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Análise da participação do sistema renina angiotensina em pacientes com fibrose pulmonar idiopática

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“O sucesso nasce do querer, da determinação e persistência em se chegar a um objetivo. Mesmo não atingindo o alvo, quem busca e vence obstáculos, no mínimo fará coisas admiráveis”

José de Alencar.

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LISTA DE ABREVIATURAS

Ang-(1-7) – Angiotensina-(1-7)
AngI – Angiotensina I
AngII – Angiotensina II
ATS – American Thoracic Society
CVF – Capacidade vital forçada
TC6 – Teste da caminhada dos 6 minutos em metros
ECA – Enzima conversora da angiotensina
ECA2 – Enzima conversora da angiotensina II
ERS – European Respiratory Society
FP – Fibrose Pulmonar
FPI – Fibrose Pulmonar Idiopática
HP – Hipertensão Pulmonar
IMC – Índice de Massa Corporal
ISCMPA - Irmandade da Santa Casa de Misericórdia de Porto Alegre
MAS – Receptor da Angiotensina-(1-7) acoplado à proteína G
MMHG – Milímetros de mercúrio
PAD – Pressão Arterial Diastólica
PAP – Pressão da Artéria Pulmonar
PAS – Pressão Arterial Sistólica
SRA – Sistema Renina Angiotensina
VEF1 – Volume expirado forçado no primeiro segundo

SUMÁRIO

RESUMO

A fibrose pulmonar idiopática (FPI) é uma doença crônica, progressiva e de etiologia desconhecida, ainda sem tratamento que acaba levando o paciente à morte. Portanto, o desenvolvimento de tratamentos alternativos para controlar a FPI bem como sua progressão para a Hipertensão Pulmonar (HP), ainda representa um grande desafio. Já se sabe que o sistema renina angiotensina (SRA) está envolvido em diversas patologias fibróticas, porém até agora nenhum estudo verificou o envolvimento desse sistema na FPI. Objetivo: verificar as concentrações plasmáticas de Angiotensina I (AngI), Angiotensina II (AngII), Angiotensina-(1-7) Ang-(1-7) e Alamandina no plasma de pacientes com FPI. Métodos: Participaram do estudo 10 pacientes com FPI, com presença ou não de HP, e 10 indivíduos controles, pareados conforme sexo e idade. Todos os participantes realizaram uma coleta de sangue. Na análise estatística as variáveis quantitativas foram descritas por média e desvio padrão ou mediana e amplitude interquartílica. As variáveis qualitativas foram descritas por frequências absolutas e relativas. O teste t de Student Newman-Keuls foi utilizado para dados paramétricos e o teste de Mann-Whitney para não paramétricos. Para comparar proporções, o teste exato de Fisher foi aplicado. As associações entre as variáveis clínicas e os peptídeos foram avaliadas pelos coeficientes de correlação de Pearson ou Spearman. $P \leq 0.05$ foram considerados estatisticamente significativos e todas as análises foram realizadas utilizando o software SPSS versão 21. Resultados: Somente as concentrações plasmáticas de Alamandina foram significativamente diferentes entre os grupos, sendo menores ($P<0.05$) no grupo com FPI. Houve uma associação positiva entre a pressão da artéria pulmonar (PAP) e as concentrações plasmáticas de Alamandina ($r=0.876$). No entanto, os demais peptídeos não diferiram significativa entre os grupos. Conclusão: Este estudo demonstrou que há um prejuízo na participação do peptídeo Alamandina em indivíduos com FPI, indicando que o eixo ECA-AngII-AT1 pode estar proporcionalmente mais ativo nesta doença.

Palavras chaves: fibrose pulmonar idiopática, sistema-renina-angiotensina, alamandina.

ABSTRACT

Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive disease of unknown etiology and without treatment that leads the patient to death. Therefore, an alternative treatment to control the IPF, and its progression to pulmonary hypertension (PH), is still a major challenge. It is known that the renin angiotensin system (RAS) is involved in several fibrotic diseases. On the other hand, there is no study that it has investigated this system in patients with IPF. Objective: to verify the plasma concentrations of Angiotensin I (AngI), Angiotensin II (AngII), Angiotensin-(1-7) Ang-(1-7) and Alamandine in patients with IPF. Methods: The study included 10 patients with IPF, with or without PH, and 10 controls matched by sex and age. All participants underwent a blood collection. Quantitative variables were expressed as mean and standard deviation or median and interquartile range. The qualitative variables were described by absolute and relative frequencies. The Student Newman-Keuls t test was used for parametric data, Mann-Whitney for nonparametric data and, to compare proportions, the Fisher exact test was performed. The associations between clinical variables and the peptides plasma concentration were evaluated by Pearson or Spearman correlation coefficients. A $P \leq 0.05$ was considered statistically significant. All analyzes were performed using SPSS software version 21. Results: The plasma concentrations of Alamandine were significantly lower in the IPF group. There was a positive association between pulmonary artery pressure and plasma concentrations of Alamandine ($r=0.876$). However, the other peptides did not differ significantly between groups. Conclusion: This study showed, for the first time, that there is a decrease in Alamandine participation in patients with IPF, indicating that ACE-AngII-AT1 axis may be proportionally more active in this disease.

Key words: idiopathic pulmonary fibrosis, system-renin-angiotensin, alamandine.

