The Human Chorionic Gonadotropin Derivate EA-230 Modulates the Immune Response and Exerts Renal Protective Properties: Therapeutic Potential in Humans

Roger van Groenendael, MD, MSc [*,†,‡,§] *Remi Beunders, MD, MSc* [*,†] *Matthijs Kox, PhD* [*,†]
Lucas T. van Eijk, MD, PhD [*,†,‡,§] *and Peter Pickkers, MD, PhD* [*,†]

Summary: The extent of the systemic inflammatory response following infectious or noninfectious insults is related to impaired patient outcome. Pregnancy is associated with immunotolerance and an increased glomerular filtration rate. EA-230 is a newly developed synthetic linear tetrapeptide derived from the "pregnancy hormone" human chorionic gonadotropin. In this review, we describe the immunomodulatory and renoprotective properties of EA-230 in preclinical animal models, phase 1 studies in humans and phase 2a studies performed during human experimental endotoxemia. In addition, details pertaining to the design of a recently completed phase 2b study in 180 patients who underwent cardiac surgery to investigate the safety and immunomodulatory and renoprotective properties of EA-230 are discussed.
Semin Nephrol 39:496−504 © 2019 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license. (http://creativecommons.org/licenses/by-nc-nd/4.0/)
Keywords: Immunomodulation, pregnancy, renal, inflammation, cytokines

S ystemic activation of the immune system in response to invading pathogens and/or tissue injury is essential for the clearance of pathogens and initiation of tissue repair mechanisms. However, several infectious (eg, sepsis) and noninfectious conditions (eg, trauma and major surgical interventions) may cause an inappropriate systemic activation of the immune system (eg, too pronounced or protracted).[1,2] This dysregulated inflammatory response often results in tissue damage, failure of one or more organ systems, and high mortality.[3,4] An example is the development of acute kidney injury, one of the first and most frequent manifestations of inflammation-induced organ failure, which occurs in over half of the critically ill patients and in up to 30% of patients undergoing elective cardiac

surgery.[5-8] Furthermore, acute kidney injury has been associated with impaired clinical outcome; even a minimal increase in serum creatinine has been shown to correlate with impaired survival in patients undergoing elective cardiac surgery.[6,9]

Although the consequences of a dysregulated immune response have a large impact on patients, current therapeutic strategies are still confined to supportive treatment. To date, all immunomodulatory interventions have failed to demonstrate clear beneficial effects in terms of preventing organ injury.[10-12] For example, large studies investigating the use of high dose corticosteroids revealed no improvements in overall outcome of patients with systemic inflammation following cardiac surgery.[13,14] However, nonspecific anti-inflammatory effects and/or the broad spectrum of adverse reactions may have contributed to the absence of overall beneficial effects. Therefore, novel pharmaceutical strategies that aim to more safely and more specifically target the dysregulated inflammatory response to prevent organ failure are highly warranted.

The unique immunologic adaptation of the maternal immune system during pregnancy has inspired research into new immunomodulatory strategies. In pregnancy, a remarkable symbiosis of two major histocompatibility complex incompatible individuals enables the semi-allogeneic fetus to be tolerated by the maternal immune system, whereas the ability to combat invading pathogens remains unaffected.[15] Strikingly, pregnancy-related rearrangements of the maternal immune system are associated with improvement of several autoimmune diseases, including rheumatoid arthritis, psoriasis, and multiple sclerosis, while relapse of these diseases usually occurs following delivery.[16-19] Nevertheless, the immune system of pregnant women is fully capable to combat pathogens. As such, the

*Department of Intensive Care Medicine, Radboud University Medical Center, Nijmegen, the Netherlands

†Radboud Institute for Molecular Life Sciences, Radboud Center for Infectious Diseases (RCI), Nijmegen, the Netherlands

‡Department of Anesthesiology, Pain and Palliative Medicine, Radboud University Medical Center, Nijmegen, the Netherlands

§Radboud Institute of Molecular Life Sciences (RIMLS), Nijmegen, the Netherlands

Drs van Groenendael and Beunders contributed equally to this work.

https://doi.org/10.1016/j.semnephrol.2019.06.009

pregnancy-induced immunologic adaptation is characterized by suppression of detrimental (auto-)immunologic processes to mother and fetus, whereas beneficial immune processes such as host defence remain unaffected. An immunomodulatory pharmacological compound that could mimic these favorable features might be of benefit for patients suffering from overzealous systemic inflammation.

Several possible underlying mechanisms via which the maternal immune system adapts during pregnancy have been postulated and investigated. Although there is no clear consensus on this topic, changes in the hormonal milieu appear to play a central role.[20] Of particular interest is the release of the "pregnancy hormone" human chorionic gonadotropin (hCG). This hormone is produced throughout pregnancy, already present at a very early gestational stage, and may increase up to 50.000-fold at the end of the first trimester. Several studies have reported hCG-mediated immunomodulatory effects.[21-26] An important role in these effects are liked to immunologically active oligopeptides originating from the β-loop of hCG, which are abundantly present throughout pregnancy.[27] As such, various β-hCG-derived oligopeptides have been studied, and were demonstrated to exert immunomodulatory effects, limit organ failure, and decrease mortality in several animal models of systemic inflammation.[27-33] Of the assessed oligopeptides, the linear tetrapeptide Alanine-Glutamine-Glycine-Valine (later named EA-230) showed the most promising effects. EA-230 strongly attenuated inflammation and substantially reduced organ failure and mortality in models of hemorrhagic[28] and endotoxemia-induced shock.[29] Furthermore, treatment with EA-230 exerted kidney-protective effects in ischemia-reperfusion (IR) and kidney transplant models.[31,34] These findings may be related to the fact that pregnancy profoundly impacts renal function by increasing renal flow and the glomerular filtration rate (GFR).[35]

This review describes the development of EA-230, ranging from experimental dose finding, pharmacokinetic profiling and efficacy studies in animals, to the clinical development of EA-230 with first-in-man and first-in-patient trials. These studies in healthy volunteers and patients include dose finding, pharmacokinetics, safety and tolerability, and efficacy assessments.

