



Original Article

Expression, purification and biological characterisation of recombinant human irisin (12.5 kDa)



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ABSTRACT

Fibronectin type III domain containing 5 (FNDC5) is a transmembrane protein. Upon cleavage, it yields a peptide called irisin that is supposedly bind to an unknown receptor and facilitates browning of white adipose tissue (WAT). Increased levels of irisin are associated with increased levels of energy expenditure markers PGC-1 α , UCP-1, besides abundance of beige adipocytes in WAT. Though varied sizes of irisin were reported in humans and rodents it is not yet clear about the actual size of the irisin produced physiologically. Hence, we cloned and expressed human irisin (32–143 aa of FNDC5) in *Escherichia coli* based on the proposed cleavage site that yields 12.5 kDa peptide to study its antigenicity and other biological functions *in vitro*. We purified recombinant human irisin (rh-irisin) to 95% homogeneity with simple purification method with a yield of 25 mg/g wet cell pellet. rh-irisin has been detected by commercially available antibodies from different sources with similar antigenicity. Biological activity of the rh-irisin was confirmed by using 3T3-L1 pre-adipocyte differentiation by Oil red O staining. Further, rh-irisin treatment on pre-adipocytes showed increased expression of markers associated with energy expenditure. As it is involved in energy expenditure process, it could be considered as potential therapeutic option for various metabolic diseases.

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1. Introduction

Irisin, a recently identified adipo-myokine [1], is cleaved proteolytically from Fibronectin type III domain containing 5 (FNDC5) into extracellular milieu with unknown mechanism. Increased levels of FNDC5 mRNA were reported after exercise [1]. Exercise induced beneficial effects were well documented such as decreased insulin resistance, improved functions of heart, brain, adipose tissue etc. The mouse FNDC5 consists of a signal peptide (29 amino acids (aa)), a single fibronectin type III domain (94 aa), a region of 28 aa of an unknown structure and function, a transmembrane domain (19 aa) and a cytoplasmic domain (39 aa) [2].

A putative cleavage site is proposed in the region of 'unknown structure and function' in FNDC5 [3]. After cleavage, the irisin travels through the blood and reaches the adipose tissue where it binds to an unknown receptor and facilitates the browning of white adipose tissue (WAT) [1].

While WAT is known for storage of energy, brown adipose tissue (BAT) is involved in thermogenesis. Moreover, BAT is characterized by more number of mitochondria and multilocular lipid droplets [4]. Recently, a third type of adipocytes has been identified in rodents and humans upon the thermogenic stimuli of WAT and are named as beige or brite (brown in white) adipocytes. However, the precursor cells of beige adipose cells are not similar to classical BAT but closer to WAT [5]. Above all, the thermogenic capacity is also much lower than the classical BAT [6]. However, the beige adipocytes showed increased number of mitochondria and UCP-1 mRNA levels which indicates increased energy expenditure.

Bostrom et al., suggested that the main function of irisin could be conversion of WAT into brown like adipose tissue (beige adipose

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COVID-19 Outbreak: an Update on Therapeutic Options

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Dear Editor,

Initial identification of novel coronavirus disease (COVID-19) in Wuhan was alerted by the ophthalmologist by name Li Wenliang and after the enquiry of reporting unusual cases of pneumonia by Wuhan Municipal Health Commission geared up the panic in public. Though it was believed to be linked with Wuhan Huanan seafood wholesale market initially, now it is spread to more than 166 countries in the world, and it was declared as pandemic by WHO. Whole landscape of the disease has changed from epidemic to pandemic in just 45 days. Worldwide, 191,127 confirmed cases and 7807 deaths were reported as on March 18, 2020, indicating serious global public health concern (https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200318-sitrep-58-covid-19.pdf?sfvrsn=20876712_2). Situational awareness in all levels for risk management is badly required to contain the spread of this deadly disease.

Based on SARS and MERS coronaviruses, it was believed 2–14 days as incubation period for COVID-19. The Chinese researchers found that the incubation period could be 24 days and asymptomatic carriers were evidenced to transmit the COVID-19 also. The incubation period may be as long as 0–24 days in some cases (<https://www.pharmaceutical-technology.com/news/coronavirus-study-incubation-period/>). The viral genomes, isolated from both bronchoalveolar lavage fluid and cell cultures, were sequenced using next-generation sequencing and found

COVID-19 virus was similar to betacoronavirus ‘bat-SL-CoVZC45’. However, the remarkable exception was the spike protein, which is only 80% similar to bat coronaviruses. The envelope spike protein plays major role in binding to receptor, membrane fusion, and transmission capacity. Interestingly, it was more similar to SARS-CoV with respect to receptor-binding domain [1]. Despite there are some variations in COVID-19 receptor-binding domain, it was suggested that COVID-19 might use angiotensin-converting enzyme 2 (ACE2) as cell membrane receptor similar to SARS-CoV [1]. ACE2 is expressed by epithelial cells of the lung, intestine, kidney, and blood vessels.

At present, no specific antiviral treatment and no efficient vaccines are available for COVID-19 in humans. Recently, two familiar broad-spectrum antiviral drugs remdesivir and favipiravir have been tested against clinical isolate of COVID-2019 in vitro. They found that EC₅₀ of a remdesivir, an adenosine analogue, as 1.76 μM which can be achieved in non-human primate studies. Moreover, their preliminary data showed that it inhibits COVID-19 virus infection also in human liver cancer (Huh-7) cells.

Some clinical trials with remdesivir, ritonavir-boosted lopinavir monotherapy, have been launched (ChiCTR2000029308, NCT04257656). Xia et al. developed a pan-CoV fusion inhibitor, EK1 peptide, to inhibit the infection of five human coronaviruses including SARS-CoV. The same group showed that the EK1 peptide and the peptide derived from HR2 domain in spike protein of COVID-19 virus could effectively inhibit COVID-19 pseudo virus infection and domain S2 mediated cell fusion [2]. It has been recently shown by using probative assays that SARS-CoV receptor-binding domain-specific monoclonal antibody, CR3022, could potentially bind with COVID-19 viral receptor-binding domain.