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Capítulo I – Revisão da Literatura

1. INTRODUÇÃO

1.1 Fibrose Pulmonar Idiopática

A Fibrose Pulmonar Idiopática (FPI) foi descrita inicialmente em 1935 por Hamman e Rich (Hamman & Rich, 1935). No ano de 2000, a *American Thoracic Society* (ATS) e a *European Respiratory Society* (ERS), com o intuito de reunir informações e compreender melhor a patogênese, hereditariedade, fisiopatologia, apresentação clínica, ocorrência familiar e estadiamento da FPI, realizaram uma revisão que incluiu cerca de 3.500 artigos científicos ("American Thoracic Society. Idiopathic pulmonary fibrosis: diagnosis and treatment. International consensus statement. American Thoracic Society (ATS), and the European Respiratory Society (ERS)," 2000). A partir desses, e de outros estudos, a FPI foi classificada como sendo uma doença crônica e progressiva que faz parte do grupo de pneumonias intersticiais idiopáticas. Em média, a sobrevida dos pacientes é inferior a cinco anos após seu diagnóstico, realizado tanto através de biópsia pulmonar como com base em critérios clínicos e fisiopatológicos (Raghu et al., 2011).

Embora o mecanismo da doença e sua etiologia ainda sejam desconhecidos (Raghu et al., 2011), já se sabe que a FPI está associada tanto ao envelhecimento, quanto a cicatrização progressiva do tecido pulmonar. Ocorre proliferação de fibroblastos e acúmulo excessivo de matriz extracelular, sendo mais frequente na sexta e sétima décadas de vida e em indivíduos do sexo masculino (Frankel & Schwarz, 2009; Gross & Hunninghake, 2001; Raghu et al., 2011).

Mundialmente, os dados epidemiológicos estimam que a incidência da FPI é de aproximadamente 11 casos em 100.000 para o sexo masculino e em torno de 8 casos/100.000 no sexo feminino. Por outro lado, a prevalência da doença é estimada em 20 casos em 100.000 no sexo masculino e 13 casos em 100.000 no sexo feminino (D. S. Kim, Collard, & King, 2006). Em 10 anos de estudo, dados epidemiológicos americanos apontaram taxas de mortalidade

de 64,3 óbitos por milhão em homens e de 58,4 por milhão em mulheres por FPI (Olson et al., 2007).

No Brasil, especialmente no Rio Grande do Sul (RS), há um crescente número de notificações de óbitos por FPI. Esses dados são inferiores aos dados de mortalidade de outros países como Europa e Estados Unidos, o que pode significar que a doença está sendo subestimada na nossa população. Nesse sentido, a taxa de óbito pela FPI no RS pode aumentar consideravelmente nos próximos anos (Fortuna, Perin, Cunha, & Rubin, 2003) reforçando a necessidade de avançarmos no conhecimento sobre a doença que ainda não é suficiente para adotar uma terapia eficiente que cure a FPI.

Além disso, sintomas como dispneia, fadiga, fraqueza da musculatura respiratória e esquelética (Eaton, Young, Milne, & Wells, 2005) e redução da capacidade funcional (Collard et al., 2003; Harris-Eze et al., 1996) comprometem substancialmente a qualidade de vida destes pacientes e, portanto, representam um desafio no tratamento da doença.

1.2 Progressão para Hipertensão Pulmonar

A FPI, assim como todas as doenças intersticiais, tem como consequência uma inflamação que progride para a fibrose irreversível (Cherniack et al., 1991). Esse processo induz ao remodelamento da artéria pulmonar e à vasoconstrição, culminando com o surgimento de Hipertensão Pulmonar (HP), o que piora o prognóstico destes pacientes (Caminati, Cassandro, & Harari, 2013; Ley, Collard, & King, 2011). De fato, uma doença progressiva e incurável, a HP, é caracterizada por pressão na artéria pulmonar em repouso igual ou superior a 25mmHg (Humbert et al., 2006), e está associada ao remodelamento vascular, vasoconstrição e trombose (Humbert et al., 2004), podendo levar o paciente à morte em até três anos após seu diagnóstico (Reis et al., 2010; Runo & Loyd, 2003).

Dessa forma, a HP é uma complicação comum em doenças pulmonares intersticiais (Handa et al., 2007; Lettieri, Nathan, Barnett, Ahmad, & Shorr, 2006;

Nadrous et al., 2005a, 2005b), sendo mais frequente na fibrose pulmonar idiopática (Lettieri et al., 2006).

Um estudo multicêntrico realizado em pacientes com FPI, submetidos ao cateterismo cardíaco direito e ao teste de função cardiopulmonar, demonstrou que 54% dos pacientes analisados com FPI também tinham HP (Gläser et al., 2013). A HP provoca distúrbios nas trocas gasosas (Girgis & Mathai, 2007; Nadrous et al., 2005b), gera dispneia, fadiga, diminuição da capacidade funcional, redução da qualidade de vida (Eaton et al., 2005) e aumento da mortalidade de pacientes com FPI (Hamada et al., 2007; Lettieri et al., 2006; Nadrous et al., 2005b). Além disso, ainda não está claro na literatura as implicações clínicas da associação entre a FPI e a HP. O que se sabe é que não há um tratamento eficaz para a HP (Galiè et al., 2009), e que o surgimento desta patologia, pode ser um fator de risco independente para a mortalidade de pacientes com FPI (Lettieri et al., 2006).

