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Intravenous anakinra can achieve experimentally effective concentrations in the central nervous system within a therapeutic time window: results of a dose-ranging study

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The naturally occurring antagonist of interleukin-1, IL-1RA, is highly neuroprotective experimentally, shows few adverse effects, and inhibits the systemic acute phase response to stroke. A single regime pilot study showed slow penetration into cerebrospinal fluid (CSF) at experimentally therapeutic concentrations. Twenty-five patients with subarachnoid hemorrhage (SAH) and external ventricular drains were sequentially allocated to five administration regimes, using intravenous bolus doses of 100 to 500 mg and 4 hours intravenous infusions of IL-1RA ranging from 1 to 10 mg per kg per hour. Choice of regimes and timing of plasma and CSF sampling was informed by pharmacometric analysis of pilot study data. Data were analyzed using nonlinear mixed effects modeling. Plasma and CSF concentrations of IL-1RA in all regimes were within the predicted intervals. A 500-mg bolus followed by an intravenous infusion of IL-1RA at 10 mg per kg per hour achieved experimentally therapeutic CSF concentrations of IL-1RA within 45 minutes. Experimentally, neuroprotective CSF concentrations in patients with SAH can be safely achieved within a therapeutic time window. Pharmacokinetic analysis suggests that IL-1RA transport across the blood–CSF barrier in SAH is passive. Identification of the practicality of this delivery regime allows further studies of efficacy of IL-1RA in acute cerebrovascular disease.
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Introduction

Cerebral ischemia in stroke and subarachnoid hemorrhage (SAH) is a major cause of death and disability (Rabinstein *et al*, 2004). Although there have been in excess of 110 trials of potentially neuroprotective drugs (O'Collins *et al*, 2006), few have shown promising results and only one, recombinant tissue plasminogen activator, is currently licensed for administration. The use of recombinant tissue plasminogen activator is limited to within 4.5 hours of stroke after the onset of symptoms and requires that the criteria for patients with ischemic stroke who could be treated with recombinant tissue plasminogen activator are fully satisfied (Adams *et al*, 2007). Although the proportion of patients arriving to hospital with 4.5 hours of stroke onset is ~30% (Deng *et al*, 2006), recombinant tissue plasminogen activator is administered to only 6% to 25% for a variety of reasons (Huang *et al*, 2006*b*). Apart from the use of calcium channel blocker nimodipine in SAH (Dorhout Mees *et al*, 2007), no pharmacological agent has been proven to improve outcome in hemorrhagic stroke, intracerebral hemorrhage, or

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SAH. Moreover, side effects resulting from administration of nimodipine occasionally limit its use (Topcuoglu and Singhal, 2006).

There is an increasing body of evidence to suggest that inflammation has an important function in the pathological events arising as a consequence of cerebral ischemia (Huang *et al*, 2006*a*) and that the proinflammatory cytokine interleukin-1 (IL-1) is particularly implicated as an important mediator (Boutin *et al*, 2001; Buttini *et al*, 1994; Hara *et al*, 1997; Yamasaki *et al*, 1995). The IL-1RA is the naturally occurring antagonist of IL-1 and is the most widely studied putative neuroprotective agent in acute cerebrovascular disease (Banwell *et al*, 2009). It is a potent inhibitor of experimentally induced ischemic brain damage when given intracerebroventricularly (Loddick and Rothwell, 1996), intraperitoneally (Ohtsuki *et al*, 1996), intravenously (Clark *et al*, 2008), and subcutaneously (Relton *et al*, 1996) up to 3 hours after induction of ischemia (Mulcahy *et al*, 2003). A recombinant formulation of IL-1RA, anakinra (Kineret) (Amgen Inc, Thousand Oaks, CA, USA) is manufactured and licensed as a secondary-line treatment for rheumatoid arthritis. Clinically, it is effective in reducing peripheral markers of inflammation in stroke (Emsley *et al*, 2005) and has been shown to have a good safety and tolerability profile in SAH (Clark *et al*, 2008), stroke (Emsley *et al*, 2005), and other diseases such as rheumatoid arthritis (Nuki *et al*, 2002) and diabetes mellitus (Larsen *et al*, 2007). In SAH, IL-1 potentially contributes to the development of cerebral ischemia through various mechanisms. It may cause vasospasm by the upregulation of potent vasoconstrictors such as endothelin and induction of IL-6 that has been shown to induce vasospasm in experimental studies. The IL-1 contributes to blood–brain barrier (BBB) dysfunction, worsens cerebral edema, and leads to increased intracranial pressure (Blamire *et al*, 2000). It may enhance cytotoxicity caused by NMDA-mediated cortical spreading depression, another potential cause of delayed cerebral ischemia after SAH (Obrenovitch and Zilkha, 1996). The IL-1 may also be a primary driver of a systemic acute phase response, including an increase in plasma IL-6 (McMahon *et al*, 2010). Finally, it acts as an endothelial activator and is induced during the coagulation cascade triggered by tissue injury (Cicala and Cirino, 1998).

