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Molecular Metabolic Fingerprinting Approach to Investigate the Metabolic Alterations in Heart, Aorta and Renal Tissues of Nitric Oxide Deficient Hypertensive Rats

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Abstract:

Hypertension is the leading cause of cardiovascular diseases (CVD) globally. It is well known that the incidence of several CVDs, including stroke, coronary heart disease, and peripheral artery disease, is closely linking to hypertension. Nitric oxide is the imperative regulator of the vascular system and its deficiency leads to elevated blood pressure and metabolic alterations in the tissues such as liver, kidney heart and aorta. Fourier transform infrared spectroscopy (FTIR) is a vibrational spectroscopic technique that uses infrared radiation to vibrate molecular bonds with in the sample that absorbs it and different vibrational spectra can be used to explore the qualitative and quantitative constituent of macromolecules. The aim of this study was to compare molecular changes in the tissues of normal control rats with nitric oxide deficient hypertensive rats. Hypertension was induced in male albino Wistar rats by oral administration of L-NAME (40mg/kg body weight) dissolved in drinking water daily for four weeks. Results indicate that FTIR can successfully identify the molecular changes in hypertensive groups. Overall, the findings demonstrate that in

nitric oxide-deficient animals, the heart, kidney and aortic tissues metabolic programs were altered through an increase in structural modifications in proteins and triglycerides and a quantitative alteration in proteins lipids and transcription factors.

Key words: Hypertension; metabolites; FTIR spectroscopy; nitric oxide

Introduction

Cardiovascular diseases (CVDs) are the leading cause of death worldwide and hypertension continues to be a leading risk factor for disease burden worldwide, despite the availability of several preventive and therapeutic approaches [1]. Hypertension substantially increases the risk of stroke, heart disease, chronic kidney disease and cognitive decline. The chronic administration of Nω-nitro-L-arginine methyl ester (L-NAME) through inhibition of endothelial nitric oxide synthase (eNOS) causes vasoconstriction and systemic arterial hypertension [2]. In the vasculature, chronic blockade of NO synthesis induces oxidative stress, which activates transcription regulatory proteins and induces the expression of various genes, including those encoding adhesion molecules and inflammatory cytokines [3].

Hypertension is commonly associated with panoply of metabolic derangements. Hyperinsulinemia, insulin resistance and hyperglycemia have each been implicated as contributing factors in the pathogenesis of hypertension and atherosclerosis both in obese as well as in non-obese hypertensives. The use of metabolites profiling in hypertension has been generating increasing interest since it was first established that there is an association between serum metabolite profiles and blood pressure in clinically hypertensive patients [4-6]. Several attempts have been made at understanding the association between genetic and metabolic features as well as blood pressure attenuation. This is with a view to discovering biomarkers useful for predicting and diagnosing hypertension using the spontaneously hypertensive rats (SHR) model in comparison with their normotensive Wistar Kyoto (WKY) rats [7, 8].

Fourier Transform Infrared (FTIR) spectroscopy is an accurate label free method, in which infrared radiations interact with matter and are selectively absorbed by it according to their chemical composition, thus creating a molecular fingerprint [9]. As different samples contain different molecular bonds or different configurations of molecular bonds, FTIR allows us to obtain chemical information on molecules within the

sample [10]. Biological tissue is essentially made up of proteins, nucleic acids, carbohydrates and lipids all of which have characteristic absorption bands in the infrared frequency domain [11]. The frequency shifts in the FTIR spectrum show the molecular alteration of macromolecules such as protein, lipid, carbohydrate and nucleic acid which can be considered for analysis [12]. In this study, we used this molecular fingerprinting approach to investigate metabolic alterations in the control and L-NAME induced hypertensive rat kidney, heart and aortic tissues.

Materials and methods

Animals

Male albino Wistar rats, 7–10 weeks old (weighing150–200 g) were procured from the Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College and Hospital, Annamalai University and maintained in an air-conditioned room (25 ± 3°C) with a 12 h light/12 h dark cycle. Feed and water were provided ad libitum. All the experimental studies were carried out in Annamalai University, in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH 1985); the experimental study was approved by the Ethical Committee of Rajah Muthiah Medical College and Hospital (Register No. 160/1999/CPCSEA, Pro.No. 597), Annamalai nagar, Tamil Nadu, India.

Chemicals and reagents

L-NAME was purchased from Sigma-Aldrich Chemical Company, St. Louis, Missouri, USA. All other chemicals used in this study were of highest analytical grade obtained from Sisco Research Laboratories and Himedia, Mumbai, India.

Treatment schedule

The rats were randomly divided in to two groups of six animals each as given below. The first group (Group I) served as a control and received tap water only. The second group (Group II), L-NAME group, received drinking water to which L-NAME was added at a concentration of 50 mg/kg body weight (BW)/day. The initial and final BP was measured by tail cuff plethysmograph method (results not shown).

