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Safety, pharmacokinetics and pharmacodynamics of recombinant human tumour necrosis factor-binding protein-1 (Onercept) injected by intravenous, intramuscular and subcutaneous routes into healthy volunteers

Abstract

The safety, pharmacokinetics and pharmacodynamics of recombinant human tumour necrosis factor-binding protein-1 (r-hTBP-1, Onercept) were investigated after intravascular and extravascular injection, in three studies in healthy volunteers. Subjects received Onercept as single intravenous doses of 5, 15, 50 and 150 mg, or single IV, IM, SC injection of 50 mg, or six repeated SC injections of 50 mg. Based on vital signs, hematology and blood chemistry, antibodies to study drug and local tolerability, r-hTBP-1 exhibited a remarkably safe profile. There was no evidence of alteration of hepatic oxidative metabolism. Recombinant-hTBP-1 showed linear pharmacokinetics that could be described by a triexponential model, and exhibited an initial half-life of 30 min, an intermediate half-life of 4 hours and a terminal elimination half-life of about 15 hours, although it was prolonged to 21 hours after repeated SC injections. The total clearance was estimated at 4 l/h. The initial (Vc) and steady state (Vss) volumes of distribution were approximately 4 l and 10 l, respectively. Renal clearance was minimal, representing around 2.5% of the total clearance, and remained constant after increasing doses of r-hTBP-1. The absorption was slow and biphasic. The immunoactivity of r-hTBP-1 was closely related to its biological activity, although the assessment was limited to only some of the samples. As anticipated in normal healthy volunteers, the pharmacodynamic response was generally not different from placebo. Total TNF-alpha serum levels increased slightly, 1 hour following IV administration of 50 mg and 150 mg r-hTBP-1. However, no major increase in the active entity levels (free TNF-alpha) was observed. In addition, no TNF-alpha-driven biological response was observed, i.e. C-reactive protein, IL-6 and fibrinogen remained almost constant, as did transferrin and albumin. Its safety profile and pharmacokinetic characteristics make Onercept a candidate drug suitable for antagonising pathologically high levels of TNF-alpha as reported in inflammatory, immune and cardiovascular diseases.

Full text

INTRODUCTION

Tumour necrosis factor-binding protein (TBP-1) is a soluble glycoprotein corresponding to the extracellular portion of the human TNF receptor type I (p55). This soluble receptor, which comprises the domain binding to TNF-alpha and TNF-beta, is naturally shed by enzymatic cleavage from the cell membrane

into the circulation where it can be measured. It is excreted in the urine where it was first identified and characterised [1-7].

Cloning of the TNF receptor type I mRNA [8] has permitted the manufacture of recombinant human TBP-1 (r-hTBP-1) by genetic engineering in mammalian cells (Chinese hamster ovary cells). It is a 20-kD molecular weight glycoprotein reproducing an identical amino acid sequence and glycosylation pattern to the natural form excreted in the urine. By specifically binding to the bioactive trimeric form of both TNF-alpha and TNF-beta, r-hTBP-1 neutralises their bioactivities.

Preclinical investigations both *in vitro* [9] and *in vivo* in animal disease models [10-14] as well as toxicology, have shown the activity and safety of r-hTBP-1. Therefore, r-hTBP-1 is being developed as an antagonist to TNF-alpha and beta in clinical indications where TNF's pathophysiological role has been demonstrated. TNF-alpha and beta are cytokines involved in the initiation, expansion and effector phase of the immune and inflammatory responses. Excessive systemic or local tissue release of both TNFs has been shown to be detrimental, for example in rheumatoid arthritis, severe sepsis, Crohn's disease and ischemia-reperfusion syndrome [15-17].

We report here on the safety, the pharmacokinetics and pharmacodynamics, and the absence of immunogenicity of escalating doses of r-hTBP-1 injected by three different routes of administration into a total of 36 healthy volunteers.

