Development and effect of antibodies to anakinra during treatment of severe CAPS: sub-analysis of a long-term safety and efficacy study

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Received: 19 April 2018 / Revised: 26 June 2018 / Accepted: 28 June 2018 / Published online: 7 July 2018
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Abstract

Anakinra is an effective, well-tolerated, long-term anti-inflammatory treatment for cryopyrin-associated periodic syndromes (CAPS), yet evidence shows that it can induce the development of anti-drug antibodies (ADA). This analysis aims to determine ADA occurrence in CAPS patients and elucidate their effects on anakinra dosing and drug efficacy. A post hoc analysis was performed on data from a long-term safety and efficacy study in patients with severe CAPS. Patients were initiated on an anakinra dose of 1.0–2.4 mg/kg once daily subcutaneously, which was increased (in 0.5–1.0 mg/kg increments) to 2.0–5.0 mg/kg/day according to clinical need (median 3.1 mg/kg/day). ADA, serum amyloid A (SAA), and C-reactive protein (CRP) levels were measured at various time points, and pharmacokinetic (PK) parameters at 1 and 3 months. Efficacy was evaluated using a diary symptom sum score (DSSS), and SAA and CRP levels were evaluated as proxies of efficacy. Safety was evaluated by an analysis of adverse events (AEs). Anakinra dose levels were unrelated to ADA status. A high proportion of patients with at least one post-baseline assessment developed ADA (83%), the majority (79%) within 3 months. However, anakinra treatment markedly improved symptoms and was effective regardless of the presence of ADA; the annual rates of AEs were comparable between ADA-negative and ADA-positive patients. While ADA are likely to occur in CAPS patients treated with anakinra, our evidence shows that chronic daily subcutaneous treatment with anakinra is safe and effective regardless of the development and presence of ADA.

Keywords Anakinra · Anti-drug antibody (ADA) · Cryopyrin-associated periodic syndromes (CAPS) · Dosing · Neonatal-onset multisystem inflammatory disease (NOMID) · Pharmacokinetics (PK)

Introduction

Cryopyrin-associated periodic syndromes (CAPS) are a group of ultra-rare inherited autoinflammatory diseases encompassing three clinical phenotypes with increasing severity: familial cold autoinflammatory syndrome, the mildest of the three; Muckle-Wells syndrome (MWS), which is of intermediate severity; and neonatal-onset multisystem inflammatory disease (NOMID), the most severe form, involving continuous multiple organ inflammation [1]. CAPS results from

mutations in the gene *NLRP3*, which leads to activation of the enzyme caspase-1 and subsequent interleukin (IL)-1 β -mediated inflammatory response [2–5].

Anakinra, a recombinant human IL-1 receptor antagonist, blocks the inflammatory effects of IL-1 β in CAPS patients by competitively inhibiting the binding of IL-1 β to the IL-1 receptor [6]. It has been approved for the treatment of CAPS in the EU and Australia, and for the treatment of NOMID in the USA and Canada. Anakinra has also been used for over 15 years for the treatment of rheumatoid arthritis (RA) in the EU, the USA, Canada, and Australia, where its safety profile is well documented in adult patients [7–12]. While anakinra has been found to control active inflammation in patients with severe CAPS [13], it has also been shown to induce anti-drug antibodies (ADA) in patients with RA and juvenile idiopathic arthritis (JIA) [9, 14, 15]. In this report, we aim to evaluate the impact of ADA on the pharmacokinetic (PK) profile, overall efficacy and safety, and effective dose of the drug.

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s10067-018-4196-x>) contains supplementary material, which is available to authorized users.

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Materials and methods

A post hoc analysis was performed on data from a prospective, open-label, single-center, clinical cohort study conducted at the National Institutes of Health (NIH) in the USA from 2003 to 2010. This study investigated the efficacy and safety of anakinra treatment for up to 5 years in patients with severe CAPS [1, 16].

Patients

A total of 43 patients were included in the study; 25 (58.1%) were female and 36 (83.7%) were white. All patients were diagnosed with CAPS, 36 with NOMID, and seven with characteristics overlapping between MWS and NOMID. The age of patients at the start of treatment ranged from 0.7 to 46.3 years, with an overall mean of 10.3 years; 36 patients (83.7%) were children.

Dosing

Patients were initiated on an anakinra dose of 1.0–2.4 mg/kg once daily administered via subcutaneous injection. Over the course of the study, in patients who were not in clinical remission, the maintenance dose was adjusted (in 0.5–1.0 mg/kg increments) to 2.0–5.0 mg/kg/day (median 3.1 mg/kg/day). A total of eight patients received doses > 4.5 mg/kg/day at some time during the study, and the highest dose administered was 7.6 mg/kg/day, temporarily to one patient only.