Studying the pharmacokinetics of IL-1RA is pivotal to the process of development of the drug as a potential neuroprotective agent, is a prerequisite for pharmacodynamic studies and one of the main Stroke Therapy Academic Industry Roundtable (STAIR) criteria drafted in effort to maximize the potential success of any potential stroke treatment (STAIR, 2001). Investigating the pharmacokinetics of IL-1RA within the central nervous system is technically challenging in stroke given the lack of access to both CSF and intraparenchymal tissue. The SAH patients who undergo insertion of an external ventricular drain (EVD) to control intracranial pressure provide ready access to CSF and therefore present an ideal cohort in which to investigate IL-1RA pharmacokinetics across the BBB. The BBB integrity influences the transfer of IL-1RA across the BBB and there is inter- and intracondition heterogeneity in the extent of BBB disruption. Radiological evidence of BBB disruption is present in stroke patients, cerebral small vessel disease, and lacunar stroke (Topakian *et al*, 2010). In poor-grade SAH, contrast-enhanced computed tomography and isotope brain scintigraphy studies have also shown that there is widespread BBB dysfunction (Doczi, 1985). Moreover, in SAH patients who experience overt delayed cerebral ischemia (DCI) leading to cerebral infarction, the extent of BBB dysfunction within the infarct would be expected to be similar to that in primary acute stroke affecting the same vascular distribution.

In preclinical studies, a cerebrospinal fluid (CSF) concentration of 100 ng/mL of IL-1RA within 1 hour of transient middle cerebral artery ischemia was observed to correspond to its neuroprotective effect (Clark *et al*, 2008). A pilot study in SAH patients showed that intravenous IL-1RA given as a bolus of 100 mg followed by an infusion of 2 mg per kg per hour was safe and penetrated the BBB to achieve this experimentally therapeutic concentration within 3 hours of commencement of infusion (Clark *et al*, 2008). Given that an average of 1.9 million neurones are destroyed per minute (Saver, 2006), and the delay from onset of symptoms to commencement of drug administration (Lacy *et al*, 2001), it is essential that therapeutic concentrations are achieved within the shortest time possible. We therefore set out to identify a safe, tolerable, practical administration regime that could achieve experimentally therapeutic CSF concentrations of IL-1RA within a reasonable time window.

Materials and methods

The study protocol was submitted and approved by Amgen Inc who provided the drug, anakinra, free of charge. Medicine and Healthcare Products Regulatory Agency and National Research Ethics Committee approvals were obtained before the study commenced.

Patient Recruitment

Eighty-eight patients were screened and 30 were recruited from the Intensive Care and High Dependency units at Salford Royal NHS Foundation Trust, UK and Cambridge University Hospitals NHS Foundation Trust, UK between December 2007 and December 2008 (Figure 1). Patients were Caucasian, aged 37 to 76 years (mean age = 53 years), with a mean weight of 73 kg (range, 58 to 110 kg). Patients eligible for the study had radiologically confirmed SAH and an external ventricular drain inserted to manage ensuing hydrocephalus. Exclusion criteria for study entry included a clinically significant concurrent medical

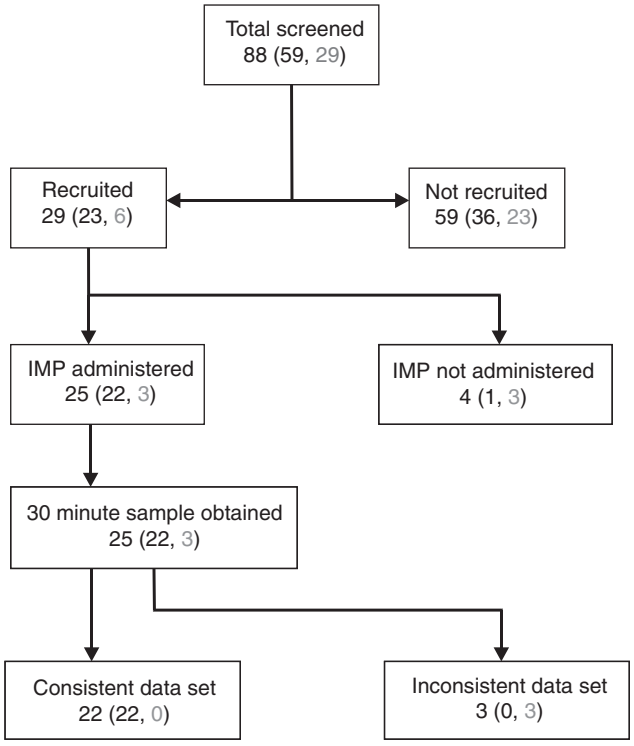

Figure 1 Screening and recruitment schema (black = SRFT, gray = Cambridge). IMP, investigational medicinal product.

Table 1 General patient characteristics for patients receiving anakinra

Male:female ratio	7:18
Smokers	64%
Hypertensive (diagnosed preictus)	24%
Ethnicity	100% Caucasian
Weight (mean, range)	73.4 kg (58–110 kg)
Right handed	100%

condition, participation in another study in the last 30 days or concurrent immunosuppressant medication. Pregnant or breast-feeding patients, those with a history of sensitivity to products derived from *Escherichia coli* and patients suffering from malignancies were also excluded.

Written consent was obtained from the next of kin for all patients as none were deemed fit to consent at the time of recruitment. Two patients improved cognitively during the course of the study and were subsequently approached for consent. Four patients recruited into the study did not receive the investigational medicinal product for various reasons including accidental removal of the EVD and death. Of the 25 patients receiving the drug, 22 were recruited from Salford and 3 from Cambridge. General patient characteristics are shown in Table 1.

Clinical history and demographic data were collected and a physical examination was performed on each patient. At presentation, 17 patients were poor grade (World Federation of Neurosurgeons Score IV and V) and 8 were good grade (World Federation of Neurosurgeons Score I–III). The distribution of blood on computed tomography scan varied but all had diffuse clots of blood (Fisher grade III) or intraventricular blood (Fisher grade IV). Of the 25 patients receiving the investigational medicinal product, 20 had solitary aneurysms and 5 had multiple aneurysms. In all, 76% of the aneurysms were located within the anterior circulation and the majority (21) were coiled.

