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PERSPECTIVE

IL-1Ra and its Delivery Strategies: Inserting the Association in Perspective

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ABSTRACT Interleukin-1 receptor antagonist (IL-1Ra) is a naturally occurring anti-inflammatory antagonist of interleukin-1 family of pro-inflammatory cytokines. The broad spectrum anti-inflammatory effects of IL-1Ra have been investigated against various auto-immune diseases such as diabetes mellitus, rheumatoid arthritis. Despite of its outstanding broad spectrum anti-inflammatory effects, IL-1Ra has short biological half-life (4–6 h) and to cope with this problem, up till now, many delivery strategies have been applied either to extend the half-life and/or prolong the steady-state sustained release of IL-1Ra from its target site. Here in our present paper, we have provided an overview of all approaches attempted to prolong the duration of therapeutic effects of IL-1Ra either by fusing IL-1Ra using fusion protein technology to extend the half-life and/or development of new dosage forms using various biodegradable polymers to prolong its steady-state sustained release at the site of administration. These approaches have been characterized by their intended impact on either *in vitro* release characteristics and/or pharmacokinetic and pharmacodynamic parameters of IL-1Ra. We have also compared these delivery strategies with each other on the basis of bioactivity of IL-1Ra after fusion with fusion protein partner and/or encapsulation with biodegradable polymer.

KEY WORDS delivery strategies · encapsulation of IL-1Ra · fusion of IL-1Ra · sustained release of IL-1Ra · thermosensitive gel

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ABBREVIATIONS

AlbudAbs	Albumin binding human binding domain antibodies
ELP	Elastin-like peptide
HSA	Human serum albumin
IL-1Ra	Interleukin-1 Receptor antagonist
PF127	Pluronic F127
PLGA	Poly-lactic-co-glycolic acid
RFVP	Amino acid residues of *Streptococcus gordonii*

INTRODUCTION

From the last few decades, the clinical achievements regarding interleukin-1 receptor antagonist (IL-1Ra) for the prevention and treatment of various auto-immune diseases have been admired significantly by the modern medical care in the field of pharmaceutical biotechnology. IL-1Ra merges several unique properties such as high binding affinity with interleukin-1 receptor I (IL-1RI), outstanding target specificity, low toxicity and relatively small molecular weight (17 kDa) in comparison to other therapeutic proteins. IL-1Ra is a naturally occurring anti-inflammatory antagonist of interleukin-1 family of pro-inflammatory cytokines such as interleukin-1β (IL-1β) and interleukin-1α (IL-1α). These pro-inflammatory cytokines are decisively involved in the underlying mechanism of various chronic inflammatory diseases such as rheumatoid arthritis (1–3) and type 2 diabetes mellitus (T2DM) (4–6) neuropathological diseases such as stroke (7,8), Alzheimer's disease (9,10), Parkinson's disease (11,12) and epilepsy (13–17). Various experimental studies have confirmed that pro-inflammatory cytokines provoke numerous inflammatory mechanisms (4,5) whereas, IL-1Ra has been proven to be effective for ameliorating the detrimental effects of these pro-inflammatory cytokines (4,18,19). The outstanding therapeutic potential of

IL-1Ra has created a significant interest to develop new anti-inflammatory therapeutic modalities to target pro-inflammatory cytokines (IL-1β and IL-1α).

The broad spectrum anti-inflammatory therapeutic outcomes of IL-1Ra against various chronic inflammatory diseases has already been well described (1,2,4,20) . There are several therapeutic characteristics that make IL-1Ra safe and effective therapeutic agent against different auto-immune diseases. Despite of its outstanding anti-inflammatory effects and commercial success, there are certain limitations which need attention to make IL-1Ra an ideal anti-inflammatory therapeutic agent. These limitations are rapid clearance from kidney and short biological half-life (4) but to cope with these problems, up till now, many delivery strategies have been applied to prolong the half-life of IL-1Ra with a decelerate clearance from the body.

Here in our present article, we have reported different delivery strategies that have been adopted up till now to extend the biological half-life and prolong the therapeutic potential of IL-1Ra. The main purpose of our present article was to summarize all delivery strategies along with their methodologies that were adopted to extend the biological half-life and prolong the steady-state release of IL-1Ra.

STRATEGIES FOR THE EXTENSION OF IL-1RA BIOLOGICAL HALF-LIFE

Small molecular weight proteinous drugs are quickly cleared by degradation and renal filtration (21). The main reasons for rapid elimination of proteinous drugs from circulation are enzymatic degradation, biochemical and/or biophysical clearance mechanisms. Therefore, IL-1Ra being a small molecular weight proteinous drug is rapidly cleared from kidney by biochemical and biophysical clearance mechanisms (22). There are several mechanisms that are involved in the clearance of IL-1Ra from the body including rapid distribution into the organs, hepatic metabolism, and rapid elimination from the kidney (22) that ultimately cause the variation in standard therapeutic dosage regimen for therapeutic agents (23). In summary, the attempts made and the strategies that have been applied to extend the half-life and/or sustained release of IL-1Ra can be grouped firstly in to, those in which IL-1Ra has been fused and expressed with other proteins or peptides that directly increased the biological half-life of IL-1Ra, secondly, those in which IL-1Ra was encapsulated with drug delivering polymers that prolonged the sustained release of IL-1Ra at target site (Fig. 1).

FUSION OF IL-1RA WITH DIFFERENT FUSION PROTEIN AND PEPTIDE PARTNERS

IL-1Ra is finding its ways into the multiplicity of applications for the treatment of several life threatening diseases which are not curable by using traditional and conventional medicinal agents due their limitations in therapeutic efficacy and possible potential hazardous effects (3,24). To cope with the problem of half-life of IL-1Ra, fusion protein technology has been applied to fuse IL-1Ra with suitable fusion protein and/or peptides. Up till now, various methodologies have been applied to fuse IL-1Ra with fusion proteins that are discussed below.

Fusion of IL-1Ra with Elastin-Like Polypeptides (ELP)

Elastins are extracellular matrix (ECM) proteins in conjunction with glycosaminoglycans (GAGs) and collagens which have distinctive mechanical property to allow repetitive extensibility followed by elastic recoil. These are most commonly found in various body parts such as elastic ligaments, skin tissues, arteries and lung parenchyma tissues (25–27). Characteristically, elastins are insoluble proteins that are synthesized as soluble precursor protein named as tropoelastin (MW 70 kDa). Tropoelastin is composed of alternatively hydrophobic and hydrophilic crosslinking domains. During elastogenesis, it is secreted from the extracellular cells and create fibrils of insoluble elastins (28,29) that enzymatically crosslinked with lysine residues (28). The biological role of elastin in ECM has made it a versatile protein for therapeutic delivery of various proteinous drugs and tissue engineering (29–31). ELPs are artificial replicative polypeptides that are resultant of amino acids sequences found in the hydrophobic domain of tropoelastin. The most frequently used motif for ELPs consist of replicates of the sequence (VPGXG)n where "X" can be the guest residue having any amino acid other than proline and "n" represents the number of pentapeptide replicates in the ELPs (27). There are some other variants of ELPs that are composed of pentapeptide replicate sequences KGGVG (32) or LGGVG (33) to heptapeptides with the sequence LGAGGAG and nonapeptides with the sequence LGAGGAGVL (34). Although, all of these ELPs exhibit elastin-like properties but among all of them, the most important and most commonly used ELPs is (VPGXG)n.