MATERIALS AND METHODS

Three randomised studies were conducted in healthy volunteers, and were aimed at safety, pharmacokinetics and pharmacodynamics in different settings. In the placebo-controlled study 1, 12 male subjects received increasing intravenous administrations of r-hTBP-1 at 5, 15, 50 and 150 mg diluted in 10 ml saline and injected over 1 min; in each dose group, placebo was given in a blinded manner to one-third of the subjects. In study 2, the absorption and bioavailability of r-hTBP-1 were investigated in a Latin square, crossover design after single administrations to 6 males and 6 females of 50 mg: intravenously (short IV infusion of 10 ml over 1 min), subcutaneously (1.7 ml into the anterior abdominal wall) and intramuscularly (1.7 ml into the buttock). In both studies, the washout period between injections was 14 days. Study 3 involving 6 males and 6

females investigated the steady state pharmacokinetics of 50 mg r-hTBP-1 injected SC (1.7 ml into the buttock), six times, 48 hours apart. All studies were conducted according to the Declaration of Helsinki, were approved in writing by the local Research Ethics Committee of Cambridge Health Authority, and written informed consent was obtained from all subjects prior to inclusion in the studies.

Experimental subjects

Subjects aged between 26 and 50 years (weight 52-88 kg) participated in the studies (male volunteers only in study 1, both sexes in studies 2 and 3). All subjects were within 15% of their ideal body weight, as determined by the Lorenz formula [18], and were in good health. Female subjects were required to lack childbearing potential for at least two cycles prestudy and at least one cycle post-study.

Safety analysis

Routine safety hematology, coagulation, biochemistry and antibodies assessment were conducted at regular intervals throughout the studies. Adverse events and local tolerability were also monitored in an ongoing fashion.

Sample collection

Samples of venous blood were withdrawn through an indwelling cannula or by separate venepuncture, from the opposite arm of that injected for the first 24 hours. Samples were collected between 0 and 144 hours post-dose for measurement of serum concentrations of TBP-1, TNF-alpha and IL-6, and until 96 hours post-dose for the measurement of C-reactive protein, transferrin and fibrinogen. Samples were allowed to clot for 15 min at room temperature, stored in the refrigerator for a subsequent 1 hour, and serum was separated by centrifugation and stored at - 70° C until required for analysis.

Urine was collected by fractions up to 48 hours after administration of Onercept. The total volume and time of collection of each sample were noted, and duplicate 10 ml samples removed and stored at - 70° C prior to analysis.

All analyses were run by the accredited laboratory of LCG Bioscience (Bourn, Cambridge, UK).

Measurement of serum and urine TBP-1 and serum biological markers

TBP-1 serum and urine concentrations were assessed by an enzyme-linked immunosorbent assay (R & D Systems, Quantikine™ Human TNF-RI ELISA; LOQ: 0.16 ng/ml, reference range: 0.16-10.24 ng/ml, assay CV: < 11%) [19]. In some of the serum samples obtained after IV injection, levels of TBP-1 were also assessed by a specific bioassay [19] using the A9 murine cell line (LOQ: 7 mug/ml).

Serum concentrations of total TNF-alpha were measured by ELISA (Medgenix) [19], using monoclonal antibodies against different epitopes of TNF-alpha (LOQ: 17 pg/ml, reference range: 13-495 pg/ml, assay CV: < 3%). Serum concentrations of free TNF-alpha were measured by an ELISA (Genzyme Predicta) internally validated [19], using a mouse monoclonal antibody to TNF-alpha (LOQ: 13 pg/ml, reference range: 16-1,024 pg/ml, assay CV: < 2%).

Serum concentrations of IL-6 were measured by ELISA (R & D Systems), using a mouse monoclonal and polyclonal antibody to human IL-6 (LOQ: 0.156 pg/ml, reference range: 0.156-10 pg/ml, assay CV: < 7.5%). Serum CRP (LOQ: 5 mg/l, reference range: 5.0-181 mg/l, assay CV: < 7%) and transferrin (LOD: 0.74 g/l, reference range: 0.74-7.41 g/l, assay CV: < 7%) levels were assessed by an immunoturbidometric method. Serum levels of fibrinogen were assessed by the Clauss method (LOD: 1.18 g/l, reference range: 1.5-4.5 g/l, assay CV: < 20%), and serum albumin concentrations were measured after reaction with Bromocresol purple (LOD: 10 g/l, reference range: 10-70 g/l, assay CV: < 3%).

