In Vivo Absorption Properties of Orally Administered Recombinant Human Interleukin-11

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INTRODUCTION

Interleukin-11 (IL-11) is a cytokine with a molecular mass of 19 kDa and 178 amino acids. Recombinant human interleukin-11 (rhIL-11) contains 177 amino acids and differs from the naturally occurring protein only by the absence of the amino-terminal proline residue (1). rhIL-11 has been demonstrated to stimulate megakaryocytopoiesis and increase platelet counts in myelosuppressed animals (2). Based on the results of clinical trials, rhIL-11 has been approved for use for the prevention of severe thrombocytopenia following myelosuppressive chemotherapy in patients with nonmyeloid malignancies. In addition to its thrombopoietic activity, rhIL-11 has exhibited potent anti-inflammatory activity in a variety of pharmacological animal models including rat models of inflammatory bowel disease (3). The anti-inflammatory mechanisms of rhIL-11 have been examined at cellular and molecular levels (3,4). Key findings indicate that rhIL-11 reduces the production of proinflammatory cytokines such as tumor necrosis factor α and interleukin-12 from activated macrophage. Based on its anti-inflammatory activity, clinical studies are currently underway to assess the efficacy and safety of rhIL-11 given subcutaneously in the treatment of inflammatory bowel disease (Crohn's disease), rheumatoid arthritis, psoriasis and chemotherapy-induced mucositis.

The pharmacokinetics (PK) of rhIL-11 have been investigated in mice, rats, and humans (5–7). rhIL-11 is almost completely absorbed (100%) in mice and rats after subcutaneous (SC) injection. Bioavailability of rhIL-11 was examined in male healthy subjects at SC doses of 10 to 50 μg/kg with an estimated bioavailability of approximately 65%. In vivo disposition studies using isotope labeled rhIL-11 in mice and rats (5,6) demonstrated that rhIL-11 is predominantly eliminated via the kidneys and the liver. Higher concentrations of radioactivity were also observed in the gastrointestinal tract in addition to those observed in the liver and kidneys compared to concurrent plasma concentrations at 1.5 hours after dosing.

Recently, rhIL-11 was developed as an enteric-coated multiparticulate formulation for oral dosing of patients with inflammatory bowel disease. Therefore, we conducted a series of PK studies to examine the systemic bioavailability and biodistribution of orally administered rhIL-11 to support its proposed clinical use.

METHODS

rhIL-11 in an enteric-coated multiparticulate formulation (Genetics Institute, Cambridge, MA) was used for oral dosing. rhIL-11 was layered onto a sugar sphere, followed by subsequent layers of sealant and the enteric coating. The multiparticulates contained 1.11 mg active rhIL-11 per 100 mg pellets. rhIL-11 in lyophilized powders (Genetics, Institute, Cambridge, MA) was used for IV and SC dosing. Prior to IV and SC treatment, lyophilized rhIL-11 powders were reconstituted with 1 mL distilled water. rhIL-11 solution was further diluted to the concentration of 1 mg/mL with physiology buffer.

Bioavailability of Orally Administered rhIL-11 in Normal and HLA-B27 Rats

Female Fisher rats cannulated with the femoral venal catheters (140–150 g, Taconic, Germantown, NY) received 1 mg/kg of rhIL-11 intravenously (as liquid) or by oral gavage (as multiparticulate particles) on day 1 (n = 4, respectively). The rationale for the dose chosen was based upon doses used in prior pharmacological studies. Blood samples (0.2 ml) were taken from the venal catheters of the animals before and at 1, 5, 10, 15, 30, 60, 120, 180, 360, and 1440 minutes for the oral group and at 1, 5, 10, 15, 30, 60, 120, 180, 360, and 1440 minutes for the IV group following administration. No attempt was made to compensate blood loss. Blood samples were clotted at room temperature and centrifuged. Serum samples were then prepared and stored at −80 °C freezer until analyzed for rhIL-11 by ELISA. Similar procedure for ELISA has been described previously (7). The lowest quantifiable sample concentration was 102.0 pg/ml. In a separate study, HLA-B27 rats (271–295 g, n = 5, Taconic, Germantown, NY) were administered rhIL-11 (1 mg/kg) by oral gavage (as multiparticulate particles) in an identical protocol as outlined above.

