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Associação inversa entre a expressão pulmonar de AT1 e Mas com parâmetros espirométricos de pacientes com fibrose pulmonar idiopática

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RESUMO

Introdução: A fibrose pulmonar idiopática (FPI) é uma doença intersticial grave, de etiologia desconhecida, com sobrevida média de cerca de 2,5 a 5 anos após o diagnóstico definitivo. A sua fisiopatologia ainda é um grande desafio para a ciência. É sabido que a superativação do receptor AT1 (AT1R) pela angiotensina II (ANG II) do sistema renina-angiotensina (SRA) induz efeitos observados na FPI como fibrose, inflamação e estresse oxidativo. Em contrapartida, a ativação do receptor Mas (Mas-R) pela angiotensina 1-7 (ANG-(1-7)) desse sistema induz ações protetoras que se contrapõem aos efeitos deletérios. No entanto, nenhum estudo foi encontrado na literatura avaliando a expressão desses receptores diretamente no tecido pulmonar desses pacientes. **Objetivo:** Analisar a expressão dos receptores AT1 e Mas nos pulmões de pacientes com FPI. Adicionalmente, verificamos a associação desta expressão com os parâmetros de função pulmonar através do volume expiratório forçado no primeiro segundo (VEF_1%) e da capacidade vital forçada (CVF%), resultantes da espirometria. **Métodos:** As amostras de 1cm³ de tecido pulmonar foram obtidas de seis pacientes com FPI (tecido fibrótico) e da porção considerada saudável do pulmão de seis pacientes sem FPI submetidos à ressecção de carcinoma brônquico. As expressões dos receptores foram quantificadas pela técnica de Western Blot. **Resultados:** A expressão do AT1R foi significativamente maior (34%) no tecido dos pacientes com FPI (P=0,006), enquanto que o Mas-R estava significativamente menos expresso (54%) nos pulmões desses pacientes (P=0,046). Houve correlação positiva entre a expressão do Mas-R e os parâmetros espirométricos VEF_1% (r=0,62, P=0,03) e CVF% (r=0,58, P=0,05). Já quando comparada a expressão do AT1R com os mesmos parâmetros, houve correlação negativa, com valores de r=0,8 e P=0,002 para VEF_1% e r=0,74 e P=0,006 para CVF%. **Conclusão:** nossos resultados demonstram, de forma inédita, aumento da expressão do AT1R e redução da expressão do Mas-R no tecido pulmonar de pacientes com FPI. Também evidenciamos que o desequilíbrio entre os receptores está associado à redução da função pulmonar. Estes achados abrem novos horizontes em relação ao papel do SRA na fisiopatologia da FPI.

Palavras-chave: Fibrose Pulmonar Idiopática; Doenças Pulmonares Intersticiais; Sistema Renina-Angiotensina.

ABSTRACT

Introduction: Idiopathic pulmonary fibrosis (IPF) is a severe interstitial disease of unknown etiology with a mean survival of about 2.5–5 years after definite diagnosis. Its pathophysiology is still a major challenge for science. It is known that the AT1 receptor (AT1R) overactivation by the angiotensin II (ANG II) of the renin angiotensin system (RAS) induces effects observed in IPF such as fibrosis, inflammation and oxidative stress. In contrast, the activation of Mas receptor (Mas-R) by angiotensin 1-7 (ANG-(1-7)) of this system induces protective actions that are opposed to these harmful effects. However, no study has been found in the literature evaluating these receptors expression directly in lung tissue of these patients. **Objective:** Analyze the AT1 and Mas receptors expression in IPF patients' lungs. Additionally, we verified the association of this expression with pulmonary function parameters through forced expiratory volume in the first second (FEV_1%) and forced vital capacity (FVC%), resulting from spirometry. **Methods:** Lung tissue's samples of 1cm³ were obtained from six patients with IPF (fibrotic tissue) and from a considered healthy portion of the lung of six patients without a diagnosis of IPF who underwent bronchial carcinoma resection. Receptor expressions were quantified using Western Blot technique. **Results:** AT1R expression was significantly higher (34%) in the tissue of patients with IPF (P=0,006), while Mas-R was significantly less expressed (54%) in these patients' lungs (P=0,046). There was a positive correlation between the expression of the Mas-R and the spirometric parameters FEV_1% (r=0,62, P=0,03) and FVC% (r=0,58, P=0,05). When comparing the AT1R expression with the same parameters, a negative correlation was found, with values of r=0,8 and P=0,002 for FEV_1% and r=0,74 and P=0,006 for FVC%. **Conclusion:** In conclusion, our results have shown, in an unprecedented way, increased expression of AT1R and reduced expression of Mas-R in lung tissue of patients with IPF. We also showed that the imbalance between the receivers is associated with reduced lung function of patients. These findings open new horizons in the role of RAS peptides in the pathophysiology of IPF.

Keywords: Idiopathic Pulmonary Fibrosis; Lung Diseases, Interstitial; Renin-Angiotensin System.

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LISTA DE ABREVIATURAS E SIGLAS EM PORTUGUÊS

ANG-(1-7)	Angiotensina 1-7
ANG I	Angiotensina I
ANG II	Angiotensina II
DLCO	Capacidade de difusão do monóxido de carbono
CVF	Capacidade vital forçada
CEA	Células epiteliais alveolares
DATASUS	Departamento de informática do Sistema Único de Saúde
DPI	Doenças pulmonares intersticiais
ECA	Enzima conversora de angiotensina
ECA2	Enzima conversora de angiotensina 2
TGF β	Fator de transformação e crescimento beta
KGF	Fatores de crescimento que controlam o comportamento epitelial
FPI	Fibrose Pulmonar Idiopática
MEC	Matriz extracelular
MMP	Metaloproteinases de matriz
LAP	Peptídeo associado à latência
PIU	Pneumonia intersticial usual
JNK	Proteína quinase
AT1R	Receptor de angiotensina tipo 1
Mas-R	Receptor Mas
SRA	Sistema renina-angiotensina
TCAR	Tomografia computadorizada de alta resolução
VEF_1	Volume expiratório forçado no primeiro segundo

LISTA DE ABREVIATURAS E SIGLAS EM INGLÊS

ANG-(1-7)	Angiotensin 1-7
ACE	Angiotensin converting enzyme
ACE2	Angiotensin converting enzyme 2
ANG I	Angiotensin I
ANG II	Angiotensin II
AT1R	Angiotensin receptor type 1
AEC	Apoptotic alveolar epithelial cells
BMI	Body mass índex
ECM	Extracellular matrix
FDA	Food and Drug Administration
FEV_1	Forced expiratory volume in the first second
FVC	Forced vital capacity
IPF	Idiopathic pulmonary fibrosis
Mas-R	Mas receptor
mRNA	Messenger RNA
JNK	Protein kinase C-Jun N-terminal
RAS	Renin-angiotensin system
TGFβ 1	Transforming growth factor beta 1

LISTA DE SÍMBOLOS

B	Beta
ºC	Grau Celsius
=	Igual
±	Mais ou menos
®	Marca registrada
-	Menos
%	Percentual
<	Símbolo matemático, menor que

SUMÁRIO

1 REVISÃO DA LITERATURA

1.1 DEFINIÇÃO

A fibrose pulmonar idiopática (FPI) está entre os distúrbios fibróticos que levam a perda de estrutura e função dos órgãos. É definida como uma pneumonia intersticial crônica de etiologia desconhecida e caracteriza-se por lesão pulmonar, infiltração de fibroblastos e deposição de matriz extracelular (MEC) rica em colágeno. Essa cascata de eventos culmina em destruição irreversível da arquitetura alveolar normal e leva ao comprometimento das trocas gasosas (1,2). Além de apresentar sintomas como tosse e dispneia progressiva, a doença está associada à debilidade da musculatura respiratória e esquelética, o que leva a fadiga aos mínimos esforços (3,4) e à redução da capacidade funcional dos indivíduos (5,6). Esses efeitos possuem impacto devastador na qualidade de vida do paciente (7,8). Devido à característica fibrosante da doença e às limitações terapêuticas, a FPI possui um prognóstico muito ruim, com sobrevida média de cerca de 2,5 a 5 anos após o diagnóstico definitivo (9,10).

1.2 FISIOPATOLOGIA

Por muitos anos, a FPI foi considerada uma doença de caráter essencialmente inflamatório devido ao aumento de células inflamatórias nos pulmões. Porém, a falta de sucesso dos pacientes em responder à terapia com corticosteróides, bem como a não visualização de linfócitos nas biópsias desses, levaram ao enquadramento da FPI como uma doença de origem não inflamatória (11). No entanto, contrariando a ideia inicial, estudos verificaram que, tanto na fibrose pulmonar experimental quanto na idiopática (12,13), há um reconhecimento do papel da inflamação. Além disso, dados publicados apoiam o conceito de que as células mesenquimais pulmonares são imunologicamente ativas e podem atuar como reguladores potentes do infiltrado inflamatório pulmonar (14,15). Esse conjunto de evidências demonstra, pois, que a inflamação está envolvida no processo fibrótico da FPI, apesar desta não ser mais considerada uma doença de origem inflamatória.

Atualmente, a fisiopatologia conhecida indica que a doença tenha origem a partir de lesões epiteliais repetitivas sobrepostas ao envelhecimento alveolar acelerado. Em condições normais, quando há uma lesão epitelial, o reparo leva ao restabelecimento do epitélio. Na fibrose pulmonar, no entanto, o reparo epitelial é falho (16). Uma vez ativado de forma descontrolada, o epitélio pulmonar produz mediadores de migração de fibroblastos, proliferação e diferenciação de fibroblastos em miofibroblastos ativos. Estes miofibroblastos secretam quantidades exageradas dos componentes da MEC na área lesada (como o colágeno dos tipos I, III e V e fibronectina) (17) e tornam-se resistentes à apoptose (18), o que resulta em alterações profibróticas e no remodelamento da MEC (19,20).

Um importante mediador desse processo é o TGFβ (Fator de Transformação do Crescimento Beta), uma proteína que, quando ativada, liga-se a receptores que propagam o sinal e ativam o processo descrito acima (21), desempenhando, portanto, um papel importante na fibrose pulmonar progressiva. Estudos demonstram que pacientes com fibrose pulmonar idiopática (22,23) e alguns modelos animais de fibrose apresentaram aumento da produção pulmonar de TGFβ (24). Quando ligado ao peptídeo associado à latência (LAP), o TGFβ latente pode ser liberado e ativado por forças mecânicas celulares (25) e pode ser ativado também por metaloproteinases de matriz (MMP) (26). A proliferação induzida por TGFβ também pode ser dependente da proteína quinase JNK, responsável pelo desencadeamento do processo de apoptose de células epiteliais alveolares (27), que, quando ocorre de forma patológica, pode induzir a proliferação de fibroblastos (28). Além disso, a expressão de fatores de crescimento que controlam o comportamento epitelial (KGF) em fibroblastos de pulmão normal é dependente da JNK e a expressão de KGF é prejudicada em fibroblastos de FPI (29).

Paradoxalmente, os fibroblastos nos pulmões de FPI demonstram pouca apoptose (18), apesar do pulmão ser um ambiente hostil à sobrevivência celular (30). Em resumo, esses dados mostram que, na FPI, os fibroblastos proliferam-se e evitam a apoptose por mecanismos que incluem a estimulação por fatores de crescimento, interações com a matriz e ativação de vias pró-sobrevivência (16). O acúmulo de MEC resultante na região alveolar é a última característica patológica da fibrose, que remodela a arquitetura pulmonar e leva ao declínio progressivo da sua função (17).

1.3 EPIDEMIOLOGIA

Os dados epidemiológicos da FPI no mundo são esparsos e, além disso, mudanças na classificação das pneumonias intersticiais idiopáticas e novos critérios diagnósticos dificultam a comparação entre dados passados e atuais. De fato, devido a diferenças importantes nas fontes de dados e a variações entre regiões geográficas existem incertezas sobre as taxas de incidência e mortalidade da FPI (31-34). Estudos usando critérios menos restritivos para definir FPI sugerem incidência que varia de 2 a 30 casos por 100.000 pessoas/ano e prevalência variando de 10 a 60 casos por 100.000 pessoas (35,36); cerca de 3 milhões de pessoas afetadas em todo o mundo. Uma revisão sistemática publicada em 2015 sugere que, em estimativa conservadora, a incidência da doença gire em torno de 2,8 a 9,3 casos por 100.000 pessoas/ano para a América do Norte e Europa (37).

No Brasil, dados de incidência e mortalidade disponíveis no site do Departamento de Informática do Sistema Único de Saúde (DATASUS) relativos ao período entre 1996 e 2010 demonstraram um aumento progressivo dos dois parâmetros. No ano de 2010, a incidência de FPI registrada foi de 4,84 casos por 1.000.000 de habitantes, enquanto a mortalidade foi de 12,11 óbitos por 1.000.000 de habitantes. Além disso, os dados apontaram aumento na mortalidade de 0,65 por 100.000 habitantes em 1996 para 1,21 por 100.000 em 2010. Entretanto, o DATASUS não reflete a prática da medicina privada, podendo, portanto, não refletir a realidade brasileira (38).

1.4 FATORES DE RISCO

Estudos indicam que os mais afetados por essa doença são homens na sexta ou sétima décadas de vida (39-42), porém, ainda não está claro se a associação com o sexo masculino sugere uma relação biológica ou é resultado de fatores comportamentais e ambientais (43). Como a incidência e a prevalência da FPI aumentam com a idade, pode-se pensar que seja uma doença associada à disfunção das células epiteliais alveolares relacionada ao envelhecimento (44), de forma que a senescência dessas células parece ser um fenótipo central que promove fibrose pulmonar (45). O envelhecimento celular precoce pode prejudicar a função pulmonar através de vários mecanismos, tais como: interferência específica

nos mecanismos de reparo tecidual após o dano, indução da alteração sistêmica e/ou local do sistema imunológico e comprometimento dos mecanismos de defesa pulmonar contra infecções, que podem levar a complexas características de remodelamento pulmonar, como no caso da FPI (46).

A literatura demonstra que os fatores de risco ambientais mais importantes para o desenvolvimento da FPI são o tabagismo e a exposição aos pós de metal, madeira, pedra e sílica (1,2,31). O hábito de fumar está fortemente associado à FPI, principalmente em indivíduos com histórico de tabagismo superior a 20 maços/ano (41,31). A natureza desses estímulos apoia o conceito de fibrose a partir de uma lesão, uma vez que o epitélio respiratório está continuamente exposto às partículas dispersas do ambiente externo.

Além das causas ambientais, as causas genéticas são as que parecem estar mais intimamente associadas aos fenótipos das células epiteliais pulmonares. Alguns estudos demonstram que mutações em genes envolvidos na manutenção do comprimento dos telômeros estão associadas a um risco aumentado de FPI (47,48), devido à deficiência na capacidade de reparo celular causada por defeitos nessa região do cromossomo (47). Outros estudos demonstraram que variações em genes que são responsáveis pela adesão, integridade e mecanotransdução celular também conferem uma predisposição para a FPI (49,50). Esse conjunto de dados parece direcionar para a importância genética no desenvolvimento da FPI, de modo que o fator genético juntamente com o tempo e as interações ambientais contribui para a patogênese da doença. Em suma, quando há uma predisposição genética, o estresse cumulativo causado ao epitélio por fatores ambientais pode favorecer o encurtamento acelerado dos telômeros e a senescência celular alveolar prematura, aumentando o risco para o desenvolvimento da FPI (51,52,46).