PRECLINICAL PHARMACOKINETIC STUDIES

Several animal studies have evaluated the pharmacokinetics of EA-230, of which a detailed description is described in supplementary material elsewhere.[36] Briefly, the distribution and metabolism of EA-230 was examined following a single intravenous (IV) injection of 50 mg/kg radiolabeled EA-230 (Ala-Gln-[1-14C]Gly-Val) in mice. EA-230 was shown to be rapidly removed from the blood, as metabolite profiles in blood plasma and urine obtained 10 minutes after administration

revealed no intact EA-230 peptides. This indicates that systemic EA-230 clearance is high (eg, exceeding liver blood flow). In rats, IV administration of EA-230 (50, 100, or 200 mg/kg/day) for 5 days, again revealed rapid (within minutes) elimination of EA-230. The volume of distribution of EA-230 was high (range, 2-50 L/g), systemic clearance was swift (range, 14-43 L/g/h), and as a consequence, elimination half-life was short (<30 minutes). Furthermore there was no evidence of accumulation on repeated dosing. Finally, a 2-week repeated dose toxicity study with EA-230 administrations up to 200 mg/kg/day was performed in dogs. Besides the maximum tolerated dose of 200mg/kg/day that was established, a pharmacokinetic profile similarly to the studies in mice and rats was observed: rapid elimination of EA-230 from the circulation, a large volume of distribution and a short elimination half-live (< 50 minutes). Furthermore, more than dose-proportional increases of plasma concentrations over time were observed.

PRECLINICAL EFFICACY STUDIES

EA-230 has been evaluated in multiple animal models of renal injury. Among various oligopeptides tested in a murine model of renal IR damage (25 minutes of renal pedicle clamping), EA-230 (5 mg/kg, administered 1 minute before clamping and 1 minute before reperfusion) showed high efficacy in preservation of renal function and prevention of mortality. No animals died within 72 hours after clamping versus 50% mortality in the control group.[31] Improved survival and renal preservation were confirmed in subsequent experiments using ascending dosages of EA-230 (0.3-30 mg/kg).[31] Furthermore, IR-induced systemic inflammation was significantly attenuated, which was reflected by attenuation of circulating levels of cytokines interleukin 6 (IL-6), interleukin 10 (IL-10), interferon gamma, and tumor necrosis factor alpha (TNF-α), reduced neutrophil influx into renal tissue, and a decreased expression of the leukocyte adhesion molecule E-selectin.[31] Notably, EA-230 improved IR-related survival even when administered late: initiating therapy (30 mg/kg EA-230 twice daily for 4 consecutive days) at 24 hours after 35 minutes of renal clamping, resulted in 70% survival after 3 days versus no survival in the control group. The deterioration of renal function was prevented and the increase in serum concentrations of the cytokine IL-6 and monocyte chemoattractant protein-1 was attenuated. A more elaborate renal IR study (35 minutes of pedicle clamping) using ascending dosages of 20-50 mg/kg twice daily for 4 consecutive days confirmed the previously observed improved survival after treatment with EA-230. Interestingly, this study also showed EA-230 to mitigate acute tubular injury, to enhance tubular epithelial cell proliferation, and to attenuate the upregulation of markers related to postischemic fibrosis.[34] EA-230 improved renal blood flow and the

GFR at 28 hours after IR damage.[34] A different murine model of renal injury was used in which an allogeneic kidney was transplanted, recipients treated with EA-230 (50 mg/kg) showed markedly improved survival and preservation of renal function, accompanied by enhanced allograft survival and a decrease in acute tubular necrosis compared with the control group.[34]

Parallel to these IR studies, preclinical work has focused on the immunomodulatory properties of EA-230 in models of systemic inflammation. In a rat model, 60 minutes of hemorrhagic shock was used to induce pronounced systemic inflammation with subsequent hepatic failure.[28] Rats treated with a single bolus administration of EA-230 (5mg/kg 30 minutes after the initiation of shock) displayed an attenuated systemic inflammatory response compared with saline-treated animals. At organ level, EA-230 administration resulted in reduced hepatic mRNA expression of TNF-α, IL-6, and E-selectin.[28] Furthermore, a nonsignificant trend toward preservation of hepatic function and prevention of neutrophil infiltration into the liver was observed.[28] Likewise, in mice infected with *Listeria Monocytogenes*, EA-230 (50 mg/kg every 24 hours for 3 days) attenuated systemic cytokine levels and reduced tissue influx of neutrophils.[32] Immunomodulatory effects of EA-230 have also been explored in murine models of sepsis. These include administration of endotoxin (lipopolysaccharide [LPS]), a specific Toll-like receptor-4 (TLR-4) ligand. In this model, treatment with EA-230 (both at 2 and 24 hours post-LPS administration) resulted in a significant decrease in sickness scores and attenuation of splenocyte proliferation compared with placebo-treated animals.[29] Furthermore, EA-230 completely prevented endotoxemia-induced mortality 84 hours after LPS administration (100% versus 0% survival).[29] As LPS models solely induce TLR-4-mediated inflammation, cecal ligation and puncture (CLP) model has been used to examine the effects of EA-230 on polymicrobial sepsis. CLP more accurately reflects polymicrobial sepsis and is therefore considered the gold standard animal sepsis model. After CLP, mice treated with escalating dosages of EA-230 (5-50 mg/kg, 4 and 16 hours after CLP and thereafter daily over 4 days) showed improved survival (50% in EA-230 50 mg/kg treated animals versus 0% in placebo animals), attenuated cytokine responses, and reduced influx of leukocytes into the peritoneal space. Furthermore, renal function was preserved in the EA-230 group, illustrated by an attenuated increase of serum creatinine levels and a less pronounced decline in GFR and renal blood flow. Of interest and relevant to the clinical situation: the latter results were confirmed in experiments in which EA-230 was administered late and only twice (4 and 16 hours following CLP induction).

