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RESEARCH PAPER

Sustained Delivery of IL-1Ra from Pluronic F127-Based Thermosensitive Gel Prolongs its Therapeutic Potentials

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ABSTRACT

Purpose Pluronic F-127 (PF127) has previously shown to prolong the sustained release of various proteinous drugs and their serum half-lives. Subsequently, we have extended this approach to look at *in vitro* release, *in vivo* efficacy and pharmacokinetics of interleukin-1 receptor antagonist (IL-1Ra).

Methods Various concentrations of PF127 gels were prepared using cold method. *In vitro* drug release kinetic studies were performed using membraneless dissolution method. Stability of IL-1Ra was assessed by SDS-PAGE. *In vivo* studies and *in vivo* bioactivity of IL-1Ra were also performed on wistar rats.

Results IL-1Ra loaded PF127 gels showed *in vitro* sustained release of IL-1Ra, depending on the concentration of gel used. SDS-PAGE confirmed the stability of protein during its *in vitro* release. PF127 gel also exhibited prolonged release of IL-1Ra in rats as compared to that of IL-1Ra aq. solution. *In vivo* bioactivity of IL-1Ra loaded in gel was confirmed by its ability to inhibit IL-1β-stimulated induction of IL-6.

Conclusions When compared directly, IL-1Ra loaded PF127 gel exhibited prolonged *in vitro* and *in vivo* release, greater efficacy to induce hypoglycemia and inhibited IL-1β-stimulated production of IL-6 as compared to IL-1Ra aq. solution. We believe that this methodology for sustained delivery of IL-1Ra probably be suitable for the convenience of patients to achieve desired therapeutic potentials without exceeding dose limits and frequent administration.

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KEY WORDS Akaike information criterion · controlled release · interleukin-1 receptor antagonist · *in vitro* study · *in vivo* study · pharmacokinetics

INTRODUCTION

Along with the advancements and developments in the field of biotechnology, proteinous drugs have attained considerable attention to treat many incurable and life-threatening diseases. Besides their beneficial therapeutic effects, they have some limitations due to their short biological half-life, thereby; large amount of dose is usually required to obtain their desired therapeutic potentials. Recently, scientists and researchers are emphasizing more on improving the proficient delivery of proteins and bioactive peptides with shorter biological half-life.

Various techniques and methodologies are being investigated to overcome the problem of short biological half-life which is usually faced during the delivery of proteinous drugs into the body. IL-1Ra, a naturally occurring anti-inflammatory agent of IL-1 cytokine family (1), binds to interleukin-1 receptor-I (IL-1RI) without triggering any intracellular response (2). Recently, IL-1Ra has been used in many auto-immune diseases (3–7) but due to short biological half life (8), higher doses with shorter dosing intervals are required (9,10). The basic reason for short half-life of IL-1Ra is its low retention time in peripheral tissues except kidneys and its rapid excretion via urine (11,12). Due to high tissue-to-plasma ratio of IL-1Ra, kidneys work as the preferential route for its elimination from the body.

Since the last decade, various methodologies have been utilized to develop the existing parenteral dosage form of IL-1Ra (13–20) intended for patient compliance but most of these techniques are depicted to be complicated. Thereby, such techniques and methodologies are required to be investigated in which the desired outcomes may be achieved without facing complications. Among them, PF127, which

has a thermal reversible gelation property in aqueous solutions in the range of 20–35% concentration, (21) has been known for its ability to extend the stability of proteins (22) that are loaded into the gel. These proteins are completely recovered when the gel is dissolved in excess buffer even at body temperature (23). PF127 is known to have dual characteristics as it exists in solution form at room temperature but rapidly transforms into gel at body temperature. Due to this characteristic, after administration, PF127 rapidly forms a network of rigid semisolid gel inside the body. Besides convenience in administration, the foremost advantage of PF127 is its simple preparation; as it only requires a simple mixing process without undergoing extreme conditions such as overheating that may result in denaturation of proteinous drugs. Numerous previous studies have already confirmed that PF127 has the ability to achieve the sustained release of various proteinous drugs (21,24,25) but till now no one reported the use of PF127 for sustained delivery of IL-1Ra.

Thereby, the purpose of our present research was to develop PF127 based thermosensitive gel of IL-1Ra, and to investigate its effect on biological half-life of IL-1Ra during its release from PF127 gel. Moreover, we aimed to detect the probable effect of PF127 concentrations on the biological half-life of IL-1Ra, and the efficacy of IL-1Ra for blocking the secretion of IL-1β-induced IL-6. We hypothesized that the therapeutic effects of IL-1Ra loaded in gels might probably be more prolonged and sustained as compared to IL-1Ra aq. solution.

MATERIALS AND METHODS

Materials

IL-1Ra was kindly gifted by (Zhejiang Hisun Pharmaceutical Co., Ltd. Taizhou China). Pluronic PF127 (Shanghai Yunhong Pharmaceutical aids & technology Co. Ltd), Micro BCA protein assay reagent kit (Beyotime, Jiangsu Province, China), Human IL-1Ra/IL-1F3 Quantikine ELISA Kit, Rat IL-6 ELISA Quantikine kit, and recombinant rat IL-1β/IL-1F2 (R&D System, Inc., USA), glucose kit (Shanghai Rongsheng Biotech Co., China), microplate reader (Model 680, Bio-Rad, Japan). Other materials and reagents were at least analytical grade and used without further purification.

Preparation of Different Concentrations of PF127

We used various concentrations of pluronic PF127 (20%, 25%, 30% w/w) to investigate the optimal amount of pluronic for the development of thermosensitive gel having IL-1Ra by using cold method as previously described (26). Calculated amount of pluronic for each gel formulation was added carefully in sufficient amount of 0.9% NaCl solution at 4°C with continuous stirring of 100 RPM (BG-Stirrer4D1, Baygen Biotech Co.) and left overnight with continuous gentle mixing until complete dissolution occurred. Then specific amount of IL-1Ra (1 mg/g of gel) was added with continuous mixing. All formulations were kept at 4°C in refrigerator until further analysis.

