

Novel Drug Delivery Systems of Methotrexate for Treatment of Rheumatoid Arthritis

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ABSTRACT

Rheumatoid arthritis (RA) is an autoimmune disorder of joints that is one of major problem among the various orthopaedic problems. Various medications used for the treatment of RA includes non-steroidal anti-inflammatory drugs (NSAIDs), disease modifying anti-rheumatic drugs (DMARDs), steroids and biological products. Methotrexate is used as first line drug for the treatment of RA, either as monotherapy or in combination with NSAID's or steroids. Conventional preparations of methotrexate like tablets, capsules and injections are currently available in the market. Novel drug delivery systems exhibit numerous advantages over the conventional formulations. Liposome, dendrimers, hydrogels and nanoparticles are the main technologies that are used for making targeted preparations. Other approaches like carriers substance like albumin, erythrocytes and gold shells are also used now a days to give direct effect on the target site. Side effects are very less with these preparations as distribution in organs other than target organs does not occur. These formulations will definitely prove to be a very effective and superior approach in treatment of rheumatoid arthritis.

Keywords: Methotrexate, Rheumatoid arthritis, Liposomes, Novel drug delivery systems

1. Introduction

Rheumatoid arthritis (RA), an autoimmune inflammatory joint disorder, mainly affect 1-2% of world population [1]. The name came from rheumatic fever that is a fever which includes joint pain. The diseases has been first ever described by Dr. AJ Landre-Beauvais in 1800 [2]. Most common joints involved are hands, feet and cervical spine besides it can also affect larger joints like shoulder and knee. Prolonged manifestation causes severe inflammation and pain of joints and ultimately results in deformities of joints. Other body organs that can be included are skin, lungs, heart and blood vessels in 15-20% of individuals. The disease affects people of all age group and both sexes, but is most prevalent in elderly people. Women are more likely to be affected than men [3]. Osteoporosis is a key feature of long term rheumatoid arthritis but it is also significant in early patients of disease [4]. Approximately half of the RA are not able to perform daily activities within 10 years of onset of disease [5].

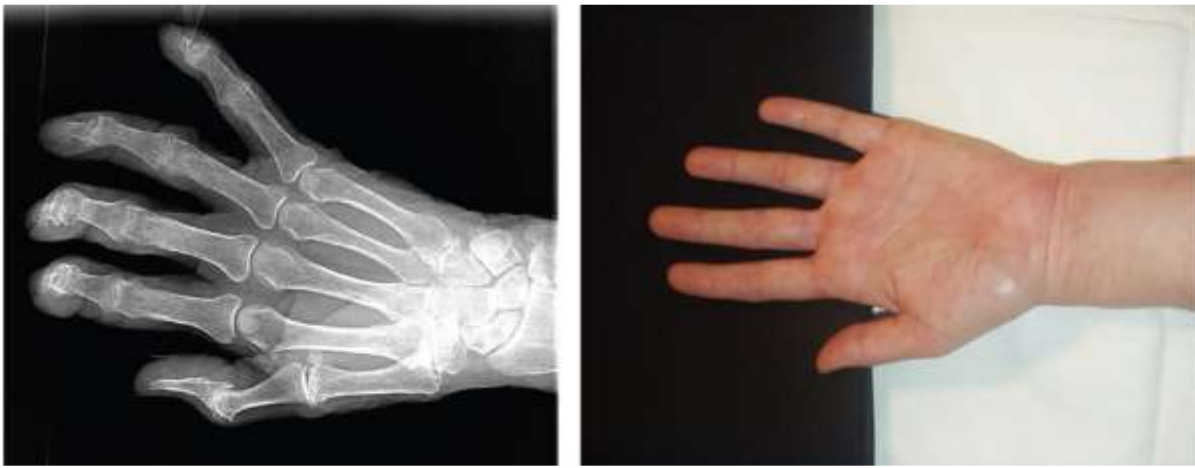


Figure 1: Deformed hand of a rheumatoid arthritis patient [6]

2. Criteria for classification of a person as RA patient

The Committee for Therapeutic Criteria of the New York Rheumatism Association has drafted a revised criteria for the classification of RA. As per the criteria, RA patient can be classified based on daily, self-care, vocational and avocational activities. Class I patients are able to perform usual daily activities while class II patients perform self-care and vocational activities but perform limited avocational activities. Class III patients perform only self-care activities but limited in vocational and avocational activities while class IV patients are limited to perform all the activities [7].

