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The serum and cerebrospinal fluid pharmacokinetics of anakinra after intravenous administration to non-human primates

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Abstract

Anakinra improves the central nervous system manifestations of neonatal-onset multisystem inflammatory disease, which is mediated by IL-1 β oversecretion. The cerebrospinal fluid (CSF) penetration of the IL-1 receptor antagonist anakinra was studied in rhesus monkeys after intravenous doses of 3 and 10 mg/kg. Drug exposure (area under concentration-time curve) in CSF was 0.28% of that in serum. The average CSF concentration at 3 mg/kg was 1.8 ng/mL, which is 30-fold higher than endogenous CSF levels of IL-1Ra. The CSF penetration was not dose-dependent, indicating that the CSF penetration was not saturated in the 3 to 10 mg/kg dose range.

Introduction

Interleukin-1 (IL-1), implicated in the pathogenesis of neonatal-onset multisystem inflammatory disease (NOMID), mediates pro-inflammatory effects through binding to the IL-1 receptor which is present on many nucleated cells.(Dinarello, 2004) The recombinant IL-1 receptor antagonist, anakinra (IL-1Ra, KineretTM), improves the clinical and laboratory manifestations of NOMID in patients treated with 1-2 mg/kg daily by subcutaneous injection. (Goldbach-Mansky et al., 2006, Lovell et al., 2005) In addition to the disappearance of the cutaneous manifestations, an improvement in the central nervous system (CNS) manifestations was observed, including alleviation of headache, reduction in increased intracranial pressure, and decreased leptomeningeal and cochlear enhancement on MRI. The median (interquartile range) serum IL-1Ra concentration in patients with NOMID was 0.36 ng/mL (0.23-1.26 ng/mL) pretreatment and 43 ng/mL (8.8-200 ng/mL) after 3 months of anakinra. In CSF, IL-1Ra concentration was 0.21 ng/mL (0.077-0.35 ng/mL) and increased 5-fold on anakinra to 1.14 ng/mL (0.50-1.69 ng/mL).(Goldbach-Mansky et al., 2006)

The clinical improvement in the CNS manifestations of NOMID and the increase in CSF IL-1Ra concentrations measured at a single time point post-treatment in children treated with anakinra prompted us to more fully characterize the CSF pharmacokinetics of anakinra in a

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non-human primate model, which is highly predictive of CNS pharmacology in humans. (Bacher et.al., 1994; McCully et.al., 1990; Jacobs et.al. 2005; Meany et.al. 2007; Muscal et.al. 2010) Animals have chronically indwelling Ommaya reservoirs that permit serial atraumatic sampling of ventricular CSF to assess the time course of CSF drug concentrations after a systemic dose of drug. Drug exposure in CSF is compared to serum using the area under the concentration-time curve (AUC) rather than single time point measurements. CSF drug penetration in this model is a surrogate for blood-brain barrier penetration.(Fox et al., 2002)

Materials and methods

Drug

Anakinra (100 mg/0.67 ml ; Biovitrum, AB, Stockholm, Sweden) was purchased from commercial sources. Anakinra (17.3 KD) differs from native human IL-1Ra (23-25 KD) by the addition of a methionine at the amino terminus and the absence of glycosylation. Two dose levels (3 and 10 mg/kg) were studied. Each dose was diluted in normal saline to a final concentration of 25-50 mg/mL in 1-3 mL and administered as an intravenous (IV) bolus.

Animals

Three adult male rhesus monkeys (*Macaca mulatta*), weighing 10.2-14.4 kg, were used for this study. Animals were group housed in accordance with the Guide for the Care and Use of Laboratory Animals (<http://oacu.od.nih.gov/regs/guide/guide.pdf>). The experimental protocol was approved by the National Cancer Institute's Animal Care and Use Committee. IV bolus anakinra was delivered through a jugular venous port, and blood samples were drawn from a saphenous venous catheter on the side contralateral to drug administration. CSF samples were obtained from an indwelling Pudenz silicon catheter attached to a subcutaneously implanted Ommaya reservoir.(Bacher et al., 1994)

Experiments

Blood samples (3 mL) were collected before, and 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10, 24, and 48 h after anakinra administration. The blood was allowed to clot for 30 minutes and serum was separated by centrifugation and stored at -70° C. CSF samples (0.3 ml) were collected at the same time points as blood. The Ommaya reservoir was pumped 3 times prior to collection of each sample to insure adequate mixing of CSF. CSF samples were immediately frozen and stored at -70° C. Animals RQ3633 and RQ3588 were studied after 3 and 10 mg/kg of anakinra with >2 month break between experiments. Animal 15398 was studied after 10 mg/kg.