1.5 DIAGNÓSTICO

Para estabelecer o diagnóstico da doença e descartar outras causas ou condições sobrepostas, características clínicas (como exame físico de tórax), imagem do tórax e, em alguns casos, histopatologia pulmonar são necessárias. O diagnóstico geralmente é alcançado através da discussão entre uma equipe multidisciplinar, prática recomendada por diretrizes internacionais (2,53). Os testes de função pulmonar geralmente identificam doenças restritivas que levam a

capacidade pulmonar total reduzida e trocas gasosas anormais (2), porém, a FPI em estágio inicial pode demonstrar espirometria normal (54).

Para um correto diagnóstico, a tomografia computadorizada de alta resolução (TCAR) do pulmão revela-se essencial. Os critérios radiológicos são classificados em três categorias: padrão de PIU (Pneumonia Intersticial Usual), possível PIU e inconsistente com o padrão da PIU. O diagnóstico de um padrão de PIU que indica FPI requer predominância subpleural e basal, anormalidades reticulares, faveolamento com ou sem bronquiectasia de tração e ausência de características inconsistentes com padrão de PIU (2). Quando o padrão da TCAR é indeterminado ou inconsistente com a PIU, uma biópsia pulmonar cirúrgica deve ser considerada. Apesar dos avanços no diagnóstico por TCAR, alguns estudos demonstram que a biópsia continua sendo método importante para o diagnóstico de FPI, sobretudo em um grande subconjunto de pacientes em que as características clínicas e de imagem não bastam (55,56).

1.6 PARÂMETROS FUNCIONAIS

A progressão ou estabilidade da doença são definidas por critérios de função pulmonar (5). Um dos parâmetros funcionais utilizados na prática clínica para monitoramento e avaliação da doença consiste na espirometria, expressa em valor percentual da capacidade vital forçada (CVF) e do volume expiratório forçado no primeiro segundo (VEF_1), além da relação VEF_1/CVF, sendo a CVF% o parâmetro da função pulmonar que melhor prediz a mortalidade (1). Juntamente com a restrição dos volumes pulmonares, a outra característica importante das doenças pulmonares intersticiais (DPI) é a troca gasosa prejudicada. A capacidade de difusão de monóxido de carbono (DLCO) normalmente é reduzida em DPI, em maior extensão do que o volume pulmonar em que é medido (57).

Alguns estudos relataram taxas de mortalidade mais altas quando a CVF% está gravemente comprometida, porém os limiares para prever maior mortalidade variam. Para a CVF%, os valores associados à maior mortalidade vão de menos de 83% a menos de 60% da capacidade prevista (58-60). Além disso, foi demonstrado que um declínio na CVF maior do que 5% (do seu valor absoluto) e maior do que 10% (do seu valor absoluto ou relativo) nos seis a doze meses de seguimento foi

associado à diminuição da sobrevida (59,5). Um aumento da relação VEF_1/CVF também foi associado ao aumento nas taxas de mortalidade (61).

1.7 TRATAMENTO E IMPACTO ECONÔMICO

Os mecanismos envolvidos na patogênese e progressão da FPI são complexos, dificultando o desenvolvimento de um tratamento específico. Além disso, a progressão e os sintomas variam entre os pacientes devido à patologia da doença, o que exige uma personalização dos tratamentos. Para um tratamento eficaz da doença, mais investigações são necessárias para entender a patogênese e desenvolver novas terapias para prevenir a sua progressão (16). Nas últimas décadas, muitos conceitos terapêuticos para FPI mostraram-se fracassados. Em particular, o tratamento imunossupressor não mostrou nenhum benefício (62). Embora um estudo inicial tenha mostrado melhora na sobrevida de pacientes sob terapia de anticoagulação durante a exacerbação aguda (63), um estudo posterior mostrou aumento da mortalidade com essa terapia a longo prazo (64).

Em novembro de 2014, a Food and Drug Administration (FDA) dos EUA aprovou a pirfenidona e o nintedanibe como tratamentos para a FPI (65,66). Esses medicamentos, de forma independente, demonstraram diminuir a taxa de deterioração da função pulmonar medida pela CVF (67). Knüpel et al. (2017) (68) demonstraram eu seu estudo que, tanto o nintedanibe quanto a pirfenidona exercem efeitos antifibróticos por meio da inibição da formação de fibrilas do colágeno tipo I, que reduz o tamanho e o número de feixes de fibras colágenas. Os autores também demonstraram que o nintedanibe reduziu significativamente o colágeno tipo V e fibronectina; e que, tanto o nintedanibe quanto a pirfenidona impactaram a formação de fibrilas de colágeno nos fibroblastos. Utilizando microscopia eletrônica de varredura, os autores demonstraram que as drogas encurtaram o tamanho da fibra e a espessura da fibrila em comparação com os controles. Finalmente, com base nesses resultados, os autores sugerem que ambos os fármacos atuam em importantes etapas regulatórias da síntese e do processamento de colágeno, porém, o nintedanibe foi mais efetivo na regulação negativa da expressão gênica profibrótica e da formação de colágeno.

Os mecanismos descritos acima não excluem os efeitos potencialmente deletérios das substâncias (69,70). Atualmente, os dois medicamentos utilizados

para o tratamento da FPI foram bem-sucedidos em fornecer alívio sintomático e produzir pequena redução no declínio da função pulmonar (67,2,71). Porém, nenhuma terapia pode modificar a história natural da FPI, com exceção do transplante pulmonar (2). Mais de 2000 transplantes de pulmão são realizados nos Estados Unidos a cada ano. Destes, aproximadamente metade das indicações é para doença pulmonar intersticial (72).

O encaminhamento para um centro de transplante especializado deve ser feito imediatamente após o diagnóstico, uma vez que o processo de avaliação e o tempo de espera podem durar meses ou anos (73). Apesar de ser muitas vezes a única alternativa de tratamento, o transplante pulmonar está associado a complicações inerentes ao procedimento, como infecções devido ao uso de medicamentos imunomoduladores e rejeição do órgão (74). Apesar de poder prolongar a sobrevida do paciente e melhorar a qualidade de vida (75,76), apenas 66% dos pacientes transplantados com FPI sobrevivem por mais de três anos após o transplante e 53% por mais de cinco anos (72).

Além de reduzir a qualidade de vida do paciente, a FPI gera encargo substancial aos serviços de saúde (77) haja vista o grande número de comorbidades que esses indivíduos apresentam e a necessidade de empregarem-se recursos complexos para manejá-las (78,79). Dentre eles, destacam-se a utilização de medicamentos de alto custo, a realização do transplante pulmonar e a oxigenoterapia por longo prazo (80). Como a incidência e a prevalência da FPI são maiores entre os idosos e a população tende ao envelhecimento, projeta-se elevação dos gastos com a doença no futuro e consequente impacto na economia (36).

1.8 ASSOCIAÇÃO ENTRE O SISTEMA RENINA-ANGIOTENSINA E A FPI

Neste contexto, um dos importantes sistemas que desempenha papel-chave sobre a homeostase de diversos órgãos como coração, vasos sanguíneos e principalmente pulmões, está o sistema renina-angiotensina (SRA). Classicamente, sabemos que a ativação do SRA ocorre pela conversão do angiotensinogênio em um decapeptídeo pouco vasoconstritor conhecido como angiotensina I (ANG I) através da ação da enzima renina, liberada pelas células justaglomerulares dos rins. Em seguida, ANG I é convertida no octapeptídeo angiotensina II (ANG II) através da

enzima conversora de angiotensina (ECA), localizada nos pulmões. O receptor de angiontensina tipo 1 (AT1R) é o mais envolvido nos efeitos da ANG II, compondo o importante eixo ECA-ANGII-AT1R (81).

O AT1R através da ANG II promove regulação da pressão arterial e homeostase de sódio e água (81). Porém, quando ativado de forma exagerada, o mesmo induz efeitos deletérios como fibrose, angiogênese, vasoconstrição, estresse oxidativo e proliferação em diversos tipos celulares (82-85). Além disso, foi demonstrado que o AT1R está envolvido na fosforilação da proteína JNK, processo que desencadeia a apoptose de células epiteliais alveolares (CEA) (27). Importante ressaltar que a apoptose patológica das CEA pode induzir a proliferação de fibroblastos (28), evento envolvido na patogênese da fibrose pulmonar. Há evidências de que a expressão do AT1R apresenta-se aumentada no tecido pulmonar de pacientes com FPI (86) e que há uma alta concentração da ECA no líquido do lavado broncoalveolar desses pacientes (87).

A ANG II desempenha um papel importante na fibrose pulmonar experimental, evidência demonstrada por estudos que utilizaram inibidores da ECA e de AT1R e encontraram resultados como redução da fibrogênese e atenuação subsequentemente os níveis de TGFβ no tecido (88-90). In vitro, foi verificado que a estimulação com ANG II aumentou a síntese de TGFβ em fibroblastos e que os seus efeitos profibróticos ocorrem pela indução da produção de procolágeno em fibroblastos do pulmão humano através da ativação do AT1R e, pelo menos em parte, através da ação do TGFβ (82,85). Apesar das concentrações plasmáticas de ANG II apresentaram-se semelhantes entre pacientes com e sem FPI (91), estudos demonstraram que o parênquima pulmonar possui capacidade de geração local (extravascular) de ANG II através da clivagem do angiotensinogênio disponível no tecido e sugerem que as CEA apoptóticas e os miofibroblastos são as fontes principais desses peptídeos (92,93). Em conjunto, esses dados demonstram que, atuando em condições anormais, o eixo ECA-ANGII-AT1R está negativamente envolvido com o desenvolvimento da FPI.

Em contrapartida, por uma via alternativa, a enzima conversora de angiotensina 2 (ECA2) exerce o papel de converter a ANG I em angiotensina 1-7 (Ang-(1-7)) (94), a qual se liga ao receptor Mas (Mas-R) (95), compondo o eixo ECA2-ANG-(1-7)-Mas-R. Agindo como um peptídeo antagonista dos efeitos do eixo representado pela ANG II, a ANG-(1-7) é vasodilatadora, antiproliferativa,

antifibrótica e anti-inflamatória (96-100). De fato, segundo Li X (2006), antagonistas da ANG II exercem um efeito que atenua o aparecimento de fibrose pulmonar em modelos animais (92). Além disso, estudos demonstraram que a expressão da ECA2 é reduzida em pulmões de pacientes com FPI e de modelos animais com fibrose induzida por bleomicina (101,102). De uma forma promissora, foi demonstrado também que ANG-(1-7) inibe a fosforilação da proteína JNK, evitando o processo de apoptose das células epiteliais alveolares (27). Esses dados sugerem, portanto, que peptídeos que antagonizem os efeitos da ANG II poderiam ter um papel importante na FPI.

Para tornar o SRA ainda mais interessante no contexto da FPI, recentemente foi descoberta a alamandina: um heptapeptídeo gerado pela ação catalítica da ECA2 sobre a angiotensina A ou diretamente sobre a ANG-(1-7), que atua via receptor MrgD. A alamandina está intimamente relacionada ao vasodilatador ANG-(1-7), com apenas uma diferença de aminoácidos: o primeiro aminoácido de ANG-(1-7) é o aspartato e o da alamandina é a alanina (103). Além disso, semelhante a ANG-(1-7), a alamandina possui propriedades anti-hipertensivas, vasodilatadoras e antifibróticas (103-105). Recentemente, um estudo demonstrou que as concentrações plasmáticas de alamandina são reduzidas em pacientes com FPI (91), estreitando ainda mais a relação dos peptídeos antagonistas da ANG II com o efeito pulmonar protetor. Dessa forma, ambas vias (ANG-(1-7)/Mas-R e Alamandina/MrgD), compõem um eixo contrarregulatório para a ANG II muito importante para a homeostase tecidual. Esse conjunto de evidências demonstra a importância do equilíbrio entre os dois eixos do SRA no manejo dos pacientes com FPI, de modo que, talvez mais importante do que bloquear o eixo fibrosante, seja estimular o eixo antagonista.

Mesmo com os avanços na compreensão dos mecanismos envolvidos no desenvolvimento e progressão da FPI, a doença continua sendo um problema de saúde pública com lacunas que necessitam de mais pesquisas com novas perspectivas. Apesar do grande e evidente envolvimento do SRA com a doença, nenhum estudo foi encontrado na literatura demonstrando a expressão dos receptores desse sistema diretamente no tecido pulmonar de pacientes com FPI. Uma das possíveis explicações para essa ausência é o fato de que o uso de inibidores do eixo profibrótico não tenha demonstrado benefício considerável no tratamento da FPI (106). No entanto, a identificação de novos componentes do SRA

formando eixos que se contrapõem às ações clássicas da ANG II nos desafia a verificar qual a participação desse sistema diretamente no tecido pulmonar de pacientes com a doença. Nesse sentido, acreditamos que o equilíbrio dos componentes do SRA possa representar uma alternativa promissora de tratamento da doença.

REFERÊNCIAS DA REVISÃO DA LITERATURA

1. American Thoracic Society. Idiopathic pulmonary fibrosis: Diagnosis and treatment. International consensus statement. Am J Respir Crit Care Med. 2000;(161):646–64.

2. Raghu G, Collard HR, Egan JJ, Martinez FJ, Behr J, Brown KK, et al. An Official ATS/ERS/JRS/ALAT Statement: Idiopathic pulmonary fibrosis: Evidence-based guidelines for diagnosis and management. Am J Respir Crit Care Med. 2011;183(6):788–824.

3. Kim HJ, Perlman D, Tomic R. Natural history of idiopathic pulmonary fibrosis. Respir Med. 2015;109(6):661–70.

4. Eaton T, Young P, Milne D, Wells AU. Six-minute walk, maximal excercise tests: reproducibility in fibrotic intersticial pneumonia. Am J Respir Crit Care Med. 2005;171:1150–7.

5. Collard HR, King TE, Bartelson BB, Vourlekis JS, Schwarz MI, Brown KK. Changes in clinical and physiologic variables predict survival in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2003;168(5):538–42.

6. Harris-Eze AO, Sridhar G, Clemens RE, Zintel TA, Gallagher CG, Marciniuk DD. Role of hypoxemia and pulmonary mechanics in exercise limitation in interstitial lung disease. Am J Respir Crit Care Med. 1996;154(4 I):994–1001.

7. Bárczi E, Erdélyi T, Bohács A, Eszes N, Tárnoki AD, Tárnoki DL, et al. Quality of life of idiopathic pulmonary fibrosis patients. Eur Respir J. 2017 Sep 1;50(61):3813.

8. De Vries J, Kessels BLJ, Drent M. Quality of life of idiopathic pulmonary fibrosis patients. Eur Respir J. 2001;17(5):954–61.

9. Du Bois RM. An earlier and more confident diagnosis of idiopathic pulmonary fibrosis. Eur Respir Rev. 2012;21(124):141–6.

10. Funke-Chambour M, Geiser T. Idiopathic pulmonary fibrosis: the turning point is now! Swiss Med Wkly. 2015;145:1–13.

11. Selman M, King J, Pardo A. Idiopathic pulmonary fibrosis: Prevailing and evolving hypotheses about its pathogenesis and implications for therapy. Vol. 134, Annals of Internal Medicine. 2001. p. 136–51.

12. Herazo-Maya JD, Noth2 I, Duncan SR, Kim S, Ma S-F, Tseng GC, et al. Peripheral Blood Mononuclear Cell Gene Expression Profiles Predict. Sci Transl Med. 2013;5(205):205ra136.

13. Reilkoff RA, Peng H, Murray LA, Peng X, Russell T, Montgomery R, et al. Semaphorin 7a+ regulatory T cells are associated with progressive idiopathic pulmonary fibrosis and are implicated in transforming growth factor-β1-induced pulmonary fibrosis. Am J Respir Crit Care Med. 2012;187(2):180–8.

