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Toxicity, Pharmacokinetics, and Dose-Finding Study of Repetitive Treatment with the Humanized Anti-Interleukin 6 Receptor Antibody MRA in Rheumatoid Arthritis. Phase I/II Clinical Study

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ABSTRACT. ***Objective.*** To evaluate the safety and pharmacokinetics of multiple infusions of a humanized anti-interleukin-6 (IL-6) receptor antibody, MRA, in patients with rheumatoid arthritis (RA).

Methods. In an open label trial, 15 patients with active RAwere intravenously administered 3 doses (2, 4, or 8 mg/kg) of MRA biweekly for 6 weeks, and pharmacokinetics were assessed. Patients continued on MRAtreatment for 24 weeks, and were then assessed for safety and efficacy.

Results. The treatment was well tolerated at all doses with no severe adverse event. Increased total serum cholesterol was detected as an MRArelated reaction in 10/15 (66%) patients. There was no statistically significant difference in the frequency of adverse events among the 3 dose groups. There were no new observations of antinuclear antibody or anti-DNAantibody, and no anti-MRAantibody was detected. The $T_{1/2}$ increased with repeated doses and as the dose increased. $T_{1/2}$ after the 3rd dose of 8 mg/kg reached 241.8 ± 71.4 h. In 12/15 (80%) patients whose serum MRA was detectable during the treatment period, objective inflammatory indicators such as C-reactive protein, erythrocyte sedimentation rate, and serum amyloid A were completely normalized at 6 weeks, although there was no statistically significant difference in efficacy among the 3 dose groups. Nine of 15 patients achieved ACR 20 at 6 weeks. At 24 weeks, 13 patients achieved ACR 20 and 5 achieved ACR 50.

Conclusion. Repetitive treatment with MRA was safe and normalized acute phase response in patients with RA. Optimal dosing schedule was not defined in this small study, but maintenance of serum MRAconcentration seemed important to achieve efficacy. (J Rheumatol 2003;30:1426–35)

Key Indexing Terms:
RHEUMATOID ARTHRITIS INTERLEUKIN 6 THERAPY
HUMANIZED ANTI-IL-6 RECEPTOR ANTIBODY

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by persistent synovitis and progressive destruction of cartilage and bone with the presence of rheumatoid factors. RA is also associated with systemic inflammatory manifestations in addition to local inflammation of multiple joints. Although the causes are not fully understood, many cytokines with inflammatory and joint destructive properties are involved in the development of RA[1-3]. These inflammatory cytokines are thought to be a potential therapeutic target for treatment.

Interleukin 6 (IL-6) was originally identified as an antigen-nonspecific B cell differentiation factor produced by activated mononuclear cells[4], and it has been shown to be produced from RAsynovial fibroblasts stimulated by tumor necrosis factor (TNF) or IL-1[3]. Most clinical abnormalities in RA can be accounted for by the unregulated hyperproduction of IL-6[1]. It may induce activation of autoreactive T cells and polyclonal hypergammaglobulinemia and emergence of autoantibodies as a result of B cell differentiation[5–7]. IL-6, as a hepatocyte-stimulating factor, may induce acute phase proteins, resulting in elevation of serum fibrinogen, C-reactive protein (CRP), and amyloid A (SAA) concentrations, and a decrease in serum albumin[8-11]. Further, hyperproduction of IL-6 may cause bone absorption through activation of osteoclasts, resulting in osteoporosis and bone destruction[12]. IL-6 may induce thrombocytosis by acting as

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a megakaryocyte differentiation factor to produce platelets[13,14]. Indeed, elevation of IL-6 concentrations has been observed in both serum and synovial fluid of patients with RA[15,16]. Correlation has been observed between serum IL-6 levels and clinical and laboratory indices of RA[17]. Wendling, *et al* reported that administration of mouse monoclonal anti-IL-6 antibody to 5 patients with RA for 10 consecutive days resulted in clinical and biological (CRP) improvement although the improvement was transitory[18]. Therefore, interference with the action of IL-6 may constitute a new therapeutic strategy for RA.

The IL-6 signal is mediated via the 80 kDa IL-6 receptor (IL-6R) molecule on the cell surface or the soluble form of IL-6R (sIL-6R), followed by dimerization of the 130 kDa signal transducer gp130, which is bound to the IL-6/IL-6R complex[19,20]. MRAis a humanized anti-human IL-6R monoclonal antibody (Mab) that inhibits the binding of IL-6 to IL-6R or sIL-6R. The effect of MRA was examined in the collagen induced arthritis model with cynomolgus monkeys, because MRA crossreacts with monkey IL-6R but not with rodent IL-6R. MRA inhibited the development of arthritis and improved such inflammatory indicators as CRP, fibrinogen, and erythrocyte sedimentation rate (ESR)[21]. In a SCID mouse model into which synovial tissues from RA patients were implanted, MRA treatment resulted in shrinkage of the implanted tissue and significant reductions in the numbers of inflammatory cells and osteoclasts[22].

