ARA290 : a novel treatment for neuropathic pain in sarcoidosis

Heij, L.

CHAPTER 4

Population pharmacokinetic analysis of intravenous and subcutaneous ARA290

Subcutaneous ARA 290 administered to healthy volunteers: a safety and pharmacokinetic analysis

Lara Heij,[1] Marieke Niesters,[1] Monique van Velzen,[1] Anthony Cerami,[1,2] Ann Dunne,[2] Albert Dahan,[1] Michael Brines[2]

1. Department of Anesthesiology, Leiden University Medical Center, 2300 RC Leiden, The Netherlands

2. Araim Pharmaceuticals Inc. Tarrytown, NY 10591, USA

Abstract

Background: ARA290 is an 11-amino acid peptide with tissue protective properties. It is effective in the treatment of neuropathic pain in patients with sarcoidosis and diabetes mellitus type 2. Since ARA290 requires frequent parenteral administrations, various routes of administration are being examined that allow treatment outside the hospital setting with high patient compliance. In this study we determined the safety and pharmacokinetics of subcutaneous (SC) compared to intravenous (IV) ARA290 administration.

Methods: 10 healthy volunteers received on one occasion 2 mg IV ARA 290, and on another occasion 2 mg SC ARA290. On a third occasion, 5 subjects received 4 mg SC and the five others 6 mg SC ARA290. Serial plasma samples were obtained to determine the time-dependent plasma concentrations of ARA290. Safety parameters were obtained; the pharmacokinetics were assessed by non-compartmental analysis.

Results: No safety issues were identified. The mean peak plasma concentration after IV dosing was estimated at 111 ng/mL. After SC administration peak concentrations were observed between 12 and 15 min and were greater than 1.25 ng/mL for all SC doses, the minimum concentration believed to be necessary to trigger the receptor mediating ARA 290 biological effects. The terminal half-life of the IV and SC doses were 1.1 min and 17-26 min, respectively. Based on the area-under the plasma-concentration curve the bioavailability of SC ARA290 ranged from 11 to 25%.

Conclusions: ARA 290 is safe and well tolerated up to 6 mg SC. All three SC doses exhibited peak concentrations greater than the assumed minimum effective concentration.

Introduction

Tissue injury causes an inflammatory response, driven by pro-inflammatory cytokines (most importantly TNF-α) and results in tissue destruction and systemic disease.[1-3] Simultaneous to the pro-inflammatory response an anti-inflammatory reaction is initiated by the release of anti-inflammatory cytokines. Important mediators of the anti-inflammatory response are erythropoietin (EPO) and its receptor, the innate repair receptor (IRR). The IRR is composed of two classical EPO receptors and two β-common receptor subunits (CD131).[1,2] Activation of the IRR by EPO activates multiple anti-inflammatory, tissue protective and regenerative pathways. While it seems attractive to treat patients with an inflammatory disease with EPO, its side effect profile prohibits its (long-term) use.[4] Side effects include thrombotic events, hypertension and myocardial infarction. Recently, a series of non-hematopoietic EPO analogues have been constructed that mimic the anti-inflammatory effects of EPO without any of its deleterious side effects. One such analogue is ARA290, a pyroglutamate helix B surface peptide that mimics the

spatial configuration of the EPO surface that interacts with the IRR.[1-3] Various experimental and clinical studies show that ARA290 effectively reduces symptoms of tissue injury. For example, in sarcoidosis and diabetic mellitus type 2 patients with severe neuropathic pain (pain caused by a lesion or disease of the peripheral somatosensory nervous system) show a prolonged reduction of neuropathic pain symptoms and an improved quality of life.[5-7] ARA290 is administered intravenously and requires frequent (daily to two-three times weekly) injections. Daily intravenous injections are difficult to manage outside the hospital setting. Subcutaneous injections, however, comparable to insulin injections, are easily accomplished by the patient at home. To test the feasibility of subcutaneous ARA290 injections, we performed this phase 1 study in healthy volunteers to assess the safety of ARA290 injections and determine its pharmacokinetics.

