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THE EFFECTS OF TACROLIMUS ON POTENTIAL DRUG TARGETS FKBP65 AND FKBP22 IN FIBROSIS

Bachelorscriptie pathofysiologie

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Abstract

Collagen is one of the most abundant proteins in the human body. The over-stimulation of collagen is called fibrosis. One possible group of drug targets for fibrosis are FK506-binding proteins (FKBP). FKBP65 and FKBP22 are involved in the biosynthesis of collagen as molecular chaperones and folding enzymes. Mutations of *FKBP10* and *FKBP14* (genes encoding FKBP 65 and FKBP22) have been shown to be a cause of several connective tissue disorders. Recently, this gained them attention as potential drug targets.

FK506 (tacrolimus) is a immunosuppressive drug used in organ transplantations which interacts with FKBPs. It has been shown to have an anti-fibrotic effect in several fibrotic diseases, mainly working through inhibiting TGF-B and TGF-B activated pathways. TGF-B is a known fibrosis inducing cytokine and has been extensively researched in association with fibrosis. Research investigating the effect of tacrolimus on FKBPs in relation to fibrosis is, however, less common.

In this report it was tried to establish what is known about the potential of tacrolimus as an anti- fibrotic drug effecting FKBP65 and FKBP22. Because the interest in this field only recently came up, the literature on this subject is sparse, but the cautious conclusion that mainly FKBP65 is not a good drug target for tacrolimus is drawn. This does not negate the potential of FKBP65 and FKBP22 as potential drug targets but does imply that tacrolimus might not be the drug to exploit this potential.

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Introduction

Collagen is one of the most abundant proteins in the human body. It makes up a large part of the extracellular matrix (ECM) and plays an important role in maintaining the framework of the human body. The ECM composition is highly regulated and maintained by fibroblasts. When this process is disturbed and collagen secretion is over-stimulated, a pathological situation called fibrosis will ensue. While the over-secretion of ECM proteins is not necessarily pathological, in wound healing for example, fibrosis is one of the most fatal diseases in the western world, with few treatment options and a poorly understood mechanism of action.

One possible group of drug targets for fibrosis which has recently gotten more attention of researchers are FK506-binding proteins (FKBP). The FKBP family is a subfamily of immunophilins, which are distinguished by being molecular chaperones with peptidyl-prolyl isomerase (PPIase) activity and binding the immunosuppressive drug FK506 (tacrolimus) (Staab-Weijnitz CA et al., 2015). In the case of collagen there are several FKBP's important for its synthesis. The PPIase activity of the FKBP's are essential for the correct folding of collagen, while they also play an important role in collagen trafficking (Patterson CE et al., 2005). Two FKBP's essential for correct biosynthesis of collagen are the rough endoplasmatic reticulum (rER) resident FKBP65 (encoded by *FKBP10*) and FKBP22 (encoded by *FKBP14*).

Recently the interest in tacrolimus as a potential drug for fibrosis has increased. This compound has been shown to affect FKBPs like FKBP65 and FKBP22 (Seo J et al., 2016, Lee C et al., 2016, Lan CCE et al., 2013) and can therefore influence the collagen secretion in fibroblasts.

In this report, we will look at what is known in the literature of the function and working mechanisms of FKBP65 and FKBP22 and what happens when there is a mutation in one of the corresponding genes. After this, the working mechanism of tacrolimus and what is currently known about its effects on fibroblasts will be investigated and finally there will be looked at the presence of *in vivo* evidence of the workings of tacrolimus.

The roles of FKBP65 and FKBP22 in collagen synthesis

As said before, collagen makes up the major part of the structural frameworks of the human body, such as bone, tendon, cartilage and skin. There are 29 different kinds of collagen, each with its own function and distribution throughout the body. The process of collagen synthesis takes place in the rER, after which the collagen is excreted to the ECM through the Golgi complex. This process is aided by molecular chaperones, protein foldases and other posttranslational modifying enzymes. These aiding enzymes are, however, not universal for every different type of collagen (Engel J et al., 2005). FKBP65 and FKBP22 are two proteins which play a role in collagen biosynthesis.