For a given analyte, no cross-reactivity was reported with relevant substances.

The immunological response was assessed by analysing IgM, IgG and IgE, as well as CD4/CD8 ratio pre-study, pre-last dose and post-study. Antibodies to the study drug were assessed by an internally validated radio-immunoprecipitation, where ^{125}I-r-hTBP-1 reacts with antibodies to r-hTBP-1 to form an immunocomplex which is then precipitated and the radioactivity measured in a gamma counter.

Phenotyping

Liver enzyme activity was compared for a series of cytochrome P-450 isoenzymes using a substrate-specific cocktail of drugs. This cocktail was administered to the volunteers 7 days before the first drug administration and 4 days after the sixth and last injection of r-hTBP-1, and their urine collected. The metabolic ratios of the drugs and their metabolites were compared before and after the study. The drugs (and corresponding CYP-450) are: mephenytoin (CYP2C19), tolbutamide (CYP2C9), dextrometorphan (CYP2D6 and CYP3A4), caffeine (CYP1A2) and chlorzoxazone (CYP2E1).

Pharmacokinetic analysis

Pharmacokinetics of r-hTBP-1 following IV, IM and SC administration of Onercept, were determined by non-compartmental analysis (NCA) and mathematical modeling. In addition, numerical deconvolution was used to describe the drug absorption in studies 2 and 3. Only the results from the NCA and numerical deconvolution are reported here, and modeling results will be described elsewhere.

Individual pharmacokinetic and deconvolution analyses [20, 21] were performed using Kinetica® v. 2.01 (Innaphase, France), WinNonLin v. 3.0 (Pharsight Corporation, USA) and Microsoft® Excel 97 SR-1 (Microsoft Corporation, USA). Pharmacokinetic parameters were calculated using standard formulas [22].

Model-independent method (NCA)

Endogenous TBP-1 serum levels were detected in all subjects before drug administration. Before all calculations, the baseline was subtracted from measured TBP-1 concentrations to assess the net increase due to drug administration. The baseline was estimated as being the average between the value observed at time 0 and the value observed at time 144 hours. If baseline subtraction resulted in a "reduced concentration" below zero, it was set equal to zero. C_{max} represented the serum maximum reduced concentration, and its time of occurrence (t_{max}) was the observed value.

The area under the serum concentration *versus* time curve (AUC) and under the serum concentration *versus* time first moment curve (AUMC) were calculated by

the log-linear and linear trapezoidal rules, respectively, and extrapolated to infinity.

The elimination constant ke was obtained for the log-transformed reduced concentrations by linear regression of the terminal phase. The terminal half-life $t_{1/2}$ was calculated as

$$t_{1/2} = 0.693/ke \text{ - Total serum clearance}$$

was calculated as:

$$CL = Dose / AUC$$

Mean residence time was

$$MRT = AUMC / AUC,$$

and the volume of distribution at steady state was estimated as: $V_{ss} = MRT.CL$. Renal clearance was estimated over 48 hours, from the ratio of the amount of drug excreted unchanged in the urine, A_e(corrected for the endogenous TBP-1 by subtraction of the average excretion in those subjects having received the placebo) to the AUC. The fraction excreted unchanged in urine was expressed as the ratio of corrected $A_e$to the dose of r-hTBP-1. Creatinine clearance was calculated for each subject after r-hTBP-1 and placebo administrations as

$$CL_{creatinine} = U.V / P$$

(where U is the urinary concentration, V the urinary flow rate and P the plasma creatinine concentration).