There are some medical conditions, which are being treated by ACE inhibitors and angiotensin II type-I receptor blockers, such as type-1 and type-2 diabetes and hypertension that result in the increased expression of ACE2 [3]. It was hypothesised that treatment with ACE2-stimulating

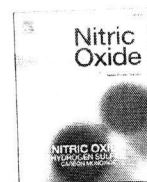
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Review

Electrophilic nitrated fatty acids are potential therapeutic candidates for inflammatory and fibrotic lung diseases

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ABSTRACT

Several types of exposures can cause acute or chronic inflammatory reactions in the lungs often leading to asthma, pulmonary fibrosis, chronic obstructive pulmonary disease (COPD), acute lung injury, lung cancer, and other deleterious health outcomes. Current therapy, with inhaled or oral glucocorticoids, successfully targets inflammation but also produces adverse effects that limit their enthusiastic use. Accordingly, the need remains for interventions that are safer and more effective. Nitrated fatty acids (NFAs) are highly electrophilic and are produced endogenously by non-enzymatic reactions of nitric oxide with conjugated unsaturated fatty acids. The literature indicates that NFAs are detected in humans at the nanomolar range and are produced more robustly under inflammatory conditions. Recent studies on novel NFAs report antiinflammatory, antioxidant, and anti-fibrotic effects, while also acting as partial agonists of peroxisome proliferator-activated receptor-gamma (PPAR- γ). Furthermore, these functions of NFAs occur via reversible electrophilic alkylation of cysteine residues and regulation of antiinflammatory, antioxidant signaling through modulation of transcription factors, including nuclear factor E2-related factor 2 (Nrf2), PPAR- γ , and NF- κ B. Here, we review and update the role of NFA signaling mechanisms and their therapeutic potential in various lung diseases. As NFAs display strong electrophilic interaction with multimechanistic pathways, they can be considered promising drug candidates for challenging lung diseases.

1. Introduction

The lung continuously functions, working from birth to death. During one's lifetime, the airway epithelium is constantly exposed to external stimuli and pathogens. These exposures can lead to many disease conditions, particularly allergic and inflammatory diseases. As a natural first-line defence, the epithelium secretes compounds such as mucins, lysozyme, and synthesizes nitric oxide [1], to act as a non-specific shield against microbial attack. Lungs also secrete reactive oxygen species, chemokines, and PDGF to trigger inflammatory response. These compounds stimulate goblet cells and result in the production of mucus which then clears inhaled agents adhered to the epithelium [2]. Despite these different layers of protection in the lung, immune cell dysfunction is the major culprit of many lung diseases [3, 4]. Recent studies show that individuals are predisposed to many of these diseases during their embryonic stage itself [5]. Additionally, mortality due to lung diseases like asthma and chronic obstructive pulmonary disease (COPD), has doubled since 1980 [6]. Corticosteroids

remain the first-line drugs used to reduce the symptoms of lung diseases [7]. However, drugs used for the underlying causes of these diseases are often ineffective, show mixed results in trials, and sometimes cause severe side-effects [8–10]. Hence, there is an urgent need for efficient therapies with minimal side effects.

Nitrated fatty acids (NFAs) such as nitro-oleic acid (OA-NO₂) and nitro-linoleic acid are endogenously generated to resolve the inflammation. Nitric oxide ($\bullet$ NO), a free radical, was originally studied in the environment, but later found to mediate redox-signaling reactions endogenously [11–13]. In contrast, superoxide anion radical ($\bullet$ O₂⁻) is a toxic compound that inactivates iron-sulfur-cluster-containing enzymes and leads to the production of a highly reactive hydroxyl radical. Although it displays less reactivity with many biological molecules, it does react with $\bullet$ NO to rapidly form peroxynitrite anion [14]. Oxidative inflammatory conditions lead to nitration of free and esterified unsaturated fatty acids by $\bullet$ NO and nitrite (NO₂⁻)-dependent reactions [15] (Fig. 1). This yields a wide array of regioisomers with unique chemical reactivities and signaling properties [16]. Studying the biological

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RESEARCH ARTICLE

Biochanin A Ameliorates Ovalbumin-induced Airway Inflammation through Peroxisome Proliferator-Activated Receptor-Gamma in a Mouse Model**BENTHAM
SCIENCE**Madhavi Derangula¹, Kalpana Panati² and Venkata R. Narala^{1,*}¹Department of Zoology, Yogi Vemana University, Kadapa, 516 005, Andhra Pradesh, India; ²Department of Biotechnology, Government College for Men, Kadapa, Andhra Pradesh, India

Abstract: Objective: Asthma is an inflammatory airway disease affecting most of the population in the world. The current medication for asthma relieves airway inflammation but it has serious adverse effects. Biochanin A (BCA), a phytoestrogen, is an active component present in red clover, alfalfa, soy having anti-oxidant and anti-inflammatory properties. BCA was identified as a natural activator of peroxisome proliferator-activated receptor-gamma (PPAR γ).

Methods: The study aims to evaluate the effects of BCA in ovalbumin (OVA)-induced murine model of asthma and to study the role of PPAR γ .

Results: We found that BCA administration reduced the severity of murine allergic asthma as evidenced histologically, and measurement of allergen-specific IgE levels in serum as well as in BAL fluid. BCA also reversed the elevated levels of inflammatory cytokines, cell infiltration, protein leakage into the airways and expression of hemoxygenase-1 in OVA-induced lungs. Further, we confirmed that BCA mediated inhibitory effects are mediated through PPAR γ as assessed by treatment with PPAR γ antagonist GW9662.

Conclusion: Our results suggest that BCA is efficacious in a preclinical model of asthma and may have the potential for the treatment of asthma in humans.

Keywords: Allergic airway disease, biochanin A, IgE, inflammation, isoflavones, PPAR γ .

1. INTRODUCTION

Asthma is a chronic airway inflammatory disease, wherein many cell types are involved, particularly, eosinophils, macrophages, neutrophils, T-lymphocytes, mast cells and epithelial cells in the pathogenesis [1]. It is characterized by airway hyperresponsiveness to a variety of allergens. Symptoms include wheezing, coughing, chest tightness, and excess production of mucus by goblet cells [2]. The severity of asthma is correlated with the number of eosinophils [3]. Currently, β_2 -adrenoceptor agonists [4], methylxanthines [5], leukotriene modifiers [6], corticosteroids [7] and non-steroidal anti-inflammatory drugs [8] are being used as a treatment for asthma. However, long term use of these may cause adverse effects like stunted growth, osteoporosis, anxiety and tachycardia [9, 10]. Therefore, a safe and secure alternative drug is needed to manage asthma. In general, plant extracts are natural products, which usually have less adverse effects compared to synthetic drugs and are being investigated as a therapy for various conditions [11].

