### Transport study of interleukin-1 inhibitors using a human *in vitro* model of the blood-brain barrier

Elisabet O. Sjöström a,1, Maxime Culot b, Lisa Leickt a, Mikael Åstrand a,1, Erik Nordling a, Fabien Gosselet b, Christina Kaiser a,*

**a** Swedish Orphan Biovitrum AB (publ), SE-112 76, Stockholm, Sweden

**b** Univ. Artois, UR 2465, Laboratoire de la Barrière Hémo-Encéphalique (LBHE), F-62300, Lens, France

#### A R T I C L E   I N F O

**Keywords:**
Interleukin-1
Interleukin-1 receptor antagonist
Anakinra
*In vitro* blood-brain barrier
Stroke
Neuronal injury
Acute brain injury
Inflammation

#### A B S T R A C T

The proinflammatory cytokine interleukin-1 (IL-1), with its two isoforms α and β, has important roles in multiple pathogenic processes in the central nervous system. The present study aimed to evaluate and compare the blood-to-brain distribution of anakinra (IL-1 receptor antagonist), bermekimab (IL-1α antagonist) and canakinumab (IL-1β antagonist).

A human *in vitro* model of the blood-brain barrier derived from human umbilical cord blood stem cells was used, where isolated CD34+ cells co-cultured with bovine pericytes were matured into polarized brain-like endothelial cells. Transport rates of the three test items were evaluated after 180 min incubation at concentrations 50, 250 and 1250 nM in a transwell system. We report herein that anakinra passes the human brain-like endothelial monolayer at a 4.7-fold higher rate than the monoclonal antibodies tested. Both antibodies had similar transport rates at all concentrations. No dose-dependent effects in transport rates were observed, nor any saturation effects at supraphysiological concentrations. The larger propensity of anakinra to pass this model of the human blood-brain barrier supports existing data and confirms that anakinra can reach the brain compartment at clinically relevant concentrations. As anakinra inhibits the actions of both IL-1α and IL-1β, it blocks all effects of IL-1 downstream signaling. The results herein further add to the growing body of evidence of the potential utility of anakinra to treat neuroinflammatory disorders.

### 1. Introduction

Inflammatory processes are implicated in the pathophysiology of both acute and chronic diseases affecting the central nervous system (CNS), influencing neurodegenerative processes, tissue injury, repair, and recovery. Inflammation is necessary for adequate response to injury, but responses are often exacerbated and lead to untoward effects (DiSabato et al., 2016). CNS manifestations are common in the most severe clinical phenotypes of a spectrum of autoinflammatory disorders termed cryopyrin-associated periodic syndromes (CAPS) (Sibley et al., 2012). Dysregulation of inflammatory pathways in cerebral ischemia and stroke are well documented, with sustained inflammation in subacute and chronic phases (Gerhard et al., 2005) being independently associated with worse functional outcome (Whiteley et al., 2009). Likewise, traumatic brain injury (TBI) has a large inflammatory component, where both acute and long-lasting inflammation are present (Kumar et al., 2015; Webster et al., 2017). Also, growing evidence suggests an important role for inflammatory pathways in refractory seizure disorders and epileptogenesis (Koh et al., 2021; Vezzani et al., 2011; Webster et al., 2017).

A key mediator of inflammatory processes is the interleukin-1 (IL-1) pathway driven by the cytokines IL-1α and IL-1β. Both ligands act via the IL-1 type I receptor (IL-1RI), which is expressed on many cell types in the periphery as well as in the brain (Allan et al., 2005; Basu et al., 2002; Pintaux et al., 2002). Under normal conditions, IL-1 is expressed at very low levels in the brain, but production of both IL-1α and IL-1β increases significantly during ischemic brain injury (Murray et al., 2015). Upregulation of IL-1α is a critical step in the ischemia-induced inflammatory cascade (Brough and Denes, 2015), while IL-1β production is believed to play an important role in sustaining local inflammation later in the response (Allan et al., 2005; Murray et al., 2015). A naturally occurring competitive inhibitor to IL-1α and IL-1β is the IL-1 receptor antagonist (IL-1Ra), which by binding to IL-1RI without inducing intracellular...
downstream signalling, antagonizes all known functions of the two IL-1 isoforms (Dinarello, 1996; Dinarello et al., 2012; Hannum et al., 1990).