Pharmacokinetics

An empirical pharmacokinetic (PK) model for intravenous anakinra has already been published by our research group (Gueorguieva *et al*, 2008), with population pharmacokinetics (PPK) fitting a linear two-compartment model for plasma with elimination from the central compartment and a one-compartment model for CSF. Before using this model to inform the design of our dose-ranging study, the raw data for the model-making study were reviewed and the parameter estimates for the published model were refined using the NONMEM computer program, version VI (ICON Development Solutions, Dublin, Ireland).

Using revised parameter estimates, simulation of different permutations of intravenous boluses (ranging from 100 to 1000 mg in 100 mg steps) and infusions (ranging from 1 to 10 mg per kg per hour for 4 hours in 1 mg per kg per hour steps) of anakinra were performed. Simulations were performed using a custom script in GNU-R. The administration regime that would potentially achieve our target CSF concentration within 30 minutes of start of infusion of IL-1RA with the lowest peak plasma concentration (C_{max}) was determined to be 500 mg intravenous bolus followed by an intravenous infusion of 10 mg per kg per hour. Although our initial aim was 60 minutes, we have chosen 30 minutes to safeguard against possible deviations of actual from predicted CSF concentration profiles of anakinra. For safety reasons, four other regimes were chosen, each reflecting a stepwise increase in C_{max} (Table 2).

The published PPK model was based on a single-dose regime using a much lower bolus dose and infusion rates of intravenous IL-1RA. Given that earlier experimental data had indicated that the IL-1 family (IL-1α, IL-1β, and IL-1RA) is transported through a saturable mechanism across the BBB (Banks *et al*, 1991; Gutierrez *et al*, 1994), it was important to determine whether data generated during this study would match the concentrations profiles predicted by the model. Prediction intervals for plasma and CSF for each of the administration regimes were therefore calculated. To further optimize the efficacy and reduce the risk of introducing central nervous system infection by unnecessary repeated sampling, the optimal sampling time points for plasma and CSF were determined by pharmacometric analysis using published techniques (Ogungbenro *et al*, 2005).

Patients received anakinra within a mean of 4 days (range, 2 to 12 days) from ictus. Baseline blood and CSF samples were obtained within 5 minutes before anakinra administration for measurement of IL-1RA concentrations and determination of preinfusion biochemical and hematological profiles. Participants were administered a bolus

Table 2 Summary of proposed treatment regimes including estimated peak plasma concentrations and estimated times to reach 100 ng/mL in the CSF

Regime no.	*Bolus (mg)*	*Infusion rate (mg/h)*	*Mean predicted peak plasma concentration (ng/mL)*	*Total dose (mg)*	*Estimated time taken to reach 100 ng/mL of IL-1RA in CSF (minutes)*
1	100	4	34,772	1220	104
2	200	2	41,152	760	141
3	300	2	61,728	860	96
4	400	6	82,304	2080	43
5	500	10	102,880	3300	30

CSF, cerebrospinal fluid; IL, interleukin.

of anakinra over 1 minute through a dedicated intravenous line that was immediately followed by a continuous infusion of anakinra diluted in 500 mL of clinical grade 0.9% saline over 4 hours through a volumetric pump (Graseby, Watford, UK). Five patients were allocated to each of the five regimes sequentially.

Samples of up to 3 mL of CSF and up to 5 mL of blood were obtained using a sterile nontouch technique after removal and discard of ~2 mL of CSF or blood. At 10, 20, 30, 45, 60, 90, 120, 240, 300, 360, 540 minutes and at days 1, 2, 3, and 7 ± 1, CSF and plasma samples were collected in EDTA and anticoagulant-free tubes, respectively, and kept at 0°C to 4°C. Samples were centrifuged at 2000 *g* and 4°C for 15 minutes. Plasma and CSF were decanted into 2 mL freeze vials and stored at −70°C until analysis. The IL-1RA assays were performed using an 'in-house' immunoassay, calibrated against National Institute for Biological Standards Control (South Mimms, UK) standards, as described earlier (Emsley *et al*, 2005). The technicians were masked to the infusion rate.

The occurrence of adverse events was monitored by the research team. Full clinical examinations were performed at days 1, 2, 3, and 7 ± 1 after infusion. Hematological, biochemical, and microbiological results were monitored on a daily basis until day 7 after commencement of infusion. The CSF was sent for microscopy, culture and antibiotic sensitivity whenever sampled for research purposes. The EVD infections were defined as the presence of a ratio of white cells: red blood cells of >500 in the CSF or the presence of organisms in two consecutive CSF samples. They were documented as being expected if the drains had been *in situ* for longer than 10 days, if there had been a documented significant CSF leak around the exit site or if there had been a documented breach of sterility of the drainage systems before investigational medicinal product was administered. All adverse events were classified and logged into the case-report forms and reported to the study sponsor and the Independent Data Monitoring and Safety Committee for review. Interim analyses of these adverse events were performed after each regime to review data quality and safety before proceeding to the next dose regime.

