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Research Article

Can NMR (Nuclear Magnetic Resonance) spectroscopy serve as a diagnostic tool for Rheumatoid Arthritis?

Abstract

Rheumatoid arthritis (RA) is a general, chronic, systemic and autoimmune inflammatory disease. This disease represents the inflammatory response of the synovium resulting to hyperplasia of synovial cells along with associated destruction of cartilage, bone and ligaments, eventually leading to deformity of the joints. There is a considerable alteration observed in the metabolism. Chronic inflammation is responsible for altering the metabolism and also the metabolic profile of an individual patient with early inflammatory arthritis expects the following route of disease. These metabolic changes would identify biomarkers, which may be useful for the diagnostic purposes. NMR (nuclear magnetic resonance) spectroscopy based metabolomics studies of serum, urine and synovial fluid of RA were performed. These studies reflected the possibility of the development of metabolomics based diagnostic methods for RA. So, our question "Can NMR (Nuclear Magnetic Resonance) spectroscopy serve as a diagnostic tool for rheumatoid arthritis?" will definitely get an answer with the development of NMR based diagnostic method for RA.

Abbreviations

NMR: Nuclear Magnetic Resonance; RA: Rheumatoid Arthritis; CIA: Collagen-Induced Arthritis; GPC: Glycerophospho-Choline.

Introduction

Rheumatoid arthritis (RA) is an extremely widespread chronic disease, at present affecting around 1% of the world's population. Patients suffering with RA characteristically have damage of joint cartilage and bone accompanied by joint stiffness, hyperplasia, microvascular injury, swelling and pain [1-2]. The RA pathogenesis is started with the secretion of cytokines such as interleukins (e.g., IL-1 and IL-6), tumor necrosis factor (TNF) and interferon gamma (IFN- γ). There is an observation of enhancement in several pro-inflammatory activities of the nuclear factor (NF)- κ B pathway, which hampers apoptosis in immune cells, also responsible for RA progression [1-3]. Activation or dysregulation of several cellular immune responses are carried out by increased cytokine levels in RA [4-5]. With the degradation of skeletal muscle proteins and energy expenditure, almost two-thirds of patients with RA develop cachexia and sarcopenia [6-7]. Such disturbance in the catabolic processes created the body into a state of negative energy balance, which further responsible for

skeletal muscle atrophy, loss of muscle strength and decreased physical activity [8].

Systemic inflammation is responsible for alteration in metabolism. Several researchers have examined the resulting changes in low molecular weight metabolites in patients and animal models of inflammation [9-13]. Nuclear magnetic resonance (NMR) based metabolomics analysis has been used to recognize variations in a broad range of different metabolites concurrently and from such studies numerous key metabolites have been recognized, providing insights into the mechanisms of disease [14-15]. In RA, noteworthy influences on metabolism is also recognized and investigated. In the light of several studies, we would like to search the answer of our question "Can NMR (Nuclear Magnetic Resonance) spectroscopy serve as a diagnostic tool for rheumatoid arthritis?" Here, metabolomics, NMR and NMR spectroscopy based metabolomics studies of RA must be described and discuss to search the answer of the above described question.

Metabolomics

In 1998, the term "metabolome" was first applied. The term metabolome is originated from the combination of the words "metabolite" and "chromosome". The reason behind the use of this term was to reveal that metabolites are indirectly encoded by genes. The term "metabolome" is specified to the entire set of

little molecule found within a biological sample. The study of the metabolome is known as metabolomics [16-17].

NMR (Nuclear Magnetic Resonance) spectroscopy

NMR spectroscopy is a phenomenon which occurs when the nuclei of certain atoms are immersed in a static magnetic field and exposed to a second oscillating magnetic field. Some nuclei experience this phenomenon, and others do not, depending upon whether they possess a property called definite magnetic moment [18].

NMR spectroscopy is one of the most important and widespread analytical methods in academic and industrial research. It facilitates an exclusive and, in principle, quantitative determination of the relative amount of molecular groups, thus offering a tool to quantify entire substances even in mixtures [19].

NMR spectroscopy is a potential instrument for the analysis of metabolites in biofluids, such as plasma, serum, CSF (cerebrospinal fluid), pus, saliva, cervicovaginal secretions and urine, and tissue extract [20]. This is a non-destructive and rapid technique which requires minimum sample processing. This property makes it the most efficient method for qualitative as well as quantitative analysis of metabolites with outstanding repeatability and reproducibility [20]. NMR spectroscopy is successfully applied to metabolic profiling in various diseases such as inflammatory bowel disease [21], ocular inflammatory disease [13], neurological diseases [12], coronary heart disease [14] and RA [22].

