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Review Article

Systemic Lupus Erythematosus (SLE): Non-Biologic and Biologic Therapeutic Agents against the Disease, their Quantification in Biological Fluids

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Abstract

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease which affects multiple organs. Both innate and adaptive immune responses are involved. Complex interaction of environments, genetics, and hormones leads to immune dysregulation, tolerance disintegration to self-antigens, resulting in autoantibody production, inflammation, and end organs destruction. Aberrations in cellular metabolism, abnormal phenotype, subsets, function of T and B lymphocytes have been identified as important factors in the underlying pathophysiology.

Symptoms associated with the disease are arthritis (joint pains and swelling), hemolytic anemia (destruction of red blood cells), leucopenia (low levels of white blood cells), neurological manifestations (psychosis, seizures), oral ulcers, renal failure, skin rashes, and thrombocytopenia (low platelet numbers). All these will further increase morbidity and mortality. However, early and accurate diagnosis of systemic lupus erythematosus will be of great significant in the prevention of morbidity and mortality. Non-biologic (conventional) or biologic (monoclonal antibodies, mAbs) therapeutic agents used alone, in combination or sequentially have enhanced the achievement of both short-term and long-term treatment objectives namely prevention of flares and organ damage, optimization of health-related quality of life, and long-term patient survival. Although enzyme-linked immunosorbent assay (ELISA) is a reasonable method for monoclonal antibodies quantifications, however chromatographic analyses in particular hyphenated systems (for example LC/MS/MS), have become more valuable approaches for both non-biologic and biologic therapeutic agents in biological fluids.

Keywords: *Systemic Lupus Erythematosus; Non-Biologic and Biologic Therapeutic Agents; Quantification in Biological Fluids*

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease in which the immune system attacks its own tissues leading to widespread inflammation and tissue damage in the affected organs [1,2]. As the prototype of autoimmune diseases, it is a highly heterogeneous disease affecting all organ systems. Blood vessels, brain, heart, joints, kidneys, lungs and skin are amongst the tissues and organs to be affected by the disease. The course of the disease is unpredictable and variable ranging from mild to severe or fatal disorder [3], chronic or pathway of a relapsing and remitting course [4].

The pathophysiology is characterized by B cell involvement in antibody-dependent and antibody-independent mechanisms [5,6]. Autoantibodies produced by B cells circulate in the body, deposit into tissues, self-react and trigger an inflammatory response through multiple mechanisms, contributing to tissue damage. In addition, cell-mediated mechanisms by innate immune cells (such as neutrophils, monocytes, macrophages) have been linked with SLE disease initiation and progression, as well as with tissue damage [7]. These defects of innate immune cells have contributed significantly in the pathogenesis of SLE, inflammatory cytokine and interferon production [8], self-antigen presentation [9], loss of regulatory function, and ineffective apoptotic debris clearance [10].

A number of factors contribute to the cause of the disease, namely:

(a) Genetic factors- genetic mutations.

(b) Environmental factors- pollution, sunlight exposure, medications, infectious agents.

(c) Hormones- body reactions to certain hormones (especially estrogen).

(d) Smoking, stress level and other health challenges.

The disease causes a number of symptoms which appear or disappear in waves (flare-ups) throughout the body depending on the organs or systems it affects. Such symptoms include blood clots, confusion, dyspnea (shortness of breath), fatigue, fever, hair loss, mouth sores, rashes, swollen glands, swollen arms, face or legs [11].

Its diagnosis is occasionally difficult since it affects many parts of the body accompanied by different symptoms (serious cardiovascular, musculoskeletal, and ocular manifestations). Currently, the diagnosis is usually part of the differential diagnosis carried out and may involve personal and family history, complete physical examination, blood and urine tests (the antinuclear antibody test), and skin or kidney biopsy [12].