Biochanin A (BCA) (5,7 dihydroxy-4-methoxy isoflavone) (Fig. 1A), is an isoflavone extracted from various sources like red clover [12], chickpea [13, 14], fruits of *Cassia fistula* etc. [15]. Its structure is shown in Fig. (1A) and it has been classified as a phytoestrogen [16]. BCA possesses many beneficial roles including anti-inflammatory, anti-allergic, anti-diabetic, and anti-cancer effects [17-20]. A recent review highlighted the potential use of BCA for human health and its limitation [21]. Previous studies reported the anti-inflammatory potential of BCA for the treatment of asthma and chronic obstructive pulmonary disease (COPD) by selective inhibition of phosphodiesterase 4 (PDE4) [19]. BCA has been recently identified as a transactivator of PPAR γ [22]. PPAR γ belongs to the nuclear hormone receptor family and plays a major role in inflammation. Activation of PPAR γ inhibits IL-2 secretion from T-cells [23], LPS-induced TNF- α in human alveolar macrophages [24], nitric oxide (NO) and matrix metalloproteinase (MMP)-13 from human chondrocytes [25], IL-1 β , IL-6, and MMP-9 from human bronchial epithelial cells [26], and inducible nitric oxide synthase (iNOS) from cardiac myocytes [27]. Earlier, it has been reported that treatment with PPAR γ agonists decreases asthma in preclinical models [28-30]. Hemoxygenase (HO)-1 is a rate-

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RESEARCH ARTICLE

An Overview on COVID-19 Pandemic: from Discovery to Treatment

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Abstract: Recently, novel coronavirus infection (COVID-19) emerged in Wuhan, China has been declared as pandemic by WHO. Until now, no evidence is documented regarding its wild animal reservoir or intermediary host, but, human-to-human transmission, asymptomatic carriers were very much observed. The number of confirmed cases and death toll have been increased almost all over the world indicating its potential threat to public health. Though the phylogenetic analysis shows some similarity of SARS-CoV2 to bat betacoronaviruses, it exhibited significant variation in S1 domain of spike protein from bat-derived viruses. S1 domain plays an important role in receptor binding and it can be a target for the development of therapeutics and vaccines. In this review, we have discussed the updates on transmission, diagnosis, genome analysis and comparison, treatment options and clinical trials of COVID-19.

Keywords: Antivirals, coronavirus pandemic, COVID-19, SARS-CoV, angiotensin converting enzyme.

1. INTRODUCTION

World Health Organization (WHO) has developed a special tool to determine which diseases and pathogens to be prioritized for research and development in the perspective of public health emergency. The Blueprint list of priority diseases, released by WHO before the end of 2019, include Middle East Respiratory Syndrome coronavirus (MERS-CoV), Severe Acute Respiratory Syndrome (SARS) and Disease X etc. 'Disease X' represents a serious international epidemic, unknown to cause human disease, however, the WHO seeks to enable preparedness for the unknown disease (<https://www.who.int/activities/prioritizing-diseases-for-research-and-development-in-emergency-contexts>).

2. OUTBREAK OF COVID-19

Hints of trouble were floated up when the Wuhan Municipal Health Commission asked the hospitals to report unusual cases of pneumonia on 30th December 2019 and turned up to 27 cases in the city linked to the wholesale Huanan Seafood Market [1]. Initially, it was believed that this epidemic was linked to the Wuhan Huanan Seafood Wholesale Market and the presumed mechanism of transmission was a wild animal-to-human transmission. However, it was found later that it also transmitted human-to-human. Probably this Wuhan pneumonia might be the Disease X of WHO. Then the causative agent of this Wuhan pneumonia was identified and named as 2019-nCoV by WHO [2] and later on it was re-named as Coronavirus disease 2019 (COVID-19) and the causative virus as SARS-CoV-2. This epidemic was declared as "pandemic" by WHO as it infected many countries

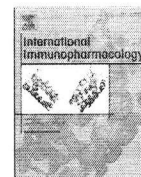
world over within 45 days. Globally, as per the WHO report, 32730945 confirmed cases and 991224 deaths were recorded as on September 27, 2020. Though animal source of the current pandemic is unknown, the human infection of COVID-19 through the seafood market in Wuhan has been reported [3]. The phylogenetic analysis revealed bat as the reservoir for COVID-19, but the following facts are taken into consideration i) most of the bat-species were hibernating during December 2019 in Wuhan, ii) no bats were sold at the Huanan seafood market, iii) less sequence similarity (<90%) between COVID-19 and bat-derived viruses, iv) though bats acted as natural reservoirs for both SARS-CoV and MERS-CoV, some other animals acted as intermediate hosts, etc., and speculated that another unknown wild animal may act as an intermediary host between bats and humans [4]. The detailed demographic, clinical characteristics, prevention and control strategies to COVID-19 pandemic were discussed elaborately elsewhere [5].

3. TRANSMISSION, SYMPTOMS AND DIAGNOSTIC TESTS OF COVID-19

The reported infected cases have been increased continuously indicating its rapid spreading and transmissibility of COVID-19 is higher than initially expected. This is, partly, because Wuhan is a crucial hub linking many cities with high-speed transport system [6]. Human to human transmission is also evidenced [7]. Though the early symptoms include fever, dry cough, malaise that are non-specific and common laboratory findings include lymphopenia and bilateral ground-glass opacity or consolidation in chest CT scans, diarrhoea was also reported in a few cases [8].

Based on SARS and MERS coronaviruses, 2-14 days was believed to be incubation period for COVID-19 [9], but the Chinese researchers found the incubation period could

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Nitrated fatty acid, 10-nitrooleate protects against hyperoxia-induced acute lung injury in mice

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ABSTRACT

The antioxidant and anti-inflammatory effects of electrophilic nitrated fatty acid (NFA); 10-nitrooleate, have been reported. The present study investigated whether 10-nitrooleate has a protective role against hyperoxia-induced acute lung injury (HALI). Using a C57BL/6 mice model of HALI, we investigated the protective effect of 10-nitrooleate. C57BL/6 mice were administered with NFA intratracheally, exposed to hyperoxia for 48 h to induce HALI, and kept at room air for 24 h. Bronchoalveolar lavage (BAL) fluid and lung samples were collected after 24 h of post hyperoxia to analyze markers associated with HALI. Intratracheal (IT) and intraperitoneal (IP) administration of NFA notably attenuated hyperoxia-induced infiltration of inflammatory cells, alveolar-capillary leakage, upregulation of proinflammatory cytokine levels (IL-6 and TNF α) into the BAL fluid, and resolution of inflammation in the lung. Western blot analyses showed that 10-nitrooleate reduced the expression of the inflammatory transcription factor NF- κ B p65 subunit and increased antioxidant proteins HO-1 and NQO1 expression in the lung tissues compared to vehicle-treated animals. Moreover, 10-nitrooleate reversed the hyperoxia-induced expression of mitophagy-associated markers (PINK1 and p62/SQSTM1), thereby protecting the HALI/ acute respiratory distress syndrome (ARDS). IT and IP delivery of 10-nitrooleate reduces hyperoxia-induced ALI/ARDS by regulating the antioxidant pathways and restoring the mitochondrial homeostasis by regulating mitophagy. It is suggested that NFAs can be further evaluated as supplementary therapy for critically ill patients like COVID-19/ARDS.