Concentration profiles of anakinra in plasma and CSF were analyzed to ensure that they did fit within prediction intervals calculated *a priori*. Safety data were analyzed descriptively. Nonlinear mixed effects modeling using NONMEM version IV was used to remodel the cumulative data obtained to date on PK of intravenous administered anakinra in patients with SAH. Student's *t*-test using SPSS version 16 (SPSS Inc, Chicago, IL, USA) was used to investigate the effect of anakinra administration on neutrophil count, temperature, and creatinine. General linear mixed model analysis was used to analyze the possible effect of covariates including dose regime used and time since administration on these variables. The half-life of anakinra was calculated using the formula $t_{\frac{1}{2}} = 0.693/k$, where k is the first-order elimination constant. Simulation and graphical representation of the data were performed using GNU-R, Matlab version 2008a (MathWorks, Natick, MA, USA) and CorelDraw X4 (Corel Corp, Ottawa, ON, Canada). Area under the curve analysis was performed using Graphpad Prism Software (Graphpad, San Diego, CA, USA).

Results

Safety

All patients completed the 4-hour infusion of anakinra. Pharmacokinetic data for CSF for the three patients recruited from Cambridge had to be omitted because of contamination with plasma resulting in aberrant IL-1RA concentrations. There were four deaths during the 7-day study period and all of these were related to cerebral ischemia. One of these patients had demonstrable absence of filling of the ipsilateral middle cerebral artery at the time of initial angiography before commencing the infusion.

The infection rate for EVDs was 3/25 or 12%, with two of these being expected. An independent audit performed by the neurosurgical department during the same period showed an overall infection rate for EVDs in the neurosurgical unit of 20%. The complete list of adverse events is listed in Table 3. All adverse events were reported to the Independent Data Monitoring and Safety Committee but none were deemed attributable to the study drug.

Effect of Hematological and Biochemical Parameters

There was a statistically significant reduction in neutrophil counts between before administration and day 1 after administration of anakinra (*t*-test, $P = 0.04$) (Figure 2). None of the patients experienced neutropenia. There was no significant difference

Table 3 List of adverse events reported to the IDMSC during the study

Category	*Adverse event*	n
Infection	Chest infection	9
	CSF infection	3
	Gastroenteritis	1
	PUO	3
	UTI	2
Cerebrovascular	Spasm/DCI	4
CSF drainage	EVD malfunction	3
	MODS	1
	Arrhythmia	2
	Rectal prolapse	1
Total		29

CSF, cerebrospinal fluid; DCI, delayed cerebral ischemia; EVD, external ventricular drain; IDMSC, Independent Data Monitoring and Safety Committee; MODS, multiple organ dysfunction syndrome; PUO, pyrexia of unknown origin; UTI, urinary tract infection.

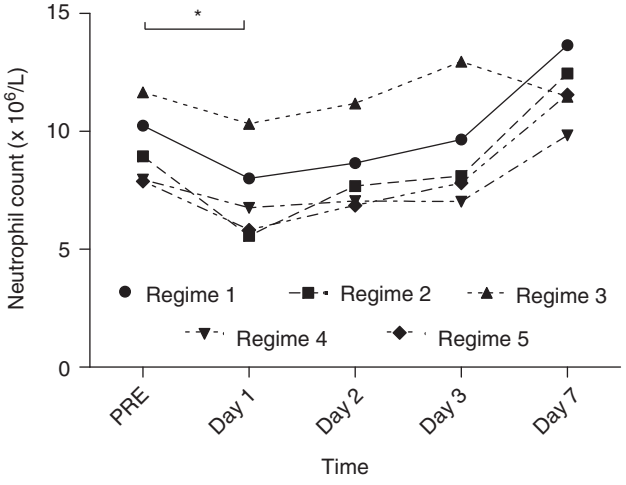

Figure 2 Neutrophil count pre and days 1, 2, 3, and 7 after administration of anakinra (*$P < 0.05$).

in the change in neutrophil counts between the regimes. For the duration of the study, anakinra had no significant effect on hemoglobin concentration or platelet numbers. In addition, there was no significant alteration in biochemical, renal, or hepatic parameters related to infusion of anakinra and no effect on heart rate, blood pressure, or brain temperature during the course of the study.

Pharmacokinetics of Anakinra

All IL-1RA plasma concentrations from the five regimes fitted the prediction intervals that were calculated *a priori* (see Figure 3). The IL-1RA CSF concentrations for all patients except for those recruited in Cambridge (patients 2, 4, and 5) also fitted the prediction intervals. The mean baseline concentration of IL-1RA was 476 pg/mL (± 664 pg/mL). The concentration of IL-1RA in plasma after infusion increased to peak values within the first 10 minutes reflecting the predictable importance of the bolus dose to achieve early high concentrations. After cessation of intravenous infusion, concentrations in plasma decreased rapidly with a half-life of 33 minutes (± 9 minutes) (see Figure 4).

The mean baseline concentration of IL-1RA in CSF was 606 pg/mL (± 1300 pg/mL). The concentration of IL-1RA in CSF after intravenous infusion of anakinra increased slowly over the 4-hour period and continued to rise for a variable period beyond the cessation of infusion. Steady state concentrations were not reached. Concentrations in CSF decreased slowly with an initial half-life of 238 minutes for regime 5. Area under the curve analysis for each of the regimes comparing plasma and CSF concentrations showed that 1.6% (± 0.4%) of intravenous administered anakinra crossed into the CSF of patients with SAH. There was no relationship between the administration regime and amount of IL-1RA entry into CSF.

The cumulative pharmacokinetic data from the model-making study and the current study fit a two-compartment and a one-compartment linear model for plasma and CSF, respectively. The IL-1RA concentration achieved in CSF in regime 5 at 30 minutes was 70,403 ± 23,417 pg/mL and at 60 minutes was 135,909 ± 45,963 pg/mL. The concentrations observed at 30 minutes were less than those predicted. Simulations using this updated PPK model would suggest that a regime consisting of an intravenous bolus of 1 g of anakinra followed by 10 mg per kg per hour would potentially achieve 100 ng/mL of anakinra in CSF within 30 minutes.

