International Journal of Biomedical Research
ISSN: 0976-9633 (Online); 2455-0566 (Print)
Journal DOI: 10.7439/ijbr
CODEN: IJBRFA

Review Article

Therapeutic Role of Relaxin in the Treatment of Fibrosis

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Abstract

Relaxin is a polypeptide hormone of the insulin superfamily. It acts as a pleiotropic hormone and has many physiological roles. Relaxin signals through the Relaxin Family Peptide 1 (RXFP1) receptor, a G protein-coupled receptor that triggers multiple signaling pathways to achieve functions including remodeling of extracellular matrix. The polypeptide plays a role in the prevention and treatment of fibrosis in a number of tissues and organs including liver. The ongoing research on relaxin have generated interested in its use as antifibrotic agent. Their roles in remodeling extracellular matrix, inhibiting collagen deposition in experimental models suggest its therapeutic role in treating established fibrosis.

Keywords: Relaxin, Relaxin Family Peptide Receptor 1 (RXFP1), fibrosis, Protein Kinase A (PKA).

1. Introduction

1.1 Relaxin

Relaxin was discovered in 1926 by Frederick L. Hisaw. He found that serum injection from pregnant guinea pigs or rabbits induced a relaxation of pubic ligaments in virgin guinea pigs (Hisaw 1926). Relaxin at the time of its discovery was thought to be a reproductive hormone, being produced in the reproductive tract of many pregnant mammals including the corpus luteum, placenta and uterus. In pregnancy it was found to reduce spontaneous uterine contraction by softening of the cervix and elongation of the interpubic ligament. In the 1960s, experiments using porcine relaxin showed relaxin to be a promising therapeutic agent to treat scleroderma, a disease of skin stiffness, because application of the hormone increased skin elasticity [1]. Recent discoveries of the diverse action of relaxin and the presence of relaxin receptors in tissues such as brain, kidney, heart, lung, and liver demonstrates the intriguing fact that relaxin has a role in numerous physiological processes far beyond pregnancy and reproduction [2, 3]. Relaxin is now known to have effects on the central nervous system, and its binding sites have been found at many areas of the brain [4]. It promotes tissue remodeling via increased collagen turnover, and shows promise as an anti-fibrotic agent [5-7].

The relaxin-like family of peptides present in humans includes relaxin 1, relaxin 2 (referred as relaxin throughout this article), relaxin 3, and the insulin like peptides- InsL3, InsL4, InsL5 and InsL6 [1, 8]. The insulin-relaxin family is a group of evolutionary related proteins which possess a variety of hormonal activities. It is postulated that their respective genes had divergent evolution with time. The seven peptides have high structural but low sequence similarity but despite their structural similarity, relaxin and insulin have no common cellular effects (Bennett 2009). These family members are synthesized as prepro-hormones compromised of a B-C-A domain configuration. The C domain peptide is removed to process the pro-hormone to the mature active peptide that consists of two interchain and one intra chain disulphide bonds between highly conserved cysteine residues on the A and B chains.

2. Receptors of the relaxin family peptides

After the discovery of relaxin family peptides, the cognate receptors for these peptides remained elusive for many years. In 2002, they were shown to be leucine-rich G-protein-coupled receptors (GPCRs) [9]. The leucine-rich GPCR receptor (LGR) family includes FSH, LH, TSH and relaxin receptors. The receptors for relaxin and INSL3 contain leucine rich repeats in the extracellular domain and share 60% homology. These receptors were named LGR7 (now relaxin family peptide receptor; RXFP1) [9, 10] and LGR8 (now RXFP2) [11] respectively.

Relaxin 3 and InsL5 interact with GPCRs that are unrelated to LGRs. Relaxin 3 and InsL5 activate GPCR135 (now RXFP3) and GPCR142 (now RXFP4) [12, 13]. Relaxin, relaxin 3 and InsL3 bind with different affinity to activate leucine rich receptors, RXFP1 and RXFP2. Relaxin activates

both RXFP1 and RXFP2 but InsL3 activates only RXFP3. Relaxin3 activates RXFP1, 3 and 4 but InsL5 activates only RXFP4. We will focus on the characteristics, distribution and signaling of relaxin receptors RXFP1 and RXFP2.

Relaxin receptors:

2.1 RXFP1 and RXFP2- Structure and Distribution

2.1.1 Receptor structure:

RXFP1 and RXFP2 are multidomain protein receptors with a relatively large ectodomains that connect with a seven transmembrane (TM) spanning region, and finally an intracellular C-terminal tail [14]. The ectodomain makes up over half of the receptor size and facilitates relaxin binding. Both RXFP1 and RXFP2 contain unique N-terminal low density lipoprotein receptor type A (LDLa) modules. The LDLa module at the N-terminus is connected to ten leucine rich repeats (LRRs) by a short linker. The LRRs are "capped" at each end by cysteine-rich regions [15]. These cysteine-rich regions are important to maintain the protein structure of LRRs [15, 16]. The LRRs are connected by another linker to connect to the seven TM helices. The amino acid sequence of these receptors suggests the receptors to be N-terminally glycosylated, with glycosylation sites in ten leucine rich repeats and phosphorylated at several sites in 7 TM and C terminal tail. The post translational glycosylation modification of the receptors is shown to be important for receptor functional maturation and trafficking to cell surface, receptor activation and receptor signaling [17].

