A Novel Peptide Agonist of Formyl-Peptide Receptor-Like 1 (ALX) Displays Anti-Inflammatory and Cardioprotective Effects

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ABSTRACT

Activation of the formyl-peptide receptor-like (FPRL) 1 pathway has recently gained high recognition for its significance in therapy of inflammatory diseases. Agonism at FPRL1 affords a beneficial effect in animal models of acute inflammatory conditions, as well as in chronic inflammatory diseases. TIPMFVPESTSKLQKFTSWFM-amide (CGEN-855A) is a novel 21-amino acid peptide agonist for FPRL1 and also activates FPRL2. CGEN-855A was discovered using a computational platform designed to predict novel G protein-coupled receptor peptide agonists cleaved from secreted proteins by convertase proteolysis. In vivo, CGEN-855A displays anti-inflammatory activity manifested as 50% inhibition of polymorphonuclear neutrophil (PMN) recruitment to inflamed air pouch and provides protection against ischemia-reperfusion-mediated injury to the myocardium in both murine and rat models (36 and 25% reduction in infarct size, respectively). Both these activities are accompanied by inhibition of PMN recruitment to the injured organ. The secretion of inflammatory cytokines, including interleukin (IL)-6, IL-1β, and tumor necrosis factor-α, was not affected upon incubation of human peripheral blood mononuclear cells with CGEN-855A, whereas IL-8 secretion was elevated up to 2-fold upon treatment with the highest CGEN-855A dose only. Collectively, these new data support a potential role for CGEN-855A in the treatment of reperfusion-mediated injury and in other acute and chronic inflammatory conditions.

Uncontrolled inflammation is a major component in the etiology of many diseases and pathological conditions. Abundant evidence substantiates a critical role for neutrophils in myocardial ischemia-reperfusion (I/R)-mediated injury (Vinten-Johansen, 2004). Neutrophils are recruited to the myocardial area at risk by proinflammatory signals during the very early phase of reperfusion. These activated neutrophils contribute to tissue damage by releasing proteolytic enzymes, cytokines, and reactive oxygen species. In accord- ance with these findings, several experimental therapies targeting neutrophil activation and/or recruitment reduced myocardial I/R injury in animal models. Among these, agonists of formyl-peptide receptor-like (FPRL) 1 display cardioprotective effects in models of I/R, in part by negative regulation of PMN activity (Leonard et al., 2002; Gavins et al., 2003, 2005; Bannenberg et al., 2004).

FPRL1, also known as ALX (lipoxin A₄ receptor) or CCR12, belongs to the formyl-peptide receptor (FPR)-related family of G protein-coupled receptors (GPCRs) that also includes FPR and FPRL2. It is expressed primarily on neutrophils and monocytes, and it is activated by a variety of endogenous and exogenous ligands, most of which are nonspecific (Le et al., 2002; Chiang et al., 2006). The prominent endogenous FPRL1 ligands are derivates of lipoxin, i.e., lipoxin A₄ (LXA₄) and the aspirin-triggered lipoxins (Bannenberg et al., 2004), as well as the glucocorticoid-regulated protein annexin 1 and its N-terminal-derived peptide Ac2-26 (Perretti et al., 1993). These ligands display anti-inflammatory properties via the FPRL1 pathway in various experimental animal models of acute and chronic inflammation, hence substantiating the therapeutic potential of FPRL1 agonists. Lipoxin- and an-
nexin 1-related molecules reduced inflammation induced by zymosan A in the air pouch (Perretti et al., 2002) and peritonitis (Bannenberg et al., 2004) models, and afforded protection against I/R-related damage in various organs, including heart, lung, kidney, bowel, cerebrum, and mesentery (Cuzzocrea et al., 1999; La et al., 2001; Leonard et al., 2002; Gavins et al., 2003, 2005; Bannenberg et al., 2004). In addition, these ligands are efficacious in models of asthma and pleurisy (Bandeira-Melo et al., 2000; Bandeira-Melo et al., 2005), whereas lipoxin derivates also ameliorated colitis induced by various agents, including dextran sulfate sodium, trinitrobenzene sulfuric acid, or aspirin (Fiorucci et al., 2002, 2004; Gewertz et al., 2002). The mechanism underlying the anti-inflammatory activity afforded upon FPRL1 activation by these ligands involves resolution of inflammation through differential regulation of leukocyte activity and life span. Activation of FPRL1 leads to inhibition of PMN migration, hence preventing neutrophil-mediated tissue injury while promoting nonphlogistic monocytes emigration that is not accompanied by degranulation, thereby allowing clearance of apoptotic cells by macrophage phagocytosis (Chiang et al., 2006).