ELPs that are composed of VPGXG pentapeptide repeats demonstrate to be thermally responsive polypeptides that exhibit a reversible inverse temperature phase transition (27). ELP reveals up to 25-fold increase in its intraarticular biological half-life when compared to that of soluble non transitioning ELP (35). Moreover, systemic exposure of ELP is also decreased due to its phase transitioning property (35). The phase transitioning property of ELP conjugated to drug

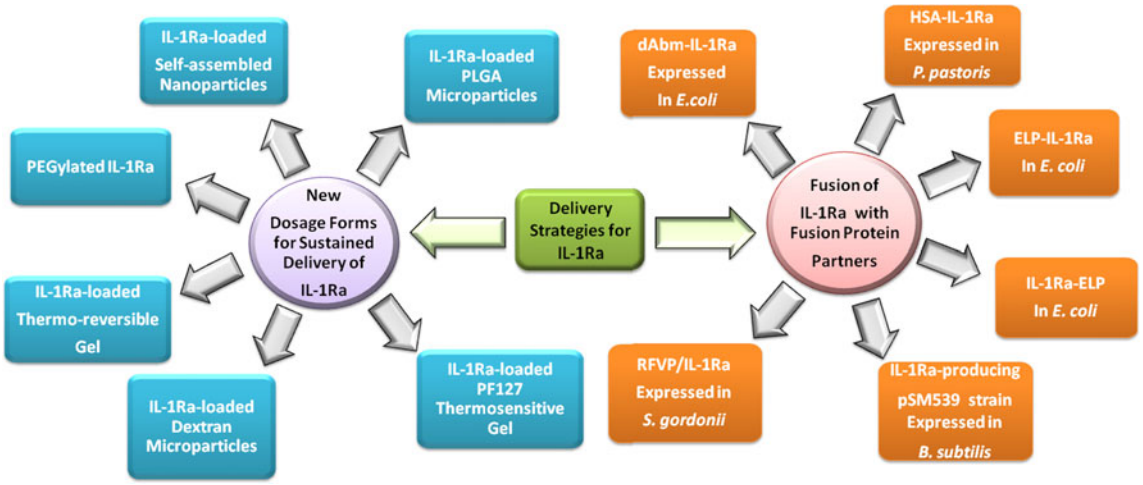

Fig. 1 Schematic representation of different delivery strategies for IL-1Ra. Fusion of IL-1Ra with different fusion protein and/or peptide partners (in *orange*). Different dosage forms for sustained release of IL-1Ra using various biodegradable polymers (in *blue*). *Abbreviations*: *IL-1Ra* Interleukin-1 receptor antagonist, *ELP* Elastin-like peptide, *HSA* Human serum albumin, *dAbm* Human domain antibodies, *ELP-IL-1Ra* IL-1Ra fused at N-terminus of ELP, *IL-1Ra-ELP* IL-1Ra fused at C-terminus of ELP, *HSA-IL-1Ra* IL-1Ra fused at N-terminus of HSA, *dAbm-IL-1Ra* IL-1Ra fused with dAbm, *RFVP/IL-1Ra* IL-1Ra fused with amino acid residue of *Streptococcus gordonii*, *PF127* Pluronic F127, *PLGA* Poly-lactic-co-glycolic acid.

makes it suitable for generating potentially valuable intra-articular drug depot at the site of injection. The formation of drug depot allows slow resolubilization of drug at the site of administration that would ultimately extend the drug longevity at targeted site (36).

Keeping in view the biological role of ELPs, first attempt was made to fuse IL-1Ra with two distinct sequences and molecular weights of ELP (37). In their study, they placed ELP at N-terminus of IL-1Ra (ELP-IL-1Ra). The genes of both proteins (IL-1Ra and ELP) were overexpressed in *Escherichia coli* (*E. coli*). Surface plasmon resonance (SPR) analysis was done to find the ELP-IL-1Ra and the IL-1R binding interaction. They observed the phase-transitioning behavior of the fusion protein (ELP-IL-1Ra) utilizing UV–vis spectrophotometry and dynamic light scattering (DLS). They estimated the bioactivity of IL-1Ra by evaluating the antagonism of IL-1-mediated lymphocyte and thymocyte proliferation *in vitro*. Moreover, by using human intervertebral disc fibrochondrocytes, they also observed the expressions of IL-1-induced TNF α, matrix metalloproteinase 3 (MMP-3) and ADAMTS-4 messenger RNA expression. Immunoreactivity of ELP-IL-1Ra fusion protein and degradation products relative to commercial IL-1Ra was also assessed. Though, they observed the anti-IL-1 activity *in vitro*, but they found a reduced association interaction of ELP-IL-1Ra fusion protein and IL-1R when compared with that of the commercial IL-1Ra. On the other hand, they observed that the peptide liberated as a result of ELP domain proteolysis showed equivalent bioactivity against lymphocyte proliferation when compared to the commercial IL-1Ra. Shamji *et al.* suggested that ELP-IL-1Ra fusion protein can retain the characteristics of ELP along with the *in vitro* bioactivity of IL-1Ra.

Correspondingly, to enhance the half-life of IL-1Ra, another group also expressed the fusion of IL-1Ra and ELP (IL-1Ra-ELP) in *E. coli* (38). In their study, instead of placing ELP at N-terminus of targeted protein, they placed ELP at the C-terminus of IL-1Ra (IL-1Ra-ELP). The bioactivity of IL-1Ra-ELP was compared with the commercial IL-1Ra by determining the inhibition of IL-1β-proliferated RPMI 1788 cells. Although, both proteins (IL-1Ra-ELP and IL-1Ra) showed significant inhibition of the stimulatory effects of IL-1β but the EC_{50} value for IL-1Ra-ELP was found to be significantly higher (211.21 ± 0.31pM) as compared to the EC_{50} value of IL-1Ra (20.21 ± 0.85pM). Stability of immobilized IL-1Ra-ELP on the self-assembled monolayers (SAM) in RPMI 1640 media was determined and found that IL-1Ra was strongly stable on SAM surface. The half-life of soluble IL-1Ra-ELP was determined with and/or without THP-1 monocytes. It was found that no IL-1Ra was detected at early time points when IL-1Ra-ELP was cultured with THP-1 monocytes whereas, when cultured without THP-1 monocytes the half-life of soluble IL-1Ra-ELP appeared to be for only few hours. It was due to the reason that when IL-1Ra-ELP was cultured with THP-1 monocytes, IL-1Ra was scavenged initially by IL-1Rs on the membrane of THP-1 monocytes and these THP-1 monocytes secreted IL-1Ra at later time points. The expression levels of various pro-inflammatory cytokines (IL-1β, TNF-α, IL-6 and IL-8), chemokines (MIP-1α, MCP-1 and IFN-γ) and pro-wound healing cytokines (VEGF, IL-1Ra and IL-4) were also investigated using IL-1Ra-ELP fusion protein. Most interestingly, it was found that the expression levels of pro-inflammatory cytokines and chemokines were significantly attenuated by IL-1Ra-ELP whereas, the expression levels of pro-wound healing cytokines were significantly enhanced by IL-1Ra-ELP.

