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A Review

Downmodulation of lysophosphatidic acid by Berberine loaded folate-conjugated glycol chitosan nanoparticles (BFGCN) to mitigate Rheumatoid arthritis (RA) & Cardio-vascular disease(CVD): Current knowledge and future perspectives

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The perils of cardiovascular diseases (CVD) are enhanced by systemic chronic inflammation in autoimmune disorders like Rheumatoid arthritis (RA), in which the patients generally exhibit a high inflammatory burden, dyslipidemia causing 50-60% of RA patients susceptible to CVD dependent mortality. Lysophosphatidic acid (LPA) is a polar, pleiotropic lipid molecule that is water soluble and present in the synovial fluid that can be exploited as an effective biomarker for lipid-signalling. Current research on alternative medicine has recognized various new molecular targets of Berberine (BBR) and established novel signals in support of the efficacy and therapeutic potential of BBR to fight CVD. Therefore, BBR, an alkaloid with poor aqueous solubility could be foreseen as a therapeutic strategy for the reduction of inflammation induced lipidemia by targeting the macrophages and modulating their functions. Hence, a novel BBR loaded folate-conjugated glycol chitosan nanoparticles (BFGCN) could be hypothesized as a three-pronged approach to target activated macrophages, fibroblasts of synovial fluid for downmodulation of LPA. The greatest challenge is the heterogeneity, complexity and interdependence of RA and CVD. Investigation of prognostic and predictive biomarkers is urgently required. Therefore, an improved understanding of the pathogenesis of RA would facilitate identifying an improved targeted treatment and management of RA patients.

Keywords: BBR-FGCN, Berberine, Cardiovascular disease, Lysophosphatidic acid, Macrophage polarization, Rheumatoid arthritis

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disorder primarily affecting minor joints, continuing to larger joints and eventually to the eyes, heart, lungs, kidneys and skin. Common symptoms of RA comprise of morning stiffness of the inflamed joints for more than 30 min, fever, fatigue, loss of weight, tender and swollen joints and presence of rheumatoid nodules beneath the skin. Characteristically, RA exhibits symmetric poly-arthritis, inflammation of synovium leading to hyperplasia, gradually damaging the cartilage and subsequently damaging the bone[1]. Occurrence of RA is higher among the adult population which accounts for 1% in Europe, USA and 0.5% in other geographical area[2]. The validate prevalence in India is 0.28-0.70%[3] with higher incidence in women than in men with an appropriate ratio of 3:1[4]. Cardio vascular diseases (CVD is the major cause of mortality in RA patients, with reports of approximately 50-60% increase in death rate by CVD in RA patients as compared to normal people[5]. Enhanced atherosclerosis leads to premature mortality and unexpected death[6]. RA and CVD share similar inflammatory pathophysiology so far. The enhanced inflammation levels that characterize RA dysfunction offer a "natural experiment" to elucidate the inflammatory mechanisms that speeds progression of CVD. Whereas, the confined pathogenic mechanism of RA is still not very clear, numerous evidences suggest that imbalance in immune cells play a key role in disease progression.

Disorders of lipid metabolism, involving alterations in levels of lipid and their circulation, have been revealed during RA progression[7]. Atherosclerosis

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Abbreviations: AIA, Adjuvant induced arthritic; ATX, Autotoxin; BBR, Berberine; BFGCN, Berberine loaded folate-conjugated glycol chitosan nanoparticles; CIA, Collagen-induced arthritis; CVD, Cardiovascular diseases; CVR, Cardiovascular risk; DMARDs, Disease modifying antirheumatic drugs; FLS, Fibroblast-like synoviocytes; GIT, Gastro-Intestinal Tract; GPCRs, G-protein coupled receptors; LDL, Low-density lipoprotein; LPA, Lysophosphatidic acid; MAPK, Mitogen-activated protein kinase; MMPs, Metal matrix protease; NSAIDs, Nonsteroidal anti-inflammatory drugs; RA, Rheumatoid arthritis