Determinations of Gelation Temperature, Gelling Rate and Viscosity

Gelation temperature (GT) was measured as previously described (27). To calculate the gelling rate, 5 ml of sample solution and a magnet bar were transferred into a transparent vial with continuous stirring of 100 RPM, and placed in water bath at 37°C. The time (seconds) and temperature at which the solution lost its fluidity and magnet bar did not move further due to gelation, the gelling rate was calculated. The gelling rate for each solution was recorded in triplicate. The viscosity of all formulations was measured using Brookfield viscometer (Rotational viscometer (NDJ-1), spindle type 3, speed 30 RPM) at 25±0.5°C with programmable rheometer and ice-bath to maintain the temperature at 25°C (28).

In Vitro Release of IL-1Ra from PF 127 Gels

The *in vitro* release of IL-1Ra from thermosensitive gel was studied in release medium (0.9% NaCl) using membraneless dissolution method as previously described (24). Temperature of dissolution media was kept at 37±0.5°C. The cold PF127 (2 g) solutions containing IL-1Ra (1 mg/g of gel) were transferred into clear transparent vial and incubated at 37°C until clear gel was formed. 5 ml of 0.9% NaCl pre-equilibrated at 37°C was layered over the surface of gel in each vial; these were then kept in thermostatic shaker (100 RPM, 37°C). At pre-determined time intervals, the release medium was totally replaced with the fresh medium and same process was repeated until complete dissolution occurred. Prior to assay, the samples were diluted with fivefold of 0.9% NaCl and the concentration of IL-1Ra released in medium was then analyzed using BCA kit according to the manufacturer's directions. SDS-PAGE (BIO-RAD) with 8–10% polyacrylamide gel was also performed for *in vitro* release samples at different time intervals in order to confirm the stability of IL-1Ra in gel formulation.

In Vitro Erosion Profile of PF127 Gel

The vials were weighed after the removal of each release medium at the same pre-determined time intervals and differences in the weight of vials were calculated to determine the amount of gel dissolved. The erosion profile of all

formulations was then acquired by plotting the cumulative weight of each pluronic gel dissolved *versus* time (29), and their coefficient of determination (R^2) was also determined using zero order release kinetic model.

Drug Release Kinetic Study

Several mathematical models were applied to investigate the mode of IL-1Ra release from thermosensitive gel using membraneless dissolution method. DDSolver (30) was used to calculate the *in vitro* drug release kinetics of IL-1Ra *via* zero order, first order and Higuchi release kinetic models. To validate the goodness of fit from above mentioned models, we also applied Akaike information criterion (AIC) (31) to our *in vitro* data using DDSolver. The best fit release kinetic model was selected on the basis of regression analysis. Similarly, the mechanism of IL-1Ra release from thermosensitive gel was also studied by calculating the value of "n" using Korsmeyer-Peppas equation (32). If the value of n= 0.45, it usually represents that the mode of drug release is following fickian diffusion (case I); while the value of "n" >0.45 but <0.89 would indicate that the mode of drug release is non-fickian (anomalous); conversely, if n >0.89 it represents super case II type of drug release. Generally case II refers to the mode of drug release by the erosion of polymer used while anomalous (non-fickian) represents the combination of erosion and diffusion controlled mechanism of drug release (33).

Similarly all gel formulations were also evaluated to predict any difference in their release mechanisms by calculating MDT and similarity factor (f_2) using DDSolver. 20% thermosensitive gel was considered as reference profile to calculate the similarity factor (f_2). According to FDA guidelines, value of f_2 >50 represents the similarity of dissolution profiles between the formulations and the difference between them would be insignificant (P>0.5) while f_2<50 represents that the two dissolution profiles are not identical to each other and they may have a significant difference (P< 0.5). Time of 50% IL-1Ra release ($t_{50\%}$) was also calculated from the regression analysis of zero order kinetics (32).

In Vivo Study on Wistar Rats

All procedures and protocols used for animals were in accordance with the ethics committee for the use of experimental animals at Zhejiang University, China. Male wistar rats (n=15) were obtained from Academy of Medical Science, Zhejiang province, China and their average body weight was 200 g±20. Animals were caged for one week before the start of experiments in rooms with controlled temperature, humidity and light (12 h cycles) and were fed with water *ad labitum*. All animals were divided into 3 equal groups for further study.

As the viscosity of 30% PF127 gel was considerably high, we selected only 20% and 25% PF127 gel formulations for further *in vivo* experiments. A volume of 200 μl of each gel formulation was administered subcutaneously into the loose skin over the shoulder/neck into the rats and the amount of IL-1Ra administered in each rat was 2 mg. Approximately 0.2 ml blood sample was collected from each group 5 min before the administration of different gel formulations and then subsequent blood samples were collected at 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24 h for IL-1Ra group and 0.5, 1, 2, 4, 8, 12, 24, 36 48 h for 20% and 25% PF127 respectively after dose administration. The blood samples were centrifuged at 5000 rpm for 15 min at 4°C to collect the serum which was kept frozen at −20°C until analysis. The serum concentrations of IL-1Ra, and glucose were measured accordingly.

Pharmacokinetic Analysis of IL-1Ra

Pharmacokinetic analysis for all groups was performed using kinetica (version 4.4) by linear trapezoidal rule. Previously calculated data was tabulated in MS excel windows professional XP and plasma drug concentration-time curve was plotted. Maximum concentration of IL-1Ra in plasma (C_{max}) and time to reach maximum concentration (T_{max}) were determined by visual inspection of plasma concentration *vs.* time profiles. The pharmacokinetic parameters for 20% and 25% PF127 gel were compared with that of IL-1Ra aq. solution group after subcutaneous injection into wistar rats. Area under concentration time curve ($AUC_{0-\infty}$) was calculated by linear trapezoidal rule. Mean residence time (MRT), elimination rate constant (K_e), volume of distribution (V_d), clearance (CL) and biological half-life ($t_{1/2}$) were also calculated by non-compartmental model.