In above mentioned two studies (37,38), although IL-1Ra was successfully fused with ELP at two different positions *i.e.* N- and C-terminus respectively but the expression levels and yields of these two purified proteins (ELP-IL-1Ra and IL-1Ra-ELP) were not directly compared. Later on, another study was conducted in which the role of position of ELP in the fusion of IL-1Ra on the expression level and yield of purified recombinant ELP fusion protein was investigated with three other proteins (39). From the results, they found that the bioactivity of IL-1Ra-ELP was ~100-fold lower (38) than that of IL-1Ra. On the other hand, the activity of ELP-IL-1Ra was 500-fold lower than that of IL-1Ra (37). The highly significant difference in the bioactivity of these two fusion proteins (IL-1Ra-ELP and ELP-IL-1Ra) compared to their native protein (IL-1Ra) exhibit that the specific activities of these two fusion proteins are dependent upon the position in fusion construct (N- and/or C-terminus). Although, both the fusion proteins exhibited comparatively low specific activity when directly compared with that of commercial IL-1Ra but ELP-IL-1Ra showed only ~25% of activity of IL-1Ra-ELP. From their results, it had been concluded that IL-1Ra-ELP had greater level of expression than that of ELP-IL-1Ra (39).

Although, Shamji *et al.* (37) and Kim *et al.* (38) fused and overexpressed IL-1Ra with ELP at N- and C-terminus respectively and investigated the characteristics of fusion proteins (ELP-IL-1Ra and IL-1Ra-ELP) *in vitro* only. No *in vivo* studies had been conducted with these fusion proteins (ELP-IL-1Ra and IL-1Ra-ELP).

Fusion of IL-1Ra with Human Serum Albumin (HSA)

HSA is a naturally occurring abundant protein in plasma with long biological half-life of about 19 days. HSA performs multifunctions within the body (21). During the past decade, albumin fusion technology has also been used for the extension of half-lives of various proteins (40–44). The targeted proteins can be fused with either N- or C-terminus of HSA or with both ends of HSA (45–47). After fusing with target protein, HSA is widely distributed within the body having negligible potential for confounding immunological function. HSA also improves the half-lives of targeted proteins, reduces the dosing intervals and frequency, and improves their stability (41,42,48).

On the basis of therapeutic advantages of albumin fusion technology, Dai *et al.* (49) fused IL-1Ra with HSA and expressed in *Pichia Pastoris* (*P. pastoris*). In their study, they investigated the expression phenomenon of fusion protein (HSA-IL-1Ra) by removing glycosylation and different ways of fusion by using different clones and different expression hosts. From their results, they found that although glycosylation might had its impact on expression of HSA-IL-1Ra but different clones and different expression hosts could also influence the heterogeneity of HSA-IL-1Ra. Correspondingly on the basis of this study, another study was conducted (50) in which HSA-IL-1Ra was purified by using affinity chromatography and ion-exchange chromatography techniques and found 98% pure HSA-IL-1Ra. The *in vitro* bioactivity of HSA-IL-1Ra was also detected by using IL-1-induced A375.S2 apoptotic cells. The *in vitro* bioactivity of HSA-IL-1Ra was found to be almost similar to that of IL-1Ra alone.

P. pastoris system that provides rapid growth and expression of fusion proteins is currently being used for the expression of many albumin fusion proteins (40,43,44). It has also been previously noticed that several factors may influence the production of fusion protein from *P. pastoris* such as properties of nucleotide sequences, gene copy number, and protein folding (51–53) but the most important one is decreased bioactivity (41) and in turn, higher doses of albumin fusion proteins are usually required to achieve desired therapeutic effects (54–56). Therefore to increase the production of albumin fusion protein of IL-1Ra in *P. pastoris*, an attempt was made to augment the expression of IL-1Ra and HSA fusion protein (IH) in *P. pastoris* by increasing IH gene copy number (57). Furthermore, they also investigated the influence of co-expression of protein disulfide isomerase (PDI) and immunoglobulin binding protein (BiP). From their results, they concluded that the expression of albumin fusion protein (IH) of IL-1Ra was significantly increased by increasing the gene copy number of IH gene when directly compared with that of single copy number of IH gene strain. Similarly, the expression of IH protein with high copy number of IH gene was also increased when it was co-expressed with PDI as compared to that of single copy number of IH gene strain whereas, the expression of IH protein was significantly decreased when it was co-expressed with BIP.

Although, the expression of albumin fusion protein of IL-1Ra was augmented by increasing the gene copy number of albumin fusion protein of IL-1Ra and co-expressed PDI but *in vitro* and/or *in vivo* have not been investigated yet.

Fusion of IL-1Ra with Albumin Domain Antibodies (AlbudAbs)

Human domain antibodies (dAbs) are known for their presence in cultures of mammalian cell and bacteria. dAbs are recognized to have small molecular weight of about 11–13 kDa but are considered to be highly stable (58) and functional binding units of human immunoglobulins contain only a single heavy or light chain of variable domains (59). In contrast to non-serum albumin-binding dAbs, Albumin-binding domain antibodies (AlbudAbs) are highly specific for mouse, rat and human serum albumin and the half-lives of these AlbudAbs are identical to that of serum albumin (60). When compared with other fusion technologies (48,61), AlbudAbs fusion technology use smaller size of dAbs fusion partner for extending the serum half-life of proteinous drugs.

On the basis of advantages of AlbudAbs fusion technology, Holt *et al.* (60) used two AlbudAbs (dAbm16 and dAbh8) with MSA reactivity to create genetic fusion of IL-1Ra. Both forms of AlbudAbs were overexpressed in *E.coli*. *In vitro* SA binding on Biacore and *in vitro* bioactivity of fusion proteins were performed by testing their ability to neutralize the induction of IL-1α-stimulated IL-8 in MRC-5 cells. For both fusion proteins, the SA binding and IL-1Ra activity was unaffected due to the presence of fusion partner. dAbm16-IL-1Ra was further subjected to investigate the *ex vivo* serum stability by immunoblot analysis and pharmacokinetics in mice to test the activity of the SA-binding dAb *in vivo*. They found that the fusion of dAbm16-IL-1Ra stayed over 90% intact when incubated at 37°C in mouse serum for 1.5 days and *in vivo* half-life of dAbm16-IL-1Ra was significantly high ($t_{1/2}$=4.3 h) when compared directly with that of IL-1Ra alone ($t_{1/2}$=2 min). From the value of half-life, they concluded that the half-life of dAbm16-IL-1Ra was not significantly as long as for either AlbudAb alone or for other AlbudAb fusion proteins. It might be due the reason that some other mechanism specific for IL-1Ra may be involved too for the clearance of fusion protein (dAbm16-IL-1Ra) from blood stream. Additionally, they also compared the biodistribution of dAbm16-IL-1Ra with IL-1Ra alone and/or MSA in mice. From their results, they found that IL-1Ra was detected in renal cortex and bladder confirming rapid elimination from the blood *via* glomerular filtration whereas, dAbm16-IL-1Ra adopted a pattern of tissue distribution that was approximately indistinguishable to that of MSA. Furthermore, the *in vivo* bio-efficacy of dAbm16-IL-1Ra was also investigated in the collagen induced arthritis (CIA) rat model. dAbm16-IL-1Ra exhibited greater efficacy for the treatment of CIA as compared to that of IL-1Ra alone at same dose despite that fact that dAbm16-IL-1Ra provided that half moles of IL-1Ra for CIA treatment as compared to IL-1Ra alone.

