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Intravenous Human Recombinant Tumor Necrosis Factor Receptor p55-Fc IgG1 Fusion Protein Ro 45-2081 (Lenercept): A Double Blind, Placebo Controlled Dose-Finding Study in Rheumatoid Arthritis

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ABSTRACT. ***Objective.*** To determine the optimal dose regimen for intravenous Ro 45-2081 (lenercept) in patients with rheumatoid arthritis (RA) by evaluating efficacy, safety, tolerability, and pharmacokinetic and pharmacodynamic characteristics.

Methods. Adult patients with longstanding RA who were taking stable doses of nonsteroidal antiinflammatory drug and/or low dose corticosteroids but who had stopped their previous disease-modifying antirheumatic drug were randomly assigned to receive 3 intravenous infusions, one every 4 weeks, of placebo or Ro 45-2081 in a double blind, placebo controlled, parallel group multicenter trial. Patients received one of the following: (1) placebo, (2) low dose Ro 45-2081 (0.05 mg/kg, maximum 5 mg), (3) middle dose (mid-dose) Ro 45-2081 (0.2 mg/kg, maximum 20 mg), or (4) high dose Ro 45-2081 (0.5 mg/kg, maximum 50 mg). Efficacy measures included change from baseline in number of swollen joints and tender joints, scores on physician and patient assessments of disease activity, and patient assessment of pain, as well as acute phase reactants.

Results. Patients treated with Ro 45-2081 exhibited improvement after one day of the first intravenous infusion. This treatment benefit maximized by 2 weeks but diminished thereafter. After the second and third infusion, improvement was of shorter duration as non-neutralizing anti-Ro 45-2081 antibodies developed and accelerated clearance of Ro 45-2081. There were no antibodies after the first infusion. This made efficacy transient in the mid-dose group and modest in the low and high dose groups at 12 weeks of treatment, resulting in no statistical differences at most time points or doses of Ro 45-2081. The majority of adverse experiences were mild or moderate, and were not related or only remotely related to study drug. No clinically relevant changes in mean laboratory values were reported. The third dose pharmacokinetic measurements showed that the average Ro 45-2081 clearance rate more than doubled compared with the first dosing interval, thus reducing the average Ro 45-2081 AUC by 36%.

Conclusion. Intravenous Ro 45-2081 every 4 weeks proved to be well tolerated and transiently effective in the mid-dose group and modestly effective in the low and high dose groups in patients with longstanding RA. The interactions between Ro 45-2081, its non-neutralizing anti-Ro 45-2081 antibody, and the clinical benefit remain complex, but affected efficacy over the 12 weeks of treatment as Ro 45-2081 concentrations fell. (J Rheumatol 2003;30:680–90)

Key Indexing Terms:
TUMOR NECROSIS FACTOR RECEPTOR FUSION PROTEIN Ro 45-2081
RHEUMATOID ARTHRITIS CONTROLLED TRIAL ANTIBODY FORMATION

Rheumatoid arthritis (RA) is a common disease characterized by inflammatory synovitis followed by joint erosions involving both cartilage and bone. Since many patients with RA endure unsatisfactory therapeutic response to conventional disease modifying antirheumatic drugs (DMARD) with incomplete inhibition of structural damage[1-3], and

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treatment often has to be discontinued due to adverse experiences, new treatment modalities are welcome.

Strategies directed against the proinflammatory cytokines involved in the pathogenesis of RA are a promising therapeutic approach. There is substantial evidence that cytokines such as interleukin 1ß (IL-1ß) and IL-6 and tumor necrosis factors (TNF) are involved in the induction and maintenance of synovial inflammation similar to that observed in RA[4-7]. Several clinical trials with monoclonal anti-TNF-α antibody treatment [as monotherapy or in combination with methotrexate (MTX)] have shown significant efficacy in patients with RA[6,8-13]. Also, a soluble TNF receptor p75 fusion protein has proved effective in refractory RA as monotherapy[14,15] and in combination with MTX[16]. This compound has been shown to have advantages regarding efficacy and tolerability over MTX in early RA[17]. Moreover, trials with infliximab, etanercept, and adalimumab over one year have shown a significant inhibition of progression of structural damage seen on radiographs in patients with active RA[13,17,18]. This was confirmed in etanercept treated patients over 2 years[19].

Ro 45-2081 (lenercept) is a fusion protein molecule combining the extracellular domain of the p55 kDa TNF receptor and immunoglobulin heavy chain sequences. The cDNA encodes the complete extracellular domain of the human 55 kDA receptor (residues 1–182). The combination of the 2 TNF receptors with immunoglobulin IgG1 heavy chain provides a longer *in vivo* half-life of 176 hours following the first dose and higher TNF-binding (neutralizing) capacity than endogenous TNF receptors[20,21]. The drug is made through a fermentation process in CHO (Chinese hamster ovary) cells. Its potential therapeutic actions in RA are thought to derive from its neutralization of excess TNF. Preliminary efficacy data, based on a single intravenous (IV) infusion, indicated that Ro 45-2081 is well tolerated with a long-lasting amelioration of clinical measures reflecting inflammatory synovitis. Significant pain relief, reduction in counts of swollen and tender joints, and improvement according to both physician rated and patient rated disease activity assessment instruments was evident within 24 hours of treatment and was maintained for at least 3 weeks[22]. An extension of this study of over one year duration[23] continued to demonstrate efficacy. Due to its long half-life, Ro 45-2081 administered intravenously every 4 weeks might maintain antirheumatic efficacy. At the end of 3 years the sponsor discontinued the extension trial, with patients still receiving benefit from IV Ro 45-2081 despite having anti-Ro 45-2081 antibodies (unpublished data).