Studies investigating penetration of IL-1RA past the BBB have been performed in rodent models using radiolabeled substrates (Banks *et al*, 1991; Gutierrez *et al*, 1994). These studies have shown that the transport mechanism is saturable by virtue of varying degrees of mutual cross-inhibition of entry of IL-1α, IL-1β, and IL-1RA across the BBB. The percentage transfer of IL-1RA across the blood–CSF barrier in our study ranged from 0.97% to 1.27% and did not differ significantly in our five regimes, suggesting that transport of IL-1RA using administration regimes up to 500 mg bolus and 10 mg per kg per hour is largely passive.

Discussion

This dose-ranging study has determined the optimal dose regime required to safely achieve an experimentally therapeutic IL-1RA concentration in CSF within a time window that would be appropriate for the treatment of acute cerebral ischemia. This regime has been identified as an intravenous bolus of 500 mg administered over a minute followed by intravenous infusion of 10 mg per kg per hour. The study has also explored the effects of altering the bolus dose and the infusion rate on the time to reach steady state and

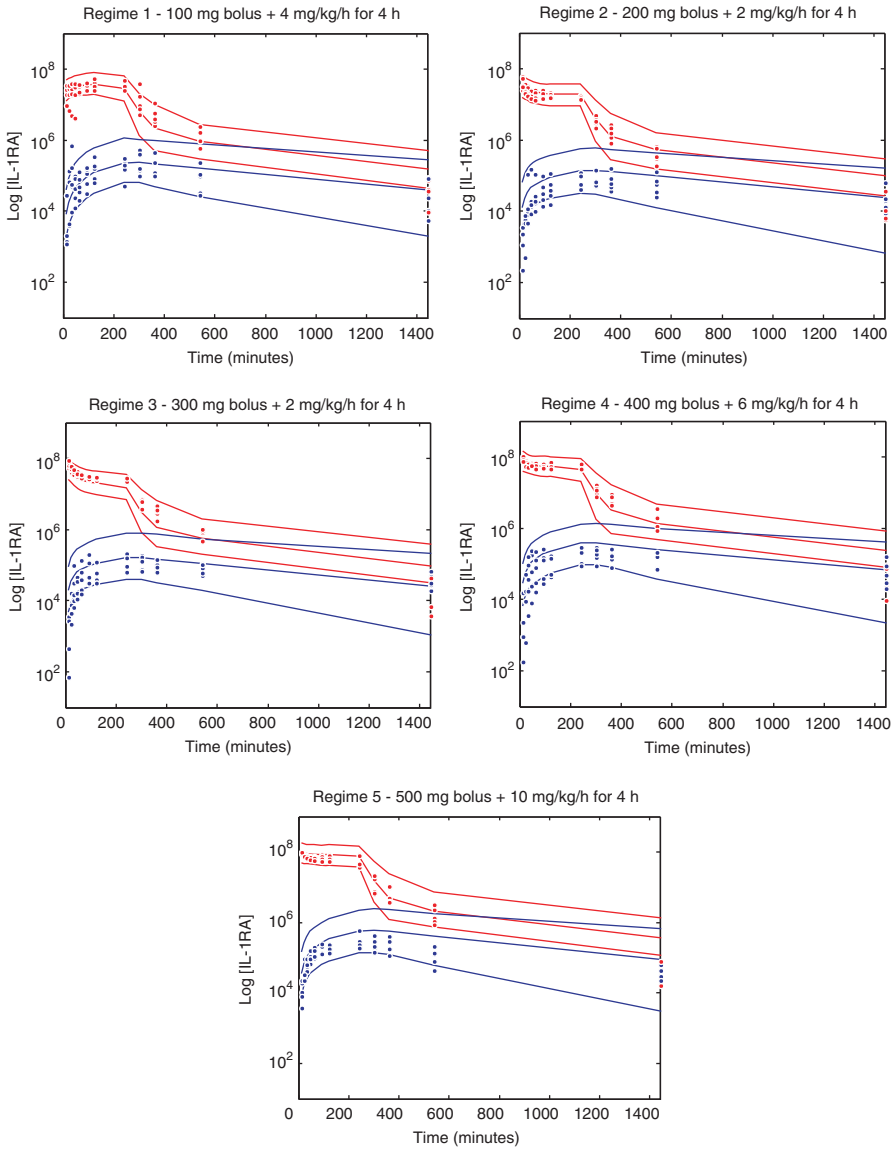

Figure 3 Predicted plasma (red lines) and cerebrospinal fluid (CSF) (blue lines) mean and interval concentration profiles, and actual plasma (red points) and CSF (blue points) for each regime. IL, interleukin.

C_{max} and tested the validity of our published PPK model.