The growing evidence supporting the anti-inflammatory and tissue-protective effects of FPRL1 ligands prompted us to search for novel ligands for this receptor. This was achieved using a computational biology discovery platform using machine learning algorithms designed to predict novel GPCR peptide ligands cleaved from secreted proteins (extracted from the Swiss-Prot protein database) by convertase proteolysis. Therefore, the ligands identified might also exist endogenously due to naturally occurring proteolysis. The predicted ligands were synthesized and screened for activation of 152 GPCRs by calcium flux and AMP assays. After intense screening efforts, a novel peptide agonist of FPRL1 and FPRL2 was discovered and designated CGEN-855A. CGEN-855A has no significant homology to known GPCR ligands and is highly specific to FPRL1 and FPRL2, of the 152 GPCRs screened, that also included the other member of the family, FPR (Shemesh et al., 2008). Herein, we investigated the FPRL1-CGEN-855A interaction focusing on anti-inflammatory and cardioprotective activities.

Materials and Methods

Peptide Synthesis. Peptide CGEN-855A was synthesized and purified in acetate salt by Sigma-Aldrich (Rehovot, Israel).

Radioligand Competition Binding Assay. The assay was performed by MDS Pharma Services (Taipei, Taiwan). In brief, purified membranes of CHO cells transiently transfected with FPRL1 were incubated at room temperature for 90 min, with 0.025 nM 125I-membranes of CHO cells transiently transfected with FPRL1 were formed by MDS Pharma Services (Taipei, Taiwan). In brief, purified anti-inflammatory and cardioprotective activities. member of the family, FPR (Shemesh et al., 2008). Herein, we to known GPCR ligands and is highly specific to FPRL1 and peptide agonist of FPRL1 and FPRL2 was discovered and des-

Calcium Mobilization Assay. CHO-K1 cells were transiently cotransfected with pcDNA3.1 constructs encoding Go16, and either FPR or FPRL1, using a lipid technique. Five hours later, the cells were replated into 96-well plates (60,000 cells/well), grown over-night, and loaded with Fluo4-NW (Invitrogen, Carlsbad, CA) according to the manufacturer’s recommendations. Fluorescence was mon-
tored by FlexStation plate reader (Molecular Devices, Sunnyvale, CA). Seventeen seconds following initiation of reading, cells were stimulated with the indicated agonist (prepared in PBS + 0.1% BSA) in triplicate.

Aequorin Assay. The assay was carried out by Euroscreen (Gosselies, Belgium). CHO-K1 cells stably expressing FPRL2, Go16, and mitochondrial apaoequorin were plated at 10^6 cells/ml in assay medium (Dulbecco’s modified Eagle’s medium-F12 medium + 0.1% BSA) and incubated with 5 mM coelenterazine H (Molecular Probes, Burlington, ON, Canada) overnight at room temperature. Cells were then washed in assay medium, resuspended, and plated onto 96-well plate at 10^5 cells/ml. The ligand was prepared in assay medium and added to the cells. Emission was recorded over 60 s by using an FDBS reader (Hamamatsu Photonics, Hamamatsu, Japan).

Neutrophil Infiltration into Murine Air Pouch. Male out-bred Swiss Albino mice (T.O. strain; Harlan UK Limited, Bicester, Oxon, UK), weighing −25 g, were used. Dorsal air pouches were raised by subcutaneous injection of 2.5 ml of sterile air 6 and 3 days before treatment. CGEN-855A and Ac2-26 (Perretti et al., 1993) were dissolved in sterile pyrogen-free PBS (Invitrogen, Carlsbad, CA) and administered intravenously at 200 µl (n = 8), followed immediately by an intrapouch challenge with 1 mg of zymosan A (Sigma-Aldrich, Steinheim, Germany). Alternatively, CGEN-855A or vehicle was administered into the pouch (in situ) in the absence of zymosan A challenge. Four hours later, lavage fluids were washed with 2 ml of ice-cold PBS containing 3 mM EDTA and kept on ice. An aliquot of the lavage fluid was stained for neutrophils with phcoerythrin-conjugated anti-Gr-1 monoclonal antibody (BD Biosciences Pharmingen, San Jose, CA) or iso-type control (rat IgG2b) and analyzed using FACSscan analyzer (BD Biosciences, Bedford, Cowley, UK).