The blood-brain barrier (BBB) plays a key role in maintaining the specialized environment required for neuronal functioning. The monolayer of tightly sealed endothelial cells within brain capillaries surrounded by basement membranes, pericytes and astrocytes, together referred to as the neurovascular unit, provides a well-regulated gate for influx and efflux of molecules to cells of the brain, protecting it from systemic toxic insults, high protein loads, inflammatory mediators and immune cells (Abbott et al., 2006; Banks, 2016; Pardridge, 2012). Modelling of transport across the BBB in polarized endothelial monolayers in vitro allows for comparison and evaluation of transport mechanisms of relevant molecules (Cecchelli et al., 2014; Helms et al., 2016).

Anti-IL-1 molecules are an emerging treatment approach for selected CNS disorders, including stroke, TBI and seizure disorders (Helmy et al., 2014; Galea et al., 2018; Kenney-Jung et al., 2016; Koh et al., 2021). Therapeutic monoclonal antibodies targeting IL-1α (canakinumab) and IL-1β (bermekimab) have been developed. The recombinant version of IL-1Ra (rhIL-1Ra), anakinra, has been in clinical use for more than 20 years. Canakinumab and anakinra are approved for several auto-inflammatory disorders, some of which have CNS manifestations, while bemekimab is being investigated in clinical trials. These three drugs differ in terms of their target (soluble factors versus receptor), their size (148 kDa vs 17 kDa) and their pharmacokinetic properties. It is of high relevance to understand their access to the CNS compartment and in a broader perspective, it is pertinent to consider and compare the therapeutic effects of blocking the entire IL-1 downstream pathway (through receptor blockade) versus selective cytokine neutralization.

The current study aimed to evaluate and compare the passage of canakinumab, bemekimab and anakinra into the brain using a transwell in vitro model of the human BBB.

2. Materials and methods

Two independent experiments were performed in this study, referred to as Experiment 1 (Exp. 1) and Experiment 2 (Exp. 2). In Exp. 1, anakinra was compared head-to-head with canakinumab and bemekimab at three concentrations. In Exp. 2, canakinumab and anakinra were explored under the same conditions as those in Exp. 1, but with an additional test item as a size control to anakinra.

2.1. Test compounds

The test items for the experiments were provided as pharmaceutical drug products (DP), for canakinumab (Ilaris®) and anakinra (Kineret®). The monoclonal antibody bemekimab (MABp1, Xilonix) was produced based on information provided in patent EP2285409 (A1/9 and A1/11). The recombinant full-length antibody was produced in ExpiCHO cells, purified and analyzed according to standard methods and formulated in NaP–NaCl buffer (pH 7.0). As a size control to anakinra, an experimentally produced single domain antibody (VHH) of Llama origin (devolved of human binding specificity or interaction partner) of approximately 15 kDa was used (D08-568P). The VHH was expressed with a C-terminal hexahistidine tag in E. coli periplasm using a pelB secretion signal peptide and purified using immobilized metal ion affinity chromatography. The VHH was fluoroscence labeled with Promofluor 568 (PK FS568-1-05, PromoCell, Heidelberg, Germany), according to the manufacturer’s instructions.

2.2. In vitro BBB model

An in vitro model of the human BBB using endothelial cells (ECs) derived from human umbilical cord blood stem cells (Cecchelli et al., 2014) was used to study the rate of transport of the test items. Infants’ parents signed an informed consent form allowing the collection of the umbilical cord blood, in compliance with the French legislation. The protocol was approved by the French Ministry of Higher Education and Research (CODECOH Number DC2011-1321). All experiments were performed in accordance with the approved protocol.

2.3. Cell culture

CD34+ cells isolated from human umbilical cord blood were differentiated into ECs, as described previously (Pedroso et al., 2011). Briefly, CD34+ cells were cultured in EC growth medium supplemented with serum and growth factors for 15–20 days and expanded until confluence. ECs were then trypsinized and seeded onto Matrigel coated transwell inserts (0.4 μm pore size) at a density of 8 × 10^4, and co-cultured with bovine brain pericytes for 6 days (Fig. 1A). Bovine pericytes were isolated and characterized as described in (Vandenhaute et al., 2016). Pericytes grown on the bottom well were removed prior to the experiment and replaced by empty lower chambers (Fig. 1B).