Sample analysis

Serum and CSF anakinra concentrations were measured with a human IL-1Ra ELISA (Invitrogen, BioSource™). Spectrophotometric endpoint detection (OD 450nm) of the biotin-streptavidin horseradish peroxidase product was quantified using a SpectraMax M5 (Molecular Devices Sunnyvale, CA). Standard curves were generated using anakinra rather than the supplied native IL-1Ra standard. CSF standards were prepared by diluting anakinra in phosphate buffered saline with 1% bovine serum albumin. All standards, controls and samples were analyzed in duplicate and the assay validated according to FDA guidelines.(US DHHS, 2001) Standard curves using a four parameter logistic fit for serum and CSF anakinra concentrations ranged from 0.062 to 4.0 ng/mL. The median (range) R² for standard curves generated on 9 days of sample analysis was 0.999 (0.998-1.000). The lower limit of detection was 0.032 ng/mL and the lower limit of quantification was 0.062 ng/mL. For low (0.062 ng/mL), mid (0.50 ng/mL) and high (2.0 ng/mL) serum controls the day to day coefficient of variation was 8%, 2 %, and 3%, respectively. The day to day coefficient of variation for CSF controls was 3%, 1% and 1% at low, mid and high concentrations, respectively.

Pharmacokinetic analysis

The serum and CSF pharmacokinetics of anakinra were analyzed by non-compartmental methods. (Gibaldi, M and Peirrer,; 1982) The area under the concentration-time curve (AUC_{0-last}) was calculated with the linear trapezoidal method and extrapolated to infinity ($AUC_{0-\infty}$) using the terminal rate constant derived from the slope of the natural log-transformed concentrations and times on the terminal elimination phase of the decay curve. Average concentration (C_{ave}) at steady state was derived from the $AUC_{0-\infty}/24$ h. Terminal half-life was calculated by dividing 0.693 by the terminal rate constant, which was derived from the slope of the natural log transformed concentrations and times on the terminal elimination phase of the plasma and CSF curves. Anakinra clearance was calculated by dividing the dose by the $AUC_{0-\infty}$. Volume of distribution at steady state was determined from the anakinra area under the moment curve and $AUC_{0-\infty}$ with correction for the short duration of drug infusion. CSF penetration of anakinra was calculated from the $AUC_{CSF}:AUC_{serum}$ ratio.

Results

Pretreatment serum and CSF native IL-1Ra concentrations were detectable (>0.032 ng/mL) but not quantifiable (<0.062 ng/mL). Serum and CSF pharmacokinetic parameters for anakinra are provided in Table 1, and the mean serum and CSF concentration-time profiles for the 3 and 10 mg/kg doses are shown in Figure 1. After the bolus dose, serum anakinra concentrations declined by 5 logs over 24 to 48 h with a mean ($\pm$ SD) terminal half-life in serum of 2.9 ± 1.3 h. The $AUC_{0-\infty}$ was $16,200 \pm 1,800$ ng•h/mL at 3 mg/kg and $58,200 \pm 12,600$ ng•h/mL at 10 mg/kg, yielding C_{ave} of 670 and 2,420 ng/mL at 3 and 10 mg/kg. Mean ($\pm$ SD) clearance was 3.0 ± 0.5 mL/min/kg and V_{dss} was 0.45 ± 0.36 L/kg.

CSF anakinra concentration peaked 1-2 h post-dose. The CSF $AUC_{0-\infty}$ was 43 ± 24 ng•h/mL at 3 mg/kg and 164 ± 11 ng•h/mL at 10 mg/kg, and the C_{ave} in CSF at 3 and 10 mg/kg were 1.8 and 6.8 ng/mL, respectively. Anakinra exposure in the CSF was substantially lower than serum ($AUC_{CSF}:AUC_{serum}$ was $0.28\% \pm 0.10\%$). However, the decline in CSF anakinra concentrations was slower ($T_{1/2}$, 4.7 h) than in serum, such that by the last measurable time point the CSF anakinra concentration was 56% of the serum concentration.

