Review

Biological Actions of Alamandine: A Scoping Review

Juliane Flor , Andresa Thomé Silveira , Isabel Amaral Martins , Laura Bastos Otero Scherer , Flávia Moraes Silva , Adriana Fernanda K Vizuete , Márcia Rosangela Wink * , Katya Rigatto *

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Biological Actions of Alamandine: A Scoping Review

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Abstract

This scoping review aims to comprehensively map the existing literature on the mechanisms of action of Alamandine (ALA), a peptide within the renin-angiotensin system, and its effects across various physiological systems. **Materials and Methods:** Utilizing the Joanna Briggs Institute methodology, a thorough search of databases including PubMed, Embase, Scopus, and Web of Science was conducted up to January 30, 2025. The review focused on identifying studies that explore the biological and therapeutic roles of ALA in different contexts, incorporating in vivo, in vitro, and in silico research. **Results**: A total of 590 records were initially identified, with 26 meeting the eligibility criteria for inclusion in this review. China emerged as the leading contributor to research in this area, with a significant focus on the cardiovascular system. The studies revealed that ALA exhibits a range of beneficial effects, including anti-inflammatory, vasodilatory, antifibrotic, and antiapoptotic actions. These effects are primarily mediated through the inhibition of the Mitogen-Activated Protein Kinase (MAPK) signaling pathway and modulation of the nitric oxide pathway. The review also highlighted ALA's potential in mitigating oxidative stress and its implications in treating cardiovascular diseases, fibrosis, and cancer. **Conclusions:** The findings suggest that ALA holds significant therapeutic potential, offering antihypertensive, anti-inflammatory, antifibrotic, and anticancer benefits without notable adverse effects. This underscores its promise as a therapeutic agent in various clinical settings, warranting further research to explore its full potential and mechanism of action.

Keywords: Alamandine; Mechanism of action; Therapeutic potential; AMPK; Nitric oxide; MAPK

1. Introduction

Alamandine (ALA) was identified and characterized in 2013 by a research team led by Dr. Robson Santos during an investigation into the function of angiotensin-converting enzyme 2 (ACE2), a component of the renin-angiotensin system (RAS) [1]. This discovery, alongside the identification of Angiotensin-(1-7) [Ang-(1-7)] in 1988 [2], marks a significant milestone in the interpretation of the RAS.

These findings reinforced the concept of two axes within the RAS that have seemingly opposing functions, which balance each other in maintaining health. The classical axis, represented by ACE-AngII-AT1, coexists with a novel axis involving ACE2-Ang-(1-7)/ALA-Mas/MrgD, which plays a central role in the pathophysiology of various diseases. However, despite these advancements, clear gaps persist in our understanding of this system.

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The primary mechanism by which ALA functions is through the activation of G protein-coupled receptor signaling, specifically involving the Mas receptor (MrgD). This activation leads to various downstream effects, including vasodilation, anti-inflammatory actions[3], and antifibrotic effects [4], which closely resemble those of Ang-(1-7) [5].

Literature data demonstrate that ALA induces vasodilation both in vitro and in vivo, likely through the activation of nitric oxide synthase (NOS) to produce nitric oxide (NO) [6]. Furthermore, ALA exhibits antifibrotic effects, likely through the inhibition of transforming growth factor-β (TGF-β) signaling, thus reducing collagen deposition in the heart [7] and kidneys [8]. These results revealed the importance of the new axis in counteracting the harmful effects of excessive angiotensin II (Ang II) action.

Building on this understanding, we propose a scoping review using an emerging methodology to synthesize existing knowledge on the topic. Although not all articles present the highest quality, considering the limited literature available, all studies discussing ALA's mechanisms of action were included. This method also identifies gaps, opportunities, and research priorities to guide future studies on its mechanisms of action in the body.

2. Materials and Methods

This scoping review employs the Joanna Briggs Institute methodology [9] and is registered on the Open Science Framework (OSF, DOI 10.17605/OSF.IO/CG9U4). The review follows five stages: identifying research questions, finding relevant studies, selecting eligible studies, collecting and recording data, and summarizing and reporting results. Detailed descriptions of each stage are provided below [9].

2.1. Stage 1: Identification of the Research Question

The main research question was formulated using the 'Population,' 'Concept,' and 'Context' (PCC) framework, covering both in vivo and in vitro studies. This approach focuses on identifying the primary signaling pathways that explain the ALA's mechanism of action. In this context, we considered all body systems, including the kidneys, brain, cardiovascular system, heart, eyes, lungs, and others.