indicates a direct correlation with LPA, which is a ubiquitous minor lipid molecule that binds to its specific cell surface G protein–coupled receptors (LPA1–6). LPA gains importance as it progressively increases in RA patients. Additionally, LPA is an active substance present in slightly oxidized low-density lipoprotein (LDL) and the negligibly modified LDL (Fig. 1). The LPA signalling is recommended to be involved in the inflammatory response of fibroblast like synoviocytes (FLS)[5]. RA is categorized by synovial hyperplasia with proliferation of FLSs, which are particularly responsible for inflammation by the production of chemokines and cytokines[8]. Additionally, activating the LPA receptor along with the mitogen-activated protein kinase (MAPK) signalling was achieved, as LPA encourages growth factor-like answers during the proliferation and apoptosis of FLS-RA. It is an active phenotypic modulator of vascular smooth muscle cells encouraging the de-differentiation, proliferation and movement of these cells that is important for the growth of intimal hyperplasia[5]. Extracellular LPA is synthesized by the enzymatic reaction of autotoxin (ATX)[9]. ATX, can be detected in circulating blood and body fluids, and can be found extensively in the synovial fluid of arthritic patients as well as in collagen-induced arthritis model. The pro-inflammatory cytokine, TNFα, the main culprit in RA, induces ATX expression from FLS, that may be responsible for the occurrence of additional LPA in the plasma of RA patients[10]. Involvement of LPA in CVD is high due to its firm role as a pro-inflammatory stimulus for vascular cells, platelets activator as well as modulator of vascular smooth muscles. All this initiates LPA role in thrombosis, hypertension, and the commencement of hyperplasia that attends vascular responses to injury and the development of atherosclerosis. Furthermore, the interface between inflammation and LPA may quicken RA development thereby enhancing the risk of CVD in RA[11]. Identification of such an ideal metabolic marker in sera could immensely benefit patients with certain pathogenesis or predisposition of response to a specific therapy.

Pathophysiology of RA involves polyarticular inflammation along with hyperplasia of the synovial lining cells. Macrophages are the principal innate immune effector cells involved in the RA pathogenesis. They produce pro-inflammatory cytokines (TNF-α, IL-6, IL-β, *etc.*) and chemokines that contribute to the destruction of cartilage and bone. As macrophages are employed into tissues, they developed into "activated macrophages" having two different phenotypes associated to different stimuli: M1 (classically activated) and M2 (alternatively activated)[12]. M1 phenotype produces increased amount of pro-inflammatory cytokines and iNOS due