Systemic Acute-Phase Reactants After Dosing with rhIL-11

Female Fisher rats (130–150 g, n = 6, respectively for each group, Taconic, Germantown, NY) were administered rhIL-11 (1 mg/kg) orally (as multiparticulate particles) or intravenously (as liquid). A separate group of rats (n = 6) was also treated orally with multiparticulate particles (without rhIL-11) as control group. Blood samples were taken before and at 3 and 6 hours after dosing. The blood samples were collected in the tube with sodium citrate (10%), and plasma samples were then prepared. The rats were sacrificed at 6 hours after dosing and liver samples were removed and immediately frozen in liquid nitrogen. The blood samples were stored at 4°C and analyzed for plasma fibrinogen within 24 hours. Liver tissues were stored at −80°C until being analyzed for fibrinogen and alpha-2 macroglobulin RNA. Plasma fibrinogen was analyzed as a pyrogallol red complex after heat precipitation of protein by

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spectrophotometry (Hitachi 717). Hepatic fibrinogen and alpha-2 macroglobulin RNA were monitored by TaqMan analysis.

Biodistribution Studies

rhIL-11 was labeled with ^{111}Indium (^{111}In) using a modification of the method described previously (8). The ^{111}In-labeled rhIL-11 was purified by size-exclusion HPLC, and the ^{111}In-labeled rhIL-11 fraction was collected. ^{111}In-labeled rhIL-11 fraction was used for the preparation of drug solution containing labeled and non-labeled rhIL-11 at the concentration of 1 mg/mL.

Female Fisher rats (120–140 g, Charles River, Andover, MA) cannulated with intraduodenal catheters were used in this study. The description for placement of the intraduodenal catheter can be obtained from Charles River. The rats were fasted overnight before the experiment. On the day of the experiment, rats were administered rhIL-11 solution (1mg/kg) intraduodenally followed by 200 μL saline and sacrificed at 1, 3, 6, and 24 hours after dosing (n = 2 for 24 hours and n = 3 for the rest of time points; total n = 11). Different segments of the intestine, i.e., the duodenum, ileum, jejunum, cecum and colon were collected. Mesenteric lymph nodes, liver, kidney and blood were also collected. A separate group of rats (n = 3) was treated with rhIL-11 (0.1 mg/kg) subcutaneously and sacrificed at 6 hours after dosing. Tissue samples were then prepared as addressed above. All the samples were measured for radioactivity using a Wizard Gamma Counter (Wallac, Gastherburg, MD) immediately after sample preparation.

Data Analysis

Data were expressed as mean ± S.D. The differences among dosing groups were evaluated by ANOVA with the post hoc Fisher test. Significance was determined at $P < 0.05$.

RESULT AND DISCUSSION

Figure 1 shows the time course of serum rhIL-11 in normal rats (n = 4) after intravenous administration (1 mg/kg). The concentration of rhIL-11 followed the biexponential decline after IV dosing. This PK profile is in agreement to those observed previously in mouse, rat, monkey and man (5,7). There was no increase in rhIL-11 level after dosing for the rats treated rhIL-11 orally (n = 4). One rat of the oral group before dosing showed detectable rhIL-11 (543 pg/mL), possibly because ELISA can not distinguish rhIL-11 from endogenous IL-11. After dosing, rhIL-11 level was not increased for this rat.

In order to examine if the inflammatory state of the gastrointestinal tract would influence the intestinal absorption and, consequently, the systemic exposure of rhIL-11, we tested the systemic bioavailability of orally administered rhIL-11 using a well-established animal model for inflammatory bowel disease, i.e., HLA-B27 rats which usually have severe diarrhea due to the inflammatory state of the intestine (3). The results show no detectable rhIL-11 in serum of HLA-B27 rats (n = 5) after oral dosing. Therefore, intestinal inflammation may not change absorption of rhIL-11 and orally administered rhIL-11 is therefore not systemically bioavailable in the presence of inflammatory bowel disease.