1. Introduction

Acute lung injury (ALI) and its severe manifestation, acute respiratory distress syndrome (ARDS), are the most devastating conditions of fatality in critically ill patients [1]. The most prevalent reason for the hospitalization of ALI/ARDS and COVID-19 patients is hypoxemic respiratory failure. High concentrations of inspired oxygen ($FI_{O_2} > 0.8$) are frequently required to sustain adequate oxygenation in patients with significantly reduced lung function, such as those with COVID-19-

induced ALI/ARDS [2–4]. The injured lungs' exposure to hyperoxia may also be considerably extended, as observational studies of COVID-19/ARDS found mean mechanical ventilation durations exceeding two weeks [5]. However, prolonged oxygen (>65% O_2) exposure to the lungs can lead to increased exacerbation of lung symptoms and cause hyperoxic acute lung injury (HALI) and respiratory failure [6–8].

HALI initiates an immediate inflammatory response that generates proinflammatory cytokines, immune cell infiltration, edema, and alveolar epithelial cell damage [9–11]. Thus, the hyperoxia model, a model of

Abbreviations: ALI, acute lung injury; ANOVA, analysis of variance; ARDS, acute respiratory distress syndrome; BAL, Bronchoalveolar lavage; COPD, chronic obstructive pulmonary disease; COVID-19, Coronavirus disease – 2019; DMSO, dimethyl sulfoxide; EBD, Evans blue dye; ELISA, enzyme-linked immunosorbent assay; H&E, hematoxylin and eosin; HALI, hyperoxia-induced acute lung injury; HETE, hydroxyecosatetraenoic acids; HO-1, heme oxygenase 1; IL-6, Interleukin 6; IP, intraperitoneal; IT, intratracheal; LC3, microtubule-associated protein 1 light chain 3; LPS, lipopolysaccharide; LTB4, leukotriene B4; MPO, myeloperoxidase; NFA, nitrated fatty acid; NF- κ B, Nuclear factor-kappa-B; NQO1, NAD(P)H Quinone Dehydrogenase 1; Nrf2, nuclear factor erythroid 2-related factor 2; p62/SQSTM1, p62/sequestosome 1; PBS, phosphate-buffered saline; PINK1, PTEN-induced kinase 1; PPAR γ , peroxisome proliferator-activated receptor gamma; ROS, reactive oxygen species; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2; SDS-PAGE, sodium dodecyl-sulfate polyacrylamide gel electrophoresis; TNF α , Tumour necrosis factor-alpha.

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Anticancer Activities of Biogenic Silver Nanoparticles Targeting Apoptosis and Inflammatory Pathways in Colon Cancer Cells

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Abstract

Colorectal cancer (CRC) progression is a complex process, with an interplay of multiple molecular signaling pathways. Cyclooxygenase-2 (COX-2) and NF- κ B are important hallmark proteins responsible for the transition from inflammation to colon cancer. Due to the adverse effects of chemotherapeutic drugs, there is an imperative need to develop new drugs, and recently, nanoparticles found to be promising strategy in tumor detection, prevention and treatment of cancer. Biosynthesis of silver nanoparticles (AgNPs) was achieved with the help of *Eucalyptus globulus* L. leaf extract. Using a typical XRD pattern, NanoSight and TEM technique, the size and shape of the biogenic AgNPs were determined as ~ 20 nm and spherical. The cytotoxicity study exhibited a dose-dependent effect against HCT116 cells, with an inhibitory concentration (IC₅₀) of 1.152 μ g/ml. In addition, AgNPs effectively inhibited the proliferation, colony formation, with increased ROS production compared to untreated cells. Further, mechanistic analysis revealed that AgNPs arrested the cell cycle, downregulated the expression of antiapoptotic, inflammatory, stem cell markers, and upregulated the apoptotic genes in HCT116 cells. In conclusion, for the first time, we report the green synthesis of AgNPs using *E. globulus* leaf extract that has potential anticancer activity with dual inhibitory action on COX-2 and NF- κ B expression.

Keywords Anticancer activity · Colorectal cancer · Nanomedicine · Silver nanoparticles · Toxicity

Introduction

Incidence of colorectal cancer (CRC) continue to increase globally, due to unhealthy food habits and lifestyles and accounts for the third most cancer deaths in men and women [1]. In the CRC progression, multiple molecular signaling pathways are involved. Cyclooxygenase-2 (COX-2) is a critical hallmark protein in carcinogenesis and inflammatory diseases. Besides, prostaglandin E2 (PGE2)

shows an immunosuppressive effect in the tumor microenvironment, promoting tumor progression [2].

Numerous studies have implicated that PGE2, the metabolite of the COX-2 enzyme, is a potent mitogen and contributes to colon cancer development [3]. Overexpression of COX-2 initiates and promotes human colorectal adenocarcinomas and it acts as a powerful biomarker for early clinical detection [4, 5]. Interestingly, when COX-2 is targeted through either gene knockouts or COX-2 specific inhibitors, a significant reduction in the number of tumors was observed, suggesting that COX-2 plays a key role in colon tumorigenesis [6]. One more important pathway in colorectal cancer is NF- κ B signaling [7]. Recent studies showed that NF- κ B acts as a central molecule responsible for the transition from inflammation to cancer [8]. NF- κ B activation promotes the release of proinflammatory cytokines, and thus it has been suggested to promote tumorigenesis [9]. Yu et al. reported that the increased expression of NF- κ B in colorectal cancer plays a vital role in the pathogenesis of colon cancer in humans by mediating the transition from colorectal adenoma with low-grade dysplasia to adenocarcinomas [10]. Studies in humans

Vydyanath R. Narasimha and T. Sree Latha have contributed equally to this study.

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Angiotensin-converting enzyme gene polymorphism may be a risk factor for COVID-19 clinical outcome

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Abstract

Angiotensin-converting enzyme 1 (ACE1) and ACE2 play a major role in regulation of blood pressure and electrolytic balance. They are known to express in epithelial cells of various tissues. SARS-CoV2 uses ACE2 as one of the receptors to enter into the host cells. Coronavirus infection-associated decrease in the expression of ACE2 is known to associate with vasoconstriction, hypertension and other cardiovascular problems. Patients who are on ACE1 inhibitors show increased ACE2 expression, which is known to protect the lung from acute lung injury. ACE1 and ACE2 polymorphisms might be associated with the infectivity, severity and recovery from the COVID-19. Association studies of ACE gene polymorphisms in affected population may suggest the clinical outcome of the COVID-19.