Numerical deconvolution

In the second study, a numerical deconvolution was applied to both the IM and SC observed concentrations of each subject using each individual's IV data [20], Individual drug absorption rate and extent were computed, as well as the absolute bioavailability (F). Mean maximum absorption rate (V_{max}), the corresponding t_{max}, and the time at which 90% of the drug is absorbed ($T_{90\%}$)

were calculated. Individual graphical representations of the individual absorption profiles were visually inspected to characterise the absorption process.

Pharmacodynamic analysis

As the variations with time of the markers were minor, only graphical and descriptive representations were performed for all biomarkers, except for total TNF-alpha.

Total TNF-alpha variations were best approached by calculating the relative increase over the baseline as follows:

$$delta\,\% = [\ (TNF_t - TNF_0) / TNF_0\ ] - 100$$

with TNF_t = concentration of TNF-alpha at time t and TNF_0 = concentration of TNF-alpha at time 0

Statistical analysis

In all studies, descriptive statistics were performed on the data. In addition, in study 1, an ANOVA (with factors: treatment; period; group; and subject-within-group) was applied to the creatinine clearance to detect whether r-hTBP-1 administration could influence the subject's renal function. The same analysis was also run on the percentage change in total TNF-alpha, and statistical contrasts regarding the factor treatment were tested (95% simultaneous confidence intervals) using Tukey's approach. Regarding the phenotyping, statistical analysis of the difference in metabolic ratios between day 14 and day 7 was run using the SAS univariate procedure (paired t-test). In addition, 95% confidence intervals were calculated for each mean difference.

RESULTS

The monitoring of safety and local tolerability in 36 volunteers through vital signs, ECG, heart rate, oral body temperature, routine hematology and chemistry analysis, adverse event monitoring and antibodies measurement showed that the overall safety profile of Onercept was very good. Three volunteers showed a slight decrease in either the systolic or diastolic blood pressure without

associated symptoms. When compared to norms intended to highlight individual values of potential clinical relevance, these were judged as insignificant. Intra- and extravascular administrations of r-hTBP-1 did not increase oral body temperature significantly, as illustrated by a maximal rise to 37.8° C 3 hours after the sixth SC injection, which returned to normal at the next time point (4 hours). No variations of other vital signs were recorded. During ECG monitoring, none of the PR, QRS, QT and QTc intervals was outside the volunteers' safety reference ranges, except in one volunteer who showed QTc interval measurements above 450 msec both after placebo and the dose of 150 mg IV, which was therefore not related to the drug. With regards to hematology, there were some occurrences of decreased hemoglobin, red blood cell count or packed cell volume, but most of them (8/10) were related to values lower than normal before drug administration. The decrease in the red cell series appeared to be correlated with the time pattern of blood collection, and was more frequent in females than in males. Coagulation parameters were not influenced by the repeated administration of Onercept. Also, volunteers presented some isolated occurrences of decreased white blood cells or decreased total protein counts. Isolated cases of decreased calcium, cholesterol, bilirubine, creatinine and uric acid were considered anecdotal. Urinalyses were normal.

Thirty percent of the volunteers did not report any adverse events. Altogether, after 136 administrations, 69 adverse drug events were recorded: none of them was serious, and were mainly graded as mild in intensity. The majority of them resolved spontaneously, and consisted of headache, abdominal pain, diarrhoea, cold sensation, sleepiness, tiredness and restlessness. CD4/CD8 ratios as well as IgM, IgG and IgE measurements were normal, and regular measurements did not show any changes throughout the study. Importantly, none of the volunteers developed any antibody to Onercept.