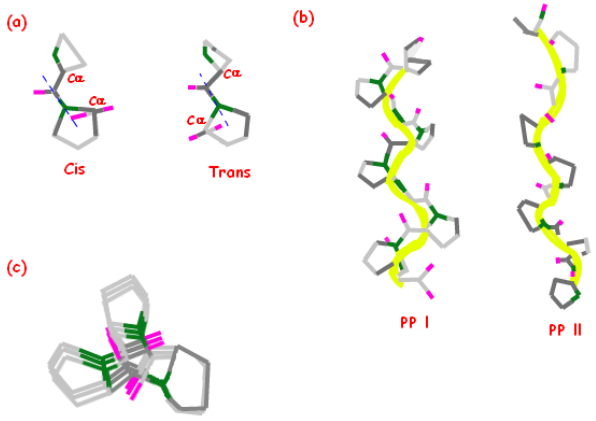

Figure 1. A schematic depiction of the collagen molecule with proline in its cis *and* trans *formation, regulated by the activity of PPIases like FKBP65 and FKBP22 (Proline [Website]).*

FKBP65

FKBP65 plays a diverse role in the biosynthesis of collagen. The first function consists of its function as an enzyme with PPIase activity. Peptidyl-prolyl isomerases are enzymes which catalyse the isomerization of proline amino acids in proteins. Collagen is a protein consisting of three individual chains which together form a triple helix and contain a high number of proline amino acids. when the individual chains are being translated and are deposited in the rER, the proline amino acids will find a

natural balance of *cis* and *trans* configuration. For the collagen triple helix to be correctly formed, all of the proline amino acids have to be in the *trans* configuration shown in figure 1 (Engel J et al., 2005, Ishikawa Y et al., 2015). FKBP65 is a PPIase resident in the rER and can therefore catalyse this step and provide the post-translational modification of the individual collagen chains needed for correct folding of collagen.

Secondly, FKBP65 acts as a molecular chaperone for collagen I and III in its triple helix form (Lietman CD et al., 2014). It has the ability to stabilize the triple helices and prevent the premature association of the individual collagen chains (Ishikawa Y et al., 2008).

FKBP22

The PPIase activity of FKBP proteins is attributed to the FKBP domain these proteins have in common. Naturally every protein does however, have a different structure. FKBP65, for example has four FKBP regions, whereas FKBP22 has only one (Boudko SP et al., 2013). Herein lies the reason for the substrate specificity, because while FKBP65 shows activity with collagen I and III substrates (Lietman CD et al., 2014), FKBP22 shows an interaction with type III, VI and X collagen (Ishikawa Y et al., 2014).

The first role of FKBP22 consists of its PPIase activity with collagen, like FKBP65, except FKBP performs its activity on collagen chains which form type III, VI and X collagen. It also acts as a molecular chaperone for these collagen types, but it has a rather weak interaction compared to heatshock protein 47 (Hsp47), a known collagen chaperone. This weaker interaction causes FKBP22 to be unable to stabilize the triple helix, but it is still strong enough to prevent the premature interactions of the individual chains (Ishikawa Y et al., 2014).

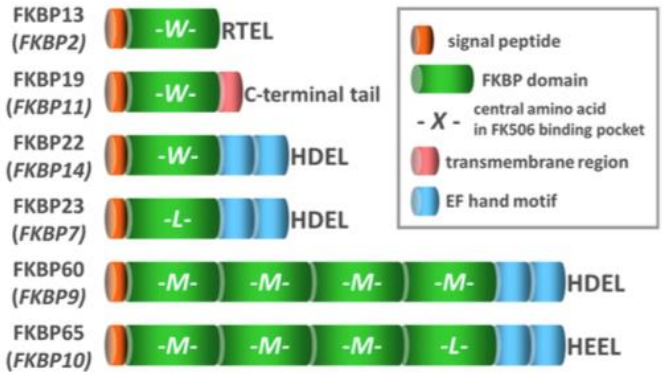

Figure 2. Schematic representation of domain structures of rER resident FKBPs. (Ishikawa Y et al., 2015)

Mutations in FKBP65 and FKBP22

There are several pathological situations associated with mutations in FKBP proteins. Mutations causing the protein to dysfunction prevent proper collagen synthesis, causing several connective tissue disorders.