Sampling and analysis

Baseline values for ADA evaluation were obtained using samples taken from 32 of the 43 patients enrolled in the study and 4 CAPS patients who were not enrolled in the study; these additional four samples were used to improve the statistical validity of the cut-point for ADA assessment (see [Supplementary Material](#) for information about why the additional samples were obtained). The presence of ADA was assessed in blood samples that had been taken prior to anakinra dosing at baseline and months 1, 3, 6, 12, 36, 54, and 60 from 32 (plus four non-study patients; see above), 21, 28, 9, 28, 22, 9, and 6 patients, respectively, with at least one sample taken from each of the 43 patients. Analysis of anakinra-specific ADA was performed using a bridging format immunoassay and a tiered approach, including, as a first step, screening of samples followed by a second confirmatory testing of screened positive samples. Screening and confirmatory cut-points were calculated using baseline data following a statistical approach. The assay had a sensitivity of

0.8 ng antibody/mL. Low and high positive control samples used in the ADA assessments were prepared from a polyclonal affinity purified goat anti-IL-1 receptor antagonist (anti-IL-1Ra) antibody preparation (R&D Systems) at 2.19 and 67.8 ng/mL in negative control serum. Although the presence of anakinra reduced the assay sensitivity, potentially clinically relevant ADA concentrations were still detectable at anakinra concentrations of up to 20 µg/mL (see [Supplementary Materials](#) for details).

Levels of serum amyloid A (SAA) had been determined in blood samples taken prior to dosing at baseline and months 1, 3, 6, 12, 36, and 54/60 from 29, 17, 22, 6, 22, 19, and 12 of the patients who had ADA assessments, respectively. Because of the low number of samples at months 54 and 60, the data have been pooled together for the analysis. Analysis of SAA was performed by high-sensitivity latex-enhanced nephelometry with an analytical sensitivity of 0.75 mg/L (at the National Amyloidosis Centre, UCL Medical School, Royal Free Hospital, London, UK). For C-reactive protein (CRP), blood samples were taken prior to dosing at baseline and at months 1, 3, 6, 12, 36, and 54/60 from 32, 18, 23, 7, 25, 21, and 14 of the patients who had ADA assessments, respectively. Analysis was performed at NIH, USA, using a high-sensitivity CRP assay.

Clinical efficacy

Clinical efficacy was evaluated using a diary symptom sum score (DSSS), with severity of the main symptoms of the disease scored daily on a scale from 0 (no symptoms) to 4 (highest severity). The five key symptoms included in the DSSS were fever, headache, rash, joint pain, and vomiting. A mean value for each of the five symptoms was calculated for a period preceding the visit: days 5–30, days 14–30, and the last 30 days at baseline, month 1, and months 3–60, respectively. The DSSS for each visit was then calculated as the sum of the mean values for each of the five symptoms, with a possible range of 0–20.

Adverse events

The classification and analysis of adverse events (AEs) are described in the [Supplementary Material](#).

Bioanalysis and pharmacokinetics

The analysis of endogenous IL-1Ra levels prior to the first dose with anakinra, as well as anakinra concentrations following dosing, was performed at NIH using a kit-based sandwich enzyme-linked immunosorbent assay (ELISA), run according

Fig. 1 Anakinra dosage in ADA-negative and ADA-positive CAPS patients. Not present = ADA negative; present = ADA positive; dose levels (mean $\pm$ SEM) of anakinra in ADA negative and ADA positive patients at baseline and at subsequent visits during anakinra treatment; N = number of ADA negative patients/number of ADA positive patients at respective visit. ADA, anti-drug antibodies; CAPS, cryopyrin-associated periodic syndromes; SEM, standard error of the mean

to the manufacturer's protocol (ELISA, IL-1Ra Cytoscreen assay kit, BioSource) (see [Supplementary Materials](#) for more information about this analysis). To assess PK parameters, samples were obtained from 13 patients after the first dose and at 3 months. Blood samples were collected pre-dose and at 2, 4, 8, and 24 h post-dose at each time point, with an analysis performed on serum concentration data. The PK analysis was based on individual serum concentration data, and the PK parameters, C_{max} , C_{24h} , and AUC were calculated using WinNonlin Professional (Pharsight Corporation, Mountain View, CA, USA) version 4.1, using noncompartmental methods.