With patients' informed consent and approval of the Ethical Committee and the Advanced Medical Treatment Review Board of Osaka University, we treated some patients with refractory RA with MRA. The patients received MRA with stepwise dose escalation, mostly up to 50 mg/patient twice a week, with monitoring for safety. The results showed a rapid decrease in CRPto the normal range, and alleviation of joint swelling and tenderness[23].

Based on these findings, we performed a phase I/II open label, dose-ascending trial to evaluate the safety, pharmacokinetics, and efficacy of repetitive intravenous treatment with MRA in patients with established and active RA.

MATERIALS AND METHODS

Patients. The study began in August 1999 and ended in August 2000. Sixteen patients (median age 55 yrs, range 32–72), diagnosed with RAin accord with the 1987 American College of Rheumatology (ACR) criteria and with a history of disease activity for more than 6 months, were enrolled (Table 1). They had failed to respond to at least one of the disease-modifying antirheumatic drugs (DMARD) or immunosuppressants, or were unable to continue the treatments due to adverse reactions. We required patients to have at least 3 swollen joints and at least 6 tender joints, ESR ≥ 30 mm/h, serum CRP ≥ 2.0 mg/dl, a white blood cell count ≥ 3500/μl, and platelet count ≥ 10^5/μl. Pregnant women, nursing women, and women of childbearing potential not using an effective method of contraception were excluded. Patients were also excluded if they had severe disability (Steinbrocker Class IV)[24], a history of a serious allergic reaction, any other concurrent collagen disease, significant cardiac, blood, respiratory, neurological, endocrine, renal, hepatic or gastrointestinal disease, or an active intercurrent infection. DMARD and immunosuppressants were discontinued at least 4 weeks before the initial MRAadministration. Stable doses of nonsteroidal antiinflammatory drugs and prednisolone (10 mg daily maximum) were allowed. Use of parenteral and/or intraarticular steroid within 4 weeks before the initial MRAadministration and during the study period were not permitted. Written informed consent was obtained from each patient before enrollment. The study was approved by the Ministry of Health, Labour and Welfare of Japan and the local ethics committees. Patients were indemnified by the sponsor of the study, Chugai Pharmaceutical Company Ltd., Tokyo.

Study medication and administration. MRAis a humanized anti-human IL-6R Mab of the IgG1 subclass. The antibody was produced by Chugai Pharmaceutical Co. Ltd. by continuous fermentation of Chinese hamster ovary cells, which had been transfected with cloned DNAcoding for MRA, and was purified from culture supernatant by a series of column chromatography steps. The MRAretains specificity for human IL-6R and is of high affinity. The antibody was stored at 4°C in 50 ml vials containing 2.5 mg MRA/ml.

The appropriate amount of MRAwas diluted to a total volume of 500 ml in sterile saline and administered intravenously with a 0.2 μm in-line filter. The drug was infused at a rate of about 0.3 ml/min over the first 15 min of infusion, while the patient's condition was closely monitored. If there was no sign of anaphylactic reaction, the rate of infusion was increased. The infusion was performed over a period of 2 h. To ensure safety, patients were carefully monitored during infusion and for at least 1 h after completion. During the first 3 doses, patients were under supervision of the investigator or coinvestigator for at least 24 h after MRAinfusion.

This was an open label, dose-ascending study with 3 dose groups, 2, 4, and 8 mg/kg. For each dose, MRAwas administered biweekly for 6 weeks, and pharmacokinetics and safety data were collected up to 6 weeks after the first dose. The study was started from the lowest dose, 2 mg/kg. Escalation to the next dose level was permitted if the previous dose level was satisfactory in terms of safety and tolerance as determined by the sponsor after discussion with the sponsor's medical expert and the investigators or coinvestigators. The next higher dose was examined with a group of newly recruited patients. With patients' consent and if MRA treatment was well tolerated and showed an improvement of CRP or ESR compared to baseline, patients were allowed to continue MRAtreatment until 24 weeks and were then further assessed for safety and efficacy.

