

Relaxin as a Cure for Fibrosis

Sarah Akilov

Sarah Akilov graduated in June of 2018 with a B.S. degree in Biology and is attending the SUNY College of Optometry.

Abstract

Until recently, relaxin has been known as a hormone that pertained to the female reproductive system. Its ability to remodel the extracellular matrix is responsible for its known reproductive effects. Current research has indicated that it may be useful as a drug to combat fibrosis. Relaxin has been proposed as an antifibrotic drug to target a variety of organs, including the skin, lung, kidney, liver, and heart. Studies done using the relaxin null knockout mouse have given scientists insight into the workings of this hormone. Human studies have also been done to test the efficacy of relaxin in its reversal of fibrosis. With more research, perhaps relaxin can be used as a drug in the future.

Introduction

Fibrosis is a hardening or scarring of tissue that results from the repair of injuries in the body. It is estimated that 45-50% of deaths in the Western world are caused wholly or in part by fibrosis in various organs in the body. Currently, there is no available cure for damage caused by fibrosis (Summers, 2016).

Relaxin is a peptide hormone usually associated with the reproductive system. Relaxin has been found to be pleiotropic, meaning that it is active in many varying roles in the body. It is produced in both pregnant and non-pregnant females as well as in males (Pallardy, 2016). It appears that relaxin can be manipulated to target injured organs in order to reverse fibrosis and prevent its further progression. There have been studies conducted that point to relaxin as a possible cure for fibrosis in various organs, including the skin, lung, kidney and heart (Tan, et. al. 2016). Fibrosis is a part of the pathology of many different human diseases and thus is said to account for 45-50% of deaths in the western world (Summers, 2016).

Methods

Information in this paper is based primarily on research accessed through the Touro Library Databases. The peer reviewed, scholarly journal articles were retrieved through the Proquest or Pubmed databases.

Fibrosis and its Causes

Fibrosis is an exaggeration of the scarring and hardening of tissue usually caused by the body's normal response to injury. It is characterized by a build-up of extracellular matrix (ECM) components, or scar tissue, in place of normal tissue. Particularly, there is a buildup of fibrillar collagens, such as types I and III. Fibrogenesis is the body's mechanism of healing and repairing wounds. What occurs in fibrogenesis is that myofibroblasts rush to the site of the injury and release cytokines. This causes fibroblasts to proliferate and build up the ECM producing fibrillar collagen creating a scar in place of the damaged tissue (Baum, et. al. 2011). Excessive build-up of connective tissue can be harmful to underlying tissue and organs. This is because the ECM is the framework for the cells and extracellular structures in the body. Therefore, if the normal form of the ECM is disrupted, the tissue in which it is present will not continue to function as it should (Bathgate, et. al. 2003). Fibrosis is often caused by chronic inflammatory reactions that can be a result of various stimuli among which are persistent bacterial and viral infections, autoimmune and allergic reactions, and tissue injury (Wynn, 2008).

The body has a system that works to clear accumulated extracellular matrix materials. Matrix Metalloproteinases (MMP's) are a group of enzymes that break down extracellular matrix proteins, such as collagen. Part of the body's normal way of repairing itself after tissue injury is to inhibit the production of these enzymes and to increase the secretion of Matrix Metalloproteinase Inhibitors (MMPis), and more specifically, Tissue Inhibitors of Metalloproteinases (TIMPs). Thus, scar tissue replaces the injured tissue, and the body can heal itself. This too is favorable in a normal situation, but if this process continues unceasingly, it can result in disruption of normal organ function as the matrix builds up without anything present to break it down. An imbalance between collagen degrading enzymes will also cause fibrosis (Bennett, et. al. 2009).

What is Relaxin?

Relaxin was first discovered in 1926 by Frederick Hisaw as a hormone present in pregnant guinea pigs. The function of relaxin observed at the time, was that it relaxed the pelvic ligaments and the cervix of the uterus to make it possible to carry the fetus and to prepare the area for parturition. Later, it was discovered that it was through collagen remodeling that relaxin was able to play this important role in pregnancy and birth (Pallardy, 2016). Upon closer inspection of the relaxed ligaments it was seen that the collagen was remodeled from dense bundles to looser, less structured fibers (Bennett, et. al. 2009).

