Pharmacokinetics and lung distribution of a humanized anti-RAGE antibody in wild-type and RAGE−/− mice

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Key words: monoclonal antibody, ADME, tissue distribution, sepsis, pharmacokinetics, advanced glycation end products, RAGE, autoimmunity

A neutralizing antibody to the receptor for the advanced glycation end products (anti-RAGE Ab) was developed as a potential treatment of acute and chronic inflammatory conditions. Previous pharmacology studies demonstrated efficacy of the anti-RAGE antibody in a mouse model of sepsis. We examined pharmacokinetics and lung distribution of [125I]anti-RAGE Ab in RAGE+ and wild-type (129S5) mice following single IV administration. Serum pharmacokinetics of [125I]anti-RAGE Ab was similar in RAGE+ and 129S5 mice, with the total body clearance of 0.3 mL/hr/kg and the elimination half-life of 11–12 days, suggesting the target expression had limited impact on overall elimination of [125I]anti-RAGE Ab from mice. [125I]Anti-RAGE Ab accumulated in the lung of 129S5 mice, with ~4% of total dose retained in the lung at days 6–27 and the lung AUC0-∞ of ~300% of that in serum. The SDS-PAGE analysis suggested that most of retained lung radioactivity was attributed to intact antibody. No accumulation of radioactivity was observed in the lung of RAGE−/− mice, indicating that lung uptake of [125I]anti-RAGE Ab was target-dependent in wild-type mice. These data suggest that the anti-RAGE Ab was able to localize to the site of RAGE expression, the lung, and support the findings in the previous pharmacology studies.

Introduction

The receptor for the advanced glycation end products (RAGE) is a multiligand membrane receptor that interacts with multiple unrelated types of ligands.1–3 The receptor binds advanced glycation end products (AGEs), that form under diverse conditions including aging, diabetes, sepsis and kidney failure.2–5 RAGE ligands also include high mobility group protein 1 (HMGB1), S100/calgranulin family proteins, amyloid beta (Aβ) peptide, as well as Collagen I and IV.3,4,6 In healthy animals and humans, RAGE expression is most prominent in the lung, including alveolar type I and type II epithelial cells, alveolar macrophage, endothelia and some bronchiolar epithelia.7–10

The activation of RAGE by its ligands triggers several signal transduction pathways involved in acute and chronic inflammation, including the NFκB and MAP kinase pathway.11,12 In turn, NFκB upregulates RAGE expression, leading to the amplification of the pro-inflammatory cascade.12–14 Several lines of evidence suggest an important role of RAGE-mediated signaling in the pathogenesis of sepsis. The soluble isoform of RAGE (sRAGE) is elevated in plasma of septic patients and relatively high concentrations of sRAGE are negatively correlated with the survival.15 RAGE−/− mice are protected from lethal septic shock compared to wild-type mice in the cecal ligation and puncture (CLP) model.16 RAGE-dependent activation of NFκB in the lung is though to be the major signaling pathway that modulates outcome in the CLP model.17

Several therapeutic approaches aimed at limiting RAGE interaction with its ligands have been reported, including neutralizing anti-RAGE antibodies, sRAGE and low anticoagulant heparin.16–18 One antibody, designated XT-M4, a rat-derived antimouse RAGE antibody, binds the extracellular region of RAGE and inhibits the interaction of RAGE with multiple ligands. XT-M4 has broad species cross-reactivity, a binding affinity of 0.3 nM for murine dimeric RAGE and was protective in the mouse CLP model of sepsis.16 The humanized XT-M4 antibody (referred to as anti-RAGE Ab) maintains all the inhibitory and binding properties of the parental rat XT-M4 antibody and also showed efficacy in the mouse model of Pneumococcal Pneumonia (Christaki el al., unpublished observations).