Bioactivity of IL-1Ra

The bioactivity of *in vivo* IL-1Ra released from gel was evaluated by measuring its ability to inhibit the production of IL-1β stimulated IL-6 (13). The rats were administered IL-1Ra in 25% gel (n=4) and IL-1Ra aq. solution (n=4), and after 48 h of administration the rats received intraperitoneal injection of 100 ng IL-1β, further 3 h later, rats were bled and serum was separated. IL-6 levels were measured by ELISA using antibodies specific to rat IL-6. Serum levels of IL-6 in these groups were compared to those demonstrated in control groups that received either injection of 25% pluronic gel without drug (n=4), or no treatment (n=4).

In-Vitro-In-Vivo Correlations (IVIVC) Analysis

An IVIVC for IL-1Ra was calculated by plotting the percent dissolved of IL-1Ra from 25% PF127 *in vitro versus* percent absorbed (F_a) of same formulation *in vivo*. Percent dissolved

data were taken from *in vitro* release data whereas percent absorbed data were determined by Wagner-Nelson method using following equation (34):

$$F_a(\%) = \left[\left(C_p + K_e \cdot AUC_{0-t}\right)/K_e \cdot AUC_{0-\alpha}\right] \times 100$$

Where F_a is the fraction of drug absorbed, C_p is plasma concentration of drug at time t, K_e is elimination rate constant and AUC is area under curve. Linear regression analysis was applied to fit the data and the value of correlation coefficient (R^2) was calculated.

Statistical Analysis

Unless otherwise described, data were represented as mean ± SD of three separate experiments and compared using one way ANOVA using SPSS (version 16). Significant differences between the values were evaluated using student's unpaired *t*-test. A level of significance difference was set at 0.05 in all cases.

RESULTS AND DISCUSSION

Effect of Concentrations of PF127 on GT, Gelling Rate and Viscosity

In our present study, we used three different concentrations (20%, 25% and 30%) of PF127 for preparation of IL-1Ra thermosensitive gel. Initially, we determined the effect of pluronic concentrations on GT, gelling rate and viscosity. From the results, we observed that there was significant difference among GT, gelling rate and viscosity for all formulations (Table I). It was observed that GT, gelling rate and viscosity were dependent on concentration of PF127 used. Increasing concentration of PF127 showed the ability to decrease gelling rate (quick gel formation) while, the viscosity of solution increased promptly, which corresponded with already published results (28,35,36). It has been well known that PF127 when placed in water above certain critical concentration has the ability to convert its structure from individual unimers (block copolymers) to self assembling polymer micelles, this phenomenon is known as micellization (36). The temperature at which these micelles are formed is known as critical micelles temperature (CMT). At elevated temperature, the phase transformation completely changes due to dehydration of block polymers (36). In general, they are liquid at low temperature but are transformed quickly into gel at elevated temperature and their GT decrease. Similarly, as the concentration is increased, they transform into the gel more quickly as compared to the less concentrated PF127 solutions.

In Vitro Release of IL-1Ra

The *in vitro* release of IL-1Ra was studied in 0.9% NaCl solution using membraneless dissolution model. The samples were analyzed using BCA kit to determine the amount of IL-1Ra released at predetermined time points. From Fig. 1, it was clearly observed that the release rate of IL-1Ra was prolonged as the concentration of PF127 increased that was mainly due the delayed erosion of the gel (29,36). As evident from *in vitro* dissolution profiles, the time for 50% drug release ($t_{50\%}$) illustrates the retarding effect of PF127 on the release behavior of IL-1Ra (Table I). This could be due to the erosion of gel and dehydration of propylene oxide and ethylene oxide blocks (37). The value of $t_{50\%}$ for all formulations was increased proportionally as the concentration of PF127 increased. Inverse relation between the rate of drug release and the concentration of the PF127 was found. There was a significant difference ($P<0.5$) between the release rates of all gel formulations and these results were further verified from zero order release rate constants of all formulations (Table II). With the increase in concentration of PF127, the crosslinking of unimers also increased and the availability of free space for the escape of drug to release from the gel decreased. Attributable to higher crosslinking, the penetration of dissolution medium into the gel was also decreased along with the decrease in the drug release from gel (24,29,35). This phenomenon is highly supported by the

Table I Rheological and Dissolution Parameters of Various Concentrations of PF127 gel. Data are Represented as Mean ± SD (n=3)

Formulations	Parameters					
	GT (°C)	Gelling rate[a] (Sec.)	Viscosity (cP)[b]	$t_{50\%}$ (h)	f_2	MDT (h)
20% PF127	37.0 ± 0.85	50 ± 0.65	38.50	1.99 ± 0.28	Reference	1.86 ± 0.14
25% PF127	34.0 ± 1.35	15 ± 0.47	75.00	3.97 ± 0.44	26.15	2.05 ± 0.19
30% PF127	29.0 ± 1.50	5 ± 0.08	1000.00	6.25 ± 0.19	18.26	3.15 ± 0.13

GT gelation temperature, *cP* centipoises, $t_{50\%}$ time required for the release of 50% of IL-1Ra, f_2 similarity factor, *MDT* mean dissolution time

[a] Gelling rate was measured at 37°C

[b] viscosity was measured at 25°C

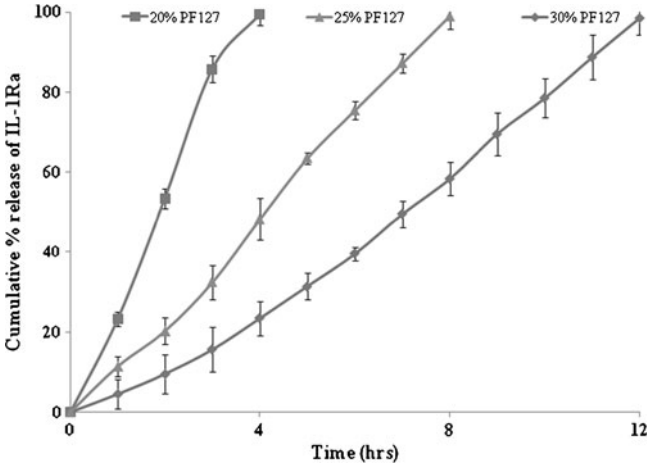

Fig. 1 *In vitro* release kinetics of IL-1Ra from PF127 gel as a function of time, using membraneless dissolution model at 37°C. Data are represented as mean ± SD (n=3).

value of release rate constants of zero order kinetic model for all formulations (Table II).