Assessment of safety and efficacy. Safety was monitored until 4 weeks after the last dose. Frequency and severity of adverse effects and adverse drug reactions were observed. Clinical and laboratory tests were performed at screening, at baseline, on dosing day, at 1 week after every dose, and at 4 weeks after last dose. For the first 3 doses, clinical and laboratory tests were also performed on the day after each dose and 2 days after each dose. Laboratory measurements including a complete blood cell count and ESR were performed at each study site. Other laboratory tests were undertaken by the central laboratory, SRL Co., Ltd. Serum levels of MRA were measured with an enzyme immunoassay using MT18 Mab specific for another binding site on IL-6R than that detected by MRA in combination with the sIL-6R. The captured MRAwas detected using a biotinylated Mab specific for an epitope in the variable region of MRA, at a dose that does not inhibit the binding of IL-6R. The lowest concentration that could be reliably detected was 1.0 μg/ml.

The primary efficacy measurements were the changes in CRPand ESR over time, up to 6 weeks after the first infusion. Other efficacy measures were ACR 20, 50, and 70 improvement[25] and the change over time in ACR components up to 4 weeks after the last dose.

Statistical methods. For safety analysis, the number of patients who reported adverse events and number of adverse events were recorded for each adverse event for each dose group. Incidence rates of adverse events were calculated with 95% confidence intervals. Pharmacokinetic parameters were calculated from serum MRA concentration data, based on the non-compartment analysis method.

Table 1. Characteristics of the patients at entry.

	MRADose, mg/kg			
	2	4	8	Total
No. of patients	5	5	5	15
Age, yrs, median (range)	55 (40–61)	54 (40–63)	55 (32–72)	55 (32–72)
Sex, M:F	1:4	2:3	1:4	4:11
Duration of disease, yrs, median (range)	10 (4–16)	6 (1–8)	4 (2–25)	7 (1–25)
No. of failed DMARD, median (range)	5 (3–7)	4 (2–6)	4 (2–6)	4 (2–7)
Tender joint counts, mean ± SD*	26 ± 17	26 ± 16	20 ± 11	24 ± 14
Swollen joint counts, mean ± SD*	19 ± 10	23 ± 12	19 ± 9	21 ± 10
ESR, mm/h, mean ± SD	92 ± 24	92 ± 27	76 ± 24	87 ± 25
CRP, mg/dl, mean ± SD	6.9 ± 4.5	5.3 ± 2.4	5.4 ± 1.8	5.9 ± 3.0
WBC, per µl, mean ± SD	8646 ± 3068	10722 ± 1619	10506 ± 2853	9958 ± 2587
Platelets, 10^4/µl, mean ± SD	30.8 ± 4.5	32.8 ± 11.5	48.0 ± 13.7	37.2 ± 12.7

*Tender joint count was assessed with 49 joints (maximum joint count was 49). Swollen joint count was assessed with 46 joints (maximum joint count was 46). All values were mean ± SD. DMARD: disease modifying anti-rheumatic drugs, ESR: erythrocyte sedimentation rate (Westergren); CRP: C-reactive protein, WBC: white blood cell count.

For the efficacy analysis, changes in each of the ACR components, such as CRP, ESR, swollen joint counts, tender joint counts, modified Health Assessment Questionnaire score, physician's global assessment, patient's global assessment, and patient's pain assessment, from baseline for each dose group were analyzed by paired t tests, and mean changes from baseline among the dose groups were analyzed by t tests. The dose relationship was analyzed by appropriate statistical procedures such as Jonckheere's test for trends. Significance was set at $p < 0.05$.

RESULTS

Patients. Sixteen patients were enrolled in the study; their disposition is illustrated in Figure 1. After enrollment, one patient in the 8 mg/kg group was found to have a chest radiograph abnormality and was thus ineligible and was withdrawn. A total of 15 patients were included in the analysis. Demographic and clinical data at the entry period are summarized in Table 1. The median age was 55 years (range 32–72 yrs) and the median duration of RA was 7 years (range 1–25). The patients had a mean of 24 tender joints (range 8–41) and 21 swollen joints (range 10–35). There were no clinically significant differences among all the dose groups.

Safety. Treatment tolerance of MRA was good. A total of 132 adverse events were reported in all 15 patients analyzed for safety (Table 2 describes adverse events appearing in more than 2 patients). In the 2, 4, and 8 mg/kg groups, there