Relaxin is produced by various structures in the female's reproductive tract including the placenta, corpus luteum and uterus, though this varies by species. During the first trimester of pregnancy, relaxin rises from practically undetectable levels to 1ng/mL and then slowly wanes as delivery draws near. Relaxin is also present in non-pregnant human females and males (Pallardy, 2016). In males, relaxin is produced by the prostate gland and can be found in the seminal fluid, though it is not usually detected in circulation (Bennet, 2009).

Relaxin is a peptide hormone whose two-chain structure is similar to that of insulin. In humans and higher primates, three genes for relaxin have been discovered, which include encoding proteins known as H1, H2 and H3 relaxin. In rodents, only two relaxin genes have been discovered: relaxin-1 and relaxin-3, which are equivalent to the H2 and H3 in humans, respectively. The H3 relaxin gene was discovered in 2002 and is known to act mainly in the brain. H2 relaxin in humans- the product of the RLN2 gene, and relaxin-1 in rodents- the product of the RLN1 gene, are the major circulating forms of relaxin and are the ones

that are thought to have an effect on fibrosis (reference to relaxin for the duration of the paper will refer to these forms of relaxin) (Samuel, et. al. 2007).

There has been a lot of research in the past few years that has tried to find more uses for relaxin in the body. There have been identified relaxin-binding sites in various places in the body in both males and females, including in the heart and brain. This suggests that relaxin may also influence nonreproductive areas of the body and leads scientists to do further research into these possible uses (Samuel, et. al. 2016).

Knockout Mouse Studies

To aid scientists in the understanding of relaxin, a relaxin-null knockout mouse was created by The Howard Florey Institute in Melbourne, Australia. A knockout mouse is a mouse that was genetically modified in order to remove or “knockout” a specific gene. Using such a mouse helps give scientists insight into the biology of a particular gene (Austin, et. al. 2004). The mouse established to research relaxin is the Rln1-KO mouse, meaning that the Rln1 gene, that codes for the major circulating form of relaxin in animals, was removed. However, the Rln3 gene was not removed. Monitoring the development of these mice showed that the reproductive organs, in both males and females, were underdeveloped. In pregnant females, the mammary glands, nipples, and pubic symphysis were underdeveloped, and lactation was prevented. In the male mice, the testis, epididymis and prostate did not mature properly. Later, it was discovered that abnormal development in knock-out mice was due to excess collagen. In addition to this, as the mice aged, there was a buildup of interstitial collagen in the heart, lung, kidney, and skin. This eventually caused malfunction in these organs, appearing more prominently in the male mice.

Scientists then administered recombinant human (H2) relaxin to the knockout mice and saw that it was helpful in reversing the fibrosis and restoring organ function. The relaxin was helpful in both early and late stages of the fibrosis, but worked to different extents depending on the organ. These findings point to a use of relaxin as a drug to reverse and prevent fibrosis in human pathological situations (Bathgate, et. al. 2003 and Samuel, et. al. 2005b).

In 2009 a study was done on relaxin null knockout mice to test the efficacy of using recombinant relaxin as a treatment for the fibrosis that developed in them. The study also aimed to test if it was significant when in the progression of the fibrosis the relaxin was administered. There were two groups of mice, in one group, relaxin was given to 9 month-old mice, during early stages of fibrosis. The other group was given relaxin when their fibrosis was more progressed, at 12 months of age. There was less fibrosis in the 9-month group, and no difference in the 12-month group when compared to the untreated controls. This points to the fact that administered relaxin will be more effective in earlier stages of fibrosis (Giannakis, et. al. 2009).

Relaxin null knockout mice were used to test the effects of relaxin on scleroderma, a form of dermal fibrosis. It is a connective tissue disease that causes fibrosis or various internal organs in addition to skin thickening. Untreated, scleroderma can cause irreversible damage. The study indicated that relaxin is more effective in treating dermal fibrosis in its early stages. (Samuel, et. al. 2005a).

How does Relaxin Help Reverse Fibrosis?

