly, that enhancement does not occur through the inhibition of a calcineurin-mediated pathway [20]. Therefore, low-dose tacrolimus might function in MG not only through the inhibition of calcineurine but also through an enhancement of CS effects. Combination therapy with low-dose tacrolimus and low-dose CSs might be a rational therapeutic strategy for minimizing side effects and simultaneously for producing synergistic therapeutic effects on MG. Some patients have been able to discontinue CS [25], suggesting that tacrolimus might replace CS as a sole immunosuppressive agent for MG treatment [22, 24, 26, 27].

In a preliminary open trial with tacrolimus, an immunosuppressant and enhancer of ryanodine receptor (RyR)-related sarcoplasmic calcium release, the authors observed sustained benefits in anti-RyR-positive MG patients [28]. Tacrolimus acts on RyR-related sarcoplasmic Ca²⁺ release to potentiate excitation-contraction coupling in skeletal muscle [29], as shown by an increased muscle contractile force using an isometric strain gauge [30]. Due to these dual actions but not immunological mechanisms, tacrolimus treatment in MG might have early benefits in MG patients, especially in cases with anti-RyR antibodies.

5. TOLERABILITY

The principal adverse effects associated with tacrolimus treatment in major transplant trials have included nephrotoxicity, neurotoxicity, disturbances in glucose metabolism, gastrointestinal tract disturbance, and hypertension [10-13]. Hyperglycemia is a well-recognized complication of immunosuppression with tacrolimus [31]. Tacrolimus may reduce insulin secretion from β cells of Langerhans islet of pancreas. Susceptibility to infection and malignancy is also increased. However, low-dose tacrolimus treatment in MG appears to be associated with a much lower incidence of such adverse side effects than in transplant cases.

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