Due to its small molecular weight, AlbudAbs fusion technology may only slightly increase the molecular weight to a target protein which allows more moles of therapeutic moiety in the form of given dose. AlbudAbs fusion technology is especially important for small molecular weight proteins in contrast to other fusion technologies (62) without the size and manufacturing disadvantages (60). Despite of its advantages on other fusion technologies, further *in vivo* and pre-clinical studies are also required to investigate the therapeutic effects of AlbudAbs-IL-1Ra fusion protein against different autoimmune diseases.

MUCOSAL DELIVERY OF IL-1RA BY SPORULATING RECOMBINANT BACTERIA

There are some limitations for the parental delivery of therapeutically active proteins such as stability and high cost for their final purified materials. These problems can be evaded by the direct administration of recombinant bacteria that may acts as simultaneously cell factory and delivery system for proteinous drugs. This delivery system is most commonly known as mucosal delivery that delivers therapeutic proteins *via* sporulating bacteria and exhibits many advantages such as cheap and can be developed easily within friendly environment. The use of live bacteria is most commonly used for the delivery of vaccines. Purified bacterial components may have a chance to cause immune response whereas, live bacteria do not cause such type of immune reactions as these bacteria totally depend on the host for their ability to colonize and then enter the host organs with same pattern as their virulent counterparts. Similarly, gene coding for targeted protein in mucosal delivery is incorporated in bacterial carrier rather than in host cells. The incorporation of targeted protein gene in bacterial carrier allows the complete control of its durability in body. Mucosal delivery system has already been applied on local delivery of antibodies (63,64), cytokines (65,66),vaccines (67), and anti-inflammatory cytokines (68–70).

An attempt was made to deliver IL-1Ra *via* sporulating bacteria by Ricci and colleagues (70). They used *Streptococcus gordonii* (*S. gordonii*) for the delivery of IL-1Ra at mucosal target sites. They constructed the *S. gordonii* strain secreting IL-1Ra by composing four amino acid residues (RVFP) of *S. gordonii* at its N-terminus with IL-1Ra. The *in vitro* bioactivity of RFVP/IL-1Ra was assessed on IL-1β-induced lymphocyte proliferation and then compared with that of commercial IL-1Ra. The *in vitro* inhibitory activity of RFVP/IL-1Ra was 1.25×10^6 Antagonist Units (AU)/mg whereas, the *in vitro* inhibitory activity IL-1Ra was 1.0×10^6 AU/mg. furthermore, in order to verify that the presence of residue amino acids (RFVP) in RFVP/IL-1Ra did not influence the bioactivity of RFVP/IL-1Ra produced by *S. gordonii*, RFVP/IL-1Ra was expressed in *E.coli* and then compared it's *in vitro* inhibitory activity with that of IL-1Ra. Dose dependent inhibitory effects of RFVP/IL-1Ra expressed in *E.coli* was equivalent to that of IL-1Ra with same inhibitory activity as measured when RFVP/IL-1Ra expressed in *S. gordonii*. Similar bioactivity of RFVP/IL-1Ra expressed in *E.coli* and/or *S. gordonii* signified that there was no role of RFVP in the bioactivity of IL-1Ra produced by *S. gordonii*. The *in vivo* bioactivity of IL-1Ra released from *S. gordonii* was also assessed at vaginal and gastrointestinal mucosa of mice. IL-1Ra released from *S. gordonii* was detected up to day 11 from the last inoculum in the stomach and caecum. The beneficial effects of IL-1Ra released from *S. gordonii* was also assessed on ulcerative colitis represented in murine model of IBD in IL-2 −/− mice and found that recombinant *S. gordonii*, that is able to release IL-1Ra in the GI tract, could recover the symptoms of ulcerative colitis.

Another attempt was made to deliver IL-1Ra *via* sporulating bacteria by Porzio and colleagues (71) but they used

Bacillus subtilis (*B. subtilis*) instead of *S. gordonii* to produce IL-1Ra. They cloned cDNA coding for IL-1Ra in pSM539 strain of *B. subtilis* and instilled recombinant *B. subtilis* in the distal colon of rats and rabbits. They detected intact IL-1Ra in intestinal lavage and serum of treated animals up to significant extent. Similarly, recombinant *B. subtilis* and purified IL-1Ra was also administered intra-colonically to compare the pharmacokinetic parameters. IL-1Ra released by recombinant *B. subtilis* exhibited shorter C_{max} (0.136 μg/ml) when compared directly with C_{max} (0.482 μg/ml) of purified IL-1Ra whereas, the value of T_{max} for IL-1Ra released by recombinant *B. subtilis* was significantly high (200 min) as compared to the T_{max} of purified IL-1Ra (60 min). The AUC/dose for both type of IL-1Ra was similar to each other. The *in vitro* bioactivity of IL-1Ra released from *B. subtilis* was also measured on IL-1β-proliferated thymocytes and then compared with that of purified IL-1Ra. The *in vitro* bioactivity of IL-1Ra released by *B. subtilis* was identical with that of purified IL-1Ra. The *in vivo* bioactivity of IL-1Ra released by *B. subtilis* was assessed by measuring the ability of IL-1Ra released from intra-colonic administration of recombinant bacteria (*B. subtilis*) to inhibit the IL-1β-stimulated induction of fever, neutrophilia, hypoferremia and hypoglycemia. IL-1Ra released by *B. subtilis* attenuated the systemic effects of IL-1β more significantly as compared to that of saline and/or control treated groups.

Although, in these two studies, two different strains of bacteria were used and significant therapeutic outcomes were achieved in both studies (70,71) but the main advantage of using *B. subtilis* over *S. gordonii* was that *B. subtilis* allowed rapid release of IL-1Ra followed by absorption into the blood stream as compared to that of IL-1Ra released from *S. gordonii*. The recombinant *B. subtilis* permitted an accurate release of IL-1Ra into the blood stream to achieve desired therapeutic effects whereas, similar type of effects could not be achieved with *S. gordonii* as the amount of IL-1Ra released might be influenced by dissimilarity of colonization capacity that was dependent on discrepancy of environmental conditions of the host tissues (71).

DEVELOPMENT OF NEW DOSAGE FORMS FOR SUSTAINED RELEASE/DELIVERY OF IL-1RA

Biodegradable- and biocompatible-based delivery strategies have gained considerable attention for the sustained delivery (72–74) of short half-life proteinous drugs (75–77) without losing their effectiveness and bioactivity (78–80). The advantage of sustained delivery of proteinous drugs over protein fusion technology is that this delivery strategy avoids resistance to microorganisms (81–83). Therefore, in order to reduce the dosing frequency and intervals, sustained release and steady-state delivery of IL-1Ra were investigated using various techniques and biodegradable polymers.

Development of IL-1Ra-Loaded PLGA Microspheres

PLGA a biodegradable and biocompatible polymer that has been approved for use in human from FDA (84) is one of the most extensively used polymer for the development of sustained steady-state delivery of therapeutic proteins (85–87). PLGA particles are produced by polymerization of lactic acid with glycolic acid residues through the formation of ester linkage (88). Upon administration of PLGA into the body, the esterase enzymes in the blood hydrolyze ester linkage of PLGA and allow the polymer to release drug into the blood stream and after releasing the drug, polymer acids are eliminated from the body *via* metabolism (89) to toxicologically acceptable metabolites.