The preferred mode of treatment entails a combination of medications since the primary objective is to minimize damage to organs as well as reduce patient's daily afflictions. Such treatment strategy of SLE involves the use non-biologic and biologic therapeutic agents [13]. The determination of patient compliance to this treatment strategy is assessed by therapeutic drug monitoring. Therapeutic drug monitoring requires development of a reliable analytical method for the determination of these active agents in biological fluids-whole blood, plasma, serum, saliva, urine, cerebrospinal fluid, and tears etc. Literature survey has revealed a number of such reliable analytical methods, namely immunoassays, spectroscopic, electrochemical, electrophoretic and chromatographic analytical methods [14]. Chromatographic methods namely high performance liquid chromatography or gas chromatography is often employed either as hyphenated or non-hyphenated system. Hyphenation is an on-line combination of a chromatographic system and one or more spectroscopic detection techniques. These analytical methods vary in terms of assay time, compatibility, robustness, sensitivity, specificity and therefore should be selected based on these parameters for determining therapeutic agent concentrations in biological fluids.

Discussion

Non-biologic therapeutic agents

1. Antimalarial agents-hydroxychloroquine is the drug of choice in the treatment of systemic lupus erythematosus patients [15]. The mechanisms of action involve (i) modulating the immune response by blocking toll-like receptors on dendritic cells [16], (ii) modulating macrophage and other antigen presenting cell function [17], (iii) improving glucose metabolism and lipid levels and thus preventing subclinical atherosclerosis [18].
2. Nonsteroidal anti-inflammatory drugs (NSAIDs)-reduce inflammations (swellings), joints and muscles pain. They act primarily by inhibiting prostaglandin biosynthesis through the blockade of the cyclooxygenase (COX) enzymes COX-l and COX-2 [19]. Typical examples are ibuprofen and naproxen.

3. Glucocorticoids-are utilized in SLE to reduce disease activity (inflammation, pain and tenderness) and disease burden accumulation. Large doses may be used as needed (in cases of disease flare or target organ involvement) and small doses as maintenance treatment [20]. They act through (i) genomic pathway (transactivating and transrepressive modes of action on the cell nucleus), and (ii) a non-genomic pathway [21]. Typical example is prednisone.
4. Immunosuppressants-prevent inflammation and tissue damage in systemic lupus erythematosus. Typical examples are (a) Azathioprine: aids in steroid sparing, maintenance treatment in renal disease in lupus and in lupus flares [22], (b) Methotrexate: contributes to steroid sparing [23], and is indicated in lupus patients showing inadequate response to hydroxychloroquine as well as patients with cutaneous and articular involvement without renal disease [24], (c) Tacrolimus and cyclosporine: are calcineurin inhibitors. Both modulate the immune response by inhibiting T cell activation [25], (d) Therapeutic intravenous immunoglobulin (IVIg): a product containing human multi-specific immunoglobulin G and found to be effective for various manifestations in SLE patients such as renal disease, target organ manifestations (lupus myocarditis, refractory neuropsychiatric lupus, thrombocytopenia) [26]. The intravenous immunoglobulin is a safe and beneficial mode of treatment for SLE patients resistant to or non-responsive to other types of treatment [27]. Inhibition of autoreactive B lymphocytes is one of its mechanisms of action [28].

Biologic therapeutic agents

The objective of using biologic therapeutic agents to treat systemic lupus erythematosus is to establish self-tolerance and achieve disease remission [29]. Flare occurrence, inadequate response of some SLE patients to conventional immunosuppressive agents, target organ involvement and side effects of broad immunosuppressive agents, necessitate the application of these agents in the treatment of the disease [30]. These biologic therapeutic agents target the B cells, B-cell signaling, CD-20 negative cells, T cells, and interferons.

Those that target B-cells

Despite being antibody producers and antigen-presenting cells, B cells are important mediators of organ inflammation. Due to multiple B cells abnormalities (for example B cell lymphopenia, B cell hyperactivity), modulating B cell function is seen as a vital therapeutic approach in SLE treatment [31]. They inhibitory agents include:

(a) Tocilizumab-a monoclonal antibody targeting the interleukin-6 receptor to treat refractory hemolytic anemia in an SLE patient [32].