14. Song JS, Kang CM, Kang HH, Yoon HK, Kim YK, Kim KH, et al. Inhibitory effect of CXC chemokine receptor 4 antagonist AMD3100 on bleomycin induced murine pulmonary fibrosis. Exp Mol Med. 2010;42(6):465–76.

15. Li M, Riddle SR, Frid MG, El Kasmi KC, McKinsey TA, Sokol RJ, et al. Emergence of Fibroblasts with a Proinflammatory Epigenetically Altered Phenotype in Severe Hypoxic Pulmonary Hypertension. J Immunol. 2011;187(5):2711–22.

16. Willis MS, Yates CC, Schisler JC. Fibrosis in disease: An Organ-Based Guide to Disease Pathophysiology and Therapeutic Considerations. Press H, editor. 2019. 91-239 p.

17. King TE, Pardo A, Selman M. Idiopathic pulmonary fibrosis. Lancet. 2011;378:1949–61.

18. Thannickal VJ, Horowitz JC. Evolving Concepts of Apoptosis in Idiopathic Pulmonary Fibrosis. Proc Am Thorac Soc. 2006;3(4):350–6.

19. Xia H, Diebold D, Nho R, Perlman D, Kleidon J, Kahm J, et al. Pathological integrin signaling enhances proliferation of primary lung fibroblasts from patients with idiopathic pulmonary fibrosis. J Exp Med. 2008;205(7):1659–72.

20. Li Y, Jiang D, Liang J, Meltzer EB, Gray A, Miura R, et al. Severe lung fibrosis requires an invasive fibroblast phenotype regulated by hyaluronan and CD44. J Exp Med. 2011;208(7):1459–71.

21. Massagué J. TGFβ signalling in context. Nat Rev Mol Cell Biol. 2014;13(10):616–30.

22. Khalil N, O'Connor RN, Flanders KC, Unruh H. TGF-β1, but Not TGF-β2 or TGF-β3, Is Differentially Present in Epithelial Cells of Advanced Pulmonary Fibrosis: An Immunohistochemical Study. Am J Respir Cell Mol Biol. 1996;14(2):131–8.

23. Broekelmann TJ, Limper AH, Colby T V., McDonald JA. Transforming growth factor β1 is present at sites of extracellular matrix gene expression in human pulmonary fibrosis. Proc Natl Acad Sci U S A. 1991;88(15):6642–6.

24. Chun Geun Lee, Homer RJ, Zhu Z, Lanone S, Wang X, Koteliansky V, et al. Interleukin-13 induces tissue fibrosis by selectively stimulating and activating transforming growth factor β1. J Exp Med. 2001;194(6):809–21.

25. Munger JS, Huang X, Kawakatsu H, Griffiths MJD, Dalton SL, Wu J, et al. The integrin αvβ6 binds and activates latent TGFβ1: A mechanism for regulating pulmonary inflammation and fibrosis. Cell. 1999;96(3):319–28.

26. Yu Q, Stamenkovic I. Cell surface-localized matrix metalloproteinase-9 proteolytically activates TGF-β and promotes tumor invasion and angiogenesis. Genes Dev. 2000;14:163–76.

27. Uhal BD, Li X, Piasecki CC, Molina-Molina M. Angiotensin signalling in pulmonary fibrosis. Vol. 44, International Journal of Biochemistry and Cell Biology. 2012. p. 465–8.

28. Hagimoto N, Kuwano K, Miyazaki H, Kunitake R, Fujita M, Kawasaki M, et al. Induction of Apoptosis and Pulmonary Fibrosis in Mice in Response to Ligation of Fas Antigen. Am J Respir Cell Mol Biol. 1997;17(3):272–8.

29. Marchand-Adam S, Plantier L, Bernuau D, Legrand A, Cohen M, Marchal J, et al. Keratinocyte Growth Factor Expression by Fibroblasts in Pulmonary Fibrosis. Am J Respir Cell Mol Biol. 2005;32(5):470–7.

30. Bridges RS, Kass D, Loh K, Glackin C, Borczuk AC, Greenberg S. Gene expression profiling of pulmonary fibrosis identifies Twist1 as an antiapoptotic molecular “rectifier” of growth factor signaling. Am J Pathol. 2009;175(6):2351–61.

31. Taskar VS, Coultas DB. Is Idiopathic Pulmonary Fibrosis an Environmental Disease? Proc Am Thorac Soc. 2006;3(4):293–8.

32. Nalysnyk L, Cid-Ruzafa J, Rotella P, Esser D. Incidence and prevalence of idiopathic pulmonary fibrosis: Review of the literature. Eur Respir Rev. 2012;21(126):355–61.

33. Kaunisto J, Salomaa ER, Hodgson U, Kaarteenaho R, Myllärniemi M. Idiopathic pulmonary fibrosis - a systematic review on methodology for the collection of epidemiological data. BMC Pulm Med. 2013;13(1):1–11.

34. Samet JM, Coultas D, Raghu G. Idiopathic pulmonary fibrosis: tracking the true occurrence is challenging. Eur Respir J. 2015;46(3):604–6.

35. Baddini-Martinez J, Pereira CA. Quantos pacientes com fibrose pulmonar idiopática existem no Brasil? J Bras Pneumol. 2015;41(6):560–2.

36. Raimundo K, Chang E, Broder MS, Alexander K, Zazzali J, Swigris JJ. Clinical and economic burden of idiopathic pulmonary fibrosis: A retrospective cohort study. BMC Pulm Med. 2016;16(1):1–8.

37. Hutchinson J, Fogarty A, Hubbard R, McKeever T. Global incidence and mortality of idiopathic pulmonary fibrosis: A systematic review. Eur Respir J. 2015;46(3):795–806.

38. Rufino RL, Costa CHD, Accar J, Torres GR, Silva VL, Barros NP, et al. Incidence And Mortality Of Interstitial Pulmonary Fibrosis In Brazil. In American Thoracic Society; 2013. p. A1458–A1458. (American Thoracic Society International Conference Abstracts).
39. Gribbin J, Hubbard RB, Le Jeune I, Smith CJP, West J, Tata LJ. Incidence and mortality of idiopathic pulmonary fibrosis and sarcoidosis in the UK. Thorax. 2006;61(11):980–5.

40. Raghu G, Freudenberger TD, Yang S, Curtis JR, Spada C, Hayes J, et al. High prevalence of abnormal acid gastro-oesophageal reflux in idiopathic pulmonary fibrosis. Eur Respir J. 2006;27(1):136–42.

41. Iwai K, Mori T, Yamada N, Yamaguchi M, Hosoda Y. Idiopathic pulmonary fibrosis: Epidemiologic approaches to occupational exposure. Am J Respir Crit Care Med. 1994;150(3):670–5.

42. Coultas. D., Z.umwalt. R, Black. W., Sobonya. R. The epidemiology of interstitial lung diseases. Am J Respir Crit Care Med. 1994;150:967–72.

43. Martinez FJ, Collard HR, Pardo A, Raghu4 G, Richeldi L, Selman M, et al. Idiopathic pulmonary fibrosis. Nat Rev Dis Prim. 2017;3:17074.

44. Wolters PJ, Collard HR, Jones KD. Pathogenesis of Idiopathic Pulmonary Fibrosis. Annu Rev Pathol Mech Dis. 2013;9(1):157–79.

45. Selman M, Pardo A. Revealing the pathogenic and aging-related mechanisms of the enigmatic idiopathic pulmonary fibrosis: An integral model. Am J Respir Crit Care Med. 2014;189(10):1161–72.

46. Chilosi M, Carloni A, Rossi A, Poletti V. Premature lung aging and cellular senescence in the pathogenesis of idiopathic pulmonary fibrosis and COPD/emphysema. Transl Res. 2013;162(3):156–73.

47. Armanios MY, Chen JJ-L, Cogan JD, Alder JK, Ingersoll RG, Markin C, et al. Telomerase Mutations in Families with Idiopathic Pulmonary Fibrosis. N Engl J Med. 2007;356(13):1317–26.

48. Tsakiri KD, Cronkhite JT, Kuan PJ, Xing C, Raghu G, Weissler JC, et al. Adult-onset pulmonary fibrosis caused by mutations in telomerase. Proc Natl Acad Sci U S A. 2007;104(18):7552–7.

49. Fingerlin TE, Murphy E, Zhang W, Peljto AL, Brown KK, Steele MP, et al. Genome-wide association study identifies multiple susceptibility loci for pulmonary fibrosis. Nat Genet. 2013;45(6):613–20.

50. Allen RJ, Porte J, Braybrooke R, Flores C, Fingerlin TE, Oldham JM, et al. Genetic variants associated with susceptibility to idiopathic pulmonary fibrosis in people of European ancestry: a genome-wide association study. Lancet Respir Med. 2017;5(11):869–80.

51. Zglinicki T, Martin-Ruiz C. Telomeres as Biomarkers for Ageing and Age-Related Diseases. Curr Mol Med. 2005;5(2):197–203.

52. Tsuji T, Aoshiba K, Nagai A. Cigarette smoke induces senescence in alveolar epithelial cells. Am J Respir Cell Mol Biol. 2004;31(6):643–9.

53. Travis WD, Costabel U, Hansell DM, King TE, Lynch DA, Nicholson AG, et al. An official American Thoracic Society/European Respiratory Society statement: Update of the international multidisciplinary classification of the idiopathic interstitial pneumonias. Am J Respir Crit Care Med. 2013;188(6):733–48.

54. Lama VN, Martinez FJ. Resting and exercise physiology in interstitial lung diseases. Clin Chest Med. 2004;25(3):435–53.

55. Yagihashi K, Huckleberry J, Colby T V., Tazelaar HD, Zach J, Sundaram B, et al. Radiologic-pathologic discordance in biopsy-proven usual interstitial pneumonia. Eur Respir J. 2016;47(4):1189–97.

56. Sverzellati N, Wells AU, Tomassetti S, Desai SR, Copley SJ, Aziz ZA, et al. Biopsy-proved idiopathic pulmonary fibrosis: Spectrum of nondiagnostic thin-section CT diagnoses. Radiology. 2010;254(3):957–64.

57. Plantier L, Cazes A, Dinh-Xuan AT, Bancal C, Marchand-Adam S, Crestani B. Physiology of the lung in idiopathic pulmonary fibrosis. Eur Respir Rev. 2018;27(147):1–14.

58. Jezek V, Fucik J, Michaljanic A, Jezkova L. The prognostic significance of functional tests in cryptogenic fibrosing alveolitis. Bull Eur Physiopathol Respir. 1980;16(6):711—720.

59. Latsi PI, Du Bois RM, Nicholson AG, Colby T V., Bisirtzoglou D, Nikolakopoulou A, et al. Fibrotic idiopathic interstitial pneumonia: The prognostic value of longitudinal functional trends. Am J Respir Crit Care Med. 2003;168(5):531–7.

60. Erbes R, Schaberg T, Loddenkemper R. Lung function tests in patients with idiopathic lung fibrosis. Pneumologie. 1997;51(9):925.

61. Schwartz DA, Helmers RA, Galvin JR, Van Fossen DS, Frees KL, Dayton CS, et al. Determinants of survival in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 1994;149(2 I):450–4.

62. Raghu G, Anstrom KJ, Talmadge E. King J, Lasky JA, Martinez FJ. Prednisone, Azathioprine, and N-Acetylcysteine for Pulmonary Fibrosis. N Engl J Med. 2012;366:1968–77.

63. Kubo H, Nakayama K, Yanai M, Suzuki T, Yamaya M, Watanabe M, et al. Anticoagulant therapy for idiopathic pulmonary fibrosis. Chest. 2005;128(3):1475–82.

64. Noth I, Anstrom KJ, Calvert SB, De Andrade J, Flaherty KR, Glazer C, et al. A placebo-controlled randomized trial of warfarin in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2012;186(1):88–95.

65. Pollack A. F.D.A. approves first 2 drugs for treatment of a fatal lung disease. New York Times. 2014.

66. Bahudhanapati H, Kass DJ. Unwinding the Collagen Fibrils: Elucidating the Mechanism of Pirfenidone and Nintedanib in Pulmonary Fibrosis. Am J Respir Cell Mol Biol. 2017;57(1):10–1.

67. Richeldi L, Bois RM du, Raghu G, Azuma A, Brown KK, Costabel U, et al. Efficacy and safety of nintedanib in advanced idiopathic pulmonary fibrosis. N Engl J Med. 2014;370(22):2071–82.

68. Knüppel L, Ishikawa Y, Aichler M, Heinzelmann K, Hatz R, Behr J, et al. A novel antifibrotic mechanism of nintedanib and pirfenidone: inhibition of collagen fibril assembly. Am J Respir Crit Care Med. 2017;57:77–90.

69. Nakazato H, Oku H, Yamane S, Tsuruta Y, Suzuki R. A novel anti-fibrotic agent pirfenidone suppresses tumor necrosis factor-α at the translational level. Eur J Pharmacol. 2002;446(1–3):177–85.

70. Didiasova M, Singh R, Wilhelm J, Kwapiszewska G, Wujak L, Zakrzewicz D, et al. Pirfenidone exerts antifibrotic effects through inhibition of GLI transcription factors. FASEB J. 2017;31(5):1916–28.

71. King TE, Bradford WZ, Castro-Bernardini S, Fagan EA, Glaspole I, Glassberg MK, et al. A Phase 3 Trial of Pirfenidone in Patients with Idiopathic Pulmonary Fibrosis. N Engl J Med. 2014;370(22):2083–92.

72. Valapour M, Lehr CJ, Skeans MA, Smith JM, Carrico R, Uccellini K, et al. OPTN/SRTR 2016 Annual Data Report: Lung. Am J Transplant. 2018;18:363–433.

73. Weill D, Benden C, Corris PA, Dark JH, Davis RD, Keshavjee S, et al. A consensus document for the selection of lung transplant candidates: 2014--an update from the Pulmonary Transplantation Council of the International Society for Heart and Lung Transplantation. J Hear Lung Transpl. 2015;34(1):1–15.

74. Rafii R, Juarez MM, Albertson TE, Chan AL. A review of current and novel therapies for idiopathic pulmonary fibrosis. J Thorac Dis. 2013;5(1):48–73.

75. Titman A, Rogers CA, Bonser RS, Banner NR, Sharples LD. Disease-specific survival benefit of lung transplantation in adults: A national cohort study. Am J Transplant. 2009;9(7):1640–9.

76. Singer JP, Katz PP, Soong A, Shrestha P, Huang D, Ho J, et al. Effect of Lung Transplantation on Health-Related Quality of Life in the Era of the Lung Allocation Score: A U.S. Prospective Cohort Study. Am J Transplant. 2017;17(5):1334–45.

77. Lee AS, Mira-Avendano I, Ryu JH, Daniels CE. The burden of idiopathic pulmonary fibrosis: An unmet public health need. Respir Med. 2014;108(7):955–67.

78. Collard HR, Ward AJ, Lanes S, Cortney Hayflinger D, Rosenberg DM, Hunsche E. Burden of illness in idiopathic pulmonary fibrosis. J Med Econ. 2012;15(5):829–35.

79. Wu N, Yu YF, Chuang C-C, Wang R, Benjamin NN, Coultas DB. Healthcare resource utilization among patients diagnosed with idiopathic pulmonary fibrosis in the United States. J Med Econ. 2014;18(4):249–57.

80. Raghu G. Idiopathic pulmonary fibrosis: guidelines for diagnosis and clinical management have advanced from consensus-based in 2000 to evidence-based in 2011. Eur Respir J. 2011;37(4):743–6.