Considerando que a HP é frequente na fisiopatologia da FPI (Lettieri et al., 2006), e que seu tratamento ainda permanece elusivo, o entendimento mais aprofundado da FPI e da HP pode fornecer conhecimentos que contribuam para melhorar a sobrevida dos indivíduos acometidos por essas doenças. Curiosamente, apesar de o pulmão ser um dos importantes sítios que participam da ativação do sistema renina angiotensina (SRA), ativando a angiotensina II (AngII) pela enzima conversora da angiotensina (ECA), muito pouco se sabe a respeito da participação desse sistema nas doenças pulmonares, especialmente na FPI.

1.3 Sistema Renina Angiotensina e a Fibrose Pulmonar Idiopática

Os primeiros estudos relacionados ao SRA foram realizados por Tiegerstedt e Bergman em 1898, que descobriram uma substância sintetizada nos rins, com efeitos pressóricos, chamada “Renina” (Tiegerstedt & Bergman, 1898). A partir desta descoberta outros estudos foram sendo realizados com o intuito de elucidar o complexo SRA. Em 1940, Braun-Mendes e colaboradores e Page & Helmer, descobriram que o peptídeo pressórico, não era a renina, e sim um produto da ação enzimática dessa substância sobre uma proteína plasmática (Braun-Menendez,

Fasciolo, Leloir, & Muñoz, 1940; Page & Helmer, 1940), sendo denominada posteriormente de Angiotensina (Braun-Menendez & Page, 1958). Estes estudos possibilitaram a descoberta de duas formas de Angiotensina, a Angiotensina I (AngI) e a AngII (SKEGGS, KAHN, & SHUMWAY, 1956). Desde então, diversos estudos foram realizados, permitindo assim, a ampliação dos conhecimentos sobre esse complexo e desafiante sistema que até hoje surpreende a comunidade científica com o surgimento de novos componentes.

Hoje está bem estabelecido na literatura que o SRA é um importante mecanismo de manutenção da homeostase, participando da modulação de órgãos-alvo, como o coração, vasos sanguíneos, e pulmões (A. J. Ferreira et al., 2012). A ativação do SRA inicia através da conversão do angiotensinogênio em AngI, que é clivada pela ECA para formar AngII. As ações da AngII são mediadas por dois receptores acoplados à proteína G (GPCR), AT_1 e AT_2 (A. J. Ferreira et al., 2012).

As principais funções fisiológicas de AngII são mediadas pelos receptores AT_1, cuja ativação exagerada induz efeitos deletérios, tais como, vasoconstrição, retenção de líquidos, além de fibrose, crescimento e proliferação celular em diversos tipos celulares (S. Kim & Iwao, 2000; Mehta & Griendling, 2007).

Por outro lado, a visão mais atual do SRA trouxe à tona novos componentes desse sistema, entre eles a enzima conversora de angiotensina 2 (ECA2), que tem como função primordial a conversão de AngII em Angiotensina-(1-7) [Ang-(1-7)] (Ferrario, Chappell, Tallant, Brosnihan, & Diz, 1997).

A Ang-(1-7), ao se ligar ao receptor Mas (Santos et al., 2003), é conhecida por seu efeito anti-hipertensivo, com capacidade de proteger os pulmões (Donoghue et al., 2000) e ainda se contrapõem aos efeitos vasoconstritores, proliferativos, inflamatórios e fibróticos da AngII (Grobe et al., 2007).

De acordo com Rubin (1997), a administração de XNT, que ativa a enzima ECA2, atenua a fibrose pulmonar e o engrossamento da parede vascular (Rubin, 1997). Por outro lado, esses benefícios foram inibidos, quando administrado o antagonista da Ang-(1-7), o A779, demonstrando a importância da ativação da ECA2 (A. J. Ferreira et al., 2009; Silva et al., 2011). Tudo indicava que a Ang-(1-7) era a protagonista das ações benéficas de um novo eixo com ações que contra-regulavam as conhecidas ações da AngII na doença.

Evidências *in vitro* sugerem que a AngII está envolvida na fibrose pulmonar (FP). Estudos genéticos de polimorfismos de genes do SRA, demonstraram a presença de genes desse sistema em amostras de biópsia do pulmão de pacientes com fibrose pulmonar (Uhal, Li, Piasecki, & Molina-Molina, 2012). De fato, em estudos envolvendo modelos animais, os antagonistas da angiotensina bloquearam a fibrose pulmonar, demonstrando assim, que a AngII desempenha um papel muito importante na fibrogênese do pulmão (Li et al., 2007).

Além disso, resultados significativos demonstram que o estímulo ao eixo ECA2-Ang-(1-7)-Mas atenuam tanto a FP (Hampl et al., 2015) quanto a HP (Rigatto, Casali, Shenoy, Katovich, & Raizada, 2013). Nesse contexto, reforçando os achados desses autores, estudos envolvendo a transferência de genes endotraqueal (Shenoy et al., 2014), mini-bomba osmótica (Chen, Xiao, Li, & Ma, 2011) ou administração oral de Ang-(1-7) (Shenoy et al., 2014) demonstraram que este peptídeo atenua a FP e a HP em diversos modelos experimentais.

Para tornar o desafio ainda mais estimulante, em 2013 foi descoberta a Alamandina, um heptapéptido endógeno, gerado pela ação catalítica da ECA2 sobre a Angiotensina A ou diretamente sobre a Ang-(1-7) por descarboxilação (Lautner et al., 2013). Esta descoberta veio enriquecer a gama de atuação do SRA e trouxe novas esperanças para a compreensão do envolvimento do SRA na fisiopatologia das doenças.