Methods

Subjects

This is a single-center, open-label study performed in 10 healthy volunteers. Five male and five female subjects were enrolled in the study after the protocol was approved by the local human ethics committee and after the subjects had given written informed consent. Subjects were included if they were 18 to 65 years (inclusive), had a body mass index of 18-30 kg/m^2 (inclusive) and body weight 50-90 kg (inclusive), and were able to give informed consent. Exclusion criteria included: a history of physical and/or mental disease, hypertension (systolic blood pressure > 150 mmHg and/or diastolic blood pressure > 90 mmHg), a history of substance abuse (incl. alcohol use > 21 units/week (male) or > 14 units/week (female)), positive pregnancy test, use of nicotine containing products within 3 months prior to screening, use of xanthine containing products during the study, presence of allergies to prescription and non-prescription drugs or food, vaccination or immunization within the last month, participation in other trials in the 3 months prior to administration of the initial dose of the study drug or more than 4 times per year, donation or loss of blood (> 500 mL) within 3 months prior to screening, any other condition that in the opinion of the investigator could complicate or compromise the study, or the well-being of the subject.

Study Design

All subjects were submitted to the laboratory on three separate occasions, at least 1 week apart. They received on one occasion 2 mg intravenous (IV) ARA290 (in 6 mL saline, conc. 0.33 mg/mL, administered over 2 min using an infusion pump) and on the other 2 mg subcutaneous (SC) ARA290 (in 0.25 mL saline, conc. 8 mg/mL). On the third occasion, 5 subjects received 4 mg SC ARA290 (in 0.5 mL saline, conc. mg/mL), while the other 5 received 6 mg SC ARA290 (in 0.75 mL saline, conc. 8 mg/mL).

Blood samples were obtained from an IV access line in the left or right cubital vein (opposite to the line used for the intravenous infusion) at t = 1, 2, 3, 4, 6, 8, 10, 14, 18 and 24 min post dosing on occasion 1 and t = 1, 2, 4, 6, 10, 15, 20, 30, 45 and 60 min post dosing on occasions 2 and 3. The volume drawn was 3 mL; less than 30 mL blood was drawn per visit. Blood samples were collected in 6 mL EDTA-coated tubes. Within 30 min after collection, the samples were centrifuged at 3000 rpm for 10 minutes at 4˚C. The collected plasma was transferred to

duplicate transport tubes (approximately 1 mL per tube) and stored at -80˚C. One set of samples was shipped for analysis; the other set stored as backup.

Subjects were monitored for adverse effects at LUMC until 1-2 hours after administration of study drug and collection of the plasma samples.

ARA29

ARA290 (L pyroglutamyl-L glutamyl-L glutaminyl-L leucyl-L glutamyl- L arginyl-L alanyl- L leucyl-L asparaginyl-L seryl-L serine-OH, ARAIM Pharmaceuticals, Tarrytown, NY) is an 11-amino acid, linear peptide with a molecular weight of 1257 Daltons. ARA290 was manufactured by standard F-moc solid phase peptide synthesis, purified by HPLC and ion-exchange chromatography, and stored as a lyophilized powder. The drug was stored by the local pharmacy and manufactured by Bachem Distribution Services GmbH, Weil am Rhein, Germany. The drug was stored in the local pharmacy at -20 °C; the syringes were prepared by the investigators according to pharmacy guidelines.

ARA 290 plasma concentrations were determined by Charles River Laboratories in Halifax, Canada, using a validated high-performance liquid chromatography with tandem mass spectrometric (LC-MS/MS) detection. The lower limit of quantitation was 0.1 ng/mL.

Pharmacokinetic Analysis

A non-compartmental PK analysis was performed using WinNonlin software (version 5.3, Pharsight Corporation, Mountain View, CA). For the single IV infusion the following parameters were estimated: terminal half-life (t½), area-under the plasma-concentration curve ($AUC_{0\text{-}t}$ and $AUC_{0\text{-}\infty}$), total plasma clearance (CL), apparent volume of distribution at steady state (VSS). For the single SC injection the following parameters were estimated: observed maximum ARA 290 plasma concentrations (C_{MAX}), time to maximum ARA 290 plasma concentrations (TMAX), t½ and $AUC_{0\text{-}t}$ and $AUC_{0\text{-}\infty}$. The absolute systemic bioavailability was calculated as F = (SC $AUC_{0\text{-}t}$ /IV $AUC_{0\text{-}t}$) × (IV dose/SC dose). Values given are mean ± SD and coefficient of variation (%CV).