Based on the PCC framework, our main research question was: What mechanisms of action of ALA are described in the literature? This led to the following sub-questions:

- In what clinical conditions and outcomes has ALA been described?
- What are the effects of ALA?

2.2. Stage 2: Identification of Relevant Studies (Search Strategy)

All studies that evaluated the mechanism of action of ALA, regardless of the body system studied, were eligible for this scoping review. We did not impose restrictions on publication date or language. However, qualitative studies, reviews, editorials, comments, abstracts, and conference proceedings were excluded.

Initially, a search was conducted in the PubMed database to identify potential keywords for the search strategy, based on the titles, abstracts, and keywords of retrieved articles. Subsequently, a final search was performed across the PubMed, Embase, Scopus, and Web of Science databases (see Supplementary Table S1 for all search strategies). All databases were searched on April 14, 2023, and updated on January 30, 2025.

2.3. Stage 3: Selection of Studies for the Review

Endnote® was employed as reference management software to facilitate data management. Duplicate studies were removed using the software's automated deduplication function. Subsequently, Rayyan® software was utilized, and two independent reviewers (ATS and JF) assessed the eligibility of each report through a two-step process. Initially, they reviewed the titles and

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abstracts to select all potentially eligible articles. Later, ATS and JF read the full texts to confirm eligibility. Any discrepancies between the reviewers were resolved through consensus, and if necessary, a third reviewer (IAM) was consulted.

2.4. Stage 4: Data Extraction

The same two reviewers (ATS and JF) independently extracted data from all eligible studies using a data extraction spreadsheet created in Microsoft Office Excel®. A pilot test of this spreadsheet was conducted using five randomly selected full texts before proceeding with data extraction, ensuring consistent data extraction by the reviewers and avoiding ambiguities and errors. Supplementary Table S2 summarizes the extracted data.

2.5. Stage 5: Summary of Data and Synthesis of Results

We used descriptive statistics to outline the characteristics of studies on ALA mechanisms of action, incorporating tables and graphs to present the collected data. This scoping review is presented in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses for Scoping Reviews (PRISMAScR) checklist (Supplementary Table S3) [10].

3. Results

3.1. Selection of Studies and General Characteristics of Selected Studies

Figure 1 presents the study selection flowchart. A total of 590 records were identified across all databases. After automated deduplication and screening of titles and abstracts, 67 records remained for full-text examination and data extraction. Of these, 41 studies were excluded for the following reasons: 26 did not address the research question, 13 were posters, and 2 were abstracts. Consequently, 26 studies were deemed eligible [4,6,7,11–33]. Supplementary Table S4 provides a list of excluded studies along with justifications. Detailed characteristics of each study are presented in Supplementary Table S5. Most studies were published in 2022 (n=6) [7,11,13–16].