(b) Rituximab-a B cell-depleting anti-CD20 monoclonal antibody. It depletes B cells via antibody-dependent and complement-mediated cytotoxicity and may be used to treat refractory SLE with renal and neuropsychiatric manifestations [33].

(c) Belimumab-is a fully humanized monoclonal antibody approved for the treatment of seropositive, moderate SLE. It significantly reduces disease flares, serologic activity and steroid dosage [34]. It is also effective in lupus patients without renal involvement, responding inadequately to hydroxychloroquine, glucocorticoids and immunosuppressants [35].

(d) Blisibimod-a moiety that inhibits the B lymphocyte stimulator (BlyS) and shows characteristics of peptide as well as antibody. Elevated BlyS levels are found in the circulation of lupus patients and are associated with disease activity [36].

Other monoclonal antibodies targeting B-cell include (i) binutuzumab [37], (ii) tabalumab [38], (iii) atacicept [39], (iv) epratuzumab [40], (v) daratumumab [41], (vi) ocrelizumab [42], (vi) obinutuzumab [43], (vii) ofatumumab [44], (viii) obexelimab [34].

Those that target B-cell signaling

B-cell signaling has also become target for B-cell treatment in SLE patients. Typical examples of these inhibitors that have shown beneficial effects are tyrosine kinase inhibitors namely fenebrutinib and ibrutinib. Both are irreversible highly selective oral inhibitors of Bruton's tyrosine kinase [45].

Those that target proteasome

Proteasome inhibitors target CD-20 negative cells by inhibiting the proteasome. Inhibition of proteasome results in accumulation of defective immunoglobulin chains, induction of stress in the endoplasmic reticulum, ending in plasma cell apoptosis [46]. Typical example of proteasome inhibitor is bortezomib. The inhibitor has exhibited good results in SLE and may be used in SLE patients with very active disease, who have previously been treated with rituximab [47].

Those that target T cells

Abnormal T cell phenotype in SLE patients has been found to arise from biochemical and molecular defects in T cells coupled to aberrations in gene regulation. These multiple subsets of T cells (CD4+, CD8+, double-negative) found in SLE patients are aberrantly activated, provide help to B cells, mediate inflammatory responses, and are unable to produce enough amounts of the crucial cytokine interleukin 2 (IL-2) [48]. Examples are rigerimod and interleukin-2. Rigerimod is a peptide which acts by blocking antigen presentation to T cells thus inhibiting B cell function [49].

Those that target interferons

Interferons have been found to be involved in the pathogenesis of SLE [50]. Typical example of interferon inhibitor is sifalimumab. Sifalimumab is a fully human monoclonal antibody against IFN-α subtypes which has shown significant improvement in moderate-to-severe systemic lupus erythematosus [51].

To monitor the effectiveness of these active agents as well as patients' compliance to therapy, a number of analytical techniques have been utilized to assay them in biological fluids and they include:

1. Quantification of non-biologic therapeutic agents:

A. Hydroxychloroquine:

(i) Human whole blood- hyphenated HPLC system (LC-MS/MS method) [52]; (LC-HRMS method) [53].

(ii) Human plasma- hyphenated HPLC system (LC-HRMS method) [53].

(iii) Human serum- hyphenated HPLC system (LC-MS/MS method) [54].

B. Prednisone:

(i) Human whole blood- non-hyphenated HPLC system (HPLC method) [55].

(ii) Human plasma- non-hyphenated HPLC system (HPLC method) [55]; hyphenated HPLC system (LC-MS/MS method) [56]; electrochemical (voltammetric method) [57].

(iii) Human serum- non-hyphenated HPLC system (HPLC method) [58]; hyphenated HPLC system (LC-MS/MS method) [59].

(iv) human urine- non-hyphenated HPLC system (HPLC method) [60].

C. Azathioprine:

(i) Human plasma- hyphenated HPLC system (LC-MS/MS method) [61]; spectroscopic system (spectrofluorometric method) [62].

(ii) Human serum- non-hyphenated HPLC system (HPLC method) [63].

(iii) Human urine- hyphenated capillary electrophoresis system (CE/MS method) [64].