Results

The subject's age ranged from 20 to 23 years (mean ± SD 21.3 ± 1.2 years), weight from 51 to 90 kg (70.8 ± 12.1 kg), height 164 to 198 cm (180 ± 10 cm) and body mass index from 18.7 to 26.0 $kg.m^{-2}$ (22.3 ± 2.5 kg/m^2). Since the ARA290 dose was not corrected for body mass, the average doses given were 29.0 ± 5.0 µg/kg for the 2 mg dose, 58.5 ± 4.5 µg/kg for the 4 mg dose and 86.4 ± 21.4 µg/kg for the 6 mg dose.

All subjects completed the study without any adverse events. No local symptoms or signs were observed after either the intravenous or subcutaneous injection sites.

The individual PK data for the 4 treatment levels are given in Figure 1; the mean ± SEM data are given in Figure 2. Following the IV injection, ARA290 plasma concentration peak concentration was at the first ARA290 measurement at t = 1 min post dosing (54 ± 14 ng/mL) followed by a rapid decline in plasma concentration reaching values < lower limit of quantitation after ~10 min post infusion. In contrast, following SC administration, peak concentrations are observed after 8

min. The elimination phase is slower than that observed after the IV injection with mean concentrations > 0.3 ng/mL after 60 min.

IV ARA290. Plasma concentrations of ARA 290 following IV administration declined in a mono-exponential fashion with a mean t½ of ~1.1 min (Table 1). The extrapolated ARA290 plasma concentration at the end of the 2-min infusion was ~111 ng/mL. The mean total plasma clearance was high at 10.6 L/min and this value exceeded organ blood flows and cardiac output. This finding is highly suggestive of ARA 290 being rapidly metabolized or degraded in the circulation. The volume of distribution at steady-state (V_{SS}) was large with a mean value of approximately 21 L.

Table 1. Pharmacokinetic parameter estimates

	2 mg IV	2 mg SC	4 mg SC	6 mg SC
C_{MAX} ng/mL	-	1.8 ± 1.4 (75)	2.9 ± 1.7 (59)	8.3 ± 4.0 (48)
t½ min	1.1 ± 0.1 (13)	20.6 ± 10.1 (49)	17.6 ± 10.8 (61)	16.9 ± 5.6 (33)
CL L/min	10.6 ± 5.9 (55)	*	*	*
V_{SS} L/min	21.1 ± 13.4 (63)	*	*	*
F %	-	22 ± 13 (60)	18 ± 7 (38)	37 ± 24 (66)
AUC_{0-t} ng.min/mL	274 ± 202 (74)	53 ± 36 (68)	82 ± 51 (61)	223 ± 51 (23)
$AUC_{0-\infty}$ ng.min/mL	274 ± 202 (74)	64 ± 48 (76)	95 ± 46 (49)	275 ± 42 (15)

* not estimated; C_{MAX} is the observed maximum ARA290 concentration in plasma; t½ is the terminal half-life; CL is total plasma clearance; V_{SS} is the apparent volume of distribution; F is the absolute systemic bioavailability ; AUC is the area-under-the-curve.

SC ARA290. ARA 290 plasma concentrations, C_{max} and AUC increased with increasing dose of SC ARA 290 (Table 1). The mean peak plasma concentrations occurred at 6 min and were greater than 1.25 ng/mL for all SC dose levels, the minimum concentration believed to be necessary to trigger the receptor mediating ARA 290 biological effects. After reaching maximal ARA 290 plasma concentrations within 12-15 min, ARA 290 plasma concentrations declined in a mono-exponential fashion with a mean t½ of 17 to 21 minutes. The relatively high value of t½ after SC infusion indicates "flip-flop" kinetics, *ie.* the terminal phase of decline of ARA290 does not represent the elimination phase. The slower PK process governing the decline in concentrations is related to the absorption of the drug from the subcutaneous injection site. The mean absolute bioavailability (F%) was estimated as 22%, 18% and 37% for the single 2 mg, 4 mg and 6 mg SC ARA 290, respectively.

Considerable between-subject variability was observed in ARA290 plasma concentrations for both the intravenous and subcutaneous routes of administration (Fig. 1); the %CV ranged from 24% to 138% at any given sampling time point for the IV and SC routes of administration. The %CV in PK parameters was greatest for the exposure parameters of C_{MAX} and AUC ranging from 15% to 82%.