Firstly, Lavi *et al.* (90) encapsulated IL-1Ra within PLGA microspheres and investigated the *in vitro* and *in vivo* sustained steady-state release of IL-1Ra from IL-1Ra-loaded PLGA microspheres. They enhanced the stability of encapsulated IL-1Ra by co-encapsulating IL-1Ra-loaded PLGA microspheres with albumin and trehalose. *In vitro* release of IL-1Ra was assessed by BCA protein assay kit and found initial burst release followed by continuous release up to 16 days. Similarly, the *in vitro* bioactivity of IL-1Ra loaded in PLGA microspheres was assessed by mouse embryo fibroblast culture and found to be effective to inhibit IL-1β-induced secretion of IL-6 from embryo fibroblast culture after 7 days. Then, they administered IL-1Ra-loaded microspheres subcutaneously into BALB/c mice and compared the serum levels of IL-1Ra released from PLGA microspheres with that of IL-1Ra administered either subcutaneously and/or intravenously. Serum levels of IL-1Ra released from PLGA microspheres were significantly high (when compared with that of IL-1Ra) and maintained this level for up to 8 days. Furthermore, *in vivo* bioactivity of IL-1Ra-loaded PLGA microspheres was also assessed on mice by measuring the ability of IL-1Ra to attenuate IL-1β-induced secretion of IL-6. Sustained release of IL-1Ra from PLGA microspheres was found to be effective in inhibiting the IL-1β-induced production of IL-6 over the course of 2 weeks.

Lavi *et al.* (91) performed another study in which they characterized IL-1Ra-loaded PLGA microspheres by using some additional techniques. In their results, they determined similar pattern of *in vitro* protein content measured by Micro BCA protein assay reagent kit as in their previous study (90) but they used ELISA and MALDI-TOF to predict the levels of IL-1Ra release from PLGA microspheres. By using ELISA and MALDI-TOF, the *in vitro* release pattern of IL-1Ra was found to be almost similar to that of their previous study (90). The *in vitro* bioactivity of IL-1Ra released from IL-1Ra-loaded PLGA microspheres was assessed by measuring

its ability to inhibit IL-1β-induced cell proliferation in melanoma B16 cell cultures. PLGA microspheres could sufficiently allow the sustained steady-state release of IL-1Ra on daily basis that could effectively block cell proliferation till one week. Serum levels of IL-1Ra released from IL-1Ra-loaded PLGA microspheres was also determined and compared with that of IL-1Ra alone after subcutaneous administration of both forms of IL-1Ra. Similar pattern of serum levels of IL-1Ra released from PLGA microspheres was also observed in previous study (90). *In vivo* bioactivity of IL-1Ra released from IL-1Ra-loaded PLGA microspheres was also found to be similar as in their previous study (90). Sustained steady-state delivery of IL-1Ra released from IL-1Ra-loaded PLGA microspheres was also found to be effective to attenuate the B16 melanoma tumor growth in mice. After inoculation with B16 melanoma tumor cells, mice treated with IL-1Ra-loaded PLGA microspheres had significantly improved survival as compared with control- and/or non-treated groups. Sustained steady-state delivery of IL-1Ra released from IL-1Ra-loaded PLGA microspheres also reduced the tumor vascularization and number of lung metastasis in tumor-amputated mice more significantly as compared to that of control- and/or non-treated groups.

Another attempt was also made to encapsulate IL-1Ra with PLGA microspheres by Gorth *et al.* (92) but they used 75:25 PL/GA copolymer ratio of PLGA instead to 50:50 PL/GA copolymer ratio that was used by Lavi *et al.* in their study for the development of IL-1Ra-loaded PLGA microspheres (90,91). The *in vitro* release profile of IL-1Ra released from PLGA microspheres initially showed burst release followed by an approximate decrease to linear and sustained steady-state release up to first 10 days. Furthermore, the *in vitro* therapeutic activity of IL-1Ra released from IL-1Ra-loaded PLGA microspheres was assessed for the sustained attenuation of IL-1β-mediated degradation in the nucleus pulposus (NP). IL-1Ra released from IL-1Ra-loaded PLGA microspheres could sufficiently block the degenerative effects of IL-1β in NP that was further confirmed by mechanical properties of NP, glycosaminoglycans and collagen content, nitrites release and mRNA expression of inflammatory mediators for 7 days.

From the results of IL-1Ra-loaded PLGA microspheres (90–92), although, PLGA microsphere technology has significantly increased the half-life of IL-1Ra and its overall therapeutic efficacy but this technique may have some drawbacks such as the microspheres of PLGA which are usually hydrophobic in nature are usually prepared from emulsification process with proven safety (93) but the therapeutic proteins are usually hydrophilic in nature and difficult to encapsulate within these hydrophobic polymer microspheres (94,95) which may cause the loss of proteins bioactivity due to its hydrophobic nature and total protein loss during loading of protein in microencapsulation process (88,96,97) and result in initial burst release (98,99).

Development of PEGylated IL-1Ra

Polyethylene glycol (PEG) is a polyether compound that is most widely used in pharmaceutical applications. PEG has also been approved from FDA for delivery of therapeutic agents (100). PEGylation is a phenomenon in which therapeutic protein is attached with PEG (101). PEGylated therapeutic proteins have prolonged half-lives, reduced immunogenicity and improved pharmacokinetic and pharmacodynamic properties as compared to their native proteins (100,102). Various therapeutic proteins have been successfully PEGylated with PEG which had significantly prolonged their biological half-lives (103–106).

Yu *et al.* (107) extended this approach to PEGylate IL-1Ra using two PEG targets *i.e.* lysine-target (SCM-PEG) and thiol-target (MBS-PEG). In SCM-PEG conjugate of IL-1Ra, the conjugation site was lysine residues whereas in MBS-PEG conjugate of IL-1Ra, the conjugation site was cysteine residues. After PEGylation, they investigated the *in vitro* binding activity of these two conjugates on receptor using EL-4/CTLL-2 cells. SCM-PEG conjugate of IL-1Ra exhibited only 9.8% of binding activity while MBS-PEG conjugate of IL-1Ra exhibited 40% binding activity. Decreased binding activity of SCM-PEG conjugate of IL-1Ra was because of random attachment of PEG at receptor site. This decreased binding activity of SCM-PEG conjugate of IL-1Ra was in consistent with PEGylated interleukin-15 (108) and lysozyme (109). PEGylation directly influences the binding affinity of therapeutic proteins to cellular receptors which ultimately results in decrease *in vitro* bioactivity of these proteins however; this negative effect of PEGylation may be offset in biological systems by increasing the residence time of drug in serum (101). Yu *et al.* (107) performed only *in vitro* studies therefore; it is rather difficult to predict the application of PEGylated strategy of IL-1Ra in *in vivo* environment.

Development of IL-1Ra-Loaded Complex Coacervation Thermo-Reversible Gel

In order to achieve desirable sustained steady-state therapeutic effects of IL-1Ra, thermo-reversible and coacervation gel technology was applied for sustained delivery of IL-1Ra by Jun *et al.* (110). This technique has already been applied for sustained delivery of therapeutic proteins (111–113). Two oppositely charged macromolecules form complex coacervation by electrostatic interaction. The formation of complex coacervation increases the stability of protein inside the gel and prolongs sustained release of proteins (113). Jun *et al.* (110) prepared complex coacervation thermo-reversible gel with IL-1Ra and cationic macromolecules followed by co-formulation with methylcellulose as negative thermosensitive macromolecule. They inserted the gel containing IL-1Ra in the upper part of transwell system and in lower part, they

placed human osteoarthritis (OA) chondrocytes having IL-1β. Then they stimulated the OA chondrocytes with IL-1β and investigated the sustained steady-state effects of IL-1Ra released from complex coacervation by measuring the expression of matrix metalloproteinases (MMPs) with RT-PCR and ELISA. IL-1Ra released from complex coacervation system followed a sustained release pattern for extended period of time with minimal initial burst release. From the results of RT-PCR and ELISA, they found that IL-1β increased the expression of MMPs whereas, IL-1Ra released from complex coacervation system significantly inhibited the ameliorating effects of IL-1β by inhibiting IL-1β-induced expression of MMPs in OA chondrocytes.