Keywords : Angiotensin-converting enzyme, polymorphism, COVID-19, spike protein

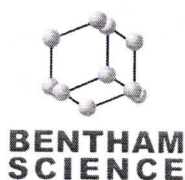
Contracting a disease is dependent on individual's genetic makeup in several cases. It is true in metabolic disorders and infectious diseases too. Even in case of viral infections, some people showed inherited resistance against HIV infection (Zimmerman et al. 1997). Among coronaviruses, SARS-CoV and MERS-CoV resulted in 10% of case-fatality rate during 2003 epidemic (Barnard et al. 2011) and up to 40% mortality rate in some regions during 2012 (Al Awaidy et al. 2019) respectively. This is due to, partly, extreme genetic variability of RNA viruses

such as coronaviruses, increased transmission rate and recombination that occur in viruses when they are in reservoir animals (Barnard et al. 2011). The recent pandemic, COVID-19, accounts for 5103006 infected cases and 333401 deaths as on May 23, 2020 (https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200523-covid-19-sitrep-124.pdf?sfvrsn=9626d639_2). Though good supportive care was given and maintaining social distancing, the infective cases and death toll is being increased continuously. The SARS-CoV (Li et al. 2003) and COVID-19 (Zhou et al. 2020) were found to use angiotensin-converting enzyme 2 (ACE2) as one of the receptors to enter into host cell (Panati et al. 2020). In recent COVID-19 pandemic, older individuals and the individuals with cardiovascular diseases were observed to be at the high risk in China (Wu et al. 2020) and other places.

The renin-angiotensin system (RAS) is a key player in maintaining the homeostasis of blood pressure and electrolyte balance and is a marker for essential hypertension (Nicholls et al. 1998) and COVID-19 (Roncati et al. 2020). ACE1 gene is mapped to chromosome 17q23 and consisting of 25 exons and 26 introns. A 287 bp *A/u* repeat sequence inclusion plays altered levels of ACE1 and its activity. The insertion/deletion (I/D) polymorphism was found in intron 16 of ACE1 gene. The homozygous deletion genotype (DD) results in higher levels of ACE1 protein in plasma. Heterozygous condition shows moderate levels

ACE gene polymorphism in COVID-19

RESEARCH ARTICLE



Thiamine: A Natural Peroxisome Proliferator-Activated Receptor Gamma (PPAR- γ) Activator

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Abstract: Background: There has been increasing evidence of the correlation between thiamine deficiency and type 2 diabetes (T2D). T2D is a condition in which an individual's insulin sensitivity is highly compromised. Peroxisome proliferator-activated receptor gamma (PPAR- γ) is a ligand-activated transcription factor etiologically relevant to T2D. We hypothesized that thiamine could be a PPAR- γ ligand and thus activate PPAR- γ and ameliorate T2D.

Objective: This study aims to establish thiamine as a PPAR- γ ligand via molecular docking and dynamics simulations (MDS) and thiamine's ability to induce adipogenesis while upregulating PPAR- γ and AP-2 genes using *in vitro* assays.

Methods: Thiamine/PPAR- γ binding was studied using Schrödinger's Glide. The bound complex was simulated in the OPLS 2005 force field using Desmond. 3T3-L1 preadipocyte cells were differentiated in the presence of thiamine and rosiglitazone and stained with Oil Red O. Nuclear protein from the differentiated cells was used to study the binding of the PPAR- γ response element (PPRE) using an ELISA-based assay. mRNA from differentiated cells was used to study the expression of genes using quantitative RT-PCR.

Results: *In silico* docking shows that thiamine binds with PPAR- γ . MDS indicate that the interactions between thiamine and PPAR- γ are stable over a significant period. Thiamine induces the differentiation of 3T3-L1 preadipocytes in a dose-dependent manner and enhances the PPRE-binding activity of PPAR- γ . Thiamine treatment significantly increases the mRNA levels of PPAR- γ and AP-2 genes.

Conclusion: Our results show that thiamine is a PPAR- γ ligand. Animal studies and clinical trials are required to corroborate the results obtained.

Keywords: PPAR- γ , thiamine, type 2 diabetes, molecular dynamics simulations, adipocytes differentiation, natural ligand.

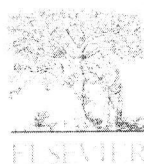
1. INTRODUCTION

Type 2 diabetes (T2D) is becoming a pressing problem for developing countries. A 2019 study estimated that the number of T2D patients will increase by 51% worldwide and that nearly 11% of the human population will be affected by this disease by 2045 [1]. Causal agents of T2D include age [2], diet [3], genetics [4], and lifestyle [5]. Triggers for T2D, including depression, sedentary behavior [6], consumption of sweetened beverages [7], and intake of fast food rich in lipids [8], are increasing due to the urban lifestyle and peer pressure. Maintaining a healthy lifestyle, good eating habits, and regular exercise can prevent T2D [9].

Thiamine, vitamin B₁, is commonly found in cereals such as rice [10]. All animals have to acquire thiamine through their diet since they cannot synthesize it on their own [11]. Enzymes important for carbohydrate metabolism have thiamine as a cofactor, and thiamine deficiency was found to be a risk factor for T2D [12, 13]. Administration of thiamine along with other nutritional supplements has been shown to lessen T2D markers such as blood glucose level [14]. Mutations in the thiamine transporter gene *SLC19A2* were shown to cause T2D [15, 16]. A number of researchers have reported that thiamine could cure diabetic symptoms [17-21]. Thiamine deficiency is common among people consuming alcohol [22]. Thiamine deficiency leads to several downstream clinical syndromes and finally causes diabetic coma [23]. The molecular pathway behind the relationship between thiamine deficiency and T2D is largely unknown. Researchers implicate various molecules that may play a vital role in this pathway. In this study, we show for the first time that thia-

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Code inside the codon: The role of synonymous mutations in regulating splicing machinery and its impact on disease

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ABSTRACT

In eukaryotes, precise pre-mRNA processing, including alternative splicing, is essential to carry out the intricate protein translation process. Both point mutations (that alter the translated protein sequence) and synonymous mutations (that do not alter the translated protein sequence) are capable of affecting the splicing process. Synonymous mutations are known to affect gene expression via altering mRNA stability, mRNA secondary structure, splicing processes, and translational kinetics. In higher eukaryotes, precise splicing is regulated by three weakly conserved *cis*-elements, 5' and 3' splice sites and the branch site. Many other *cis*-acting elements (exonic/intronic splicing enhancers and silencers) and *trans*-acting splicing factors (serine and arginine-rich proteins and heterogeneous nuclear ribonucleoproteins) have also been found to enhance or suppress the splicing process. The appearance of synonymous mutations in *cis*-acting elements can alter the splicing process by changing the binding pattern of splicing factors to exonic splicing enhancers or silencer motifs. This results in exon skipping, intron retention, and various other forms of alternative splicing, eventually leading to the emergence of a wide range of diseases. The focus of this review is to elucidate the role of synonymous mutations and their impact on abnormal splicing mechanisms. Further, this study highlights the function of synonymous mutation in mediating abnormal splicing in cancer and development of X-linked, and autosomal inherited diseases.