2.1.2 Receptor distribution:

Molecular techniques such as northern blotting and RT-PCR suggest that in humans RXFP1 mRNA is expressed in female reproductive organs such as the cervix [18], ovary, uterus, and placenta [9]. It is also expressed in the nipple and breast [18, 19]. It is expressed in male reproductive organs such as testis and prostate [9]. RXFP1 mRNA was also identified in adrenal, brain, bone marrow, heart, kidney, liver, lung, muscle, peripheral blood vessels, salivary glands, skin, thyroid [9] and in the monocyte cell line THP1 [20]. In addition, rats and mice also express RXFP1 in the oviduct and intestine [21].

The RXFP2 mRNA in humans is expressed in brain, bone marrow, kidney, muscle, pituitary, peripheral blood cells, thyroid, testis and uterus myometrium [9, 22, 23]. In addition to these, rat and mice also express RXFP2 in the ovary and gubernaculum [11, 17]. Normal lipid droplet storing, quiescent hepatic stellate cells (HSC) express low levels of RXFP1 but not RXFP2 [24]. Activated HSC express both receptors. In normal rat liver, RXFP1 but not RXFP2, is expressed at low levels, but in cirrhotic liver, expression of both receptors increases significantly. Thus, with progression of fibrosis, there is an increased expression of both receptors.

2.2 RXFP1 and RXFP2 signaling

Relaxin has been shown to induce cAMP as demonstrated by Braddon in the mouse symphysis [25]. The relationship between relaxin and a cAMP increase were established by Sanborn *et al* in 1980 [26], and later confirmed in rat myometrial cells [27] and in breast cancer cells [28]. The recent discovery of the relaxin cognate receptors, RXFP1 and RXFP2, showed that in spite of relaxin's structural similarity with insulin, its signaling through RXFPs is unlike insulin signaling through a tyrosine kinase receptor [14]. The discovery of relaxin receptors has helped to study its different signaling pathways and its functional role in more depth. Based on the conserved mechanisms identified in constitutively activated LH and TSH receptors, studies of putative gain-of-function point mutants of RXFP1 showed that this receptor could mediate signaling through the protein kinase A-dependent pathway [9, 14]. Further studies lead to a general consent that relaxin binding to RXFP1 and RXFP2 elicits bioactivity by stimulating adenylate cyclase to increase cAMP that stimulates downstream effector molecules such as PKA [17, 29-31]. In some cell types such as human macrophages (THP-1) and human breast adenocarcinoma cells (MCF-7), recent studies provide evidence that relaxin stimulates a biphasic cAMP response through RXFP1 with an initial phase of cAMP rise by activated by Gαs that lasts 10-15 minutes before inhibition by GαoB [20, 32]. The second, delayed phase of cAMP increase is through Gαi activation, that releases Gβ subunits, and these subunits then activate phosphatidylinositol 3 kinase (PI3K) to activate and translocate PKCζ to the cell membrane and activate adenylate cyclase for second, delayed round of cAMP production [20, 32, 33]. Contrary to the complex-biphasic signaling of RXFP1, RXFP2 signaling seems not to be biphasic. INSL3 or relaxin 2 stimulates RXFP2 to activate Gαs resulting in cAMP accumulation whereas, GαoB and Gβγ mediates the inhibition of RXFP2 mediated cAMP accumulation [32]. In addition to the cAMP pathway, there is evidence that relaxin signals through multiple pathways [2, 29]. In human uterine cells and THP-1, RXFP1 also initiates tyrosine kinase activation [34,35]. Relaxin is shown to signal through MAPK in human endometrial stromal cells and epithelial (HeLa cells) [2]. RXFP1 activation of ERK1/2 depends on the cell type. In THP1 cells and human pulmonary artery smooth muscle primary culture, ERK1/2 is quickly activated, within 5 minutes of RXFP1 activation [36] whereas in HeLa cells the response is noted after 45 to 90 minutes [37]. Bani and co-workers showed Relaxin to work through increased nitric oxide generation in guinea pig hearts, human breast cancer cells, mouse uterus and many other organs [38-40]. This NO production might use either a PI3K activated Akt pathway or IkB inactivation to increase in nitric oxide synthase 2 gene transcription via nuclear factor kB (NFkB) [41]. This diverse signaling of relaxin on different tissues results in various cellular and physiological processes including tissue remodeling, wound healing, cardiac protection, allergic responses and fibrosis [1, 3].