2.4. In vitro BBB transwell assay

Before commencement of the experiment, BLECs (on inserts) were washed twice with Ringer HEPES (RH) buffer (NaCl 150 mM, KCl 5.2 mM, CaCl2 2.2 mM, MgCl2 0.2 mM, NaHCO3 6 mM, glucose 2.8 mM, HEPES 5 mM). At t = 0 min, the test items, with or without fluorescently
labeled human serum albumin (hSA-647; equimolar to test item (cat. # 009-600-051, Jackson Immunoresearch Europe Ltd, Ely, United Kingdom) were added to the donor (luminal) compartment at concentrations of 50, 250 and 1250 nM (in RH buffer supplemented with 0.5% hSA and containing 1 μM sodium fluorescein (NaF, MW 376, CAS 518-47-8, Sigma-Aldrich, Merck, Darmstadt, Germany), to verify tightness of the cell layer. Lower chambers were filled with 1.5 mL RH buffer plus 0.5% hSA. Plates were incubated at 37 °C at slow shaking velocity for 3 h.

At t = 180 min, samples were taken from the donor and receiver chambers (see Fig. 1B). BLECs were washed three times with RH buffer, scraped and lysed in 0.5 mL of radio-immunoprecipitation assay (RIPA) buffer. All experimental conditions were run in triplicate wells.

To evaluate the ability of test compounds to cross Matrigel-coated filters without cells and to control for the adsorption to plastics and/or Matrigel, the assay was also run without cells at the lowest assay concentration (50 nM). Mass balance (M.B %) was calculated from the amount of compound recovered in both compartments at the end of the experiment divided by the total amount added to the donor compartment at the start of the experiment. Samples from the assay, at t = 0 and t = 180 were immediately dispensed into 96-well plates and frozen to -70 °C. Samples were analyzed for fluorescence using a Synergy H1 (BioTek, Vinooski, USA) set to detect wavelengths 488, 568 and 647 and the presence of test items with customized immunoassays. Note: hSA-647 was not used in combination with the fluorescent test item (D08-568P), due to the risk of confounding fluorescence interference.

2.5. Immunoassays of test items

2.5.1. Method 1 (used in Exp. 1)

Analysis of the monoclonal antibodies (mAbs), bermekimab and canakinumab in the test samples was made using an enzyme-linked immunosorbent assay (ELISA) method established to detect the human IgG Fc domain. Briefly, microtiter plates (P96 MaxiSorp Nunc Immuno-plate 442404, ThermoFischer Scientific, Waltham, US) were coated with anti-human IgG (F(ab) specific fragment (cat # I5154, Sigma Aldrich, Merck Darmstadt, Germany), washed and blocked with 5% bovine serum albumin (BSA). Samples and standards were added, and bound protein was detected using an HRP conjugated anti-human IgG F(ab)2 fragment (Sigma A22920). TMB (Sigma T0440) was used as substrate and the absorbance was measured on a Tecan Infinite i3 reader (Molecular Devices, San Jose, CA, USA) at 450 nm (540 nm background correction).

2.5.2. Method 2 (used in Exp. 2)

A human IgG ELISA assay from Cygnus Technologies (Southport, NC, USA; Cat #F160) was applied to measure levels of canakinumab. Samples were added to microtiter strips coated with a capture antibody and containing a horseradish peroxidase (HRP) enzyme labeled anti-hIgG antibody, resulting in the formation of a sandwich complex of solid phase antibody-human IgG-enzyme labeled antibody, followed by a TMB substrate reaction. The amount of hydrolyzed substrate was measured on a Tecan Infinite M200 Pro reader (Männedorf, Switzerland).

2.5.3. Method 3 (used for anakinra in Exp.1)

Microtiter plates were coated with anti-IL1Ra antibody (AF280 R&D Systems, Minneapolis, MN, USA) and blocked with 1% BSA. After a wash step, plates were incubated with samples or standards followed by a wash step and incubation with biotinylated anti-IL1Ra antibody (BAF280 R&D Systems). The plates were washed and incubated with streptavidin (SA)-HRP, followed by another wash step and finally incubation with TMB (EC-Blue Enhanced, Medicago, Uppsala, Sweden). Results were read on a SpectraMax i5 reader.

2.5.4. Method 4 (used for anakinra in Exp. 2)

The second method for detection of anakinra involved an ECL (electrochemiluminescence)-based method employing an SA-coated MSD (Meso Scale Diagnostics; Rockville, MA, USA) plate that was blocked and incubated with biotinylated anti-IL1-Ra antibody. After a wash step, diluted samples were added and incubated on the plate. After another wash step, the plates were incubated with a sulfo-tagged anti-IL1Ra antibody solution. After a final wash step, read buffer was added to produce a chemiluminescent signal when an electrical voltage is applied. The plates were read on MSD QuickPlex SQ 120 (Meso Scale Diagnostics).