NMR spectroscopy based metabolomics studies of RA (Rheumatoid arthritis) and its relevance

Genetic and environmental factors are responsible for causation of RA. NMR spectroscopy based metabolomics may be one of the useful methods for exploring the causation of RA because the disease is responsible for the alteration in the levels of certain metabolites [22-23].

Metabolic profiling of synovial fluid and serum was performed in patients with RA [22-26]. Similarly, metabolic profiling was also performed on serum/plasma and urine samples of CIA (collagen induced arthritis) rats. The procedure of the development of CIA rat model is represented in figure-1 [27-30].

In 1993, NMR spectroscopy based profiles of synovial fluid and corresponding serum samples were studied [22]. The profiles of synovial fluid were noticeably dissimilar from their corresponding serum samples. High levels of lactate and low levels of glucose were found in the synovial fluid as compared to the serum. These alterations are constant with the hypoxic status of the rheumatoid joint [22]. This study is extremely helpful for distinguishing between patients with RA and osteoarthritis (OA) [31]. The occurrence of these metabolites recommends that nucleic acid metabolism may be extremely affected in RA and the cause of this incident may be an association with oxidative stress [32]. The metabolic profiles of plasma of patients with RA were exclusively different from

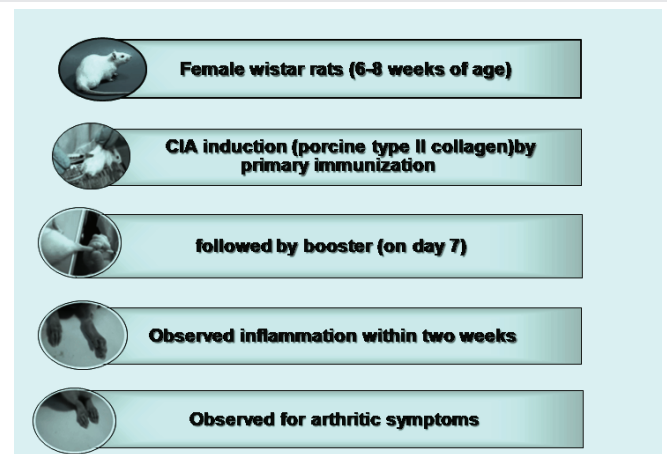


Figure 1: Representation of the stepwise procedure for the development of CIA rat model.

healthy controls [33]. The cholesterol, lactate, acetylated glycoprotein and lipids were identified, which discriminated between the patients with RA and healthy controls [33]. The lactate level is an indicator for oxidative damage and thus indirectly reflected active inflammation. This outcome put forward the idea that the inflammatory processes perform a considerable change in the metabolism that can be measured in the peripheral blood [33]. Metabolic profiling of joint tissue of CIA rats was analyzed by NMR spectroscopy [30]. Identification of biomarker from metabolic pattern associated with RA was observed in serum from mice with RA [32]. Uracil, xanthine and glycine could be used to make a distinction arthritic from control animals [32].

NMR spectroscopy based metabolomics analysis of lipid components in plasma and synovial fluid of arthritic patients as well as animal models of arthritis was performed for the assessment of lipid metabolism [22,32-36]. Plasma and joint tissue of CIA rats represented the alteration in the quantity of lipid components. Quantitative alteration in the phospholipids, triglycerides and total cholesterol (free cholesterol and cholesterol esters) was observed in the plasma as well as joint tissue of CIA rat. The level of phospholipids, triglycerides and total cholesterol (free cholesterol and cholesterol esters) was decreased in the plasma of CIA rat (Figure-2). But, the level of phospholipids and triglycerides was increased in the joint tissue of CIA rat. There was a slight reduction observed in the quantity of triglycerides in the joint tissue of CIA rat (Figure-3) [30].

Lower levels of chylomicron and very-low-density-lipoprotein associated triglycerides were found in synovial fluid as compared to corresponding serum samples. The synovial fluid had high levels of ketone bodies as compared to corresponding serum samples. These results suggest that the metabolic pattern was altered in the RA [22-23]. Lipid profile was investigated in synovial fluid from patients with RA [37] and identified approximately 70 different lipid components. In all these lipid components, few were associated with anti-inflammatory and others were with pro-resolving properties [37]. Inflammatory disease activity in CIA rats with synovitis carried out a noteworthy change in the lipid metabolism.