Taken together, EA-230 has been demonstrated to attenuate the release of systemic inflammatory mediators, to reduce tissue leukocyte infiltration, and to mitigate organ damage (particularly renal failure) in various animal models of renal injury, as well as during sterile and infectious systemic inflammation. Because of these promising results, EA-230 has been advanced into clinical development.

CLINICAL STUDIES

Several human studies in both healthy subjects and patients have been conducted. These include three phase 1 studies in healthy volunteers to investigate safety, tolerability and pharmacokinetics of EA-230. To investigate the immunomodulatory potential in humans, two phase 2a studies were performed in healthy volunteers undergoing experimental human endotoxemia, a standardized, controlled and reproducible model of systemic inflammation. Finally, a phase 2b study on safety, tolerability and efficacy of EA-230 in patients undergoing cardiac surgery is currently being conducted. These studies are discussed in the current paragraph and an overview of all clinical studies performed to date is provided in Table 1.

Phase 1 Studies

The first-in-man studies to investigate the pharmacokinetics, safety and tolerability of EA-230 were conducted using several dosing schemes: escalating single and multiple dosages and escalating single continuous dosages.[36]

A single dosage study was conducted in 32 healthy male volunteers. Subjects were assigned to one of four dosage groups: 1, 3, 10, and 30 mg/kg. Each group consisted of 8 subjects (EA-230: n = 6, placebo: n = 2), randomized in a double-blind fashion. Subjects were hospitalized for 24 hours and followed-up for a period of 14 days in which vitals signs were monitored and blood samples were taken regularly.

A 3-day multiple dosage study was conducted in 24 healthy male volunteers using escalating dosage groups of 10, 20, and 30 mg/kg. Each dosage group consisted of 8 subjects (EA-230: n = 6, placebo: n = 2), randomly assigned in a double-blinded fashion. 3 boluses were administered in 10 minutes with intervals of 8 hours for 3 consecutive days (9 dosages in total). Subjects were hospitalized and monitored until 12 hours after the last dosage administration and returned after 14 days for follow-up.

Finally, a continuous dosage study was conducted in 24 healthy male and female subjects who were assigned to one of three dosage groups: 15, 45, or 90 mg/kg with an IV infusion duration of 2 hours. Subjects were randomly assigned to receive EA-230 (n = 6) or placebo (n = 2) in a double-blinded fashion. Subjects were monitored until 6 hours after cessation of study drug administration and followed-up for a period of 14 days.

Table 1. Overview of Study Procedures in Clinical Studies with EA-230

Study	Year	Dosage of EA-230	Methods of Administration	Frequency of Administration	PK Sampling (in minutes)	Endpoints	Follow-Up
Phase 1 single dose	2005	1, 3, 10, or 30 mg/kg	Bolus	Once	−1, 5, 10, 15, 20, 30, 45, 60, 120, and 240 minutes after bolus	Safety, PK	7 days
Phase 1 multiple dose	2006	10, 20, or 30 mg/kg	3 Bolus	Trice for 3 consecutive days	−1, 5, 10, 15, 20, 30, 45, 60, 120, and 240 minutes after first bolus on every day	Safety, PK	14 days
Phase 1 continuous dose	2016	30, 45, or 90 mg/kg/hr	Continuous	Once for 2 hours	−1, 15, 30, 60, 90, 120, 180, 240, 360, 480, and 1440 minutes after start of infusion	Safety, PK	14 days
Phase 2a endotoxemia 1	2006	10 mg/kg	Bolus	Once, simultaneous with LPS administration	−1, 5, 10, 15, 20, 30, 45, 60, 120, and 240 minutes after bolus	Safety, PK immunology	7 days
Phase 2a endotoxemia 2	2016	30, 45, or 90 mg/kg/hr	Continuous	Once, for 2 hours, starting simultaneous with LPS administration	−1, 15, 30, 60, 90, 120, 180, 240, 360, 480, and 1,440 minutes after start of infusion	Safety, PK immunology, renal function	14 days
Phase 2b patient trial	2016-2018	90 mg/kg/hr	Continuous	Once, for a maximum of 4 hours (depending on surgery duration), starting simultaneous with surgeon's first incision	Before start and stop of CBP and −1, 2, 5, 10, 20, 30, 60, 120, 240, and 360 minutes after cessation of infusion	Safety, PK immunology, renal function, clinical endpoints	90 days

Abbreviations: CBP, cardiopulmonary bypass; LPS, lipopolysaccharide; PK, pharmacokinetics.