The choice of an appropriate model should be considered essential during the assessment of characteristics of drug release and various dissolution profiles *via* model-dependent approaches. Numerous efficient statistical principles for selecting the best model are provided by DDSolver, one of these is Akaike Information Criterion (AIC). The model which has the lowest AIC values as compared to other models is considered best according to this criterion. AIC has been recognized as a finest criterion for analyzing the dissolution data of a drug (38). We have utilized AIC to verify and elucidate the goodness of fit model among various models used in the present study. According to the values of AIC (Table III), zero order was found to be the best fit model, thereby, the AIC values also validated the highest value of coefficient of determination (R^2) for zero order.

Drug Release Kinetic Study

In vitro dissolution profiles were expressed using zero order, first order and higuchi kinetic models while Korsmeyer-Peppas kinetic model was used to explore the mechanism of drug release from gel. Dissolution data was plotted according to these models and the curves were plotted in accordance with kinetic models which were used to predict the mechanism of IL-1Ra release from gel on the basis of coefficient of determination (R^2). Among all release kinetic models, the highest value of R^2 was obtained in zero order kinetics for all formulations (Table II) which was further verified by the lowest value of AIC (Table III) for zero order among other kinetic models, revealing that the drug release was independent of IL-1Ra concentration used, while other models produced curvilinear curves with low values of regression coefficients (R^2) in all cases. Similarly, zero order drug release kinetics for PF127 gel has also been found in several previous reports for paclitaxel (36), chlorhexidine (33), recombinant hirudin (24), ceftiofur (29), insulin (21) and IL-2 (25). However, previous studies have also reported first order and Higuchi model for drug release *via* PF127 (26,39,40). This divergence in the results may be due to the differences in experimental conditions, drug used and/or the properties of gel. The erosion profiles of different concentrations of PF127 gel formulations were elaborated by plotting the cumulative percent of gel dissolved *versus* time (Fig. 2). The values of R^2 calculated from zero order release kinetics showed linearity for all PF127 gel formulations along with a decrease in their rate of gel dissolution with the increase in PF127 concentration. In our preliminary experiments, we also observed that the rate of gel dissolution was dependent on some factors such as; a) concentration of PF127 used, and b) volume of the dissolution media used. We also noticed that concentration of PF127 used showed a direct effect on the rate of gel dissolution whereas volume of the dissolution media used showed an inverse effect on the rate of gel dissolution.

The IL-1Ra release profile in all gel formulations were compared with that of reference product (20% PF127 gel formulation) to evaluate the effect of pluronic concentration on the release mechanism of IL-1Ra and the concentrations were compared with each other using similarity factor (f_2). Similarity factor f_2 was calculated using DDSolver to compare different dissolution profiles. The value of $f_2 > 50$ represents the similarity of two dissolution profiles; similarly, $f_2 < 50$ represents the dissimilarity of two dissolution profiles. From the calculated values of f_2 (Table I), it is therefore, evident that value of f_2 for all dissolution profiles was less than 50 which indicated the dissimilarity of dissolution

Table II Applications of Kinetic Models to Access Release Behaviour of IL-1Ra from PF127 Gel

Formulations	Parameters								
	Zero order		First order		Higuchi		Korsmeyer-Peppas		
	K_o	R^2	K_1	R^2	K_H	R^2	K_{KP}	R^2	n
20% PF127	0.418	0.999	0.007	0.931	5.527	0.909	0.518	0.999	0.995
25% PF127	0.21	0.999	0.004	0.927	3.8	0.879	0.249	0.999	0.971
30% PF127	0.133	0.997	0.002	0.909	2.909	0.835	0.083	0.999	1.075

Table III Values of Akaike Information Criterion for Kinetic Models

Formulations	Akaike Information criterion		
	Zero order	First order	Higuchi model
20% PF127	9.66	32.2	33.625
25% PF127	19.695	60.61	65.23
30% PF127	46.98	93.2	100.945

profiles between all gel formulations and these formulations were independent with each other having significant difference ($P<0.5$). These results indicated the significant effect of PF127 concentrations on the release behavior of IL-1Ra.

MDT was used to characterize the ability of drug release and efficacy of polymer to retard the release of drug from dosage form (41,42). From our results (Table I), it was clearly observed that the higher concentrations of PF127 had increased MDT value and *vice versa*.

Correlation Between *In Vitro* Gel Dissolution *Versus In Vitro* Drug Release

To validate, whether the gel dissolution has any effect on the rate of drug release, we also performed further experiments by calculating the weight of gel dissolved at the same time intervals at which the samples were taken to calculate the rate of *in vitro* drug release. The remaining weight (%) of the gel also followed the zero order release kinetics representing a good linear correlation between the percent drug released *vs*. percent gel dissolved for all gel formulations (Fig. 3). This correlation indicates that the gel dissolution process controls the mechanism of drug release and our results are highly supported by already published data (24,29,36). The membraneless dissolution model which follows zero order release kinetics, allows the release medium directly contact to the surface of gel resulting in controlled gel dissolution mechanism. As evident from Fig. 4, the release rate of IL-1Ra was dependent on the rate of gel dissolved which prolonged the release rate of IL-1Ra release significantly with the increase in gel concentration.