A Alamandina atua através do receptor denominado MrgD, sendo considerada um peptídeo vasoativo com ações de proteção semelhantes aos da Ang-(1-7), tais como ações antifibróticas, anti-hipertensivas vasodilatadoras e ações com efeitos centrais. Além disso, evidências indicam que a Alamandina apresenta um efeito direto sobre a remodelação tecidual, diminuindo colágeno I, III bem como a acumulação de fibronectina em ratos com fibrose cardíaca (Lautner et al., 2013).

No entanto, por ser um peptídeo identificado recentemente, ainda há pouca informação sobre o seu envolvimento tanto na saúde quanto na doença. É sabido que a sua concentração plasmática está elevada em paciente nefropatas, sugerindo que a Alamandina pode participar em condições patológicas (Lautner et al., 2013) possivelmente compensando essa situação. Por outro lado, ainda não há dados na literatura indicando a participação da Alamandina na FPI.

De fato, a Alamandina pode representar um peptídeo envolvido na contra-regulação do eixo do ECA-AngII-At$_1$ do SRA, conforme representado na Figura 1. Ela pode ser formada tanto a partir da descarboxilação da Ang-(1-7), quanto da Angiotensina A pela ECA$_2$, perdendo um peptídeo.

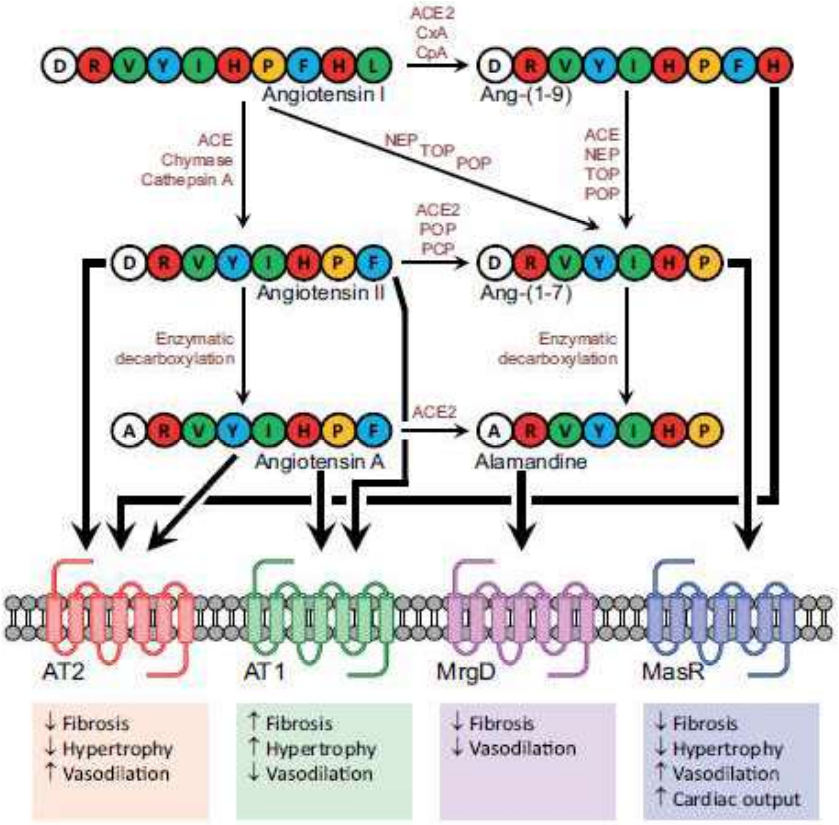

Figura 1: Ações do SRA. Legenda com abreviaturas.
Enzima conversora da angiotensina (ECA); Enzima conversora da angiotensina-2 (ACE2; Receptor da angiotensina 2 (AT2R); Carboxipeptidase A (CxA); Catepsina A (Cpa); Endopeptidase neutra (NEP); Receptor Mas (Masr); Proteína relacionada ao L-Mas acoplado ao receptor do membro D (MrgD); *Polly* Carboxipeptidase (PCP); *Prolil* endopeptidase (POP); *Thimet* oligopeptidase (TOP). Fonte: E Mendoza-Torres et al. (2015).

No entanto, ainda não está claro na literatura a relação do SRA e suas implicações clínicas na fisiopatologia da FPI. Todos os tratamentos até o momento estão focados no bloqueio do eixo ECA-AngII-AT$_1$, não levando em conta a importância do(s) eixo(s) contra-regulatório(s).

Inúmeros estudos da literatura demonstram que na doença o eixo ECA-AngII-AT$_1$ está geralmente superativado e que possivelmente a relação entre os eixos ECA2-Ang-(1-7)-Mas e/ou ECA2-Alamandina-MrgD versus ECA-AngII-AT$_1$ é mais

baixa. Por outro lado, nenhuma investigação até o momento procurou esclarecer o papel do SRA de uma forma mais ampla, considerando uma possível contra-regulação entre os eixos desse sistema, sobretudo na FPI.

Nossa meta é obter resultados que chamem a atenção sobre a importância de aumentar o conhecimento sobre as diversas ações entre os eixos do SRA em uma doença que apresenta foco no pulmão, como a FPI. Com isso, pretendemos desencadear ações para melhorar a qualidade de vida e a sobrevida desses pacientes, estimulando os eixos que se contrapõem à fibrose e ao desenvolvimento de HP, entre outras ações.

2. OBJETIVO

Verificar as concentrações plasmáticas de Angiotensina I (AngI), Angiotensina II (AngII), Angiotensina-(1-7) Ang-(1-7) e Alamandina no plasma de pacientes com FPI.

