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Development and validation of an IL-6 immuno-receptor assay based on surface plasmon resonance

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Abstract

Interleukin-6 (IL-6) is a pleiotropic cytokine which interacts with the specific IL-6 receptor at the surface of the T lymphocytes. A combined immuno- and receptor-assay has been developed and validated to characterize the biological activity of recombinant IL-6 (rIL-6). This assay is based on Surface Plasmon Resonance (SPR) technology. From each experiment two successive interactions were monitored: anti-IL-6 antibody/rIL-6 and rIL-6/IL-6 soluble Receptor (sIL-6R). Based on the first interaction an immuno-assay for rIL-6 was optimized and validated. Based on the second interaction a receptor-assay for rIL-6 biological activity was optimized and validated. The assays were validated by performing three different assays on three different days. The intra- and inter-day precisions (%CV) for the immuno-assay were respectively 0.9% and 1.7%. The overall recovery of the immuno-assay was 98.9% ± 1.6. Intra- and inter-day precisions for the receptor-assay were respectively 1.1% and 1.4%. The overall recovery of the receptor-assay was 99.4% ± 1.1. This immuno-receptor assay has allowed to compare the rIL-6 stability after storage at different temperatures. The results did not show significant difference between the three lower storage temperatures (−70, −20 and 5°C). However, results obtained for the aliquot stored at 25°C have shown a drastic denaturation of the rIL-6. These results illustrate the advantage of this method combining the evaluation of the immunological and biological integrity of the drug and high reproducibility and precision of the biosensor based technology.

Keywords: Immuno-assay; Receptor-assay; SPR; Biosensor; Interleukin-6

1. Introduction

Interleukin-6 is a multifunctional cytokine which regulates the growth and differentiation of various cell types. This cytokine induces megakaryocyte maturation, regulation of T cell growth, immunoglobulin production in B cells, induction of acute phase proteins in hepatocytes, and activation of the hematopoietic stem cells [1–3].

IL-6 is a 20 kDa molecule with two N-glycosylation sites. Human IL-6 cDNA encodes a 212 amino acid residue precursor polypeptide with a

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28 amino acid residue signal peptide [4,5]. IL-6 binds to a cell surface receptor consisting of two chains: a 80-kDa glycoprotein including the IL-6 binding site and a 130-kDa glycoprotein called gp-130 involved in the transmembrane signalling [6,7].

The extra-cellular 80 kDa chain of the IL-6 receptor is cleaved and released in blood as a soluble form of receptor, sIL-6R. This soluble form of receptor has been cloned and produced in large amount [8]. This molecule provides a convenient tool to analyze IL-6 biological activity.

Recombinant Interleukin-6 has been produced by expression systems after genetic recombination. This in vitro process involving protein synthesis and post-transduction mechanism could induce some structure variations. These variations could affect the biological activity of the molecule. Thus, the characterization of this biological activity of the drug is a requirement before its use in animal or human studies. A previous method was based on a bio-assay to characterize the recombinant Interleukin-6 production lots [9,10]. The object of this study was the development of an immuno-receptor assay based on Surface Plasmon Resonance (SPR) technology [11–13] to compare the biological activity of different batches of rIL-6.

This method is based on the interaction, on one hand between an anti-IL6 antibody and rIL-6, and on the other hand between rIL-6 and the human IL-6 soluble receptor. These bindings were monitored in real time and without labelling on a optical biosensor, BIAcore®. This document summarizes the analytical validation of the method.

2. Materials and methods

2.1. Equipment and chemicals

BIAcore® upgraded instrument, CM5 sensorchips and the amine coupling kit containing *N*-hydroxysuccinimide (NHS), *N*-ethyl-*N*'(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), and ethanolamine hydrochloride were obtained from BIACORE AB (Uppsala, Sweden). The precision balance AT261 was from Mettler-Toledo AG (Greifensee, Switzerland).