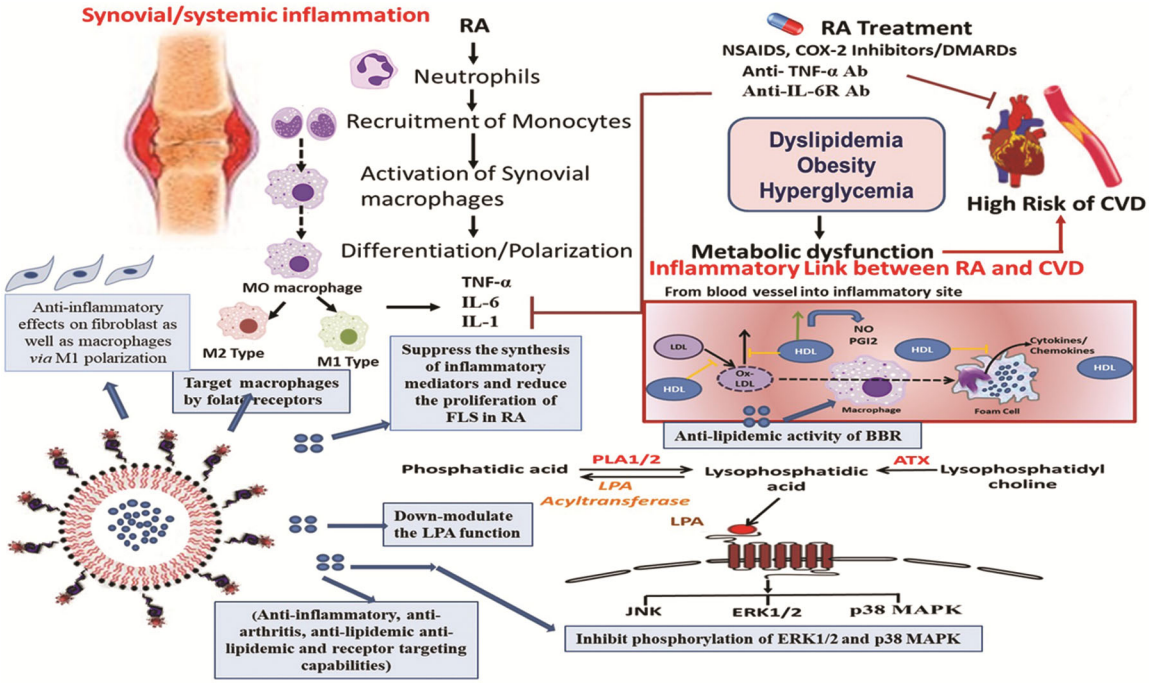

Fig. 1 — Overview of the therapeutic potential of BBR Loaded Folate Conjugated Glycol Chitosan nanoparticles (BFGCN). PLA: Phospholipase A1/2, ATX: Autotoxin, HDL: High Density Lipid, LDL: Low Density lipid, GPCR: G-Protein Cell Receptor

to the polarisation of macrophages towards the pro-inflammatory M1[13]. This increased levels of proinflammatory cytokines, such as TNF-□, IL-6 and as high-sensitive Creative protein (hs-CRP), will amplify joint destruction and influence other CVD risk factors such as metabolic syndrome insulin resistance, endothelial dysfunction and dyslipidaemia (imbalance of blood lipid)[5,14]

RA management has experienced major advances in past few years, both in respect to therapeutic strategy and drugs collection. Increasing evidences have revealed that early diagnosis, early treatment initiation and prompt achievement of remission are the foremost predictors of continuing clinical, radiographic, and functional outcomes. Although numerous modern drugs are available to treat such disorders, their extended use may lead to severe adverse effects on chronic administration such as peptic ulcers and gastrointestinal bleeding[15,16]. It has been observed that anti-rheumatoid therapy upsurges the conventional risk factors of CVD, like the elevation in LDL and triglycerides. Meta-analysis has proven that corticosteroids and non steroidal anti-inflammatory drugs (NSAIDs) have increased CVD effects on RA patients[5]. Therefore, there is a need to develop novel anti–inflammatory agents with low side effects. In recent years interest in herbal drugs has been increased significantly both in India and abroad, as herbal medicines are supposed to be relatively less toxic than the synthetics.

BBR, an isoquinoline alkaloid which is present in multiple medicinal plants, such as *Coptis chinensis*, *Phellodendron japonicum*, and *Berberis aquifolium* and has been reported to ameliorate various autoimmune diseases including RA[17]. It is a BCS class IV drug, havinglow aqueous solubility and low permeability with minimum side effects. As per modern pharmacological research, BBR also possess antibiotic and lipid-regulating effects. Wang *et al*., 2019 reported that BBR remarkably decrease arthritis in rats having bovine type II collagen-induced arthritis (CIA)[5]. MAPK signalling pathway is down-regulated by BBR that further regulates the function of LPA, this signalling thereby execute anti-inflammatory actions on arteriosclerosis, thus minimizing the risk of CVD in RA patients. Effective protection against myocardial ischemia-reperfusion injury by BBR was observed probably through attenuation of JNK and NF-kappa B signalling pathways, signifying the mediation ofMAPK pathway by BBR in RA[18]. It possesses anti-insulin resistance, anti-heart failure, anti-hyperlipidemia, anti-hypertension, anti-arrhythmias, anti-platelet aggregation, anti-inflammatory and anti-arthritic activities[7]. The anti-arthritic impact of BBR was mediated by apoptotic induction of dendritic cells and inhibition of proliferation of fibroblast-like synoviocytes. BBR executes various pharmacological anti-rheumatism activities by acting on multiple targets. It inhibits M1 macrophage polarization in RAW267.4 cells and in adjuvant induced arthritic (AIA) rat model[17]. BBR-activated AKT1 expression, reduces the activation of cytokine signalling (SOCS1) by suppressing NF-κB phosphorylation. Additionally, it was reported that BBR induced AKT1/SOCS1 signallingpathway but also inhibit p65 phosphorylation in macrophages[19].