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Alamandine is reduced in patients with pulmonar idiopathic fybrosis

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Abstract: Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive disease of unknown etiology and without treatment that leads the patient to death. Therefore, an alternative treatment to control the IPF, and its progression to pulmonary hypertension (PH), is still a major challenge. It is known that the renin angiotensin system (RAS) is involved in several fibrotic diseases. On the other hand, there is no

study that it has investigated this system in patients with IPF. Objective: to verify the plasma concentrations of Angiotensin I (AngI), Angiotensin II (AngII), Angiotensin-(1-7) Ang-(1-7) and Alamandine in patients with IPF. Methods: The study included 10 patients with IPF, with or without PH, and 10 controls matched by sex and age. All participants underwent a blood collection. Quantitative variables were expressed as mean and standard deviation or median and interquartile range. The qualitative variables were described by absolute and relative frequencies. The Student Newman-Keuls t test was used for parametric data, Mann-Whitney for nonparametric data and, to compare proportions, the Fisher exact test was performed. The associations between clinical variables and the peptides plasma concentration were evaluated by Pearson or Spearman correlation coefficients. A $P \leq 0.05$ was considered statistically significant. All analyzes were performed using SPSS software version 21. Results: The plasma concentrations of Alamandine were significantly lower in the IPF group. There was a positive association between pulmonary artery pressure and plasma concentrations of Alamandine ($r=0.876$). However, the other peptides did not differ significantly between groups. Conclusion: This study showed, for the first time, that there is a decrease in Alamandine participation in patients with IPF, indicating that ACE-AngII-AT1 axis may be proportionally more active in this disease.

Key words: Idiopathic Pulmonary Fibrosis; Alamandine, Renin-Angiotensin System.

INTRODUCTION

Idiopathic pulmonary fibrosis (IPF) is more prevalent in males[1,2] than females and it is characterized by progressive scarring of lung tissue[3]. It is considered the

most severe form of pulmonary fibrosis, without treatment and after diagnosis, the survival is around three years[4].

Although its etiology remains unknown, it is very well known that IPF is associated with aging, collagen deposition, fibroblast proliferation and excessive accumulation of extracellular matrix[5]. In addition, pulmonary hypertension (PH) is a common complication in IPF[6] and its treatment also remains elusive[7]. Thus, the understanding of IPF physiopathology, as well as to slow or even control the onset of PH, could improve the survival of these patients.

Results from studies in animals[8,9] and human lung tissue[9,10] demonstrated that the renin angiotensin system (RAS) plays an important role in lung fibrosis. It has been demonstrated that angiotensin II (AngII) is involved in the fibrosis promotion[11]. Thus, considering this involvement, and the importance of ACE-AngII-AT_1 axis to provoke vasoconstriction, cell migration, proliferation[12,13] and fibrosis[11], and mainly considering that the lungs are an important site of AngII formation, our objective was to study the involvement of this system in IPF.

Angiotensin I (AngI), converted into AngII by angiotensin converting enzyme (ACE), can be also cleaved by angiotensin converting enzyme II (ACE2) to form Ang-(1-7)[14]. In addition, the imbalance between ACE-AngII-AT_1 and ACE2-Ang-(1-7)-Mas plays an important role in the pathogenesis of many diseases[15,16] including fibrotic disorders[9,17,18]. Collectively, these findings demonstrate the importance to keep the balance in favor to the ACE2-Ang-(1-7)-Mas axis. In fact, studies[17,18] have shown that ACE2-Ang-(1-7)-Mas axis may have a role in pulmonary fibrosis. Li et al. (2008) found in mice that ACE2 inhibition induced an increase in tissue expression of AngII and provoked pulmonary fibrosis[9], indicating that ACE2-Ang-(1-7)-Mas axis might, at least, reduce the fibrosis progression. Therefore, it is reasonable to believe that the imbalance between the ACE-AngII-AT_1/ACE2-Ang-(1-7)-Mas axes participates in the physiopathology of IPF.

Currently, to make the challenge even more interesting, the discovery of a new component of this system, Alamandine[19], has further enriched the role of RAS in this disease. It is known that Alamandine, acting through the MrgD receptor, has antifibrotic and antihypertensive effects, reduces collagen I, III and fibronectin accumulation in mice with cardiac fibrosis[19].

On the other hand, there are no studies in the literature focused on the role of RAS in the physiopathology of IPF. Numerous studies have shown that in the disease ACE-AngII-AT_1 axis is overactive. Moreover, to the moment, all treatments are focused only on blocking the ACE-AngII-AT_1 axis and did not taking into account the importance of the counter-regulatory axis. Possibly the ratio between ACE2-Ang-(1-7)-Mas axis and/or ACE2-Alamandine-MrgD versus ACE-AngII-AT_1 is lower during disease.

However, although having opposite effects, the importance of the balance between these axes has been underestimated, emphasizing the importance of more studies in this direction. It is possible that, more important than exclusively block the ACE-AngII-AT_1 axis, to stimulate the ACE2-Ang-(1-7)-Mas and/or ACE2-Alamandine-MrgD axes, could improve the efficiency of the diseases treatment.

Thus, our goal is to draw the attention to the importance of those balances and to give more information of RAS participation in IPF and, in the future, to have contributed to improve the quality of life and survival of IPF patients.

MATERIALS AND METHODS

Participants

This study used a cross-sectional convenience patients sample recruited from the Irmandade Santa Casa de Misericórdia de Porto Alegre (ISCMPA).