2.2. Immunological reagents

The Protein A coupled on the sensor surface was purchased by Pharmacia (Uppsala, Sweden). Recombinant Interleukin-6 (rIL-6) was provided by Dr K. Tullberg (TRD-Biotechnology, Novartis Pharma, Basel). The monoclonal anti-IL-6 antibody was produced by Dr R. Papoian (DS-DMP, Novartis Pharma, Basel). Rabbit anti-mouse IgG antibody (RAM) was obtained from Pierce (no 31190) and the recombinant human IL-6 soluble receptor (sIL-6R) from R&D Systems (no 227-SR-025).

2.3. Preparation of the sensor surface

Immobilization of Protein A on the sensor surface was performed according to the method described by Johnsson et al. [14]. BIAcore® system was equilibrated in running buffer (PBS, 0.05% Tween 20®, 1 mM EDTA, pH 7) at a flow rate of 5 μl/min. The carboxymethylated dextran matrix was activated by injecting 35 μl of a solution containing NHS (0.05 M)–EDC (0.2 M) (50:50, v/v). Thirty five microliters of Protein A at 300 μg/ml in citrate buffer (pH 4; 0.01 M) were injected. The deactivation of the remaining NHS-ester groups was performed by injecting 35 μl of ethanolamine hydrochloride (pH 8.5; 1 M). A regeneration of the sensor surface was done by the injection of 5 μl HCl (0.1 M).

2.4. Assay procedure

The experiments were performed at a constant flow rate of 5 μl/min of running buffer. All the reagents were prepared by dilution in the running buffer. Fig. 1a and b show the design of the assay and a corresponding sensorgram. First, 15 μl of rabbit anti-mouse antibody (20 μg/ml) were injected on Protein A followed by the injection of 15 μl of monoclonal anti-IL-6 antibody (25 μg/ml). Fifteen microliters of standard, quality control or unknown solution were then injected. The last injection was 15 μl of recombinant human

IL-6 soluble receptor (1.25 μg/ml). The sensor surface was regenerated by injecting 5 μl of HCl (0.1 M). The measurements were made in duplicates for standards and in triplicates for quality control and unknown samples.

2.5. Standard curve elaboration and quality control preparation

Standards and quality controls were made by successive dilutions of the rIL-6 Reference batch in running buffer. All the dilutions were performed by weighing in order to calculate the exact concentrations and reduce all the variations introduced by pipetting.

Five different concentrations of rIL-6 Reference batch were injected in duplicates to built the standard curves. A change in rIL-6 concentration involves changes in rIL-6 and IL-6 soluble receptor relative responses, thus from each experiment two values were measured. The signal measured after the rIL-6 injection corresponds to the antibody/antigen interaction and has allowed to plot the standard curve of the immuno-assay (Fig. 2a). The second standard curve (Fig. 3a) was drawn from the signal obtained after the sIL-6R injection which corresponds to the receptor-assay. The five rIL-6 concentrations of the standard solutions were: 68.9, 136.4, 270.7, 539.3 and 1071.8 ng/ml as calculated by weighing. Both standard curves were fitted with RIAcalc 2.57 software using a 4 parameter logistic algorithm. For each, a precision profile was established with the same software (Fig. 2b and Fig. 3b). The three quality controls were injected in triplicates to validate the standard curves. These solutions were prepared by weighing and their exact concentrations were 163.5, 217.5 and 324.4 ng/ml.

2.6. Validation procedure

The validation of the method was done by three independent assays on three different days. The three quality control concentrations measured in triplicates have allowed to calculate an intra-day precision from nine values and an inter-day precision from 27 values corresponding to the 3 days of the validation. The intra- and inter-day precisions were calculated for both immuno- and receptor-assays. The third parameter used to characterize the method was the recovery calculated on the quality control values and defined as:

$$\text{Recovery} = (\text{calculated concentration}/\text{nominal concentration}) \times 100$$