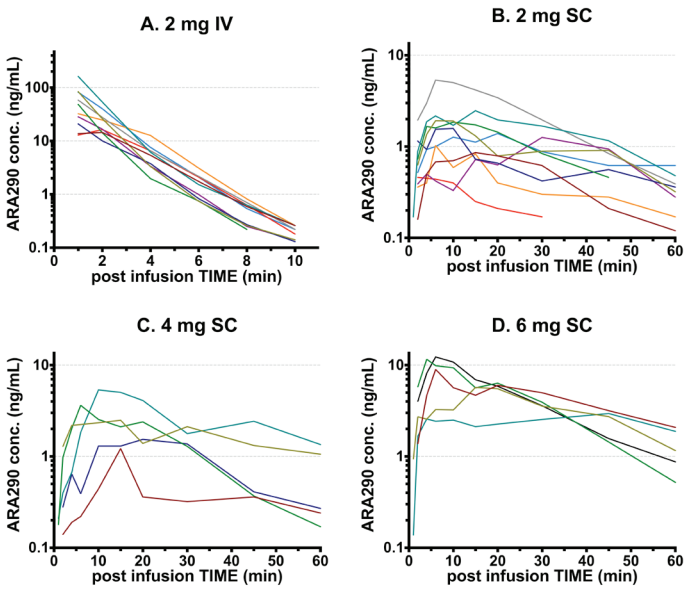

Figure 1. A. Individual plasma concentrations following the administration of 2 mg intravenous (IV) ARA290. **B-D.** Individual plasma concentrations following the administration of 2-6 mg subcutaneous (SC) ARA290.

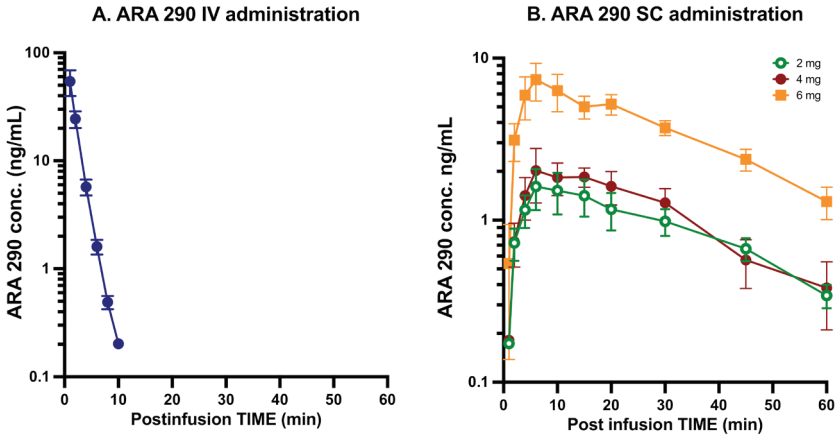

Figure 2. A. Mean ± SEM ARA 290 plasma concentration following 2 mg intravenous administration. **B.** Mean ± SEM ARA 290 plasma concentration following 2, 4 and 6 mg subcutaneous administration.

Discussion

ARA 290 administered in the dosage range of 2-6 mg SC and 2 mg IV was not associated with any adverse events. Specifically, there were no complaints of local pain, redness, or cutaneous sensitivity and no systemic effects reported. On average, all three subcutaneous doses exhibited a C_{MAX} greater than the assumed minimum effective concentration of 1 nM obtained from preclinical studies.

The doses used in the current study were based on previous studies. A preclinical model of neuropathic pain in the rat showed that for a sustained therapeutic effect of ARA290, a minimum dose of 30 $\mu g.kg^{-1}$ twice weekly administered via the intraperitoneal route is required.[8] Results of previous clinical studies of ARA 290 in patients with diabetes or sarcoidosis and small fiber neuropathy have shown that IV dosing (2 mg or approximately 30 $\mu g.kg^{-1}$) administered three days weekly was associated with a significant improvement in pain in a majority of patients, consistent with the preclinical observations.[5-7] Repeated intravenous dosing is, however, not feasible for chronic treatment of disease, as the assistance of a health professional is required. A desirable therapeutic paradigm is one of patient self-administration. An additional consideration of chronic dosing is the frequency required. Although 3 doses of ARA 290 appear to be efficacious on the basis of small, proof of concept trials, in practice, such a dosing paradigm is difficult for patients to attain, as frequently doses are inadvertently omitted. Daily dosing as a routine will likely be associated with a better compliance. Therefore, a subcutaneous formulation of ARA 290 is highly desirable.