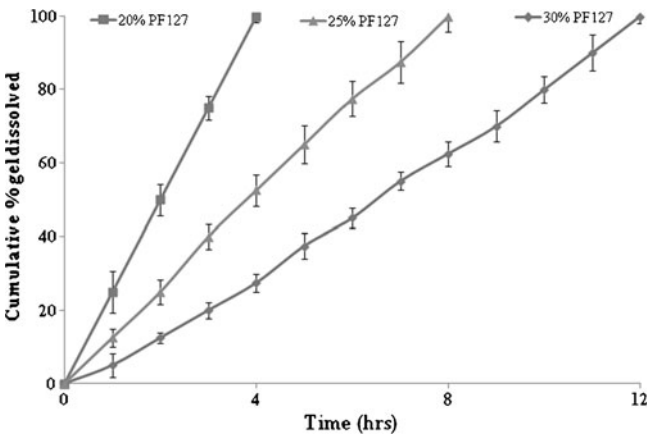

Fig. 2 *In vitro* gel dissolution (dissolved wt %) of PF127 gel as a function of time, using membraneless dissolution model at 37°C. Data are represented as mean ± SD ($n=3$).

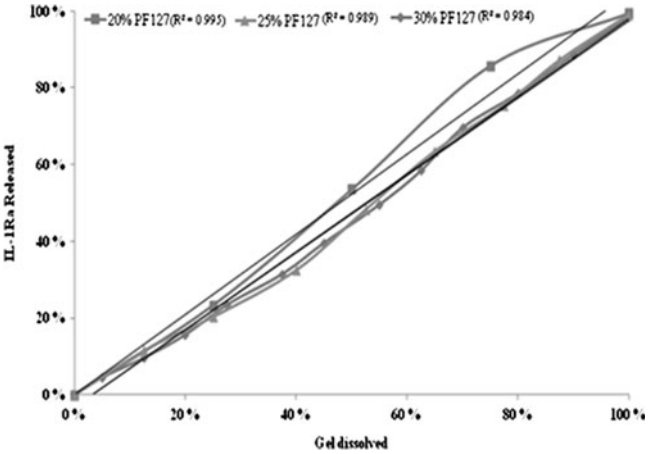

Fig. 3 Correlation between cumulative % PF127 gel dissolved *vs*. cumulative % IL-1Ra released.

Stability of IL-1Ra in Gel Formulation

We also performed SDS-PAGE to evaluate the stability of IL-1Ra during its *in vitro* release from 25% gel and according to the Fig. 5, the major band for IL-1Ra released from gel at different time points appeared at 17 kDa (Lane 3-7) in correspondence to the protein marker (Lane 1) and standard IL-1Ra aq. solution (Lane 2) which clearly indicated that IL-1Ra remained stable during the observation period.

Changes in Serum Levels of IL-1Ra and Glucose

Type 2 diabetes mellitus (T2DM) has been considered among top 5 major causes of the death, affecting millions of people every year (43). IL-1β is a main factor for the induction of inflammation in β-cells of pancreatic islets (20,44) that may impair the normal function of β-cells to secrete insulin. IL-1Ra competitively blocks the binding of IL-1β with IL-1RI (45) to neutralize its inflammatory effects. Taking patient convenience into account, various therapeutic strategies have been applied to treat this disease without any potential side effects but IL-1Ra is considered best among these strategies that may help the patient to achieve desired therapeutic potentials. Although, IL-1Ra has broad spectrum anti-inflammatory effects against various autoimmune diseases (3–7) but due to its short biological half-life, frequent administration with higher dose of IL-1Ra is required (20). As shown in Fig. 6, after the subcutaneous administration, the IL-1Ra aq. solution showed an acute and little hypoglycemic effect as compared to the effects of

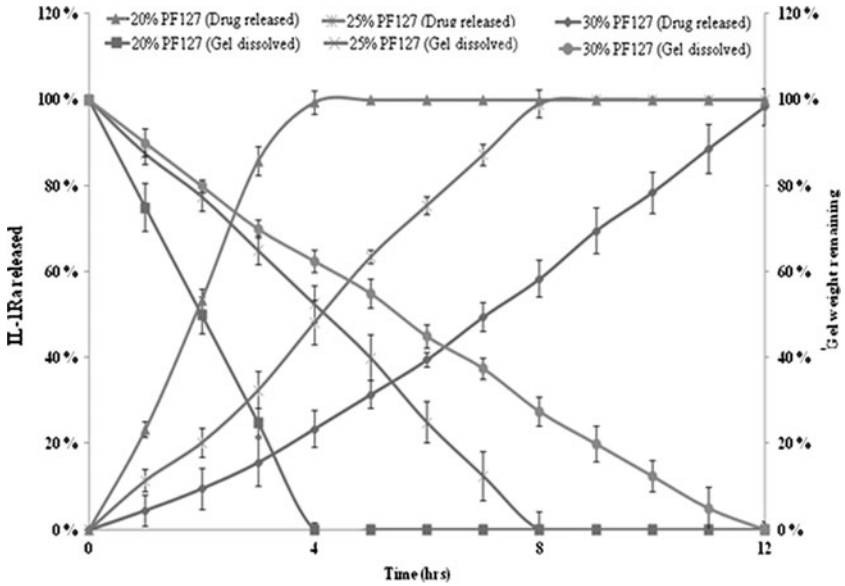

Fig. 4 *In vitro* drug Release (%) *vs. in vitro* gel dissolution (remaining weight %) as a function of time (hours).