Relaxin binds with its endogenous receptor, relaxin family peptide receptor 1 (RXFP1), which is also known as LGR7. This inhibits the actions of major profibrotic factors such as transforming growth factor beta 1 (TGFβ1), and angiotensin II in several organs. As a result of this, there will be a decreased expression of types I, III, and V collagens, interstitial collagens and type IV basement membrane collagen. There will also be an increase in the breakdown of collagen via the activation of MMPs and an inhibition of the TIMPs (Samuel, et. al. 2016, fig 1)

Mechanisms

The mechanisms involved in the antifibrotic effects of relaxin are not currently well known by the scientific community. The primary receptor for relaxin is LGR7, or RXFP1, and was only recently discovered in 2002. Before this was known, the LGR7 and LGR8 relaxin receptors were known as orphan G-protein receptors. (Bennet, 2009). It is interesting to note that these receptors exist in organs outside the reproductive tract, pointing to the fact that relaxin has other functions than it was historically thought. Perhaps as the understanding of the pathway used by relaxin is enhanced, we will better be able to use relaxin as a drug for the treatment of fibrosis (Samuel, et. al. 2005a).

Discussion: Clinical Trial for Relaxin

In the 1950's, there was the emergence of the idea that relaxin could be used to treat fibrosis, and it was clinically tested then and again in the 1990's. These studies failed to reach the levels of effectiveness that were required for it to pass as a drug, but shed a lot of light onto the antifibrotic actions of relaxin (Samuel, et. al. 2005a).

A randomized, double-blind, placebo-controlled study was conducted using recombinant human relaxin in the treatment of systemic sclerosis. This was a phase III trial for testing relaxin as a possible drug to reverse fibrosis. Systemic sclerosis, also called systemic scleroderma, is a disease in which there is a buildup of collagen in various organs in the body. This is an ideal disease with which to test for the efficacy of relaxin, as the hallmark of this disease is ongoing fibrosis. The patients were divided into 2 random groups, one of which was given the actual drug, and the other group was given a placebo. In addition to this, neither those being treated, nor the researchers administering the medication knew who belonged to which group.

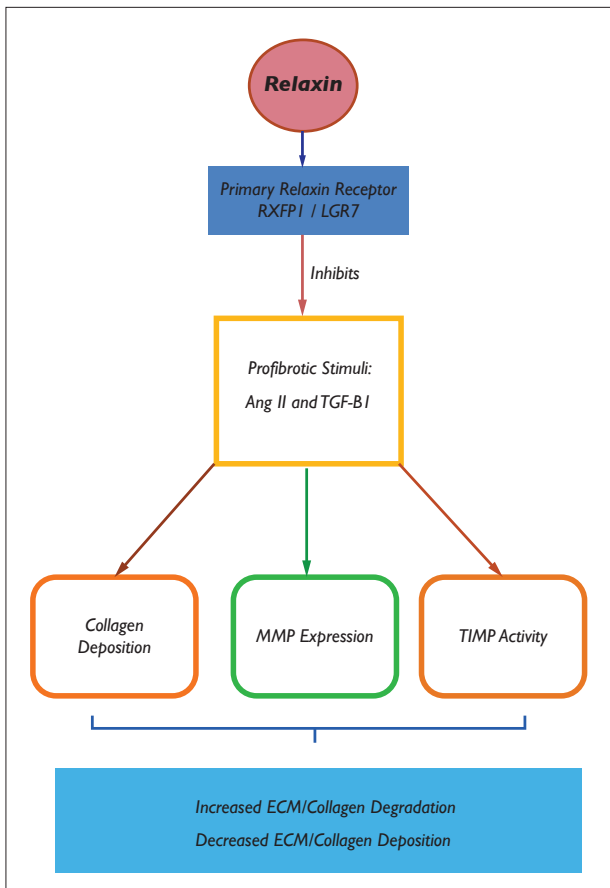


Fig. 1 Mechanism for relaxin's role in inhibiting fibrogenesis. (Modified from Samuel, et. al. 2016.)

There was not a significant difference in the levels of fibrosis between the two groups. In addition, it seems that there were negative side effects to those that were administered the relaxin drug. The forced vital capacity in those patients given relaxin was decreased, and there were adverse renal effects primarily after they stopped giving them the relaxin. The study concluded that if relaxin is ever to be used in the reversal of fibrosis, there must be intensive monitoring of blood pressure and of renal function (Khanna, et. al. 2009).

