The Hsp90-binding immunophilin FKBP52 enhances neurodifferentiation and neuroregeneration in murine models

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The term immunophilin involves a family of proteins whose domain shows peptidyl-prolyl-(*cis*/*trans*)-isomerase (PPIase) enzymatic activity, i.e., the reversible *cis*/*trans* interconversion of Xaa-Pro bonds (Annett et al., 2020). The PPIase domain of these proteins usually binds to immunosuppressive drugs, such as the macrolide FK506 (referred to as the FKBP subfamily) or the cyclic undecapeptide cyclosporine A (called CyP subfamily). The binding of the drug implies the inhibition of the PPIase enzymatic activity. Those members of each subfamily that show the smallest molecular weight (i.e., FKBP12 and CyP17/CyPA) are the only proteins responsible for the immunosuppressive action of the cognate drug due to the abrogation of calcineurin (or protein-phosphatase 2B) biological action. This prevents the nuclear translocation of phospho-NFAT, a transcription factor that induces the expression of interleukins and interferon-γ, both factors being critical components of the cell-mediated immune response. In contrast to those two low molecular weight immunophilins, larger members of the family such as the heat-shock protein of 90 kDa (HSP90)-binding immunophilins are not related to immunosuppression and are characterized by the additional presence of degenerate sequences of 34 amino acids repeated in tandem arrays, the TPR domains, through which they interact with dimers of the molecular chaperone HSP90 and the associated cochaperone p23 (**Figure 1A**). Among them, FKBP51 and FKBP52 are studied well since they were first described associated with the HSP90-based chaperone heterocomplex of steroid receptors (Storer et al., 2011). Both immunophilins share 75% of amino acid similitude and bind FK506 with equivalent *Ki*. They play regulatory roles in the steroid-dependent retrotransport of corticosteroid receptors, the translocation of the receptor through the nuclear pore complex, and the hormone-dependent transcriptional regulation (Zgajnar et al., 2019; Mazaira et al., 2020). Usually, both immunophilins show antagonistic action. Thus, FKBP52 favors glucocorticoid binding to the glucocorticoid receptor as well as the active transport of glucocorticoid receptor and other factors (NF-kB, nuclear factor kappa-light-chain-enhancer of activated B cells; hTERT, human telomerase reverse transcriptase; p53, tumor protein of 53-kDa) throughout the cytoplasm to the nucleus using dynein/dynactin motors to power that movement. The motor complex interacts with the PPIase domain of FKBP52, but not with that of FKBP51 (Storer et al., 2011). In addition to its role in protein trafficking, FKBP52 also enhances the steroid-dependent transcriptional activity of steroid receptors and NF-kB, whereas its partner FKBP51 shows inhibitory effects (Lagadari et al., 2016; Daneri-Becerra et al., 2019). In the nervous system, the expression of FKBP51 and FKBP52 is noteworthy in both neurons and glial cells.

Previous studies by the Bruce Gold Laboratory revealed that the macrolide FK506 (also called tacrolimus) increases neurite outgrowth of undifferentiated cells. The mechanism was unclear and remained poorly understood. Recently, although it was evident that it was irrelated to the immunosuppression mechanism since neurodifferentiation also occurred when FKBP12-KO mice were assayed, as well as when FK506 synthetic derivatives lacking immunosuppressive action were tested (Gold et al., 2004). This excluded the implication of the immunosuppression axis and demonstrated that the effect is independent of calcineurin inactivation. More recently, it was observed that in undifferentiated neurons, the FK506-binding immunophilin FKBP52 locates in perinuclear structures associated to HSP90 (**Figure 1B**). Upon cell stimulation with FK506 in a medium without trophic factors, including serum, that ring-like perinuclear structure disassembles and FKBP52, HSP90 and p23 rapidly spreads throughout the cytoplasm. This very early event of the neurodifferentiation process has two consequences for the undifferentiated cell: a) those genes located in the perinuclear ring area become transcriptionally active (as shown by the accumulation of early mRNAs), which suggests a repressive on the architecture of that chromatin for the p23•$(HSP90)_2$•FKBP52 heterocomplex, and b) microtubules become depolymerized in those areas where FKBP52 concentrates, such that the cytoplasm becomes more "fluid" and consequently versatile in areas where the nascent axon or incipient dendrites are developed (Quintá et al., 2010; Quinta and Galigniana, 2012). Interestingly, the mere overexpression of FKBP52 already stimulates the spontaneous differentiation of cells not exposed to any trophic factor, whereas the overexpression of FKBP51 antagonizes the FKBP52 action at all levels. These original observations *in vitro* in cell culture systems gained more relevance when FKBP51-KO and FKBP52-KO mice were recently used as *in vivo* model (Daneri-Becerra et al., 2020). As expected, the FKBP52 knock-out impaired the action of the immunophilin ligand and significantly delayed the spontaneous recovery of the locomotor activity of mice (**Additional file 1**). On the other hand, the knock-out of the inhibitory immunophilin FKBP51 greatly enhances both neurodifferentiation and neuroregeneration upon mouse treatment with as a low dose as 10 µg FK506/kg of body weight. This suggests that the inhibitory neurotrophic action of FKBP51 is also triggered by FK506 binding, such that in a normal situation, the final biological effect is the outcome of the effects of both immunophilins, the prevailing effect being neuroregeneration (see FK506-treated wild-type mice *versus* vehicle-treated mice in **Additional file 1**). This key observation opens the possibility to design a potential combined treatment where each immunophilin is properly targeted, i.e., FKBP52 with a neuroregenerative ligand