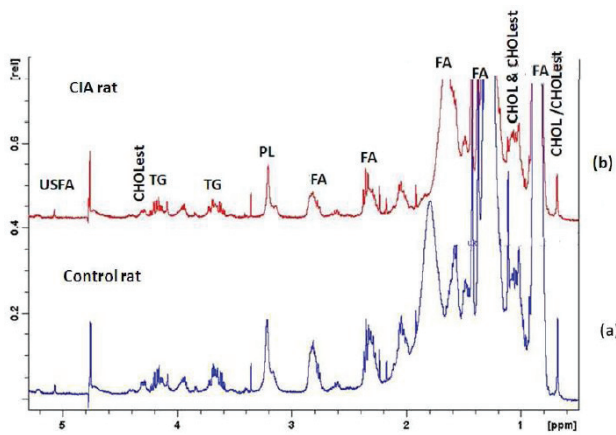


Figure 2: One-dimensional proton NMR spectra of the plasma lipid extract of (a) control rat as compared to (b) CIA rat [FA = Fatty acids; USFA = Unsaturated fatty acids; CHOL = Free cholesterol; CHOLest = Esterified cholesterol; TG = Triglycerides; PL = Phospholipids].

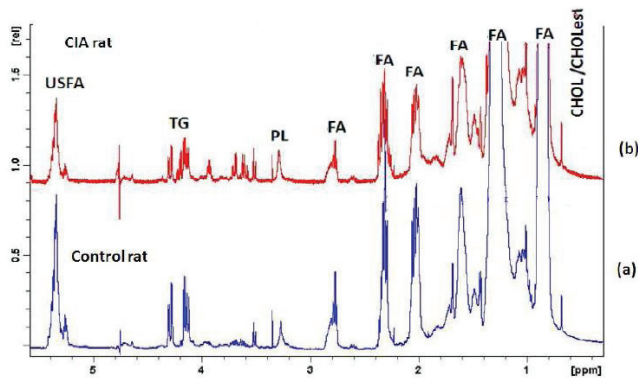


Figure 3: One-dimensional proton NMR spectra of the joint tissue lipid extract of (a) control rat as compared to (b) CIA rat [FA = Fatty acids; USFA = Unsaturated fatty acids; CHOL = Free cholesterol; CHOLest = Esterified cholesterol; TG = Triglycerides; PL = Phospholipids].

Metabolomics based analysis of the lipid component delineating a possible pathway of altered lipid metabolism in CIA rat model, which contributed to an understanding of the pathophysiology of rheumatoid arthritis (RA) [38].

In this way, the metabolomics may be helpful as a measure of the degree of disease and potentially separating low disease activity states from patients in proper diminution. Metabolomics based study was performed with traditional Chinese medicine for categorization of different types of patients with RA [35]. The metabolic profile of urine and plasma of 39 patients with RA was investigated and demonstrated significant biochemical differences between different subgroups of patients with RA. The outcome of this study showed the different mechanisms of disease progression and treatments could be adapted accordingly [35]. The metabolic profiling of serum of 38 patients with active RA showed considerable differences for certain metabolites who demonstrated a clinical response to methotrexate monotherapy compared to those who did not respond [36].

An enhancement in the production of TNF is responsible for increased energy requirement in patients with RA [39].

Xanthine showed the discrimination of RA from controls in mice [32]. Discrimination between septic and non-septic RA was carried out by the analysis of lactate level in the synovial fluid [24]. There is a clear distinction observed for the serum metabolic profile in patients with RA as compared to healthy controls. Lactate, histidine and lipid levels are also play a role for the discrimination of patients with RA as compared to healthy control [25–26]. The metabolic profile of joint tissue showed the GPC (Glycerophospho-choline), carnitine, acetate, and creatinine were important discriminators of CIA rats as compared to control rats [40].

Conclusion

From the above descriptions, there are several strong evidences which noticeably demonstrated the possibility for the development of the NMR spectroscopy based (metabolomics) diagnostic approach for RA. So, the answer of the question “Can NMR (Nuclear Magnetic Resonance) spectroscopy serve as a diagnostic tool for rheumatoid arthritis?” is yes. Here, a strong need to perform the systematic study of the development of this method of diagnosis for RA.

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