Title	Efficiency and safety studies of a humanized antibody to human interleikin-6 receptor in primates
Author(s)	SHINKURA, Hirofumi
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enhanced CA secretion induced by ACh (50 μM) at lower concentrations (0.1–20 μM), but decreased it at higher concentrations (100–200 μM).
3. CA secretion induced by carbachol (50 μM) was not enhanced by low concentrations, but was markedly decreased by high concentrations of tacrine and Phys.
4. Tacrine and Phys inhibited cholinesterase activities of adrenal homogenate in a dose-dependent manner, and the effects reached a maximum at 10 μM and 20 μM, respectively.
5. In perfused adrenal glands, CA secretion induced by nicotine (50 μM) was inhibited by high concentrations of tacrine (100 μM) and Phys (200 μM). Nicotine-induced CA secretion from dispersed adrenal chromaffin cells was also inhibited dose-dependently by tacrine and Phys, and was almost abolished by 100 μM tacrine and Phys.
6. In dispersed adrenal chromaffin cells, CA secretion induced by veratridine (20 μM) was significantly decreased by tacrine (100 μM) and Phys (1 mM).
7. Secretory response to high K^+ (46.2 mM) was not affected by tacrine and Phys (1-100 μM).
8. In voltage-clamped cells, tacrine and Phys dose-dependently inhibited membrane currents in a following order; inward currents induced by nicotine (50 μM) > voltage-dependent sodium currents > voltage-dependent calcium currents.
9. Nicotinic currents were inhibited by both drugs with a similar potency, and were almost abolished by 100 μM tacrine and 200 μM Phys. On the other hand, voltage-dependent sodium and calcium currents were more sensitive to tacrine than Phys.
10. These results demonstrate that low concentrations of tacrine and Phys increase ACh-induced CA secretion by their anti-cholinesterase actions in guinea-pig adrenal chromaffin cells. On the other hand, high concentrations of both drugs inhibit nicotine- and veratridine-induced CA secretion by probably blockade of nicotinic receptor channels and sodium channels, respectively. Inhibitory effects of tacrine and Phys on nicotinic receptor channels appear to be one of the major mechanisms of inhibition of CA secretion induced by ACh in guinea-pig adrenal chromaffin cells.

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Efficiency and safety studies of a humanized antibody to human interleikin-6 receptor in primates

Hirofumi Shinkura

Fuji-Gotemba Research Laboratories, Chugai Pharmaceutical Co. Ltd., 1–135 Komakado, Gotemba, Shizuoka 412–0038

A humanized antibody, hPM-1, was constructed by grafting the complementarity determining regions to human interleukin-6 receptor (IL-6R), raised in mouse, onto a human antibody backbone. hPM-1 is expected to be useful as a therapeutic agent for IL-6-related diseases such as multiple myeloma and rheumatoid arthritis.

In order to investigate the efficacy and safety of hPM-1 preclinically, we first selected suitable species IL-6R of which cross-reacts with hPM-1. We examined the binding activity of hPM-1 to IL-6R with peripheral blood lympho-

cytes of primates by flow cytometry and found that hPM-1 recognized the IL-6R on lymphocytes of cynomolgus and rhesus monkeys but did not those of marmoset. The homology of IL-6R between human and cynomolgus monkey was 97.3% in extracellar domain of the amino acid sequence determined by DNA sequencing of PCR product from peripheral blood lymphocytes. Furthermore, hPM-1 inhibited B cell IgG production and T cell proliferation provoked by IL-6 in cynomolgus monkey in vitro. Thus, it was shown that hPM-1 not only bound to IL-6R but also blocked IL-6 function in cynomolgus monkey in vitro.

Next, we examined the ability of hPM-1 to block in vivo functions of IL-6 and its associated serum concentration profile with cynomolgus monkeys. Cynomolgus monkeys were intravenously administered with hPM-1 at doses of 0 (vehicle) or 5 mg/kg, then subcutaneously injected with human IL-6 at a dose of 5 μg/kg, once a day for 7 days. The injections of IL-6 increased blood platelet counts twofold and elevated serum C-reactive proteins to levels of 0.15 to 0.17 mg/ml. These IL-6-induced responses were completely inhibited by single pretreatment with hPM-1. Serum concentrations of hPM-1 were maintained for a long period ; those, even at one week after its administration, were high enough to inhibit the IL-6 functions such as promotion of myeloma cell growth in vitro. The circulating half-life ($\beta t_{1/2}$) of hPM-1 ranging from 6.3 to 10.5 days was calculated. These findings suggest that hPM-1 may be effective in the treatment of IL-6-related diseases, and provide valuable information on the dose and dosing interval of this antibody for its future preclinical and clinical studies.

Finally, we investigated the toxicological properties of hPM-1 in healthy cynomolgus monkeys administered with hPM-1 at doses of 0 (vehicle), 4 or 40 mg/kg once a week for 13 weeks. In addition, we also examined the kinetics and antigenicity of hPM-1. Upon toxicological examination, there were no changes in clinical signs, food consumption, body weights, urinalyses, body temperatures, electrocardiograms, hematological and biochemical parameters, and pathological findings. In kinetic study, serum concentrations of hPM-1 showed a linearity between doses of 4 and 40 mg/kg. The serum concentrations, even at a dose of 4 mg/kg, were maintained at high enough levels to inhibit the IL-6 functions throughout the period of the study. Concentrations of hPM-1 in bone marrow were almost equal to those in serum, demonstrating that hPM-1 was well distributed in bone marrow wherein myeloma cells proliferate. The antibodies against hPM-1 were detected only in one of four monkeys receiving hPM-1 in spite of repeated administration of hPM-1. The reduced antigenicity might have been gained by humanization of antibody. These results indicate that blockage of IL-6 functions by hPM-1 does not induce any influence on a healthy living body, and hPM-1 is not toxic under the conditions of this investigation.

Collectively, the present studies in primates suggests that hPM-1 may be useful as a therapeutic agent for IL-6-related diseases.

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