3. Pathophysiology

Till now the exact etiology of RA has not completely deciphered, but the involvement of proinflammatory cytokines has been reported in various scientific literature. The various cytokines involved in the pathogenesis of disease are tumour necrosis factor- α (TNF- α), interleukins (IL-1, IL-6), prostaglandin E2 (PGE2) and transforming growth factor- β (TGF- β) [8-10]. When antigen attacks HLA-DR (MHC class II cell surface receptor), CD4 T-cells are activated due to which cytokine like TNF- α , interferons and ILs are also activated. These factors activate B-cells which results in immune response against antigen. Antibodies IgG and IgM fight against the antigen and also causes damage to the synovial joint as shown in figure 2. Activated macrophages further causes activation of cytokines which ultimately results in bone and cartilage damage and ultimately results in fibrosis and joint deformity [8].

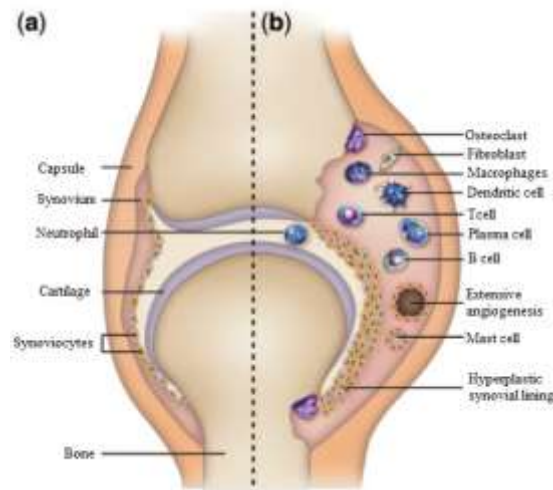


Figure 2: Schematic view of normal joint (a) and a joint affected by RA (b).

RA is best characterized as an immune mediated inflammatory disease (IMID) and cytokines (as mediators) play vital role in the pathphysiology of RA [11]. It is necessary to study the nature and role of these cytokines to identify potential therapeutic targets. IL-1 and TNF- α cytokines up-regulate the production of the receptor activator of nuclear factor-kappa B ligand, which enhances osteoclastic bone resorption. Although there is overlap actions of IL-1 and TNF- α , however, blockade of either cytokine results in clinical improvement and less radiographic progression in patients with active RA [12].

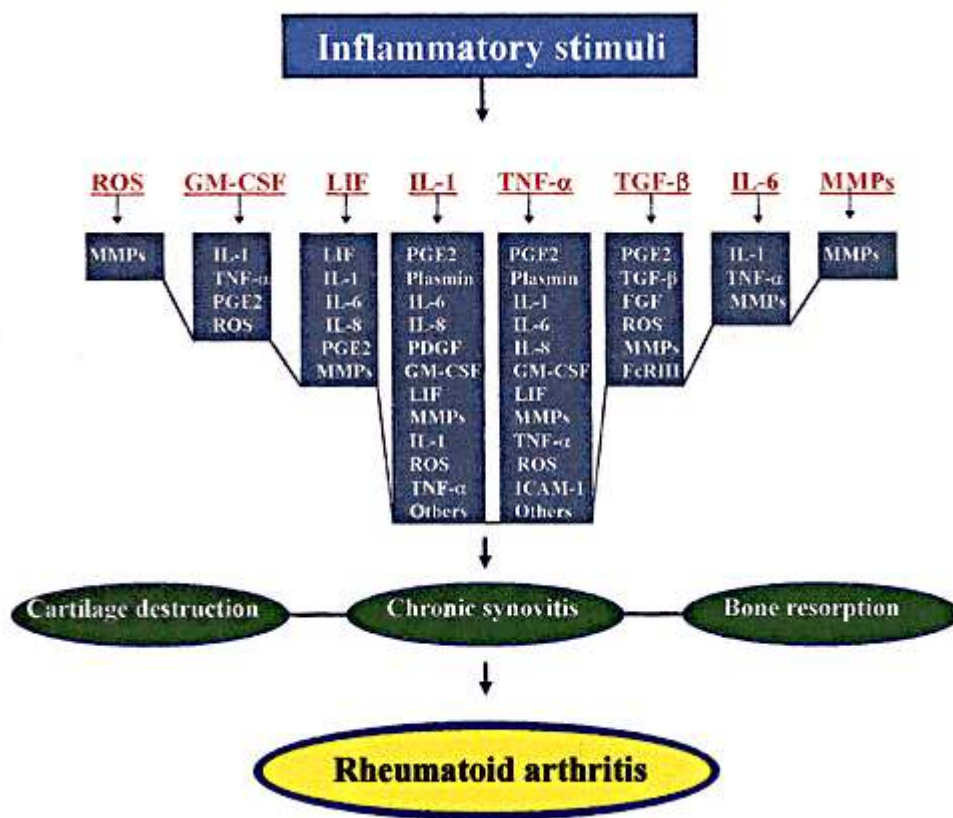


Figure 3: Inflammatory mediators involved in rheumatoid arthritis

4. Diagnosis of RA

Being a serious disease, timely diagnosis of RA is necessary. Accurate diagnosis in early stages is usually difficult. Nowadays, there are many tools which can predict the disease accurately [13].