Glycol chitosan has various properties such as higher water solubility and functional groups for further chemical modifications making it as a potent drug carrier[20]. Glycol-chitosan nanoparticles is an unquestionable choice for drug delivery due to easy surface modification, biocompatibility, pH responsive behaviour and biodegradability. Glycol chitosan have pH-sensitive nature that will release the free drug in the acidic microenvironment of the inflamed arthritic tissue[21]. Excessive expression of folate receptors β (FR-β) on activated macrophages has also been reported in RA patients. By utilizing the self-assembling nature of glycol-chitosan and presence of folate receptors on the surface of activated macrophages, folic acid can be conjugated to glycol chitosan for target drug delivery to the specific site of inflammation[22]. Since low aqueous solubility, low permeability and poor bioavailability of the BBR limit the use of drug, therefore, surface modified nanoparticle holds promising applications for intravenous drug delivery as they will prevent drug degradation in the gastro-intestinal tract (GIT), enhance permeability and bioavailability and will lead to uptake of drug by macrophages specifically through receptor-mediated active targeting.

BFGCN can be a potential nano-therapeutic to target macrophages polarization from M1 to M2 in RA

In pathophysiology of RA, macrophages are actively participating and play very significant role in inflammatory reactions that are responsible for the restoration process of musculoskeletal tissue and immune defence. According to the microenvironment of inflammatory joints, macrophages can be shifted from M1 to M2 phenotypes. The cytokine interferon-γ

(IFN- γ) and bacterial lipopolysaccharides (LPS) can activate M1 macrophages (classically activated) through the stimulation of toll-like receptors (TLRs). M1 macrophages are responsible for high secretion of pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), IL-6, IL-12, IL-1β, inducible nitric oxide synthase (iNOS) and reactive oxygen species (ROS). However, M2 macrophages are responsible for the secretion of mediators including IL-13, IL-10, IL-4, IL-10 and arginase-1 (Arg-1) to activate immunosuppressive and anti-inflammatory responses[37].

BBR may down-regulate the role of M1 macrophages in dextran sulphate sodium (DSS) induced colitis through AKT1/SOCS1/NF0κB pathway[38]. BBR attenuated inflammation in the early phage by interacted with TLR4 *via* TLR4/Myd88/ NF-κB signalling pathway in LPS stimulated macrophages[39]. It also inhibits the migration and invasion in colorectal tumor cell line by regulating polarization of macrophages suggesting that BBR may attenuates intestinal tumorigenesis[40]. BBR could improve the glucose tolerance, energy metabolism and UCP1 expression by regulating the macrophages polarization in white adipose tissue of mice model[41]. Concentration dependent doses of BBR in mice can interfere with the polarization of macrophages through TLR4/MyD88/NF-κB signalling pathway[42]. BBR could promote the protein expression of apoE and their binding to VLDLR in RAW264.7 macrophages by shifting M1 macrophages to anti-inflammatory M2 macrophages[43]. Gold nanoparticles conjugated with BBR have exceptional anti-apoptotic and anti-inflammatory effect and it could also polarize macrophages from M1 to M2 phenotypes to improve the motor function in rat[44]. In RA, the potential role of BBR was indicated as a prophylactic supplement and it can be arbitrated through the suppression of T cells [45]. In adjuvant induced arthritis (AIA), treatment of BBR self-processed the phagocytic function of macrophages and reinstated the balance of M1/M2 polarization. Ratio of Th17/Treg cells was also downmodulated by BBR treatment. Further, studies have reported that BBR reduced the expression of phospho-NF-κB, COX-2 and phosphor-p65 while enhanced the activity of AMPK through AMP-AMPK/NF-κB signalling pathway[46]. Moreover, BBR also down-regulated the expression of hypoxia inducible factor 1α (HIF-1α) and up-regulated the expression of AMP-activated protein kinase phosphorylation (p-AMPK) in synovial macrophages of AIA rats. Also, studies have reported that the glycolysis of M1 macrophages were suppressed by BBR and antiarthritic effect of BBR was induced by regulating energy metabolism of macrophages *via* AMPK/HIF-1α signalling pathway[47]. In joint inflammation, BBR modulated various signalling pathways such as P13K/Akt, AMPK/lipogenesis, LPA/LPA1/ERK/p38 MAPK and Wnt1/β-catenin, that can inhibit inflammation and activate dendritic cells to prevent bone and cartilage destruction[48]. In LPS stimulated macrophages, BBR has suppressed the phosphorylation of MAPK including JNK, p38, ERK and ROS level to down-regulate the pro-inflammatory reactions in LPS-induced macrophages *via* AMPK pathway activation[49] (Fig. 2).