 doi:10.20944/preprints202506.1985.v1

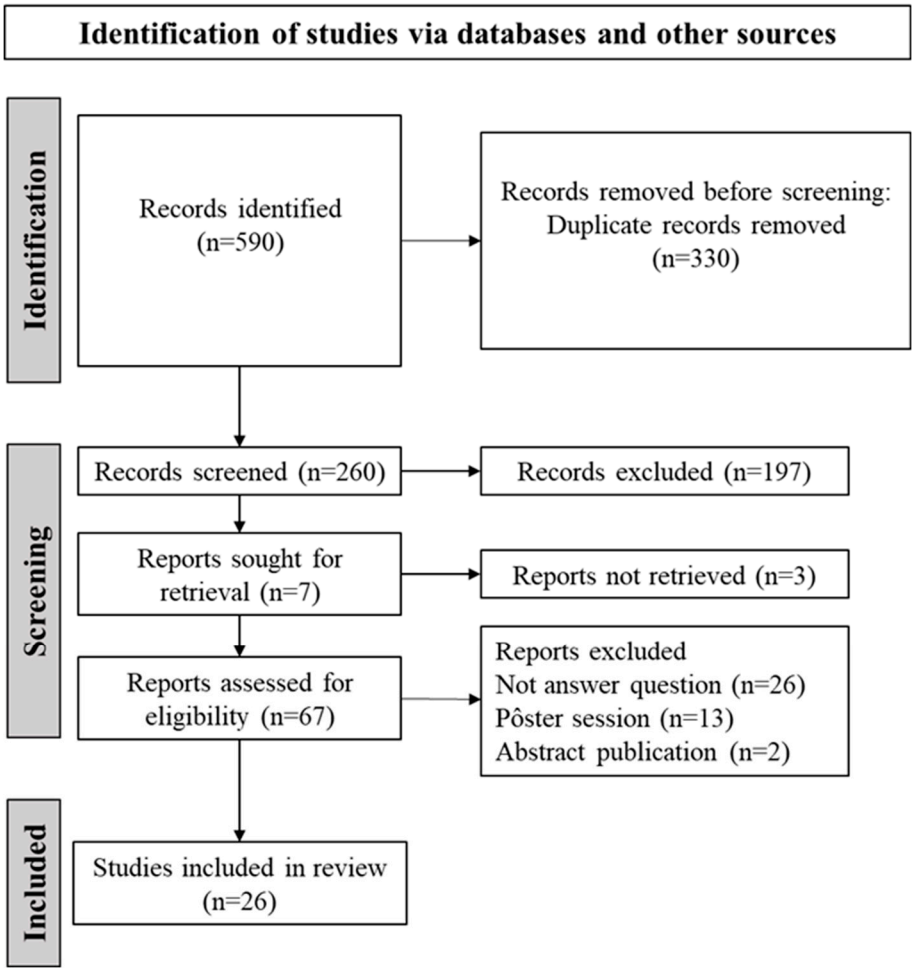

Figure 1. Visual Representation of Study Selection for the Current Scoping Review.

Figure 2 provides a summary of the study features, including publication year, country, impact factor, and body system. All studies were described in English and were experimental in nature (100%). The studies were published in journals with an impact factor above 4.5 (n=11), and the cardiovascular system was the most studied body system (n=12). Figure 3 illustrates the timeline of ALA's mechanisms of action, beginning with its discovery in 2013.

 doi:10.20944/preprints202506.1985.v1

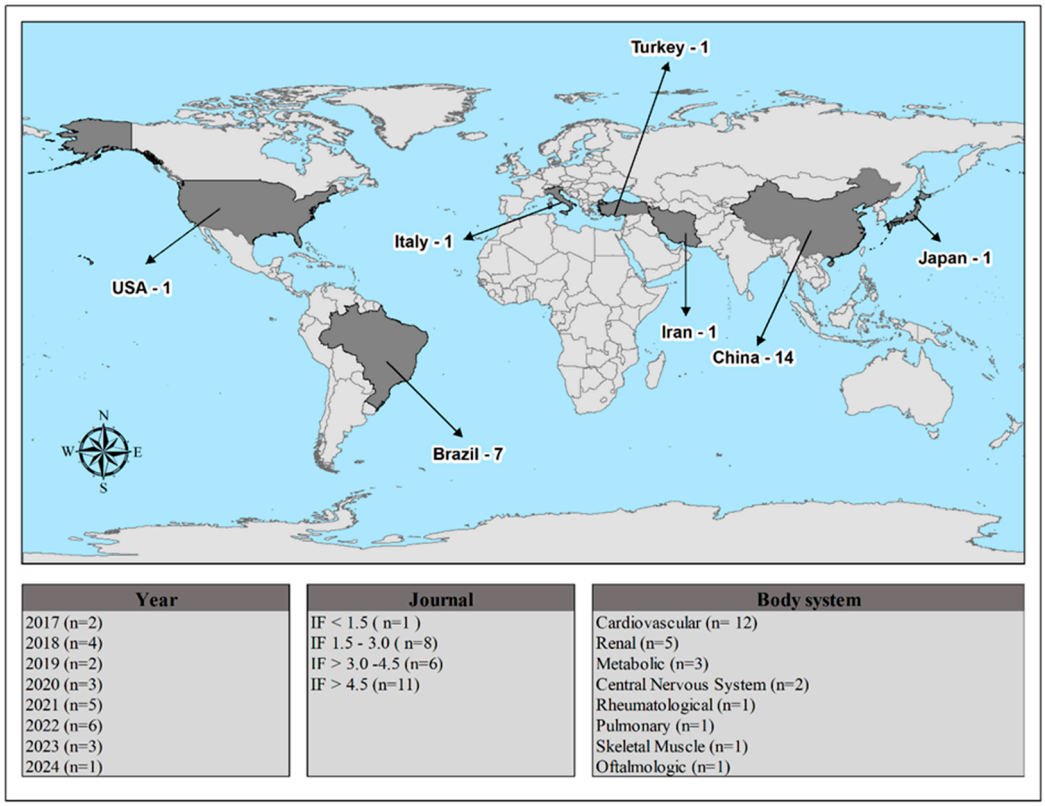

Figure 2. Distribution and Impact of Scientific Articles by Country. IF = impact factor.