Serum concentrations of rhIL-11 in these two aforementioned bioavailability studies were monitored by ELISA. To determine if undetectable levels of rhIL-11 in serum might be bioactive, a functional assay using the acute-phase response was performed. The acute-phase response is a sequence of processes induced in the early and immediate stages of inflammation. A large number of plasma proteins of hepatic origin involving acute-phase response have been grouped together as the acute-phase proteins (9). These proteins and their RNA are collectively referred to the acute phase reactants. A variety of cytokines have been shown to regulate the production of acute-phase reactants. IL-11 and the rest of IL-6-type cytokines (IL-6, leukemia inhibitory factor, oncostatin, ciliary neutrophic factor) have been recognized to regulate the production of most acute-phase reactants, including fibrinogen and α2-macroglobulin (10). A clinical study showed that acute-phase reactants were significantly increased with rhIL-11 even at very low dose of 10 μg/kg/day (11). Therefore, acute-phase reactants can be used as sensitive surrogate markers for the systemic exposure of rhIL-11. Three acute-phase reactants, i.e., plasma fibrinogen, liver fibrinogen RNA, liver alpha-2 macroglobulin RNA, were used as surrogate markers to examine the systemic exposure of rhIL-11 administered orally.

Plasma fibrinogen levels were analyzed following rhIL-11 administration (Fig. 2). ANOVA analysis for the acute phase

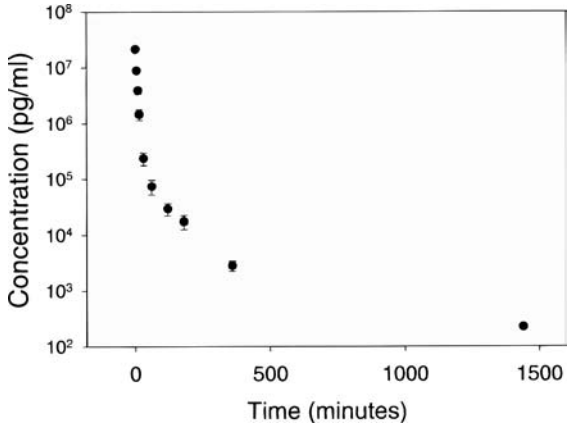

Fig. 1. Time courses of serum rhIL-11 in normal rats (n = 4) after intravenous administration (1 mg/kg). Error bars for some time points are too samll to be seen.

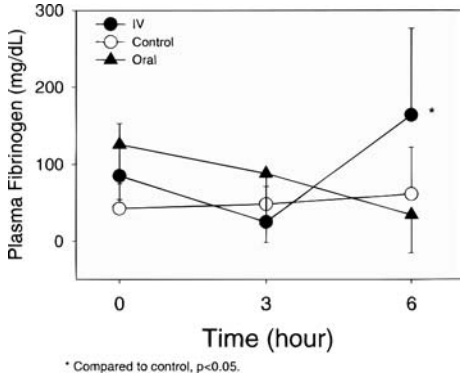

Fig. 2. Time course of plasma fibrinogen (mean ± S.D.) of the rats (n = 6/group) treated with multiparticulate particles (without rhIL-11) orally, rhIL-11 (1 mg/kg) intravenously (as liquid) or orally (as multiparticulate particles).