In the aforedescribed first-in-man studies, the preclinical pharmacokinetic profile of EA-230 was reconfirmed. The pharmacokinetical profile of EA-230 in humans is characterized by a large volume of distribution, very rapid clearance, and a short half-life of less than 15 minutes. This very rapid plasma clearance exceeds both the renal and portal flow, implying proteolysis, hydrolysis, tissue/protein binding, and/or cellular uptake of the peptide. Furthermore, conform earlier preclinical work, subjects in the highest dosage group showed lasma concentration higher than proportional to the administered dosage. This may suggest dose-dependent metabolism, elimination, and/or a shift in distribution.

Importantly, no safety issues emerged across all dosage groups as no serious adverse events (SAEs) were reported. Overall, all AEs were transient and mild and no dose-dependent increase in the number or intensity of AEs was observed. Moreover as 50% of the AEs were observed in placebo-treated subjects, the likelihood that AEs were related to the compound was therefore considered low. Notably, in the continuous infusion study, with accordingly the highest plasma concentrations and the longest exposure, no possibly related AEs were reported. No effects of EA-230 on vital parameters were found.

In conclusion, all available data on pharmacokinetics, safety and tolerability in a total of 80 healthy volunteers show a pharmacokinetic profile of EA-230 that is in line with the results of preclinical studies; a short half life, large volume of distribution, no accumulation after multiple dosing and a more-than-proportional increase in plasma concentration. Furthermore these phase 1 studies reveal EA-230 to be well tolerated without the occurence of any safety issues.

Phase 2a Experimental Human Endotoxemia Studies

The encouraging results from phase 1 studies prompted the design of studies to assess immunomodulatory effects of EA-230 in humans. These studies used experimental human endotoxemia as a model for systemic inflammation. In this model, endotoxemia is induced in healthy volunteers by administering purified *Escherichia coli*-derived LPS, which elicits a systemic inflammatory response. LPS activates the immune system only through ligation of TLR-4, which is in contrast to the plethora of pathways that are activated in patients with systemic inflammation related conditions such as sepsis. However, this temporary inflammatory response captures several hallmarks of systemic inflammation in patients. The LPS-induced inflammatory response shows considerable overlap in terms of cytokine production and leukocyte activation. Moreover, LPS administration induces comparable but less pronounced clinical symptoms such as an increase in heart rate, a decrease in blood pressure, an increase in body temperature and the development of

flu-like symptoms.[37] This LPS induced inflammatory response is short-lived, relatively mild, not associated with clinically relevant organ dysfunction and well-tolerated by numerous subjects that underwent experimental human endotoxemia worldwide.[37] Furthermore, these endotoxemia studies can be conducted in a highly standardized manner, using a homogenous population that can be intensively monitored. Experimental human endotoxemia therefore represents a very suitable model to assess the immunmodulaory behavior of a drug such as EA-230.

In the first study that evaluated safety and the immunomodulatory effects of EA-230 during human endotoxemia, 12 subjects were enrolled who all received an IV bolus administration of 4 ng/kg LPS. After randomization, eight subjects were administered 10 mg/kg EA-230 (IV bolus), whereas four received placebo. Primarily, administration of EA-230 was well tolerated in this study without any safety concerns.