BFGCN can be a potential nano-therapeutic to downmodulate the expression of LPA to detect CVD in RA disease progression

As per the study of epidemiology, CVD is one of the foremost reasons of death in RA patients. Mortality rate of RA patients with CVD risk is 50 to 60% as compared to healthy patients. Additionally, studies have suggested that increased CVD risk is associated with hyperlipidemia and increased inflammatory load in RA patients. During RA disease progression, lipid metabolism disorders such as compositional dispersal and changes in lipid level have been reported. Conventionally, the level of lipids could change due to inflammation, but the so far the consequence of these changes on RA is still not very clear[50].

Lysophosphatidic acid (LPA), has been reported as an effective lipid-signalling molecule which has also been quantified in RA and directly correlated with CVD such as arthrosclerosis. LPA is a small ubiquitous lipid molecule which is mainly formed by the conversion of lyso-phosphatidylcholine (LPC) in presence of autotoxin (ATX) in body fluid. It has been suggested that inflammation in fibroblast-like synoviocytes (FLS) of RA is due to the involvement of LPA signalling. In addition, apoptosis and proliferation in FLS has been attained by inducing the growth factor-like response through the activation of MAPK signalling and LPA receptors. Moreover, RA progression may be accelerated by the interaction of inflammation and LPA which may increase the risk of CVD in RA patients[50].

Recent pharmacological studies have reported that BBR has anti-lipidimic and antibiotic activities. It can also improve arthritis in collagen-induced arthritic (CIA) rats. However, it is still unclear that, whether

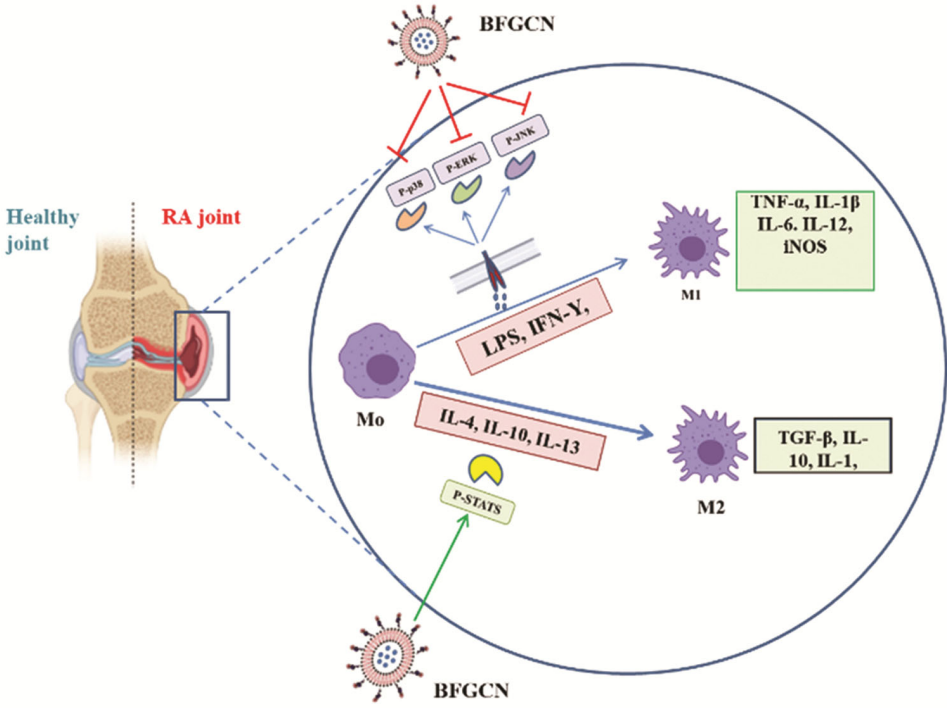

Fig. 2 — Illustration of macrophage polarization in inflammatory arthritis by BFGCN. P-STATS: phosphorylated- signal transducer and activator of transcription protein