4.1 Clinical diagnosis

The small joints of hands and feet are normally first to get infected. Shoulders and elbows are less involved in initial stages. Main clinical diagnosis includes morning stiffness, arthritis of joints, rheumatoid nodules and rheumatoid factor which is found in blood test [14].

4.2 Blood test

A specific test to RA is estimation of levels of antibodies that bind selectively to citrulline modified proteins. It has been observed that the level of these antibodies is elevated in patients suffering from RA or those who are at the initial stage of development of RA [15]. Rheumatoid factor, an antibody in RA patients, can be used as marker for detection of RA which is positive in 70-80% of patients. This test is not very specific as rheumatoid factor may also be elevated in other autoimmune diseases besides RA [16]. Other blood tests include the erythrocyte sedimentation rate test and C-reactive protein that are elevated in inflammatory conditions [17]. These are some blood tests that can be used for diagnosis but these are not exact as they may be increased in any other inflammatory disorder also.

4.3 Radiographical studies

In the early phase, radiographical examination of joints show normal or marginal swelling of soft tissues which aggravates to bone erosions as the disease progresses. During bone scanning, a small amount of a radioactive substance is used to show the inflamed joints. Magnetic resonance imaging can also be used to demonstrate soft tissue injuries as well as joint damage [18].

5. Treatment

Diagnosis and timely management of RA is very critical, as 30% of patients are not able to perform daily activities within 3 years of disease if there is a delay in the treatment [19]. The objectives of RA treatment are to provide symptomatic relief and to delay the progression of the disease [20]. Currently available drugs for the treatment of RA can be classified as:

- Non-steroidal anti-inflammatory drugs (NSAIDs)
- Disease-modifying anti-rheumatic drugs (DMARDs)
- Glucocorticoids
- Biological agents

5.1 NSAIDs

NSAIDs are recommended to provide symptomatic relief from pain and to reduce minor inflammation. They act by inhibiting the cyclooxygenase (COX) enzymes i.e., COX-1 and COX-2, thus interfere with the production of inflammatory mediators [21]. Non-selective inhibition of COX is associated with several side effects such as gastrointestinal bleeding, dyspepsia, peptic ulcer etc [22]. The various NSAIDs used for the management of RA are aspirin, indomethacin, diclofenac, ibuprofen etc.

5.2 DMARDs

DMARDs substantially reduce the inflammation, reduce or prevent joint damage, preserve joint structure and function. They mainly act on immune system which slows the progression of disease [23]. The clinically used DMARDs are methotrexate, hydroxychloroquine, cyclosporine, sulfasalazine etc. They are used either as monotherapy or in combination with NSAIDs or glucocorticoids [24].

5.3 Steroids (Glucocorticoids)

Glucocorticoids, orally as well as parenterally, are recommended to relieve the pain, stiffness as well as to decrease the swelling in RA. They act by dual mechanisms such as inhibition of accumulation of macrophage and by decreasing capillary permeability [25]. They are commonly prescribed in combination with DMARDs. The long term use of steroids is associated with well-known side effects such as weight gain, diabetes complications, osteopenia, osteoporosis and an increased risk of infection [26]. The use of minimum dose of glucocorticoids for the shortest period of time reduces the risk of these complications many times. Examples: prednisone and methyl prednisone, hydrocortisone and dexamethasone [27].

5.4 Biologicals

Biologicals/biologics belong to the class of DMARDs that have been designed to prevent or reduce the inflammatory response. Biologics are often recommended to patients who are un-responsive to traditional DMARDs [28]. In addition to the high cost, treatment with biologicals increases the chances of infections caused by bacteria and fungi [29]. Some of the biologicals recommended in developed countries are adalimumab, abatacept, infliximab etc [30].