Our study was designed to investigate the magnitude and duration of decreased inflammatory synovitis following infusions of Ro 45-2081 or placebo every 4 weeks for 3 months. Data from this study, conducted in Europe and the United States, were published in abstract form[24-26].

We believe it is worthwhile to publish this study years after its completion since it represents the first clinical experience with a TNF receptor fusion protein in a controlled trial and demonstrates the influence on efficacy of non-neutralizing antibodies.

MATERIALS AND METHODS

In a double blind, placebo controlled, parallel group, multicenter trial, adult patients with longstanding RA were randomly assigned to receive slow IV infusions of either placebo or one of 3 doses of Ro 45-2081. Previous DMARD had to be stopped at least 4 weeks before the first dosing of Ro 45-2081. Treatment with stable doses of nonsteroidal antiinflammatory drug (NSAID) and/or low dose corticosteroids was continued.

Inclusion criteria. Patients must have been at least 18 years old and women of childbearing potential must have had a negative pregnancy test and used reliable means of contraception. Entry criteria were as follows: (1) American College of Rheumatology 1987 diagnostic criteria for RA; (2) more than 13 tender joints; (3) more than 9 swollen joints, and 2 of the following: (a) C-reactive protein (CRP) > 30 mg/l or erythrocyte sedimentation rate (ESR) > 28 mm/h, (b) patient global assessment of disease activity at least 50 mm (on 100 mm visual analog scale, VAS), (c) physician global assessment of disease activity at least 50 mm (on 100 mm VAS), and (d) patient pain assessment at least 50 mm (on 100 mm VAS).

Exclusion criteria. Patients with evidence of clinically relevant cardiovascular disease, alcohol or drug abuse within the preceding 6 months, joint surgery within the preceding 2 months, or major infection within the preceding one month were excluded. Also excluded were patients exhibiting substantially elevated serum creatinine (> 1.5 mg/dl), AST or ALT (more than twice the upper limit of normal for the testing laboratory), or bilirubin (≥ 3 mg/dl), as well as substantially subnormal hemoglobin (< 9.5 g/dl males, < 9.0 g/dl females), total white blood cell count (< 3×10^9/l), or platelet count (< 150×10^9/l).

Prestudy lead-in. Qualified patients were stabilized on specified background RA therapies for 4 weeks prior to the first dose of study medication. Only stable doses of NSAID or ≤ 10 mg/day prednisolone, but not DMARD, were permitted during this period and throughout the study. Intraarticular corticosteroids were not allowed during the 6 weeks prior to study entry and throughout the study. Analgesics except for paracetamol (acetaminophen), propoxyphene, or codeine were not permitted.

Dosing. Following this 4 week prestudy lead-in each study patient was administered one of the following regimens: slow IV infusions once every 4 weeks of (1) placebo or (2) low dose Ro 45-2081 (0.05 mg/kg, maximum 5 mg), (3) middle dose Ro 45-2081 (0.2 mg/kg, maximum 20 mg), or (4) high dose Ro 45-2081 (0.5 mg/kg, maximum 50 mg). Patients returned to the study center weekly after the first and third IV infusions as well as one week after the second IV infusion.

Primary efficacy measure. Change from baseline in the number of swollen joints was the primary efficacy measure. At every study visit, 48 joints per patient were assessed and classified as either swollen or not swollen: temporomandibular, sternoclavicular, acromioclavicular, shoulder, elbow, wrist, metacarpophalangeals (MCP) 1–5, interphalangeal (IP) thumb, proximal interphalangeals (PIP) 2–5, hip, knee, ankle mortise, ankle tarsus, and metatarsophalangeals (MTP). In addition, a mean swollen joint count was calculated for each patient for the first and third dosing intervals (4 visits within each interval). When this mean swollen joint count was reduced by more than 50% from baseline, a patient was considered a responder for purposes of statistical evaluation of primary efficacy. Any patient who discontinued the study prematurely due to poor efficacy was counted as a nonresponder for analysis of the primary efficacy variable. However, missing values for all other analyses were imputed using the last observation carried forward method.

Secondary efficacy measures. Secondary measures included change from baseline in the number of tender joints, scores on physician and patient

assessments of disease activity, and patient pain assessment; the latter 3 assessment instruments were 100 mm VAS. Tender joint counts were based upon a 50 joint assessment (including hips) by pressure and manipulation; each joint was classified as either tender or not tender. In addition, laboratory indicators of inflammation, ESR, and CRP were measured. The WHO/ILAR core set was used to determine the WHO-20[27]. WHO 20 is defined as a 20% reduction in both swollen and tender joint counts as well as a 20% improvement in 2 of the following measures: physician global assessment of disease activity, patient assessment of disease activity, patient assessment of pain, ESR, or CRP.

Adverse experiences. Adverse experiences were monitored throughout the study. An adverse experience was defined as any adverse change from a patient's baseline condition that occurred after study medications (including placebo) had been administered.

Vital signs. Supine blood pressure, pulse rate, and body temperature were measured prestudy, predose, at 1 and 8 h postdose, 24 h after the first dose, and during study visits at Weeks 1, 4, 5, 8, 9 and 12. A 12 lead electrocardiogram was taken 3 times during the study: (1) during prestudy lead-in, (2) 4 h after the first infusion, and (3) at the end of treatment (Week 12).