FTIR sample preparation

Animals were sacrificed by cervical dislocation and the kidney, heart and aorta was excised. The samples of all two groups were homogenized and freeze dried. Samples were stored under-80° C until used. For FTIR analysis, the samples were mixed with KBr at ratio of 1:100. The mixture was then subjected to a

pressure of 1,100 kg/cm2 to produce KBr pellets for use in FTIR spectrometer. Pellets of the same thickness were prepared by taking the same amount of sample and applying the same pressure. Consequently, in corresponding functional groups.

FTIR spectra and data analysis

FTIR spectra of the region 4,000–400 cm-1 were recorded at the temperature of 25 ± 1_C on a Nicolet-Avatar-360 FTIR spectrometer equipped with an air-cooled DTGS (deuterated triglycine sulfate) and purged with nitrogen. Each sample was scanned with three different pellets under identical conditions. These replicates were averaged and then used. The spectra were analyzed using ORIGIN 6.0 software (OriginLab Corporation, Massachusetts, USA). NIH Image J software was used for band area measurement for quantitative analysis.

Statistical analysis

Values are given as means ± SD for six rats in each group. Data were analyzed by one-way analysis of variance followed by Duncan's multiple range tests using SPSS version11.5 (SPSS, Chicago, IL). The limit of statistical significance was set at P\0.05.

Results

Figure 1. FTIR spectrum of aortic tissue

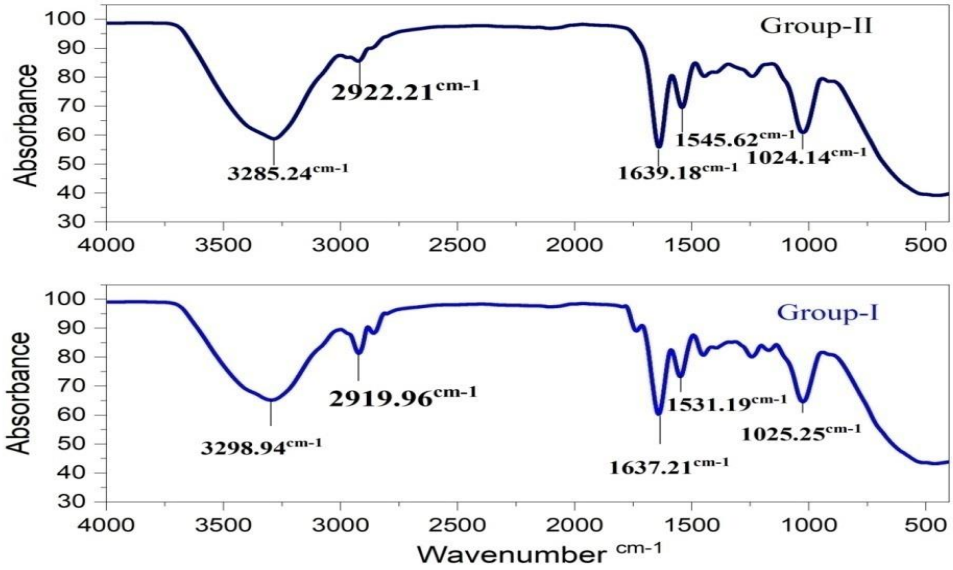

Table 1. General wave number assignment of FTIR spectrum of aortic tissue

Wave number alterations	Wave number (cm^{-1})	
	Control	**L-NAME**
N-H stretching (proteins)	3298.94	3285.28
Olefinic=CH (lipid unsaturation	2919.96	2922.21
CH2 symmetric (lipids–fatty acids) Ester(triglycerides)	1637.21	1641.89
Amide I (Protein)	1531.19	1545.62 cm
Amide II (protein)	1025.25	1024.61