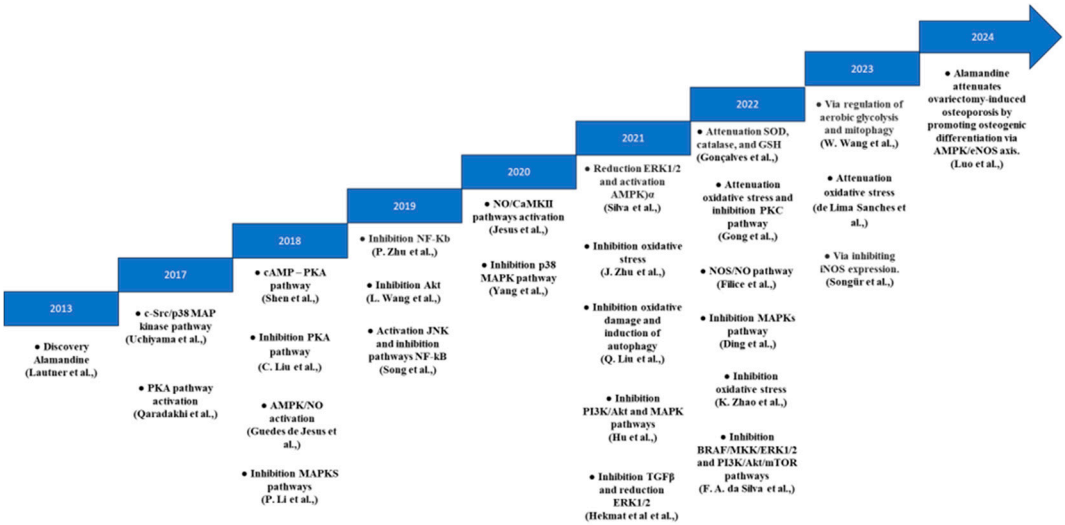

Figure 3. Chronological Discoveries of Alamandine's Mechanisms of Action.

4. Discussion

Identified in 2013, ALA, closely related to Ang-(1-7) and distinguished by a single amino acid [34], dramatically alters its biological activity. ALA can be derived from angiotensin A through the action of ACE2 [35] or by the decarboxylation of Ang-(1-7) [36] (Figure 4). This axis, comprising ACE2-Ang-(1-7)/ALA-Mas/MrgD, plays a critical role in counterbalancing the effects of Ang II, offering promising therapeutic avenues for various diseases.

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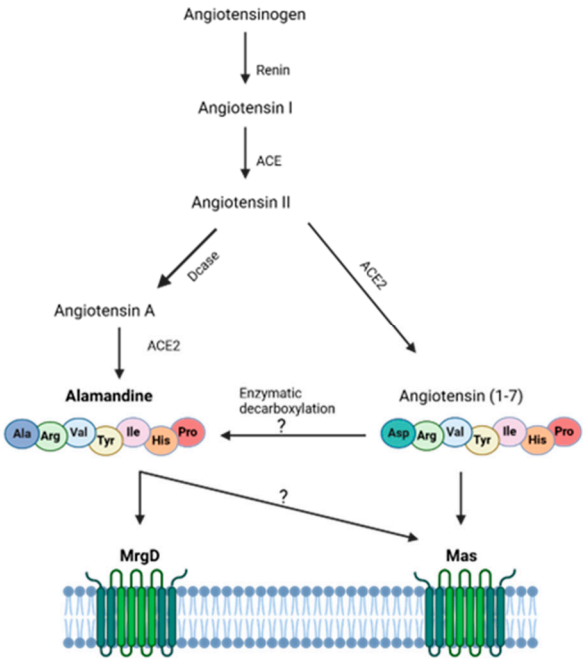

Figure 4. The classic renin-angiotensin system cascade. ACE: an-giotensin-converting enzyme; ACE2: angiotensin-converting enzyme 2; Dcase: decarboxylase.