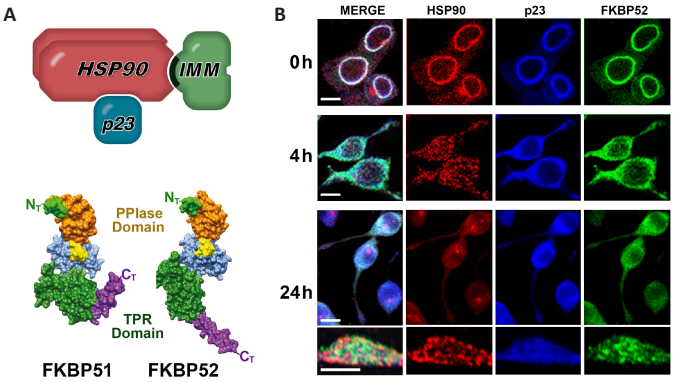

Figure 1 | **HSP90-binding immunophilins (IMM) rule the early events of neurodifferentiation.**
(A) IMM form heterocomplexes with a dimer of the molecular chaperone HSP90 and its associated cochaperone, p23. Binding to HSP90 dimers occurs via TPR (tetratricopeptide repeats) sequences located at the C-terminal end of the IMM. The macrolide FK506 (tacrolimus) binds to the IMM at the peptidyl-prolyl isomerase (PPIase) domain located at the N-terminal end. The molecular surface depictions of the X-ray crystallographic structures for the highly homologous hFKBP51 and hFKBP52 are shown at the bottom of the panel. (B) Confocal microscopy images showing the subcellular localization of the p23•$(HSP90)_2$•FKBP52 heterocomplex (40× magnification) in undifferentiated N2a cells (0 hour) and its cell redistribution after 4 and 24 hours of exposure to 1 µM FK506 in a medium lacking any other trophic factor (including serum). Note the rapid acquisition of a neuron-like phenotype of the cells. This property is observed in both undifferentiated cell lines such as N2a or SH-SY5Y and in primary cultures of undifferentiated hippocampal embryonic cells. The lower panel (100× magnification) shows the distribution of the heterocomplex in a ramification body. Note the central distribution of FKBP52 with respect to HSP90. All scale bars: 10 µm. Figure 1B is reprinted with the permission from Quinta et al. (2010). FKBP51: FK506-binding protein of 51-kDa; FKBP52: FK506-binding protein of 52-kDa; HSP90: 90-kDa heat-shock protein.

that is uncapable to interact with FKBP51, and a specific ligand for FKBP51 capable of preventing its inhibitory action without affecting the stimulant action of FKBP52.

From the mechanistic viewpoint, the activation of the ERK pathway is a clear requirement that follows cell stimulation with FK506 (Gold et al., 2004; Quinta and Galigniana, 2012). Upon peripheral nerve injury, the distal nerve segment undergoes Wallerian degeneration, a process where axons and myelin degenerate creating an empty tube for axons and Schwann cells in the proximal segment of the injured nerve. Treatments with FK506 favour reinnervation of the target muscle through this segment by increasing GAP43 expression, its phosphorylation, and concentration in growth cones and Schwann cells, where it plays a key role in nerve growth cone motility and modulation of new synapses (Saffari et al., 2019). TGF-β1-pathway is also activated favoring the synthesis of NGF, accelerating the Wallerian degeneration, and enhancing GAP43 phosphorylation.

Actually, the availability of a compound like FK506 to prevent graft rejection and the parallel efficiency for nerve regeneration is a great advantage. The clinical use of alternatives to autologous nerve grafts for the treatment of extensive nerve injuries in cases where enough autologous nerve grafts may be difficult to obtain, and regardless of type of graft used, the rate of recovery is normally slow. The synergistic impact of an immunosuppressive and, at the same time, neuroregenerative drugs may provide novel methodologies for bridging nerve gaps after peripheral nerve injury. The current clinical use of FK506 is almost exclusively as immunosuppressive agent to prevent allograft rejection after solid organ transplantation. This broad clinical experience also revealed that, unfortunately, FK506 shows toxicity side-effects ranging from hand tremor and seizures to insomnia, headache, and stupor. Most of these symptoms show good correlation with high plasma levels of the drug. Opportunistic infections are also possible. The lack of knowledge about the mechanism of action of FK506 and the undesirable side effects have consequently discouraged the widespread utilization of FK506 in peripheral injuries to improve nerve regeneration. Nonetheless, the recent findings (Saffari et al., 2019; Daneri-Becerra et al., 2020) evidencing the *ying-yang* roles of FKBP52 and FKBP51, the fact that the neuroregenerative actions of FK506 are dose-dependent and these doses are commonly sub-immunosuppressive, and the successful experimental studies on axonal regeneration after peripheral nerve lesions showing that treatments could be effective when the drug is administered subcutaneously, make feasible this unconventional therapeutic strategy. In this regard, the clinical design of the treatment should consider maintaining a therapeutic concentration of FK506 while avoiding immunosuppression, and even better, the design of neuroregenerative ligands devoid of immunosuppressive action is also possible and there are several ongoing endeavors in this regard.