Furthermore, EA-230 significantly attenuated the LPS-induced increase in plasma concentrations of proinflammatory cytokines IL-6 and IL-8 levels over time compared with the placebo group ($P = .04$ and $P = .004$, respectively, Fig. 1), with a decrease in area under the time-concentration curve of 46% for IL-6 and 36% for IL-8. Also, a significantly attenuated increase in body temperature was observed in the EA-230 group 4 hours after LPS administration (temperature change [minimal-maximal] in the EA-230 group of +0.6°C [0-2] versus +1.6°C [1-2] in the placebo group, $P = .02$). There were no between-group differences in total leukocyte counts or subsets over time. The observerd pharmacokinetic profile of EA-230 was similar to those observed in phase 1 studies.
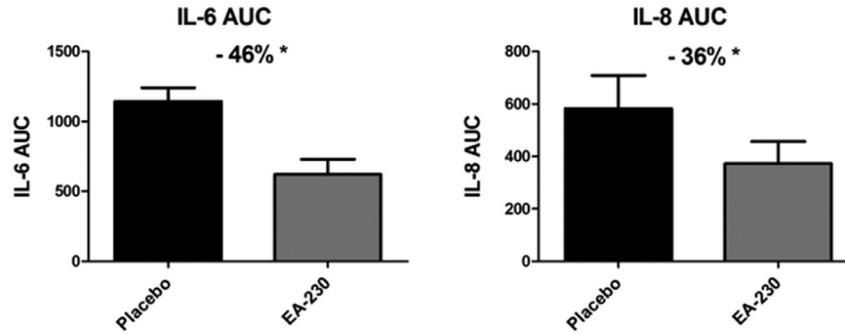
In a slightly different setup, the effect of continuous administration of EA-230 on LPS-induced systemic inflammation was investigated in 36 healthy volunteers.[38] Briefly, in this study EA-230 was administered continuously considering its short half-life. Subjects were randomly assigned to one of three dosage groups: 15, 45, or 90 mg/kg/h. In each dosage group, eight subjects received EA-230 IV and four subjects received placebo. LPS administration (IV bolus of 2 ng/kg) was directly followed by continuous study drug infusion for a period of 2 hours. The primary endpoint was safety and tolerability, whereas the primary efficacy endpoint was EA-230-induced modulation of the inflammatory response. In total, 19 mild and transient AEs were reported that were equally distributed over the treatment and placebo groups. AEs were assessed as having no or an unlikely relation with the study drug. No SAEs were reported. The highest dosage of EA-230 (90mg/kg/h) exerted significant effects on plasma levels of several proinflammatory cytokines and chemokines, reducing the area under the time-concentration curve of IL-6 by 48%, IL-8 by 28%, and monocyte chemoattractant protein-1 by 28% (an overview of cytokine data is provided in Fig. 2). Although levels of proinflammatory cytokines were attenuated in subjects who received EA-230, plasma concentrations of the anti-inflammatory cytokine IL-10 were not significantly affected. The LPS-induced increase in body temperature was significantly less pronounced in the EA-230 90mg/kg/h group compared with placebo (maximum increase of 1.3 ± 0.2°C versus 1.8 ± 0.1°C in placebo-treated subjects, $P = .003$). Also flu-like symptoms (headache, nausea, shivering, muscle and back pain, and vomiting scored per item on a 0-5 Likert scale) were significantly less pronounced in this group (maximum score of 4.0 ± 1.2 points versus 7.4 ± 1.0 points in placebo-treated subjects, $P < .0001$). The LPS-induced increase in leukocyte counts was enhanced in the EA-230 group compared with the placebo group,
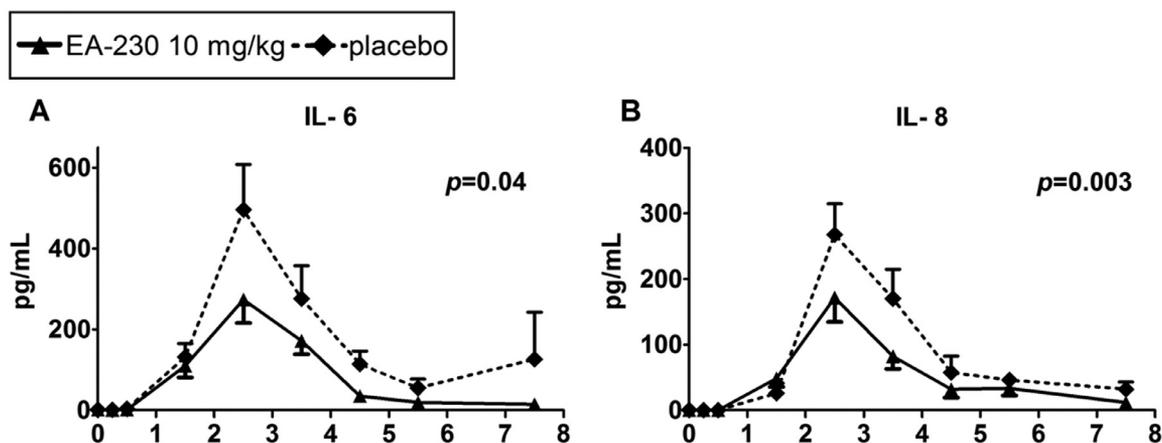
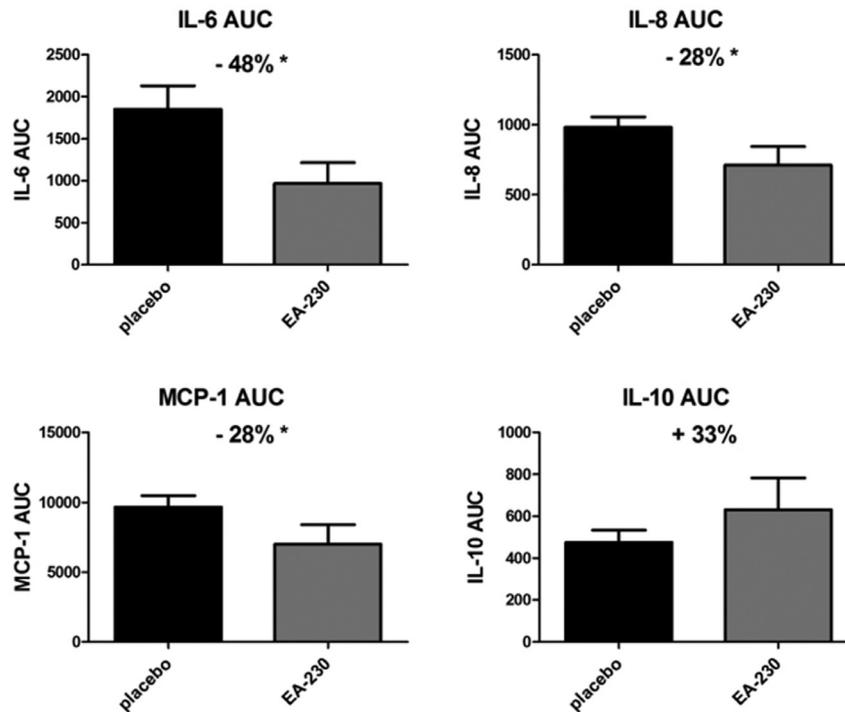**Figure 1.** Plasma levels of cytokines during experimental human endotoxemia. (A) IL-6 and (B) IL-8. Data are represented as means with standard error of the mean. N = 8 in the EA-230 group, n = 4 in the placebo group. Lipopolysaccharide and study drug or placebo were infused on T = 0. *P* values between groups were calculated using repeated measures 2-way analysis of variance (interaction term). Abbreviation: IL, interleukin.