ALA interacts with widely expressed MrgD and Mas receptors. Activation of the ALA-MrgD axis is associated with beneficial effects, including vasodilation, anti-inflammatory, and anti-fibrotic actions. These effects are mediated by signaling pathways (Figure 5), particularly Mitogen-Activated Protein Kinases (MAPKs) and Adenosine Monophosphate-activated protein kinase (AMPK), regulating NO production and cellular energy homeostasis. Understanding ALA's role in the RAS system opens new research opportunities for treating cardiovascular, renal, and oncological conditions.

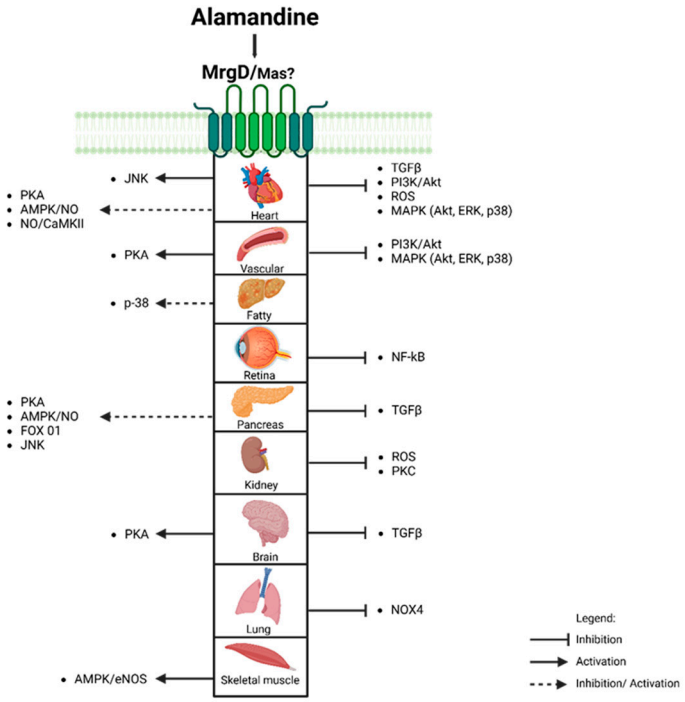

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Figure 5. Organs and Mechanisms Activated by Alamandine. JNK - c-Jun N-terminal; PKA - protein kinase A; AMPK - adenosine monophosphate-activated protein kinase; NO - nitric oxide; AMPK - adenosine monophosphate-activated protein kinase; JNK - c-Jun N-terminal; TGFβ - transforming growth factor-β; PI3K - Phosphoinositide 3-kinase; Akt - protein kinase B; ROS; MAPKs - Mitogen-Activated Protein Kinases; ERK - Extracellular Signal-Regulated Kinases; NF-kB- nuclear factor kappa B ; PKC - Protein Kinase C.

4.1. Mas and Mrgd Receptors: ALA's (un)Specificity

The MrgD and Mas receptors are expressed in the dorsal root ganglia of the nervous system [37] and various organs, including the heart [6,20,38], brain [39–41], lungs [21], adipose tissue [42], vascular endothelium, arterial smooth muscle cells [43], and retina [32]. ALA's affinity for the Mas receptor indicates a secondary receptor role [36,14].

The Mas and MrgD receptors may interact to form a functional complex. ALA binds to MrgD, promoting the dimerization of Mas and MrgD receptors, which leads to anti-inflammatory responses like reduced interleukin-6 and interleukin-1β secretion in lipopolysaccharide-activated THP-1 macrophages and M1 macrophages. Moreover, ALA promotes antiproliferative effects [44].

The complexity of this interaction is evident in cells using compounds like as PD123319, D-PRO7-ANG-(1-7), and A779, exposing the challenge of targeting specific receptors [45]. This suggests ALA's actions may involve MrgD and AT2 receptors, not just the Mas receptor [1,31,46]. Furthermore, ALA's potential to promote MasR and MrgDR dimerization opens possibilities for enhanced anti-inflammatory and antiproliferative effects.

4.2. Mapks and Ampk Signaling Pathways

ALA-MrgD interaction in cardiomyocyte cultures enhances NO production [6] and phosphorylates AMPK and LKB1, key regulators of energy and cardioprotection [47]. AMPK activation is crucial for preventing cardiac injury progression to heart failure [48,49] and is linked to metabolic diseases [50,51], with bone tissue research supporting these findings [22].