2.6. Calculation of permeability coefficients

The endothelial permeability coefficient (Pe) of the test items was calculated as described by Dehouck et al. (1992). The cleared volume was obtained by dividing the amount of test items in the receiver compartment at the end by its concentration in the donor compartment at the onset of experiment, divided by the duration of the experiment (180 min) to elicit the permeability surface area product (PS in μL per minute). In this calculation, both the permeability of filters without cells (PSf = insert filter + coating) and filters with cells (PS = filter + coating + ECs) were taken into account, according to the formula:

$$ P_{Se} = \frac{1}{P_{St}} + \frac{1}{P_{Sf}} \left( \frac{1}{P_{St}} - \frac{1}{P_{Sf}} \right)^{-1} $$

$P_{Se}$ is the permeability surface area product of the ECs monolayer (in μL per minute) which is divided by the surface area of the filter (S, which is 1.13 cm² for a 12-well format inserts) to generate the endothelial permeability coefficient (Pe) in centimeters per minute.

$$ Pe = P_{Se}/S $$

2.7. Analysis & statistics

As Exp. 1 and Exp. 2 were run at different time points and from different primary cells, results are not compared side by side as absolute values. Moreover, the immunoassays used to quantify the test items differed with respect to methodology, materials and location. Within each experiment, Pe values were compared with two-way ANOVA and Tukey's test of multiple comparisons for groupwise comparisons (test items). Significance threshold level was set at p < 0.05. Data analyses and statistics were performed using Graph Pad Prism 9 (San Diego, California, USA).

3. Results

3.1. Recovery/mass balance without cells

To ensure that passage of the test compounds was not critically limited by the filter or Matrigel coating, each test item was run in triplicate at 50 nM in well inserts and buffer, without BLECs, for 3 h. Among the tested compounds, the recovery and mass balance ranged from 88% to 101% in Exp. 1 and from 93% to 123% in Exp. 2 (Table 1). Passage of the test items, i.e. the amount diffused (% from the upper (donor) to lower (receiver) chamber after 3 h across the inserts without cells, were in the same range between test compounds 27.3–33.6% and 19.2–26.4% for Exp. 1 and 2, respectively. The amount diffused was slightly higher for anakinra (17 kDa) than for canakinumab (148 kDa), Table 1.

3.2. Cellular integrity, permeability of NaF and hSA

Cellular tightness and consequent permeability (Pe) of the BLEC monolayer to the small integrity marker NaF was overall low and stable in both experiments, with no signs of toxicity or untoward effect of the added test items at any concentration when compared with controls without added protein (Fig. 2A and B). The two studies used different primary cell sources and reagent batches (such as serum), and consequently a clear difference in basal cell permeability was observed. Within
each of the two experiments (Exp.1 and Exp. 2), the range of the endothelial permeability to NaF (Pe to NaF) obtained in the presence or absence of the test compounds at different concentrations was narrow (Exp. 1: 0.70-1.08 \( \times 10^{-3} \) cm/min and Exp 2: 0.27-0.38 \( \times 10^{-3} \) cm/min). We have previously validated the use of fluorescent hSA (hSA-647) as size control in the assay system to not affect cellular integrity at any concentration used herein. Endothelial permeability (Pe) to hSA-647 was stable at all concentrations (50, 250 and 1250 nM) and in a similar range for all test item conditions (Fig. 2C and D). A slightly elevated Pe to NaF was observed in the hSA 1250 nM control (without test item co-incubation, Exp. 1, Fig. 1B “hSA-647”). This result was not observed in a parallel plate run performed at the same time from another donor (data not shown), or in Exp. 2. In Exp. 2, hSA-647 was omitted in all wells with D08-568P, to avoid the risk of fluorescence interference, and hence was not determined (ND).

### 3.3. Endothelial permeability of test items

In a first experiment, the difference between IL-1 targeting biologics in transport rates across the BLEC was investigated. Bermekimab and canakinumab showed very low, dose-dependent, transport rates across the endothelial monolayer at all concentrations (Pe 0.74-0.94 \( \times 10^{-3} \) cm/min and Pe 0.53-0.75 \( \times 10^{-3} \) cm/min, respectively), Fig. 3A. The transport rate for anakinra was 5-6-fold higher than bermekimab and canakinumab (Pe 3.32-4.44 \( \times 10^{-3} \) cm/min; \( p < 0.0001 \), two-way ANOVA), with no dose-dependent effects on permeability rates (Fig. 3A). To validate the findings, the experiment was repeated with the two drugs that are used clinically, canakinumab and anakinra, as well as a non-IL-1 targeting size control (D08-568P, 15 kDa), which is devoid of specific human binding interactions. As expected, the size control behaved similarly to that of anakinra with transport rates across BLEC over 3 h at all concentrations tested (Fig. 3B), and rates of transport were in the same range for D08-568P (Pe 2.20-3.01 \( \times 10^{-3} \) cm/min) as for anakinra (Pe 2.85-3.14 \( \times 10^{-3} \) cm/min). As previously observed (Exp. 1), canakinumab had lower transport rates across BLEC compared to