2.3 Liver fibrosis

Fibrosis is the accumulation of unwanted extracellular matrix in response to organ injury resulting in loss of tissue function [42]. Fibrotic disease irrespective of cause is generally associated with the accumulation of matrix. As the liver injury progresses, the scarring becomes more extensive, leading to cirrhosis and hence liver failure [43]. Chronic liver disease and cirrhosis was one among the 15 leading causes of mortality in 2007. Mortality due to chronic liver disease and cirrhosis was 1.2 % of total deaths (National Vital Statistics Reports, Vol. 58, No. 19, May 20, 2010). Later research has also shown that genetic determinants and environmental factors influence the rate of fibrosis progression. Fibrotic scarring changes the normal architecture and function of the liver. Unrestricted scarring progresses to cirrhosis and loss of the hepatic parenchyma due to increases in collagen deposition. This diseased liver includes altered vasculature resulting in portal hypertension, ascites, encephalopathy and finally disruption of metabolic functions of the liver as a whole. Research to understand the underlying mechanism of liver injury has seen progress in recent years, but still the removal of the causative agent or liver transplantation are currently the only treatments available [43]. HSCs are the major contributor to injury-induced collagen production.

2.3.1 Hepatic Stellate Cells

These cells are major mediators of the fibrotic process in liver during the wound healing process. HSC reside in the space of tissue between hepatocytes and the heapatic sinusoids [44]. In normal uninjured liver, HSC are quiescent cells that store vitamin A. As a result of injury, HSCs activate or transdifferentiate to a myofibroblast-like cell that is characterized by having a different phenotype and properties, including loss of normal retinoid-storing capacity, changes in cellular morphology and cytoskeletal organization, enhanced cell migration, adhesion, contractibility and proliferation, expression of α-SMA and acquisition of fibrogenic properties [45-47]. The expression of matrix-degrading enzymes such as matrix metalloproteinases (MMP)-1 and MMP 13 that degrade fibrillar collagen and MMP2 and MMP9 that degrade basement membrane collagen is reduced. Simultaneously, expression of tissue inhibitors of metalloproteinases (TIMPs) is increased [48]. Hence chronic liver injury leads to overall changes in expression of enzymes involved in matrix degradation resulting to a state towards accumulation of collagen and hepatic fibrosis. Relaxin and activators of PPAR have been shown to prevent the activation of activated HSC, bringing them back to quiescent stage and thus preventing progressive hepatic fibrosis [49, 50].

2.3.2 Relaxin and fibrosis

Many of relaxin's effects are due to its involvement in widespread remodeling of the extracellular matrix that involves altered secretion and degradation of matrix components [51]. Relaxin is responsible for widespread extracellular remodeling of vagina, cervix and in some species, the pubic symphysis during pregnancy [1]. These findings were later confirmed using the relaxin-null mouse model [52].

Relaxin has been shown to prevent fibrosis in many major organs including skin, lung, and heart. Relaxin was shown to reduce fibrotic lesions and skin thickness, characteristics of scleroderma, by decreasing collagen secretion and increasing collagen degradation [7, 53, 54]. Relaxin was reported to decrease the synthesis of TIMP-1 in dermal fibroblasts and enhance the expression of collagenase in culture of dermal fibroblasts [2]. The RXFP1-null and relaxin-null mouse models demonstrate age-associated pulmonary fibrosis [55]. Relaxin treatment of the relaxin null mice that have knocked out relaxin gene and experimental models of lung fibrosis in the mouse model was enough to reverse pulmonary fibrosis [51, 55]. Relaxin has also been found to be a potent regulator of collagen in the ECM of heart [56]. Relaxin inhibits the transition of cardiac fibroblasts to active myofibroblasts, decreases collagen I and III, and increases MMP secretion [56].

In liver fibrosis, HSCs are the major collagen secreting cells. The quiescent hepatic cells express RXFP1 at low levels, but not RXFP2. During progression to the activated phenotype, expression of RXFP1 and RXFP2 increases significantly. These changes are also seen in the cirrhotic liver, where expression of the receptors is increased, suggesting the role of these receptors and their ligands during fibrosis [24]. In activated HSC, relaxin treatment decreased the expression of smooth muscle actin, type I collagen and total collagen and decreased the synthesis of new collagen. Furthermore, relaxin decreased TIMP-1 and TIMP-2 and increased interstitial collagenase levels [50]. The *in vivo* effect of relaxin treatment on rat CCl_4 model resulted in significant decrease in liver weight, hepatic hydroxyproline levels and reduction in collagen deposition [57]. The progression of liver fibrosis with increased HSC activation is accompanied with decreased PPAR expression that makes PPAR ligands less useful in advanced liver fibrosis[58]. But in the early stages of HSC activation, many of the effects of relaxin on activated HSCs are similar to those seen with agonists of PPARγ in reducing liver fibrosis [10, 30, 31, 59].

3. Conclusion

Relaxin studies in the last two decades have made remarkable advances in the area of investigating relaxin as an antifibrotic agent. Not only were the relaxin receptor discovered but in depth studies have shown the complex signaling pathways to effect many physiological functions of body including many non-reproductive actions. The development of the relaxin-null animal models has provided strong evidence that one of relaxin functions is its role as antifibrotic agent in a number of organs. Relaxin treatment has reduced fibrosis in a variety of *in vivo* models, suggesting

that this may be a valuable area for translational research benefitting patients with fibrosis. Moreover, relaxin research as well as its combined role with other therapeutic drugs could possibly help treating established fibrosis and revert the progression of disease that would otherwise lead to organ failure.

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