81. Hall JE, Guyton AC. Tratado de Fisiologia Médica. 10ª. Koogan G, editor. Rio de Janeiro; 2002. 190-193 p.

82. Marshall RP, Gohlke P, Chambers RC, Howell DC, Bottoms SE, Unger T, et al. Angiotensin II and the fibroproliferative response to acute lung injury. Am J Physiol Cell Mol Physiol. 2004;286(1):L156–64.

83. Wang R, Zagariya A, Ibarra-sunga O, Gidea C, Ang E, Deshmukh S, et al. Angiotensin II induces apoptosis in human and rat alveolar epithelial cells. 1999;(18):885–9.

84. Yamakawa T, Tanaka SI, Numaguchi K, Yamakawa Y, Motley ED, Ichihara S, et al. Involvement of Rho-kinase in angiotensin II-induced hypertrophy of rat vascular smooth muscle cells. In: Hypertension. 2000. p. 313–8.

85. Marshall RP, McAnulty RJ, Laurent GJ. Angiotensin II is mitogenic for human lung fibroblasts via activation of the type 1 receptor. Am J Respir Crit Care Med. 2000;161(6):1999–2004.

86. Königshoff M, Wilhelm A, Jahn A, Sedding D, Amarie OV, Eul B, et al. The angiotensin II receptor 2 is expressed and mediates angiotensin II signaling in lung fibrosis. Am J Respir Cell Mol Biol. 2007;37(6):640–50.

87. Specks U, Martin WJ, Rohrbach MS. Bronchoalveolar lavage fluid angiotensin-converting enzyme in interstitial lung diseases. Am Rev Respir Dis. 1990;141(1):117–23.

88. Li X, Rayford H, Uhal BD. Essential roles for angiotensin receptor AT1a in bleomycin-induced apoptosis and lung fibrosis in mice. Am J Pathol. 2003;163(6):2523–30.

89. Waseda Y, Yasui M, Nishizawa Y, Inuzuka K, Takato H, Ichikawa Y, et al. Angiotensin II type 2 receptor antagonist reduces bleomycin-induced pulmonary fibrosis in mice. Respir Res. 2008;9(43):1–9.

90. Otsuka M, Takahashi H, Shiratori M, Chiba H, Abe S. Reduction of bleomycin induced lung fibrosis by candesartan cilexetil, an angiotensin II type 1 receptor antagonist. Thorax. 2004;59(1):31–8.

91. Taís Salvi S, Robson Augusto Souza dos S, Katya R. The Renin-Angiotensin System: Alamandine is reduced in patients with Idiopathic Pulmonary Fibrosis. J Cardiol Cardiovasc Med. 2019;4(3):210–5.

92. Li X, Molina-Molina M, Abdul-Hafez A, Ramirez J, Serrano-Mollar A, Xaubet A, et al. Extravascular sources of lung angiotensin peptide synthesis in idiopathic pulmonary fibrosis. Am J Physiol Lung Cell Mol Physiol. 2006;291(5):L887-95.

93. Wang R, Alam G, Zagariya A, Gidea C, Pinillos H, Lalude O, et al. Apoptosis of lung epithelial cells in response to TNF-alpha requires angiotensin II generation de novo. J Cell Physiol. 2000;185:253–9.

94. Ferrario CM, Chappell MC, Tallant EA, Brosnihan KB, Diz DI. Counterregulatory actions of angiotensin-(1-7). Hypertension. 1997;30(3 Pt 2):535–41.

95. Santos RAS, e Silva ACS, Maric C, Silva DMR, Machado RP, de Buhr I, et al. Angiotensin-(1-7) is an endogenous ligand for the G protein-coupled receptor Mas. Proc Natl Acad Sci U S A. 2003;100(14):8258–63.

96. Raffai G, Durand MJ, Lombard JH. Acute and chronic angiotensin-(1-7) restores vasodilation and reduces oxidative stress in mesenteric arteries of salt-fed rats. Am J Physiol - Hear Circ Physiol. 2011;301(4):H1341-52.

97. da Silveira KD, Coelho FM, Vieira AT, Sachs D, Barroso LC, Costa VV, et al. Anti-Inflammatory Effects of the Activation of the Angiotensin-(1–7) Receptor, Mas, in Experimental Models of Arthritis. J Immunol. 2010;185(9):5569–76.

98. Pei Z, Meng R, Li G, Yan G, Xu C, Zhuang Z, et al. Angiotensin-(1-7) ameliorates myocardial remodeling and interstitial fibrosis in spontaneous hypertension: Role of MMPs/TIMPs. Toxicol Lett. 2010;199(2):173–81.

99. Pei N, Wan R, Chen X, Li A, Zhang Y, Li J, et al. Angiotensin-(1-7) decreases cell growth and angiogenesis of human nasopharyngeal carcinoma xenografts. Mol Cancer Ther. 2015;15(1):37–47.

100. Kostenis E, Milligan G, Christopoulos A, Sanchez-Ferrer CF, Heringer-Walther S, Sexton PM, et al. G-protein-coupled receptor Mas is a physiological antagonist of the angiotensin II type 1 receptor. Circulation. 2005;111(14):1806–13.

101. Li X, Molina-Molina M, Abdul-Hafez A, Uhal V, Xaubet A, Uhal BD. Angiotensin converting enzyme-2 is protective but downregulated in human and experimental lung fibrosis. AJP Lung Cell Mol Physiol. 2008;295(1):L178–85.

102. Wang L, Wang Y, Yang T, Guo Y, Sun T. Angiotensin-converting enzyme 2 attenuates bleomycin-induced lung fibrosis in mice. Cell Physiol Biochem. 2015;36(2):697–711.

103. Villela DC, Passos-Silva DG, Santos RAS. Alamandine: A new member of the angiotensin family. Vol. 23, Current Opinion in Nephrology and Hypertension. 2014. p. 130–4.

104. Qaradakhi T, Matsoukas MT, Hayes A, Rybalka E, Caprnda M, Rimarova K, et al. Alamandine reverses hyperhomocysteinemia-induced vascular dysfunction via PKA dependent mechanisms. Cardiovasc Ther. 2017;35(6).

105. Soares ER, Barbosa CM, Campagnole-Santos MJ, Santos RAS, Alzamora AC. Hypotensive effect induced by microinjection of Alamandine, a derivative of angiotensin-(1–7), into caudal ventrolateral medulla of 2K1C hypertensive rats. Peptides. 2017;96(September):67–75.

106. Kreuter M, Lederer DJ, Molina-Molina M, Noth I, Valenzuela C, Frankenstein L, et al. Association of Angiotensin Modulators With the Course of Idiopathic Pulmonary Fibrosis. Chest. 2019;156(4):706–714.

2 OBJETIVOS

2.1 OBJETIVO GERAL

Analisar a expressão dos receptores AT1 e Mas no pulmão de pacientes com fibrose pulmonar idiopática.

2.2 OBJETIVOS ESPECÍFICOS

- Analisar a expressão dos receptores AT1 e Mas em pulmões de pacientes com fibrose pulmonar idiopática em comparação com o grupo controle;
- Correlacionar a expressão dos receptores AT1 e Mas com os parâmetros de função pulmonar dos pacientes.

3 ARTIGO CIENTÍFICO

O presente artigo foi elaborado em língua estrangeira e está formatado conforme as normas da revista Peptides (Fator de impacto 2018/2019: 2.659).

Inverse Association between AT1 and Mas pulmonary expression with spirometric parameters of patients with idiopathic pulmonary fibrosis

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Abbreviations: IPF, idiopathic pulmonary fibrosis; RAS, renin-angiotensin system; ACE, angiotensin converting enzyme; ACE2, angiotensin converting enzyme 2; ANG I, angiotensin I; ANG II, angiotensin II; ANG-(1-7), angiotensin 1-7; AT1R, angiotensin receptor type 1; Mas-R, Mas receptor; BMI, body mass index; ECM, Extracellular matrix; FEV_1, forced expiratory volume in the first second; FVC, forced vital capacity; JNK, protein kinase c-Jun N-terminal; TGFβ 1, transforming growth factor beta 1.

Highlights:

- Idiopathic pulmonary fibrosis pathophysiology is still a major challenge for Science;
- Renin-angiotensin system has been neglected in pulmonary pathophysiology;
- The patients' functional capacity is negatively associated with AT1 expression;
- But positively associated with Mas receptor expression.

ABSTRACT

Idiopathic pulmonary fibrosis (IPF) is a severe interstitial disease with a mean survival of about 2.5–5 years after diagnosis. Its pathophysiology is still a major challenge for science. It is known that angiotensin II (ANG II) binds AT1 receptor (AT1R) and its over activation induces fibrosis, inflammation and oxidative stress. In contrast, the activation of Mas receptor (Mas-R) by angiotensin 1-7 opposes the harmful effects induced by ANG II. Thus, our innovative objective was to analyze, in patients' lung with IPF, the balance between AT1 and Mas receptors expression and their possible association with pulmonary spirometric parameters: the forced expiratory volume in the first second (FEV_1%) and the forced vital capacity (FVC%). It was used 1cm³ of fibrotic lung tissue from IPF patients (n=6) and from patients without IPF (n=6) who underwent bronchial carcinoma resection. Receptor expressions were quantified using Western Blot. AT1R expression was significantly higher (34%) in patients with IPF (P=0.006), while Mas-R was significantly less expressed (54%) in these patients' lungs (P=0.046). There was also a positive correlation between Mas-R expression with FEV_1% (r=0.62, P=0.03) and FVC% (r=0.58, P=0.05). Conversely, AT1R expression was negatively correlated with FEV_1% (r=0.80, P=0.002) and FVC% (r=0.74, P=0.006). In conclusion, our results demonstrated an increased expression of AT1R and reduced expression of Mas-R in the lung of patients with IPF. The dominance of AT1R expression is associated with a reduced lung function, highlighting the role of the renin-angiotensin system peptides in the pathophysiology of IPF.

Keywords: Idiopathic Pulmonary Fibrosis; Lung Diseases, Interstitial; Renin-Angiotensin System.

3.1 INTRODUCTION

Idiopathic pulmonary fibrosis (IPF) is defined as chronic fibrosing interstitial pneumonia of unknown etiology with a poor prognosis. The mean survival is about 2.5–5 years after diagnosis [1,2]. IPF is more common in men, current or former smokers, and it usually occurs between the sixth and seventh decades of life [1,3]. The known pathophysiology of IPF indicates that the disease originates from repetitive injuries in the pulmonary epithelium overlapped the accelerated aging of alveolar cells, which triggers failed repair mechanisms [4]. When activated in an uncontrolled manner, pulmonary epithelium produces mediators of fibroblast migration, proliferation and fibroblasts into active myofibroblasts differentiation [5]. Once in the injured areas, these myofibroblasts secrete exaggerated amounts of extracellular matrix (ECM) components and become resistant to apoptosis [5,6]. This results, in ECM and fibrosis remodeling [7,8].

Due to the progressive loss of lung function caused by IPF, individual's quality of life decreases as the disease progresses [9]. In addition, due to the large number of associated comorbidities and the need to apply complex resources to manage them, IPF generates a substantial burden on health services [10,11]. Currently, the two drugs being used to treat the disease are expensive and promote only a small reduction in the lung function decline [12,13]. However, no therapy can modify IPF natural history, except for transplantation [1]. Therefore, understanding the IPF pathophysiology is a major challenge for science and becomes extremely important in the search for new therapeutic targets not only to IPF but also to the new COVID-19 that causes fibrosis [14,15].

In this context, one of the important systems that plays a key role on the homeostasis of different organs such as heart, blood vessels, and especially lungs, is the renin-angiotensin system (RAS). Classically, it is known that the conversion of angiotensin I (ANG I) to angiotensin II (ANG II) occurs by angiotensin converting enzyme (ACE) located also in the lungs [16]. ANG II is a potent vasoconstrictor that acts mainly via angiotensin receptor type 1 (AT1R), forming the ACE-ANGII-AT1R axis [17]. When excessively activated, AT1R may induce deleterious effects such as fibrosis, apoptosis, angiogenesis, hypertrophy, oxidative stress and cell proliferation in several cell types [18-22].

On the other hand, angiotensin converting enzyme 2 (ACE2) plays the role in cleaving ANG I into angiotensin 1-7 (ANG-(1-7)), which binds to Mas receptor (Mas-R), forming the ACE2-ANG-(1-7)-Mas-R axis. Acting as an antagonist peptide to the effects of ANG II, ANG-(1-7) has a vasodilating, antiproliferative, antifibrotic and anti-inflammatory action [23-27]. Thus, the axis represented by ANG-(1-7) possibly induces effects that are opposite to those generated by the ACE-ANGII-AT1R axis [28,29].

Surprisingly, although ANG II has a well-described fibrotic effect [30-32], be activated in the lung [16], and ANG-(1-7) has actions that antagonize ANG II effects [27,29], no study has been found in the literature demonstrating the expression of their receptors directly into lung tissue of patients with IPF. A possible reason for this lack of studies is due to the fact that using ANG II blockers has not demonstrated effective clinical results in disease improvement or in survival [33-35].

However, considering the therapeutic potential of ANG-(1-7), shown in many tissues fibrosis [36-38], it is reasonable to believe that an imbalance between the axis receivers of the RAS may be involved in the IPF progression. More important than blocking the components of the fibrous axis, represented by ANG II, it seems to be due to stimulation of the protective axis represented by ANG-(1-7).

Despite scientific advances, fibrotic diseases remain an important public health problem and further research is essential to improve the understanding of peptides and mechanisms involved in the pathophysiology of those diseases. Hence, our objective was to analyze the expression of AT1 and Mas receptors in the lungs of patients with IPF and verify whether there is an association with the pulmonary function parameters.

3.2 MATERIALS AND METHODS

3.2.1 IPF patients and controls

Lung tissue samples were obtained from six patients with IPF who underwent lung transplantation and six patients who underwent bronchial carcinoma resection (control group). The control group included individuals without a diagnosis of IPF, preferably with age and sex similar to the group with IPF members, but not necessarily respecting a perfect pairing. Patients diagnosed with heart failure were

excluded. Considering the complexity of the disease and the number of medications these patients could be taking at the time of surgery, exclusion criteria were not strict, even allowing the inclusion of patients who were using drugs that interfere with RAS in both groups.

The study was approved by the research ethics committees of *Irmandade Santa Casa de Misericórdia of Porto Alegre* and of the Universidade Federal de Ciências da Saúde de Porto Alegre, approval numbers 2.691.887 and 2.619.738, respectively. The informed consent form was applied to all patients.

3.2.2 Tissue Collection

1 cm^3 of lung tissue from each patient was collected and frozen in liquid nitrogen and then stored at -80ºC. IPF was later diagnosed through anatomopathological testing. Sample collection from the control group was carried out in the safety margin of the removed lung carcinoma, allowing analysis of tissue with similar characteristics to the lungs of a healthy individual.

3.2.3 Pulmonary function test

Spirometry was performed at the health service in the preoperative period and data collected through medical records. The spirometric parameters evaluated for analysis were: forced expiratory volume in the first second (FEV_1%), forced vital capacity (FVC%) and FEV_1/FVC% ratio.

3.2.4 Protein extraction

Protein was extracted from samples by manual homogenization in 50µL of lysis buffer containing protease inhibitor (10 mM Tris-HCl, pH 7.5; 1 mM MgCl2; 1mM ethylenediaminetetraacetic acid [EDTA]; 0.1mM phenylmethylsulfonyl fluoride [PMSF]; 5 mM 2-mercaptoethanol; 0.5% of 3 - [(3-Cholamidopropyl) dimethylammonio] -1-propanesulfonate (CHAPS) and 10% glycerol). To homogenize, the samples were vortexed for 30 seconds (four times with an interval of 10 minutes each) and then, centrifuged for 1h, at a speed of 13000rpm at 4ºC. After centrifugation, only the supernatant was carefully collected and frozen at -12ºC for

further analysis. The protein quantification of the samples was done through spectrophotometry.