### Table 1

| Test item     | Approximate MW | Experiment 1 | Experiment 2 |
|---------------|----------------|--------------|--------------|
|               | Mean (SD)      | Mean (SD)    | Mean (SD)    |
| Anakinra      | 17 kDa         | 33.6 (9.7)   | 26.4 (2.1)   |
| Bermekimab    | 148 kDa        | 32.5 (4.3)   | 24.0 (3.8)   |
| Canakinumab   | 148 kDa        | 27.3 (4.2)   | 19.2 (11.2)  |

Table 1. Amount diffused (%) after 180 min, mass balance (amount recovered) and passage (or diffusion) rate of test items at 50 nM without cells (PsF); kDa, kilo daltons, MW; Molecular weight, nM; nanomolar, min; minutes, SD; standard deviation, \( \mu \)L; microliters.

Fig. 2. Endothelial permeability (Pe) of integrity and size controls, detected by fluorometric analysis, in Experiment 1 (left panels) and Experiment 2 (right panels). (A–B) Permeability to the integrity marker sodium fluorescein (NaF) at 1 \( \mu \)M in all experimental conditions. (C–D) Permeability of human serum albumin Alexa-647 (hSA-647), added at equimolar concentration to test items or alone as control (1250 nM). N.D; not determined.
Table 2

Volume cleared and mass balance for test items in experiments with cells.

| Condition (test item) | Pst $\mu$L/min (SD) | Mass balance (% recovery) |
|----------------------|---------------------|--------------------------|
| Anakinra 50 nM       | 0.047 (0.012)       | 56 (18)                  |
| Anakinra 250 nM      | 0.037 (0.005)       | 99 (1)                   |
| Anakinra 1250 nM     | 0.049 (0.004)       | 69 (6)                   |
| Bermekimab 50 nM     | 0.011 (0.003)       | 85 (8)                   |
| Bermekimab 250 nM    | 0.009 (0.003)       | 96 (1)                   |
| Bermekimab 1250 nM   | 0.008 (0.002)       | 93 (5)                   |
| Canakinumab 50 nM    | 0.006 (0.002)       | 92 (7)                   |
| Canakinumab 250 nM   | 0.007 (0.002)       | 93 (4)                   |
| Canakinumab 1250 nM  | 0.008 (0.001)       | 87 (2)                   |
| D08-568P 250 nM      | 0.006 (0.001)       | 92 (1)                   |
| D08-568P 1250 nM     | 0.005 (0.001)       | 91 (1)                   |

4. Discussion

To gain an understanding of gross transport characteristics of different pharmaceuticals employed in the clinic towards the interleukin-1 pathway, an unbiased evaluation of directional transport was performed in a human in vitro BBB system, namely BLECs. To our knowledge, this is the first reported comparative study of three clinically relevant drugs targeting the IL-1 pathway. Anakinra, a recombinant form of the human IL-1Ra (rHuIL-1Ra; 17 kDa), was compared with the two recombinant human monoclonal IgGs (148 kDa) that block IL-1α (bermekimab) and IL-1β (canakinumab). Results from the present study demonstrate that anakinra displays the highest rate of transport across the BLECs, from the luminal to the abluminal compartment. The transport rate provides estimates for a theoretical comparison of brain exposure after systemic dosing between the drugs. Doses tested herein were in relevant ranges for a pharmacological $C_{\text{max}}$ in plasma. For canakinumab, $C_{\text{max}}$ in patients (adults, 150 mg dose) is reported to be 16 ± 3.5 μg/mL (corresponding to 110 nM) occurring at 7 days (Chakraborty et al., 2012). Anakinra has a reported $C_{\text{max}}$ of 3.6 μg/mL (range 0.7–8.5 μg/mL) occurring after 3–7 h (Kineret SPC “Kineret Summary of Product Characteristics,” 2020). Despite apparent differences in pharmacokinetic profiles between anakinra and mAbs, a $C_{\text{max}}$ reflects the concentration exposed to the BBB and hence, indicates the amounts readily available for brain uptake.