This study was conducted to investigate the potential link between the pharmacological action of anti-RAGE antibodies and the localization to the target (RAGE) in the lung. A previous pilot biodistribution study in wild-type (CD-1) mice suggested that the [125I]-labeled anti-RAGE Ab specifically accumulated in the mouse lung after a single intravenous (IV) dose.19 To demonstrate that accumulation of [125I]anti-RAGE Ab in the lung of wild-type mice is target-dependent, we investigated pharmacokinetics (PK) and lung distribution of [125I]anti-RAGE Ab in...
(RE) serum concentration of [125I]\(^{-}\)anti-RAGE Ab declined bi-exponentially with a short initial distribution phase and long elimination phase (Fig. 1A), in accordance with serum concentration-time profiles observed for other human IgG1 antibodies in mice.\(^{20-22}\) [125I]\(^{-}\)anti-RAGE Ab was eliminated slowly from serum of RAGE\(^{-}\) or 129S5 mice, with total body clearance (CL) of \(-0.323\) and \(0.303\) mL/hr/kg, respectively and the elimination half-life (t\(_{1/2}\)) of \(-11.7\) and \(10.9\) days, respectively (Table 1). The RE serum concentration of [125I]\(^{-}\)anti-RAGE Ab at the first sampling time point after IV administration (C\(_{5\ min}\)) was \(86.6\) and \(90.9\) \(\mu\)g eq./mL and the exposure (AUC\(_{0-\infty}\)) was \(15,476\) and \(16,494\) \(\mu\)g eq. \(\cdot\) hr/mL in RAGE\(^{-}\) and 129S5 mice, respectively (Table 1). Thus, based on non-compartmental analysis, serum pharmacokinetics of [125I]\(^{-}\)anti-RAGE Ab after a single IV dose appeared similar in RAGE\(^{-}\) and wild-type 129S5 mice. When serum RE concentrations from RAGE\(^{-}\) and 129S5 mice were fitted into the two-compartment model, the estimated distribution half-life (~5 hr) was similar between these two strain of mice, which was consistent with the observed serum concentration-time profiles (Fig. 1A). Overall, serum pharmacokinetic profiles suggested the target expression had no detectable impact on elimination of [125I]\(^{-}\)anti-RAGE Ab from mice. Serum PK parameters of [125I]\(^{-}\)anti-RAGE Ab observed in this study for RAGE\(^{-}\) and wild-type 129S5 mice were also similar to serum pharmacokinetics in CD-1 mice,\(^{19}\) as well as to serum PK profile of unlabeled anti-RAGE Ab dosed BALB/c mice (examined by an ELISA; data not shown).

**Figure 1.** Radioactive equivalent (RE) concentrations and total radioactivity, as percentage of administered dose (% dose) of [125I]\(^{-}\)anti-RAGE Ab in serum, lung and spleen of RAGE\(^{-}\) and wild-type (129S5) mice following a single 5 mg/kg IV dose. Serum and tissue RE concentrations (A, B and D) and radioactivity in tissues (expressed as % dose; C and E) were monitored by gamma-counting up to \(-5\) mo (132 days) in RAGE\(^{-}\) (open circles) and wild-type (wt) 129S5 (open triangle) mice. The insert in (A) shows serum RE concentrations up to day 9 post-dose. Data show mean ± SD values, with \(n = 6\) per time point. Individual animal concentration values that were less than the limit of quantitation (LOQ, defined as \(3\) \times background cpm) were treated as zero for calculations of the mean and the standard deviation.

RAGE deficient (RAGE\(^{-}\)) and wild-type (129S5) mice following a single IV dose.