The assessment of serum concentrations of hTBP-1 after increasing IV doses (5-150 mg) (Figure 1), single IV, IM, SC and repeated SC injections of 50 mg allowed the calculation of the pharmacokinetic parameters reported in Table 1. Regardless of the route, r-hTBP-1 presented with an absolute SC or IM bioavailability of around 80% (Figure 2). The disposition profile after IV injection is illustrated in Figure 1, and a non-compartmental approach to these data resulted in the following half-lives: 0.5, 2.5 and 12 hours. Non-compartmental analysis of the data after the last subcutaneous dose estimated the terminal half-life to be 21 hours. There were no gender differences. The absorption was

apparently biphasic, with a rapid onset, and was subsequently sustained for over 1 day. Pharmacokinetics were shown to be dose-independent at least up to 150 mg (Figure 1, Table 1). Renal excretion was minimal as it was less than 2.5%. After six doses of 50 mg at 48-hour intervals, the accumulation was low, estimated to be less than 1.5 (Figure 3).

Although bioassays have known sensitivity limitations, the immunoactivity correlated well with the biological activity (Figure 4) as attested by a coefficient of correlation of 0.97.

Single and repeated administrations were not associated with any significant variation from the normal ranges of most of the pharmacodynamic markers. Total TNF-alpha levels increased slightly 1 hour after the IV dose of 50 and 150 mg, however, no such increase could be detected for the active moiety, free TNF-alpha. This increase in TNF-alpha was not reproduced after the other IV injections, nor was it observed after the extravascular doses. IL-6 serum levels (and CRP to a lesser extent) exhibited some fluctuations after IV, IM or single and repeated SC injections, but remained within the normal range.

Finally, Table 2 summarises the effect of repeat doses of Onercept on the main cytochrome P450 isoforms. The statistical comparison of the urinary metabolic ratios of the parent drug to metabolite did not show significant changes in of any of the P450 isoenzymes studied, although for CYP2D6 and CYP3A4 the ratios tended to decrease after r-hTBP-1.

DISCUSSION

In these studies, Onercept was injected, for the first time in man, into 36 healthy human male and female volunteers, aiming at evaluating the safety and tolerability of r-hTBP-1, and at assessing its pharmacokinetics. Pharmacodynamics were also tested through the monitoring of free and total TNF-alpha, IL-6, fibrinogen, C-reactive protein, transferrin and albumin.

The overall safety and local tolerability profile was very good. Safety laboratory tests did not reveal any trends, and variations were often judged as related to study conditions, or subject normal variability. This observation is well known for studies run in healthy subjects [23, 24]. The same pattern was found after examining the vital signs or the adverse events recorded throughout the

studies. This safety profile was not modified by repeated injections of the drug, and supports chronic administration of r-hTBP-1.

From this safety pattern, there is no indication that any specific population should be excluded or special caution exercised based on pharmacokinetics if Onercept is to be administered to patients with impairment of the hepatic or renal function, or even cardiac function. This is also supported by the assumption that the elimination of r-hTBP-1 mainly occurs through the peptidic pathway, as renal clearance was shown to be negligible (this was not expected, as hTBP-1 was first discovered in urine). The need for therapeutic adjustment is therefore not expected to be a limitation to the administration in humans, although the hepatic metabolic exploration needs further characterisation. Sex did not play a discriminating role in these studies, at least from a pharmacokinetic and safety standpoint, which should allow administration in both males and females. This confirmation is important to document, as some pathologies such as rheumatoid arthritis have a greater incidence in the female population.

Recombinant-hTBP-1 was not found to be immunogenic after three doses weekly for 2 weeks, which is usually sufficient to trigger the immune system. This may occur later, as the number of subjects was low, as was the number of injections and the time of follow-up was limited. However, this is not expected as the molecule is well characterised and is identical to its natural counterpart as assessed with the current analytical methods.

In addition, the results of urine phenotyping on the main isoenzymes of P450 cytochrome indicated that Onercept would not act as an inhibitor or an inducer of the main drug-metabolising enzymes. This is likely to enable the combination of r-hTBP-1 with other therapies in clinical indications such as autoimmune [15], inflammatory disorders [16] or cardiovascular diseases [17].