FKBP65

One of these disorders is in fact a group of disorders called Osteogenesis Imperfecta (OI). Also known as brittle bone disease, OI is marked by bone fragility and low bone mass leading to increased fracture risk (Schwarze U et al., 2013). This disorder was initially believed to be solely caused by defects in type I collagen, but recent discoveries showed that there are many different, mainly recessive, genes with different roles in collagen synthesis that can cause OI (Forlino A et al., 2016). One of these genes that causes recessive OI is *FKBP10* (the gene translating to FKBP65). Mutations in *FKBP10* also cause Bruck syndrome (Schwarze U et al., 2013), a disorder with severe OI and congenital joint contractures, and Kuskokwim Syndrome, which is characterized by showing only the congenital joint contractures. Recessive OI caused by *FKBP10* mutations differ from other causes in that its working is based on the

loss of telopeptide hydroxylysine crosslinking in type I collagen and show phenotypic similarities in mutations in *PLOD2* (the gene encoding lysyl hydroxylase 2), which incidentally also indicates that FKBP65 has more functions than only being a molecular chaperone as was originally thought (Lietman CD et al., 2014).

There have also been studies which researched the effect of mutations in *FKBP10* by looking at *FKBP10* knockdown mice. Mice with a mutation that caused a complete loss of function of the *FKBP10* gene did not survive birth. The embryos also showed a delay in growth and a general tissue fragility. This lethality seen in mice stands in contrast to patients with loss of FKBP56 function, for reasons yet unknown. It was suggested that humans have other genes which take over FKBP65 functions. The mice with the *FKBP10* mutation also showed an ER that was dilated and had increased amount of procollagen, probably caused by wrongly folded procollagen which form aggregates due to the loss of chaperone function of FKBP65. The difference in lethality between mice and humans was also reported in mutations of SERPINH1, which translates to HSP47 and has similar activity in collagen synthesis to FKBP65 (Lietman CD et al., 2014).

FKBP22

The research of mutations in *FKBP14* (the gene encoding FKBP22) in the context of pathological situations has only recently started to attract attention. The first description of *FKBP14* mutations being associated with a disorder originates from 2012, where it was reported that it might be a cause of an autosomal-recessive variant of the Ehlers-Danlos syndrome (EDS) (Baumann M et al., 2012). EDS comprises a group of connective tissue disorders characterized by skin hyper elasticity, joint hypermobility and increased tissue fragility. The kyphoscoliotic type of EDS is known to be caused by incorrect functioning of the enzyme lysyl hydroxylase 1 (LH1) caused by mutations in the corresponding PLOD1 gene. This deficiency results in an abnormal amount of lysyl pyridinoline (LP) and hydroxylysyl pyridinoline (HP) in the urine. This LP/HP ratio is one of the diagnostics of kyphoscoliotic EDS.

The researchers from the 2012 report found a form of kyphoscoliotic EDS characterized by a normal LP/HP ratio, sensorineural hearing loss, myopathy and joint hypermobility. The researchers identified mutations in *FKBP14* the be the cause (Baumann M et al., 2012). In accordance with (Ishikawa Y et al., 2014), where it was demonstrated that FKBP22 interacted with type III, VI and X collagen, (Baumann M et al., 2012) showed phenotypical symptoms corresponding to the types of collagen FKBP22 interacts with. Recently reported vascular (Murray ML et al., 2014) and skin (Baumann M et al., 2012) symptoms in EDS caused by *FKBP14* mutations correspond to its role in proper functioning of type III collagen. Patients also showed signs of myopathy, which indicates interaction of FKBP22 with type VI collagen (Foley AR et al., 2013). The binding of FKBP22 to type X collagen (Ishikawa Y et al., 2014) can be connected to the joint hypermobility observed in patients. Further research about the role of *FKBP14* mutations is necessary to understand the exact mechanisms, but as of yet it has only been studied in the context of observational patient studies. No known studies have been published where the effects of *FKBP14* mutations are studied in mice knockdown models.

The workings of tacrolimus on FKBP family members

Tacrolimus has been shown to be a compound with an anti-fibrotic effect (Seo J et al., 2016, Lee C et al., 2016, Lan CCE et al., 2013), through inhibition of FKBP proteins. To understand how tacrolimus can be used as an anti-fibrotic drug we must first establish the way this compounds inhibits the FKBP function.