Figure 2. FTIR spectrum of renal tissue

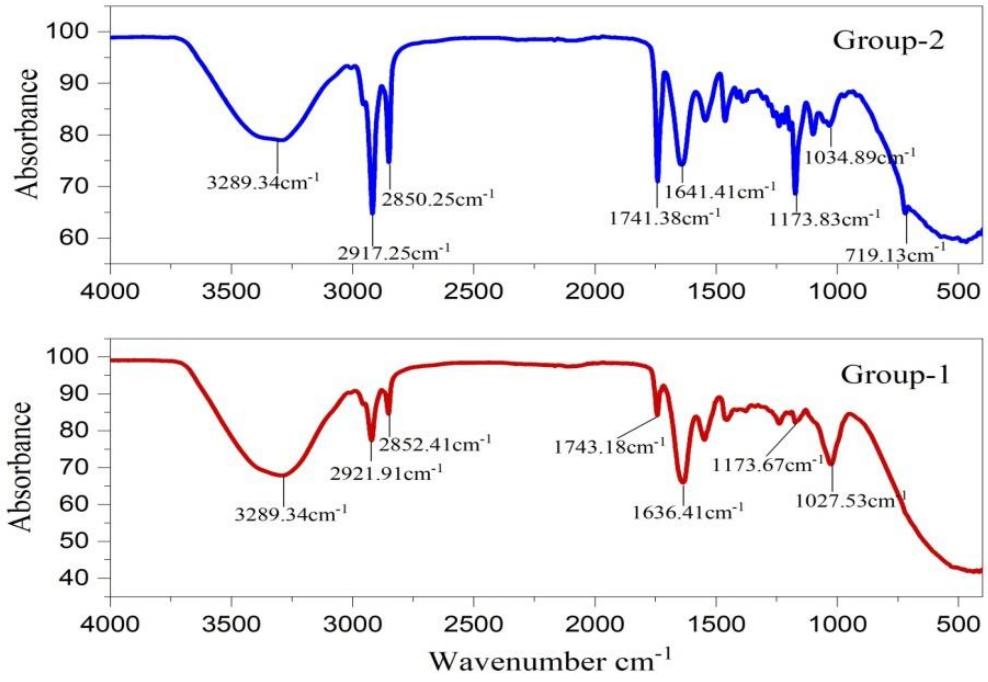

Table 2. General wave number assignment of FTIR spectrum of renal tissue

Wave number alterations	Wave number (cm^{-1})	
	Control	L-NAME
N-H stretching (protein)	3289.34	3294.99
CH_3 asymmetric (Mainly lipids)	2921.91	2917.25
C=H stretching (unsaturated fatty acid	2852.41	2850.25
C=O stretching (esters),triglycerides	1743.18	1741.39
Amide 1	1636.41	1646.40
Amide II	1027.53	1173.84

Figure 3. FTIR spectrum of heart tissue

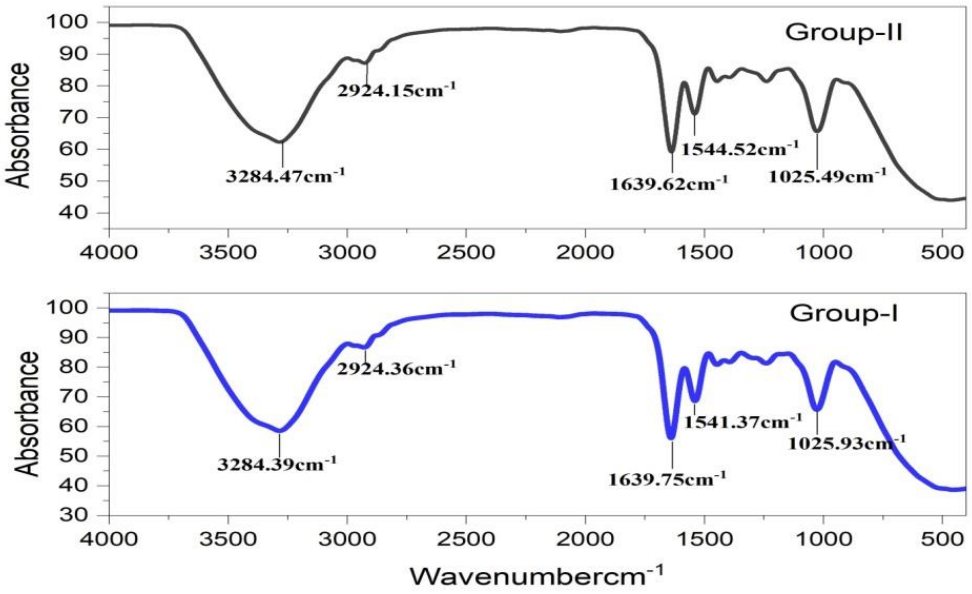

Table 3 General wave number assignment of FTIR spectrum of heart tissue

Wave number alterations	Wave number (cm^{-1})	
	Control	L-NAME
N-H stretching (protein)	3284.47	3284.39
Olefinic C=H (Lipid unsaturation)	2924.16	2924.36
C=C Asymmetric mainly lipids	1634.62	1639.75
Amide I	1025.49	1025.93
Amide II	467.09	451.05

Table1. shows the significantly shifted frequency value in L-NAME (P\0.05) rats aorta compared with control. The bands in this region arise from N–H and O–H stretching modes of proteins, polysaccharides and intermolecular hydrogen bonding. N-H (Protein) amide appeared at 3298.94 cm^{-1} in control and 3285.24 in L-NAME treated aortic tissue. The peak area value of amide A band also decreased (15.1%) significantly from 20.620 ± 2.423 to 12.556 ± 2.028 between control and L-NAME aorta tissue respectively. Absorption bands observed at 3064 cm^{-1} corresponds to N–H stretching of amide B proteins. The peak observed at 1025.25cm^{-1} was assigned to asymmetric.