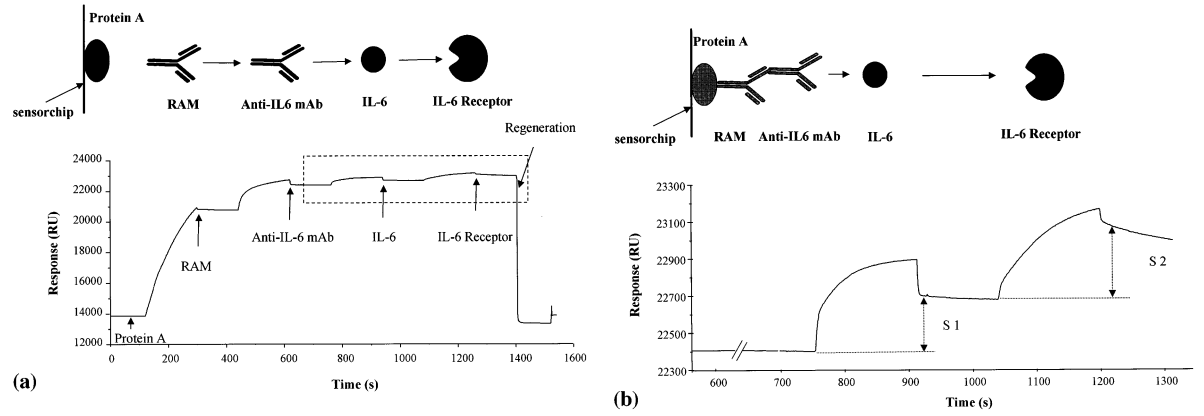
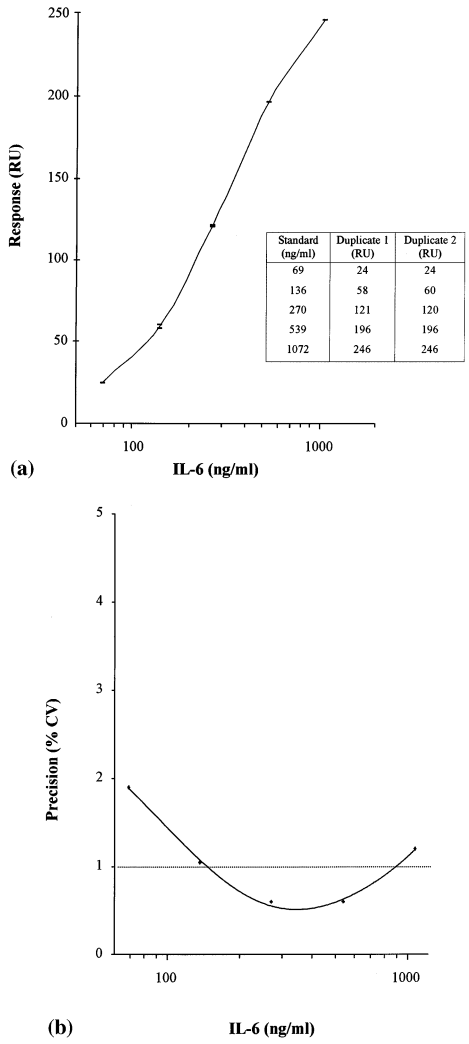


Fig. 1. (a) Immuno-assay receptor: Final experiment design. Sensor surface was prepared with Protein A. The immuno-receptor assay is composed by successive injections of rabbit anti-mouse antibody (20 μg/ml), monoclonal Anti-IL-6 antibody (25 μg/ml), rIL-6 standards or quality controls and IL-6 soluble receptor (1.25 μg/ml). The corresponding sensorgram shows the successive associations of the different reactants. The regeneration of the sensor surface with 5 μl of HCl (0.1 M) has allowed to recover the initial baseline. The surrounded part of the sensorgram is enlarged in Fig. 1b. (b) Extension of the surrounded part of Fig. 1a. Rabbit anti-mouse and Anti-IL-6 antibodies are already bound to the sensor surface. S1 and S2 are the relative signals corresponding, respectively, to the successive bindings of rIL-6 and sIL-6R.



