Pharmacokinetics and Pharmacodynamics of ASKP1240, a Fully Human Anti-CD40 Antibody, in Normal and Renal Transplanted Cynomolgus Monkeys

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Background. The purpose of this study was to evaluate the serum concentration of ASKP1240 (pharmacokinetics [PK]) and the CD40 occupancy of ASKP1240 (pharmacodynamics [PD]) in normal and renal transplanted Cynomolgus monkeys to clarify the PK/PD relationship.

Methods. In a 70-day study, two ASKP1240 doses (2 and 5 mg/kg) were evaluated in normal and transplanted monkeys. Full doses were administered during the induction phase, and half doses were administered during the maintenance phase. The PK and PD were assessed using ELISA and FACS assays.

Results. The serum concentration and receptor occupancy of ASKP1240 reached their maximum levels rapidly after the first dose and remained at an almost saturated rate during the induction phase. They then decreased gradually during the maintenance phase in all of the groups. The serum concentration and duration of full receptor occupancy were dose dependent in the normal and transplanted monkeys. On day 70 after therapy with 5 mg/kg ASKP1240, the transplanted monkeys presented a significantly lower occupancy of the CD40 receptors compared with the normal animals (5.5%±14.1% vs. 72.8%±3.4%). The serum concentration of ASKP1240 was also strongly correlated with the occupancy of the ASKP1240 receptors.

Conclusion. This study showed strong positive PK/PD relationships in renal transplanted and normal monkeys. The results may thus serve as a guide for optimal dosage and timing of ASKP1240 therapy in clinical trials and will propel the translation of ASKP1240 therapeutics from the bench to preclinical and clinical trials.

Keywords: Co-stimulation, CD40-CD40L, Kidney transplantation, Nonhuman primates, Pharmacokinetics, Pharmacodynamics.

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RESULTS

Monkey Survival

The survival time for all of the monkeys is shown in Table 1. Monkey 0403019C was excluded from group 4 because of ureteral stenosis with severe hydronephrosis. The survival time was longer than 70 days for all of the nontransplanted monkeys in the low- or high-dose groups. The 70-day survival rates for the transplanted monkeys were 66.7% and 60.0% in the low- and high-dose groups, respectively.

PK Study

Before the first dose, ASKP1240 was undetectable in all serum samples, but it was detected in the serum samples collected from day 1 to 70 after administration. The serum ASKP1240 concentrations for all of the groups reached the maximum level immediately after the first dose and remained at this steady state level, that is, 10^4 to 10^5 ng/mL (low-dose) and 10^5 to 10^6 ng/mL (high-dose), during the induction stage (Fig. 1A). During this maintenance period, the serum concentration decreased gradually until the termination of the study in an ASKP1240 dose-dependent manner. The serum concentrations were higher in the high-dose group than in the low-dose group in normal monkeys (Fig. 1B–E). In monkeys treated with the same dose, the serum ASKP1240 concentration was lower in transplanted monkeys than in normal monkeys. High interindividual variation was observed in the transplanted monkey group that received 2 mg/kg ASKP1240. These concentration-time curves indicate that the serum concentration of ASKP1240 was higher in normal monkeys and depended on the dose of ASKP1240.

PD Study

The ASKP1240 occupancy-time results (Fig. 2A) show that occupancy for the ASKP1240 receptor was increased immediately after the first dose, reached an almost saturated state in all of the monkeys from the four groups, and remained stable during the induction phase. During the maintenance treatment, receptor occupancy decreased slowly until the next administration. In normal monkeys, the low- and high-dose groups were significantly different. At the same dose, an earlier decrease in occupancy was found in transplanted monkeys compared with normal monkeys (Fig. 2B–E).