Tacrolimus initially became known as an immunosuppressive drug used in human organ transplantations. It works as a calcineurin inhibitor in T-cell activation, in particular inhibiting gene expression of interleukin-2 (IL-2) in CD4+ T-helper lymphocytes. It binds with FKBP12 in the cytoplasm, forming a complex which interacts with additional substrates like calcineurin. When this enzyme is inhibited, the signalling pathway which activates IL-2 gene transcription is interrupted and IL-2 release is inhibited, causing the immunosuppressive effect (Ivery MT et al., 1997).

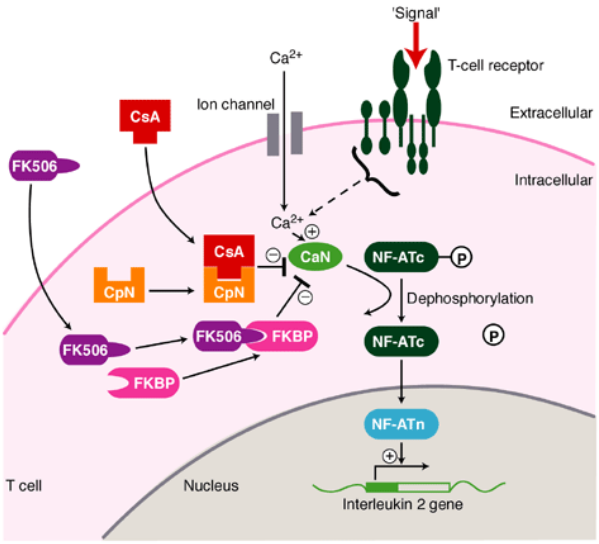

Figure 3. Mechanism of action of tacrolimus in its traditional immunosuppressive role. Tacrolimus binds to FKBP, forming a complex which binds to and blocks calcineurin. This prevents activation of the pathway needed for translation of the IL-2 gene, thus inhibiting T-cell activation. (Stepkowski SM et al., 2000)

In regard to the anti-fibrotic effect of tacrolimus the binding to the FKBPs is essential. FKBPs are not only involved in the biosynthesis of collagen, but also in other cellular pathways that influence collagen production (Lan CCE et al., 2013, Wu CS et al., 2012).

Tacrolimus as an anti-fibrotic drug

Fibrosis

Fibrosis is a disease caused by fibroblasts. These are cells which control the composition of the ECM. In normal wound healing, fibroblasts are activated by cytokines which are released into the system with tissue injury and inflammation. After activation fibroblasts release ECM components to create scar tissue and repair the injured tissue. In case of fibrosis this process of ECM secretion is uncontrolled and ultimately leads to organ failure (Manojlovic Z et al., 2013). One of the cytokines involved in fibroblast activation is TGF-B.

TGF-B is known to activate fibroblasts into myofibroblasts, the cells responsible for fibrosis. The TGF-B family, with TGF-B1 being the most well studied, transduce their signal from the membrane to the nucleus through transmembrane type I and II receptors (TGF-BR1, TGF-BR2) and the effector Smad proteins (Massagué J, 2000). Another Smad protein independent pathway activated by TGF-B is the mitogen-activated protein kinase (MAPK) pathway (Lan CCE et al., 2013).

Known effects of tacrolimus in fibrosis

Tacrolimus has shown to have an anti-fibrotic effect in studies concerning idiopathic pulmonary fibrosis (IPF) (Seo J et al., 2016). Most studies that look at tacrolimus as a drug for fibrosis, describe its effect to its immunosuppressive function. It inhibits T-Cell activation, preventing the cells from secreting cytokines like TGF-B and in this manner prevents fibrosis formation, because for TGF-B to activate the fibrotic response, it needs to bind to the TGF-b receptors. According to (Seo J et al., 2016) tacrolimus strongly inhibits the activation of p38 MAPK, which prevents the completion of the pathway needed for TGF-B activation and thus the increase of type I collagen expression in pulmonary fibrosis.