1. Introduction

Mutation is a permanent alteration involving the deletion, insertion, or substitution of nucleotides, resulting in a change in DNA sequence [1]. A nucleotide alteration that changes the corresponding amino acid in the protein is called a nonsynonymous mutation, whereas a nucleotide alteration that does not modify an amino acid sequence of a protein due to codon degeneracy is referred to as a synonymous mutation [2]. As the amino acids in the peptide chain are triplet codon reflections, and mRNA contains four nucleotides [A, U, G and C], a total of 64 codons are generated, of which 61 encode the 20 standard amino acids and the remaining three function as stop signals [3]. Except for methionine and tryptophan, which have single codons, the remaining 18 amino acids are encoded by two or more codons. Leucine, arginine and serine each have

six codons owing to codon degeneracy. Different codons that encode the same amino acid are known as synonymous codons. The translation of different synonymous codons has no impact on the amino acid composition of a polypeptide chain, as the main polypeptide chain includes the same amino acid, whether encoded by a single or multiple synonymous codons [4]. Therefore, synonymous mutations are ignored in investigations of protein expression.

Although numerous diseases have been shown to be associated with synonymous mutations during the last two decades, only recently scientists have investigated the impact of synonymous mutations with respect to the molecular etiology of diseases.

While it is believed that nonsynonymous mutations are subject to natural selection, synonymous mutations were believed to be evolutionary neutral [5]. However, the presence of synonymous mutations in

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Indigenous knowledge on medicinal plants used by local villagers associated with Sadasivakona—A sacred grove of Chittoor district, Andhra Pradesh, India

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Yarramreddy Manjunatha Reddy⁴

Abstract

BACKGROUND: The local villagers residing at Sadasivakona sacred grove are associated with medicinal plants and utilize many medicinal plants to cure ailments. Because of this, the present study was intended to document the traditional uses of medicinal plants used by local villagers residing at Sadasivakona—a sacred grove of Chittoor district, Andhra Pradesh, India.

METHODS: The documentation of indigenous knowledge on medicinal plants used by local villagers of Sadasivakona sacred grove was carried out with a structured questionnaire. The documentation of indigenous knowledge was made by conducting several tours and personal interviews with their local dialect from 2019 to 2020.

RESULTS: Sixty-seven potential medicinal plants belonging to 59 genera and 36 families were documented from Sadasivakona—a sacred grove of Chittoor district, Andhra Pradesh, India. For the preparation of medicine, leaves (49%) among the plant parts, paste (36%) among the form of medicine, internal administration (53%) among the intake of medicine, and cough (16%) among the ailment treatment were noticed as the highest percentages. Altogether, 41 types of ailments were successfully documented from the herbal practitioners of Sadasivakona sacred grove.

CONCLUSIONS: There is no documentation on the indigenous knowledge of local villagers of Sadasivakona sacred grove so far. Hence the present study focuses on the documentation of perishing knowledge of this sacred grove. Among the documented plants, the therapeutic uses of 28 medicinal plants were matched with Dr. Duke's Phytochemical and Ethnobotanical database and 10 medicinal plants with Foundation for Revitalization of Local Health Traditions database. The documented information may be helpful to the pharmaceutical industries in the preparation of novel drugs.

Keywords:

Indigenous knowledge, medicinal plants, sacred grove, Sadasivakona

Introduction

Despite advances in biomedicine, rural or tribal communities still practice the use of medicinal plants to cure different ailments. Rural communities have a long tradition of using medicinal plants for various human

ailments, which are pivotal in providing primary health needs. The herbal practice of healing diseases by herbal practitioners is locally called "Naatuvaidhyalu." In recent years, the traditional approach of healing diseases with medicinal plants has been gaining importance worldwide because of its safety and affordability. According to

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Occurrence of *Crinum lorifolium* (Amaryllidaceae) in Eastern Ghats, Andhra Pradesh, India

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ABSTRACT

The present paper records the occurrence of *Crinum lorifolium* from Eastern Ghats (Andhra Pradesh). A detailed description, photographs and a key to *Crinum* species are provided for easy identification.

INTRODUCTION

Crinum L. (Amaryllidaceae), comprising of 114 species, is distributed in tropics and sub-tropics (Africa, America, Asia and Australia) (Govaerts et al., 2012; POWO, 2022). They are valued as ornamentals due to their showy flowers and also traded for traditional medicines (Refaat et al., 2012). In India, it is represented by 16 species (Karthikeyan et al., 1989; Gaikwad, Garad & Gore, 2014; Patel & Patel, 2019), of which 6 species namely, *C. brachynema* Herb. (Maharashtra), *C. eleonora* Blatt. & McCann (Maharashtra), *C. malabaricum* Lekhak & S.R. Yadav (Kerala), *C. reddyi* M. Patel & H. Patel (Gujarat), *C. solapurens* Gaikwad, Garad & Gore (Maharashtra) and *C. woodrowii* Baker (Maharashtra) are endemics. In Eastern Ghats, four species of *Crinum* (*C. amoenum* Roxb. ex Ker Gawl., *C. asiaticum* L., *C. latifolium* L., *C. viviparum* (Lam.) R. Ansari & V.J. Nair) have so far been recorded (Pullaiah & Karuppusamy, 2020), of these two species, *C. asiaticum* and *C. latifolium* are widely used as potential ornamentals. While collecting wild ornamentals in Seshachalam hills of Andhra Pradesh (Eastern Ghats), the authors collected a *Crinum* species growing in rock

crevices. After critical study, the collected specimens were identified as *Crinum lorifolium* Roxb. ex Ker-Gawl. Perusal of literature revealed that this species has not been reported from Eastern Ghats and Andhra Pradesh to date (Pullaiah & Karuppusamy, 2018, 2020). Hence, it is reported here as an addition to the Eastern Ghats from Andhra Pradesh state. Detailed description, photo plate and key to the species of Eastern Ghats is provided to facilitate easy identification.