The study enrolled patients with IPF (n=10), diagnosed by lung biopsy. All patients were evaluated for weight, height, body mass index (BMI), blood pressure, medications and previous illness. Seven patients performed spirometry test and all patients were submitted to the six-minute walk test, according to the guidelines of the American Thoracic Society[20]. In addition, the pulmonary artery pressure was measured in five patients.

The control group (n=10) consisted of healthy age and gender matched. The health status was determined through anamnesis and all individuals self-declared without disease.

Smokers, alcohol users or with neurological and/or musculoskeletal disorders were excluded. Moreover subjects/patients with heart or kidney failure taking antibiotics, ACE inhibitors and/or beta-blockers were also excluded of the study.

ETHICAL ASPECTS

The Ethics Committee of ISCMPA approved the project (number 1293420). All participants signed a consent form.

STUDY PROTOCOL

The study was conducted in patients with IPF from ISCMPA. Circulating concentration of RAS peptides were measured in both groups.

The evaluation of circulating RAS: in the morning and after resting for 5 minutes, blood samples (5ml) were collected through peripheral venipuncture after a fasting period of 12 hours. The same trained professional performed the blood collection.

Blood collection and plasma extraction

The five milliliters of blood were stored in a BD Vacuette® EDTA tube containing a protease inhibitor cocktail (Complete™, Mini-Protease Inhibitor Cocktail Tablets, Roche®-Germany) prepared according to the manufacturer's instructions.

The blood was centrifuged for 10 minutes, 3,500 rpm at 4°C. The plasma was separated and frozen at -80°C to measure AngI, AngII, Ang-(1-7) and Alamandine.

Peptides measurements

The plasma renin activity as well as AngI, AngII and Ang-(1-7) concentrations were determined through radioimmunoassay, as detailed elsewhere[21]. The recovery of ^{125}I-labeled AngI, AngII, and Ang-(1-7) was 79.2% ± 2.3%, 86.9% ± 0.8% and 83.5% ± 0.9%, respectively. Results were expressed as nanograms of AngI

generated per milliliter of plasma per hour (ng AngI/mL per hour) for plasma renin activity and pg/mL of plasma for peptides measurements. Alamandine detection was performed as described previously[22].

STATISTICAL ANALYSIS

The qualitative variables were described by absolute and relative frequencies.

Student's t test was used to compare means between groups. In the case of asymmetry, the Mann-Whitney test was performed.

The Fisher exact test was used to compare proportions. Pearson or Spearman correlation coefficients were used to detect associations.

Quantitative variables were expressed as mean and standard deviation or median and interquartile range. When $P \leq 0.05$ the data were considered statistically different. All analyzes were performed using SPSS version 21.

RESULTS

The weight and body mass index (BMI) were significantly different between groups (Table 1). On the other hand, regarding to age, sex, height, as well as the hemodynamic data such as systolic blood pressure (SBP) and diastolic blood pressure (DBP) there were no difference between groups.

Regarding to clinical data, there were no significant difference in the parameters analyzed (Table 2). In addition, the plasma concentration of AngI, AngII and Ang-(1-7) were also not different between groups.

However, the group with IPF showed a significant reduction in Alamandine plasma concentration (Table 3), and also there was a positive correlation of this concentration with the pulmonary artery pressure (PAP; Table 4). However, the AngI, AngII and Ang-(1-7) have no association with any result.

DISCUSSION

The most important observation of this study was that patients with IPF showed a significant decrease in Alamandine plasma concentrations, while AngI, AngII and Ang-(1-7) were similar to controls. In addition, there was also an association of Alamandine concentration with the pulmonary artery pressure.

Our results showed that body weight and BMI of IPF-patients were significantly lower than controls. These results are probably due to a pronounced increase in body weight, seen in the control group, that it is not observed in IPF-patients, who usually have higher metabolic expenditure. In fact, these results agree with those found by Alakhras et al. (2007), who demonstrated that the majority of IPF patients present a BMI and weight within or above the normal range[23]. In fact, it would be expected that patients with pulmonary diseases had a decrease in body weight due to hypoxemia found when there is a respiratory injury. According to Bunn & Poyton (1996), the hypoxemia modifies the muscles by generating a sharp decline in protein synthesis to adapt the oxygen supply to the respiratory demand in these patients[24]. Weight loss is also a serious problem in patients with cystic fibrosis[25,26]. These clinical signs probably exasperate the loss of muscle mass through oxidative stress and inflammatory responses[27].

On the other hand, although in vivo studies have shown that AngII plays an important role in lung fibrogenesis[11] and that there is an increase in this peptide in pathological situations associated to fibrosis[28-30], our results showed that AngI, AngII and Ang-(1-7) plasma concentration were not different between groups. This unexpected finding remains to be explained, particularly regarding to AngII plasma concentration that it is similar in PIF-patients and controls.

A possible explanation could be related to where the AngII was measured. The majority of the data from the literature are from tissues and also from different species. Our results reflect the plasma concentration in human being. In addition, the literature data have shown that the use of ACE inhibitors attenuate experimental pulmonary fibrosis[8,31-33]. Regarding to ACE inhibitor or beta-blockers, it is important to emphasize that, in this study, patients and controls were not taking them.