**Results and Discussion**

Serum pharmacokinetics. After a single 5 mg/kg IV dose to RAGE\(^{-}\) or wild-type 129S5 mice, the radioactive equivalent

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Table 1. Serum and tissue pharmacokinetic parameters of [125I]anti-RAGE Ab in RAGE-/- or wild-type 129S5 mice after a single 5 mg/kg IV dose

| Tissue | Strain | C_{max} or C_{max} | T_{max} | AUC_{0-∞} | CL | Vdss | t_{1/2} |
|--------|--------|---------------------|---------|------------|-----|------|--------|
|        |        | (μg eq/mL or μg eq/g) | (hr) | (μg eq • hr/mL or μg eq • hr/g) | (mL/hr/kg) | (mL/kg) | (day)  |
| Serum  | RAGE-/- | 86.6 | 0.08 | 15476 | 0.323 | 101 | 11.7   |
|        | 129S5  | 90.9 | 0.08 | 16494 | 0.303 | 98.4 | 10.9   |
| Spleen | RAGE-/- | 3.45 | 1  | 1186 | NA | NA | 21.7   |
|        | 129S5  | 3.38 | 1  | 1268 | NA | NA | 24.3   |
| Lung   | RAGE-/- | 10.7 | 1  | 2084 | NA | NA | 21.5   |
|        | 129S5  | 29.6 | 144 | 42519 | NA | NA | 22.8   |

PK parameters were determined based on mean RE concentrations (n = 6 per time point), using non-compartmental modeling. Individual animal concentration values < the limit of quantitation (LOQ, defined as 3 x background cpm) were treated as zero for calculations of the mean. NA, Not applicable; CL, Serum clearance; Vdss, Volume of distribution at steady-state; AUC, Area under the curve; t_{1/2}, Terminal phase half-life.

For serum, C_{max} (concentration at 5 minutes, the first sampling time point for serum) is shown. *The first tissue collection time point for tissues.

Table 2. Mean (±SD) tissue-to-serum radioactive equivalent concentration ratios (T/S ratios) after a single 5 mg/kg IV dose of [125I]anti-RAGE Ab to RAGE-/- or wild-type 129S5 mice

| Tissue | Strain | 1 hr | 1 day | 2 days | 6 days | 14 days | 27 days | 41 days | 62 days | 97 days | 132 days |
|--------|--------|------|-------|--------|--------|---------|---------|---------|---------|---------|----------|
|        |        | 1 hr | 1 day | 2 days | 6 days | 14 days | 27 days | 41 days | 62 days | 97 days | 132 days |
| Lung   | RAGE-/- | 0.125 ± 0.046 | 0.124 ± 0.075 | 0.055 ± 0.024 | 0.063 ± 0.032 | 0.202 ± 0.160 | 0.612 ± 0.893 | 0.142 ± 0.053 | NC | NC | NC |
|        | 129S5  | 0.153 ± 0.013 | 0.238 ± 0.13 | 0.302 ± 0.132 | 0.792 ± 0.218 | 1.46 ± 0.59 | 6.84 ± 6.08 | 57.9 ± 82.8 | 28.4 ± 22.6 | 40.8 ± 28.3 | NC |
| Spleen | RAGE-/- | 0.040 ± 0.007 | 0.031 ± 0.005 | 0.061 ± 0.006 | 0.096 ± 0.051 | 1.52 ± 3.14 | 0.076 ± 0.037 | NC | NC | NC |
|        | 129S5  | 0.044 ± 0.007 | 0.044 ± 0.009 | 0.060 ± 0.010 | 0.056 ± 0.023 | 0.102 ± 0.071 | 1.25 ± 0.88 | 0.304 ± 0.233 | 0.663 ± 0.430 | NC |

NC, Not calculated, when serum concentrations from at least four of 6 mice were below the limit of quantitation (LOQ, defined as 3 x background cpm). Only two of six 129S5 mice designated for 1 hr serum and tissue collection were dosed (due to animal health status prior to dosing); therefore n = 2 for the 1 hr time point.

RAGE Ab was target-dependent.