| TABLE 1. Survival days and histopathology results of the ASKP1240 pharmacology study |
|---------------------------------|-----------------|---------------------|--------------------------|
| **Group** | **Administration** | **Animal ID** | **Survival day** | **Pathologic evaluation** |
| 1 | ASKP1240 2 mg/kg | 0310155C | >70 | Intact renal tissue |
| | | 04C7297 | >70 | Intact renal tissue |
| | | 04C9251 | >70 | Intact renal tissue |
| 2 | ASKP1240 5 mg/kg | 0301111C | >70 | Intact renal tissue |
| | | 0212101C | >70 | Intact renal tissue |
| | | 04C7773 | >70 | Intact renal tissue |
| 3 | ASKP1240 2 mg/kg | 0203063C | >70 | Mild interstitial inflammation (i1/t1) |
| | | 0302081C | 7 | Acute allograft rejection type III (i1/v3/i2) |
| | | 03C1097 | 45 | Acute allograft rejection type IIa with chronic nephropathy grade Ia (i1/v1/i2/cv1/ci1) |
| | | 05C1321 | >70 | Chronic allograft nephropathy grade Ia (i1/cv1/i2/cv1/ci1) |
| | | 05C1839 | >70 | Severe interstitial inflammation (i3/cv1/i2) |
| | | 05C2159 | >70 | Borderline changes (i1/i2) |
| 4 | ASKP1240 5 mg/kg | 0305157C | 45 | Moderate interstitial inflammation (i2/cv2) |
| | | 0209089C | 54 | Chronic allograft nephropathy grade Ib (i3/cv3/cv1/i2) |
| | | 04C0119 | >70 | Mild interstitial inflammation (i1/i2) |
| | | 05C0011 | >70 | Chronic allograft nephropathy grade Ia (i1/cv1/i2/cv1) |
| | | 04C0127 | >70 | Chronic allograft nephropathy grade Ia (i3/cv1/ci2) |

*Renal abscesses.
Especially on day 70, the transplanted monkeys had a significantly lower occupancy rate than the normal animals (5.53%±14.13% vs. 72.80%±3.44%) when receiving 5 mg/kg ASKP1240. Figure 2F shows the representative FACS data from the four different groups. These PD profiles indicate that ASKP1240 receptor saturation was higher for normal monkeys than for transplanted monkeys during the maintenance treatment in a dose-dependent manner.

**PK/PD Relationship**

The PK/PD relationship was investigated with regard to the serum concentration and receptor occupancy for ASKP1240. The profiles for PK and PD revealed a very close PK/PD relationship at 2.0 mg/kg ASKP1240 (Fig. 3A) and 5.0 mg/kg ASKP1240 (Fig. 3B) in the normal and transplanted monkeys.

**Anti-ASKP1240 Antibody**

In the ELISA assay, anti-ASKP1240 antibodies were not detected in any sample from the four groups of experimental monkeys, except one monkey (Monkey ID 0403019C) in group 3 that had a reactive screening assay (before dosing on day 42); however, the serum of this monkey was found to be negative in the immunodepletion assay.
FIGURE 2. PD study. Rate of ASKP1240 receptor occupancy in the low- and high-dose ASKP1240-treated normal and transplanted monkeys (A). Rate of ASKP1240 receptor occupancy in the 2.0 mg/kg ASKP1240-treated normal monkey group (B), 5.0 mg/kg ASKP1240-treated normal monkey group (C), 2.0 mg/kg ASKP1240-treated transplanted monkey group (D), and 5.0 mg/kg ASKP1240-treated transplanted monkey group (E). The representative MFI data show the ASKP1240 occupancy in the four different groups; the green line represents the controls (F).
Change in Peripheral CD20+ Cell Counts

There was no change in CD20+ cell levels in treated normal monkeys. In transplanted monkeys that received low- or high-dose ASKP1240, the CD20+ cell counts remained stable until 6 weeks post-transplantation, they were found to be slightly elevated.

Renal Graft Function

The results for sCr showed that all of the normal monkeys in groups 1 and 2 had normal renal function until the end of the study. Stable renal function was also found in renal transplanted monkeys in groups 3 and 4.

Histopathology

A systematic histologic examination of the main organs was performed as part of the routine autopsy for all of the monkeys from the four experimental groups. The left kidneys, which were the intact original kidneys in groups 1 and 2, showed no significant histopathologic changes, whereas all the recipient renal grafts from groups 3 (ASKP1240 2.0 mg/kg) and 4 (ASKP1240, 5.0 mg/kg) were observed to have different degrees of lesions (Table 1). Types II and III acute rejection (34%) and Grade I chronic nephropathy (17%) were found in group 3 (ASKP1240, 2.0 mg/kg). The acute or chronic renal lesions observed in the other three monkeys in group 3 (50%) were classified as mild/moderate changes. In group 4 (ASKP1240 5.0 mg/kg), acute rejection was not found in any of the monkeys. Three renal grafts developed chronic nephropathy (60%), and two grafts (40%) showed mild/moderate/borderline changes. No obvious pathologic changes were observed in other organs, including the liver, pancreas, spleen, heart, lungs, stomach, small bowel, thoracic aorta, mesentery lymph nodes, and pancreas.