Although, Jun *et al.* (110) developed a novel drug delivery system for the sustained steady-state delivery of IL-1Ra to its target site but in this study, they did not performed *in vivo* experiments. Therefore, it cannot be commented that whether this system will be helpful inside the *in vivo* environment or not and as well as for other auto-immune diseases.

Development of IL-1Ra-Loaded Self-Assembled Nanoparticles

Therapeutic delivery of IL-1Ra is a big challenge to maintain its plasma concentration up to desired levels. Although, microencapsulation of IL-1Ra with biodegradable polymers have overcome the shortcomings of IL-1Ra (90–92,110) but this technology may have some limitations for IL-1Ra as the polymers used in these studies are hydrophobic in nature, having low drug loading efficiency and worse chemical conditions during processing (88,96,97). Keeping in view the drawbacks of these delivery technologies, Whitemire *et al.* (114) designed a new block copolymer that assembled in to the submicron-scale particle after modifying the commercially available μ-RAFT agent with a paranitrophenol (pNP) and then used to polymerize the tetraethylene glycol methacrylate (TEGM) monomers to create the hydrophilic block copolymer. They confirmed the hydrophilic block polymer cytotoxicity by MTT assay and found to be non-toxic. Then IL-1Ra was tethered on to the surface of these hydrophilic block copolymers for controlled intra-articular delivery of IL-1Ra. They confirmed the binding efficiency of IL-1Ra with block copolymer using dot blot analysis with IL-1Ra antibody. The target specificity of IL-1Ra-tehtered nanoparticles was assessed on target HIG-82 synoviocyte cells *via* surface IL-1 receptors. The *in vitro* bioactivity of IL-1Ra-tethered nanoparticles was confirmed by measuring the effectiveness of IL-1Ra-tethered nanoparticles by blocking IL-1β-induced activation of NF-kB and found almost similar with that of IL-1Ra alone. The *in vivo* retention time of IL-1Ra-tethered nanoparticles was also measured by administering IL-1Ra-tethered nanoparticles and/or IL-1Ra alone in to the stifle joint *via* intra-articular injection and then compared with that of IL-1Ra alone. Dramatically, IL-1Ra-tethered nanoparticles augmented the half-life of IL-1Ra (3.01 days) significantly very high as compared to that of IL-1Ra alone (0.96 days). They also confirmed the retention time of IL-1Ra-tethered nanoparticles with confocal microscopy. IL-1Ra-tethered nanoparticles did not cause any alterations in cartilage structure and morphology.

In this study, significant therapeutic outcomes have been attained by the authors (114) more importantly the half-life of tethered IL-1Ra was significantly prolonged for up to 3 days as compared to that of IL-1Ra alone but on contrary, a complex mechanism is involved for the generation of hydrophilic block copolymers for the tethering of IL-1Ra on their surface. Further studies are also required to investigate the stability and long term therapeutic effects of tethered IL-1Ra on various auto-immune diseases.

Development of IL-1Ra-Loaded Dextran Microparticles

In order to prolong the therapeutic outcomes of IL-1Ra, although, IL-1Ra has been extensively studied after encapsulation with biodegradable polymers and confirmed its steady-state release from microspheres (90–92,110,114) but however, these methodologies may cause the loss of bioactivity of IL-1Ra due to its exposure to water/oil interfaces (90,91). To keep the bioactivity of IL-1Ra, Liang *et al.* (115) developed a new method which protected the IL-1Ra from water/oil interfaces. They prepared IL-1Ra-loaded dextran microparticles by dissolving IL-1Ra in water along with dextran and PEG. Subsequently, IL-1Ra-loaded dextran microparticles were obtained after lyophilizing the mixture by freeze drying and then washed the dried powder with dichloromethane to remove the PEG. They also prepared controlled dextran microparticles by water-in-oil-in-water (W/O/W) emulsion method. IL-1Ra-loaded dextran microparticles exhibited spherical shape, smooth surface and 1–5 μm diameters whereas, controlled dextran microparticles showed holes on its surfaces with wide size distribution. The encapsulation efficiency of IL-1Ra-loaded dextran microparticles was checked by ELISA and found 98.5% while controlled dextran microparticles showed only 70.5% encapsulation efficiency using W/O/W method. SEC-HPLC analysis was used to check the aggregation of IL-1Ra after encapsulation in dextran microparticles and was compared with that of controlled dextran microparticles. IL-1Ra-loaded dextran microparticles showed same phenomenon of aggregation as in original IL-1Ra solution whereas, the monomer contents of IL-1Ra in controlled dextran microparticles were less as compared to that in IL-1Ra-loaded dextran microparticles. The *in vitro* bioactivity of IL-1Ra-loaded dextran microparticles was determined to assess the inhibitory effects of IL-1Ra on IL-1β-induced proliferation

of melanoma B16 cancer growth cells. The bioactivity of IL-1Ra released from IL-1Ra-loaded dextran microparticles was found to be almost similar to that of original IL-1Ra solution whereas, the bioactivity of IL-1Ra released from controlled dextran microparticles was only 30% than that of IL-1Ra-loaded dextran microparticles.

From the above results, it has been clearly observed that the dextran microparticles not only increased the encapsulation efficiency of IL-1Ra and reduced the rate of aggregation but also significantly increased the *in vitro* bioactivity of IL-1Ra as compared to that of IL-1Ra released from controlled dextran microparticles. Although, Liang *et al.* (115) obtained desirable outcomes after loading the IL-1Ra into dextran microparticles but further studies are still required to investigate its *in vivo* therapeutic effects against various autoimmune diseases.

Development of PF127-Based Thermosensitive Gel

Apart from all above mentioned strategies for the sustained delivery of IL-1Ra, recently our group has also developed a new dosage for sustained delivery of IL-1Ra using PF127-based thermosensitive gel (116–118). PF127 is biodegradable polymer and has been approved from FDA for use in human. PF127 has thermoreversible and thermosensitive gelation property at concentration of 20–35% (119) and has its ability to prolong the stability and extend the half-life of proteins (76,77,119–121). PF127 also has unique property to be dissolved in excess buffer at body temperature (75). The most important advantage of PF127-based thermosensitive gel over the other sustained release dosage form formulations for proteins is that it involves simple mixing and prevents the exposure of proteins to overheating and chemical exposure during gel preparation.