In (Asano Y et al. 2005) the effect of tacrolimus in systemic sclerosis (SSC) was investigated. SSC is one of the most complex systemic autoimmune diseases. It targets the vasculature, connective tissue-producing cells (namely fibroblasts/myofibroblasts), and components of the innate and adaptive immune systems (Pattanaik D et al., 2015). The researchers looked at the expression of the human a2(I) collagen gene (the gene translating to one of the collagen chains needed for collagen I synthesis) in human and in SSC fibroblasts. It showed that tacrolimus decreased TGF-B induced expression of the type I procollagen and a2(I) collagen gene in normal fibroblasts by reducing mRNA stability, while basal expression remained the same. This effect was, according to the researchers, due to lack of TGF-B induced stability of human a2(I) collagen mRNA, caused by tacrolimus.

Tacrolimus also had an effect on FKBP12. This FKBP binds to TGF-BR1 and prevents spontaneous ligand-independent activation of TGF-BR1 by TGF-BR2. In the presence of tacrolimus, the two bind and FKBP12 is dissociated from TGF-BR1, causing TGF-BR2 to activate TGF-BR1 and induce a TGF-B response in normal fibroblasts. In SSC fibroblasts however, tacrolimus did not affect the activation state of TGF-B signalling. Even though the TGF-B response was not inhibited by tacrolimus, the SSC fibroblasts still showed reduced expression of the a2(I) collagen gene, indicating tacrolimus reduces the expression of the a2(I) collagen gene without mediating its activation effect on TGF-B signalling in SSC fibroblasts (Asano Y et al. 2005). This is one of the few reports where tacrolimus is shown to have an anti-fibrotic effect which does not work through inhibiting TGF-B activation.

In (Lan CCE et al., 2013) the effect of Atopic dermatitis (AD) was investigated. AD is a chronic relapsing skin disease characterized by inflammation and lichenification (a thickening of the skin). Due to chronic inflammation in chronic lichenified skin lesions, this tissue often undergoes remodelling caused by dermal fibroblasts. One of the working mechanism of the anti-fibrotic effects of tacrolimus discussed in this report concerned its influence on the functioning of TGF-B. It was reported that TGF-B plays an important role in fibroblast activation and proliferation in dermal fibroblasts, mainly through the autocrine positive feedback loop, which stimulates TGF-BRI and TGF-BRII expression. It was shown that tacrolimus interrupted this feedback loop and suppressed TGF-BR expression. In this report tacrolimus was again shown to inhibit p38MAPK (this time in dermal fibroblasts, where earlier it was in lung fibroblasts), which causes TGF-B signalling to be interrupted, which caused the inhibitory effect of tacrolimus on TGF-BR expression.

Another anti-fibrotic effect of tacrolimus described in (Lan CCE et al., 2013 and Gaglianoa N et al., 2008) involves its effect on matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs (TIMPs). MMPs are endopeptidases which degrade various ECM proteins and TIMPs are proteins that block the activity of MMPs (Brew K et al., 2010). Regulation of MMPs and TIMPs is important in the process of wound healing and tissue remodelling. An imbalance in MMPs and their TIMPs is often observed in scleroderma and keloids, and has been suspected to play a role in AD. TGF-B was shown to induce a decrease in MMP and an increase in TIMP gene expression. (Lan CCE et al., 2013) found that treatment with tacrolimus inhibited this effect and increased MMP and decreased TIMP activity, showing yet another different mechanism of the anti-fibrotic function of tacrolimus although it still functions through its effect on TGF-B.

Other research done with gingival fibroblast also described an effect of tacrolimus on MMPs. However, where up until now all the effects of tacrolimus seem to be through influencing TGF-B signalling, in contrast to other fibroblasts discussed tacrolimus did not influence TGF-B in gingival fibroblasts, but exclusively induced an increase in MMP protein expression. This, in turn, prevented the occurrence of gingival overgrowth, a common problem in patients treated with immunosuppressive drugs (Gaglianoa N et al., 2008). This is the second indication after (Asano Y et al. 2005) found of tacrolimus having an effect on fibroblasts that does not work through TGF-B, but it has only been observed in gingival fibroblasts.

Now that it is established that tacrolimus does indeed have an anti-fibrotic effect, we can see if there are other functional mechanisms where it works that does not involve TGF-B. We also know that FKBP65 and FKBP22 are proteins which play important roles in the biosynthesis of collagen. With this information in mind it seems plausible that FKBP65 and FKBP22 could be drug targets in the

treatment of fibrosis. With both of them binding tacrolimus it seems probable that it can also display an anti-fibrotic effect through FKBP65 and FKBP22.