In addition, Jiang et al. (2016) demonstrated in a mouse model of Alzheimer's disease that there was a significant decrease in plasma concentrations of Ang-(1-7)[34]. However, we did not find a significant difference in Ang-(1-7) plasma concentration between groups. A possible explanation could be the fact that the balance among peptides plasma and/or tissue concentrations is more important than the absolute values of each alone. The predominance of one, and its respective actions, could be more important in the pathology of IPF to counteract the detrimental effects of the disease.

Although Lautner et al. (2013) showed an increase in plasma concentration of Alamandine in nephropathic patients[19], it is important to consider the known anti-fibrotic effect of this peptide[19]. It would be reasonable that patients with IPF showed a reduction in Alamandine plasma concentration. This apparent contradiction could be probably due to the difference in the physiopathology of the diseases. Nephropathic patients present hemodynamic changes that could justify the need for vasodilation to offset the reduction in renal filtration. Accordingly, the increased Alamandine could be promoting vasodilation to compensate the volume overload, counteracting that effect.

In contrast, patients did show a positive association between Alamandine plasma concentration and PAP. This result indicates that Alamandine could be attempting to minimize the effects on pulmonary artery pressure. In fact, our study was the first to demonstrate in patients with IPF that Alamandine plasma concentrations is decreased, but positively associated to PAP. On the other hand, there was no association of any peptide with any other clinical parameter. These findings could be important to understand the pathology of IPF. Both Ang-(1-7) and Alamandine may represent a new counter-regulatory axis to the deleterious effects of AngII[35]. An important point of criticism of this investigation is the absence of data showing the peptides tissue expression. It would be very interesting to show the RAS involvement with the lung tissue injury. Future investigation in animals might clarify this participation. More studies are needed to demonstrate the magnitude of RAS effects in patients with IPF and the real possibilities to develop an effective treatment to this disease.

CONCLUSION

This study showed, for the first time, that there is a decrease in Alamandine participation in patients with IPF, indicating that ACE-AngII-AT1 axis may be proportionally more active in this disease.

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Table 1 – Sample characterization.

Variables	Control (n=10)	IPF (n=10)	P
Age (years)	58±13	58±13	0.973
Male	6	6	1.000
White breed	10	8	0.474
Weight (kg)	80±13	67±9.4	0.023*
Height (m)	1.68±0.10	1.63±0.08	0.299
BMI (kg/m^2)	28±4	25±1.9	0.041*

Values expressed as mean ± standard deviation; IPF: idiopathic pulmonary fibrosis; BMI = body mass index.*P<0,05.

Table 2 – Patients clinical characterization

Variables	Mean ± SD
FVC (ml)[a]	1.97±0.69
FVC (% do previsto)[a]	56±22
VEF_1 (ml)[a]	3.05±3.45
VEF_1 (% do previsto)[a]	58±19
PAP (mmHg)	50±13
Diagnostic time (years)	2.15±1.45
Oxygen Use	6
Distance in 6MWT (m)	421±171
Distance in 6MWT (%)	74±31

[a]Pulmonary function tests pre-bronchodilator; FVC: forced vital capacity;$FEV_{1:}$ force expiratory volume in one second; PAP: pulmonary artery pressure; 6MWT:Distance covered in the walk test of six minutes. Values expressed as mean ± standard deviation.

Table 3 - Renin-Angiotensin-System peptides plasma concentration (pg/mL).

Variables	Control (n=10)	IPF (n=10)	P
Angiotensin I	22 (16.6 – 33.4)	37 (22.9 – 81.9)	0.089
Angiotensin II	7.78 (4.95 – 15.6)	9.84 (4.56 – 20.4)	0.780
Angiotensin-(1-7)	31 (20.5 – 36.3)	28 (25.8 – 34.6)	0.684
Alamandine	618±169	474±100	0.033*

Values expressed as median for dosages Angiotensin I, Angiotensin II and Angiotensin-(1-7) and mean±standard deviation for the dosages of Alamandine. *P <0.05.

Table 4 - Associations between clinical variables and Renin –Angiotensin-System peptides.

Variables	AngI	AngII	Ang-(1-7)	Alamandine
FVC (%)	r_s=-0.042	r_s=-0.134	r_s=-0.393	r=0.106
VEF_1 (%)	r_s=-0.059	r_s=-0.176	r_s=-0.427	r=0.115
PAP (mmHg)	r_s=0.400	r_s=0.000	r_s=-0.200	r=0.876*
Diagnostic time (years)	r_s=0.217	r_s=0.093	r_s=0.093	r_s=0.106
Distance in 6MWT (%)	r_s=-0.333	r_s=-0.236	r_s=-0.018	r=-0.249

FVC: forced vital capacity; FEV_1: forced expiratory volume in one second; PAP: pulmonary artery pressure; 6MWT (%): Estimated Percentage of distance covered in the walk test of six minutes in meters. Amounts in Pearson correlation coefficients (r)and Spearman (rs).* $P<0.05$.

ANEXOS

ANEXO 1

Normas de formatação da revista The International Journal of Biochemistry & Cell Biology

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Citations may be made directly (or parenthetically). Groups of references should be listed first alphabetically, then chronologically.
Examples: 'as demonstrated (Allan, 2000a, 2000b, 1999; Allan and Jones, 1999). Kramer et al. (2010) have recently shown ....'
List: References should be arranged first alphabetically and then further sorted chronologically if necessary. More than one reference from the same author(s) in the same year must be identified by the letters 'a', 'b', 'c', etc., placed after the year of publication.
Examples:
Reference to a journal publication:
Van der Geer, J., Hanraads, J.A.J., Lupton, R.A., 2010. The art of writing a scientific article. J. Sci. Commun. 163, 51–59.
Reference to a book:
Strunk Jr., W., White, E.B., 2000. The Elements of Style, fourth ed. Longman, New York.
Reference to a chapter in an edited book:
Mettam, G.R., Adams, L.B., 2009. How to prepare an electronic version of your article, in: Jones, B.S., Smith , R.Z. (Eds.), Introduction to the Electronic Age. E-Publishing Inc., New York, pp. 281–304.
Reference to a website:
Cancer Research UK, 1975. Cancer statistics reports for the UK. http://www.cancerresearchuk.org/aboutcancer/statistics/cancerstatsreport/ (accessed 13.03.03).