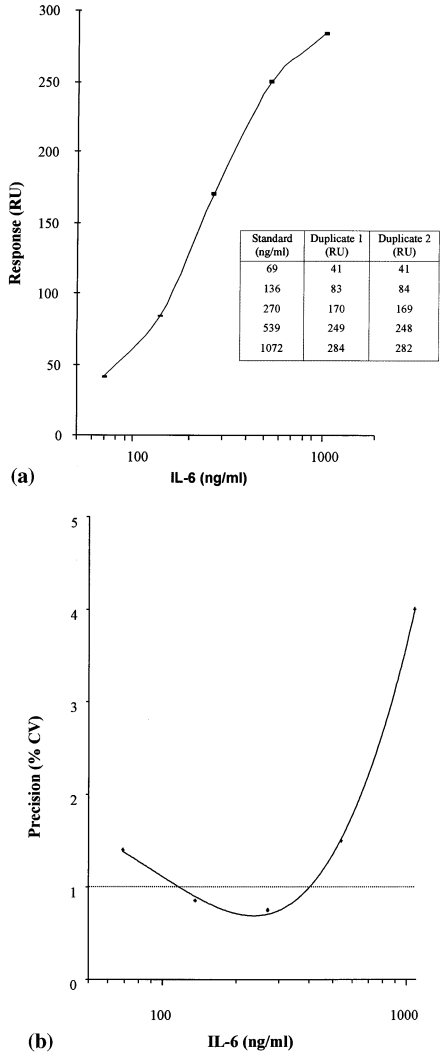
Standard (ng/ml)	Duplicate 1 (RU)	Duplicate 2 (RU)
69	24	24
136	58	60
270	121	120
539	196	196
1072	246	246

Fig. 2. (a) Immuno-assay standard curve. Standard curve obtained for the binding of rIL-6 to the anti-IL-6 antibody. Measurements were done in duplicates. Working range was 70–1070 ng/ml. The curve was fitted with a 4 logistic parameter fitting on RiaCalc 2.57 sofware. (b) Precision profile of the immuno-assay standard curve (calculated with RiaCalc 2.57). Results gave a precision higher than 1% between 120 and 900 ng/ml corresponding of the linear part of the standard curve.

The nominal concentration of the rIL-6 Reference batch was 4320 μg/ml. For each day the average calculated concentration was reported to the nominal concentration to obtain an intra-day recovery for both the immuno- and receptor-assays. The overall recovery of the assay was the mean of the three intra-day recovery values.

2.7. Determination of the limit of detection

Lower IL-6 concentrations (0, 3.75, 7.5, 15 and 30 ng/ml) than for standard curve elaboration were injected to settle the limit of detection (LOD) of this IL-6 immuno-receptor assay. Owing the very low signals each concentration was



Standard (ng/ml)	Duplicate 1 (RU)	Duplicate 2 (RU)
69	41	41
136	83	84
270	170	169
539	249	248
1072	284	282

Fig. 3. (a) Receptor-assay standard curve. Standard curve obtained for the binding of IL-6 soluble receptor to rIL-6. Measurements were done in duplicates. Working range was 70–1070 ng/ml. The curve was fitted with a 4 logistic parameter fitting on RiaCalc 2.57 sofware. (b) Precision profile of the receptor-assay standard curve (calculated with RiaCalc 2.57). Results gave a precision higher than 1% between 120 and 400 ng/ml corresponding of the linear part of the standard curve.

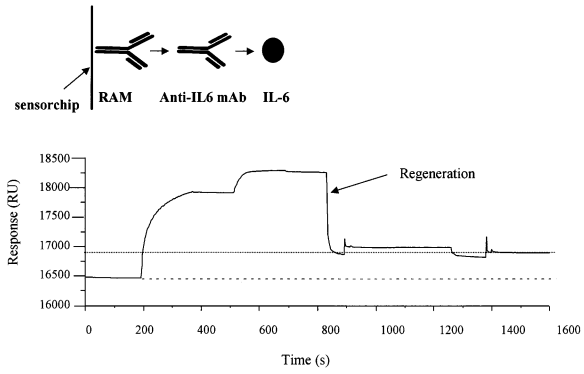


Fig. 4. Polyclonal rabbit anti-mouse antibody (RAM) was directly immobilized on the sensor surface. Monoclonal anti-IL-6 antibody (Anti-IL-6 mAb) and rIL-6 were then injected on this surface. The corresponding sensorgram shows that sensor surface regeneration did not allow to recover the initial baseline.