FKBP65 and FKBP22 as drug targets in fibrosis

While an effect of tacrolimus has been demonstrated working through binding to FKBPs (Asano Y et al. 2005, Manojlovic Z et al., 2013), FKBP65 and FKBP22 are relatively new as potential drug targets. In (Staab-Weijnitz CA et al., 2015) it is suggested that tacrolimus might have an effect on *FKBP10* expression, but their research also showed that *FKBP10* is at least partly regulated by TGF-B making it likely that tacrolimus effects FKBP65 through TGF-B.

As of yet there are no studies published which specifically investigate the effect of tacrolimus binding to FKBP65 and FKBP22 in relation to fibrosis, but there are some clues to the possibility of FKBP65 and FKBP22 as potential drug targets.

One report determining the chaperone functions of FKBP65 showed an effect of tacrolimus on FKBP65 and FKBP22, although the possibility of these FKBPs being potential drug targets was not investigated. It was shown that when tacrolimus was added the chaperone function of FKBP65 was increased. The researchers speculated that the changed conformation of FKBP65 strengthened the recognition and effective folding of the substrate. In contrast, the addition of tacrolimus to FKBP22 completely blocked its chaperone function. This difference was ascribed to FKBP65 having four FKBP domains, compared to FKBP2 which has only one (fig. 2), of which tacrolimus can only bind one (Ishikawa Y et al., 2008). Although this study did not look at the FKBPs in the context of fibrosis, it does make it less likely that tacrolimus has anti-fibrotic effect through inhibiting FKBP65 function. The results concerning FKBP22 however, do seem promising with regard to FKBP22 as a potential drug target.

Tacrolimus effects in vivo

To determine the potential of tacrolimus as an anti-fibrotic drug, the *in* vivo effects of the compound have to be examined. Tacrolimus has been used for some time as an immunosuppressive drug in medical operations like kidney and liver transplants. As such has many known adverse side effects like post-transplantational diabetes mellitus, nephrotoxicity, neurotoxicity and gastrointestinal effects. In spite of this, tacrolimus remains the treatment of choice for liver and kidney transplants (Li CJ et al., 2016). Since researchers only recently started to investigate the anti-fibrotic effects of tacrolimus, it is not yet well-established whether tacrolimus might induce other adverse side-effects when used to treat fibrosis.

In cases of atopic dermatitis a topical application of a tacrolimus ointment was shown to be an effective treatment, preventing, delaying and reducing the occurrence of lichenification without any negative side-effects (Lan CCE et al., 2013, Wollenberg A et al. 2008). This result is promising, but applying tacrolimus with an ointment prevents the substance from entering other parts of the body. On its own this is a good thing, but it does prevent observations of any other side effects tacrolimus might have.

Other studies investigated induced pulmonary fibrosis (IPF) in mice and the effects of tacrolimus when it was inhaled. Mice were treated with inhaled tacrolimus-bound albumin nanoparticles (Seo J et al., 2016) and tacrolimus-loaded chitosan-coated poly(lactic-co-glycolic acid) nanoparticles (Lee C et al., 2016). These studies both reported an improvement of the pathological situation in IPF in mice, induced by the effect of the inhaled tacrolimus. Another report which studied the effects of tacrolimus on IPF found tacrolimus attenuated IPF when treatment was initiated on day 6 after bleomycin administration (the substance inducing pulmonary fibrosis) and suppressed TGF-BRI expression in vitro and in vivo. However, when treatment was initiated during bleomycin administration in the acute inflammatory phase of IPF, the researchers found increased pulmonary

vascular permeability and exudation, and a decrease of survival in the concerning mice (Nagano J et al., 2006).

Tacrolimus also has been shown to have an effect on ethanol induced hepatic fibrosis (Manojlovic Z et al., 2013). In alcoholic liver disease, alcohol metabolism in hepatocytes directly activate hepatic stellar cells. These are ECM secreting cells responsible for the excessive collagen excretion in hepatic fibrosis. It was shown that in vivo tacrolimus completely prevented development of alcohol induced hepatic fibrosis. The duration of the treatment was, unfortunately, not long enough to observe any adverse side-effects, but the use of tacrolimus as an immunosuppressive drug in humans has already clarified the adverse side-effects of prolonged tacrolimus treatment.