Specific immunohistochemical staining for ASKP1240 was observed in the following tissues/organs. In the kidney transplant group, staining specific for ASKP1240 was observed in the kidney interstitial cells (Fig. 4B), which were identified as capillary endothelial cells, macrophages, and B lymphocytes based on double staining of anti-cell marker antibodies and ASKP1240. In the normal and transplanted groups, staining specific for ASKP1240 was observed in lymphocytes in the follicles of the lymphoid organs (spleen and mesenteric lymph node), lymphocytes in the lymphoid tissue, lymphoid follicles or Peyer’s patches (kidney, stomach, jejunum, and colon), mononuclear cells (paracortex in the mesenteric lymph nodes, lung interstitium, and lamina propria in the stomach, jejunum, and colon), hepatocytes (liver), and acinar cells (pancreas). In the control group, contrary to the kidney transplant group, no specific staining to ASKP1240 was detected in the lymphocytes in the follicles of the lymphoid organs (spleen and mesenteric lymph node), lymphocytes in the lymphoid tissue, lymphoid follicles or Peyer’s patches (kidney, stomach, jejunum, and colon), hepatocytes (liver), and acinar cells (pancreas).

DISCUSSION

Our 70-day results for the PK study reveal that the serum ASKP1240 concentration was measurable in all of the serum samples, except those obtained before the first dose. In the full-dose maintenance phase in the normal and transplanted monkeys, the serum ASKP1240 concentration reached its maximum level immediately after each dose of ASKP1240 (low- or high-dose). Then, the trough level gradually decreased until the next infusion. The serum ASKP1240 concentration was considered to follow a dose-dependent pattern, although a large interindividual variation was observed in renal transplanted monkeys that received 2 mg/kg of ASKP1240.

Similarly, the PD results show that low- or high-dose of ASKP1240 was sufficient to mask almost all of the CD40 sites on the CD20+ B cells in the peripheral blood during the induction phase, and the binding capacity of ASKP1240 to CD40 was almost equal for all of the monkeys. The CD40 occupancy increased to its maximum level after each ASKP1240 infusion and decreased gradually until the next dose. These regular, indented curves were dose dependent.

During the half-dose maintenance phase, the PD study showed that the occupancy decreased at varying rates but was not significantly different between the normal and transplanted monkeys. In the high-dose (5.0 mg/kg) groups, the 56-day occupancy rate (%) after administration was 87.11%±2.28% for the normal monkeys and 89.65%±1.01% for the transplanted monkeys. However, under the same conditions, the PK results showed serum ASKP1240 concentration of 91,500±25,343 ng/mL for the normal monkeys.
When the study reached destination day 70, the transplanted monkeys had a significantly lower PD occupancy and PK serum concentration than the normal animals (5.53%±14.13% vs. 72.80%±3.44%; 2830±1866 ng/mL vs. 10.5 ng/mL).

Our results suggest that the occupancy and concentration were different for the normal and transplanted monkeys and also different relative to the full-dose treatment. These possibilities may be explained as follows. These results suggest that the differences in occupancy and serum concentration between the transplanted and normal monkeys may be related to the following factors. (i) Production of a specific anti-drug antibody: antidrug antibody production is a common reason for a reduced antibody level. However, in the present study, no anti-ASKP1240 antibodies were detected in any of the samples during the 70-day observation period. (ii) Overexpression of CD40: as previously demonstrated, the CD40 antigen is expressed by B cells, dendritic cells (DCs), T cells, macrophages, vascular endothelia, and smooth muscle cells in patients with calcified atherosclerotic lesions (24–26).

Our pathologic results (data not shown) show that ASKP1240 antibody binding occurs not only in interstitial cells but also in capillary endothelial cells, macrophages, lymphocyte follicles in lymphoid organs (spleen and mesenteric lymph nodes), lymphocytes in lymphoid tissue, lymphoid follicles or Peyer's patches (kidney, stomach, jejunum, and colon), mononuclear cells (paracortex in mesenteric lymph nodes, lung interstitium, lamina propria in the stomach, jejunum, and colon), hepatocytes (liver), and acinar cells (pancreas). In contrast, when the transplanted monkeys received allograft kidneys, the recipient's immune system was rapidly activated, possibly resulting in an increase in CD40 antigen levels in B cells, DCs, T cells, or macrophages. Thus, in this study, as a result of the immune response against allo-antigens, the number of activated cells and unoccupied CD40 sites increased may require higher dose or more frequent administration of ASKP1240.

Recent studies have shown that a high-dose (5 or 10 mg/kg) of ASKP1240 significantly prolongs the graft survival time of pancreatic islet transplants in Cynomolgus monkeys (17). These results suggest that transplanted monkeys may require a higher dose or more frequent administration of ASKP1240 to maintain effective inhibition of the CD40-CD40L activation pathway.