Phase 2a - 1

IL-6 AUC

- 46% *

IL-8 AUC

- 36% *

Phase 2a - 2

IL-6 AUC

- 48% *

IL-8 AUC

- 28% *

MCP-1 AUC

- 28% *

IL-10 AUC

+ 33%

Figure 2. Area under the time-concentration curve of both phase 2a experimental human endotoxemia studies. Depicted is the mean ± standard error of the mean and the difference in area under the time-concentration curve as a percentage. *Tested statistically significant using repeated measures 2-way analysis of variance (interaction term).

largely accounted for by higher numbers of neutrophils and monocytes. Interestingly, pharmacokinetic analysis showed that plasma concentrations were approximately two-fold higher compared with the phase 1 study using the same dosages conducted in the same institute with identical analysis methods. These data suggest that systemic inflammation may affect the volume of distribution and/or the clearance of EA-230, for which the underlying mechanisms remain elusive.

Human experimental endotoxemia studies showed EA-230 to be safe and well-tolerated under inflammatory conditions in a total of 48 healthy volunteers. The immunomodulatory effects of EA-230 were confirmed in these studies, illustrated by attenuation of circulating plasma

levels of proinflammatory cytokines and chemokines a significantly less pronounced increase in body temperature, and less development of flu-like symptoms in EA-230 treated subject compared with placebo. Furthermore, pharmacokinetic results were similar to the phase 1 studies, except for the finding that plasma concentrations of EA-230 were approximately two fold higher under LPS induced inflammatory conditions.

Phase 2b Study

The next step in the clinical development of EA-230 is represented by a currently ongoing patient trial, of which the design is described in detail elsewhere.[39] Briefly, this

study is performed in patients undergoing elective coronary artery bypass grafting with or without valve surgery and aims to investigate both safety and efficacy in a large and relatively homogeneous group of patients suffering from systemic inflammation. There are several charesteristics to a cardiac surgical procedure that makes it eligible to study the immunomodulatory effects of EA-230. First, cardiac surgery is known to elicit a systemic inflammatory response.[40,41] A combination of surgical procedures contribute to this inflammatory response, such as sternotomy, the use of a cardiopulmonary bypass, IR damage following aortic clamping and endotoxemia owing to increased gut permeability.[40-42] This systemic inflammatory response is predictable and interpatient differences are relatively limited. Second, these patients have a considerable likelihood of developing renal injury due to multiple causes, including hemodynamic changes, direct IR injury to the kidneys following aortic cross clamping, systemic inflammation, and hypoxia.[5] This is of interest in view of the previously described putative renal protective effects of EA-230. Finally, as this cardiac surgical procedures are elective and highly standardized, this group of patients conveys the opportunity to uniformly time the administration of the investigational compound relative to the inflammatory and kidney-damaging insults.

In the study, 180 patients were randomized to continuous IV administration of EA-230 (90mg/kg/h) or placebo (1:1 ratio) in a double-blinded fashion. Infusion of EA-230 started at surgical incision and stopped after cessation of the cardiopulmonary bypass. To assess safety and cytokine responses, frequent blood samples were collected untill the morning after surgery. Patients were followed-up for 90 days after surfery to monitor for adverse events. The study is currently completed and data analysis is in progress (Clinicaltrials.gov; NCT03145220; https://clinicaltrials.gov/ct2/show/NCT03145220). The primary objectives of this first-in-patient study are to study safety and tolerability of EA-230 in patients, and to demonstrate the immunomodulatory effects of EA-230. Safety and tolerability are the primary focus of this study, defined as the incidence of (S)AEs and changes in vital signs and routine laboratory parameters between active and placebo treatment. The primary efficacy endpoint is IL-6 plasma concentration over time. The key-secondary endpoint is the effect of EA-230 on renal function (the "true GFR" measured by iohexol plasma clearance[43]). Additional efficacy endpoints include the effects of EA-230 on plasma concentrations of other cytokines and chemokines (among which are IL-8, IL-10, and TNF-α), hemodynamic data, and several tubular injury markers (including urinary neutrophil gelatinase-associated lipocalin, L-type fatty acid-binding protein, N-acetyl-β-D-glucosaminidase, and interleukin-18). Several clinical endpoints are included as well, such as effects of EA-230 on vasopressor requirement, fluid balance, changes in sequential organ failure

assessment score, intensive care unit length of stay, hospital length of stay, and mortality. The study employed an adaptive design: after enrolment of 90 patients a recalculation of the sample size using the obtained IL-6 data were performed.

Several potential pitfalls related to this phase 2b trial may be of importance. First, the inflammatory response observed following cardiac surgery is not the same as that elicited by IV LPS administration due to profound differences between the inciting events. This discrepancy is for instance reflected in plasma cytokine concentrations: following coronary artery bypass grafting, cytokine concentrations will reach peak concentrations hours later, will be less pronounced, and will remain elevated for a longer period of time compared with the cytokine concentrations following LPS administration.[44] This may complicate optimal timing of the intervention as EA-230 infusion will most probably be stopped prior to the expected peak inflammatory response in patients undergoing cardiac surgery, whereas EA-230 infusion was ongoing during the peak inflammatory response following LPS administration. Furthermore, EA-230 has an extraordinary large volume of distribution and is rapidly eliminated from the circulation. This may result in a very short duration of action. Combined with the fact that the mode of action of EA-230 remains largely elusive, this may hamper accurate timing and duration of therapy. In this respect, it is noteworthy that in preclinical studies, EA-230 has shown comparable efficacy on both early (just prior to or shortly after the inflammatory insult) and delayed administration (up to 24 hours after the inflammatory insult).[29,32,34]

The results of the phase 2b patient study are expected in 2019. A favorable outcome of the trial may instigate the design of a large multicenter phase 3 trial. It would also pave the way for exploration of EA-230s applicability for other conditions in which systemic inflammation and renal damage play a central role, such as in patients with sepsis and trauma.