IL-1Ra loaded PF127 gels in normal rats. Moreover it was also observed that the hypoglycemic effect achieved by 25% gel was more pronounced as compared to the IL-1Ra aq. solution, and the intensity of hypoglycemic effect was much controlled in IL-1Ra loaded 25% gel as compared to that of IL-1Ra aq. solution. The hypoglycemic serum glucose profile attained after the administration of IL-1Ra loaded in 20% and 25% PF127 gels was found to be more prolonged and stable when compared to that of IL-1Ra aq. solution. As clearly shown in Fig. 7, the effect of concentration of PF127 exhibited an inverse dependence on the release of IL-1Ra. A delayed and more prolonged release of IL-1Ra was achieved as the concentration of PF127 increased. The serum concentrations of IL-1Ra administered in the form of IL-1Ra aq. solution and IL-1Ra loaded in 20% and 25% PF127 gels were evaluated till 24 h and 48 h respectively. However, the serum concentration for IL-1Ra aq. solution was observed to reach the baseline values after 8 h.

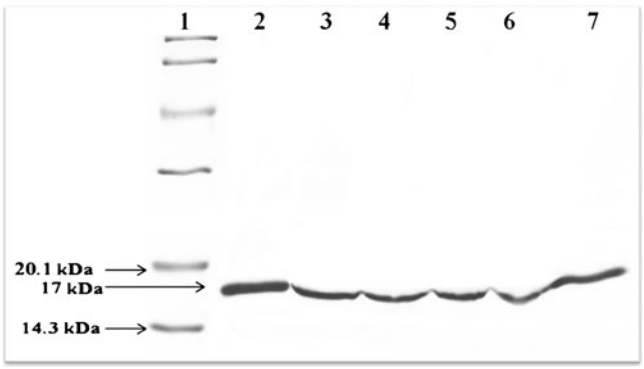

Fig. 5 SDS-PAGE results of IL-1Ra from *in vitro* release profile at different time intervals: Lane 1; Marker, lane 2; IL-1Ra standard, Lane 3-7; samples of IL-1Ra taken from 25% PF127 gel at 1 h, 2 h, 4 h, 8 h, 12 h respectively.

The prolonged hypoglycemic effects were dependant on the release rate of IL-1Ra from the gel as shown in Fig. 6. Although it is well known that subcutaneous route is well supplied of capillaries and lymphatic vessels however, it is very hard to predict that whether the absorption route for IL-1Ra is *via* capillaries and/or lymphatic vessels. Many drugs in solution form cross the subcutaneous route by passive diffusion (46). A major factor in controlling the absorption of IL-1Ra is presumed to be the surrounding polymer network of PF127 gel around IL-1Ra which might delay the absorption of IL-1Ra from subcutaneous route. In our present study, the peak hypoglycemic effect of IL-1Ra aq. solution after its subcutaneous administration was

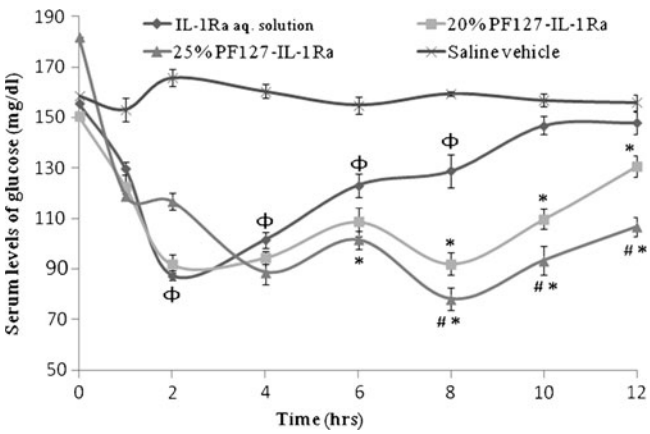

Fig. 6 IL-1Ra induces hypoglycemia. Immediately after collection of blood samples (time 0), rats were injected subcutaneously 2 mg IL-1Ra in the form of solution, 20% and 25% PF127 gels. After administration of drug, blood samples were collected at specific time intervals as indicated and serum glucose levels were measured accordingly. All points in curves demonstrate mean ± SD performed in three (with saline), and four (with IL-1Ra aq. solution, 20% and 25% PF127 gel) rats per group. *, $P < 0.05$ *vs.* saline vehicle and IL-1Ra aq. solution; #, $P < 0.05$ *vs.* 20% PF127 gel; Φ, $P < 0.05$ *vs.* saline vehicle.

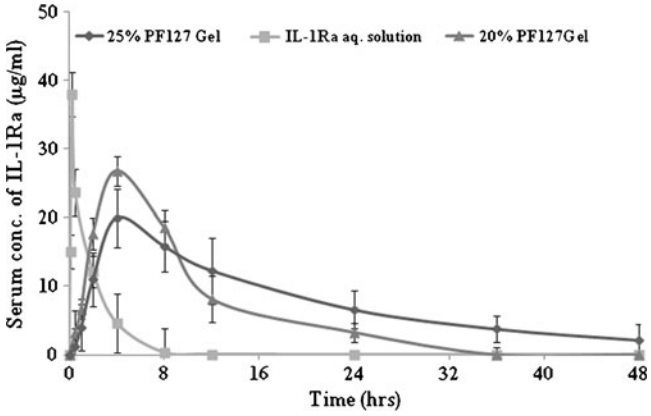

Fig. 7 Serum concentration-time profiles of IL-1Ra aq. solution, and IL-1Ra loaded 20% and 25% PF127 gels following s.c. administration in male wistar rats. All groups received equal amount of IL-1Ra. Data are represented as mean ± SD (n=5).

obtained at 2 h, similarly, although IL-1Ra loaded in 20% gel also showed the maximum hypoglycemic effects at 2 h but as compared to IL-1Ra aq. solution, the IL-1Ra loaded in 20% gel effectively maintained these hypoglycemic effects till 8 h. Conversely, the hypoglycemic effect by IL-1Ra loaded in 25% gel started appearing at 4 h whereas, the peak hypoglycemic effects were observed at 8 h. This distinct difference between the hypoglycemic effect of IL-1Ra aq. solution and IL-1Ra loaded in PF127 gels may perhaps be due to the delayed release of IL-1Ra from gels. In addition to this, absorption of drug from subcutaneous site depends on several factors such as volume of the vehicle, amount of drug used (46) and viscosity of the vehicle (47). Thereby, the difference observed between the plasma glucose profiles depends on the concentration of PF127 gel and the release rate of IL-1Ra from gel suggesting the earlier release of IL-1Ra from 20% gel as compared to the release from 25% gel. In our preliminary *in vitro* experiments, we also found that the rate of drug release from the gel was more prolonged as the amount of gel increased. These *in vivo* results are in good agreement with already described *in vitro* results.