Myocardial I/R Model in Mice. Male Albino mice (Harlan UK), weighing −30 g, were anesthetized, and left coronary artery (LCA) ligation was performed using a 7/0 silk suture (W593 7/0 BVl; Ethi-
on, Edinburgh, UK). After 25 min of myocardial ischemia, the LCA was reopened to allow reperfusion. Mice (n = 6, each group) were treated with CGEN-855A or vehicle (PBS) at 200 µl per mouse serum, and 10 µg/ml puromycin) for 2 weeks. Pools of stably trans-
fected cells were selected by puromycin resistance. Integration into the genome was verified by polymerase chain reaction using external primers resulting from the vector. Expression was validated by fluorescence-activated cell sorting analysis using anti-FPRL1 an-
tibodies (R&D Systems, Minneapolis, MN).
intravenously immediately after reperfusion. To assess the area at risk (AAR), the LCA was reoccluded 2 h after reperfusion, and Evans blue dye (1 mL of 2% w/v) was injected intravenously. The heart was cut into four to five horizontal slices. After removing the right ventricular wall, the AAR (unstained) and nonischemic (blue) myocardium were separated and weighed. The AAR is expressed as percentage of the total left ventricular (LV) weight. The infarct size was assessed by cutting the AAR into small pieces and incubating them with p-nitro-blue tetrazolium (0.5 mg/ml for 20 min at 37°C) and calculated as a percentage of necrotic tissue relative to the AAR mass.

**Plasma Troponin I Concentration.** Plasma was collected at the end of the reperfusion by centrifugation of whole blood at 4°C at 3000 rpm for 10 min. Plasma troponin I was quantified in duplicate by ELISA (BioQuant, Inc., San Diego, CA), according to manufacturer's instructions.

**Myocardial I/R Model in Rats.** Male Sprague-Dawley rats, weighing 370 to 380 g, were used. The LCA was occluded with a 6-0 prolene (Ethicon, West Somerville, NJ) ligature for 30 min and reperfused for 3 h. The rats (n = 9 or 5 for different experiments, as indicated) were treated intravenously with CGEN-855A or vehicle (saline) at 1 ml/kg, administered 5 min before reperfusion or postconditioning. Postconditioning was applied using an algorithm of 10-s reperfusion interrupted by 10 s of recollusion repeated for three cycles before full reperfusion (Kin et al., 2005). The LCA was reoccluded, and the AAR was delineated by injecting 1.5 ml of 20% Unisperse blue dye via the external jugular vein. The heart was excised and placed into 0.9% saline. The LV was separated from the remaining cardiac tissue and thinly (2 mm) cross-sectioned before separating the AAR (unstained) from the blue-stained nonischemic zone. The AAR was incubated for 10 min in a 1% solution of phosphate-buffered 2,3,5-triphenyltetrazolium chloride at 37°C, enabling assessment of the area at risk. The infarct size was calculated as a percentage of the AAR (area of necrosis/AAR).

**Detection of PMN by Immunohistochemistry.** After determination of AAR, the left ventricular tissue samples from nonischemic and ischemic zones were divided in half transversally, fixed in 4% paraformaldehyde for 1 h, and transferred to 15% sucrose overnight. The samples were embedded in optimal cutting temperature compound (O.C.T.; Sakura Finetek USA, Inc., Torrance, CA) and frozen in liquid nitrogen. Tissue samples (7 μm in thickness) were cut using a Hacker-Bright cryostat (Hacker Instruments & Industries Inc., Winnsboro, SC) and mounted onto coated VECTABOND (Vector Laboratories, Burlingame, CA) slides, refrozen, and stored at −80°C. The cryostat sections were incubated with monoclonal anti-rat CD18 antibody (BD Biosciences Pharmingen), washed in PBS, and incubated with a biotinylated horse anti-mouse IgG (Vector Laboratories, Burlingame, CA) and mounted onto coated VECTABOND (Vector Laboratories, Burlingame, CA) slides, refrozen, and stored at −80°C.

**PBMCs Preparation.** Citrated blood was obtained from healthy donors. Peripheral blood mononuclear cells (PBMCs) were isolated by centrifugation over equal volume of Histopaque 1077 (Sigma-Aldrich) at 800g for 15 min at 24°C. PBMCs were collected from the interface and washed with modified Hanks' balanced salt solution (250g for 10 min at 24°C) before resuspension in RPMI 1640 medium supplemented with 10% FCS.

**Cytokine Assays.** Freshly prepared PBMCs were suspended at 10^6 cells/ml in RPMI 1640 medium supplemented with 10% FCS, treated in duplicates with the indicated concentration of CGEN-855A or IL-1β for 24 h, and incubated at 37°C, 5% CO₂ in a humidified atmosphere. After 24 h, samples were centrifuged, and supernatants were collected and kept at −80°C until analyzed. The content of IL-6, IL-8, IL-1β, and TNF-α in the supernatants was analyzed, in duplicate, using ELISA (BioSource International, Camarillo, CA).