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ANEXO 2

Parecer Consubstanciado do CEP da ISCMPA

IRMANDADE DA SANTA CASA DE MISERICORDIA DE PORTO ALEGRE - ISCMPA

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Análise da participação do sistema renina angiotensina em pacientes com fibrose pulmonar idiopática

Pesquisador: Katya Vianna Rigatto

Área Temática:

Versão: 6

CAAE: 33228314.0.0000.5335

Instituição Proponente: Irmandade da Santa Casa de Misericordia de Porto Alegre - ISCMPA

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 1.293.420

Apresentação do Projeto:

Analisar a participação do sistema renina angiotensina composto pelos eixos (ECA-AngII-AT1) e [ECA2-Ang -(1-7)-Mas] em pacientes com fibrose pulmonar idiopática.

Objetivo da Pesquisa:

Objetivo

Analisar a participação do sistema renina angiotensina composto pelos eixos (ECA-AngII-AT1) e [ECA2-Ang -(1-7)-Mas] em pacientes com fibrose pulmonar idiopática.

Avaliação dos Riscos e Benefícios:

Apresentados e adequados.

Comentários e Considerações sobre a Pesquisa:

Solicitação de Emenda ao Protocolo com modificações no projeto original, título da pesquisa, critérios, cronograma e TCLE, todos documentos anexados e adequados.

Considerações sobre os Termos de apresentação obrigatória:

Anexados e adequados.

Recomendações:

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UF: RS **Município:** PORTO ALEGRE
Telefone: (51)3214-8571 **Fax:** (51)3214-8571 **E-mail:** cep@santacasa.tche.br

IRMANDADE DA SANTA CASA DE MISERICORDIA DE PORTO ALEGRE - ISCMPA

Conclusões ou Pendências e Lista de Inadequações:

Solicitação a Emenda ao Protocolo, aceita.

Considerações Finais a critério do CEP:

Após a avaliação da solicitação a emenda do Protocolo e demais documentos anexados, o presente comitê não encontrou óbices quanto ao desenvolvimento do estudo em nossa Instituição.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_600829_E2.pdf	20/10/2015 17:30:53		Aceito
Folha de Rosto	folhaderosto.pdf	20/10/2015 17:27:05	Tais Salvi Sipriani	Aceito
Outros	cronograma29092015.pdf	01/10/2015 19:54:23	Tais Salvi Sipriani	Aceito
Outros	emenda290915.pdf	01/10/2015 19:53:00	Tais Salvi Sipriani	Aceito
Outros	TCLEgruposemFPI.pdf	01/10/2015 19:48:38	Tais Salvi Sipriani	Aceito
Outros	TCLEgrupocomFPI.pdf	01/10/2015 19:47:51	Tais Salvi Sipriani	Aceito
Outros	projeto_ultima_versao.pdf	01/10/2015 19:47:10	Tais Salvi Sipriani	Aceito
Outros	Emenda 6-12.pdf	06/12/2014 10:00:18		Aceito
Outros	Cronograma 6-12.pdf	06/12/2014 09:59:34		Aceito
Projeto Detalhado / Brochura Investigador	Projeto FPI ULTIMA VERSAO 06-12.pdf	06/12/2014 09:58:50		Aceito
Outros	carta ao CEP 30-10.pdf	30/10/2014 17:46:35		Aceito
Outros	carta ao CEP.pdf	30/10/2014 17:28:45		Aceito
Projeto Detalhado / Brochura Investigador	Projeto FPI ULTIMA VERSAO 29-10.pdf	30/10/2014 17:26:12		Aceito
Outros	Criterios de inclusão.pdf	11/10/2014 16:50:13		Aceito
Projeto Detalhado / Brochura Investigador	Projeto FPI última versão 8-10 PDF.pdf	11/10/2014 16:48:11		Aceito
Outros	Inclusão dos colaboradores.pdf	11/10/2014		Aceito

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Outros	Inclusão dos colaboradores.pdf	16:42:44		Aceito
Outros	Carta resposta ao CEP .pdf	13/08/2014 21:10:30		Aceito
Outros	cronograma correto.pdf	12/08/2014 16:14:07		Aceito
Projeto Detalhado / Brochura Investigador	Projeto versão CORRETA CEP.pdf	09/08/2014 10:49:06		Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE.pdf	09/08/2014 10:47:57		Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	Termo de Consentimento Livre e Esclarecido.pdf	24/06/2014 21:36:05		Aceito
Outros	Doc.pdf	24/06/2014 21:28:21		Aceito
Outros	14.pdf	24/06/2014 21:27:04		Aceito
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Continuação do Parecer: 1.293.420

Situação do Parecer:
Aprovado
Necessita Apreciação da CONEP:
Não

PORTO ALEGRE, 23 de Outubro de 2015

Assinado por:
Carlos Henrique Munhoz Olea
(Coordenador)

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