Emphasizing its cardiovascular benefits, ALA exhibits a broad anti-inflammatory effect by reversing the increased phosphorylation of Phosphoinositide 3-kinase (PI3K), Protein Kinase B (Akt), Extracellular Signal-Regulated Kinase (ERK), c-Jun N-terminal kinase (JNK), and p38, reversing their increased activity in sepsis-associated renal injury [18]. This inhibition involves the protein kinase A (PKA) signaling pathway [20], underscoring cyclic adenosine monophosphate-dependent kinase's role in these processes [52]. Inhibition of PKA by ALA induces cardiac remodeling [53] and promotes vasodilation, as confirmed using KT5720, a PKA inhibitor [23]. In fact, Shen et al.(2018) demonstrated that regulation of the PKA pathway is essential for ALA's effects in the brain.

Additionally, in ischemia-reperfusion (IRI), ALA treatment significantly lowers apoptosis in myocardial cells [26], possibly by attenuating the inflammatory response through inhibition of nuclear factor kappa B (NF-κB) activation [26,32]. This suppression reduces pro-inflammatory cytokine expression [18,54], reactive oxygen species (ROS) production [32], and impacts myocardial infarction models due to pressure overload [7]. It promotes protein kinase phosphorylation [18,54], affecting the balance between cellular survival/apoptosis.

The anti-inflammatory properties of ALA also extend to synovial fibroblasts, inhibiting the MAPK pathway, and leading to a reduction in inflammatory mediators associated with rheumatoid arthritis [13]. Furthermore, ALA decreases matrix metalloproteinase-2, essential for TNF-alpha maturation and apoptosis control in cardiomyocytes [55]. It also reduces TGF-β, preventing connective tissue fibrosis [25,56], and plays a crucial role in mitigating hypertrophic and fibrotic pathways by inhibiting phosphorylated forms in the MAPK pathways [4].

Conversely, Ang II is known to promote phosphorylation of p38 MAPK, contributing to cardiac [57], vascular fibrosis [31,58], cardiac stress, and hypertrophy [59,60]. The Ang-(1-7) and ALA axis improves aortic function (61), suggesting therapeutic benefits.

ALA also shows promise in controlling blood pressure in spontaneously hypertensive rats by inhibiting PKA in the heart [20]. It improves cardiac function in models of heart failure by attenuating

 doi:10.20944/preprints202506.1985.v1

TGF-β signaling and ERK 1 and 2 (ERK1/2) phosphorylation [25]. In cardiomyocytes, PKA inhibition may reduce MAPK activation, affecting pathways like JNK and p38 [4,52,62].

4.3. Nitric Oxide Production: The Therapeutic Role of ALA in Cardiovascular Health

ALA enhances endothelial nitric oxide synthase activity at serine 1177 and threonine 495, increasing NO release and highlighting its therapeutic potential for vasodilation and cardiovascular health [23,24,53]. Studies on goldfish (Carassius auratus) show that ALA enhances cardiac contractility under normoxic conditions via the NOS/NO system, revealing its sensitivity to hypoxia and broad cardiac benefits [14,63].

Furthermore, Songür et al.(2023) and Hu et al.(2021) showed that ALA's anti-inflammatory, anti-apoptotic, and antipyretic properties protect against acute renal injury and endotoxemia by suppressing inducible NOS (iNOS) expression, highlighting its potential in mitigating inflammatory responses. In adipose tissue, ALA activates the MrgD receptor, triggering c-Src and increasing iNOS expression [28], leading to NO production that may cause mitochondrial dysfunction and stimulate lipoprotein lipase activity, adversely affecting adipocytes [64]. ALA's mechanisms are complex, showing anti-inflammatory effects by suppressing iNOS in acute renal injury, while potentially causing mitochondrial dysfunction in adipose tissue by increasing iNOS and NO production, highlighting its context-dependent biological impacts.

In a transgenic rat model with overexpressed renin, ALA enhances cardiomyocyte contractility via the NO/calcium/calmodulin-dependent kinase II (CaMKII) pathway, initiating NO production and activating CaMKII in vascular smooth muscle cells [19]. CaMKII phosphorylates the threonine 17 residue of phospholamban within the sarcoplasmic reticulum, increasing the activity of the sarcoplasmic reticulum Ca2+-ATPase and enhancing contractility [19,65].

4.4. Modulation of pi3k Enzyme Activity by ALA

The phosphoinositide 3-kinase (PI3K) enzyme family is central to numerous biological processes, including cell survival, apoptosis, and cardiac function [66]. PI3K, along with its downstream effector Akt, regulates cell growth and apoptosis, playing a significant role in cardiac hypertrophy and diastolic dysfunction associated with hypertension. In primary cardiac fibroblasts, ALA's interaction with the MrgD receptor inhibits Akt activation induced by Ang II, thereby reducing cardiac fibrosis [29,67,68].