Anakinra has an excellent safety and tolerability profile in the subcutaneous formulation that is currently licensed for use in rheumatoid arthritis and has been used in clinical trials for type 2 diabetes (Larsen *et al*, 2007), juvenile arthritis (Ilowite *et al*, 2009), Muckle Wells syndrome (Hawkins *et al*, 2004), Schnitzler's syndrome (Schneider *et al*, 2007), and non-ST segment myocardial infarction. Concerns over the potential risk of infection have occasionally arisen given the slight increase (2% in anakinra patients versus <1% in placebo) in rheumatoid arthritis patients (Nuki *et al*, 2002). This increase in infection rate occurred in the context of long-term administration in patients that were on a variety of immune-modulating drugs such as steroids and methotrexate. The intravenous formulation has already been trialled in studies of patients with acute sepsis without any safety concerns and the administration regime used had already been used by our group in the model-making study in SAH patients. In this study, however, the infusion rate and C_{max} was much higher. Recruitment was temporarily ceased after the first two regimes and after each subsequent regime to allow the Independent Data Monitoring and Safety Committee time to review and analyze the adverse events before progressing to higher dose regimes. There was, however, no suspected unexpected serious adverse reactions and none of the adverse events could be attributed to anakinra. The incidence of EVD infections was less than that generally experienced in other neurosurgical units (Arabi *et al*, 2005). This could be attributed to the meticulous sterile approach adopted, as opposed to the standard aseptic nontouch technique that is

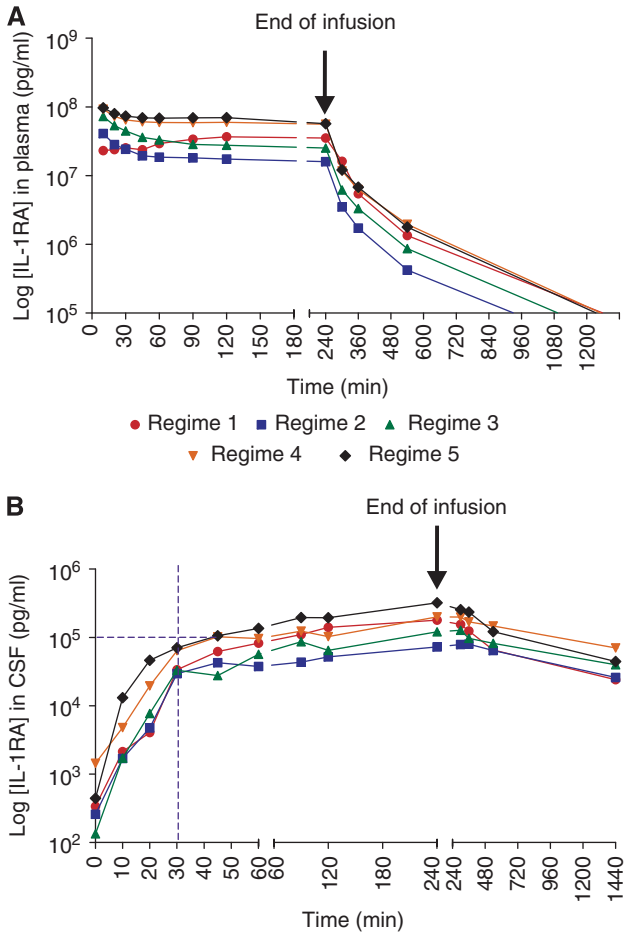

Figure 4 Mean plasma (**A**) and cerebrospinal fluid (CSF) (**B**) interleukin (IL)-1RA concentrations for patients in regimes 1–5.

standard neurosurgical practise, rather than the effect of anakinra itself. Similarly, although the occurrence of chest infections is commonplace in poor-grade SAH because of the high incidence of aspirations of gastric contents during the ictus of the bleed, our rate of respiratory infections were similar to or lower than that reported in similar studies (Berrouane *et al*, 1998). The mortality in our study (16%) compared favorably with published studies (Seifert *et al*, 1990).

Plasma concentrations of IL-1RA after intravenous anakinra administration were predicted very accurately by our previously published model and the deviation from the mean individual predicted values was low. Although CSF concentrations during the infusion and immediate postinfusion period of most patients did fit within the 2.5% to 97.5% prediction intervals, the spread around the mean individual predicted profile was much larger. This was a reflection of the relatively unconstrained nature of the original model equation predicting CSF concentrations. The CSF concentrations in the later phase (terminal half-life period) varied considerably. At such low concentrations, the endogenous production of IL-1RA significantly affects the overall (endogenous IL-1RA and anakinra) IL-1RA concentrations and probably accounts for moderate deviations from the predicted mean. The CSF infection, inflammation from mechanical irritation of brain parenchyma, and possibly inflammation as a response to delayed cerebral ischemia may account for elevated endogenous concentrations of IL-1RA.

A key question governing the pharmacokinetics of IL-1RA that could affect the applicability of our PPK data to patients with good grade SAH and patients with other forms of acute cerebrovascular disease is the degree of breakdown of compartmentalization of inflammation. We are aware that our patient cohort may not be representative of the general population suffering from SAH given those patients who require EVDs tend to represent the poorer grades, have more brain edema and may consequently exhibit a leakier BBB and blood–CSF barrier. Similarly, it may be debatable whether the pharmacokinetics of anakinra in this subpopulation of SAH patients is similar in other acute cerebrovascular disease states. It is, however, exceedingly difficult to gain repeated access to CSF in diseases such as stroke, as this can precipitate incipient tonsillar brain herniation, is not warranted for clinical reasons, and may cause unnecessary patient distress.

Another dilemma that may affect the validity of our study is the likely site of action of IL-1RA. If the main mode of action of IL-1RA is within the BBB, brain extracellular fluid concentrations associated with experimental neuroprotection may be more important than CSF concentrations. In the absence of reliable mechanisms of detecting concentrations of anakinra within extracellular fluid (unpublished studies using microdialysis of IL-1RA *in vitro* showed recovery to be very unreliable), CSF is the only measure of brain penetration available. Moreover, the intracerebroventricularly route of administration was the most effective in experimental studies.