D. Ibuprofen:

(i) Human plasma- hyphenated HPLC system (UPLC-MS/MS method) [65]; non-hyphenated HPLC system (HPLC method) [66].

(ii) Human serum- non-hyphenated HPLC system (HPLC method) [67].

(iii) Human urine- hyphenated GC system (GC-MS method) [68]; non-hyphenated HPLC system (HPLC method) [69].

E. Methotrexate:

(i) Human plasma- hyphenated HPLC system (UPLC-MS/MS method) [70]; hyphenated HPLC system (HPLC/MS method [71].

(ii) Human serum- hyphenated HPLC system (UPLC-MS/MS [72] or LC-MS/MS method) [73].

2. Quantification of biologic therapeutic agents:

A. Tocilizumab:

(i) Human plasma- non-hyphenated HPLC system (MEKC method) [74]; non-hyphenated HPLC system (HPLC- fluorescence detection method) [75].

B. Rituximab:

(i) Human plasma- hyphenated HPLC system (LC-MS/HRMS and LC-MS/MS methods) [76]; hyphenated HPLC system (LC-MS/MS method) [77]; immunoassay (ligand binding assay, ELISA method) [77]; nano-surface and molecular-orientation limited (nSMOL) proteolysis method coupled with LC/MS/MS [78].

C. Ibrutinib:

(i) Human whole blood- hyphenated HPLC system (LC-MS/MS method) [79].

(ii) Human plasma- non-hyphenated HPLC system (HPLC method) [80]; hyphenated HPLC system (LC-MS/MS method) [81].

(iii) Human serum- non-hyphenated HPLC system (HPLC method) [82].

D. Bortezomib:

(i) Human whole blood- hyphenated HPLC system (LC-MS/MS method) [83].

(ii) Human plasma- hyphenated HPLC system (LC-MS/MS method) [84].

In general, classical ligand binding assays such as enzyme linked immunosorbent assay (ELISA) is the most widely used method for quantification of therapeutic monoclonal antibodies (mAbs) in biological fluids. However, other analytical methods such as hyphenated chromatographic methods have been applied in the quantification of mAbs in biological fluids due to inability of standard ELISA to discriminate between endogenous and exogenous variants; diminution of specificity, accuracy (the error from correct value), and reproducibility of the ligand binding assays by the presence of anti-mAbs [85]. In order to escape any interference from endogenous human immunoglobulins found in plasma, nano-surface and molecular-orientation limited (nSMOL) proteolysis has been developed [86]. The nSMOL method is a solid–solid proteolysis for Fab-selective limited proteolysis. Hyphenation of nSMOL proteolysis with LC/MS/MS is considered as an optimal method for mAbs bioanalysis because it directly targets CDR peptide quantification.

Conclusion

Systemic lupus erythematosus is a chronic systemic autoimmune debilitating disease, characterized by a tendency for flares (unpredictable course of relapses) and remissions. Autoantibodies (those against double-stranded DNA) are a hallmark of lupus, and, in combination with other cellular and soluble mediators of inflammation, necessitate end-organ damage. Environmental exposures, genetic defects, hormones cause the immune system not to recognize 'self' and thus begins to attack cells and damage organs (blood vessels, brain, central nervous system, heart, joints and muscles, kidneys, lungs, and skin), leading to the disease state. Multiple failures of clinical trials (despite each biologic active agent providing the expected biological activity when administered to patients) go to suggest (i) the clinical heterogeneity of the disease, (ii) multiplicity of pathogenic processes, and (iii) lack of reliable biomarkers. Current therapeutic strategies are directed towards minimizing or halting disease progression and organ damage with the objective of improving disease activity, outcome and quality of life. Finally, although a number of reliable analytical methods (immunoassays, capillary electrophoresis spectroscopy, electrochemistry, and chromatography) have been utilized to analyze non-biologic and biologic therapeutic agents utilized in the treatment of systemic lupus erythematosus, chromatographic hyphenated system (LC/MS/MS), has been found to be a more valuable approach for such quantifications.

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