3.2.5 Western Blot Analysis

Protein samples (20 µg) were separated by one-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) at 10% and transferred to nitrocellulose membranes using buffer containing Tris 20 mM, glycine 150 mM, methanol 20% (v/v) and SDS 0.02% (w/v) (pH=8.2), in a cooled Bio-Rad transfer unit. After that, the nonspecific protein sites were blocked through 1h incubation in a blocking solution composed of 5% (v/v) skimmed milk in 0.1% phosphate-saline buffer (PBS 1X). Afterwards the membrane was stained with a 1:500 concentration of Rabbit Polyclonal anti-Human anti-Angiotensin II Type-1 Receptor antibody/AGTR1 (AAR-011 - Alomone®, Israel), Rabbit Polyclonal anti-Human anti-Angiotensin-(1-7) Mas Receptor antibody (AAR-013 - Alomone®, Israel) in a concentration of 1:250 or Mouse anti-Human β-actin Monoclonal Antibody (A2228 - Sigma Aldrich®, Germany) followed by secondary staining with 1:1000 concentration of Rabbit anti-Mouse IgG (H+L)-HRP antibody (ThermoFisher Scientific®, MA, USA).

Washing steps were carried out with Tween-20. The Western blottings were visualized by enhanced chemiluminescence (GE Healthcare Life Sciences). Band intensity was determined by densitometry analysis and for band quantification, ImageJ software was used. The results normalization was performed using Mouse anti-Human β-actin Monoclonal Antibody (A2228 - Aldrich® Sigma, Germany) with a concentration of 1:1000.

3.2.6 Statistical analysis

To identify differences between tissue concentration of the peptides, data were submitted to the Shapiro-Wilk normality test and the Student's T-test was used to compare the results. Pearson's correlation coefficient was used to detect associations. Quantitative variables were expressed as mean and standard deviation. A $P \leq 0.05$ was considered statistically significant. All analyzes were performed using the SPSS version 25.

3.3 RESULTS

There was no significant difference between age, weight, height and body mass index (BMI) between groups, indicating a homogeneous sample. Of the patients with IPF, the majority were men (83%) and only half (50%) were smokers or former smokers. As expected, lung function in IPF patients was worse than in control group, with a statistically significant difference for FEV_1% and FVC% parameters (Table 1). AT1R expression was significantly higher (34%) in the tissue of patients with IPF ($P<0.006$), whereas Mas-R expression was significantly lower (54%) in the same patients ($P<0.046$) (Figure 1).

Our data have also demonstrated a positive correlation between the expression of the Mas-R and the spirometric parameters FEV_1% ($r=0.62$, $P=0.03$) and FVC% ($r=0.58$, $P=0.05$) (Figure 2). When AT1R expression was compared to FEV_1% and FVC% parameters, a negative correlation was found (FEV_1%: $r=0.8$, $P=0.002$; and FVC%: $r=0.74$, $P=0.006$ - Figure 3). There was no correlation between the receptors expression and the FEV_1/FVC%.

3.4 DISCUSSION

In the current study, we reported increased expression of AT1R and reduced expression of Mas-R in the lung tissue of patients with IPF. In addition, we found a negative and positive correlation between spirometric parameters and AT1 and Mas receptors expression, respectively. Such evidence strengthens our hypothesis that the fibrotic process could be due to an imbalance between the RAS components in the lung in favor of the ANG II axis. In addition, these results suggest that this imbalance could be also associated with the degree of pulmonary impairment.

M. Couluris et al. (2012) demonstrated in humans the effect of Losartan, an AT1R antagonist, on the progression of IPF that lung function was stable in 12 of the 17 treated patients [39]. This finding, stretch our hypothesis that the AT1R activation might participates in the IPF pathophysiology process. On the other hand, results from our laboratory demonstrated that the plasma concentration of ANG II was similar between IPF patients and control, but alamandine plasma concentration, part of ACE2 anti-fibrotic axis, was 356% lower in these patients [40]. This rationale agrees with the increase in ANGII-AT1R participation in IPF patients.

Although the ACE blockers has not effectively improved the prognosis of patients with IPF [35], studies in an animal model has shown that the inhibition of AT1R signaling, besides attenuating pulmonary fibrosis induced by bleomycin [41], blocked ECM proteins and fibrogenic factors production, and improved respiratory compliance, demonstrating a critical role of the ACE-ANGII-AT1R axis in the development of experimental pulmonary fibrosis [31]. These results agree with Königshoff et al. (2007) who demonstrated that ANGII-AT1R stimulated cell migration and proliferation in fibroblasts [42], effects that contribute to fibrogenesis.

Considering the current knowledge of RAS, which highlights the participation of peptides with opposed effects to the ANG II [28,43], we believe that, stimulating the antagonist axis may be more important than blocking the components of the fibrosing-considered axis. Moreover, ANG-(1-7) might be a key component in the management of patients with IPF. Data found in the literature support this hypothesis, because in the culture of fibroblasts of human lung and of mice with bleomycin-induced pulmonary fibrosis, ANG-(1-7) it has been shown to inhibit the activation of the transforming growth factor-beta 1 (TGF-β1), which is responsible for the fibroblast-myofibroblast transition induced by ANG II [44,45]. Moreover, Uhal et al. (2011) observed that, in experimental pulmonary fibrosis, ANG-(1-7) prevented the protein kinase c-Jun N-terminal (JNK) activation. This protein is responsible for triggering the apoptosis process of alveolar epithelial cells [46]. Collectively, these studies suggest that TGF-β and JNK protein activation contributes significantly to the antifibrogenic effects of the ACE2/ANG-(1-7)/Mas-R axis.

A critical point of our study is the lack of results about ANG II and ANG-(1-7) plasma concentration. However, our previous study did show no significant difference in ANG I, ANG II and ANG-(1-7) plasma concentrations of IPF patients compared to the control group [40]. It is well documented that ANG II production is increased in lungs of patients with IPF, while the messenger RNA for ACE2 is reduced [47,48]. Li et al. (2006) has previously demonstrated that the lung parenchyma can control ANG II local generation (extravascular) through cleavage of angiotensinogen available in the tissue [48]. These data and our findings, demonstrate that, although there is no plasma difference between RAS peptides, the imbalance between the expression of receptors and peptides in the lung tissue could have a key role in the IPF pathophysiology.

Furthermore, our results also showed for the first time the significant functional impact of the RAS components imbalance in the lungs. In addition, patients' functional capacity was strongly associated with AT1R and Mas-R expression. The increase in AT1R is associated with a worse lung function seen by the decrease FVC% and FEV_1% while the opposite association was found with Mas-R. The importance of these results is reinforced by Bárczi et al. (2017) who studying patients with IPF, found a positive correlation between quality of life and FVC ($r=0.4$, $P<0.05$) [9], demonstrating that improving functional parameters, the patients well-being is also improved. Therefore, more studies should be conducted to confirm these important findings and contribute to improving the quality of life of those patients who will suffer for the rest of their lives with the consequences of pulmonary fibrosis.

3.5 CONCLUSION

According to these data, it is doubtless that RAS involvement in IPF is far from being clarified. However, our results demonstrated increased expression of AT1R and reduced expression of Mas-R in the lung tissue of patients with IPF. We were able to show that the imbalance between the receivers is associated with a reduced lung function of patients. Thus, these findings open new horizons in the role of RAS peptides in the pathophysiology of IPF, so that more studies need to be conducted to clarify the real implications of this system in the disease development and progression.

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REFERENCES

[1] G. Raghu, H.R. Collard, J.J. Egan, F.J. Martinez, J. Behr, K.K. Brown, T. V. Colby, J.F. Cordier, K.R. Flaherty, J.A. Lasky, D.A. Lynch, J.H. Ryu, J.J. Swigris, A.U. Wells, J. Ancochea, D. Bouros, C. Carvalho, U. Costabel, M. Ebina, D.M. Hansell, T. Johkoh, D.S. Kim, T.E. King, Y. Kondoh, J. Myers, N.L. Müller, A.G. Nicholson, L. Richeldi, M. Selman, R.F. Dudden, B.S. Griss, S.L. Protzko, H.J. Schünemann, An Official ATS/ERS/JRS/ALAT Statement: Idiopathic pulmonary fibrosis: Evidence-based guidelines for diagnosis and management, Am. J. Respir. Crit. Care Med. 183 (2011) 788–824. doi:10.1164/rccm.2009-040GL.

[2] R.M. du Bois, An earlier and more confident diagnosis of idiopathic pulmonary fibrosis, Eur. Respir. Rev. 21 (2012) 141–146. https://doi.org/10.1183/09059180.00000812.

[3] G. Raghu, D. Weycker, J. Edelsberg, W.Z. Bradford, G. Oster, Incidence and prevalence of idiopathic pulmonary fibrosis, Am. J. Respir. Crit. Care Med. 174 (2006) 810–16. doi:10.1164/rccm.200602-163OC.

[4] E.B. Meltzer, P.W. Noble, Idiopathic pulmonary fibrosis, Orphanet J. Rare Dis. 3 (2008) 8. doi:10.1186/1750-1172-3-8.

[5] T.E. King, A. Pardo, M. Selman, Idiopathic pulmonary fibrosis, Lancet. 378 (2011) 1949–61. doi:10.1016/S0140-6736(11)60052-4.

[6] V.J. Thannickal, J.C. Horowitz, Evolving Concepts of Apoptosis in Idiopathic Pulmonary Fibrosis, Proc. Am. Thorac. Soc. 3 (2006) 350–56. doi:10.1513/pats.200601-001TK.

[7] H. Xia, D. Diebold, R. Nho, D. Perlman, J. Kleidon, J. Kahm, S. Avdulov, M. Peterson, J. Nerva, P. Bitterman, C. Henke, Pathological integrin signaling enhances proliferation of primary lung fibroblasts from patients with idiopathic pulmonary fibrosis, J. Exp. Med. 205 (2008) 1659–72. doi:10.1084/jem.20080001.

[8] Y. Li, D. Jiang, J. Liang, E.B. Meltzer, A. Gray, R. Miura, L. Wogensen, Y. Yamaguchi, P.W. Noble, Severe lung fibrosis requires an invasive fibroblast phenotype regulated by hyaluronan and CD44, J. Exp. Med. 208 (2011) 1459-71. doi:10.1084/jem.20102510.

[9] E. Bárczi, T. Erdélyi, A. Bohács, N. Eszes, A.D. Tárnoki, D.L. Tárnoki, K. Karlinger, B. Fejér, V. Müller, Quality of life of idiopathic pulmonary fibrosis patients, Eur. Respir. J. 50 (2017) 3813. doi:10.1183/1393003.congress-2017.PA3813.

[10] A.S. Lee, I. Mira-Avendano, J.H. Ryu, C.E. Daniels, The burden of idiopathic pulmonary fibrosis: An unmet public health need, Respir. Med. 108 (2014) 955–67. doi:10.1016/j.rmed.2014.03.015.

[11] N. Wu, Y.F. Yu, C.-C. Chuang, R. Wang, N.N. Benjamin, D.B. Coultas, Healthcare resource utilization among patients diagnosed with idiopathic pulmonary fibrosis in the United States, J. Med. Econ. 18 (2015) 249–57. doi:10.3111/13696998.2014.991789.

[12] L. Richeldi, R.M. du Bois, G. Raghu, A. Azuma, K.K. Brown, U. Costabel, V. Cottin, K.R. Flaherty, D.M. Hansell, Y. Inoue, D.S. Kim, M. Kolb, A.G. Nicholson, P.W. Noble, M. Selman, H. Taniguchi, M. Brun, F. Le Maulf, M. Girard, S. Stowasser, R. Schlenker-Herceg, B. Disse, H.R. Collard, Efficacy and safety of nintedanib in advanced idiopathic pulmonary fibrosis, N. Engl. J. Med. 370 (2014) 2071–82. doi:10.1056/NEJMoa1402584.

[13] T.E. King, W.Z. Bradford, S. Castro-Bernardini, E.A. Fagan, I. Glaspole, M.K. Glassberg, E. Gorina, P.M. Hopkins, D. Kardatzke, L. Lancaster, D.J. Lederer, S.D. Nathan, C.A. Pereira, S.A. Sahn, R. Sussman, J.J. Swigris, P.W. Noble, A Phase 3 Trial of Pirfenidone in Patients with Idiopathic Pulmonary Fibrosis, N. Engl. J. Med. 370 (2014) 2083–92. doi:10.1056/NEJMoa1402582.

[14] F. Pan, T. Ye, P. Sun, S. Gui, B. Liang, L. Li, D. Zheng, J. Wang, R.L. Hesketh, L. Yang, C. Zheng, Time Course of Lung Changes at Chest CT during Recovery from Coronavirus Disease 2019 (COVID-19), Radiology. 295 (2020) 715–721. https://doi.org/10.1148/radiol.2020200370.

[15] W. Kong, P.P. Agarwal, Chest Imaging Appearance of COVID-19 Infection Case, Radiol Cardiothorac Imaging. 2 (2020) e200028. https://doi.org/10.1148/ryct.2020200028.

[16] J.E. Hall, A.C. Guyton. Textbook of Medical Physiology. 10th ed. Philadelphia, PA: Saunders/Elsevier; 2000.

[17] T. Unger, O. Chung, T. Csikos, J. Culman, S. Gallinat, P. Gohlke, S. Hohle, S. Meffert, M. Stoll, U. Stroth, Y.Z. Zhu, Angiotensin receptors., J. Hypertens. Suppl. 14 (1996) S95-103.

[18] R.P. Marshall, P. Gohlke, R.C. Chambers, D.C. Howell, S.E. Bottoms, T. Unger, R.J. McAnulty, G.J. Laurent, Angiotensin II and the fibroproliferative response to acute lung injury, Am. J. Physiol. Cell. Mol. Physiol. 286 (2004) L156–64. doi:10.1152/ajplung.00313.2002.

[19] R. Wang, A. Zagariya, O. Ibarra-sunga, C. Gidea, E. Ang, S. Deshmukh, G. Chaudhary, J. Baraboutis, G. Filippatos, B.D. Uhal, A. Zagariya, O. Ibarra-sunga, C. Gidea, E. Ang, S. Deshmukh, J. Baraboutis, G. Filippatos, B.D. Uhal, A. Ii, K. Flynn, G.F. Am, J.P. Lung, Angiotensin II induces apoptosis in human and rat alveolar epithelial cells, (1999) 885–89. doi: 10.1152/ajplung.1999.276.5.L885.

[20] T. Yamakawa, S.I. Tanaka, K. Numaguchi, Y. Yamakawa, E.D. Motley, S. Ichihara, T. Inagami, Involvement of Rho-kinase in angiotensin II-induced hypertrophy of rat vascular smooth muscle cells, Hypertension. 35 (2000) 313–18. doi:10.1161/01.hyp.35.1.313.

[21] R.P. Marshall, R.J. McAnulty, G.J. Laurent, Angiotensin II is mitogenic for human lung fibroblasts via activation of the type 1 receptor, Am. J. Respir. Crit. Care Med. 161 (2000) 1999–2004. doi:10.1164/ajrccm.161.6.9907004.

[22] S. Sriramula, J. Francis, Tumor necrosis factor - Alpha is essential for angiotensin II-induced ventricular remodeling: Role for oxidative stress, PLoS One. 10 (2015) 1–13. doi:10.1371/journal.pone.0138372.