One proposed reason for why relaxin was not effective in this study was because the degree of scleroderma in the patients in this trial was quite advanced. In the relaxin-null mouse model it was also seen that relaxin was less effective in more advanced stages of fibrosis (Bennet, 2009). Although this study is less optimistic about the use of relaxin as a drug in the near future, it may be possible that as research continues, and we learn more about relaxin and how it works in the body, it will seem more plausible.

Other Human Relaxin Studies

There was a study done to test the safety of administering recombinant human relaxin to people. The scleroderma patients

were administered 200mcg/kg/day for 28 days. This is approximately 50 times the relaxin present in a normal pregnant woman. The relaxin was unhelpful in reducing the fibrosis, but it was concluded that in these doses relaxin was safe and well tolerated. Some adverse side effects included; Development of a rash and pain at the site of infusion, minor bleeding in some cases, and decreased concentration of blood hemoglobin (Seibold, et. al. 1998).

It appears that the body itself uses relaxin as a mechanism to combat fibrosis. This was shown in a study of 50 patients with systemic sclerosis, an autoimmune disorder that causes fibrosis of the skin and internal organs, and 50 healthy patients in which normal relaxin levels were measured and the results were statistically analyzed. In the diseased patients, the measured levels of relaxin in the bloodstream was significantly higher than in the healthy subjects. It was hypothesized that this was the body's response and effort to reverse the fibrosis (Giordano, et. al. 2005). Thus, using relaxin as a drug for fibrotic patients is in accordance with the way nature would tackle the problem, and therefore worth further research.

Pulmonary Fibrosis

IPF or Idiopathic Pulmonary Fibrosis, is a lung disease in which there is fibrosis of the lungs for an unknown reason. It was found that the gene expression of the main relaxin receptor, RXFP1, is 2.9-fold less in lung samples taken from patients with IPF compared with lungs of normal control subjects. There was also a study done in an in vitro model, in which IPF fibroblasts were grown in media that was treated with transforming growth factor TGFb1. It was found that the expression of the RXFP1 protein was decreased in immunoblots. It was thus hypothesized that relaxin treatments will not be too effective in patients with IPF. The authors also speculate that the loss of RXFP1 expression is a common factor in fibrotic diseases and therefore may have been part of the reason that the clinical trials to treat scleroderma with relaxin failed (Tan, et. al. 2016). Royce et. al. point out that these findings are surprising because relaxin has proved to be effective in reducing fibrosis in many human fibroblast culture models in which TGFb1 is used to stimulate collagen synthesis. They argue that the functional activity of the relaxin receptor cannot be determined using gene expression studies alone and point out that there may have been other factors involved that caused these results. Therefore, they conclude, more research must be done before relaxin-based therapies for fibrosis can be either ruled out or implemented clinically (Royce, et al, 2016).

Hepatic fibrosis

Fibrosis of the liver, like forms of fibrosis in other organs, is categorized by increased collagen deposition and decreased ECM degradation. To test the effects of relaxin on hepatic fibrosis in

vivo, scientists established fibrosis in mice using carbon tetrachloride. The mice were administered the carbon tetrachloride for 4 weeks, followed by 4 weeks of administration of relaxin in addition to carbon tetrachloride. Relaxin decreased the hepatic collagen expression, increased the expression of the MMPs, and decreased the TIMPs. This suggests that relaxin might be a possible treatment for established hepatic fibrosis (Bennet, et. al. 2014).

The common endpoint of many liver diseases caused by chronic liver injury is fibrosis. The major profibrogenic cell type that is activated in cases of chronic liver injury is the hepatic stellate cell-myofibroblast (HSC-MF). This cell produces scar tissue and contributes to portal hypertension (PHT) by increasing the resistance within the hepatic vascular system. It was seen in past studies that the expression on RXFP1 was increased in human and rat HSC-MFs and in various studies on parts of the liver that were affected by fibrosis. In the model of rat fibrosis it was seen that the administering relaxin helped to reduce the fibrogenesis and reduce the PHT (McBride, et. al. 2017).