Discussion

The overall drug exposure in CSF as measured by the $AUC_{0-\infty}$ was $<1\%$ of the $AUC_{0-\infty}$ in serum. Bolus IV dosing of anakinra permitted accurate measurement of the systemic and CSF exposure and calculation of the CSF penetration as the ration of the $AUC_{CSF}:AUC_{serum}$. The half-life was similar to the 1.8 hour half-life reported for healthy humans after IV administration of anakinra.(Granowitz et.al., 1992) Unlike comparing single time point serum and CSF measurements, the $AUC_{CSF}:AUC_{serum}$ method of assessing penetration is independent of the shape of the concentration-time curves in serum and CSF. The slower decline in CSF anakinra concentrations results in higher CSF:serum ratios at later times when single time points are used to assess penetration. Despite the low penetration into CSF relative to serum, the CSF C_{ave} at the 3 mg/kg dose was 30-fold higher than pretreatment native IL-1Ra CSF concentrations and is comparable to the CSF concentration measured in patients with NOMID. (Goldbach-Mansky et al., 2006)

In humans with subarachnoid hemorrhage (SAH) treated with intravenous anakinra, the CSF penetration was 2-4%.(Gueorguieva et.al., 2008,Clark et.al., 2008) Higher concentrations of anakinra in CSF of these patients may be expected after subarachnoid hemorrhage with leakage of blood into CSF, use of an extravascular drain to collect CSF samples, and compromise of the blood brain barrier after SAH.

Proteins are generally restricted from crossing the blood-CSF barrier, as evidenced by the low concentration of proteins in normal CSF. In murine models, cytokines including IL-1 α and IL-1 β have been shown to cross the blood-brain barrier via saturable transport mechanisms. (Banks et al., 1995, Banks et al., 1991, Gutierrez et al., 1994) Gutierrez et al. demonstrated that the peak percent of an IL-1Ra IV dose per gram of brain was 0.33% to 0.65% and occurred 30 to 40 minutes after injection. However at 10 minutes after injection, the percent of the dose entering the brain was 4-8 times lower. This demonstrates the differences in T_{max} and concentration time profiles of ILRa in serum and CSF after IV injection and demonstrates the inconsistencies inherent in using individual time points to determine CNS penetration. By comparison of exposure ($AUC_{0-\infty}$) in CSF and serum, we demonstrate that the CSF penetration of anakinra in non-human primates is 0.2-0.3%. Comparing the 3 mg/kg and 10 mg/kg dose, the exposure in both serum and CSF increased by 3.7 fold at the higher dose and the CSF penetration was similar. This indicates that there was no apparent saturation of transport of anakinra into the CSF in this dose range.

Inflammation of the meninges can increase blood-CSF barrier penetration. (Nag, 2003) After systemic administration of anakinra, children with NOMID have an improvement of the CNS manifestations (decreased CSF leukocytosis and protein) and a decrease in leptomeningeal enhancement on MRI. (Goldbach-Mansky et al., 2006, Goldbach-Mansky et al., 2007) Our non-human primate model has an intact blood-CSF barrier and demonstrates that increasing the systemic dose of anakinra proportionally increased the systemic (serum) and CSF drug exposure. In addition, the CSF penetration did not change indicating that the penetration into CSF was not dose-dependent over this dose range of 3 to 10 mg/kg. These data provide a rationale to increase anakinra doses in patients to improve control of CNS inflammation. Doses of 1-5 mg/kg are currently under investigation in a clinical trial with NOMID patients to obtain better control of the CNS inflammation that seems to persist even if complete systemic inflammatory control is obtained (<http://clinicaltrials.gov/ct2/show/NCT00069329>).

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Fig. 1.
Mean serum and CSF anakinra concentration-time curves for 3 and 10 mg/kg anakinra administered as an IV bolus dose

Table 1

Non-compartmental pharmacokinetic parameters for anakinra

Animal	Dose Level [mg/kg]	Serum					Cerebrospinal Fluid				Ratio AUC _{CSF} : AUC _{Serum} [%]
		AUC _{0-24h} [ng•h/ml]	AUC _{0-∞} [ng•h/ml]	C _{max} [ng/ml]	Half-life [h]	Clearance [ml/min/kg]	Vd _{ss} [L/kg]	AUC _{0-24h} [ng•h/ml]	AUC _{0-∞} [ng•h/ml]	C _{max} [ng/ml]	Half-life [h]
RQ3588	3	14,900	14,900	25,000	2.3	3.3	0.25	59	60	16	4.6
RQ3633	3	17,400	17,400	17,700	1.7	2.8	0.54	26	27	6	1.0
RQ3588	10	59,900	59,900	114,000	2.5	2.9	0.18	158	166	17	6.0
RQ3633	10	44,700	44,800	70,300	3.3	3.8	0.25	148	152	24	5.0
15398	10	68,300	69,800	9,810	5.0	2.4	1.05	164	175	21	6.8