The results of *in vitro* studies using human endothelial cells have shown that concentrations in the 1-2 nM range provide a maximum effect of signaling pathway phosphorylation.[9] This corresponds well to minimum plasma concentrations of ARA290 required for therapeutic efficacy, which in a rat model corresponds to 30 $\mu g.kg^{-1}$ (intraperitoneally), which gives a C_{max} of approximately 1-2 nM peak plasma concen-tration. PK studies performed on normal volunteers as well as renal insufficiency/renal failure patients have shown that IV doses ranging from 70 µg to 2000 µg result in peak plasma concentrations in excess of 1-2 nM, but have a very short elimination t½ of about 2 min (ARAIM data on file); PK studies comparing the SC and IV routes were carried out in patients with chronic renal failure at a dose of 1400 µg. ARA 290 is partially cleared by the kidney, so the pharmacokinetics are modified compared to normal subjects. Specifically, a higher peak plasma concentration and a doubling of the terminal phase half-life (3.5 min *versus* 1.8 minutes) was observed in renal failure patients.

Subcutaneous dosing studies carried out in the rhesus macaque and rabbit are in agreement with our current findings (Fig. 3; ARAIM, data on file). A prompt rise to a peak at about 4 minutes and then a rapid decay is apparent. Peak plasma concen-trations for 25-30 $\mu g.kg^{-1}$ (corresponding to 8-10 $\mu g.kg^{-1}$ in humans) are in the 1-5 nM range, thus above the expected minimum effective concentration. Increasing the dose by a factor of 4 for the rabbit (equivalent to 8.4 mg in humans) did not result in excessively higher plasma peak concentrations, but rather an increased duration of plasma levels at or slightly above the expected minimum effective concentration (1-2 nM).

Similar to previous studies performed in healthy volunteers, in the current study ARA290 via the IV route had a short terminal elimination half-life. Based on data obtained in all studies to date,

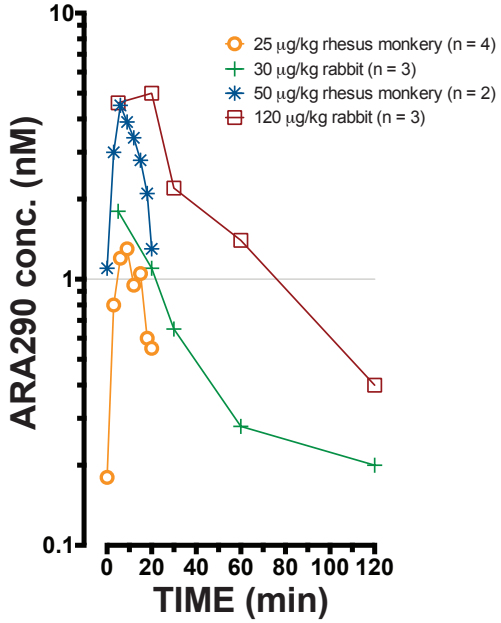

Figure 3. Mean plasma ARA290 concentrations following subcutaneous administration in rhesus monkeys and rabbits (ARAIM, data on file).

the elimination t½ of IV ARA 290 in normal volunteers has ranged from 1.1 to 2.3 minutes (standard fixe dose uncorrected for variations in body mass). Subcutaneous ARA 290 dosing peaked by 12-15 minutes and decreased with a t½ in the range of 17 to 27 minutes. In this study the concentration of ARA 290 in each injection was constant (8 $mg.mL^{-1}$) with variable injection volumes into the lateral thigh. The resulting ratios of the AUCs of the subcutaneous doses were 1:1.3:4.4 for doses of ratios 1:2:3. The observed divergence in the ratio of the observed plasma concentration may be result from, in part, variations of injection volume. Additionally, difference in body mass or other variable not adjusted for in this study could contribute variability in the pharmacokinetic results (Fig. 1). In future studies, dosing base on body weight may result in less variable PK data.