In contrast to other failed neuroprotective agents for which penetration into the site of action was not shown, there is now significant evidence that IL-1RA enters CSF and that the rate at which this occurs can be safely modulated by altering the administration regime. A functional PPK model for anakinra has potential significant clinical therapeutic implications. If IL-1RA is proven to be neuroprotective and its efficacy is maximal within a certain therapeutic time window, administration of the drug may be individually tailored to patients to ensure maximal efficacy. Phase II efficacy studies informed by safety and PPK data from this study are currently under way. We believe that our approach may be widely applicable to the evaluation of other pharmacological therapeutic strategies targeting mechanisms within the central nervous system.

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Disclosure/conflict of interest

The authors declare no conflict of interest.

References

Adams Jr HP, del Zoppo G, Alberts MJ, Bhatt DL, Brass L, Furlan A, Grubb RL, Higashida RT, Jauch EC, Kidwell C, Lyden PD, Morgenstern LB, Qureshi AI, Rosenwasser RH, Scott PA, Wijdicks EF (2007) Guidelines for the early management of adults with ischemic stroke: a guideline from the American Heart Association/ American Stroke Association Stroke Council, Clinical Cardiology Council, Cardiovascular Radiology and Intervention Council, and the Atherosclerotic Peripheral Vascular Disease and Quality of Care Outcomes in Research Interdisciplinary Working Groups: The American Academy of Neurology affirms the value of this guideline as an educational tool for neurologists. *Circulation* 115:e478–534

Arabi Y, Memish ZA, Balkhy HH, Francis C, Ferayan A, Al Shimemeri A, Almuneef MA (2005) Ventriculostomy-associated infections: incidence and risk factors. *Am J Infect Control* 33:137–43

Banks WA, Ortiz L, Plotkin SR, Kastin AJ (1991) Human interleukin (IL) 1 alpha, murine IL-1 alpha and murine IL-1 beta are transported from blood to brain in the mouse by a shared saturable mechanism. *J Pharmacol Exp Ther* 259:988–96

Banwell V, Sena ES, Macleod MR (2009) Systematic review and stratified meta-analysis of the efficacy of interleukin-1 receptor antagonist in animal models of stroke. *J Stroke Cerebrovasc Dis* 18:269–76

Berrouane Y, Daudenthun I, Riegel B, Emery MN, Martin G, Krivosic R, Grandbastien B (1998) Early onset pneumonia in neurosurgical intensive care unit patients. *J Hosp Infect* 40:275–80

Blamire AM, Anthony DC, Rajagopalan B, Sibson NR, Perry VH, Styles P (2000) Interleukin-1beta -induced changes in blood-brain barrier permeability, apparent diffusion coefficient, and cerebral blood volume in the rat brain: a magnetic resonance study. *J Neurosci* 20:8153–9

Boutin H, LeFeuvre RA, Horai R, Asano M, Iwakura Y, Rothwell NJ (2001) Role of IL-1alpha and IL-1beta in ischemic brain damage. *J Neurosci* 21:5528–34

Buttini M, Sauter A, Boddeke HW (1994) Induction of interleukin-1 beta mRNA after focal cerebral ischaemia in the rat. *Brain Res Mol Brain Res* 23:126–34

Cicala C, Cirino G (1998) Linkage between inflammation and coagulation: an update on the molecular basis of the crosstalk. *Life Sci* 62:1817–24

Clark SR, McMahon CJ, Gueorguieva I, Rowland M, Scarth S, Georgiou R, Tyrrell PJ, Hopkins SJ, Rothwell NJ (2008) Interleukin-1 receptor antagonist penetrates human brain at experimentally therapeutic concentrations. *J Cereb Blood Flow Metab* 28:387–94

Deng YZ, Reeves MJ, Jacobs BS, Birbeck GL, Kothari RU, Hickenbottom SL, Mullard AJ, Wehner S, Maddox K, Majid A (2006) IV tissue plasminogen activator use in acute stroke: experience from a statewide registry. *Neurology* 66:306–12

Doczi T (1985) The pathogenetic and prognostic significance of blood-brain barrier damage at the acute stage of aneurysmal subarachnoid haemorrhage. Clinical and experimental studies. *Acta Neurochir (Wien)* 77:110–32

Dorhout Mees SM, Rinkel GJ, Feigin VL, Algra A, van den Bergh WM, Vermeulen M, van Gijn J (2007) Calcium antagonists for aneurysmal subarachnoid haemorrhage. *Cochrane Database Syst Rev*; pp 1–50, CD000277

Emsley HC, Smith CJ, Georgiou RF, Vail A, Hopkins SJ, Rothwell NJ, Tyrrell PJ (2005) A randomised phase II study of interleukin-1 receptor antagonist in acute stroke patients. *J Neurol Neurosurg Psychiatry* 76: 1366–72

Gueorguieva I, Clark SR, McMahon CJ, Scarth S, Rothwell NJ, Tyrrell PJ, Hopkins SJ, Rowland M (2008) Pharmacokinetic modelling of interleukin-1 receptor antagonist in plasma and cerebrospinal fluid of patients following subarachnoid haemorrhage. *Br J Clin Pharmacol* 65: 317–25

Gutierrez EG, Banks WA, Kastin AJ (1994) Blood-borne interleukin-1 receptor antagonist crosses the blood-brain barrier. *J Neuroimmunol* 55:153–60