Pharmacokinetic Parameters of IL-1Ra

Despite of the great therapeutic potentials of IL-1Ra, little data has been published about the pharmacokinetics of IL-1Ra. In 1992, Granowitz with his colleagues investigated the pharmacokinetics of IL-1Ra in healthy volunteers and reported very short biological half life of IL-1Ra (21 ± 3 min) and elimination of intact IL-1Ra through kidneys (48).

Pharmacokinetic parameters of IL-1Ra aq. solution administered subcutaneously into wistar rats with IL-1Ra loaded in 20% and 25% PF127 gels are represented in Table IV. Blood sampling time point (T_{max}) was required to achieve maximum plasma concentrations (C_{max}) of IL-1Ra. Though the T_{max} for both 20% and 25% was same (4 h) but the hypoglycemic effects for 20% gel were attained earlier than that of its T_{max} as compared to 25%. The values of C_{max} and MRT attained after subcutaneous administration of IL-1Ra aq. solution and IL-1Ra loaded in 20% and 25% PF127 gels represented 25% gel to be the best formulation. The delayed and prolonged release of IL-1Ra from 25% gel can be considered as the most probable factor for depicting IL-1Ra loaded in 25% gel as the best formulation. Moreover, the values of C_{max} for gel formulations were observed to be decreased consistently with increase in the

Table IV Pharmacokinetic Parameters of IL-1Ra in Solution and in 20% and 25% PF127 gel

Parameters	Units	Formulations		
		IL-1Ra solution	20% PF127 Gel	25% PF127 Gel
C_{max}	µg/ml	37.93 ± 6.08	26.79 ± 2.17	19.97 ± 5.05
T_{max}	h	0.25	4.00	4.00
AUC(0-∞)	µg.h/ml	72.78 ± 6.53	313.69 ± 3.45*	397.72 ± 1.15**#
AUMC(0-∞)	µg.h^2/ml	104.67 ± 5.36	3829.71 ± 8.14	7931.10 ± 9.28
MRT	h	1.43 ± 3.26	12.20 ± 4.58*	19.94 ± 3.64**
$t_{1/2}$	h	1.18 ± 2.85	7.24 ± 4.25*	12.53 ± 2.48**#
CL	ml/h/kg	157.00 ± 1.68	40.00 ± 8.45*	33.00 ± 6.29**
V_d	L/Kg	10.81 ± 0.84	139.83 ± 0.47*	268.99 ± 1.27**
K_e	h^{-1}	0.588 ± 0.07	0.095 ± 1.08	0.055 ± 1.92

C_{max} maximum plasma concentration, *T_{max}* time to reach maximum plasma concentration, *AUC* area under the curve, *AUMC* area under the first moment of plasma concentration time-curve, *MRT* mean residence time, *$t_{1/2}$* biological half-life, *CL* clearance, *V_d* volume of distribution, *K_e* elimination rate constant

* significant difference ($P < 0.05$) *vs.* IL-1Ra aq. solution

** significant difference ($P < 0.05$) *vs.* IL-1Ra aq. solution

significant difference ($P < 0.01$) *vs.* 20% PF127 gel

amount of PF127 used, while, the values of MRT were noticed to be increased with the increase of gel concentration. Area under the curve (AUC) of IL-1Ra aq. solution and IL-1Ra loaded in 20% and 25% PF127 gels are represented in Table IV. Significant ($P<0.05$) increase in the values of AUC for IL-1Ra loaded in gels was observed when compared to the corresponding value of AUC for IL-1Ra in solution form. Similarly, a highly significant ($P<0.01$) increase in AUC value of IL-1Ra loaded in 25% gel (397.72) was observed when compared to the corresponding AUC value of IL-1Ra loaded in 20% gel (313.69). From Table IV, it has been clearly found that the biological half-life of IL-1Ra loaded with PF127 gel has also been increased with the increase in gel concentration. A highly significant ($P<0.01$) increase in the half-life of IL-1Ra was calculated when IL-1Ra was loaded with 25% PF127 gel (12.53 h) as compared to that of IL-1Ra aq. solution (1.18 h). This was due to the delayed absorption of IL-Ra from gel at the site of administration and delayed clearance from the body as compared to that of IL-1Ra aq. solution (Table IV). From our present results, it is obvious that desired therapeutic effects can be achieved using PF127 gel as sustained drug delivery technology.

In Vivo Bioactivity of IL-1Ra Released from PF127 Gel

We examined the *in vivo* bioactivity of IL-1Ra released from gel by measuring its ability to inhibit IL-1β-stimulated production of IL-6 in normal male wistar rats. IL-1β is a major pro-inflammatory mediator that induces the secretion and production of IL-6 by different cells within the body (49) and recently, it has been confirmed that blocking the binding of IL-1β on IL-1RI with IL-1Ra suppressed the production of IL-6 (50). The serum level of IL-1Ra having 20% PF127 gel rapidly decreased after 24 h until a baseline level was reached at 36 h whereas the serum level of IL-1Ra having 25% PF127 gel was found significantly high as compared to 20% PF127 gel and remained so for significantly high (2.25 μg/ml) up to 48 h as compared to its baseline levels.