Laboratory analyses. Laboratory testing was performed at most visits. ESR, CRP, hematology, coagulation, complement activity, clinical biochemistry, and anti-Ro 45-2081 antibodies were measured in blood samples. Protein, glucose, blood, and pH were measured in urine samples. Whenever an unexplained or unexpected laboratory value was reported, the test was repeated until the value returned to normal or an adequate explanation was determined.

Pharmacokinetics/pharmacodynamics. Blood samples (10 ml) for assessment of Ro 45-2081, TNF-α, and anti-drug antibody concentrations were taken during the prestudy lead-in period, 1 h before and immediately after each infusion, at 24 h after the first and third infusions, and at every scheduled visit. The testing of lenercept concentrations used an antibody that measured total lenercept concentrations (bound to TNF and free), with measurements carried out when lenercept was present and after it was cleared. Quantification limit for the assay was set at 5 ng/ml.

Statistical analyses. The Cochrane Armitage trend test[28] was conducted for the primary efficacy measure. For all efficacy measures at all visits, group means, standard errors of absolute values, and percentage improvements relative to baseline were calculated. The number of patients who met the WHO-20 criteria was also determined at the end of the study[29]. A closed testing procedure was used: if a significant result was seen, then the testing proceeded in a predefined stepped manner. However, if a test was found to be nonsignificant, no further testing was done. This method guaranteed that all calculated tests were interpreted on the alpha level used in the calculation without inflating the global error probability.

RESULTS

Population. A total of 100 adult patients (76% women) were randomized to 4 treatment groups (Table 1). Demographics were similar across the groups; mean ages ranging from 56 to 60 years; 64% of participants in the high dose group were women, but 80% in the other groups; rheumatoid factor positivity ranged between 76 and 84% among groups. Disease activity was documented by swelling in the majority of joints evaluated, mean baseline swollen joint count ranging between 28 and 33, mean baseline ESR ranging between 39 and 54 mm/h. Physician and patient appraisals of disease activity and pain appraisals documented active disease in all groups. Before participation, the majority of patients had been taking a variety of antirheumatic agents; NSAID were taken by 87% of patients and oral corticosteroids by 74%. DMARD (hydroxychloroquine, gold salts, sulfasalazine), including cytostatics (e.g., MTX, azathioprine), were taken by 76% of patients prior to participation (Table 2).

Efficacy. Mean swollen joint counts and CRP values for each treatment group on every assessment day are depicted in Figure 1. Mean values and degree of improvement from baseline for all efficacy measures are noted in Table 3. In contrast to patients taking placebo, whose swollen joint counts inconsistently improved from baseline, patients treated with Ro 45-2081 (all dose levels) exhibited an initial improvement over baseline, in some patients within 24 h, and this effect peaked by one or 2 weeks.

Most improvements were observed in the high dose group, where swollen joint counts improved by as much as 45% (Table 3). The treatment effect diminished over time in all groups, but especially in the mid-dose group, where almost no treatment effect was seen at the end of the 3 month period, and in the low dose group the effect was notably diminished. The high dose group maintained a 30% improvement in average swollen joint count at the end of the 3 month treatment period, 4 weeks after the third and final IV Ro 45-2081 infusion.

All other efficacy measurements including ESR and CRP reflected this pattern of improvement after the first, second, and third infusion for all dose groups of Ro 45-2081 (Table 3). By one month, using the 50% reduction in the swollen joint count responder criteria, there was no statistical difference evaluating all groups simultaneously ($p = 0.16$, Cochrane Armitage trend test). Therefore, in accordance with the closed testing procedure, no further calculations were done.

For the primary efficacy analysis, a patient was categorized as a responder if more than a 50% reduction from baseline was achieved in mean swollen joint counts averaged over the visits independently for the first and third dosing intervals. The percentage of patients qualifying as responders was relatively modest across groups (Table 4). When compared with placebo response rates, only the high dose response rate (> 50% reduction in mean swollen joint count) during the third dosing interval was significantly superior ($p = 0.02$, Cochrane Armitage trend test). Neither the mid-dose group ($p = 0.074$) nor the low dose group showed statistical difference. Although during the first dosing interval the high dose group displayed the highest response rate (24%), at one month there was no statistically significant difference between the high dose and placebo groups (chi-squared $p = 0.285$, 95% CI 0.595, 18.659 for responders).

The data were also analyzed to determine the number of patients who met the WHO-20 criteria for disease response to treatment[27]. At the end of the 3 month treatment period, 4%, 29%, 20%, and 21% of patients in the placebo, low, mid, and high dose treatment groups, respectively, met the WHO-20 criteria (none statistically significant).

Table 1. Baseline demographic and disease characteristics (mean and range, except where otherwise noted).