These findings clearly show that tacrolimus has an anti-fibrotic effect in vivo. The side-effects of tacrolimus are well described when used as an immunosuppressant, but not in the context of fibrosis. The fact that tacrolimus is a widely used immunosuppressive drug in combination with the displayed anti-fibrotic effect does make it likely that it can also be used as an anti-fibrotic drug.

Conclusions

The goal of this report was to establish what is currently known about the role of FKBP65 and FKBP22 in collagen biosynthesis and their potential as drug targets in fibrosis, and the potential of tacrolimus as an anti-fibrotic drug.

FKBP65 and FKBP22 are shown to play several different roles in the collagen biosynthesis. Their roles as molecular chaperones and folding enzymes initiated interest of their potential as anti-fibrotic drug targets. This presumption was encouraged by the results found in studies researching the consequences of FKBP65 and FKBP22 mutations. Tissues that depended on correct ECM secretion and composition showed abnormal growth in the presence of FKBP65 and FKBP22 mutations. The logical outcome of these results was a search for a compound that might inhibit their function. Because of its binding affinity to FKBPs tacrolimus was the first, and as of yet only compound researched in such a role.

Because many FKBPs are vital for proper functioning and synthesis of collagen tacrolimus was thought to exercise its anti-fibrotic effect through inhibiting these FKBPs. While this might still be the case, it has not yet been proven that tacrolimus has this effect. The main anti-fibrotic functioning of tacrolimus was found to be through inhibiting TGF-B functioning. TGF-B was known to be a fibrosis inducing cytokine and has also been extensively researched. Tacrolimus was found to inhibit the activity of the TGF-B pathway in several manners. In normal fibroblast stimulated with TGF-B it bound to FKBP12, causing the TGF-BRI to dysfunction and thus interrupting TGF-B activation (Asano Y et al. 2005). In dermal (Lan CCE et al., 2013) and in lung (Seo J et al., 2016) fibroblasts it inhibited functioning of p38 MAPK, an enzyme essential for the downstream activation of the pathway activated by TGF-B. In gingival fibroblasts tacrolimus was shown to inhibit the TGF-B induced imbalance in MMPs and TIMPs causing gingival overgrowth. These are all promising results for the future of tacrolimus as an anti-fibrotic drug, but they all work through influencing TGF-B or its induced pathways, where we had expected at least some kind of influence of tacrolimus on FKBPs involved in collagen synthesis. One paper did report an TGF-B independent anti-fibrotic function of tacrolimus in GO (Gaglianoa N et al., 2008), but this effect was ascribed to influencing MMP/TIMP ratios, not because of influencing FKBPs.

Although we did expect FKBPs involved in collagen synthesis to be researched as potential drug targets, this was not often found to be the case. There have been some recent papers doing this (Staab-Weijnitz CA et al., 2015, Ishikawa Y et al., 2008), but their results were not as promising as we expected. The first study did show an inhibiting effect of tacrolimus on FKBP65, but also reported *FKBP10* to be at least partly regulated by TGF-B, making it likely that tacrolimus affects FKBP65 through TGF-B. The second study did not investigate FKBP65 as a potential drug target, but did find that tacrolimus increased the chaperone function of FKBP65, which would mean that FKBP65 is not quite as good a

potential drug target as we had thought. This same study also reported tacrolimus to completely inhibit FKBP22 chaperone functioning, making this a more interesting potential drug target.

All in all, with the information currently available tacrolimus on its own does seem to be a promising drug for treating fibrosis. FKBPs however seem to be a less likely potential drug target of tacrolimus than initially thought. Of course, FKBPs involved in collagen synthesis as potential anti-fibrotic drug targets are a very recent field of study and there certainly has not been done enough research to make any decisive conclusions, but the anti-fibrotic effects of tacrolimus seem to be working mainly through other mechanisms. This does not necessarily make FKBP65 and FKBP22 less interesting is potential drug targets (especially the effect of tacrolimus on FKBP22, shown in Ishikawa Y et al., 2008, is promising), but it does suggest that we should perhaps investigate other compounds than tacrolimus when looking for use of FKBP65 and FKBP22 as potential drug targets in fibrosis.

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