Table 2. shows the significantly shifted frequency value in the renal tissues of L-NAME rats compared with control. The bands in region arises from N–H and O–H stretching modes of proteins, polysaccharides and intermolecular hydrogen bonding. N-H Amide A appeared at 3289.34cm^{-1} in control and 3294.99cm^{-1} in L-NAME treated renal tissue. There was a significant change in wave number between control and L-NAME treated group. The peak area value of amide A band also decreased (39.1%) significantly from 20.620 ±2.423 to 12.556 ±2.038 between control and L-NAME treated kidney tissue respectively. The CH_2 asymmetric band at 2921 cm^{-1} and CH2 symmetric band at 2924cm^{-1} is due to stretching of lipids, the wave number of these regions was not changed significantly in all the groups. The wave number region shown in Fig. 2 is mainly due to proteins, with some absorbance from lipids. The peak observed at 1743.18 cm^{1} indicates stretching of triglycerides in tissue and there was no significant change in the wave number between all groups. This region mainly originates from amide I and amide II proteins. In the present study there is no significant change in

amide I protein wave number between two groups. But the peak area value of this band decreased from 2.325± 0.192 to 1.851 ±0.138 between control and L-NAME treated kidney tissue respectively.

Table 3 shows the frequency value of control and L-NAME treated rat heart tissue. There is no significant difference in the wave number between two groups, but in band area some major biochemical changes were observed. The peak area value of amide I band increased (62.80%) significantly from 38.143 ± 3284 to 56.240 ±3284 between control and L-NAME treated heart tissue respectively. The peak area value of asymmetric stretching mode of phosphodiester groups in nucleic acids increased significantly from 2.397± 1.012 to 5.167 ± 1.361 between control and L-NAME treated heart tissue respectively.

Discussion

The present study revealed the metabolic changes in kidney, heart and aortic tissues of normal and nitric oxide deficient hypertensive rats [13]. Metabolomics has been increasingly used to characterize risk factors for cardiovascular disease, including hypertension and it appears to have significant potential for uncovering mechanisms of this complex disease. Changes in metabolites profiles have been used to accurately predict so many disease conditions in addition to identifying possible biomarkers and pathways associated in their pathogenicity. This will enable their early detection, diagnosis and treatment as well as likely complications that may arise and also assist in development of biomarkers for clinical uses [14,15]. Since metabolites represent the final products of cellular processes including genes, mRNA, protein activity and bidirectional, complex interactions between these system biology components [16], metabolomics offers an unique view of the metabolic phenotype and phenotypic perturbations associated with diseases, as well as the influence of environmental factors (e.g. diet, activity, behavior, disease and medical/surgical treatment) [17-19]. To date, however, relatively few studies have employed metabolomics in the study of hypertension in animals or humans

Chronic inhibition of nitric oxide synthase (NOS) by L-NAME is a well-established model of experimental hypertension and organ damage within the cardiovascular system and kidney. The mechanism of L-NAME-induced hypertension involves more than a simple inhibition of NO production with a consequent decrease of vasorelaxant activity. Nevertheless, attenuated vascular relaxation and enhanced contraction in different parts of the vascular tree are the first factors contributing to the increase of blood pressure. Among

the other factors, increased activity of the renin–angiotensin–aldosterone system (RAAS) and sympathetic nervous system (SNS) were demonstrated by a number of authors. Increased production of prostaglandins and reactive oxygen species (ROS) were described as additional serious factors contributing to the development of L-NAME-induced hypertension [20,21].

The FTIR spectroscopy monitors the vibration modes of functional groups present in proteins, lipids, polysaccharides and nucleic acids. Our FTIR study illustrates the shifts in peak positions, changes in bandwidths and intensities in control and hypertensive rats. This may be due to hypertensive complications involving the damage in the systems like cardiovascular, nervous system kidneys, retina etc. Several trials have demonstrated significant rise of blood pressure leading to target organ damage. Which is evidenced in our study that chronic blockade of NO synthesis by L-NAME increases oxidative stress activates transcription regulatory proteins and induces the expression of various genes including those encoding adhesion molecules and inflammatory cytokines in the vasculature which leads to the changes in the metabolites.

Conclusion

In conclusion, the findings of this study indicate that the heart, aorta and kidney metabolic programs were altered in hypertension through an increase in structural modifications in proteins and triglycerides and a quantitative alteration in proteins, lipids, and transcription factors. Therefore, this study will assist in discovering biomarkers useful for predicting and diagnosing hypertension and CVD complications.

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