Relaxin has a short in vivo half-life. This is an obstacle to using it as a drug for treatment, especially in models of disease that require long-term administration of the drug, such as liver disease. In an effort to find a new drug to combat liver fibrosis, there was an attempt to find small molecules that are similar to relaxin and that will act as agonists for the RXFP1 receptor. There are currently high throughput screening (HTS) technologies which allow scientists to explore vast libraries of compounds in a quick and efficient manner. This helps to identify molecules that can be used as starting points for creating new drugs. One compound, ML290, was found to be most promising. It binds with RXFP1 and was therefore tested extensively. Although ML290 did exhibit some relaxin-like activity, there were many differences in the way it bound to RXFP1 and its actions. Its effectiveness in combating liver fibrosis has not yet been tested and for now it does not seem that it will be a feasible replacement compound for the treatment of fibrosis. Also, being as the actions of RXFP1 differ in various organs of the body, and are not well understood in general, more research must be done before such a drug can be produced (McBride, et. al. 2017).

Cardiac Fibrosis

Fibrosis is a hallmark of hypertensive cardiac disease. The ECM has a great impact on the function of the heart, including regulating ventricular diastolic and systolic function. It also provides the framework for the cardiomyocytes and coronary vessels. There are many cardiac pathologies that result in fibrosis. Among these are ischaemic injury, myocardial infarction, hypertension, and many others. This results in a vicious cycle, as the accumulation of extracellular matrix via fibrosis leads to an increase in the amount of those negative cardiac events, which can in turn lead to more fibrosis. It was shown in relaxin knockout mouse studies that relaxin deficient mice have an elevated

collagen content in their cardiovascular system which leads to diastolic dysfunction of the left ventricle (LV).

Spontaneously hypertensive rats (SHR), in which fibrosis was not induced by having a lack of relaxin, rather it was the natural result of hypertension, were studied. When the rats were administered relaxin over a 14 day period the initially elevated collagen levels in the LV were now reduced. Fibroblast proliferation was inhibited, and MMP-2 expression increased. This means that there was a decrease in collagen synthesis as well as an increase in its breakdown. The study concluded that relaxin is a potent drug that can be used to combat hypertensive diseases. In addition to this, relaxin was compared to other hypertensive drugs such as angiotensin converting enzyme inhibitors and aldosterone inhibitors. These drugs have many side effects and only begin to help after prolonged periods of treatment. Relaxin, on the other hand, managed to work at a much faster rate to reduce fibrosis and did not have any notable side effects in this study (Lekgabe, et. al. 2005).

Renal Fibrosis

Renal fibrosis is the accumulation of collagen in the kidney. Specifically, in the renal resistance vessels, glomeruli and interstitial space. There is a very strong relation between cardiovascular diseases and renal diseases, as both diseases include high blood pressure. The SHR were also used to study renal involvement. The experimental rats exhibited renal as well as cardiac fibrosis. Administration of relaxin over a 14-day period showed to reduce the fibrosis in the kidney cortex, thus halting renal failure. (Lekgabe, et. al. 2005).

Another study treated rats with bromoethylamine, or BEA, which causes severe renal interstitial fibrosis, one week later, the rats were administered relaxin via an osmotic pump for a period of 28 days. The aim was to see whether the relaxin would inhibit the fibrosis that was caused by the BEA. The structure of the renal tubules had been affected by much collagen deposition in the rats treated with BEA alone. The rats treated with BEA in conjunction with relaxin exhibited a 75% decrease in collagen deposition and tubular structure was almost completely maintained. In addition to this, it was seen that renal function was largely restored in the relaxin-treated animals. The levels of creatinine clearance were 75% of those of the control mice. It is thus hypothesized that the reduction of the fibrosis by relaxin is the cause for the restoration of renal function (Garber, et. al. 2001).

Conclusion

Fibrosis accounts for nearly half the deaths in the western world and yet there are no known cures. Relaxin's natural antifibrotic properties make it a viable candidate for the treatment of fibrosis. Although much research has already been done, relaxin must be proven to be both safe and effective before it can begin to be implemented clinically, and thus there is a need for further research.

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