After injection, r-hTBP-1 distributes initially into the plasma volume, and quite rapidly to a steady state volume slightly greater than the volume of distribution of albumin. The total volume of distribution remains, however, less than the extracellular water, which is not unusual for a protein of this size. Onercept demonstrates a terminal half-life of around 15 hours which allows some flexibility in the frequency of administration. The biphasic absorption pattern results in serum profiles remaining elevated for more than 24 hours, a distinct advantage

when compared to the rapid distribution and elimination half-life after IV bolus injection. It is noticeable that the bioavailability of r-hTBP-1 is high after both SC and IM injections, and the variability in the exposure is no more pronounced after such extravascular administration than after IV injection. As seen following multiple injections, this allows 48-hour dosing intervals or a three-times a week dosing regimen. Onercept biodistribution may differ from other soluble anti-TNF chimeric molecules that have a much higher molecular weight.

A negligible effect was seen on the selected biological markers, which was not unexpected as these studies were performed in normal, healthy subjects. The apparent rise in total TNF-alpha could be due to the fact that the assay does not differentiate between free and TBP-1-bound TNF-alpha. After administration, the high concentration of TBP-1 could mobilise TNF-alpha, either linked to its cellular receptor, or otherwise distributed in extracellular (tissue) water space.

CONCLUSION

In these first-time-in-man studies, Onercept was demonstrated to be safe by a complete panel of tolerability parameters, it was not immunogenic and had a potentially weak influence on P450 metabolic enzymes. Its pharmacokinetic characteristics, mainly high bioavailability, sustained absorption and half-life, probably allow for less than daily extravascular administration. These advantages have to be further addressed in patient studies.

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Figures

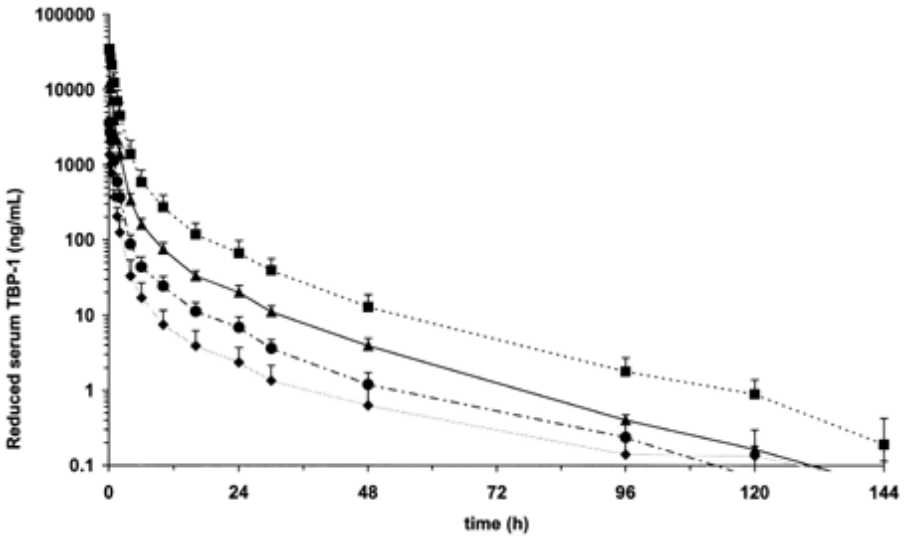


Figure 1

Geometric means (± SD) of reduced serum concentration of TBP-1 *versus* time curves after IV administration of 5 mg (n = 6), 15 mg (n = 6), 50 mg (n = 6) and 150 mg (n = 6) r-hTBP-1 (Onercept) to healthy subjects (rhomb 5 mg, round 15 mg,, triangle 50 mg,

square 150 mg, see Materials and methods for explanation).