6. Methotrexate (MTX)

Methotrexate is the drug of choice among the rheumatologists for the treatment of RA, providing initial improvement within weeks and maximal benefits generally by 6 months [31, 32]. There are various novel preparations that have been developed to deliver the target specifically to the affected site in a controlled manner. Various advantages of these preparations are:

- Direct action on target organ
- Long half life

- Sustained release effect
- Lesser side effects
- Less absorption in other organs than affected parts
- Fast onset of action

7. Targeted drug delivery approaches of methotrexate for RA

7.1 Nanoparticles

Nanoparticles can be used *in vivo* to deliver drug specifically to the target site. They also increase the sustained release properties of drug and protect the drug entity in systemic circulation. Physicochemical properties like small size, large surface area and surface charge have made nanoparticles an efficient drug delivery system [33, 34].

Multifunctional nanoparticles of MTX couple with gold shell and a targeting peptide RGD (arginine-glycine-aspartic acid) were found to be more efficient than MTX alone, thereby improved the therapeutic efficacy and minimized the dose related side effects of MTX. The same approach can be further explored for other DMARDs for RA or other inflammatory conditions [35].

Nanoparticle of Fe_3O_4 (also called Superparamagnetic iron oxide nanoparticles (SPIONs)) were loaded with MTX, then were stabilized by the chitosan. The loading of MTX and chitosan increased the size of nanoparticles, which was still in nanometer range (152 nm). These nanoparticles increased the sustained release properties of MTX effectively [36].

Lipidic nanoemulsion of MTX (LDE-MTX) was prepared and then esterified with dodecyl bromide. The therapeutic efficacy of LDE-MTX was evaluated in rabbits in comparison to MTX which exhibited 205 fold more efficacy than parent drug itself [37].

Co-administration of fumagillin nanoparticles with MTX was found to exhibit improved efficacy and sustained release profile of MTX as compared their individual administration. This combination therapy decreased the inflammatory cells, thereby exhibited minimum cartilage damage and bone erosion. Improved anti-inflammatory effect of MTX by fumagillin nanoparticles was confirmed by analysis of plasma cytokine levels [38].

7.2 Liposomes

Liposomes are the one of most extensively explored targeted drug delivery system that has been developed by Alec Bangham in 1965 [39]. The architectural features of liposomes includes aqueous core enclosed within lipid bilayer. Due to amphiphilic in nature, these are the suitable carriers for hydrophilic, lipophilic as well as amphiphilic molecules. They are similar to natural cells, thereby, exhibit low antigenicity and minimum toxicity. The architectural features of liposomes and entrapment of drugs has been shown in figure 4.

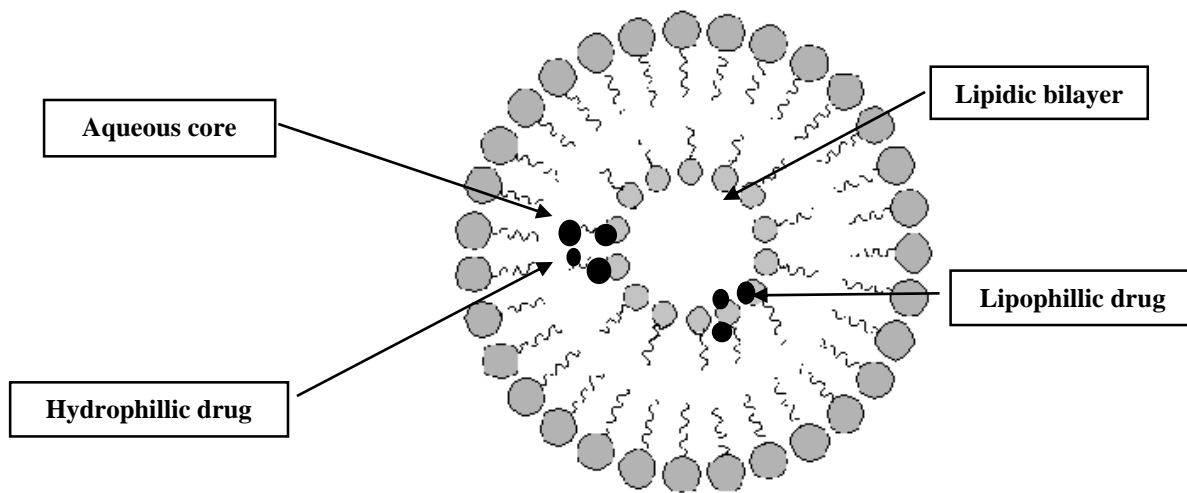


Figure 4: Structure of liposomes and entrapment of hydrophilic and lipophilic drugs

Liposomes of MTX were prepared by using dimyristoylphosphatidylethanolamine as lipid. Liposomal formulation (MTX- γ -DMPE) was very effective in curing inflammation in rats and the inflamed joint completely return to normal within 20 days. These large multilamellar liposomes were more effective in suppressing inflammation in comparison to small sized vesicles as well as suspension of MTX [40].