	Placebo	Intravenous Ro 45-2081 Low Dose (0.05mg/kg)	Mid-dose (0.2mg/kg)	High Dose (0.5mg/kg)	All Treatments
Patients/group, n	25	25	25	25	N = 100
Male/female	5/20	5/20	5/20	9/16	24/76
Age, yrs	60 (29–77)	57 (28–73)	58 (26–71)	56 (25–71)	58 (25–77)
Weight, kg	68 (51–98)	68 (52–104)	70 (46–98)	67 (43–95)	68 (43–104)
Height, cm	164 (151–180)	166 (149–177)	163 (147–179)	167 (151–187)	165 (147–187)
RA Duration, yrs	11 (1–28)	12 (3–24)	14 (3–34)	13 (3–33)	12 (1–34)
Clinical measures					
Swollen joints, /48	28 (12–44)	31 (14–44)	32 (16–46)	33 (22–46)	31 (12–46)
Tender joits, /50	33 (15–48)	33 (14–46)	38 (14–50)	36 (20–50)	35 (14–50)
Physician appraisal, /100*	72 (42–95)	74 (55–96)	76 (35–100)	77 (60–93)	75 (35–100)
Patient appraisal, /100*	71 (42–100)	67 (38–100)	74 (28–93)	71 (37–100)	71 (28–100)
Patient pain appraisal, /100*	70 (34–100)	63 (30–100)	72 (39–92)	74 (45–100)	70 (30–100)
Laboratory measures					
ESR, mm/h	39 (5–94)	41 (6–108)	51 (12–129)	53 (12–96)	46 (5–129)
CRP, g/l	36 (0–124)	45 (3–184)	50 (3–125)	53 (3–114)	46 (0–184)
Rheumatoid factor, pos/neg**	19/4[†]	21/2[‡]	23/2	19/6	82/14

* Physician/patient appraisals used 100 mm visual analog scales. ** < 8 IU/ml. [†] Two patient values missing. [‡] One patient value missing.

Table 2. Pre-entry NSAID and slow-acting antirheumatic drugs (SAARD).

	Placebo	Intravenous Ro 45-2081 Low Dose (0.05 mg/kg)	Mid-dose (0.2 mg/kg)	High Dose (0.5 mg/kg)	All Treatments
Patients/group, n	25	25	25	25	N = 100
		Total Number of Patients Using Single Medication			
Total NSAID	18	21	24	24	87
Total corticosteroids, oral	20	18	18	18	74
Methotrexate	11	17	15	11	54
Azathioprine	4	2	3	5	14
Sulfasalazine	2	1	6	4	13
Penicillamine	4	—	—	4	8
Sodium aurothiomalate	2	2	3	—	7
Hydroxychloroquine	—	2	—	1	3
Chloroquine phosphate	—	1	—	1	2
Aurothioglucose	1	—	1	—	2
Cyclosporine	—	1	—	1	2
Auranofin	—	—	—	1	1
Cyclophosphamide	—	—	—	1	1
Total DMARD	24	26	28	29	107
Total patients	15	20	19	22	(76%) 76

General safety. In this study, 81% of the study patients reported 210 adverse experiences. As shown in Table 5, the most frequently reported adverse experience was influenza, reported by 6% of the study patients, followed by headache, upper abdominal discomfort, nausea, and leg edema, each reported by 5% of patients. The majority of adverse experiences were mild or moderate and were considered unrelated or only remotely related to study medication. Aggravated arthritis was reported by 13% of patients, but was regarded as a loss or lack of efficacy to Ro 45-2081 subsequent to DMARD withdrawal rather than a true adverse event. Some investigators noted that myalgia preceded the recurrence of joint pain, indicating loss of efficacy. Similarly, hematomas were noted at the infusion site in 10% of patients, but were omitted from the list of adverse experiences.

Infections. Although TNF-α inhibitors might increase the risk of infection, no relationships between number or type of infection reported and treatment group were detected (Table 6). Patients receiving only placebo reported as many infections as did patients receiving Ro 45-2081, and types of infection appeared evenly distributed across treatment groups. For patients with an infection, there was no increase