Table 1. Tissue Distribution of rhIL-11 at Different Time Points After Intraduodenal (1 mg/kg) or SC (0.1 mg/kg) Dosing

	% Total dose				
	1 hr (n = 3)	3 hr (n = 3)	6 hr (n = 3)	24 hr (n = 2)	SC at 6 hr (n = 3)
Duodenum	0.83 ± 0.44	0.10 ± 0.02	0.14 ± 0.11	0.02 ± 0.00	0.12 ± 0.02
Jejunum	4.80 ± 6.67	0.13 ± 0.09	0.09 ± 0.05	0.01 ± 0.01	0.37 ± 0.10
Ileum	68.35 ± 6.22	1.34 ± 1.87	0.36 ± 0.12	0.31 ± 0.42	0.21 ± 0.07
Colon	0.06 ± 0.05	8.67 ± 1.88	29.88 ± 10.48	5.59 ± 2.42	0.21 ± 0.07
Lymph Node	< 0.01	0.02 ± 0.04	< 0.01	0.02 ± 0.02	0.02 ± 0.01
Cecum	0.88 ± 0.72	61.44 ± 12.11	43.96 ± 14.31	11.78 ± 5.49	0.43 ± 0.10
Liver	0.01 ± 0.01	0.03 ± 0.03	0.03 ± 0.02	< 0.01	3.55 ± 0.55
Kidney	0.13 ± 0.07	0.15 ± 0.02	0.14 ± 0.02	0.09 ± 0.02	45.16 ± 4.86
Serum	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Others	24.93 ± 9.22	28.11 ± 11.31	25.40 ± 16.68	82.19 ± 8.34	49.93 ± 5.52

response exerted by rhIL-11 indicates that the concentrations of plasma fibrinogen were not changed at three hours after dosing with either oral or IV delivery. The levels of plasma fibrinogen at 6 hours after dosing by IV route were significantly higher than those of the vehicle-treated group (approximately 2.5 fold difference), while the plasma-fibrinogen levels following oral administration were not different from that of the vehicle group.

Liver RNA for two acute phase reactants, fibrinogen and α-2 macroglobulin, were also measured 6 hours after dosing. In the IV group, higher α-2 macroglobulin RNA levels were detected compared to the control group but there is no difference between the oral and the vehicle group (IV: 166 ± 152; control: 15 ± 13; oral: 30 ± 36 μg/mL, $p < 0.05$). The levels of liver fibrinogen RNA were unchanged following IV or oral dosing (IV: 12 ± 2; control: 11 ± 5; oral: 10 ± 3 μg/mL). The data as a whole indicates the lack of systemic exposure of rhIL-11 administered orally.

Our limited sampling time points in the functional assay do not fully capture the kinetic change of acute-phase reactants. Nevertheless, we must point out that plasma fibrinogen is steadily elevated after stimulus (12), and this property may aid to identify the statistical difference in this surrogate marker between the IV and control group with limited sampling time points.

Since orally administered rhIL-11 is not systemically bioavailable, we further investigated the disposition property of rhIL-11 administered through intraduodenal catheters or subcutaneous route. The intraduodenal dosing allows rhIL-11 to bypass the stomach and therefore may mimic the release of rhIL-11 from the enteric-coated multiparticulate formulation in the intestines. Isotope labeled rhIL-11 was used for the preparation of dosing solution. The majority of radioactivity was found in the kidneys and the liver at 6 hours after SC dosing (Table 1). A minimal amount (≤ 0.2 % of total dose) of rhIL-11 after intraduodenal dosing was distributed into systemic organs/tissues, i.e., the liver, kidney, lymph node and serum at all time points examined. This observation confirms our previous conclusion that orally administered rhIL-11 is not systemically bioavailable. Furthermore, no radioactivity was observed in the mesenteric lymph nodes at any time points examined (Table 1). Therefore, the lymphatic route may not be accessible for rhIL-11 administered intraduodenally (orally).

Table 1 also shows that radioactivity distributed rapidly throughout the intestine within the time course examined. The highest intestinal radioactivity observed at 1 hour was in the ileum (≈68% of total dose), but most of radioactivity was found in the cecum (≈ 61% of total dose) and colon (9% of total dose) at 3 hours after dosing.

In conclusion, our studies using ELISA and the measurement of acute-phase reactants indicate that orally administered rhIL-11 is not systemically bioavailable. Biodistribution studies using isotope labeled material support this view.

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