On the basis of advantages of PF127 over other polymers, we (116) firstly investigated the effect of different concentrations of PF127 (20, 25 and 30%) on sustained release of IL-1Ra from PF127 gel. We also investigated the *in vivo* bioactivity and pharmacokinetic profiles of IL-1Ra-loaded PF127 gel on Wistar rats. The *in vitro* release pattern of IL-1Ra from PF127 gel followed zero-order release kinetics without showing initial burst release. On the basis of *in vitro* results, we used 20 and 25% PF127 gel for *in vivo* characteristics. PF127 exhibited the prolong release of IL-1Ra in plasma of Wistar rats as compared to that of IL-1Ra solution alone. The half-life of IL-1Ra loaded in 25% PF127 gel was significantly high (12.53 ± 2.48 h) as compared to that of IL-1Ra solution (1.18 ± 2.85 h). When directly compared with that of IL-1Ra solution alone, PF127 gel was able to maintain and prolong the *in vivo* bioactivity of IL-1Ra as assessed by its ability to inhibit IL-1β-stimulated induction of IL-6 in Wistar rats. Further, we assessed the drug-polymer interaction and stability of IL-1Ra in PF127 gel stored at different temperatures for 3 months (117). It was exhibited by the results of SDS-PAGE and DSC that IL-1Ra was absolutely stable in PF127 gel during its stability study period. Moreover, during the stability period, we also found that there was no drug-polymer interaction as confirmed by FTIR spectra.

Furthermore, on the basis of these results (116,117), we extended this approach to investigate the sustained release effects of IL-1Ra loaded in 25% PF127 thermosensitive gel against T2DM using diabetic GK-rats (118). T2DM is an auto-inflammatory syndrome in which various inflammatory responses and mechanisms are involved to induce T2DM (4,5,122). Being anti-inflammatory in nature, IL-1Ra has already been proven to be effective in reducing hyperglycemia (123–126) in diabetic rat models and human beings (127,128) however short half life of IL-1Ra remained as a limitation. Therefore, we used diabetic GK rats (129) to investigate the sustained release effects of IL-1Ra loaded in 25% PF127 gel (118). We administered IL-1Ra either alone or loaded in PF127 gel (10 mg/kg/day in 200 μl PF127 gel) through subcutaneous route for one month. In addition we monitored serum glucose level 2–3 times per week. Before the end of treatment, we also performed intraperitoneal glucose tolerance test (IPGTT). At the end of treatment, we measured various biochemical parameters and kidney function markers. Moreover, we also performed immunohistochemistry (IHC) and histological examination of the skin. IL-1Ra loaded in PF127 gel exhibited prolonged and sustained effects by reducing hypoglycemia in treated animal group. IL-1Ra loaded in PF127 gel also increased glucose tolerance, insulin sensitivity and β-cell's secretary function. IHC results confirmed that macrophage infiltration in pancreatic islets was significantly reduced in IL-1Ra loaded PF127 gel treated GK rats. Histological analysis of the skin confirmed that no significant alteration in the normal physiological functions of the skin and kidneys occurred in IL-1Ra loaded PF127 gel treated Gk rats.

FUTURE PERSPECTIVES

Up till now, delivery strategies have been investigated to extend the half-life of IL-1Ra directly using fusion protein technology and/or its sustained delivery using various biodegradable polymers (Fig. 1). In these delivery strategies, only few studies have been conducted on animals to investigate *in vivo* bioactivity of IL-1Ra either fused with other fusion protein partner and/or incorporated with biodegradable polymer (Table I). Therefore, it is necessary that these delivery strategies must be applied on various disease animal models to investigate *in vivo* therapeutic effects as without performing *in vivo* studies, it is unpredictable to conclude the pharmacological effects and release efficacy of IL-1Ra *via* these delivery strategies. Moreover, a single delivery strategy

Table I Administration Routes and Therapeutic Efficacies of Different Delivery Strategies for IL-1Ra

Sr.#	Delivery strategy	Route of administration	Investigations	References
1	ELP-IL-1Ra	Not Investigated	*In vitro* bioactivity against IL-1-induced lymphocyte proliferation and IL-1-induced proliferated RPMI 1788 cells	(37)
2	IL-1Ra-ELP	Not Investigated	*In vitro* bioactivity against IL-1β-proliferated RPMI 1788 cells. Stability of immobilized IL-1Ra-ELP on SAM cells. mRNA expression of pro-inflammatory cytokines decreased while wound healing cytokines increased	(38)
3	HSA-IL-1Ra	Not Investigated	*In vitro* bioactivity against IL-1-induced A375.S2 apoptotic cells. Expression of fusion proteins by increasing the gene copy number as well as co-expressed with PDI.	(49,50,57)
4	dAbm-IL-1Ra	Intravenous Route	*In vitro* bioactivity against IL-1α-stimulated IL-8 in MRC-5 cells. *In vivo* half-life and *in vivo* bio-efficacy of dAbm16-IL-1Ra in CIA rat model.	(60)
5	RFVP/IL-1Ra	Mucosal Route	*In vitro* Bioactivity against IL-1β-induced lymphocyte proliferation. *In vivo* bioactivity.	(70)
6	IL-1Ra-producing pSM539 strain of *B. subtilis*	Mucosal Route	*In vitro* bioactivity against IL-1β-induced lymphocyte proliferation. Pharmacokinetic parameters and *in vivo* bioactivity.	(71)
7	IL-1Ra-loaded PLGA Microspheres (50:50 PL/GA)	Subcutaneous Route	*In vitro* sustained release with initial burst release followed by continuous release up to 16 days. *In vitro* bioactivity against IL-1β- induced secretion of IL-6 from embryo fibroblast culture and melanoma B16 cell cultures. *In vivo* bioactivity against IL-1β-induced production of IL-6 over the course of 2 weeks.	(90,91)
8	IL-1Ra-loaded PLGA Microspheres (75:25 PL/GA)	Not Investigated	*In vitro* sustained release with initial burst release followed by continuous release up to 10 days. *In vitro* bioactivity against IL-1β-mediated degradation in NP.	(92)
9	PEGylated IL-1Ra	Not Investigated	*In vitro* binding activity to receptor using EL-4/CTLL-2 cells	(107)
10	IL-1Ra-loaded Coacervation Thermo-reversible Gel	Not Investigated	*In vitro* bioactivity against IL-1β-induced expression of MMPs in OA chondrocytes.	(110)
11	IL-1Ra-loaded Self-assembled Nanoparticles	Intra-articular Route	*In vitro* bioactivity against IL-1β-induced activation of NF-kB. *In vivo* half-life of IL-1Ra-tethered nanoparticles.	(114)
12	IL-1Ra-loaded Dextran microparticles	Not Investigated	Encapsulation efficiency determination. *In vitro* bioactivity against IL-1β-induced proliferation of melanoma B16 cancer growth cells.	(115)
13	IL-1Ra-loaded PF127 Thermosensitive Gel	Subcutaneous Route	*In vitro* and *in vivo* sustained release characteristics and correlation. *In vivo* bioactivity IL-1β-induced production of IL-6. Drug-polymer interaction. Stability study at different temperatures for 3 months. One month *in vivo* study on diabetic GK-rats.	(116–118)

ELP Elastin-like peptide, *IL-1Ra* Interleukin-1 receptor antagonist, *HSA* Human serum albumin, *dAbs* human domain antibodies, *RFVP* Amino acid residues of *Streptococcus gordonii*, *PLGA* Poly-lactic-co-glycolic acid, *PL/GA* Ratio of poly lactic acid to glycolic acid, *TNF-α* tumor necrosis factor-alpha, *IL-1β* Interleukin-1 beta, *PDI* Protein disulfide isomerase, *CIA* Collagen induced arthritis, *NP* Nucleus Pulposus, *MMPs* Matrix metalloproteinases, *NF-kB* Nuclear factor kappa B, *ELP-IL-1Ra* Fusion of IL-1Ra at N-terminus of ELP, *IL-1Ra-ELP* Fusion of IL-1Ra at C-terminus of ELP, *HSA-IL-1Ra* Fusion of IL-1Ra at N-terminus of HSA, *dAbm-IL-1Ra* Fusion of IL-1Ra at N-terminus of dAbm. *RFVP/IL-1Ra* IL-1Ra fused with RFVP