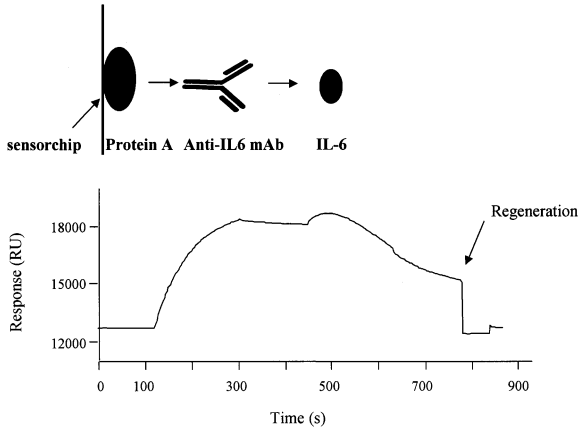


Fig. 5. Sensor surface prepared with Protein A. After immobilization of Protein A the monoclonal anti-IL6 antibody and rIL-6 have been injected successively. The injection of rIL-6 shows a drastic dissociation of both rIL-6 and the anti-IL-6 antibody.

injected five times. The limit of detection is defined as the lowest concentration which provides a signal higher than the blank signal added to three blank standard deviations (blank + 3 × SD(blank)).

2.8. Storage condition comparisons

Different temperatures of storage have been evaluated using this immuno-receptor assay. Four aliquots of a same batch of rIL-6 were placed during 6 months at −70, −20, +5 and +20°C respectively. These different aliquots were tested to evaluate a potential degradation of the rIL-6 caused by temperature. Each one was diluted at a concentration included in the working range of the assay (around 300 ng/ml). The four solutions were injected in triplicates as unknowns together with standard and quality control samples. The three quality control concentrations tested were 151.9, 202.5 and 303.75 ng/ml. For each unknown sample two concentrations were measured: one with the immuno-assay standard curve and the second with the receptor-assay standard curve. These experimental concentrations were compared with the nominal concentrations by calculating the recovery for all the tested aliquots.

3. Results

3.1. Assay procedure

Different procedures have been investigated to test several ways to immobilize the anti-IL-6 antibody. When a rabbit anti-mouse antibody was immobilized on the sensor surface followed by injections of the anti-IL-6 antibody and rIL-6, the surface regeneration was never total. Different regeneration solutions were tested but which did not allow to recover the initial baseline (Fig. 4). The second investigated format was the coupling of Protein A on the sensor surface followed by anti-IL-6 antibody and recombinant IL-6 injections. An unexpected result was obtained: during a first short part of the rIL-6 injection, recombinant Interleukin-6 has bound to its specific antibody with a maximum signal of 500 RU, and then this signal has decreased strongly until the end of the injection (Fig. 5).

Finally, the optimal result was obtained with immobilization of Protein A following by the injection of the rabbit anti-mouse antibody before that one of the anti-IL6 antibody. Fig. 1a and b show a sensorgram corresponding to this design. The baseline, 13868 Resonance Units (RU),

corresponds to the immobilization of Protein A. The injections of the rabbit anti-mouse (20 μg/ml) and anti-IL-6 (25 μg/ml) antibodies give relative responses of, respectively, 6911 RU and 1620 RU with a very good reproducibility (see below).

3.2. Reproducibility

The reproducibility of the method was related to the regeneration of the sensor surface and the individual reproducibility of the RAM and anti-IL-6 injections. The baseline after regeneration and the signals corresponding to the RAM and anti-IL-6 antibody injections have been measured during 21 successive cycles. The precisions (CV%) were 0.17% for baseline, 0.21% for RAM relative response, and 0.18% for anti-IL-6 relative response.