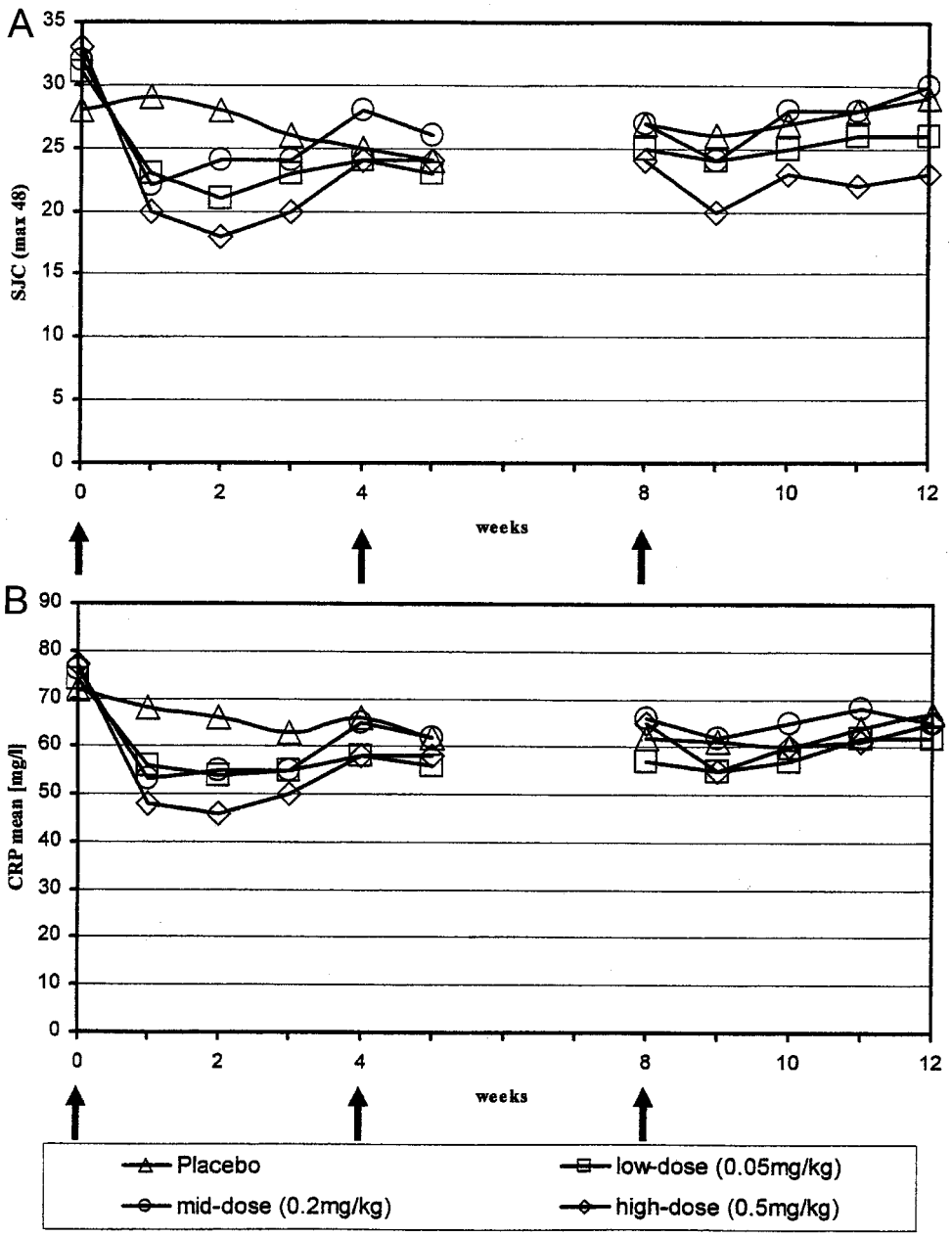

Figure 1. Mean swollen joint counts (A) and mean serum CRP (B) for each treatment group on every assessment day. Clear improvement from pretreatment baseline values (BL) is evident in all groups treated with Ro 45-2081. In contrast, swollen joint counts often worsened for patients given placebo.

in severity in those treated with Ro 45-2081 versus placebo. There was no detrimental effect of Ro 45-2081 on these measures compared to placebo.

Serious adverse events. Patients administered high dose Ro 45-2081 reported no serious adverse events in this study. Six study patients were hospitalized due to nausea, edema (2 patients receiving placebo), leg edema (one patient, low dose), back pain, erosive gastritis (2 patients, mid-dose), and anemia (one patient, high dose). One patient discontinued the study prematurely due to an adverse experience. Six patients were hospitalized for aggravated RA, 2 each from the placebo, low dose, and mid-dose groups.

Laboratory variables. There were no clinically relevant changes in mean laboratory values in this study, but transient lymphocytopenia was reported for 22 (22%) patients, among whom 4 received only placebo; lymphocytopenia was not dose-dependent and was not associated with increased rates of infection in any treatment group.

Table 3. Efficacy results. Negative values indicate deterioration from baseline.

	Placebo	Intravenous Ro 45-2081 Low Dose (0.05 mg/kg)	Mid-dose (0.2 mg/kg)	High Dose (0.5 mg/kg)
Patients/groups, n	25	25	25	25
Swollen joints, /48				
Baseline mean (range)	28 (12–44)	31 (14–44)	32 (16–46)	33 (22–46)
1 day, mean (% change from baseline)	27 (4)	27 (13)	27 (16)	28 (15)
1 wk	29 (–4)	23 (26)	22 (31)	20 (39)
2 wks	28 (0)	21 (32)	24 (25)	18 (45)
3 wks	26 (7)	23 (26)	24 (25)	20 (39)
4 wks	25 (11)	24 (23)	28 (13)	24 (27)
12 wks	29 (-4)	26 (16)	30 (6)	23 (30)
Tender joints, /50				
Baseline mean (range)	33 (15–48)	33 (14–46)	38 (14–50)	36 (20–50)
1 day, mean (% change from baseline)	30 (9)	26 (21)	28 (26)	26 (28)
1 wk	31 (6)	20 (39)	23 (39)	17 (53)
2 wks	31 (6)	21 (36)	26 (32)	16 (28)
3 wks	32 (3)	20 (39)	28 (26)	17 (53)
4 wks	31 (6)	21 (36)	31 (18)	22 (39)
12 wks	32 (3)	24 (27)	30 (21)	24 (33)
Physician global, /100				
Baseline mean (range)	72 (42–95)	74 (55–96)	76 (35–100)	77 (60–93)
1 day, mean (% change from baseline)	69 (4)	64 (14)	58 (24)	60 (22)
1 wk	68 (6)	56 (24)	53 (30)	48 (38)
2 wks	66 (8)	54 (27)	55 (28)	46 (40)
3 wks	63 (13)	55 (26)	55 (28)	50 (35)
4 wks	66 (8)	58 (22)	65 (14)	58 (25)
12 wks	67 (7)	62 (16)	65 (14)	65 (16)
Patient global, /100				
Baseline mean (range)	71 (42–100)	67 (38–100)	74 (28–93)	71 (37–100)
1 day, mean (% change from baseline)	59 (17)	53 (21)	49 (34)	48 (32)
1 wk	66 (7)	49 (27)	42 (43)	41 (42)
2 wks	68 (4)	48 (28)	46 (38)	43 (39)
3 wks	63 (11)	52 (22)	48 (35)	52 (27)
4 wks	66 (7)	54 (19)	65 (12)	58 (18)
12 wks	62 (13)	61 (9)	62 (16)	61 (14)
Patient pain, /100				
Baseline mean (range)	70 (34–100)	63 (30–100)	72 (39–92)	74 (45–100)
1 day, mean (% change from baseline)	58 (17)	50 (21)	46 (36)	47 (36)
1 wk	67 (4)	47 (25)	42 (42)	39 (47)
2 wks	67 (4)	48 (24)	47 (35)	40 (46)
3 wks	64 (9)	50 (21)	50 (31)	52 (30)
4 wks	65 (7)	50 (21)	64 (11)	58 (22)
12 wks	61 (13)	62 (2)	65 (10)	65 (12)
WHO-20, 12 wks	1 (4)	7 (29)	5 (20)	5 (21)

Table 4. Number (%) of responders*.