Hara H, Friedlander RM, Gagliardini V, Ayata C, Fink K, Huang Z, Shimizu-Sasamata M, Yuan J, Moskowitz MA (1997) Inhibition of interleukin 1beta converting enzyme family proteases reduces ischemic and excitotoxic neuronal damage. *Proc Natl Acad Sci USA* 94:2007–12

Hawkins PN, Lachmann HJ, Aganna E, McDermott MF (2004) Spectrum of clinical features in Muckle-Wells syndrome and response to anakinra. *Arthritis Rheum* 50:607–12

Huang J, Upadhyay UM, Tamargo RJ (2006*a*) Inflammation in stroke and focal cerebral ischemia. *Surg Neurol* 66:232–45

Huang P, Chen CH, Yang YH, Lin RT, Lin FC, Liu CK (2006*b*) Eligibility for recombinant tissue plasminogen activator in acute ischemic stroke: way to endeavor. *Cerebrovasc Dis* 22:423–8

Ilowite N, Porras O, Reiff A, Rudge S, Punaro M, Martin A, Allen R, Harville T, Sun YN, Bevirt T, Aras G, Appleton B (2009) Anakinra in the treatment of polyarticular-course juvenile rheumatoid arthritis: safety and preliminary efficacy results of a randomized multicenter study. *Clin Rheumatol* 28:129–37

Lacy CR, Suh DC, Bueno M, Kostis JB (2001) Delay in presentation and evaluation for acute stroke: Stroke Time Registry for Outcomes Knowledge and Epidemiology (S.T.R.O.K.E.). *Stroke* 32:63–9

Larsen CM, Faulenbach M, Vaag A, Volund A, Ehses JA, Seifert B, Mandrup-Poulsen T, Donath MY (2007) Interleukin-1-receptor antagonist in type 2 diabetes mellitus. *N Engl J Med* 356:1517–26

Loddick SA, Rothwell NJ (1996) Neuroprotective effects of human recombinant interleukin-1 receptor antagonist in

focal cerebral ischaemia in the rat. *J Cereb Blood Flow Metab* 16:932–40

McMahon CJ, Smith D, Clark SR, Hulme S, Georgiou R, Drake S, Selvarajah J, Hopkins SJ, Rothwell NJ, Tyrrell PJ, King AT (2010) Delayed cerebral ischaemia after subarachnoid haemorrhage: clinical predictors and outcome. *J Neurosurg* (submitted)

Mulcahy NJ, Ross J, Rothwell NJ, Loddick SA (2003) Delayed administration of interleukin-1 receptor antagonist protects against transient cerebral ischaemia in the rat. *Br J Pharmacol* 140:471–6

Nuki G, Bresnihan B, Bear MB, McCabe D (2002) Long-term safety and maintenance of clinical improvement following treatment with anakinra (recombinant human interleukin-1 receptor antagonist) in patients with rheumatoid arthritis: extension phase of a randomized, double-blind, placebo-controlled trial. *Arthritis Rheum* 46:2838–46

O'Collins VE, Macleod MR, Donnan GA, Horky LL, van der Worp BH, Howells DW (2006) 1,026 experimental treatments in acute stroke. *Ann Neurol* 59:467–77

Obrenovitch TP, Zilkha E (1996) Inhibition of cortical spreading depression by L-701,324, a novel antagonist at the glycine site of the N-methyl-D-aspartate receptor complex. *Br J Pharmacol* 117:931–7

Ogungbenro K, Graham G, Gueorguieva I, Aarons L (2005) The use of a modified Fedorov exchange algorithm to optimise sampling times for population pharmacokinetic experiments. *Comput Methods Programs Biomed* 80:115–25

Ohtsuki T, Ruetzler CA, Tasaki K, Hallenbeck JM (1996) Interleukin-1 mediates induction of tolerance to global ischemia in gerbil hippocampal CA1 neurons. *J Cereb Blood Flow Metab* 16:1137–42

Rabinstein AA, Friedman JA, Weigand SD, McClelland RL, Fulgham JR, Manno EM, Atkinson JL, Wijdicks EF (2004) Predictors of cerebral infarction in aneurysmal subarachnoid hemorrhage. *Stroke* 35:1862–6

Relton JK, Martin D, Thompson RC, Russell DA (1996) Peripheral administration of interleukin-1 receptor antagonist inhibits brain damage after focal cerebral ischemia in the rat. *Exp Neurol* 138:206–13

Saver JL (2006) Time is brain—quantified. *Stroke* 37:263–6

Schneider SW, Gaubitz M, Luger TA, Bonsmann G (2007) Prompt response of refractory Schnitzler syndrome to treatment with anakinra. *J Am Acad Dermatol* 56: S120–2

Seifert V, Trost HA, Stolke D (1990) Management morbidity and mortality in grade IV and V patients with aneurysmal subarachnoid haemorrhage. *Acta Neurochir (Wien)* 103:5–10

STAIR (2001) Recommendations for clinical trial evaluation of acute stroke therapies. *Stroke* 32:1598–606

Topakian R, Barrick TR, Howe FA, Markus HS (2010) Blood-brain barrier permeability is increased in normal-appearing white matter in patients with lacunar stroke and leucoaraiosis. *J Neurol Neurosurg Psychiatry* 81:192–7

Topcuoglu MA, Singhal AB (2006) Effects of common medications on cerebral vasospasm after subarachnoid haemorrhage. *Expert Opin Drug Saf* 5:57–65

Yamasaki Y, Matsuura N, Shozuhara H, Onodera H, Itoyama Y, Kogure K (1995) Interleukin-1 as a pathogenetic mediator of ischemic brain damage in rats. *Stroke* 26:676–80; discussion 81