In the normal and transplanted monkeys, the PK and PD were closely correlated (R2) for the low-dose (0.8743, 0.7515) and high-dose (0.7600, 0.8601) treatments. The concentration-receptor occupancy profile displayed a strong positive correlation between serum concentration and ASKP1240 occupancy.

Thus, the serum concentration (PK) and receptor occupancy (PD) of ASKP1240 had a very close relationship in this study, and high ASKP1240 binding depended on a high serum ASKP1240 concentration in normal and transplanted monkeys.

In conclusion, the serum ASKP1240 concentration (PK) was closely correlated with receptor occupancy (PD) and did not seem to be different between normal and transplanted monkeys. A higher dose of ASKP1240 led to a longer duration of full receptor occupancy in the normal and transplanted monkeys. To prolong the receptor occupancy in transplanted animals, increased dose or frequency of ASKP1240 administration may be necessary.

MATERIALS AND METHODS

All of the experimental procedures were approved by the Ethical Committee for Animal Experimentation at the Laboratory Animals Center of the Academy of Military Medical Sciences (AMMS) and were performed in accordance with the standards described in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health Office of Animal Care and Use.

Animals

Male *Cynomolgus* monkeys (*Macaca fascicularis*), 3 to 5 years old, weighing 3.5 to 7.2 kg, that were free from hepatitis B virus (HBV), hepatitis C virus (HCV), herpesvirus simiae (B virus), and simian immunodeficiency virus (SIV), were provided by the Laboratory Animals Center of the Academy of Military Medical Sciences in Beijing, China. All the *Cynomolgus* monkeys were screened for general health and quarantined for 2 weeks before the study. All of the monkeys were housed in individual cages and given free access to water, fruit, and monkey chow.

Reagents and Monoclonal Antibodies

A biotinylated ASKP1240 antibody and anti-ASKP1240 serum were kindly supplied by Kyowa Hakko Kirin Co., Ltd. The pooled normal *Cynomolgus* monkey sera were kindly supplied by Shin Nippon Biomedical Laboratories, Ltd. Allophycocyanin (APC)–labeled antihuman CD20 mAb...
antibody as the B lymphocyte marker. The mean fluorescence intensity (MFI) for PE-ASKP1240 in the CD20-positive B lymphocytes was detected by FACS using a FACSCalibur instrument (BD). The data were analyzed using CellQuest software (BD). The percentage of APC-CD20-positive B cells was also determined in this study. The occupancy for ASKP1240 (%) was calculated using the following formula: occupancy (%) = (1 - MFI/control) × 100.

**Monkey Antihuman ASKP1240 Antibody (MAHA) Assay**

Monkey antihuman ASKP1240 antibodies were detected using an ELISA screening assay and further confirmed using immunodepletion assays from Shin Nippon Biomedical Laboratories, Ltd. (Japan).

**Proportion of Peripheral CD20⁺ Cells**

The percentage of APC-CD20 positive B cells was determined using a FACS assay.

**Histopathology**

For routine hematoxylin and eosin (HE), PAS, and Masson’s trichrome staining, all of the animals were killed at the end of the study (day 70) or because of their moribund condition. The animals were then subjected to a full gross necropsy that included an examination of the external surface of the body, all orifices, and the thoracic and abdominal cavities and their contents. To confirm the low risk of thrombotic embolism, the lungs and brain were also checked by gross necropsy. Routine hematoxylin and eosin staining was performed on all of the paraffin-embedded sample sections, including the grafted kidney, liver, pancreas, spleen, heart, lung, stomach, small bowel (a segment of the jejunum), thoracic aorta, and mesenteric lymph nodes. PAS and Masson’s trichrome staining of the kidney tissue were performed on the paraffin-embedded sample sections. Rejection in the renal allografts was scored and diagnosed according to the 97 Banff classification (30).

**Immunohistochemical Staining**

To examine the expression of CD40 antigens in various tissues/organisms, including vascular endothelial cells in the kidney transplant animal model, a binding study for ASKP1240 was conducted immunohistochemically in 22 frozen tissues/organisms from Cynomolgus monkeys with (n = 4) and without kidney transplantation (n = 3) that were killed at the end of the study on day 5. ASKP1240 or a commercially available human IgG4 antibody at 1 and 5 μg/mL was applied to the sections as the primary antibody. Then, biotinylated antihuman IgG4 was applied to the sections as a secondary antibody. Finally, the antibody complexes were visualized using ABC (avidin-biotin complex) and diaminobenzidine (DAB).

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