Crinum lorifolium Roxb. ex Ker-Gawl., in J. Sci. Arts (London) 3(5): 110. 1817. *Crinum pratense* Herb., Amaryll. 256. 1837; Hook.f., Fl. Brit. India 6: 282. 1892; Cooke, Fl. Pres. Bombay 750.1908. Fig.1.

A bulbous, perennial herb; roots long, fleshy, thick, ca. 3 mm in diam. Bulbs ovoid or spherical, 8-12 × 11-14 cm, white, not turning pink when exposed to light, tunicate, gradually narrowing to a neck above; neck cylindrical, 8-11 cm long. Leaves contemporary with flowers, radical, linear or linear-oblong, ca. 75 × 2.5 cm, not channelled, apex truncate or obtuse or subacute; margins entire, minutely spinulose. Scape 65-72 cm long, compressed, decumbent, pale purple. Involucral bracts 2, scarious, deltoid-lanceolate, 6-8 × 2.5-3.5 cm, faintly ribbed; bracteoles linear, with broad base, 6-7 cm long, pale white. Umbels 9-14-flowered. Flowers radially symmetric, white, fragrant, subsessile or shortly pedicellate; pedicels ca. 6 mm long. Perianth tube 10-15 cm long, bluntly-angled, purplish-green; perianth segments 6, linear-lanceolate or lanceolate, 8-10 × 1-1.4 cm, shorter than perianth tube, recurved or spreading, white, purplish on keel. Stamens

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Crotalaria lamelliformis (Fabaceae: Crotalariaeae), a new species from Eastern Ghats of Andhra Pradesh, Peninsular India

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Abstract

Crotalaria lamelliformis is described and illustrated as a new species in sect. Calycinae from Eastern Ghats, Andhra Pradesh of India. The habitat ecology, distribution and conservation status are briefly discussed in this study. This new species is apparently resembled to *Crotalaria albida* B. Heyne ex Roth. (Nov. Pl. Sp., 1821:333) but shows a definite difference by showing a combination of vegetative morphology, inflorescence architecture and floral characters.

Keywords: Sadasivakona, Calycinae, Lamelliform, Callosities, India

Introduction

India is one of the main world centers of biodiversity (Rather *et al.* 2018), after Eastern and southern tropical Africa (Hamed *et al.* 2018, Sosef *et al.* 2017) and the Mediterranean Basin (García-Vega & Newbold 2020, Perrino *et al.* 2018, Wagensommer *et al.* 2017, Perrino *et al.* 2013, Naveh 1998). Mostly the diversity is concentrated in Western Ghats and North East India-Himalayan hotspots. The Western Ghats are characterized as most interesting biodiversity area in the Peninsular India and is mentioned as an important place for endemic plants (Swaroop 2013, Kuriakose & Sebastian 2016). Likewise, Eastern Ghats are also flourished well with nearly 2000 plant species, which constitute 13% plants of India (Ahmedullah and Nayar, 1986). The Fabaceae family is the third largest family in the world after Asteraceae and Orchidaceae (Lewis *et al.* 2005). It exhibits greatest ecological diversity and is well spread in tropical, desert and alpine tundra regions (Wojciechowski 2003). The genus *Crotalaria* L. (1753: 714) has distinctive characters combination, as the presence of a pair of callosities on the standard petal, five anthers (long) plus five (short), rostrate keel and inflated pod (Roux *et al.* 2013). It is one of the largest genera belongs to the tribe Crotalariae (Benth.) Hutch. of sub family Papilionoideae of Fabaceae Lindley (1836:148). It is widely distributed in tropical and sub-tropical regions of the world (Lewis *et al.* 2005).

The genus *Crotalaria* is found throughout the India including Andaman and Nicobar Islands but there is an especially high diversity is found in Peninsular India (Western and Eastern Ghats) (Krishnaraj *et al.* 2013, Subramaniam *et al.* 2015, Danada *et al.* 2016, Ninkaew *et al.* 2017 a,b, Rather *et al.* 2018, Gholave *et al.* 2019, Naik & Singh 2019, Rokade *et al.* 2019, Rokade *et al.* 2020, Herbarium 2020, Singh *et al.* 2020). In India the genus is represented by ca. 116 species of which ca. 73 is confined to Peninsular India including 38 species, 01 sub-species, 09 varieties and 02 forma are endemic (Ansari 2008:2). Most of the *Crotalaria* species were recorded from Western Ghats, whereas the Eastern Ghats of Andhra Pradesh is also considered as one of the richest and most unique regions for plant diversity in Peninsular India (Sivaramakrishna & Yugandhar 2020) with 49 *Crotalaria* species. Among them six species are endemics: *C. candicans*, *C. globosa*, *C. longipes*, *C. paniculata* var. *nagarjunakondensis*, *C. pulchra* and *C. willdenowiana* (Murthy and Pullaiah, 1998).

Preparation and Characterization of Sodium Alginate-reduced Graphene Oxide Microbeads for Co-Delivery of Doxorubicin and 5-Fluorouracil

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Abstract

Combination therapy has gained significant interest in recent years due to its potential to produce greater therapeutic success than mono-drug therapy. However, carriers capable of effective and stimuli-responsive co-delivery of several drugs are severely lacking at the time. To address this need, the study represents the fabrication of reduced graphene oxide-sodium alginate microbeads for co-delivery and controlled release of doxorubicin (DOX) and 5-fluorouracil (5-FU). The developed beads were characterized by Fourier transform infrared spectroscopy (FTIR), Differential scanning calorimetry (DSC), Thermogravimetric analysis (TGA), X-ray diffraction (XRD) and Scanning electron microscopy (SEM). Swelling and in vitro drug release experiments were performed at pH 7.4, 5.4 and 1.2 at 37 °C.

The findings indicated that the pH of the test media could be appropriate for intestinal drug delivery. The release process was studied with the release data being integrated into the Korsmeyer-Peppas equation. The presence of rGO in the polymeric matrix influences the drug release rate. The anticancer activity of the alginate beads was assessed using an MTT assay against MCF-7 cells. The findings indicate that the dual-drug containing microbeads showed good activity compared to 5-FU loaded microbeads. The results showed that the developed microbeads warrant further development for applications that require simultaneous administration of multiple bioactive agents.

Keywords: Microbeads, 5-Fluorouracil, Anti-cancer activity, Graphene oxide.