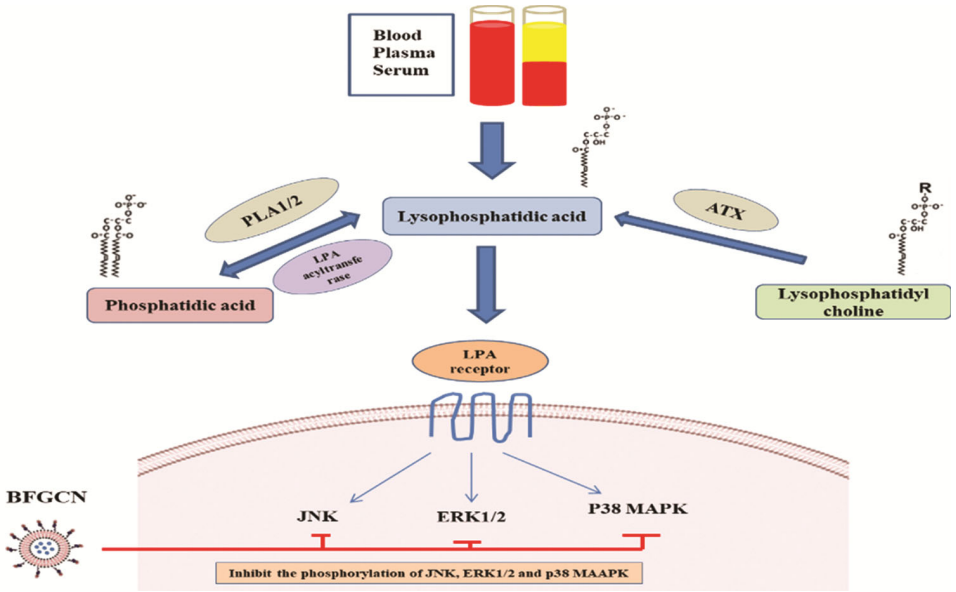

Fig. 3 — LPA can be a potential signalling molecule in RA to detect CVD risk by BFGCN. PLA: Phospholipase A1/2, ATX: Autotoxin

BBR normalise lipid level or inhibit inflammation or have direct effect on FLS or indirect effect on reducing the inflammatory cytokines in RA. It is speculated that by regulating the function of LPA signalling, BBR may down-regulate the mitogen-activated protein kinase signalling pathway as well. Also, it can reduce the risk of CVD and improve inflammation in RA patients by using their anti-lipidemic and anti-inflammatory activities[50] (Fig. 3).

In the view of above research considerations, we hypothesize the development of a folate conjugated glycol chitosan polymeric based nano-sized drug delivery system containing BBR as a pharmaceutically active molecule having anti-

inflammatory, anti-arthritis and antilipidemic activity along with receptor targeting capabilities. This study hypothesizes enhanced delivery of BBR through biopolymeric nanoparticles, thereby preventing the first pass metabolism and increasing the targeted delivery of BBR. Firstly, we have hypothesized toachieve targeting of Folate conjugated glycol chitosan nanoparticles (FGCN) to macrophages by folate/CD44 receptor mediated active-targeting. Secondly, BBR loaded in nanoparticles will downmodulate LPA owing to its anti-lipidemic activity. Finally, BBR will further exhibit its anti-inflammatory effects on fibroblasts as well as macrophages *via* M1 polarization.

BBR may also suppress synthesis of inflammatory mediators that causes unwarranted proliferation of FLSRA. It further inhibits the phosphorylation of ERK1/2 and p38 MAPK. Folate targets the activated macrophages in the synovial fluid as well as in the blood vessels. Glycol chitosan forms the biocompatible, non-immunogenic polymer shell to encapsulate BBR which is released at the target site owing to its stimuli sensitive behaviour. BBR protects against CVD (atherosclerosis) due to its lipid regulating properties and also prevented the oxidized LDL-induced down-regulation of macrophage. BBR downmodulates LPA function thatinhibits the phosphorylation of ERK1/2, JNK and p38 MAPK *via* the G-protein receptors (Fig. 1).

In RA, treatment with regular or biologic disease modifying antirheumatic drugs (DMARDs) reduces the inflammation but elevates lipid level. Although limited data suggested the limit, up to which a significant difference in lipid levels can be seen that may be distinct in various RA therapeutic strategies. This ensures a step ahead than the classical studies to confirm the correlation between reduction of inflammation and lipid increasing the cardiovascular risk (CVR). Since, role of LPA in various pathological disorders like cancer, dermal, pulmonary and renal fibrosis has been reported, its contribution in the inflammatory responses and apoptosis of FLS in RA patients *via* the ATX-LPA pathway (Fig. 1) has been demonstrated recently while monitoring the progression of experimental arthritis[23]. Hence, we propose to evolve a simple sera-based detection system to quantify LPA as a biomarker to predict the progression of RA. Our approach is to simultaneously target the macrophage polarization and FLS to improve arthritic condition, coupled with downmodulation of LPA function to reduce CVD risk. Thus, we hypothesized to develop a drug delivery system with a three-pronged approach aims to fabricate a prudent combination of drug, receptor and surface modified drug delivery system to attain asymptomatic relief in arthritic rat model by targeting activated macrophages and fibroblasts in synovial fluid as well as down modulation of LPA.