To investigate whether the accumulated radioactivity in the lung of wild-type mice could be attributed to intact [125I]anti-RAGE Ab, we conducted non-reducing SDS/PAGE analysis of lung homogenates from RAGE-/- and wild-type 129S5 mice at Day 14 post dose. For both RAGE-/- and wild-type mouse lung homogenates, the majority of the radioactivity (and ~90% or greater of total loaded dose per lane) appeared to correspond to the ~150 kDa MW band, which is the expected molecular weight of the intact anti-RAGE Ab.
weight of the intact [125I]anti-RAGE Ab (Fig. 2). In agreement with more than 10-fold higher mean RE concentration of [125I]anti-RAGE Ab in wild-type 129S5 mice compared to RAGE-/- mice at day 14 (Fig. 1B), the density of this ~150 kDa band was approximately 10-fold higher in 129S5 mice, relative to RAGE-/- strain, as examined by densitometry. In addition, TCA analysis of day 14 and day 97 lung homogenates indicated that >95% of radioactivity was TCA-precipitable, i.e., protein-associated.

In the spleen (the negative control tissue), biodistribution and PK profiles of [125I]anti-RAGE Ab were similar in RAGE-/- and wild-type 129S5 mice following a single 5 mg/kg IV dose. The mean spleen RE concentration of [125I]anti-RAGE Ab reached C_max of ~3.4–3.5 μg eq/g at 1 h, the first sampling time point for tissues, and declined during the rest of sampling period (Fig. 1D). Mean spleen RE concentrations of [125I]anti-RAGE Ab were lower than serum concentrations, resulting in low T/S ratios (in general less than 1, Table 2) and low counts, as % of total administered dose (less than 0.25%, Fig. 2E). The spleen AUC_0-∞ of [125I]anti-RAGE was 1,186 μg eq. * hr/g in RAGE-/- mice and 1,268 μg eq. * hr/g in 129S5 mice, which was ~7.7% of serum for both strains. The elimination t_1/2 of [125I]anti-RAGE in the spleen was 21.7 and 24.3 days, in RAGE-/- and 129S5 mice, respectively, which was similar to the corresponding t_1/2 values in the lung, but longer than those in serum for both strains.

In summary, our data demonstrated that following a single IV dose, serum pharmacokinetics of [125I]anti-RAGE Ab was similar in RAGE-/- and wild-type mice. In the lung, [125I]anti-RAGE Ab accumulated in wild-type but not in RAGE-/- mice. These results indicated that the lung uptake of [125I]anti-RAGE Ab in wild-type mice was target-dependent.

Lilienseik et al. showed that the protection of RAGE-/- mice from lethality in the CLP sepsis model was coincidental with an attenuation of NF-κB activation in the lung.7 Thus, NFκB activation via the RAGE-mediated signaling is believed to be important for the disease pathogenesis (including inflammation in the tissues) in the mouse models of sepsis, with the lung being one of the major affected organs.6,7 The anti-RAGE rat and humanized XT-M4 antibodies were efficacious in the murine CLP and pneumococcal pneumonia models (Christaki et al. unpublished observations).6 In the CLP studies, the histopathology analysis of animals treated with the rat XT-M4 antibody showed a reduction in pathology scores compared to control mice.6 The analysis of lungs from mice with CLP and treated with the humanized XT-M4 anti-RAGE antibody showed the induction of many genes associated with the modulation of inflammation (including several genes in the NFκB pathway) and are supportive of a protective effect of the anti-RAGE antibodies in this model (Christaki et al. unpublished observations).

The data presented in this manuscript indicates that the humanized anti-RAGE Ab localizes to its target in the lung and supports the findings in the pharmacology studies. Finally, the serum and lung pharmacokinetic data presented in this study will be useful in establishing PK-PD relationship for the humanized anti-RAGE Ab in the mouse sepsis model and guiding the dosing regimen for human studies.