[23] G. Raffai, M.J. Durand, J.H. Lombard, Acute and chronic angiotensin-(1-7) restores vasodilation and reduces oxidative stress in mesenteric arteries of salt-fed rats, Am. J. Physiol. - Hear. Circ. Physiol. 301 (2011) H1341-52. doi:10.1152/ajpheart.00202.2011.

[24] K.D. da Silveira, F.M. Coelho, A.T. Vieira, D. Sachs, L.C. Barroso, V.V. Costa, T.L.B. Bretas, M. Bader, L.P. de Sousa, T.A. da Silva, R.A.S. dos Santos, A.C. Simões e Silva, M.M. Teixeira, Anti-Inflammatory Effects of the Activation of the Angiotensin-(1–7) Receptor, Mas, in Experimental Models of Arthritis, J. Immunol. 185 (2010) 5569–76. doi:10.4049/jimmunol.1000314.

[25] Z. Pei, R. Meng, G. Li, G. Yan, C. Xu, Z. Zhuang, J. Ren, Z. Wu, Angiotensin-(1-7) ameliorates myocardial remodeling and interstitial fibrosis in spontaneous hypertension: Role of MMPs/TIMPs, Toxicol. Lett. 199 (2010) 173–81. doi:10.1016/j.toxlet.2010.08.021.

[26] N. Pei, R. Wan, X. Chen, A. Li, Y. Zhang, J. Li, H. Du, B. Chen, W. Wei, Y. Qi, Y. Zhang, M.J. Katovich, C. Sumners, H. Zheng, H. Li, Angiotensin-(1-7) decreases cell growth and angiogenesis of human nasopharyngeal carcinoma xenografts, Mol. Cancer Ther. 15 (2015) 37–47. doi:10.1158/1535-7163.MCT-14-0981.

[27] E. Kostenis, G. Milligan, A. Christopoulos, C.F. Sanchez-Ferrer, S. Heringer-Walther, P.M. Sexton, F. Gembardt, E. Kellett, L. Martini, P. Vanderheyden, H.P. Schultheiss, T. Walther, G-protein-coupled receptor Mas is a physiological antagonist of the angiotensin II type 1 receptor, Circulation. 111 (2005) 1806-13. doi:10.1161/01.CIR.0000160867.23556.7D.

[28] R.A.S. Santos, A.C.S. e Silva, C. Maric, D.M.R. Silva, R.P. Machado, I. de Buhr, S. Heringer-Walther, S.V.B. Pinheiro, M.T. Lopes, M. Bader, E.P. Mendes, V.S. Lemos, M.J. Campagnole-Santos, H.-P. Schultheiss, R. Speth, T. Walther, Angiotensin-(1-7) is an endogenous ligand for the G protein-coupled receptor Mas, Proc. Natl. Acad. Sci. U S A. 100 (2003) 8258–63. doi:10.1073/pnas.1432869100.

[29] J.L. Grobe, A.P. Mecca, M. Lingis, V. Shenoy, T.A. Bolton, J.M. Machado, R.C. Speth, M.K. Raizada, M.J. Katovich, Prevention of angiotensin II-induced cardiac remodeling by angiotensin- (1–7), Am J Physiol Hear. Circ Physiol. 292 (2007) H736–42. doi:10.1152/ajpheart.00937.2006.

[30] L.J. Song, F. Xiang, H. Ye, H. Huang, J. Yang, F. Yu, L. Xiong, J.J. Xu, P.A. Greer, H.Z. Shi, J.B. Xin, Y. Su, W.L. Ma, Inhibition of angiotensin II and calpain attenuates pleural fibrosis, Pulm. Pharmacol. Ther. 48 (2018) 46–52. doi:10.1016/j.pupt.2017.10.012.

[31] J. Wang, L. Chen, B. Chen, A. Meliton, S.Q. Liu, Y. Shi, T. Liu, D.K. Deb, J. Solway, Y. Chun Li, Chronic Activation of the renin-angiotensin system induces lung fibrosis, Sci. Rep. 5 (2015) 1–11. doi:10.1038/srep15561.

[32] M. Granzow, R. Schierwagen, S. Klein, B. Kowallick, S. Huss, A. Vogt, F.A. Schildberg, M.A. Gonzalez-, I.G.R. Mazar, G. Jan, A. Wojtalla, B. Kr, J. Nattermann, V. Siegmund, N. Werner, O.F. Dieter, W. Laleman, P. Knolle, V.H. Shah, T. Sauerbruch, J. Trebicka, Angiotensin-II Type 1 Receptor-Mediated Janus Kinase 2 Activation Induces Liver Fibrosis, Hepatology. 60 (2014) 334-48. doi:10.1002/hep.27117.

[33] H.F. Nadrous, P.A. Pellikka, M.J. Krowka, K.L. Swanson, N. Chaowalit, P.A. Decker, J.H. Ryu, Impact of angiotensin-converting enzyme inhibitors and statins on survival in idiopathic pulmonary fibrosis, Chest. 126 (2004) 438–46. doi:10.1378/chest.126.2.438.

[34] M. Kreuter, D.J. Lederer, M. Molina-Molina, I. Noth, C. Valenzuela, L. Frankenstein, D. Weycker, M. Atwood, K.-U. Kirchgaessler, V. Cottin, Association of Angiotensin Modulators With the Course of Idiopathic Pulmonary Fibrosis, Chest. 156 (2019) 706-14. doi:10.1016/j.chest.2019.04.015.

[35] K.A. Keogh, J. Standing, G.C. Kane, A. Terzic, A.H. Limper, Angiotensin ll antagonism fails to ameliorate bleomycin-induced pulmonary fibrosis in mice, Eur. Respir. J. 25 (2005) 708–14. doi:10.1183/09031936.05.00090204.

[36] Tarix Pharmaceuticals & University Of Southern California. Angiotensins for treatment of fibrosis, WO2013090833, 2013.

[37] J.S. Willey, D.N. Bracey, P.E. Gallagher, E.A. Tallant, W.F. Wiggins, M.F. Callahan, T.L. Smith, C.L. Emory, Angiotensin-(1-7) attenuates skeletal muscle fibrosis and stiffening in a mouse model of extremity sarcoma radiation therapy, J. Bone Jt. Surg. - Am. Vol. 98 (2016) 48–55. doi:10.2106/JBJS.O.00545.

[38] S. Cai, R. Yang, Y. Li, Z. Ning, L. Zhang, Angiotensin- (1-7) improve liver fibrosis by regulating the inflammasome via redox balance modulation, Antioxid. Redox Signal. (2016) 1–63. doi:10.1089/ars.2015.6498.

[39] M. Couluris, B.W. Kinder, P. Xu, M. Gross-King, J. Krischer, R.J. Panos, Treatment of idiopathic pulmonary fibrosis with losartan: A pilot project, Lung. 190 (2012) 523–27. doi:10.1007/s00408-012-9410-z.

[40] T.S. Sipriani, R.A.S. dos Santos, K. Rigatto, The Renin-Angiotensin System: Alamandine is reduced in patients with Idiopathic Pulmonary Fibrosis, J. Cardiol. Cardiovasc. Med. 4 (2019) 210–215.

https://doi.org/10.29328/journal.jccm.1001070.

[41] Y. Meng, X. Li, S.X. Cai, W.C. Tong, Y.X. Cheng, [Perindopril and losartan attenuate bleomycin A5-induced pulmonary fibrosis in rats], Nan Fang Yi Ke Da Xue Xue Bao. 28 (2008) 919–24. PMID: 18583228.

[42] M. Königshoff, A. Wilhelm, A. Jahn, D. Sedding, O.V. Amarie, B. Eul, W. Seeger, L. Fink, A. Günther, O. Eickelberg, F. Rose, The angiotensin II receptor 2 is expressed and mediates angiotensin II signaling in lung fibrosis, Am. J. Respir. Cell Mol. Biol. 37 (2007) 640–50. doi:10.1165/rcmb.2006-0379TR.

[43] R.Q. Lautner, D.C. Villela, R.A. Fraga-Silva, N. Silva, T. Verano-Braga, F. Costa-Fraga, J. Jankowski, V. Jankowski, F. Sousa, A. Alzamora, E. Soares, C. Barbosa, F. Kjeldsen, A. Oliveira, J. Braga, S. Savergnini, G. Maia, A.B. Peluso, D. Passos-Silva, A. Ferreira, F. Alves, A. Martins, M. Raizada, R. Paula, D. Motta-Santos, F. Kemplin, A. Pimenta, N. Alenina, R. Sinisterra, M. Bader, M.J. Campagnole-Santos, R.A.S. Santos, Discovery and characterization of alamandine: A novel component of the renin-angiotensin system, Circ. Res. 112 (2013) 1104–11. doi:10.1161/CIRCRESAHA.113.301077.

[44] J.P. Zhou, W. Tang, Y. Feng, N. Li, C.J. Gu, Q.Y. Li, H.Y. Wan, Angiotensin-(1-7) decreases the expression of collagen I via TGF-β1/Smad2/3 and subsequently inhibits fibroblast-myofibroblast transition, Clin. Sci. 130 (2016) 1983–91. doi:10.1042/CS20160193.

[45] V. Shenoy, A.J. Ferreira, Y. Qi, R.A. Fraga-Silva, C. Díez-Freire, A. Dooies, J.Y. Jun, S. Sriramula, N. Mariappan, D. Pourang, C.S. Venugopal, J. Francis, T. Reudelhuber, R.A. Santos, J.M. Patel, M.K. Raizada, M.J. Katovich, The angiotensin-converting enzyme 2/angiogenesis-(1-7)/Mas axis confers cardiopulmonary protection against lung fibrosis and pulmonary hypertension, Am. J. Respir. Crit. Care Med. 182 (2010) 1065–72. doi:10.1164/rccm.200912-1840OC.

[46] B.D. Uhal, X. Li, A. Xue, X. Gao, A. Abdul-Hafez, Regulation of alveolar epithelial cell survival by the ACE-2/angiotensin 1-7/Mas axis., Am. J. Physiol. Lung Cell. Mol. Physiol. 301 (2011) L269-74. doi:10.1152/ajplung.00222.2010.

[47] X. Li, M. Molina-Molina, A. Abdul-Hafez, V. Uhal, A. Xaubet, B.D. Uhal, Angiotensin converting enzyme-2 is protective but downregulated in human and experimental lung fibrosis, AJP Lung Cell. Mol. Physiol. 295 (2008) L178-85. doi:10.1152/ajplung.00009.2008.

[48] X. Li, M. Molina-Molina, A. Abdul-Hafez, J. Ramirez, A. Serrano-Mollar, A. Xaubet, B.D. Uhal, Extravascular sources of lung angiotensin peptide synthesis in idiopathic pulmonary fibrosis., Am. J. Physiol. Lung Cell. Mol. Physiol. 291 (2006) L887-95. doi:10.1152/ajplung.00432.2005.

Table 1 - Sample characterization and Pulmonary Function Test

Variables	**Control (n=6)**	**Fibrosis (n=6)**	**P**
Age (years)	55.7 ± 14.1	54 ± 12.7	0.83
Gender (male)	3	5	
Weight (kg)	79.8 ± 20.57	72.5 ± 8.94	0.67
Height (m)	1.69 ± 0.12	1.72 ± 0.13	0.34
BMI (kg/m²)	27.54 ± 3.86	24.7 ± 2.93	0.6
Previous smoking	2	3	0.6
FEV_1%	81.9 ± 14.5	46.5 ± 18.43	0.0041*
CVF%	86.3 ± 13	43.83 ± 16.9	0.0006**
FEV_1/CVF%	75.18 ± 6.36	70 ± 35.7	0.13

BMI=body mass index; kg=kilogram; m=meter; FEV_1=forced expiratory volume in the first second; FVC=forced vital capacity; %=Percentage. The data are presented as mean ± SD.

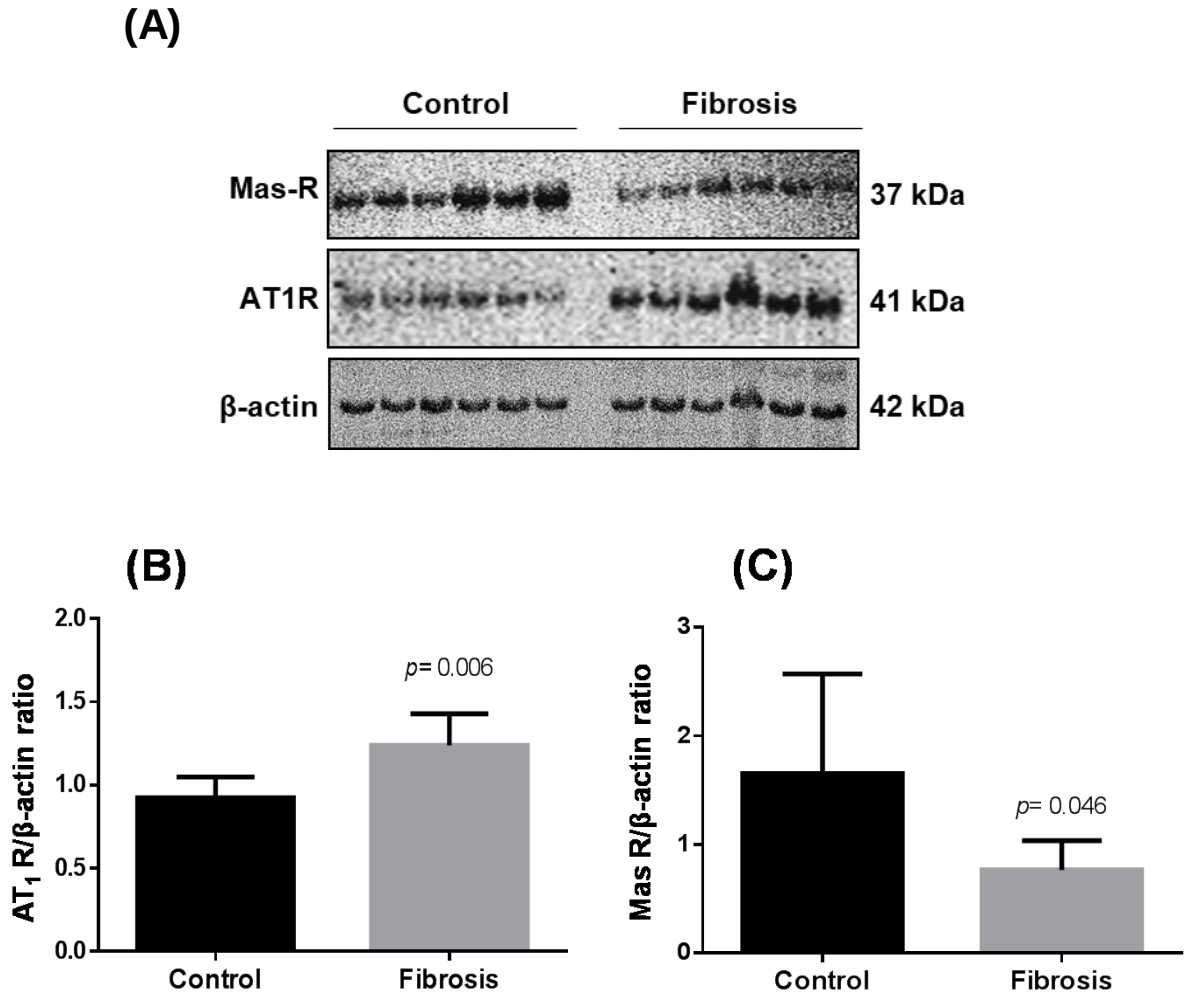

Figure 1 - Protein expression of AT1 and Mas receptors in lung tissue (n=12). A: Expression of AT1 and Mas receptors in idiopathic pulmonary fibrosis lung tissue and control evaluated by western blot. B: Protein quantification of AT1 receptor in idiopathic pulmonary fibrosis and control lung tissue.