Table II Therapeutic Outcomes and Probable Limitations of Different Delivery Strategies for IL-1Ra

Sr.#	Delivery strategy	Therapeutic outcomes	Probable limitations	References
1	ELP-IL-1Ra	Maintained *in vitro* bioactivity	Reduced bioactivity as compared to IL-1Ra alone. Can sustain the characteristics of ELP. EC_{50} value is very high as compared to that of commercial IL-1Ra. Difficult to express and purify in desired microorganism. No *in vivo* studies conducted.	(37)
2	IL-1Ra-ELP	Maintained *in vitro* bioactivity more significantly as compared to ELP-IL-1Ra. Significantly inhibited the expression of pro-inflammatory cytokines and augmented the expression of wound-healing cytokines.	Reduced bioactivity as compared to IL-1Ra alone. EC_{50} value is high as compared to that of commercial IL-1Ra. Resistance of microorganism may influence the expression of fusion protein. No *in vivo* studies conducted.	(38)
3	HSA-IL-1Ra	Maintained *in vitro* bioactivity.	Difficult to express and purify in desired microorganism. Resistance of microorganism may influence the expression of fusion protein. No *in vivo* studies conducted.	(49,50,57)
4	dAbm-IL-1Ra	Maintained *in vitro* bioactivity and prolonged *in vivo* biological half-life.	Difficult to express and purify in desired microorganism. Resistance of microorganism may influence the expression of fusion protein.	(60)
5	RFVP/IL-1Ra	Maintained *in vitro* and *in vivo* bioactivity.	Difficult to express in desired microorganism. Slow release of IL-1Ra from recombinant bacteria. Poor pharmacokinetic activity.	(70)
6	IL-1Ra-producing *pSM539* strain of *B. subtilis*	Maintained *in vitro* and *in vivo* bioactivity. Maintained serum levels of IL-1Ra for longer period of time as compared to that of IL-1Ra.	Difficult to express in desired microorganism.	(71)
7	IL-1Ra-loaded PLGA Microspheres (50:50 PL/GA)	Prolonged sustained release. Maintained *in vitro* and *in vivo* bioactivity.	Initial burst release. Loss of bioactivity during encapsulation process and chemical exposure. No drug-polymer interaction investigated.	(90,91)
8	IL-1Ra-loaded PLGA Microspheres (75:25 PL/GA)	Prolonged sustained release. Maintained its *in vitro* bioactivity.	Initial burst release. Complicated process for preparation of microparticles. No drug-polymer interaction investigated. No *in vivo* studies conducted.	(92)
9	PEGylated IL-1Ra	*In vitro* binding activity	The binding activity of PEGylated IL-1Ra was significantly very low as compared to that of IL-1Ra alone.	(107)
10	IL-1Ra-loaded Coacervation Thermo-reversible Gel	Maintained *in vitro* bioactivity.	Complicated process for preparation of microparticles. High cost. No drug-polymer interaction investigated. No *in vivo* studies conducted.	(110)
11	IL-1Ra-loaded Self-assembled Nanoparticles	Maintained *in vitro* bioactivity. Prolonged *in vivo* biological half-life.	Complicated process for preparation of self-assembled nanoparticles. No drug-polymer interaction investigated.	(114)
12	IL-1Ra-loaded Dextran microparticles	Maintained its *in vitro* bioactivity.	Complicated process for preparation of microparticles. No *in vivo* studies conducted. No drug-polymer interaction investigated.	(115)
13	IL-1Ra-loaded PF127 Thermosensitive Gel	Prolonged sustained release. Increased half-life. Prolong therapeutic effects significantly in GK rat more significantly than IL-1Ra.	Requires temperature maintenance during administration.	(116–118)

ELP Elastin-like peptide, *IL-1Ra* Interleukin-1 receptor antagonist, *HSA* Human serum albumin, *dAbs* human domain antibodies, *RFVP* Amino acid residues of *Streptococcus gordonii*, *PLGA* Poly-lactic-co-glycolic acid, *PL/GA* Ratio of poly lactic acid to glycolic acid, *ELP-IL-1Ra* Fusion of IL-1Ra at N-terminus of ELP, *IL-1Ra-ELP* Fusion of IL-1Ra at C-terminus of ELP, *HSA-IL-1Ra* Fusion of IL-1Ra at N-terminus of HSA, *dAbm-IL-1Ra* Fusion of IL-1Ra at N-terminus of dAbm. *RFVP/IL-1Ra* IL-1Ra fused with RFVP

might be effective for a specific auto-immune disease depending upon the nature of disease. For instance, in case of osteoarthritis or rheumatoid arthritis, once a week injection of IL-1Ra is suitable to prevent the symptoms of arthritis but in case of T2DM, this strategy might not be suitable because of the fluctuation of blood glucose level. In addition, the delivery strategies for IL-1Ra discussed over here have shown to possess few probable limitations as summarized in Table II. Therefore, a better understanding of the physiological and biochemical barriers faced by these strategies might help a lot in developing best suitable approaches for the appropriate delivery and effectiveness of IL-1Ra for the treatments of various auto-immune diseases.

Fusion protein technology involves high cost and more purified materials are required. Purification and expression of fusion protein in expressed media is another big challenge. Resistance of microorganism may influence the expression of fusion protein in cultured bacteria. Similarly in development of new dosage forms of IL-1Ra, there are also some limitations as PLGA is hydrophobic in nature and it may cause the loss of IL-1Ra bioactivity and/or total protein during encapsulation. Another problem is that PLGA microsphere may not prevent the initial burst release of IL-1Ra. This initial burst release may cause the fluctuation in the plasma levels of IL-1Ra. Microencapsulation process is also a complex and complicated method. IL-1Ra-loaded PF127 thermosensitive gel have been investigated in disease specific animal model (diabetic GK rats) for continuous administration up to one month and significant therapeutic effects have been obtained as compared to that of commercial IL-1Ra alone. More interestingly, PF127 did not impart its effect on therapeutic efficacy of IL-1Ra and maintained the normal physiological functions of the skin and kidneys. We have first time concluded that PF127 thermosensitive gel technology is safe in the *in vivo* environment of diabetic GK rat model. However, it only requires a careful maintenance of temperature during administration and this methodology should also be applied on other auto-immune diseases such as arthritis, inflammatory bowel disease.

CONCLUSION

The therapeutic efficacy of IL-1Ra against various auto-immune diseases has been proven and confirmed by several studies but still it has some limitations due to its short biological half-life requiring frequent dosing. To overcome this shortcoming of IL-1Ra and improve patient compliance, several attempts have been applied to fuse IL-1Ra with other fusion proteins and/or peptides, and prepare different new dosage forms using different kinds of method for extension of half-life and/or sustained steady-state release from its dosage form. In this study, we have reviewed all the techniques and methodologies that have been applied up till now on IL-1Ra for the prolongation of its biological half-life and sustained its therapeutic effects for longer period of time along with probable limitation. However, there is still a need to focus on such methodologies for successful delivery of IL-1Ra.

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