3.3. Validation of the immuno-receptor assay

Table 1 summarizes the assay parameters for the immuno-assay measurement: the average intra-day precision on the 3 days of validation was 0.93%, the inter-day precision was 1.67%, and the overall recovery was $98.93\% \pm 1.61$. Same parameters concerning the receptor-assay measurements are described on Table 2: the average intra-day precision was 1.08%, the inter-day precision was 1.41% and the overall recovery was $99.45\% \pm 1.08$.

Table 1
Validation of the IL-6 immuno-assay[a]

	Calculated IL-6 concentration (μg/ml) ($n = 9$)									Mean	Coeff. Var.%	Recovery
Day 1	4384	4370	4319	4386	4345	4345	4357	4357	4321	4353.8	0.56	100.78
Day 2	4343	4290	4291	4248	4205	4205	4160	4160	4161	4229.2	1.59	97.90
Day 3	4236	4237	4236	4229	4229	4229	4305	4239	4206	4238.4	0.63	98.11
								Mean		4273.8	0.93[c]	98.93[b]
								Std Dev.		71.6		1.61
								Coef. var%		1.67[d]		

[a] Recovery was calculated as: (calculated concentration/nominal concentration) × 100.
[b] The IL-6 immuno-assay recovery is $98.9\% \pm 1.61$. (mean value calcuated on the three independent assays).
[c] The intra-assay precision, 0.93%, was calculated as the average of the coefficients of variation obtained on the 3 days of validation.
[d] The inter-assay precision, 1.62%, was calculated as the coefficient of variation for the 27 values obtained on the 3 days.

Table 2
Validation of the IL-6 receptor-assay[a]

	Calculated IL-6 concentration (μg/ml) ($n = 9$)									Mean	Coeff. Var%	Recovery
Day 1	4367	4328	4250	4372	4338	4271	4471	4403	4336	4348.4	1.53	100.66
Day 2	4331	4254	4292	4286	4223	4256	4321	4289	4289	4282.3	0.79	99.13
Day 3	4282	4282	4247	4229	4229	4229	4345	4225	4255	4258.1	0.93	98.57
								Mean		4296.3	108[c]	99.45[b]
								Std Dev.		60.8		1.08
								Coef. Var.%		1.41[d]		

[a] Recovery was calculated as: (calculated concentration/nominal concentration) × 100.
[b] The IL-6 immuno-assay recovery is $99.45\% \pm 1.08$. (mean value calculated n the three independent assays)
[c] The intra-assay precision, 1.08%, was calculated as the average of the coefficients of variation obtained on the 3 days of validation.
[d] The inter-assay precision, 1.09%, was calculated as the coefficient of variation for the 27 values obtained on the 3 days.

Table 3
Comparison of the IL-6 storage temperatures[a]

Temperature (°C)	Signal (RU)			Mean	Calculated concentration (μg/ml)	Recovery
	RU1	RU2	RU3			
(a) Immuno-assay						
−70	164	164	162	163.33	469.8	104.9
−20	165	165	163	164.33	474.6	105.9
+5	166	164	163	164.33	474.6	105.9
+25	122	120	119	120.33	303.3	67.7
(b) Receptor-assay						
−70	186	184	181	183.67	451.7	100.8
−20	186	186	182	184.67	455.6	101.7
+5	184	182	180	182.00	445.2	99.4
+25	110	108	106	108.00	228.7	51.0

[a] Four aliquots of the same batch were stored at different temperatures during 6 months. The results obtained with the immuno-assay (a) and the receptor assay (b) were similar: The recovery rates obtained for the aliquots stored at −70, −20 and 5°C were similar to those calculated for the quality control sample (103.3 and 100.5% respectively for the immuno- and the receptor-assays). The recovery values obtained for the aliquot stored at +25°C were 67.7 and 51% and show an important degradation of the molecule.