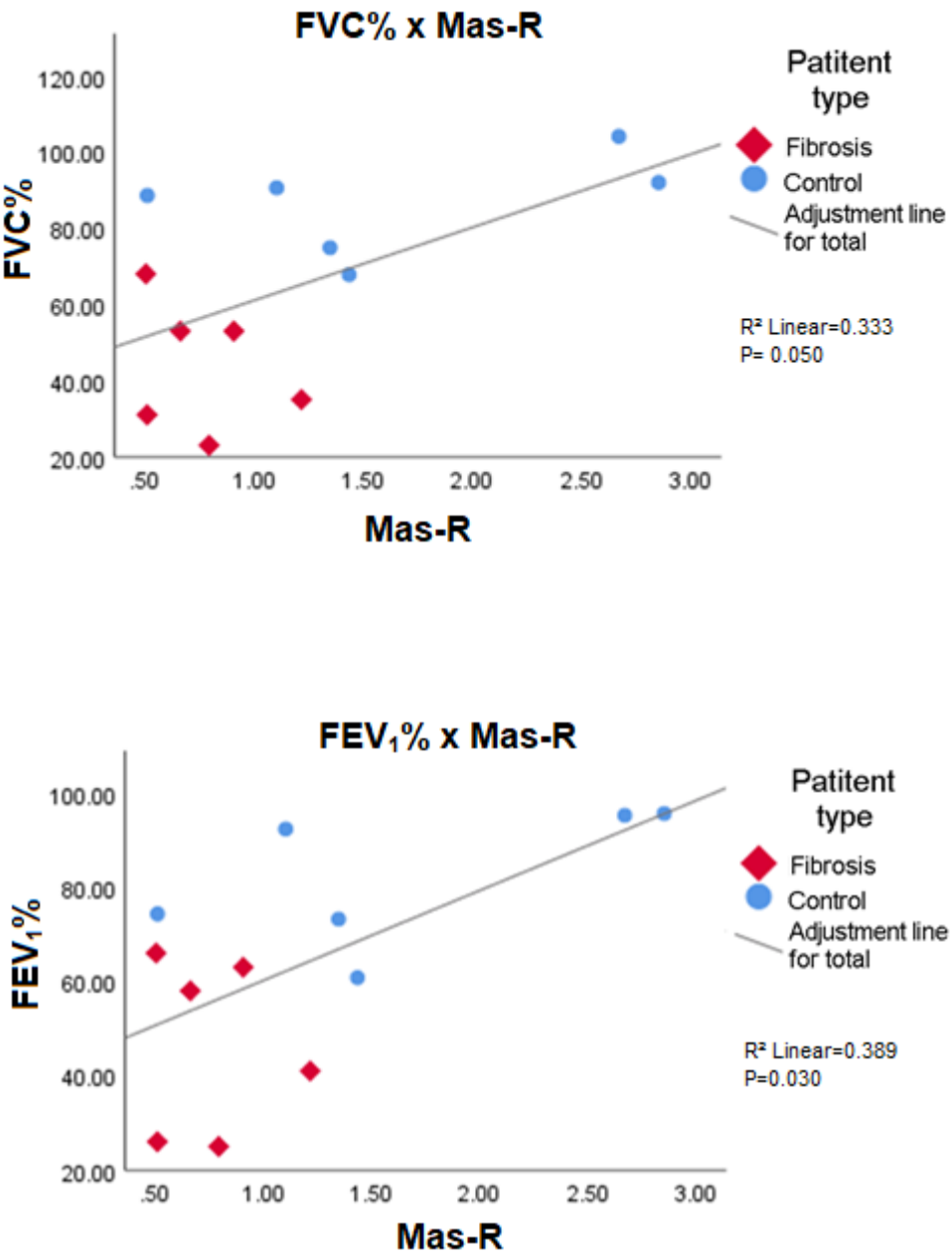

Figure 2 - Scatter plots of correlation analyzis between spirometry values and Mas receptor quantification (n=12). FEV_1=forced expiratory volume in the first second; FVC=forced vital capacity.

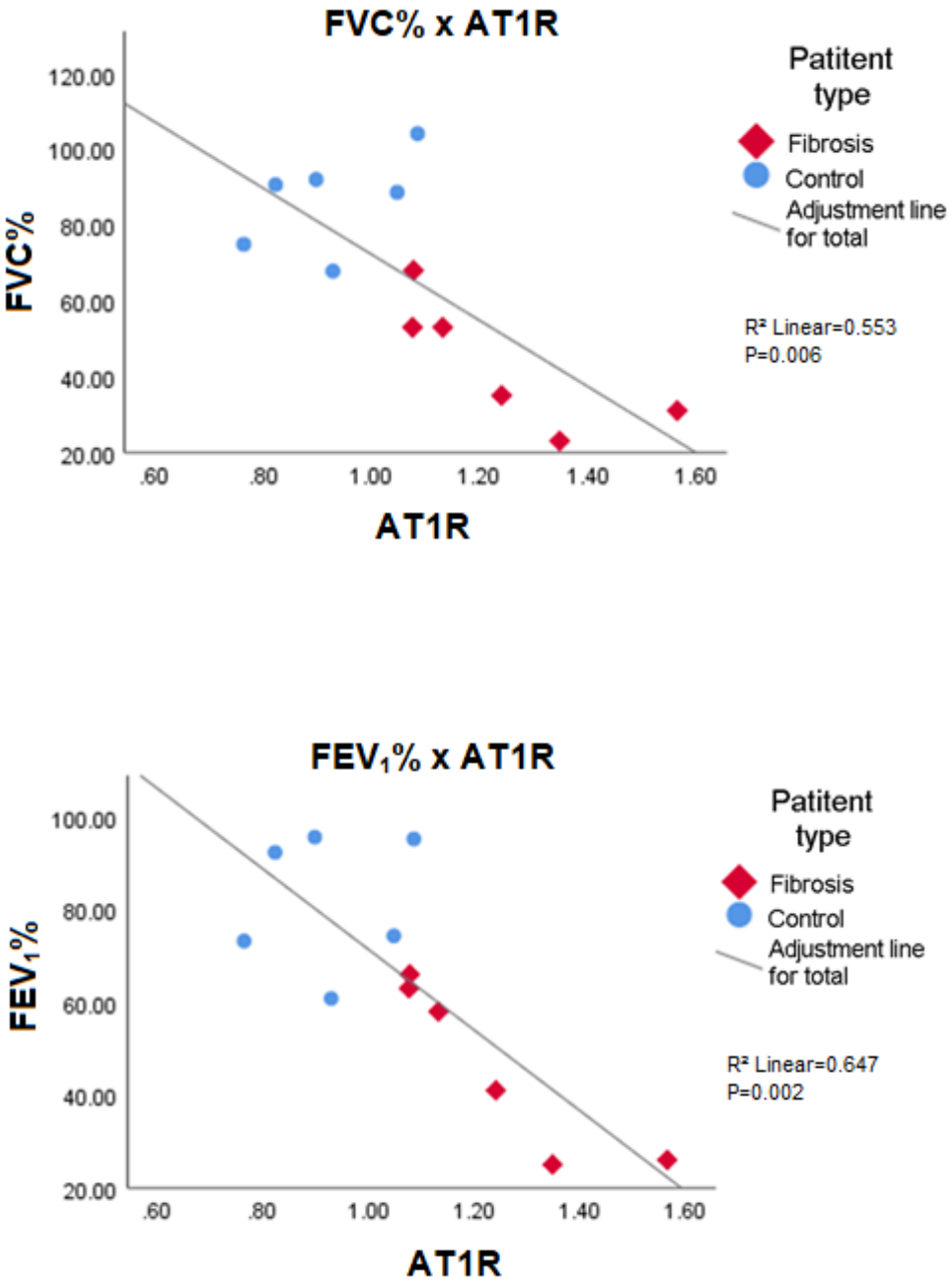

Figure 3 - Scatter plots of correlation analyzis between spirometry values and AT1 receptor quantification (n=12). FEV_1=forced expiratory volume in the first second; FVC=forced vital capacity.

4 CONCLUSÃO

Nossos achados demonstraram de forma inédita que há um desequilíbrio entre os receptores dos eixos ANGII/AT1R e ANG-(1-7)/Mas-R no tecido pulmonar de pacientes com FPI. Também evidenciamos que este desequilíbrio está associado à redução da função pulmonar. Não há dúvidas de que o envolvimento do sistema na doença está longe de ser esclarecido, porém, nossos resultados abrem novos horizontes em relação ao papel do SRA na fisiopatologia da FPI. Mais estudos precisam ser conduzidos para esclarecer as reais implicações desse sistema no desenvolvimento e progressão da doença. Nesse sentido, temos desenvolvido estudo translacional para investigar a participação dos componentes do SRA na da fibrose pulmonar idiopática. Vislumbramos contribuir para entender a fisiopatologia da doença e para identificar promissores alvos terapêuticos visando à melhora da qualidade de vida desses pacientes.

5 ANEXOS

ANEXO A - APROVAÇÃO DO COMITÊ DE ÉTICA EM PESQUISA DA UFCSPA

UNIVERSIDADE FEDERAL DE CIÊNCIAS DA SAÚDE DE PORTO ALEGRE

PARECER CONSUBSTANCIADO DO CEP

DADOS DA EMENDA

Título da Pesquisa: Análise Translacional da participação do sistema renina angiotensina e de citocinas inflamatórias na fibrose pulmonar

Pesquisador: Katya Vianna Rigatto

Área Temática:

Versão: 5

CAAE: 69947517.2.0000.5345

Instituição Proponente: Universidade Federal de Ciências da Saúde de Porto Alegre

Patrocinador Principal: Fundação de Amparo a Pesquisa do Estado do Rio Grande do Sul

DADOS DO PARECER

Número do Parecer: 2.619.738

Apresentação do Projeto:

A Fibrose Pulmonar Idiopatica (FPI) e uma doenca intersticial pulmonar com sobrevida media de tres anos, que limita a capacidade respiratoria dos pacientes e possui alta morbimortalidade devido a falta de opcoes terapeuticas. A etiologia e o mecanismo fisiopatologico da doenca sao bastante complexos e ainda desconhecidos, impossibilitando alternativas de tratamento que reduzam as internacoes e a necessidade de transplante pulmonar. Atualmente, a hipotese mais aceita para a patogenese da doenca e a fibroproliferacao e o acumulo excessivo de matriz extracelular. Tal evento no tecido pulmonar ocorre durante o reparo de lesoes epiteliais alveolares, com a liberacao de mediadores com atividade pro-inflamatoria. Fortes evidencias demonstram o envolvimento do sistema renina-angiotensina (SRA) com a Fibrose Pulmonar (FP). Dados ainda nao publicados do nosso laboratorio mostraram, em pacientes com FPI, uma significativa reducao das concentracoes plasmaticas de Alamandina nesses pacientes, sem alteracao nas concentracoes plasmaticas dos demais peptideos. De fato, esta bem estabelecido na literatura que o eixo ECA-AngII-AT1 promove vasoconstricao, proliferacao e fibrose, enquanto que o eixo ECA2-Ang-(1-7)-Mas induz intrinsecamente efeitos de protecao aos pulmoes, contrapondo-se as acoes geradas pelo eixo ECA-AngII-AT1. Acreditamos que, na FPI, ha um aumento da relacao entre a participacao do eixo ECA- AngII-AT1 versus o eixo ECA2-Ang-(1-7)-Mas ou o ECA2-Ang-(1-7)-Alamandina-MrgD, contribuindo para o desenvolvimento e o avanco da inflamacao e da fibrose. Apesar do evidente envolvimento do sistema renina angiotensina relacionados a FPI, nenhum estudo foi

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encontrado na literatura demonstrando a participacao desses eixos na fisiopatologia da doenca. Acreditamos que nos pulmoes dos pacientes com FPI ha um desequilibrio na expressao dos peptideos que compoem o SRA em favor do eixo fibrosante e constritor, representado pela ECA-AngII-AT1. Outro aspecto importante a ser pontuado e que o SRA nao tem sido considerado clinicamente no tratamento da FPI. Provavelmente isso se deva ao fato de que o uso dos inibidores da enzima conversora da angiotensina e dos bloqueadores do receptor AT1, nao trouxeram beneficios aos pacientes com FPI. No entanto, os avancos no conhecimento sobre o SRA e a descoberta recente de novos peptideos, alteram a interpretacao sobre este sistema, exigindo um novo olhar sobre a participacao desses peptideos. Nossa proposta e inedita e de grande interesse clinico, ja que pretende investigar a participacao do "novo" SRA diretamente em tecido pulmonar transplantado de pacientes com FPI e os possiveis efeitos beneficos do tratamento com Alamandina e/ou Frutose-1,6-bisfosfato (FBP). Nosso grupo tem demonstrado as acoes protetoras da FBP em uma variedade de condicoes patologicas. Um desses efeitos e a sua capacidade em diminuir a proliferacao e reverter o fenotipo de miofibroblastos hepaticos atraves da diminuicao da sintese de TGF-1, da expressao de colageno tipo 1 (Col-1) e aumento da expressao de PPAR- in vitro. Alem disso, a FBP possui acao protetora contra radicais livres in vitro com celulas expostas a peroxido de hidrogenio, impedindo a producao de malondialdeido, um indicador de peroxidacao lipidica, reduzindo a formacao de produtos de oxidacao de proteinas. Em hepatocitos lesados com galactosamina, a FBP aumenta a relacao glutationa oxidada/glutationa reduzida (GSH/GSSG) que e um importante sistema antioxidante celular e utilizado para avaliar o estado oxidativo celular. Em conjunto, esses achados nos levam a acreditar que o tratamento com FBP pode ser uma alternativa como tratamento complementar na FPI, assim como a Alamandina constitui um peptideo com acoes fisiologicas protetoras, muito convidativas do ponto de vista da fibrose. E nosso objetivo contribuir para elucidar os possiveis mecanismos envolvidos nesta patologia ainda sem cura e sem tratamento resolutivo. Se ficar demonstrado uma reducao no tecido pulmonar do eixo Alamandina na FPI, assim como observamos anteriormente no plasma de pacientes com FPI, e ainda um aumento dos efeitos inflamatorios e fibrosantes sera razoavel acreditar que mais importante do que bloquear o eixo ECA-AngII- AT1, podera ser estimular o eixo ECA2-Ang-(1-7)-Alamandina-MrgD. Alem disso, pretendemos testar os possiveis efeitos beneficos do tratamento com FBP e/ou Alamandina, tanto in vitro quanto in vivo. Assim, no presente trabalho, o efeito desses tratamentos estara sendo avaliado em modelos experimentais de fibrose pulmonar in vivo, e in vitro em fibroblastos provenientes tanto de pulmoes humanos quanto de animais. A nossa proposta e inedita, factivel, interinstitucional e, se nossas hipoteses forem demonstradas, podem gerar

conhecimentos compativeis: 1- com a melhoria do manejo da FP e da qualidade de vida dos pacientes; e 2- com a reducao do numero de internacoes, de transplantes e dos custos para o Sistema Unico de saude.

Objetivo da Pesquisa:

Contribuir para o conhecimento da fisiopatologia da Fibrose Pulmonar ao analisar, tanto em pulmoes de pacientes com fibrose pulmonar idiopatica quanto em animais com fibrose pulmonar induzida por bleomicina, se ha diferenca na razao entre os eixos ECA-AngII-AT1 e ECA2-Ang-(1-7)- MAS/Alamandina-MrgD do sistema renina angiotensina; e a participacao de citocinas inflamatorias e do estresse oxidativo.