Brain exposure of systemically dosed biologics is inherently difficult to predict in both preclinical and clinical settings. First, samples of brain interstitial fluid in the clinic are not readily available, except for microdialysis in neurocritical care (e.g. cerebral microdialysis in TBI and subarachnoid hemorrhage). Second, although there is exchange between brain interstitial fluid and cerebrospinal fluid (CSF), CSF composition does not fully reflect the contents of the brain interstitium and vice versa.
The transport of IL-1Ra into rodent brain in vivo has been previously demonstrated (Greenhalgh et al., 2010; Gutierrez et al., 1994). Further indirect evidence of central exposure comes from preclinical stroke models where systemically administered rHuIL-1Ra was found to be neuroprotective and limited functional impairment (Clark et al., 2007; Pradillo et al., 2017; Relton et al., 1996). Furthermore, clinical reports show that systemically administered rHuIL-1Ra resulted in peak CSF levels of around 100 ng/ml (Clark et al., 2007; Galea et al., 2010; Gueorguieva et al., 2008). However, it is important to consider that CSF exposure is merely a pseudomarker of brain exposure and does not confirm actual brain exposure (Pardridge, 2012). Brain exposure to anakinra has been documented in TBI, where microdialysis has been employed during neurocritical care. In a phase II randomized clinical study in TBI patients, rHuIL-1Ra was administered once daily and detected in the brain microdialysate (mean level 150–200 pg/ml; endogenous control levels 10–20 pg/ml) (Helmy et al., 2014). In another study, endogenous IL-1Ra was reported in the microdialysate of TBI patients, with increasing concentrations in relation to IL-1α and IL-1β, yielding a positive impact on edema, intracranial pressure and outcome (Hutchinson et al., 2007).

Intriguingly, in patients with febrile-infection related epilepsy syndrome (FIRES), a refractory status epilepticus affecting children and young adults without active epilepsy and without clear structural, toxic, or metabolic cause, several recent case reports have demonstrated positive outcome measures after treatment with rHuIL-1Ra. The syndrome is highly refractory and resistant to conventional treatment, resulting in high mortality and severe neurologic morbidity in those who do survive (Payne et al., 2020). Inflammatory processes are believed to be decisively involved (Kenny-Jung et al., 2016; Sakuma et al., 2015). A growing body of evidence shows encouraging long-lasting effects of IL-1 inhibition through rHuIL-1Ra (DeSena et al., 2018; Kenney-Jung et al., 2016; Westbrook et al., 2019). Of particular significance is evidence showing that certain responders to rHuIL-1Ra treatment have a functional deficit in the production of endogenous IL-1Ra, thus providing direct mechanistic support for the hypothesis (Clarkson et al., 2019).

Canakinumab and anakinra are both approved for the treatment of diseases within the autoinflammatory syndrome CAPS. The divergent treatment outcomes of the two drugs with regards to patients with CNS manifestations, with anakinra being possibly more effective, are interesting in view of the results obtained in this study (Sibley et al., 2012, 2015).

Many CNS disorders have an integral inflammatory component and correspondingly show a compromised BBB, where both IgG and smaller proteins can enter the brain parenchyma (Liebner et al., 2018). In a preclinical study where rHuIL-Ra was dosed systemically in rat, distribution of the molecule was found within the infarct area overlapping that of IgG-positivity, indicating passage over a compromised BBB (Greenhalgh et al., 2010). In epilepsy, focal BBB compromise is present within theictogenic areas (Lazovic et al., 2005). In TBI, untoward effects of IL-1β are supported by respective ligands (IL-1α and IL-1β) should not be neglected in a hyper-inflammatory state, and hence could affect the free versus the bound state of the mAbs.

Intracellular accumulation of test items, as detected from cell lysate of scraped cells diluted in buffer. (A) Experiment 1: Anakinra, bermekimab and canakinumab. (B) Experiment 2: Anakinra, D08-568P, and canakinumab. (LOD: Below limit of detection).
preclinical findings (Clausen et al., 2011; Ozen et al., 2020). In congruence, administration of rHuIL-1Ra in preclinical TBI models and to patients has shown favourable effects such as reduced neuroinflammation and improved cognition (Helmy et al., 2014; Newell et al., 2018). IL-1α has been less studied in relation to CNS disease and outcome but evidence for its role in the tissue response to stroke has emerged (Brough and Denes, 2015; Luheshi et al., 2011) and a recent experimental study demonstrated that inhibition of IL-1α alone contributed to reduced infarct size and improved neurological performance after middle cerebral artery occlusion in mice (Libera et al., 2020). Several independent preclinical studies in models of ischemic stroke, intracerebral hemorrhage (ICH) and acute subarachnoid hemorrhage (aSAH), have reported beneficial effects of rHuIL-1Ra in vivo, in both rats and mice, effects confirmed in cross-laboratory studies and meta-analyses (Maysami et al., 2015; McCann et al., 2016). Correspondingly, clinical studies of systemic administration of rHuIL-1Ra have been undertaken in acute ischemic stroke and aSAH (Clark et al., 2007; Emsley et al., 2005; Galea et al., 2018). Encouraging data have emerged, and rHuIL-1Ra is currently being tested in a Phase 3 multi-centre trial in aSAH (NCT03249207), and in a Phase 2 multi-centre trial in ICH (NCT03737344).