3.4. Limit of detection

The injection of the 'blank' solution has provided a signal of 1.6 RU with a standard deviation of 0.55 RU. Thus, the concentration corresponding to the limit of detection (LOD) must give a signal higher than 3.25 ($=1.6+3*0.55$). The lowest rIL-6 concentration injected was 3.75 ng/ml and gave a signal of 3.6 RU. Thus this rIL-6 concentration was kept as the LOD. To confirm this data a Student-Fischer statistic test was done between the two sets of results: one corresponding to the blank signals and the second corresponding to the signals obtained with the rIL-6 concentration of 3.75 ng/ml. The results have indicated, with a confidence level of 95%, that the injection of the rIL-6 solution at 3.75 μg/ml gave a signal significantly different to the blank.

3.5. Storage condition comparisons

The results obtained with the four aliquots tested in the immuno-receptor assay are summarized in Table 3. The aliquots stored at −70, −20 or +5°C did not show any degradation caused by the storage temperature. The recovery values obtained with these three aliquots were respectively 104.9, 105.9 and 105.9% for the immuno-assay and 100.8, 101.7 and 99.4% for the receptor-assay. These values are similar to those obtained with the quality control of the same order of concentration (303.75 ng/ml) injected for this comparison test: 103.3% for the immuno-assay and 100.5% for the receptor-assay. However, the recovery values obtained with the aliquot stored at +25°C were 67.7 and 51.0%, respectively, for the immuno-assay and the receptor-assay.

4. Discussion

Most of the bioanalytical methods (HPLC, ELISA…) are not related to the biological activity of the drug. The immuno-receptor assay described in this study presents the advantage to distinguish between biologically active and inactive molecules.

In addition, the choice of the Surface Plasmon Resonance (SPR) technology allows to follow all the bindings in real time and without molecular modification like labelling. In this technology one of the reactants (the ligand) is immobilized to the

matrix of the sensor surface. The analytes are then injected over the sensor surface. The interactions are observed as a change in the SPR signal. This change is a consequence of the mass accumulation on the sensor surface causing a modification of the refractive index. The data are presented on a sensorgram which shows the signal in resonance units (RU) as a function of time. In the immuno-receptor assay, sensorgrams were composed of successive reactions. Two different signals were extracted from each sensorgram. The first one corresponds to the interaction of the anti-IL-6 antibody with rIL-6. The second one corresponds to the interaction of rIL-6 with the recombinant human IL-6 soluble receptor. Thus, this immuno-receptor assay was composed of two simultaneous assays: an immuno-assay and a receptor-assay.

Different procedures have been investigated to immobilize the ligand. First, the coupling of a rabbit anti-mouse antibody was evaluated. The assay format was done by the successive injections of anti-IL-6 antibody and rIL-6. The signals obtained were sufficient. The problem occured with this design was the regeneration, different solutions were tested without success and the use of stronger regeneration solutions would be too rough and may cause a partial denaturation of the ligand.

Thus, a second method has been tested by immobilizing Protein A on the sensor surface. The first step of the assay was the injection of the anti-IL-6 antibody which gave a good and stable signal (around 5500 RU). However, the following injection of rIL-6 on this capture antibody, has shown a very unusual result. During a first short part of the injection, rIL-6 has bound to the anti-IL-6 antibody with a maximum signal of 500 RU, but then this signal has decreased strongly until the end of the injection (Fig. 5). The shape of the dissociation phase characterizes a very fast off-rate for the anti-IL-6 antibody/rIL-6 complex with a signal going down under that one of the anti-IL-6 antibody. This serious drop in the signal corresponds to a dissociation of the anti-IL-6 antibody from the surface. This loss of antibody caused by the injection of rIL-6 could be induced by a modification of the antibody Fc part, giving a low stability for its binding to Protein A.

Finally the best result was obtained by the immobilization of Protein A followed by the successive injections of rabbit anti-mouse antibody and anti-IL-6 antibody. This design has provided a minimized anti-IL-6 antibody binding variation. Furthermore, the RAM is Fc fragment specific and its use as capture molecule has allowed a good orientation of the anti-IL-6 antibody. This suitable orientation of the specific antibody has promoted the rIL-6 binding. The observation of the sensorgrams obtained with this design (as example, Fig. 1a and b) shows that rIL-6 did not lead to an unhooking of bound material, proving there was no binding of anti-IL-6 antibody directly to Protein A.