Current knowledge and future perspectives

Characteristically RA specifies chronic autoimmune disorder, with persistent, severe systemic inflammation resulting in secretion of auto antibodies. The complex heterogenous symptoms include synovial hyperplasia, intraarticular cartilage destruction, bone and joints deformities driven pain, swelling, coupled with cardiovascular and pulmonary complications[24]. RA leads to infirmity or lethality that is considered as a worry for the affected individuals and community[25,26]. Although, pathogenesis of RA is still unknown, certain immune abnormalities such as non-specific or over-expressive response towards specific stimuli may play a part for RA progression[27]. FLS, found in the synovium, is considered as the primary effector cell throughout the disease progression that releases metal matrix protease (MMPs) to orchestra the cascade of inflammatory responses. A plethora of epidemiological studies have reported CVD as the leading cause of mortality in RA patients. In the view of the above-mentioned points, it is imperative to develop novel therapeutic strategies to ameliorate inflammation along with reduction of CVD risk in RA patients.

Many relevant studies have supported that high inflammation and abnormal amount of lipid in blood (dyslipidemia) are closely related with an increased CVDs risk in RA[5]. LPA, is a phospholipid having a key role in directing several cellular processes such as survival, proliferation, differentiation, motility and its increased levels have been detected in RA. LPA binds to known G-protein coupled receptors (GPCRs) to execute a broad range of biological functions including its role in CVDs[28]. In chronic inflammatory arthritic condition, LPA receptor signalling may promote atherosclerosis development. Thus, the ATX-LPA signalling pathway (Fig. 1) is an interesting therapeutic target in diseases associated with RA and CVDs. Shen *et al*., 2019 report have suggested that the genetic deletion of ATX or inhibition of the ATX and LPA1 receptor significantly reduces symptoms in

an arthritis rat model[29]. Since, inflammation in RA is directly correlated with CVD risk by dyslipidaemia, therefore, drugs that target the macrophage polarization and modulate the LPA function could be a strong potential therapeutic strategy for treating RA and reducing the risk of CVD.

Therefore, a three-pronged treatment approach will be used to achieve the desired targets:

(a) The prolonged consumption of conventional medications for RA treatment such as glucocorticoids, NSAIDs and DMARDs often cause GIT and hepatic disorders. This necessitates the urgent requirement of a drug with reduced clinical complications, few side-effects and multiple pharmacological activities. BBR, a clinically significant natural alkaloid, has been identified based on its potent anti-diabetic, anti-inflammatory, anti-migration and anti-platelet aggregation properties (Fig. 1)[30]. Wang *et al*., 2019 have reported the anti-inflammatory activities of BBR in an experimental model of autoimmune tubulointerstitial nephritis by reducing the strength of pathological damages exhibiting an immunosuppressive result[5]. Ivanov ska *et al*., 1999 showed the efficacy of BBR in improving the clinical signs of AIA rat model. BBR employs anti-proliferative effects against FLSs-RA, basically through the modulation of cell cycle regulators and inhibition of the pro-inflammatory cytokines by acting on the activated macrophages that infiltrate at the inflammatory site[31].