5. Conclusion

We report a comparative study of three clinically relevant drugs targeting the IL-1 pathway and their transport rate across an in vitro model of the human BBB. Concordant evidence from both preclinical and clinical settings have shown that rHuIL-1Ra reaches the brain at experimentally therapeutic concentrations and has proof-of-concept biologic efficacy, while no such studies have been reported for the IL-1α and IL-1β neutralizing antibodies. Shown in this in vitro study, H+u-IL1Ra has a larger propensity to pass an in vitro model of the human BBB, compared to monoclonal antibodies. Importantly, H+u-IL1Ra inhibits the actions of both IL-1α isoforms and consequently blocks all downstream signalling. IL-1α is an early and important initiator of inflammatory processes in conjunction with acute brain injury while IL-1β propagates the inflammatory cascade and induces the production of several downstream effector molecules. Increasing evidence in support of a causative relationship between persistent, detrimental inflammation and CNS related disorders underscores the potential for targeting the IL-1 system in the brain, to alleviate symptoms and improve clinical outcomes.

Declaration of competing interest

CK, LL, EN are current employees of Swedish Orphan Biovitrum AB (publ), EOS is a former employee and current consultant contracted by Swedish Orphan Biovitrum AB (publ), MÅ is a former employee of Swedish Orphan Biovitrum AB (publ), EOS is a former employee and current consultant contracted by Swedish Orphan Biovitrum AB (publ), MÅ is a former employee of Swedish Orphan Biovitrum AB (publ), CK, LL, EN are current employees of Swedish Orphan Biovitrum AB, MÅ is a former employee of Swedish Orphan Biovitrum AB (publ), EOS is an early and important initiator of in

References

Abbott, N.J., Ronnback, L., Hansson, E., 2006. Astrocyte-endothelial interactions at the blood-brain barrier. Nat. Rev. Neurosci. 7, 41–53. https://doi.org/10.1038/nrn1824, Allan, S.M., Tyrrell, P.J., Rothwell, N.J., 2005. Interleukin-1 and neuronal injury. Nat. Rev. Immunol. 5, 629–640. https://doi.org/10.1038/nri1664.
Ozen, I., Ruscher, K., Nilsson, R., Flygt, J., Clausen, F., Marklund, N., 2020. Interleukin-1 beta neutralization attenuates traumatic brain injury-induced microglia activation and neuronal changes in the globus pallidus. Int. J. Mol. Sci. 21 (387). https://doi.org/10.3390/ijms21020387.

Pardridge, W.M. 2012. Drug transport across the brain–barrier. J. Cereb. Blood Flow Metabol. 32, 1959–1972. https://doi.org/10.1038/jcbfm.2012.126.

Payne, E., Koh, S., Wirrell, E., 2020. Extinguishing febrile infection-related epilepsy syndrome: pipe dream or reality? Semin. Neurol. 40, 263–272. https://doi.org/10.1055/s-0040-1708505.

Pedrosa, D., Tellechea, A., Moura, L., Fidalgo-Carvalho, L., Duarte, J., Carvalho, E., Ferreira, I., 2011. Improved survival, vascular differentiation and wound healing potential of stem cells Co-cultured with endothelial cells. PloS One 6, e16114. https://doi.org/10.1371/journal.pone.0016114.

Pintelas, E., Parker, L.C., Rothwell, N.J., Lubeski, G.N., 2002. Expression of interleukin-1 receptors and their role in interleukin-1 actions in murine microglial cells. J. Neurochem. 83, 754–763. https://doi.org/10.1002/jnc.10115.2002.01184.x.

Poduslo, J.F., Curran, G.L., Berg, C.T., 1994. Macromolecular permeability across the blood–nerve and blood–brain barriers. Proc. Natl. Acad. Sci. Unit. States Am. 91, 5705–5709. https://doi.org/10.1073/pnas.91.12.5705.