CONCLUSIONS

A too pronounced or protracted systemic inflammatory response may induce tissue damage and failure of organs, of which the kidneys are most likely affected. This phenomenon is observed in noninfectious as well as infectious causes of systemic inflammation. To date, immunomodulatory therapies have not led to improved outcome, and supportive treatment is therefore the only strategy in current clinical use. The β-hCG-derived tetrapeptide EA-230 has been shown to attenuate systemic inflammation, preserve renal function, and increase survival in various preclinical studies. Furthermore, EA-230 was found to be safe and well-tolerated in five independent human studies using different dosages and administration strategies. EA-230s immunomodulatory effects were subsequently confirmed in humans in two

experimental human endotoxemia studies. The results of a large double blind placebo-controlled randomized phase 2b trial in patients undergoing cardiac surgery are anticipated.

REFERENCES

1. Balk RA. Systemic inflammatory response syndrome (SIRS): where did it come from and is it still relevant today? Virulence. 2014;5:20-6.
2. Singer M, Deutschman CS, Seymour CW, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA. 2016;315:801-10.
3. Vincent JL, Sakr Y, Sprung CL, et al. Sepsis in European intensive care units: results of the SOAP study. Crit Care Med. 2006;34:344-53.
4. Dulhunty JM, Lipman J, Finfer S. Does severe non-infectious SIRS differ from severe sepsis? Results from a multi-centre Australian and New Zealand intensive care unit study. Intensive Care Med. 2008;34:1654-61.
5. Thiele RH, Isbell JM, Rosner MH. AKI associated with cardiac surgery. Clin J Am Soc Nephrol. 2015;10:500-14.
6. Hoste EA, Bagshaw SM, Bellomo R, et al. Epidemiology of acute kidney injury in critically ill patients: the multinational AKI-EPI study. Intensive Care Med. 2015;41:1411-23.
7. Rabb H, Griffin MD, McKay DB, et al. Inflammation in AKI: current understanding, key questions, and knowledge gaps. J Am Soc Nephrol. 2016;27:371-9.
8. Payen D, Lukaszewicz AC, Legrand M, et al. A multicentre study of acute kidney injury in severe sepsis and septic shock: association with inflammatory phenotype and HLA genotype. PloS One. 2012;7:e35838.
9. Liotta M, Olsson D, Sartipy U, Holzmann MJ. Minimal changes in postoperative creatinine values and early and late mortality and cardiovascular events after coronary artery bypass grafting. Am J Cardiol. 2014;113:70-5.
10. Cohen J, Vincent JL, Adhikari NK, et al. Sepsis: a roadmap for future research. Lancet Infect Dis.. 2015;15:581-614.
11. Lord JM, Midwinter MJ, Chen YF, et al. The systemic immune response to trauma: an overview of pathophysiology and treatment. Lancet. 2014;384:1455-65.
12. Landis RC, Brown JR, Fitzgerald D, et al. Attenuating the systemic inflammatory response to adult cardiopulmonary bypass: a critical review of the evidence base. J Extra Corpor Technol. 2014;46:197-211.
13. Whitlock RP, Devereaux PJ, Teoh KH, et al. Methylprednisolone in patients undergoing cardiopulmonary bypass (SIRS): a randomised, double-blind, placebo-controlled trial. Lancet. 2015; 386:1243-53.
14. Dieleman JM, Nierich AP, Rosseel PM, et al. Intraoperative high-dose dexamethasone for cardiac surgery: a randomized controlled trial. JAMA. 2012;308:1761-7.
15. Munoz-Suano A, Hamilton AB, Betz AG. Gimme shelter: the immune system during pregnancy. Immunol Rev. 2011;241:20-38.
16. Kaaja RJ, Greer IA. Manifestations of chronic disease during pregnancy. JAMA. 2005;294:2751-7.
17. Vukusic S, Hutchinson M, Hours M, et al. Pregnancy and multiple sclerosis (the PRIMS study): clinical predictors of post-partum relapse. Brain. 2004;127:1353-60.
18. Hazes JM, Coulie PG, Geenen V, et al. Rheumatoid arthritis and pregnancy: evolution of disease activity and pathophysiological considerations for drug use. Rheumatology (Oxford). 2011; 50:1955-68.
19. de Man YA, Dolhain RJ, van de Geijn FE, Willemsen SP, Hazes JM. Disease activity of rheumatoid arthritis during pregnancy: results from a nationwide prospective study. Arthritis Rheum. 2008;59:1241-8.
20. Robinson DP, Klein SL. Pregnancy and pregnancy-associated hormones alter immune responses and disease pathogenesis. Horm Behav. 2012;62:263-71.
21. Tsampalas M, Gridelet V, Berndt S, Foidart J-M, Geenen V, d'Hauterive SP. Human chorionic gonadotropin: a hormone with immunological and angiogenic properties. J Reprod Immunol. 2010;85:93-8.
22. Bansal AS, Bora SA, Saso S, Smith JR, Johnson MR, Thum MY. Mechanism of human chorionic gonadotrophin-mediated immunomodulation in pregnancy. Expert Rev Clin Immunol. 2012;8:747-53.
23. Theofanakis C, Drakakis P, Besharat A, Loutradis D. Human chorionic gonadotropin: the pregnancy hormone and more. Int J Mol Sci. 2017;18:1059.
24. Wan H, Versnel MA, Leijten LM, et al. Chorionic gonadotropin induces dendritic cells to express a tolerogenic phenotype. J Leukoc Biol. 2008;83:894-901.
25. Wan H, Versnel MA, Cheung WY, et al. Chorionic gonadotropin can enhance innate immunity by stimulating macrophage function. J Leukoc Biol. 2007;82:926-33.
26. Uzumcu M, Coskun S, Jaroudi K, Hollanders JM. Effect of human chorionic gonadotropin on cytokine production from human endometrial cells in vitro. Am J Reprod Immunol. 1998;40:83-8.
27. Khan NA, Benner R. Human chorionic gonadotropin: a model molecule for oligopeptide-based drug discovery. Endocr Metab Immune Disord Drug Targets. 2011;11:32-53.
28. van den Berg HR, Khan NA, van der Zee M, et al. Synthetic oligopeptides related to the [beta]-subunit of human chorionic gonadotropin attenuate inflammation and liver damage after (trauma) hemorrhagic shock and resuscitation. Shock. 2009;31:285-91.
29. Khan NA, Vierboom MP, van Holten-Neelen C, et al. Mitigation of septic shock in mice and rhesus monkeys by human chorionic gonadotrophin-related oligopeptides. Clin Exp Immunol. 2010; 160:466-78.
30. van den Berg JW, Dik WA, van der Zee M, et al. The beta-human chorionic gonadotropin-related peptide LQGV reduces mortality and inflammation in a murine polymicrobial sepsis model. Crit Care Med. 2011;39:126-34.
31. Khan NA, Susa D, van den Berg JW, et al. Amelioration of renal ischaemia-reperfusion injury by synthetic oligopeptides related to human chorionic gonadotropin. Nephrol Dial Transplant. 2009; 24:2701-8.
32. van der Zee M, Dik WA, Kap YS, et al. Synthetic human chorionic gonadotropin-related oligopeptides impair early innate immune responses to *Listeria monocytogenes* in mice. J Infect Dis. 2010;201:1072-80.
33. Zamorina SA, Shirshev SV. Oligopeptides of chorionic gonadotropin beta-subunit in induction of T cell differentiation into Treg and Th17. Bull Exp Biol Med. 2015;160:72-5.
34. Gueler F, Shushakova N, Mengel M, et al. A novel therapy to attenuate acute kidney injury and ischemic allograft damage after allogenic kidney transplantation in mice. PloS One. 2015;10: e0115709.
35. Odutayo A, Hladunewich M. Obstetric nephrology: renal hemodynamic and metabolic physiology in normal pregnancy. Clin J Am Soc Nephrol. 2012;7:2073-80.
36. van Groenendael R, Aarnoutse R, Kox M, van Eijk L, Pickkers P. Pharmacokinetics, safety and tolerability of the novel β-hCG derived immunomodulatory compound, EA-230. Br J Clin Pharmacol. 2019;85:1572-84.