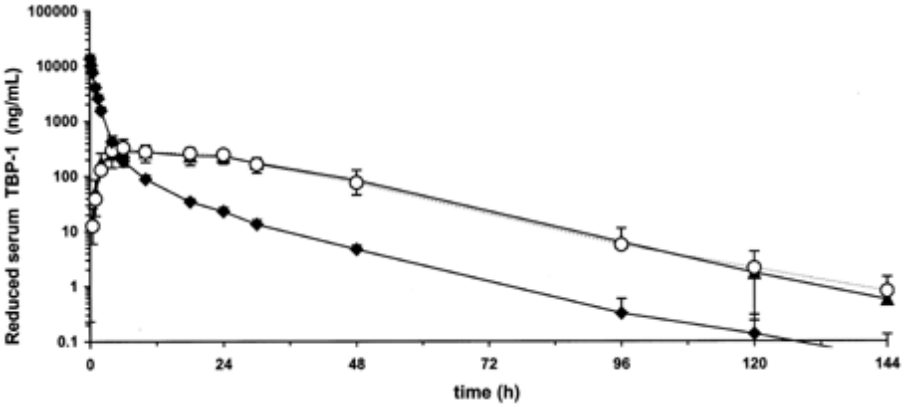


Figure 2

Geometric means (± SD) of reduced serum concentrations of TBP-1 *versus* time after IV, SC and IM administration of 50 mg r-hTBP-1 (Onercept) to healthy subjects (n = 12) (rhomb IV, round IM, triangle SC).

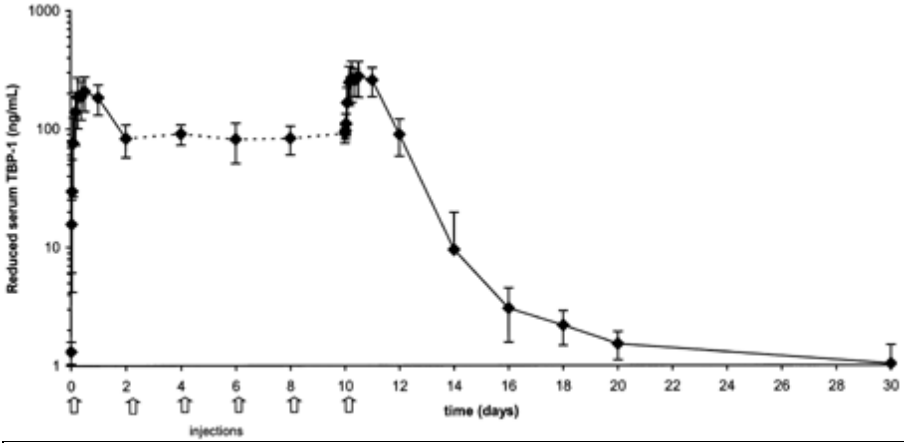


Figure 3

Geometric means (± SD) of reduced serum concentrations of TBP-1 *versus* time after six SC administrations of 50 mg r-hTBP-1 (Onercept) to healthy subjects (n = 12).