PI3K's role in cellular processes is broad. In addition to its role in cardiac hypertrophy and fibrosis, the inhibition of the PI3K/Akt pathway is also relevant in various cell types, including tumor cells. For example, in cancer cell lines such as Mia Paca-2 and A549, ALA treatment shifts energy generation from anaerobic to aerobic processes, potentially slowing cancer growth. It inhibits the PI3K/Akt/mTOR pathway, essential for cancer cell growth and survival, and induces the nuclear translocation of the FoxO1 protein, affecting various cellular processes [11].

This pathway inhibition leads to the dephosphorylation and reduced activity of components like 3-phosphoinositide-dependent kinase 1, Akt1, and the mTOR receptor, as well as the BRAF/MKK/ERK1/2 signaling pathway, resulting in cell cycle arrest and apoptosis in pancreatic cancer cells. ALA reduces the phosphorylation of ERK1/2 and its effectors, NIBAN2 and STMN1, highlighting its potential as a therapeutic target in cancer treatment [11,69].

Moreover, Wang et al. (2023) reported that ALA inhibits glycolysis in vitro, via MrgD axis and downregulates hexokinase 2 (HK2), reducing the impact of TGF-β1 on lung fibroblast (LF) [30]. ALA decreases the NADPH Oxidase 4 levels and ROS generation in fibroblasts, which are known to contribute to the development of pulmonary fibrosis [21]. Under anaerobic conditions, the glycolysis enhancement results in lactate formation from pyruvate [70], creating a feedback loop that enhances LF activation and upregulates glycolysis pathway such as HK2 activity and the allosteric effector of phosphofructokinase-1 by 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3 (PFKFB3) activity. In fact, HK2 and PFKFB3 are related to fibrosis development in several tissues: pulmonary

 doi:10.20944/preprints202506.1985.v1

[71,72], hepatic stellate cells [73,74], cardiac [75], kidney tubular cells [76] and renal fibroblast NRK-49F cells [77].

Conversely, ALA/MrgD counter-regulates this loop, reducing hexokinase activity [78], and decreasing glycolysis pathway [30]. Moreover, previous study suggests that ALA-MrgD signaling downregulates the expression of HK2 and PFKFB3 genes, reinforcing the importance of changes in energetic metabolism during fibrosis and highlighting ALA's potential role in controlling this energetic shift [30,74,76,77,79].

Mitophagy and glycolytic flux indicate a complex interaction with ALA [80]. These insights highlight ALA's therapeutic potential by inhibiting LF activation via TGF-β1 and autophagy proteins (Parkin/LC3), while counteracting metabolic reprogramming in bleomycin-induced fibrosis by suppressing HK2, PFKFB3, Parkin, and LC3 activation [30].

These findings show that in adipose tissue, ALA activates the NFκB and p38 MAP kinase pathways, which upregulate plasminogen activator inhibitor-1, a protein linked to adverse health outcomes [81]. Uchiyama et al. (2017) demonstrated that a low dose of ALA (5.76 μg/kg) modulates leptin expression and secretion via the phospholipase C, cSrc, and p38 MAPK pathways. These findings reveal ALA's intricate metabolic effects, indicating the need for further investigation to fully understand its mechanisms and potential applications.

4.5. Alamandine and Oxidative Stress

Evidence suggests that ALA plays a crucial role in reducing cardiac fibrosis resulting from oxygen and glucose deprivation. This effect is supported by reductions in oxidative stress and ischemic injury [7], as well as decreasing in apoptosis [7,82], which collectively lowers the risk of heart failure [83]. ALA administration is associated with enhanced activity of superoxide dismutase (SOD), catalase, and glutathione peroxidase (GSH), as demonstrated in the hippocampus of C57/Bl6 mice [15]. Furthermore, De Lima Sanches et al. (2023) confirmed ALA's antioxidant properties, showing that oral administration effectively reduced superoxide anion (O2•-) and normalized nuclear factor erythroid 2-related factor 2 levels in the right carotid artery following transverse aortic constriction.