An advantage of the BIAcore® technology is that the interacting analyte can be desorbed and the regenerated surface may be used for subsequent analyte binding measurements. This regeneration of the sensor surface is an important quality parameter during assay development on BIAcore®. The elimination of all the non-covalently bound reactants should be achieved. The best choice is a solution which does not degrade the immobilized ligand (by affecting the molecular structure or by breaking the covalent link to the dextran matrix) but which is strong enough to release all the adsorbed analytes. Protein A is a robust protein, the risk of degradation is very limited. The regeneration with HCl (0.1 M) has allowed to perform the 21 cycles requested for one standard curve and the corresponding quality controls on the same surface. This condition is necessary to assess a limited intra-day precision.

Five different IL-6 concentrations were injected in duplicates to build the standard curves. Within the working range, this intra-day precision of the measurements was under 1%. For all the experiments the samples were diluted to obtain concentrations within this working range. Such a precision of 1% is about five times better than those generally reached with a standard ELISA. The limit of detection (LOD) of the immuno-receptor assay was 3.75 ng/ml. These data were validated by a Student-Fischer statistic test.

The validation procedure has allowed to characterize the performances of the assay. The intra- and inter-day precisions were, respectively, 0.93

and 1.67% for the immuno-assay and 1.08 and 1.41% for the receptor-assay. The most important parameters of the validation was the global recovery: 98.93% ± 1.61 for the immuno-assay and 99.45% ± 1.08 for the receptor-assay. Thus, the performances of this immuno-receptor assay are close to those observed usually with physical bioanalytical methods like HPLC or mass spectrometry. However, the immuno-receptor assay is more attractive than these methods because it provides information about the biological activity of the drug. These informations are useful to compare different production batches of rIL-6 for their therapeutical activity which is supported by the recognition of the IL-6 soluble receptor binding site.

In this study rIL-6 storage conditions have been studied. Four aliquots of the same batch were stored at different temperatures for six months and analysed with the immuno-receptor assay. A reduction of the recovery in the results of the receptor-assay should be a consequence of a damage of the specific epitope involved in the binding with the receptor.

For the immuno-assay the three lowest tested temperatures, −70, −20 and +5°C, have given recovery values slightly higher than 100%. Nevertheless, these results were in agreement with that one of the corresponding quality control (303.75 ng/ml) which has shown a recovery of 103.3% in the immuno-assay. For the receptor-assay, the recovery values for these three aliquots were closed to 100% and the quality control of 303.75 ng/ml had a recovery of 100.5%. It could be concluded that a storage of rIL-6 at −70, −20 or +5°C did not affect the reactivity of the molecule neither with the specific anti-IL-6 antibody nor with the IL-6 soluble receptor.

The results found with the rIL-6 aliquot stored at +25°C were completely different. For the immuno- and the receptor-assays the calculated recovery was respectively 67.7 and 51.0%. Just as for the other aliquots, these values must be compared with the 103.3 and 100.5% obtained with the corresponding quality control (303.75 ng/ml). These results reflect an overall damage of the molecule. In this assay, the binding of rIL-6 to the receptor is directly dependent of its binding to the anti-IL-6 antibody. Also, a decrease in the binding level of rIL-6 to the specific antibody leads to a decrease in the binding level to the sIL-6R. However the recovery obtained with the receptor-assay (51.0%) is lower than with the immuno-assay (67.7%). Thus, we can conclude that this storage at +25°C affects both the antibody and the receptor binding sites.

5. Conclusion

In the present paper a simple procedure has been reported to assess the biological activity of recombinant Interleukin-6. This was achieved by using an optical biosensor based on Surface Plasmon Resonance detection. By developing this assay on BIAcore®, a fast system was established allowing high precision and recovery and a very good reproducibility.

We have used this system to determine the most appropriate storage temperature conditions for rIL-6. However this kind of immuno-receptor assay should be convenient for measuring the biological activity of other recombinant proteins, assessing the lot-to-lot consistency of different batches of biological products and controling the stability of drugs after prolonged storage.

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