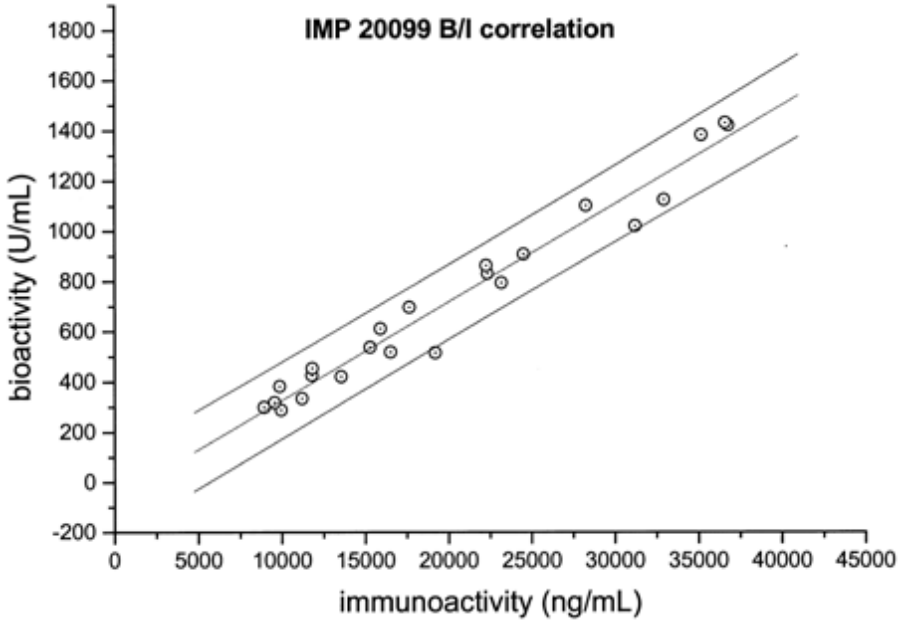


Figure 4

Correlation between immunoassay and bioassay in selected serum samples of 12 healthy male subjects receiving IV doses of 50 and 150 mg r-hTBP-1 (study IMP 20099).

Table 1
Pharmacokinetic parameters (mean ± SD) of r-hT BP-1 (Onercept) obtained by a non-compartmental approach after increasing intravenous administration (study 1, n = 12), single intravenous, intramuscular and subcutaneous injection (study 2, n = 12), and repeated subcutaneous injections (study 3, n = 12) into healthy volunteers

Dose	5 mg	15 mg		50 mg				150 mg
Study #	Study 1			Study 2			Study 3	Study 1
Route	IV			IV	IM	SC	Repeat SC	IV
AUC_{tlast}(h·mug/l)	1,428 ± 428	3,927 ± 898	13,947 ± 2,132	14,780 ± 2,065	11,302 ± 2,362	11,294 ± 2,268	‡7,395 ± 1,828	42,914 ± 11,551
CL (l/h)	2.8 ± 0.7	3.9 ± 1.2	3.4 ± 0.5	3.4 ± 0.5	–	–	–	3.7 ± 1.3
$t_{1/2}$(h)	19 ± 4	15 ± 6	14 ± 1	15 ± 3	15 ± 1	14 ± 1	21 ± 3	14.5 ± 1.9
MRT (h)	3.7 ± 0.2	3.0 ± 0.4	2.7 ± 0.3	3.0 ± 0.3	–	–	–	3.0 ± 0.6
V_{ss}(l)	10.3 ± 2.7	11.7 ± 3.5	9.3 ± 1.8	10.4 ± 1.8	–	–	–	10.5 ± 1.1
fe (%)	1.3 ± 0.8	1.4 ± 0.7	1.8 ± 0.5	0.9 ± 0.7	0.5 ± 0.3	0.4 ± 0.2	†0.5 ± 0.2	2.5 ± 1.4
F	–	–	–	–	0.76 ± 0.13	0.77 ± 0.12	–	–

AUC_{tlast}: area under the curve until the last measurable time point; CL: clearance; $t_{1/2}$: terminal half-life; MRT: mean residence time; V_{ss}: volume of distribution at steady state; fe: fraction excreted unchanged in the urine; F: absolute bioavailability.
‡AUC_{0-48}, †over the first 48-hour collection interval.

Table 2
Statistical analysis (paired-t test) of the mean differences in P450 metabolic ratios between day 14 and day-7 results in healthy subjects receiving repeated doses of 50 mg r-hT BP-1 (Onercept) (n = 12)

Metabolic ratio	Mean difference Day 14-Day 7	95% Confidence intervals Lower limit	Upper limit	p value
CYP2C19	0.914	– 0.418	2.24	0.1592
CYP2C9	99.5	– 694	893	0.7876
CYP2D6	– 503	– 1,083	76.5	0.0852
CYP3A4	– 0.140	– 0.312	0.032	0.1028
CYP1A2	– 0.503	– 1.31	0.305	0.1982
CYP2E1	– 10.5	– 3,771	3,750	0.9952

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type 1 diabetes, islets of Langerhans, beta-cells, cytokines, interleukin-1beta, interleukin-1beta receptor type I, nitric oxide, inducible nitric oxide synthase, heat shock protein 70, interferon regulating factor 1.

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microsatellites, interleukin-10 promoter, multiplex-PCR, Caucasian, African.

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