Oxidative stress has been related to caspase-3 signaling and cardiac apoptosis activation, particularly in the doxorubicin (DOX) model [84]. Hekmat et al. (2021) demonstrated increased caspase-3 activation and apoptosis in DOX-exposed groups versus controls, while ALA significantly reduces both. Zhao et al. (2022) also demonstrated that ALA inhibits increases in collagen I, α-SMA, TGF-β, Bax/Bcl2, and the caspase-3/cleaved caspase-3 ratio, processes linked to cell death from oxygen-glucose deprivation in neonatal rat cardiac fibroblasts, highlighting ALA's cardiac protective effects [7].

According to J. Zhu et al. (2021), in rats subjected to IRI, ALA treatment significantly increased SOD activity awhile reducing levels of malondialdehyde (MDA), NADPH oxidase activity, O2•- production, Nox expression, inflammation, and apoptosis. Additionally, Gong and colleagues found that in cases of renal sodium overload, ALA mitigated the decline in SOD and GSH activity, as well as the increase in MDA, 8-hydroxy-2-deoxyguanosine, and O2•- levels. This effect, likely mediated by inhibiting the protein kinase C signaling pathway [16], underscores ALA’s protective role against oxidative stress and inflammation in renal function.

ALA shows promise, but limited research prevents definitive conclusions about its pathophysiological benefits. As the mechanisms underlying ALA's effects are not fully elucidated, it is crucial to interpret these findings with caution, acknowledging that they reflect our present understanding. Continued research is necessary to fully uncover ALA's therapeutic potential and its mechanisms of action.

5. Conclusions

Despite our limited knowledge, the discovery of Ang-(1-7) and ALA marks a significant advancement in our understanding of the RAS, introducing peptides with antagonistic actions.

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Numerous studies have sought to elucidate the benefits of this contemporary axis in various pathophysiological conditions, considering it counter-regulatory to the actions of Ang II, the potentially most active and studied peptide in the classical axis.

Thus, the recognition of these new peptides sparks a new debate and reinterpretation of the RAS. In the current knowledge, ALA acts by reducing oxidative stress and the inflammatory process, is involved in hypertrophic and fibrotic pathways, may interfere with glycolysis, and even affects the spread and growth of cancer cells. Studies reveal that ALA exhibits anti-hypertensive, anti-inflammatory, anti-proliferative, anti-fibrotic, and metabolic effects through several mechanisms. Furthermore, various studies have shown that its administration, across a wide range of dosages, does not have detrimental effects. Collectively, these studies demonstrate that ALA presents impactful therapeutic potential, with the possibility of significantly contributing to improving people's health in the future.

However, it is important to note that ALA's discovery is recent, and many mechanisms require further elucidation as research continues. Thus far, part of what we know about its role in pathophysiology consists of the findings presented in this article. Moreover, the findings underscore the importance of considering dosage, tissue specificity, and the experimental conditions (in vivo vs. in vitro) when evaluating ALA's biological impacts. Further studies are necessary to elucidate ALA's diverse roles in health and disease, particularly in different pathophysiological contexts.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org, Figure S1: title; Table S1: title; Video S1: title.

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Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

ACE	Angiotensin-converting Enzyme
ACE2	Angiotensin-converting enzyme 2
Akt	Protein Kinase B
ALA	Alamandine
ANG-(1-7)	Angiotensin-(1-7)
Ang II	Angiotensin II
AMPK	Adenosine Monophosphate-activated protein kinase
CAMKII	Calmodulin-dependent kinase II
Dox	Doxorubicin
eNOS	Endothelial Nitric Oxide Synthase
ERK1/2	Extracellular Signal-Regulated Kinases 1 and 2
HK2	Hexokinase 2
iNOS	Inducible Nitric Oxide Synthase
IRI	Ischemia-Reperfusion
JNK	c-Jun N-terminal kinase

 doi:10.20944/preprints202506.1985.v1

LF	Lung Fibroblasts
MAPKs	Mitogen-Activated Protein Kinases
MDA	Malondialdehyde
MrgD	Mas-related G protein-coupled receptor member D
NF-kB	Nuclear Factor Kappa B
NO	Nitric Oxide
NOS	Nitric Oxide Synthase
O2•-	Superoxide anion
PCC	Population Concept and Context
PI3K	Phosphoinositide 3-kinase
PKA	Protein kinase A
PKFB3	Phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3
RAS	Renin-angiotensin System
ROS	Reactive oxygen species
SHR	Spontaneously hypertensive rats
SOD	Superoxide Dismutase
STMN1	Stathmin 1
TGFβ	Transforming Growth Factor-β

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