Pradillo, J.M., Murray, K.N., Couss, G.A., Moraga, A., Orzon-Gonzar, F., Boutin, H., Moro, M.A., Lizasoain, I., Rothwell, N.J., Allan, S.M., 2017. Reparative effects of interleukin-1 receptor antagonist in young and aged/co-morbid rodents after cerebral ischemia. Brain Behav. Immun. 61, 117–126. https://doi.org/10.1016/j.bbi.2016.11.013.

Relton, J.K., Martin, D., Thompson, R.C., Russell, D.A., 1996. Peripheral administration of interleukin-1 receptor antagonist inhibits brain damage after focal cerebral ischemia in the rat. Exp. Neurol. 138, 206–213. https://doi.org/10.1006/exnr.1996.0059.

Sakuma, H., Tanuma, N., Kuki, I., Takahashi, Y., Shiomi, M., Hayashi, M., 2015. Intraperitoneal overproduction of proinflammatory cytokines and chemokines in febrile infection-related status epilepticus. J. Neurol. Neurosurg. Psychiatry. 86 (820). https://doi.org/10.1136/jnnp-2014-309388.

Shah, D.K., Betts, A.M., 2013. Antithrombin biological activity: Mabs. 5, 297–305. https://doi.org/10.4161/mbas.23684.

Sibley, C.H., Chioato, A., Felix, S., Colín, L., Chakraborty, A., Plass, N., Rodríguez-Smith, J., Brewer, C., King, K., Zalewski, C., Kim, H.J., Bishop, R., Abrams, K., Stone, D., Chapelle, D., Kost, B., Snyder, C., Butman, J.A., Wesley, R., Goldbach-Mansky, R., 2015. A 24-month open-label study of canakinumab in neonatal-onset multisystem inflammatory disease. Ann. Rheum. Dis. 74 (1714). https://doi.org/10.1136/annrheumdis-2015-204877.

Sibley, C.H., Plass, N., Snow, J., Wiggs, E.A., Brewer, C.C., King, K.A., Zalewski, C., Kim, H.J., Bishop, R., Hill, S., Paul, S.M., Kicker, P., Phillips, Z., Dolan, J.G., Widemann, B., Jayaprakash, N., Pucino, F., Stone, D.L., Chapelle, D., Snyder, C., Butman, J.A., Wesley, R., Goldbach-Mansky, R., 2012. Sustained response and prevention of damage progression in patients with neonatal-onset multisystem inflammatory disease treated with anakinra: a cohort study to determine three- and five-year outcomes. Arthritis Rheum. 64, 2375–2386. https://doi.org/10.1002/art.34409.

Skinner, R., Gibson, R., Rothwell, N., Pintelas, E., Penny, J., 2009. Transport of interleukin-1 across cerebrovascular endothelial cells. Br. J. Pharmacol. 156, 1115–1123. https://doi.org/10.1111/j.1471-4148.2009.05812.x.

Vandenhaute, E., Drolez, A., Sevin, E., Mysiorek, C., Dehouck, M.-P., 2016. Interleukin-1 receptor antagonist inhibits brain damage after focal cerebral ischemia in interleukin-1 type-1 receptor immunoreactivity: relationship to patterns of inducible cyclooxygenase expression by peripheral inflammatory stimuli. J. Comp. Neurol. 472, 113–129. https://doi.org/10.1002/cne.220052.

Kumar, R.G., Boles, J.A., Wagner, A.K., 2015. Chronic inflammation after severe traumatic brain injury. J. Head Trauma Rehabil. 30, 369–381. https://doi.org/10.1097/HTR.0000000000000676.

Lazovic, J., Basu, A., Lin, H.-W., Rothstein, R.P., Kandy, J.K., Smith, M.B., Leinson, S.W., 2005. Neuroinflammation and both cytotoxic and vasogenic edema are reduced in interleukin-1 type 1 receptor-deficient mice conferring neuroprotection. Stroke 36, 2226–2231. https://doi.org/10.1161/01.STR.0000182255.08162.6a.

Boroujerdi, M.R., Boroujerdi, M.R., Ahmadi, H., Abdi, K., Dehghan, M., 2018. The role of interleukin-1 in the pathogenesis of acute myocardial infarction. Int. J. Cardiol. 256, 66–73. https://doi.org/10.1016/j.ijcard.2017.10.072.

Carr, J., Carr, J., Yamasaki, Y., Matsuura, N., Shozuhara, H., Onodera, H., Itoyama, Y., Kogure, K., 1995. Interleukin-1 α in stroke: an update. Transl Stroke Res 7, 395–406. https://doi.org/10.1523/eneuro.0385-17.2018, 2018.