Results

At baseline, none of the samples taken were found to have pre-existing ADA. Less than half (43%, $N = 9/21$) of the patients had detectable ADA at 1 month, while the majority (79%, $N = 22/28$) had detectable ADA at 3 months, a proportion that gradually decreased with time. This was in

line with the data for the subset of patients with a complete sample set, with the highest proportion of patients with ADA-positive results at 3 months (82%, $N = 9/11$ patients) and a decrease seen at later time points (the ADA response (at months 0, 1, 3, 12, 36, and 54/60) for the complete sample set subpopulation is shown in [Supplementary Material Table S1](#)).

While the average anakinra body weight-adjusted dose was increased over the study period, dose levels were comparable between patients who developed ADA (ADA positive) and those who did not (ADA negative), indicating that dose adjustments during the study were generally unrelated to a patient's ADA status (Fig. 1). Endogenous IL-1Ra and/or anakinra detected in ADA samples were at levels that would not impact ADA detection (see [Supplementary Materials](#) for details of endogenous IL-1Ra and/or anakinra levels).

The DSSS data show that patients had an almost immediate response to anakinra treatment (Fig. 2), with a marked reduction in symptom severity, independent of ADA status, which was sustained until month 60. Mean

Fig. 2 Efficacy (DSSS) in ADA-negative and ADA-positive patients during anakinra treatment. Not present = ADA negative; present = ADA positive; dose levels (mean $\pm$ SEM) of anakinra in ADA negative and ADA positive patients at baseline and at subsequent visits during anakinra treatment; N = number of ADA negative patients/number of ADA positive patients at respective visit. ADA, anti-drug antibodies; CAPS, cryopyrin-associated periodic syndromes; SEM, standard error of the mean

Table 1 Biomarkers of efficacy in ADA-negative and ADA-positive patients (SAA and CRP levels)

Month	SAA (mg/L)				CRP (mg/L)			
	ADA negative		ADA positive		ADA negative		ADA positive	
	Median (range)	N	Median (range)	N	Median (range)	N	Median (range)	N
Baseline	149 (9.0–1400)	29	–	0	53 (10–260)	32	–	–
1	12 (0.9–140)	10	97 (12–207)	7	5.6 (1.3–19)	10	25 (2.1–63)	8
3	3.3 (2.4–20)	6	8.7 (1.6–286)	16	2.8 (0.8–5.0)	6	5.5 (0.1–42)	17
6	15 (3.0–39)	4	8.5 (4.0–13)	2	7.2 (1.4–18)	4	2.8 (0.9–4.8)	3
12	11 (1.0–304)	10	7.3 (0.5–165)	12	12 (1.4–68)	12	4.1 (0.3–68)	13
36	14 (2.0–26)	8	4.0 (1.0–140)	11	4.6 (0.1–23)	9	2.5 (0.3–23)	12
54/60	15 (1.0–147)	8	7.0 (1.0–132)	4	2.5 (0.2–48)	9	7.0 (0.1–20)	5

Month 54/60: data from month 54 or month 60 were pooled

ADA, anti-drug antibodies; CRP, C-reactive protein; SAA, serum amyloid A

change from baseline in DSSS at month 1, 3, and 54/60 was similar for both ADA-negative and ADA-positive patients. Irrespective of ADA status, the median SAA and CRP levels were reduced from baseline to almost normal levels by month 3 and remained at similar levels throughout the remainder of the study (Table 1).

Part of the patients were on concomitant treatment with steroids. The proportion of ADA-positive patients at month 3 was similar among patients who at baseline either used steroids or were non-steroid users. In patients using concomitant steroids, the proportion who continued to use them after month 36 decreased to a similar level among ADA-positive and ADA-negative patients (60 and 62.5%, respectively). These data suggest that the use of steroids did not prevent ADA development and that neither continued usage nor tapering of steroids was influenced by the development of ADA. Also, the decrease in the proportion of ADA-positive patients seen beyond month 3 could not be explained by the use of steroids. A small group of patients used concomitant treatment with methotrexate that may have affected the incidence of ADA development or, alternatively, the prevalence of ADA among patients. However, the potential impact of methotrexate on ADA was not possible to evaluate due to the limited number of

patients on such treatment. The cause for change in ADA prevalence is therefore still to be identified. Immune tolerization following chronic treatment with anakinra could be a potential mechanism.

The between-patient comparison of dose-normalized PK parameters ($C_{\max}$, C_{24h} , and AUC_{0-24h}) at 3 months showed similar results among ADA-negative ($N=5$) and ADA-positive ($N=8$) patients, and the within-patient comparison of anakinra half-life in six patients showed no consistent impact of ADA at 3 months. The dose-normalized exposure of anakinra in ADA-negative and ADA-positive patients after the first dose and at month 3 is detailed in Table 2.