Avaliação dos Riscos e Benefícios:

Conforme documento de Informações Básicas do Projeto:

Riscos:

Este estudo nao oferece riscos adicionais com relacao ao procedimento cirurgico, visto que o pedaco de pulmao que sera utilizado seria retirado de qualquer forma e desprezado. Alem disso, embora todos os cuidados serao observados no sentido de preservar a identidade dos pacientes, existe a possibilidade remota de vazamento de informacoes.

Beneficios:

A nossa proposta e inedita, factivel, interinstitucional e, se nossas hipoteses forem demonstradas, podem gerar conhecimentos compativeis: 1- com a melhoria do manejo da FP e da qualidade de vida dos pacientes; e 2- com a reducao do numero de internacoes, de transplantes e dos custos para o Sistema Unico de saude.

Comentários e Considerações sobre a Pesquisa:

A pesquisadora principal encaminha emenda para acréscimo de instituição coparticipante, modificação no cronograma de pesquisa e alteração no TCLE.

Tais solicitações estão justificadas no documento encaminhado.

Foram observadas as alterações realizadas nos documentos do projeto.

Considerações sobre os Termos de apresentação obrigatória:

Todos os termos de apresentação obrigatória foram incluídos.

Recomendações:

Não há recomendações.

Conclusões ou Pendências e Lista de Inadequações:

Não há óbices éticos.

UNIVERSIDADE FEDERAL DE CIÊNCIAS DA SAÚDE DE PORTO ALEGRE

Considerações Finais a critério do CEP:

De acordo com o parecer do Relator.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_1110857_E1.pdf	10/04/2018 01:12:51		Aceito
Outros	declaracao_prontuario_publicacao.pdf	10/04/2018 01:07:52	Katya Vianna Rigatto	Aceito
Outros	declaracao_confidencialidade.pdf	10/04/2018 01:06:49	Katya Vianna Rigatto	Aceito
Outros	declaracao_isencao_onus.pdf	10/04/2018 01:06:02	Katya Vianna Rigatto	Aceito
Declaração de Manuseio Material Biológico / Biorepositório / Biobanco	declaracao_material_biologico.pdf	10/04/2018 01:02:58	Katya Vianna Rigatto	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_30_03_18.pdf	10/04/2018 01:00:54	Katya Vianna Rigatto	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_com_alteracoes_VERSAO_5.doc	10/04/2018 01:00:29	Katya Vianna Rigatto	Aceito
Outros	Carta_Resposta_05_01_2018.pdf	05/01/2018 14:56:33	Katya Vianna Rigatto	Aceito
Outros	autorizacao_chefia_responsavel_dom_vicente_scherer.pdf	05/01/2018 14:55:58	Katya Vianna Rigatto	Aceito
Outros	anuencia_Fisiologia_Translacional_assinado_por_pedro.pdf	05/01/2018 13:27:55	Katya Vianna Rigatto	Aceito
Outros	Anuencia_laboratorio_PUCRS.pdf	05/01/2018 13:23:22	Katya Vianna Rigatto	Aceito
Folha de Rosto	Folha_de_rosto.pdf	19/06/2017 14:55:12	Katya Vianna Rigatto	Aceito
Declaração de Pesquisadores	termodecompromissokatya.pdf	19/06/2017 13:10:30	Katya Vianna Rigatto	Aceito

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

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Telefone: (51)3303-8804 **E-mail:** cep@ufcspa.edu.br

Não

PORTO ALEGRE, 25 de Abril de 2018

Assinado por:
ELIANE DALLEGRAVE
(Coordenador)

Endereço: Rua Sarmento Leite ,245
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ANEXO B - APROVAÇÃO DO COMITÊ DE ÉTICA EM PESQUISA DA INSTITUIÇÃO COPARTICIPANTE

IRMANDADE DA SANTA CASA DE MISERICORDIA DE PORTO ALEGRE - ISCMPA

PARECER CONSUBSTANCIADO DO CEP

Elaborado pela Instituição Coparticipante

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Análise Translacional da participação do sistema renina angiotensina e de citocinas inflamatórias na fibrose pulmonar

Pesquisador: Katya Vianna Rigatto

Área Temática:

Versão: 1

CAAE: 69947517.2.3001.5335

Instituição Proponente: ISCMPA

Patrocinador Principal: Fundação de Amparo a Pesquisa do Estado do Rio Grande do Sul

DADOS DO PARECER

Número do Parecer: 2.691.887

Apresentação do Projeto:

Estudo clínico transversal, a ser realizado com ratos e 20 pacientes, destes pacientes 10 com Fibrose Pulmonar idiopática e 10 pacientes controle submetidos a transplante pulmonar por ressecção pulmonar para o tratamento de carcinoma brônquico. Este estudo pretende obter conhecimento da fisiopatologia da Fibrose Pulmonar ao analisar, tanto em pulmões de pacientes com fibrose pulmonar idiopática quanto em animais com fibrose pulmonar induzida por bleomicina, se há diferença na razão entre os eixos ECA-AngII-AT1 e ECA2-Ang-(1-7)-MAS/Alamandina-MrgD do sistema renina angiotensina; e a participação de citocinas inflamatórias e do estresse oxidativo, O principal resultado esperado seria que o tratamento com frutose e/ou Alamandina apresente benefícios significativos para a fibrose pulmonar.O principal resultado esperado seria que o tratamento com frutose e/ou Alamandina apresente benefícios significativos para a fibrose pulmonar.

Objetivo da Pesquisa:

O pesquisador declara sobre:

Objetivo Primário:contribuir para o conhecimento da fisiopatologia da Fibrose Pulmonar ao analisar, tanto em pulmões de pacientes com fibrose pulmonar idiopática

quanto em animais com fibrose pulmonar induzida por bleomicina, se há diferença na razão entre os eixos ECA-AngII-AT1 e ECA2-Ang-(1-7)-MAS/Alamandina-MrgD do sistema renina

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angiotensina; e a participação de citocinas inflamatórias e do estresse oxidativo.In vivo Em tecido pulmonar de pacientes com fibrose idiopática e em ratos com fibrose pulmonar induzida por bleomicina,avaliar a relação entre a expressão dos peptídeos (ECA, AngII, AT1, AT2, ECA2, Ang-(1-7), Mas, Alamandina e MrgD) do sistema reninaangiotensina

Em ratos:Avaliar o efeito do tratamento com Alamandina e/ou com frutose-1,6-bifosfato sobre:- a pressão arterial sistêmica e pulmonar;- a morfologia e a deposição de componentes de matriz extracelular do parênquima pulmonar; - o perfil inflamatório e

oxidativo; - a diferenciação, proliferação e indução de transição do epitélio mesenquimal. In vitro Em fibroblastos de pulmões de pacientes com fibrose pulmonar idiopática e em animais com fibrose pulmonar induzida por bleomicina, tratados ou não com Alamandina e/ou com frutose-1,6-bifosfato, avaliar:- a relação entre os componentes (ECA, AngII, AT1, AT2, ECA2, Ang-(1-7), Mas, Alamandina, MrgD) do sistema reninaangiotensina;-a deposição de componentes de matriz extracelular; - o perfil inflamatório e oxidativo;- a diferenciação, proliferação e indução de transição do epitélio mesenquimal.

Avaliação dos Riscos e Benefícios:

O pesquisador declara sobre:

Riscos:Este estudo não oferece riscos adicionais com relação ao procedimento cirúrgico, visto que o pedaço de pulmão que será utilizado seria retirado de

qualquer forma e desprezado. Além disso, embora todos os cuidados serão observados no sentido de preservar a identidade dos pacientes, existe a possibilidade remota de vazamento de informações.

Benefícios:

A nossa proposta é inédita, factível, interinstitucional e, se nossas hipóteses forem demonstradas, podem gerar conhecimentos compatíveis: 1- com

a melhoria do manejo da FP e da qualidade de vida dos pacientes; e 2- com a redução do número de internações, de transplantes e dos custos para

o Sistema Único de saúde.

Comentários e Considerações sobre a Pesquisa:

O pesquisador declara sobre:

Critério de Inclusão: Critérios de inclusão para o grupo com FPI (casos)1)Pacientes portadores de FPI diagnosticada através de teste anatomopatológico compatíveis com a doença, que apresentem ou não de HP;2)Idade a 18 anos;3)Pacientes que realizaram

transplante de pulmão por fibrose pulmonar idiopática.Critério de inclusão para o grupo controle

1)Indivíduos sem diagnóstico de FPI e hipertensão pulmonar (HP);2) Ser de 18 anos, preferencialmente com idade e sexo semelhante aos integrantes do grupo com FPI, mas não necessariamente respeitando um perfeito pareamento.3)Pacientes submetidos a transplante pulmonar por ressecção pulmonar para o tratamento de carcinoma brônquico.Após assinatura do Termo de Consentimento Livre e Esclarecido será priorizada a obtenção de 1 centímetro cúbico do pulmão retirado no momento do transplante, tanto dos pacientes com FPI quanto dos pacientes submetidos ao transplante pulmonar para tratar carcinoma brônquico. O tecido (1cm3) que será utilizado dos pacientes com carcinoma brônquico será coletado na área retirada ao redor do carcinoma, mas não parte do carcinoma. Esta área é sempre retirada no momento do transplante como garantia de que todo o carcinoma foi removido. Dessa forma,pretendemos garantir a utilização de um tecido controle com características semelhantes ao pulmão de um indivíduo saudável. As áreas a serem utilizadas serão definidas pelo exame anatomopatológico do tumor ressecado bem como de sua margem de ressecção considerada adequada do ponto de vista oncológico, no caso de pacientes com carcinoma brônquico. Segundo a equipe médica, liderada pela Dra Fabíola Adélia Perin que realiza os transplantes, tal fragmento apresenta dimensão irrisória, sem demandar aumento da área de ressecção ou comprometer o resultado terapêutico ou definições relevantes no diagnóstico de enfisema ou de carcinoma brônquico.Após preencherem os critérios de inclusão, e enquanto aguardam o transplante de pulmão, os pacientes serão abordados após uma consulta de rotina para apresentação da pesquisa, ocasião em que serão convidados a participar. Aqueles que concordarem, assinarão o Termo de Consentimento Livre e Esclarecido e durante o transplante será coletado 1 centímetro cúbico do pulmão, tanto dos pacientes com FPI quanto dos pacientes submetidos ao transplante pulmonar para tratar carcinoma brônquico.

Critério de Exclusão: Critérios de exclusão para ambos os grupos (casos e controles)1)Paciente com distúrbio clínico que comprometa sua participação ou conclusão neste estudo;2)Pacientes com diagnóstico de insuficiência cardíaca;3) Paciente que faça uso de antibióticos, inibidores da enzima conversora da angiotensina (ECA); beta bloqueadores e bloqueadores do receptor de angiotensina.

Considerações sobre os Termos de apresentação obrigatória:

Foram anexados e estão adequados os seguintes documentos: Formulário de Inscrição de projetos de Pesquisa – CEP da ISCMPA, Declaração de Confidencialidade no Estudo, Declaração de Autorização da Chefia responsável, Declaração de isenção de ônus à Instituição, TCLE, Declaração de utilização de dados de prontuário e uso de publicação.

IRMANDADE DA SANTA CASA DE MISERICORDIA DE PORTO ALEGRE - ISCMPA

Recomendações:

Há necessidade de adequar:

- Cronograma: tem como data inicial de captação de amostra em 24/05/2018, antes da aprovação do projeto no CEP da Santa Casa.
- Orçamento: verificar diferenças de informações sobre orçamento, na PB Informações Básicas está R$ 62.000,00 e no projeto está R$ 149.844,72.

Conclusões ou Pendências e Lista de Inadequações:

Aprovado.

Considerações Finais a critério do CEP:

Após avaliação do protocolo acima descrito, o presente comitê não encontrou óbices quanto ao desenvolvimento do estudo em nossa Instituição e poderá ser iniciado a partir da data deste parecer.

Obs.: 1 - O pesquisador responsável deve encaminhar à este CEP, Relatórios de Andamento dos Projetos desenvolvidos na ISCMPA. Relatórios Parciais (pesquisas com duração superior à 6 meses), Relatórios Finais (ao término da pesquisa) e os Resultados Obtidos (cópia da publicação).

2 – Para o início do projeto de pesquisa, o investigador deverá apresentar a chefia do serviço (onde será realizada a pesquisa), o Parecer Consubstanciado de aprovação do protocolo pelo Comitê de Ética.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1121836.pdf	03/05/2018 15:48:52		Aceito
Outros	Ficha_inscricao_ISCMPA.pdf	02/05/2018 20:31:00	Katya Vianna Rigatto	Aceito
Outros	declaracao_prontuario_publicacao.pdf	10/04/2018 01:07:52	Katya Vianna Rigatto	Aceito
Outros	declaracao_confidencialidade.pdf	10/04/2018 01:06:49	Katya Vianna Rigatto	Aceito

Endereço: R. Profª Annes Dias,295 Hosp.Dom Vicente Scherer
Bairro: 6° andar - Centro **CEP:** 90.020-090
UF: RS **Município:** PORTO ALEGRE
Telefone: (51)3214-8571 **Fax:** (51)3214-8571 **E-mail:** cep@santacasa.tche.br

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Continuação do Parecer: 2.691.887

Outros	declaracao_isencao_onus.pdf	10/04/2018 01:06:02	Katya Vianna Rigatto	Aceito
Declaração de Manuseio Material Biológico / Biorepositório / Biobanco	declaracao_material_biologico.pdf	10/04/2018 01:02:58	Katya Vianna Rigatto	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_30_03_18.pdf	10/04/2018 01:00:54	Katya Vianna Rigatto	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_com_alteracoes_VERSAO_5.doc	10/04/2018 01:00:29	Katya Vianna Rigatto	Aceito
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Outros	Anuencia_laboratorio_PUCRS.pdf	05/01/2018 13:23:22	Katya Vianna Rigatto	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

PORTO ALEGRE, 05 de Junho de 2018

Assinado por:
ELIZETE KEITEL
(Coordenador)

Endereço: R. Profª Annes Dias,295 Hosp.Dom Vicente Scherer
Bairro: 6º andar - Centro **CEP:** 90.020-090
UF: RS **Município:** PORTO ALEGRE
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ANEXO C - NORMAS DA REVISTA PEPTIDES

PEPTIDES
An International Journal
ELSEVIER

AUTHOR INFORMATION PACK

TABLE OF CONTENTS

ISSN: 0196-9781

DESCRIPTION

Peptides is an international journal presenting original contributions on the **biochemistry, physiology and pharmacology of biological active peptides,** as well as their functions that relate to gastroenterology, endocrinology, and behavioral **effects.**

Peptides emphasizes all aspects of high profile peptide research in mammals and non-mammalian vertebrates. Special consideration can be given to plants and invertebrates. Submission of articles with clinical relevance is particularly encouraged.

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Regulatory Peptides merged with *Peptides* in January 2015. The two journals now share a common aims and scope and a consolidated editorial board under the title *Peptides.*

AUDIENCE

Peptide researchers, biochemists, neuroscientists, pharmacologists.

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PubMed/Medline
Embase
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BIOSIS Citation Index
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INTRODUCTION

Peptides is an international journal presenting original contributions on the biochemistry, physiology and pharmacology of biological active peptides, as well as their functions that relate to gastroenterology, endocrinology, and behavioral effects.

Peptides emphasizes all aspects of high profile peptide research in mammals and non-mammalian vertebrates. Special consideration can be given to plants and invertebrates. Submission of articles with clinical relevance is particularly encouraged.

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Research articles Letter to the Editor. Review articles

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