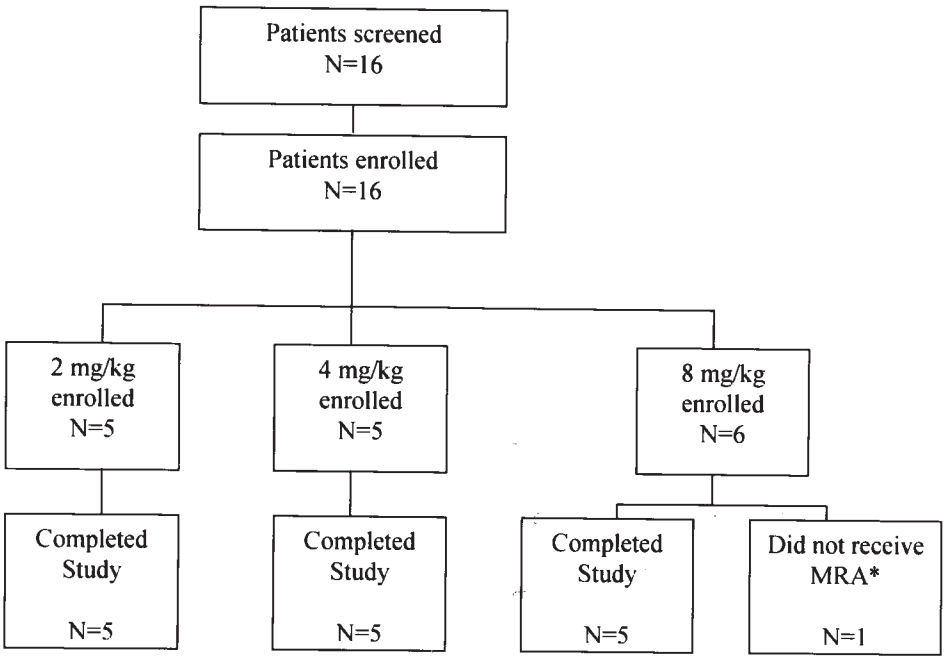

Figure 1. Disposition of patients through the stages of the study. *Patient was found to be ineligible for study because of a chest radiograph abnormality and was withdrawn before dosing.

Table 2. Adverse events (reported in more than 2 patients in this study).

	MRADose, mg/kg			
	2	4	8	Total
No. of patients	5	5	5	
Blood and lymphatic system disorder				
Iron deficiency anemia	0	1	1	2
General disorder and administration site condition				
Pyrexia	1	0	2	3
Infection and infestations				
Nasopharyngitis	3*	2**	0	5
Tinea blanca	0	2*	2*	4
Blister	0	1*	1	2
Metabolism and nutrition disorder				
Iron metabolism disorder	1	3	2	6
Musculoskeletal connective tissue and bone disorder				
Back pain	1	0	1*	2
Skin/subcutaneous tissue disorder				
Contact dermatitis	0	0	2*	2
Dermatitis NOS	1*	0	1	2
Urticaria NOS	2**	0	0	2
Investigation				
Alanine aminotransferase increased	2	1	0	3
Aspartate aminotransferase increased	1	1	0	2
Blood cholesterol increased	4*	2*	4*	10
Blood glucose increased	4	2	0	6
Blood iron decreased	0	2	0	2
Blood LDH increased	2	3	0	5
Blood pressure increased	1*	1	0	2
Blood thrombin abnormal	1	2	0	3
Blood triglyceride increased	2	1	2*	5
Blood urea increased	2	1	0	3
Glycosuria present	1	1	0	2
Hematuria present	1	1	1	3
Low density lipoprotein increased	4	1	2	7
Leukocyte count decreased	1	1	0	2
Leukocyte count increased	2**	0	0	2
White blood cells in urine	1	1	0	2

* Severity was moderate. ** Severity of one of 2 events was moderate. NOS: not otherwise specified, LDH: lactate dehydrogenase.

were 55, 51, and 26 adverse events, respectively. All adverse events were mild or moderate in severity. A single serious adverse event, herpes zoster, was reported in one patient. This was resolved by medication, and the patient continued the study.

A total of 70 adverse events for which a causal relationship with MRAcould not be ruled out (i.e., adverse reaction) were observed in 14 of the 15 patients. During the study period, 37, 20, and 13 adverse reactions were reported in the 2, 4, and 8 mg/kg groups, respectively. Some of the clinical laboratory tests showed dose-dependent changes, but no clear relationship between dose and frequency of adverse reaction was observed. There were 13 adverse events related to skin and subcutaneous tissue disorders (dermatitis, etc.), but no reactions at the injection site were reported. Symptoms associated with the common cold were reported in 5 patients.

In the abnormal laboratory findings, lipid metabolism related reactions such as an increase in blood total cholesterol, low density lipoprotein (LDL), and triglyceride were frequently observed, although they became stable at a certain level and did not continue to increase (Figure 2E–2G). The total cholesterol and LDL cholesterol levels decreased at 24 weeks in the 2 mg/kg group, but there was no statistically significant difference. There was no observation of cardiovascular complications during the study period. Leukocyte and neutrophil counts decreased after MRA administration in all dose groups, but most were within normal range. Two patients showed decrease in leukocyte counts below the normal range, and one of them, in the 2 mg/kg group, had transient, grade 3 neutropenia (neutrophil count < 1000/μl) a day after MRA infusion. There were no serious infections associated with transient neutropenia. The patient did not show neutropenia again

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