(b) Anti-arthritic drugs reduce inflammation or act as immunosuppressant with elevations in lipid levels in RA patients. Prolonged exposure to NSAIDs and corticosteroids have potential adverse effects on cardiovascular events in patients[32]. Roubille *et al*., 2015 demonstrated that both corticosteroids and the nonselective NSAIDs shows increased CVD effects in RA patients. Since, inflammation in RA clearly indicates a direct correlation between CVR and lipid levels, therefore, the need is to identify drugs delivery system that can target the macrophage polarization and simultaneously alter the LPA function, to become a robust approach for the RA treatment along with reduced CVD risk[33]. Therefore, BBR was selected as the alternative medicine with antilipidemic activity that can be a potential drug to suppress LPA activity. BBR exerts its anti-inflammatory properties *via* M1 polarization of macrophages, and affecting the fibroblast of synovium of RA patients, thereby reducing the risk of CVD as it has been documented to possess both anti-inflammatory and lipid regulating properties. Further, BBR showed improved RA symptoms in CIA rat model primarily by the polarization of macrophages *via* AMPK/NF-κB signaling cascade[17]. We propose BBR as a potential drug to limit LPA activity and executing its anti-inflammatory effect in FLS-RA amongst RA patients with enhanced CVD risk. Hence, it is concluded that BBR will act *via* down-regulation of MAPK cascade and by monitoring the LPA levels in body fluids, the anti-inflammatory and protective effects of BBR on arteriosclerosis development and RA-CVD risk can be predicted. Since, LPA possess both mitogenic and pro-inflammatory effects on FLS-RA, BBR is proposed to modulate the function of active LPA by blocking the p38/ERK MAPK pathway facilitated by LPA1, thereby inhibiting the inflammation and proliferation of FLS-RA. This shows that BBR exerts lipid-regulating and anti-arthritis activities against RA[5].

(c) Since BBR has low water solubility and low permeability there is loss of bioavailability and efficacy. Hence, the proposed nano-delivery system might prolong systemic drug circulation with enhanced efficacy of drug, simultaneously lowering the requisite dose and subsequent side effects. Thus, nanoparticle can be a directed delivery agent that can deliver drug at the target site in an adequate concentration.

Chitosan is a natural polysaccharide with many functionalized amine groups generated by chitin *via* deacetylation and having various physicochemical and structural properties such as low immunogenicity, tremendous biodegradability, good biocompatibility, and other biological activities[34]. Therefore, a vast number of chemically modified soluble derivatives of chitosan were available. Since chitosan gets precipitated in biological solution due to insolubility of chitosan in water or aqueous solutions, instead glycol chitosan (derivatives of chitosan) were used owing to their increased aqueous solubility at all pH, while retaining the unique property of chitosan[20]. To avoid biopharmaceutical challenges of BBR (low aqueous solubility and low permeability) and to deliver drug in intact form at the target site, we

proposed this biopolymer coated drug delivery system to facilitate the intravenous administration of BBR.

Macrophages are the key players in the pathophysiology of RA as they serve as key targets in regulating disease activity and drug delivery for the treatment of RA[35]. Folate receptor β (FRβ) is a glycosylphosphatidyl (GPI)-anchored plasma membrane protein that is greatly overexpressed on inflammatory mediators such as macrophages of RA patient. Folic acid has a nano-scale binding affinity with FRβ which allows folate receptor to be utilized for imaging of RA together with therapeutic targeting[36]. Activated macrophages in the synovial fluid of RA mice/rats have an enhanced expression of FRβ and therefore leads to strong folate binding activity[35]. As an important targeting ligand, Folic acid has high affinity towards its targets and the ease of surface conjugation with therapeutic agents such as BBR makes it an attractive target. Folate conjugation to the BBR loaded nanoparticle will enable the binding with FRβ receptor overexpressed on the activated macrophages, thereby, enabling BBR to reach the inflammatory site[36]. This folate conjugated glycol chitosan nanoparticle will successfully encapsulate poor aqueous soluble BBR resulting in enhanced poor bioavailability, increasedcirculation time of drug and higher accumulation at the inflammatory site owing to active targeting.

Conclusion

It is indeed extremely challenging to reduce the CVD risk with progression of RA and to improve the medical treatment of RA. Although, treatment of RA has advanced from NSAIDs to DMARDs, together with modern biologics, all drugs have multiple life-threating consequences and increased the CVD risk in RA patients. Thus, we expect to overcome the lipid paradox in RA by proposed BFGCN based antirheumatic and antilipidemic therapy. So, we propose an approach to develop a nano drug delivery system that will provide sustained localized release of the drug at the target site with less side effects and enhanced therapeutic efficacy of the drug BBR. BFGCN will reduce inflammation by inhibiting M1 macrophages polarization, and lowering lipid levels leading to reduced risk of CVD. We further proposed a simple detection method by a sera biomarker LPA that is enhanced by inflammation and is directly reported in CVD. Hence, the suppression of inflammation with lowering the lipid level is important for reducing CVD risk. Therefore, by using this three-pronged approach we can investigate the anti-inflammatory, anti-proliferative and antilipidemic effect of BBR on FLSRA through regulating LPA level.

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Conflict of interest

All authors declare no conflict of interest.

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