	Placebo	Intravenous Ro 45-2081 Low Dose (0.05 mg/kg)	Mid-dose (0.2 mg/kg)	High Dose (0.5 mg/kg)
n	25	25	25	25
First dosing interval	2 (8)	4 (16)	3 (12)	6 (24)
n	25	25	25	24
Third dosing interval†	0 (0)	1 (4)	3 (12)	4 (17)

* Responder: 50% reduction in swollen joint count averaged over the visits. † p = 0.02, Cochran Armitage test.

Table 5. Number (%) of adverse events reported by ≥ 5% of patients.

	Placebo	Intravenous Ro 45-2081			All Treatments
		Low Dose (0.05 mg/kg)	Mid-dose (0.2 mg/kg)	High Dose (0.5 mg/kg)	
Patients/group, n	25	25	25	25	N = 100
Influenza	3 (12)	3 (12)	—	—	6 (6)
Headache	2 (8)	—	1 (4)	2 (8)	5 (5)
Gastralgia	3 (12)	1 (4)	1 (4)	—	5 (5)
Nausea	1 (4)	2 (8)	1 (4)	1 (4)	5 (5)
Leg edema	1 (4)	2 (8)	1 (4)	1 (4)	5 (5)

Table 6. Number (%) of infections.

	Placebo	Intravenous Ro 45-2081			All Treatments
		Low Dose (0.05 mg/kg)	Mid-dose (0.2 mg/kg)	High Dose (0.5 mg/kg)	
Patients/group, n	25	25	25	25	N = 100
Immune system	6 (24)	4 (16)	1 (4)	2 (8)	13 (13)
Respiratory system	—	1 (4)	1 (4)	3 (12)	5 (5)
Gastrointestinal system	1 (4)	—	—	3 (12)	4 (4)
Conjunctivitis	1 (4)	1 (4)	2 (8)	—	4 (4)
Body as whole	—	—	—	1 (4)	1 (1)
Urinary system	—	—	1 (4)	—	1 (1)

Immunoglobulins were elevated very infrequently; a few patients had elevated IgE that was not accompanied by allergic reactions. Thirteen cases (4 in the placebo group) of elevated banded neutrophils and 8 cases (one placebo group) of hematuria were reported; concurrent menses was not ruled out. All these laboratory findings were considered clinically insignificant and no relationship to dose or to other reported adverse experiences was evident.

Pharmacokinetics. Although area under the curve (AUC) and peak serum concentration (C_{max}) increased with dose after first and third Ro 45-2081 infusions (Table 7), both measures decreased after the third infusion compared with the first infusion since total body clearance, especially for mid and high dose groups, increased substantially. While the average AUC reduction was 36% (844 to 530 µgh/ml), C_{max} and steady-state volume of distribution (V_{ss}) were only slightly affected, with average reductions of 8% and 16%, respectively. Following the third infusions, clearance rates increased 53%, 132%, and 129% for low, mid, and high dose groups, respectively, amounting to a mean rate increase of more than 100% (0.33 to 0.69 ml/min), and correspondingly a mean half-life (T½) reduction of nearly 50% (167 to 94 h). This increased clearance, believed due to non-neutralizing anti-Ro 45-2081 antibodies forming during repeated Ro 45-2081 treatment, resulted in a stable C_{max} between first and third infusions, but a reduced AUC after the third month compared with that after the first month (Figure 2).

Pharmacodynamics. Anti-drug antibodies. Antibodies against Ro 45-2081 were observed in all but 3 patients (low dose group) who received intravenous Ro 45-2081. Median antibody concentrations increased over time in most treatment groups receiving active drug (Table 8), but concentrations were not dose-dependent at the end of either the first

Table 7. Pharmacokinetic measurements (mean ± SD).

	First Ro 45-2081 Infusion			Third Ro 45-2081 Infusion		
	Low Dose (0.05 mg/kg)	Mid-dose (0.2 mg/kg)	High Dose (0.5 mg/kg)	Low Dose (0.05 mg/kg)	Mid-dose (0.2 mg/kg)	High Dose (0.5 mg/kg)
AUC, µg·h/ml	179 ± 53	686 ± 179	1667 ± 428	141 ± 78	300 ± 111	1150 ± 603
$C_{max,}$ ng/ml	1131 ± 187	5450 ± 1313	12,175 ± 2236	1028 ± 248	5185 ± 1302	11,030 ± 1718
T 1/2, h	169 ± 49	189 ± 53	144 ± 35	168 ± 95	52 ± 18	61 ± 63
V_{ss}, l/kg	0.058 ± 0.021	0.068 ± 0.022	0.056 ± 0.024	0.064 ± 0.019	0.038 ± 0.019	0.052 ± 0.011
CL, ml/min	0.329 ± 0.057	0.346 ± 0.086	0.329 ± 0.086	0.505 ± 0.255	0.804 ± 0.344	0.755 ± 0.480