37. van Lier D, Geven C, Leijte GP, Pickkers P. Experimental human endotoxemia as a model of systemic inflammation. Biochimie. 2019;159:99-106.

38. van Groenendael R, Kox M, Leijte G, et al. A randomized double-blind, placebo-controlled clinical phase IIa trial on safety, immunomodulatory effects and pharmacokinetics of EA-230 during experimental human endotoxaemia. Br J Clin Pharmacol. 2019;85:1559-71.

39. van Groenendael R, Beunders R, Hofland J, et al. The safety, tolerability, and effects on the systemic inflammatory response and renal function of the human chorionic gonadotropin hormone-derivative EA-230 following on-pump cardiac surgery (The EASI Study): protocol for a randomized, double-blind, placebo-controlled phase 2 study. JMIR Res Protoc. 2019;8:e11441.

40. Rossaint J, Berger C, Van Aken H, et al. Cardiopulmonary bypass during cardiac surgery modulates systemic inflammation by affecting different steps of the leukocyte recruitment cascade. PloS One. 2012;7:e45738.

41. Warren OJ, Smith AJ, Alexiou C, et al. The inflammatory response to cardiopulmonary bypass: part 1—mechanisms of pathogenesis. J Cardiothorac Vasc Anesth. 2009;23:223-31.

42. Day JR, Taylor KM. The systemic inflammatory response syndrome and cardiopulmonary bypass. Int J Surg. 2005;3:129-40.

43. Delanaye P, Ebert N, Melsom T, et al. Iohexol plasma clearance for measuring glomerular filtration rate in clinical practice and research: a review. Part 1: how to measure glomerular filtration rate with iohexol? Clin Kidney J. 2016;9:682-99.

44. Hoedemaekers CW, Pickkers P, Netea MG, van Deuren M, Van der Hoeven JG. Intensive insulin therapy does not alter the inflammatory response in patients undergoing coronary artery bypass grafting: a randomized controlled trial [ISRCTN95608630]. Crit Care. 2005;9:R790-7.