Accordingly, we administered 100 ng of IL-1β intraperitoneally into the rats immediately after 48 h of receiving either IL-1Ra aq. solution, IL-1Ra loaded in 25% PF127 gel, control gel (without IL-1Ra) or no treatment. Three hour later, the serum levels of IL-6 were measured by ELISA using antibodies specific to rat IL-6. From Fig. 8, it has been demonstrated that the serum levels of IL-1Ra released from gel were sufficient to inhibit the production of IL-6 induced by IL-1β that was administered exogenously. The serum levels of IL-6 in these animals (IL-1Ra loaded 25% PF127 gel) were nearly similar to the recorded baseline serum levels of IL-6. Contrarily, the IL-1Ra aq. solution and control gel were unable to neutralize the effects of IL-1β in respective rat groups. The elevated levels of IL-6 in animals, in which IL-1Ra aq. solution and control gel were administered, were nearly similar to the levels detected in the serums of non-treated rats after administration of IL-1β (Fig. 8). These results are in accordance with already published reports (13,15) in which they observed similar effects of sustained released IL-1Ra from PLGA microspheres on IL-1β induced IL-6. These results show that IL-1Ra loaded in PF127 gel remained biologically active during its entire period of time in the serum, also supported by our *in vitro* SDS-PAGE analysis previously described.

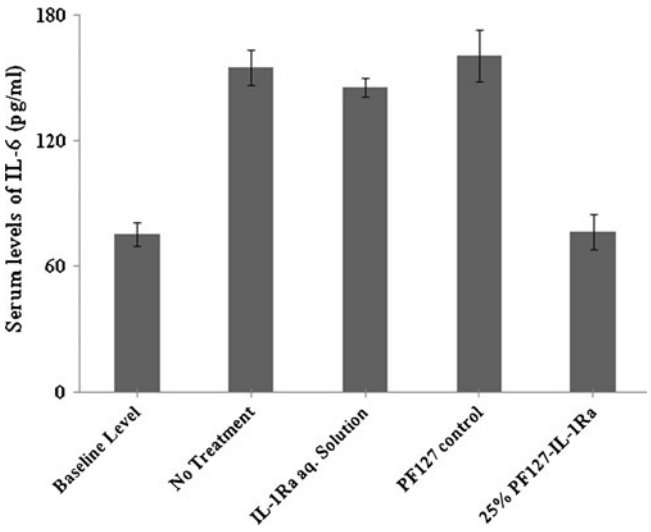

Fig. 8 Effective inhibition of IL-1β-induced production of IL-6 by sustained release of IL-1Ra from gel. 25% PF127 gel with (25% PF127-IL-1Ra group, $n=5$) or without IL-1Ra (PF127 control group, $n=3$) and IL-1Ra (IL-1Ra aq. solution group $n=4$) were administered subcutaneously. After 48 h, IL-1β (100 ng) was administered intraperitoneally to these groups as well as to another group ($n=3$) which had not been treated (named as no treatment). Three hours later, serum levels of IL-6 from all groups were measured with ELISA. *, $P<0.05$ *vs.* baseline levels of IL-6; $^{\#}$, $P<0.05$ *vs.* 25% PF127-IL1Ra.

In-Vitro-In-Vivo Correlation (IVIVC)

An IVIVC was evaluated by using Wagner-Nelson equation. The drug dissolved *in vitro vs.* drug absorbed *in vivo* for

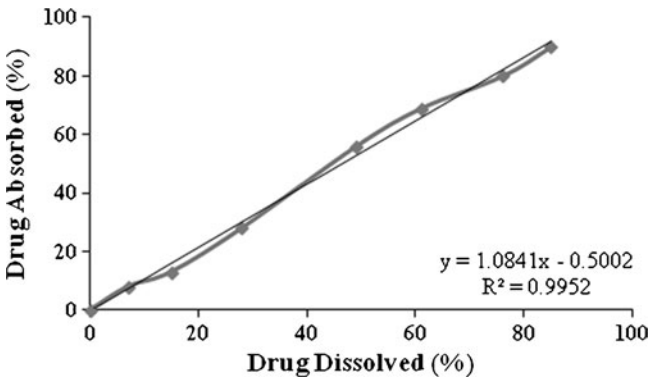

Fig. 9 Linear regression plot of drug dissolved *in vitro vs.* drug absorbed *in vivo* for IL-1Ra loaded in 25% PF127 gel.

25% PF127 gel having IL-1Ra was plotted. The value of correlation coefficient (R^2=0.9952) showed good correlation between absorption *in vivo* and drug release *in vitro* (Fig. 9). As zero order was observed to be the best fit model for all formulations; it was signified that the drug release was independent of IL-1Ra concentration used. Thereby, the use of different concentrations for *in vivo* and *in vitro* drug release (10 mg/g for *in vivo* and 1 mg/g for *in vitro*) did not affect the IVIVC results. Moreover, the shear rate of body fluid is assumed to be capable of affecting the release mechanism in similar manners as it would in *in vitro*.

CONCLUSION

Various therapeutic strategies have been utilized for the better treatment of T2DM. IL-1Ra has been long recognized best among these strategies that may help the patient to achieve desired therapeutic potentials but due to its short biological half-life, frequent administration with higher dose of IL-1Ra is required. In current study, we have used PF127-based thermosensitive gel technique to improve the pharmacokinetic and therapeutic potentials of IL-1Ra through sustained drug release. Our present results showed that IL-1Ra loaded in PF127 gel exhibited delayed and more prolonged release of IL-1Ra in *in vitro* as well as in *in vivo*. When compared directly to IL-1Ra aq. solution, IL-1Ra loaded in PF127 gel induced prolonged hypoglycemic effects and showed greater efficacy to inhibit the induction of IL-1β-stimulated IL-6.

To conclude, our results for the first time revealed that IL-1Ra loaded in PF127 gel resulted in a more sustained release of IL-1Ra from a thermosensitive gel and potentially inhibited the production of IL-1β-stimulated IL-6. These effects observed in the present study support the importance of IL-1Ra suggesting that its sustained delivery using PF127 gel might possibly be considered as an effective approach to prolong the therapeutic potentials of IL-1Ra.

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In vivo results presented here are not efficacy data. They can only be considered as animal-based pharmacokinetic and pharmacodynamic results.

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