Introduction

Cancer is a significant cause of death in humans. According to estimates, cancer is the number one leading cause of death in the present day and kills tens millions of people every year¹². Cancer incidence and mortality rates have increased in many parts of the world over the past few decades, especially in developing countries. Cancer treatments have very low therapeutic efficacy and a high level of side effects which have limited their applications due to multiple drug

resistance (MDR) effects, low efficiency of cellular uptake and nonspecific distribution in the body²⁵. Therefore, an effective strategy against cancer cells requires a combinatory approach. In recent years, a great deal of attention has been paid to the design of targeted delivery by polymer functionalized graphene oxide for cancer therapy²¹.

In recent years, there has been an immense potential in the use of polymeric matrices for targeted drug delivery due to their natural abundance, lack of toxicity, biocompatibility and biodegradability⁷. Carriers for bioactive agents (hydrogels, microparticles and nanoparticles) are often made of polymeric materials. In general, natural polymers are more biodegradable and less hazardous than synthetic polymers¹⁵. Until recently, a wide array of drug-delivery methods has been developed and published in the literature^{6,24}. Nevertheless, the majority of them are intended for single-drug delivery. They are either incapable of efficiently loading several agents or lack the capability to accurately control the release rate of each co-delivered agent^{11,20}. This limits their utility in multi-drug therapy. The purpose of this study is to design a carrier capable of addressing this technical gap and enabling the co-administration of doxorubicin (DOX) and 5-fluorouracil (5-FU).

Sodium alginate (SA) is a linear anionic polysaccharide composed of randomly 1→4 connected β-(D)-guluronic (G) and α-(L)-mannuronic (M) acids. SA is among the most commonly encountered anionic polymers in biomedical applications due to its ability to dissolve, biocompatibility, hydrophilicity, biodegradability, safety and favourable drug delivery properties^{18,19}. Despite polymers, research focus on GO has turned more significantly towards development of using the versatility of the material for medicinal purposes due to the many acid groups available on the GO surface for linking to various other compounds²¹.

Additionally, GO is two-dimensional as well as being large in area. This is absolutely essential for the adsorption of molecules on the GO sheets^{1,8}. Unlike GO, reduced graphene oxide (rGO) has a planar structure which improves the efficacy of drug encapsulation^{3,26}.

Additionally, rGO has greater bioactivity and thus can serve as a drug carrier^{9, 22}. It was shown in a previous study¹⁰. DOX has been found to interact more with rGO because

A STABILITY-INDICATING LC-MS METHOD FOR DETERMINATION OF PERINDOPRIL AND ITS PROCESS RELATED IMPURITIES

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Bondigalla Ramachandra,³ Mohammad Asif,¹
and Ahmed A. Ibrahim¹

Original article submitted April 9, 2017.

Perindopril erbumine belongs to the member of angiotensin-converting enzyme inhibitors group used in the treatment of heart failure and hypertension. A simple and highly sensitive LC-MS method has been developed for the simultaneous determination of three process-related impurities (L-norvaline, L-norvaline ethyl ester HCl, and (S)-indoline-2-carboxylic acid) in perindopril. Samples were separated using 5 mM ammonium formate (A) and acetonitrile/methanol (B) as the mobile phase on a Symmetry C₁₈ column (75 mm × 4.6 mm, 3.5 μm) using gradient elution mode at a flow rate of 0.6 mL/min. The proposed method was validated as per ICH guidelines and can be used for quality testing of perindopril and determining its process-related impurities in pharmaceuticals.

Keywords: perindopril erbumine; process-related impurities; hypertension; LC-MS; stability-indicating method.

1. INTRODUCTION

Perindopril erbumine (PER) is a *tert*-butylamine salt of perindopril, which is a pro-drug metabolized *in vivo* by hydrolysis of the ethyl ester group to form biologically active metabolite (perindoprilat). PER is an angiotensin-converting-enzyme (ACE) inhibitor successfully used in treating various cardiovascular diseases such as heart failure, hypertension, high blood pressure, and ischemic heart disease [1–3]. However, in countries of climatic zones III and IV, perindopril arginine is also used instead of PER due to therapeutic equivalence and improved stability [4].

Literature survey revealed several methods for the quantification of PER and its process-related impurities. Medenica, et al. [5] developed an isocratic HPLC method for estimating PER and its impurities [5]. A micro-emulsion LC method for the quantification of PER and impurities was reported in [6], but it was not completely validated. Zaazaa, et al. [7] reported TLC-densitometric and HPLC methods for

the impurity testing of amlodipine and perindopril in pharmaceutical formulations. The British Pharmacopeia (BP) monograph proposes a gradient RPLC method with long run time (65 min) using perchloric acid as mobile phase additive (pH 2.5) [8], which is method is not only time consuming but also produces inadequate peak shapes. Micro-calorimetry and HPLC methods for PER quantification in aqueous solutions [9] and simultaneous estimation of perindopril and indapamide in the presence of impurities and degradation products were reported [10, 11]. Prameelarani, et al. [12] developed an HPLC method for PER and the results were statistically compared by applying Student's *t*-test and *F*-test [12]. A stability-indicating RPLC method for PER was reported in [13, 14]. Darshana, et al. [15] developed absorbance-correction spectrophotometric methods for the simultaneous determination of perindopril and indapamide in pharmaceutical formulations [15]. Nevinet, et al. [16] described first-derivative spectrophotometric method with zero-crossing technique and ratio-derivative spectrophotometric technique for perindopril and indapamide in pharmaceutical dosage forms [16]. Elshanawane, et al. developed a RPLC method for separation of perindopril with indapamide and captopril with indapamide in pharmaceutical formulations [17]. Vijayalakshmi, et al. [18] reported a first-order derivative spectrophotometric method for estimating PER and losartan in formulations. A kinetic study on the isomerization

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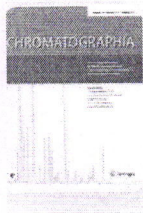
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Enantioseparation of DPP-4 Inhibitors on Immobilized Crown Ether-Based Chiral Stationary Phase

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Abstract

A group of DPP-4 inhibitors such as alogliptin, linagliptin and saxagliptin were selected for enantioseparation for this study. Crown ether columns contain a chiral crown ether ring as a chiral selector is suitable for the compounds having primary amino groups. Since the selected compounds contain free amino groups, a crown ether column was selected for enantiomeric separation of dipeptidyl peptidase-4 inhibitors. Dipeptidyl peptidase-4 inhibitors are a class of oral hypoglycemics that block dipeptidyl peptidase-4 enzyme and can be used to treat diabetes mellitus type 2. A sensitive HPLC method was developed to separate the enantiomers of each dipeptidyl peptidase-4 inhibitor. Effects of various parameters such as type of solvents, selection of additives, effect of pH and column temperature on chromatographic results were studied. The optimal conditions for the chiral separation of dipeptidyl peptidase-4 inhibitors without any derivatization were investigated.