Both for IV and SC administrations the residence time in plasma was short. Still, ARA290 has long-term pharmacodynamic effects in animals and humans. These long-term pharmacodynamic effects suggest that ARA290 initiates a cascade of events involving several steps.[1-3] For neuropathic pain, the site of action of ARA290 is within the central nervous system. Previously we showed that activation of the "Innate Repair Receptor" (a complex of the erythropoietin receptor and the β-common receptor), which is upregulated following tissue injury and inflammation, is a first step that eventually leads to the anti-inflammatory, neuroprotective and healing effects of ARA290.[10] For example, in the rodent, 1-2 weekly doses of ARA290 reduce allodynia following sciatic nerve transection for at least 15 weeks, combined with a reduction of

activated (*ie.* pronociceptive) microglia cells in the spinal cord. Anti-allodynic effects did not occur in rodents that lacked the erythropoietin receptor/β-common receptor complex.

In conclusion, we observed that the administration of SC ARA290 to healthy volunteers is safe. Furthermore, since concentrations following SC injections exceed the minimum effective concentration in the majority of subjects (and in all subjects at 4 and 6 mg SC), SC administration is an effective and reliable method to administer ARA290 to patients with neuropathic pain. The results may be used to design and compare specific dosing paradigms. Future studies should assess whether ARA290's PK obtained in specific patients populations compares to those observed in the current study in healthy volunteers.

References

1. Brines M, Cerami A. **Erythropoietin-mediated tissue protection: Reducing collateral damage from the primary injury response.** J Int Med 2008;262:405-32
2. Collino M, Thiemermann C, Cerami A, Brines M. **Flipping the molecular switch for innate protection and repair of tissues: long-lasting effects of a non-erythropoietic small peptide engineered from erythropoietin.** Pharmacol Ther 2015 [Epub ahead of print]
3. Cerami A. **Failures leading to remarkable discoveries: TNF and its natural inhibitor erythropoietin.** In Yearbook 2012. Department of Anesthesiology at Leiden University Medical Center, Leiden, 2012
4. Corwin HL, Gettinger A, fabian TC, May A, Pearl RG, Herad S, An R, Bowers PJ, Burton P, Klausner MA, Corwin MJ. **Efficacy and safety of epoietin alfa in critically ill patients.** N Eng J Med 2007; 357:965-76
5. Brines M, Dunne A, van Velzen M, Proto P, Ostenson C, Kirk R, Petropoulos I, Rayaz M, Cerami A, Dahan A. **ARA 290, a non-erythropoietic peptide engineered from erythropoietin, improves metabolic control and neuropathic pain symptoms in patients with type 2 diabetes.** Mol Med 2015; 20: 656-66
6. Dahan A, Dunne A, Swartjes M, Proto P, Heij L, Vogels O, Van Velzen M, Sarton E, Niesters M, Tannemaat M, Cerami A, Brines M. **ARA 290 improves symptoms in patients with sarcoidosis-associated small nerve fiber loss and increases corneal nerve fiber density.** Mol Med 2013; 19: 334-45
7. Heij L, Niesters M, Swartjes M, Hoitsma E, Drent M, Dunne A, Grutters JC, Vogels O, Brines M, Cerami A, Dahan A. **Safety and efficacy of ARA290 in sarcoidosis patients with symptoms of small fiber neuropathy: a randomized, double blind, pilot study.** Mol Med 2012; 18: 1430-36
8. Swartjes M, van Velzen M, Niesters M, Aarts L, Brines M, Dunne A, Cerami A, Dahan A. **ARA 290, a peptide derived from the tertiary structure of erythropoietin, produces long-term relief of neuropathic pain coupled with suppression of the spinal microglia response.** Mol Pain 2014; 10: 13
9. Ueba H, Shiomi M, Brines M, Yamin M, Kobayashi T, Ako J, Momomura S, Cerami A, Kawakami M. **Suppression of coronary atherosclerosis by helix B surface Peptide, a nonerythropoietic, tissue-protective compound derived from erythropoietin.** Mol Med 2013;19:195-202
10. Swartjes M, Morariu A, Niesters M, Brines M, Cerami A, Aarts L, Dahan A. **ARA290, a peptide derived from the tertiary structure of erythropoietin, produces long-term relief of neuropathic pain: an experimental study in rats and β-common receptor knockout mice.** Anesthesiology 2011, 115: 1084-92