The overall annual rate of treatment-emergent adverse events (TEAEs) was comparable between ADA-negative and ADA-positive patients (8.59 and 6.91 per year/per patient, respectively); the most common TEAEs were arthralgia (1.23 and 0.50 per year/per patient, respectively) and headache (0.80 and 0.61 per year/per patient, respectively). No relevant differences were observed when comparing disease-related, allergy-related, and injection-site-related annual AE rates for ADA-negative and ADA-positive patients with an exposure of 73.8 and 82.2 patient-years, respectively (Table 3).

Table 2 Dose-normalized PK parameters at first dose and at month 3 by presence of ADA

	Number of patients	$C_{\max}/\text{dose}$	C_{24h}/dose	AUC_{0-24h}/dose
First dose				
ADA negative	13	386 (210)	30.2 (31.7)	4118 (1283)
Month 3				
ADA negative	5	411 (201)	33.2 (40.6)	5448 (2892)
ADA positive	8	530 (227)	35.5 (21.8)	5465 (2167)

The unit for $C_{\max}$ and C_{24h} is ng/mL; for AUC, ng.h/mL; and for dose, mg/kg/day; values are means (SD)

ADA, anti-drug antibody; SD, standard deviation

Table 3 Annual AE rates for possible disease- or allergy-related events, and injection site-related events

Event	Annual AE rates	
	ADA negative (<i>N</i> = 39) (73.8 patient-years) ^a	ADA positive (<i>N</i> = 33) (82.2 patient-years) ^a
Total disease-related	4.38	3.04
Total allergy-related	0.80	0.90
Arthralgia	1.23	0.50
Headache	0.80	0.61
Rash ^b	0.27	0.38
Pyrexia	0.35	0.28
Ocular hyperemia ^b	0.19	0.23
Pain in extremity	0.15	0.19
Back pain	0.24	0.05
Fatigue	0.18	0.11
Total injection site-related ^c	0.11	0.09
Injection-site reaction	0.11	0.05
Injection-site erythema	0	0.02
Injection-site eczema	0	0.01

The table presents events occurring at least 0.15 times per patient-year in either group, and all injection site-related events

ADA, anti-drug antibody; AE, adverse event; NOMID, neonatal-onset multisystem inflammatory disease

^a Anakinra exposure

^b Both NOMID-related and allergy-related; all other events are only NOMID-related

^c Not NOMID-related

Discussion

Pharmacodynamic effects, efficacy, and safety of therapeutic proteins may be directly altered by neutralizing antibodies, and antibody formation may cause increased or decreased clearance of the protein. The use of clinically relevant outcome measures has been used in the present evaluation rather than applying an in vitro assay for separation of neutralizing and non-neutralizing antibodies. The results of this post hoc analysis showed that ADA had developed in the vast majority of patients at some stage during the study, with around half of these patients having detectable ADA within the first month, and the remaining patients within the first 3 months. Despite this, the presence of ADA did not have an obvious impact on the PK of anakinra, subsequent dose levels, or steroid tapering throughout the study period. In addition, ADA were deemed to have little effect on the efficacy of anakinra in reducing symptom severity (DSSS) and levels of the inflammatory biomarkers SAA and CRP. Further, the patient's ADA status did not affect the safety profile of anakinra.

While similarly high incidences of ADA development have been reported in studies of RA and JIA patients treated with anakinra (50–82%) [14, 15], a low incidence

was reported in a comparable study in RA (2.7%) [9], indicating some level of inconsistency in findings of this type. Whereas the initial studies in RA and JIA assessed antibody levels with ELISA and surface plasmon resonance-based assays, an MSD-based bridging format was applied in this study. However, apparent differences in the level of ADA development may be due to several factors, including methodological approach, differences in assay details, and differences in the patient population studied.

During the validation of the ADA assay, anakinra-binding antibodies were occasionally found in sera from drug-naïve, healthy blood donors. Such pre-existing antibodies can be found in both healthy individuals and patients in the absence of exposure to an active agent. These could be autoantibodies or antibodies induced by an unrelated compound, and their presence in patients may have clinical consequences upon drug exposure [17, 18]. However, no CAPS patients tested prior to the first dose of anakinra were found to have pre-existing ADA.

The development of ADA in patients receiving daily subcutaneous injections of anakinra appears to be common. However, evidence suggests that the presence of ADA is unlikely to have an impact on the drug's PK, safety, or efficacy.

Acknowledgements The authors extend their thanks to Raphaela Goldbach-Mansky of the Translational Autoinflammatory Disease Studies, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA, for her contribution and support for this research program.

Funding information This study has been supported by Swedish Orphan Biovitrum AB (publ).

Compliance with ethical standards

Conflict of interest All authors declare that they are currently or were previously employed by or shareholders of Swedish Orphan Biovitrum AB (publ).

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