Materials and Methods

Test article, iodination and dosing solution. Humanized anti-RAGE Ab (humanized from the rat IgG2b clone XT-M4) was generated at Pfizer (previously Wyeth Research, Cambridge, MA, USA).16,25 Iodination was performed by the IODO-BEADS method according to manufacturer’s instructions (Pierce, Rockford, IL, USA), using ~0.5 mg of test article re-formulated in phosphate buffer saline (PBS) and 5 mCi of 125-I-iodine (Perkin Elmer; Waltham, MA, USA). A dosing solution was prepared by mixing unlabeled test article, a trace amount of 125-I-labeled test article, and PBS to achieve the specific activity of ~38 μCi/mg (with <2% free iodine) and the final protein concentration of 1 mg/mL. Dosing solution was characterized by gamma-counting of trichloroacetic acid (TCA)-precipitable radioactivity and gel electrophoresis, as previously described.19

Animals and study design. Male RAGE-/- (~8 mo old) mice were generated and bred at Pfizer (previously Wyeth Research, Cambridge, MA, USA), as previously described.16 In brief, the RAGE-/- mouse was designed at Pfizer (generated at Lexicon Genetics Incorporated, Woodlands, TX, USA) as a gene targeted conditional knockout in 129SvEv-Brd mice in which Cre recombinase excises exons 2, 3 and 4. The resulting deletion resulted in a frame shift and truncation of the RAGE protein and protein was not produced. Previous studies demonstrated the absence of RAGE expression in the lung of RAGE-/- mice.16 Wild-type mice were 129S5/SvEvbrd (abbreviated as 129S5) and were also bred at Pfizer. Mice were given water containing potassium iodide (0.1 mg/mL) for 3 days prior to dosing and the dosing solution was administered as a single IV bolus dose into the tail vein (at a volume of 5 mL/kg to result in a 5 mg/kg dosage). After a whole body perfusion with heparinized (25 U/mL) PBS, serum, lung and spleen samples were taken at 1 h and then at 1, 2, 6, 14, 27, 41, 62, 97 and 132 days post dose, with six mice per time point per strain, and tissues were weighed. Additional serum samples were taken via retro-orbital bleeding during 5 min 132 day period. The Pfizer (previously Wyeth) Institutional Animal Care and Use Committee approved all aspects of these studies.
Determination of radioactive equivalent concentrations and PK calculations. Radioactive equivalent (RE) concentrations in serum and tissues were determined by gamma-counting based on TCA-precipitable and total counts, respectively, as described previously. PK parameters were calculated based on mean RE concentrations by non-compartmental analysis methods using the WinNonlin software (Pharsight, Inc., St. Louis, MO, USA; model 201 for serum and model 200 for tissues). Lung uptake clearance (CL\textsubscript{Lung}) was calculated based on the previously described method.

Homogenization of lung samples and gel electrophoresis analysis. Lung samples were homogenized in 1 mL of buffer containing 20 mM EDTA, 0.1% sodium dodecyl sulfate (SDS), 1% Triton X-100 in PBS, pH 7.4 and protease inhibitor cocktail (Roche, Indianapolis, IN), mixed with an equal volume of 2X non-reducing sample buffer (Biorad, Hercules, CA, USA), and loaded (60 μL per lane) onto a 12% SDS polyacrylamide gel Tris-HCl gel (SDS-PAGE) (Biorad). In general, lung weights were similar in RAGE\textsuperscript{-/-} and wild-type 129S5 mice. The individual animal lung weights for mice included in SDS-PAGE analysis are shown in Supplemental Table 1. The radioactive bands were visualized by autoradiography and relative band densities were quantitated by densitometry.

Acknowledgements

We thank Lioudmila Tchistiakov, Xiang Yang Tan and Nicole Piche-Nicholas for test article generation, Jennifer Spencer-Pierce for coordination of in vivo studies and Andover BioResources Group of Pfizer for help with dosing and tissue collection.

Note

Supplementary materials can be found at: www.landesbioscience.com/supplement/VugmeysterMABS2-5-sup.pdf