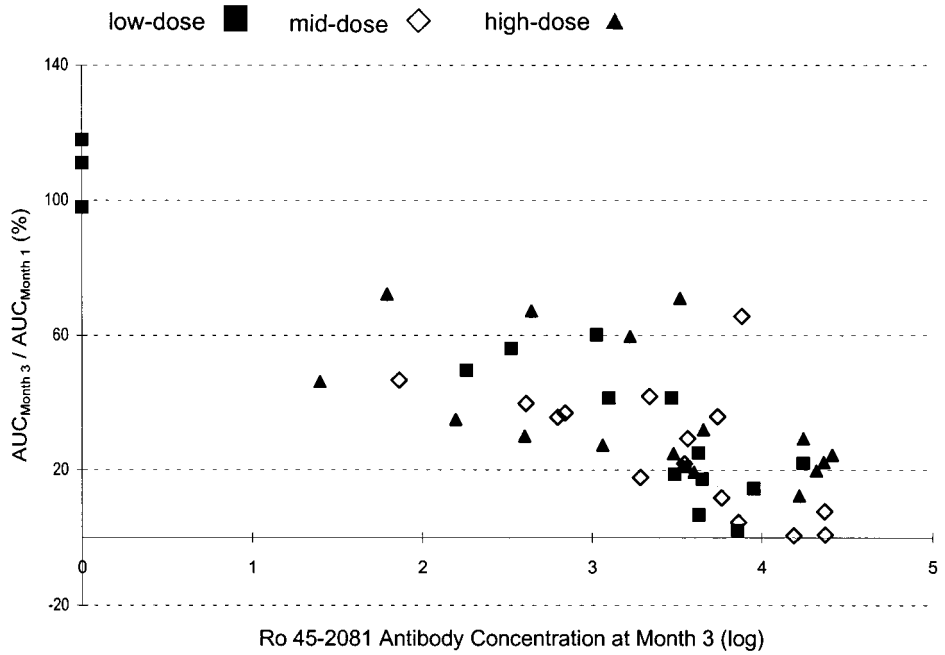

Figure 2. AUC reduction by non-neutralizing anti-Ro 45-2081 antibodies. Following the third infusions, Ro 45-2081 clearance rates increased more than 100% (0.33 to 0.69 ml/min), with mean half-life reduced nearly 50% (167 to 94 h). This antibody induced acceleration of drug clearance reduced the mean AUC by 36% (844 to 530 µg•h/ml). Only patients in the low dose group had no evidence of anti-Ro 45-2081 antibodies.

Table 8. Anti-Ro 45-2081 antibody concentrations.

	Anti-Ro 45-2081 Antibody Concentrations, ng/ml, median (range)			
	Baseline	Week 4	Week 8	Week 12
Placebo	n = 23, 0 (0–169)	n = 21, 0 (0–79)	n = 17, (0 (0–313)	n = 13, 0 (0–49)
Low dose, 0.05 mg/kg	n = 25, 0 (0–183)	n = 25, 472 (0–3639)	n = 19, 861 (0–4248)	n = 19, 2935 (0–17,558)
Mid-dose, 0.2 mg/kg	n = 25, 0 (0–159)	n = 25, 393 (1–6895)	n = 24, 873 (0–7162)	n = 21, 3662 (20–23,570)
High dose, 0.5 mg/kg	n = 25, 0 (0–243)	n = 23, 646 (0–6610)	n = 23, 951 (0–8950)	n = 22, 3161 (0–25,875)

or third dosing interval. These anti-Ro 45-2081 antibodies were non-neutralizing, as shown by subsequent analyses.

TNF-α. Total (bound plus unbound) TNF-α concentrations ranged from 3 to 36 pg/ml during the pretreatment lead-in period. These concentrations immediately and steeply increased as expected, although in a dose-independent manner, for all patients who received Ro 45-2081, in clear contrast to TNF-α concentrations of placebo treated patients, which remained stable (Figure 3). TNF bound by Ro 45-2081 is bio-inactive.

DISCUSSION

Ro 45-2081, a recombinant humanized fusion protein neutralizing excess proinflammatory TNF, binding both TNF-α and TNF-ß, was very well tolerated and produced transient efficacy for the mid-dose and modest efficacy in the low and high dose groups in this trial of severe, active RA. Adverse events occurred infrequently, were dose-independent, commonly mild or moderate, and usually not or only remotely drug related.

Although heightened susceptibility to infection was anticipated as a potential risk[30-34], intravenous Ro 45-2081 produced no apparent increase in number or type of infection. In a large trial using Ro 45-2081 versus placebo in patients with severe sepsis or early septic shock, overall mortality and organ failure were not significantly improved or impaired by Ro 45-2081[35]. In this severely ill population, substantial negative effects of Ro 45-2081 would have been expected to be prominent had immune impairment by Ro 45-2081 been an issue. This is consistent with findings in this trial and other trials using Ro 45-2081 in patients with RA and multiple sclerosis[22-26,36].