Stealth liposomes of MTX were prepared by using 1,2-distearoyl-phosphatidylethanolamine and polyethyleneglycol. The prepared formulation reached the target organ without causing significant toxicity to other organs [41].

A unique immunoliposomes for immunotherapy has been developed by attachment of human recombinant interleukin-2 on the surface of small unilamellar liposomes (46-50 nm) having methotrexate in the internal aqueous core. The developed liposomes exhibited excellent stability as more than 90% of MTX was retained in liposomes stored at 4 °C after 24 h [42].

Intra-articular administration of MTX entrapped in liposomes suppressed the joint swelling and rise in temperature in antigen induced arthritis. Liposomal formulation exhibited prolonged retention in the synovial joints even after 7 days in comparison to free drug. Longer retention of drug was responsible for less cartilage destruction and hence for better therapeutic efficacy [44, 45].

7.3 Erythrocyte carrier

Erythrocytes were taken from rats and conjugated with methotrexate (MTX-RBCs). Plasma levels of methotrexate alone and conjugated preparation was noted at various time intervals by HPLC. It had shown that conjugated preparation has three folds increase in half-life than the MTX alone. The conjugated formulation exhibited slow release properties and liver targeting characteristics also [46].

7.4 Thermosensitive hydrogels

Hydrogel is a network of polymer chains that are hydrophilic and possesses flexibility similar to natural tissue. Methotrexate hydrogel was developed for the intra-articular administration which release the drug at the target site in a controlled manner. It also decreased the clearance rate of methotrexate in joint cavity [47].

7.5 Microspheres

Microspheres are the small spheres that has diameter in micrometre range (1-1000 μm). These microspheres can be used as carriers and exhibits many advantages such as light weight, compatible with any material, high melting temperature and cost-effective [48].

Microsphere preparation of poly (l-lactic acid) loaded with methotrexate drug has been prepared. Plasma and urine concentrations of methotrexate were determined by high performance liquid chromatography (HPLC). Methotrexate microspheres showed a high burst phase followed by slow release phase. Plasma concentration of methotrexate preparation was 10 fold higher than the methotrexate used alone response. This proves a good sustained release effect of methotrexate microspheres but microspheres may decrease the clearance of drug in the joint to the blood [49].

7.6 Methotrexate coupled with albumin

Methotrexate (MTX) was associated with albumin (HSA) so to increase the solubility at targeted organ. Both drugs were given in equivalent 7.5 mg/kg intravenously twice a week. This combination worked both as synergistically and combination was superior to methotrexate alone [50].

7.7 Dendrimer

Dendrimers are polymeric materials. They are highly branched, monodisperse macromolecules [51]. Various advantages of dendrimers are:

- They have structural uniformity.
- They have better targeting power due to the presence of functional group on surface of dendrimer.

Polymers may be used as good carriers for drugs. In this preparation methotrexate was conjugated with polyamidoamine (PAMAM, generation 4). Monomethyl polyethylene glycol (MPEG) was used as a linker between MTX and PAMAM. The conjugation was confirmed by UV and NMR spectroscopy. This preparation is also called dendrimer and is superior to the methotrexate alone drug response.

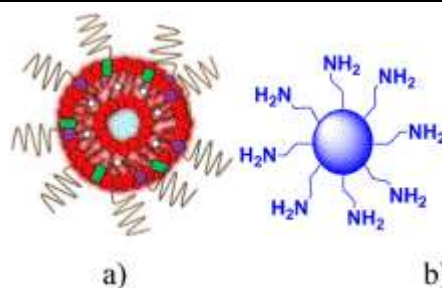


Figure 5: Nanocarrier systems (a) liposome and (b) PAMAM dendrimers [52].

Poly amidoamine dendrimer (generation 5 [G5]) nanoparticle of MTX was prepared after coupling with folic acid which exhibited potent anti-inflammatory property which was evaluated on the basis of reduction in ankle swelling, paw volume and cartilage damage [53].

8. Conclusion

In spite of development of recombinant biologicals, MTX still remains the first line drug for the treatment of RA among rheumatologist. In order to improve its therapeutic efficacy in addition to minimising side effects, a number of novel drug delivery systems such as nanoparticles, liposomes, microspheres, hydrogels, dendrimers etc. have been developed which were found to exhibit better therapeutic profile than conventional formulations in a number of pre-clinical and clinical studies.

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