Interestingly, preloading FK506 prior to nerve injury enhances nerve regeneration compared to the administration at the time or after the injury, especially when the proximal nerve stump is exposed to the drug. The optimal pretreatment is about three days prior to scheduled nerve reconstruction (Yan et al., 2012). When the therapy is initiated within 10 days post-nerve reconstruction, a short-term treatment of 2 weeks reduces the number of neural debris in nerve allografts, which also reduces the infiltration with immune cells that might mechanically obstruct the progress of regenerating axons. Actually, the treatment with FK506 itself already reduces the amount of cell debris. It has also been demonstrated that short-term treatments with FK506 also improve the functional recovery of nerves in both transection models and graft animal models. It has been postulated that the administration of FK506 should be limited to obtain optimal regenerative outcomes, whereas other studies have stated that tacrolimus is effective for as long as the drug is administered, this being dependent on the administration way. From our perspective, both systemic and locally administered FK506 appear to have beneficial effects on recovery in nerve injuries, and the successful translation of this type of treatment to human cases may be optimal if the local delivery of the drug could be developed as to minimize side effects and/or unnecessary high systemic concentrations. Although the latter is indeed an invasive methodology because drug delivery pumps or impregnated bioabsorbable carriers are required, the local drug delivery method may prevent the unwished consequences of a systemic administration of the drug while achieving efficient therapeutic levels at the local organ or tissue.

In summary, the discovery that the neurotrophic action of FK506 is dependent on FKBP52 activation, may encourage the design of novel therapies for human neuropathies to great extent. Although the macrolide has been proposed to enhance sensory and motor recovery in humans for hand- and facial-transplantation, these circumstantial studies are yet to be confirmed by more extensive clinical trials. Improved medical results are likely to be reached from a better understanding of the neurobiology of nerve repair. Due to the recent advances in the field, the potential benefits of drugs like tacrolimus in enhancing axon regeneration and neurological recovery in cases of nerve trauma are currently limited by the extent of our initiatives only.

In order to comply with space limitation rules, we apologize for not citing all the work discussed here reported by colleagues in the field.

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Additional file:
Additional file 1: *In vivo neuroregenerative effect of FK506 in a murine spinal cord injury model.*

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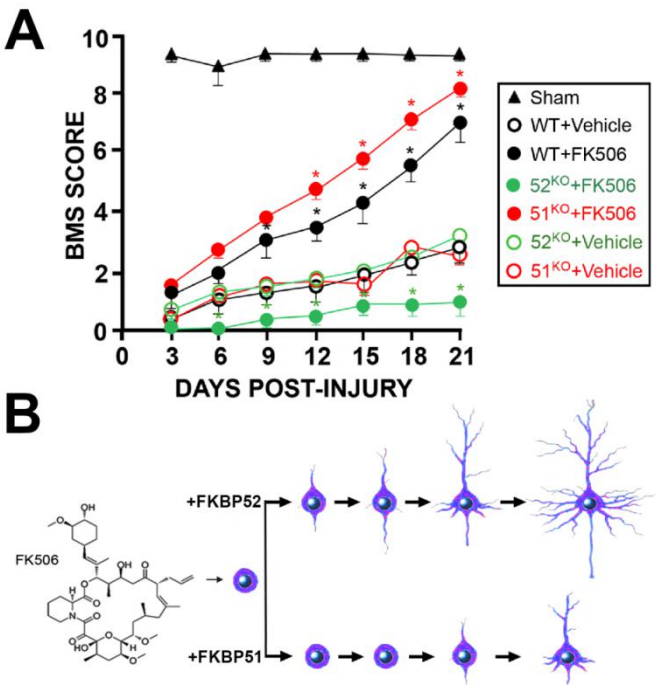

Additional file 1: *In vivo* neuroregenerative effect of FK506 in a murine spinal cord injury model.

(A) Wild-type (WT) or knock-out mice for FKBP51 or FKBP52 underwent a surgical spinal cord injury (Daneri-Becerra et al., 2020). Mice were injected during 21 days with a daily dose of 0.010 mg/kg FK506 or vehicle. The Basso Mouse Scale (BMS) score for locomotor activity was measured and shown as the mean ± SEM. Asterisks indicate the significance at $P < 0.005$. (B) The scheme depicts the neuroregenerative action of FK506 upon binding to FKBP52 and the antagonistic stimulation of FKBP51. The resultant neurotrophic effect for a treatment with FK506 is the balanced outcome of both opposite effects.