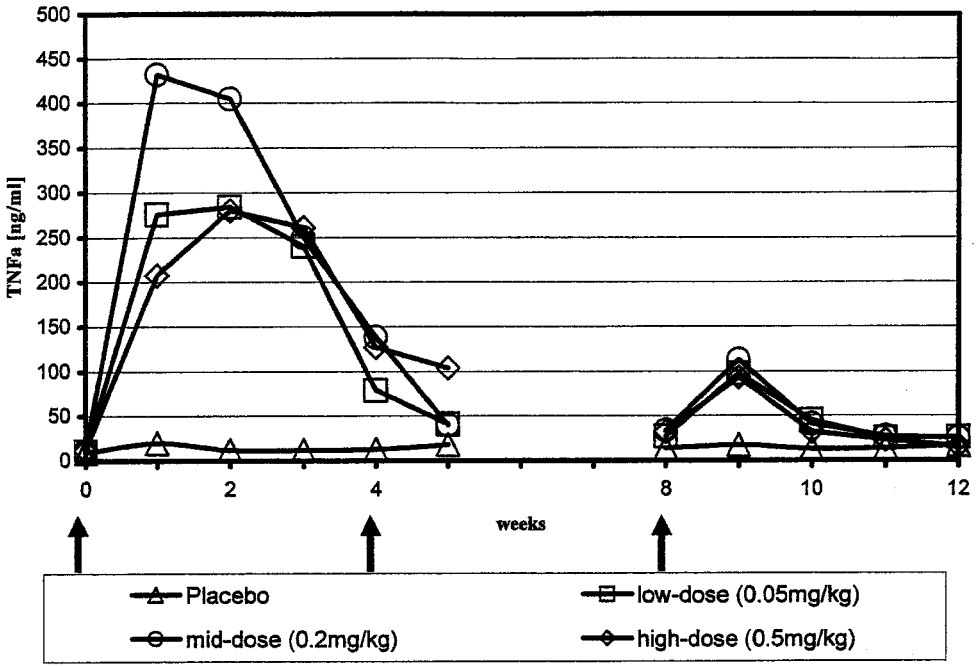

Figure 3. Total (bound plus unbound) TNF-α concentrations rapidly and steeply increased, although in a dose-independent manner, for patients treated with Ro 45-2081. In contrast, TNF-α concentrations of placebo treated patients remained unchanged.

The primary efficacy analysis in this study was based upon the number of patients achieving greater than 50% reduction in baseline swollen joint count at 2 time points, 4 weeks after the first and third infusions (Weeks 4 and 12). These time points reflected the lowest drug effects measured and therefore they proved to be a poor indicator of peak efficacy, but they do reflect the trough efficacy level that patients experienced.

All secondary efficacy variables measured at every study visit exhibited immediate treatment effects for 2 to 3 weeks after the first infusion in all active treatment groups. Maximum group mean improvements were consistently observed for the high dose treatment group for all efficacy variables, but with patient rated disease activity the mid-dose group exhibited slightly more improvement at most time points. This does indicate that there is a dose response in the range of doses explored in this trial. The formation of anti-drug antibodies complicates the interpretation of the data on the longterm dose effect.

Therapeutic effects were not maintained throughout the study: investigators observed that many patients began to lose benefits of the study drug after both second and third infusions earlier than after the first infusion, consistent with the occurrence of non-neutralizing anti-drug antibodies accelerating the clearance of Ro 45-2081. Also during this time an increased frequency of myalgias occurred, possibly indicating increased disease activity.

Pharmacokinetic characteristics of the first dose were consistent with those previously reported in healthy male volunteers[37]. All patients administered active drug exhibited similar patterns of serum Ro 45-2081 clearance following the first intravenous infusion. Following second and third infusions, however, Ro 45-2081 clearance patterns varied among patients, but were clearly accelerated. Formation of an antibody-drug complex is proposed as the most likely explanation for this phenomenon of accelerated elimination, since clearance rates increased with anti-drug antibody concentrations. In turn, increased Ro 45-2081 clearance rates would account for the diminished beneficial effect observed after repeated infusions. However, anti-Ro 45-2081 antibody titers appeared to be unrelated to adverse events, and dose-independent.

The reason Ro 45-2081 appears more immunogenic than similar recombinant proteins most likely derives from a non-human amino acid sequence at the hinge region between the p55 kDa TNF receptors and the IgG1 FC region. It appears that antibodies directed at Ro 45-2081 map to this epitope on the molecule. This was confirmed after completion of this study in additional investigations (unpublished data). This affects clearance, but does not affect binding activity of the molecule in *ex vivo* testing.

The observed TNF-α elevations following Ro 45-2081 infusion were anticipated. The analytical method used to determine serum TNF-α measured total TNF-α (both free

TNF-α and TNF-α bound to Ro 45-2081). Because the half-life of Ro 45-2081 is much greater than that of TNF-α, accumulation of total (free plus bound) TNF-α was expected. However, comparison of immunoassay results with those of a cytotoxicity assay, measuring only bioactive TNF-α, revealed that the TNF-α circulating after a dose of Ro 45-2081 was not bioactive. At least initially it was thought that there was a large excess of lenercept to TNF so that nearly all circulating TNF-α was bound in a stable and bioinactive fashion. Since TNF-α elevations subsided at the end of dosing intervals but remained higher than unbound TNF-α concentrations pretreatment, this accumulation of bound, bioinactive TNF-α appears to be counterbalanced by Ro 45-2081 clearance.

In conclusion, intravenous Ro 45-2081 every 4 weeks proved safe and effective in patients seriously afflicted with RA. Since anti-drug antibody formation accelerates drug clearance, later efficacy was compromised as Ro 45-2081 concentrations fell. Dosing regimens to address the accelerated removal of Ro 45-2081 by changing the dose, reducing dosing intervals, and/or combining with commonly used immunosuppressives, such as MTX, may allow the efficacy to be maintained while continuing the safety profile of Ro 45-2081 in patients with RA.

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