**Statistics.** All data are expressed as means ± S.E.M. All data were analyzed using SigmaStat 3.5 for Windows statistical software package (SPSS Inc., Chicago, IL). A one-way analysis of variance (infarct size, area at risk) was used, with post hoc analysis between groups using the Student-Newman-Keuls test correcting for multiple comparisons. A P value of less than 0.05 is considered significant.

**Results**

**CGEN-855A Competes with W Peptide for Binding to FPRL1.** CGEN-855A was tested for its ability to compete with 125I-WKYVMVm (W peptide), a high-affinity ligand of FPRL1 (Christophe et al., 2001), on its binding to membrane preparations from FPRL1 transiently transfected cells. CKβ8-1 (amino acids 46–137) was used as a positive control (Elagoz et al., 2004). The value of 0% inhibition (i.e., 100% binding of radioligand) was determined in the absence of either inhibitory peptide. CGEN-855A displaced the radiolabeled W peptide in a saturable manner, with an IC₅₀ value of 189 nM and a Kᵢ value of 54.1 nM (Fig. 1).

**CGEN-855A Activates FPRL1 and FPRL2 in a Dose-Dependent Manner.** A high and uniform expression of FPRL1 was detected in CHO cells that were stably transfected with FPRL1 but not in mock-transfected cells (Fig. 2A). Activation of these FPRL1-transfected cells with CGEN-855A resulted in an elevation of cell impedance index in a dose-dependent manner, with an EC₅₀ value of 381 nM (Fig. 2B). This activation was not observed after challenging mock-transfected cells with CGEN-855A.

CGEN-855A elicited a cellular response in cells expressing either FPRL1 or FPRL2 but not in FPR-expressing cells (Fig. 2, C and D). N-Formyl-L-methionyl-L-leucyl-L-phenylalanine and W peptide were included as positive controls for FPRL1 and FPR, respectively. Furthermore, CGEN-855A did not induce calcium flux in any of the other 149 GPCRs that were tested in the original screen leading to its identification, although these receptors responded to relevant positive controls (Shemesh et al., 2008).

**CGEN-855A Exhibits Anti-Inflammatory Activity in a Model of Acute Inflammation.** An intraportal challenge with zymosan A triggered a marked accumulation of neutrophils in the air pouch, as determined by fluorescence-activated cell sort-
Fig. 2. CGEN-855A specifically activates FPRL1 in a dose-dependent manner. A, CHO stably transfected with either FPRL1 (thick line) or mock vector (thin line) were stained with phycoerythrin-conjugated anti-human FPRL1 antibody or with IgG2b isotype control antibody (dashed line), and surface expression of FPRL1 was analyzed by FACScan (BD Biosciences). B, stable pools of FPRL1 were seeded on E-plates and stimulated with CGEN-855A at 25, 10, 3.3, 1.1, 0.37, and 0.12 μM. Mock-transfected cells were stimulated with 25 and 10 μM CGEN-855A. Cell impedance was recorded continuously in intervals of 71 s and presented as normalized CI. Insert presents normalized CI of FPRL1 (black bars) and mock-(white bars) transfected cells as mean ± S.D. of triplicates at one time point (12.5 min). C, CHO-K1 cells transiently transfected with either FPRL1 or FPR and G16 were loaded with Fluo4-NW. Calcium flux response was measured using FlexStation (Molecular Devices), upon cells stimulated with CGEN-855A at 1 μM. W peptide and N-formyl-L-methionyl-L-leucyl-L-phenylalanine (1 μM each) were included as positive controls for FPR and FPRL1, respectively. Assay was conducted in triplicates, mean ± S.D. is presented. D, CHO cells stably expressing FPRL2, G16, and mitochondrial apoaequorin were incubated with coelenterazine H and activated with CGEN-855A at 0.3, 1, 3, 10, 30, 100, 300, 1000, 3000, and 10,000 nM. Results are expressed as percentage of activation compared with the reference agonist.
ing analysis of Gr-1+ cells (Fig. 3). Administration of CGEN-855A at 50 and 200 μg/mouse (corresponding to 2 and 8 mg/kg) reduced the accumulation of neutrophils triggered by zymosan A by 48.8 and 23.3%, respectively (Fig. 3A). Statistical significance was achieved only for the group treated with 50 μg/mouse but not with 200 μg/mouse. Altogether, the extent of inhibition achieved after treating the mice with 50 μg/mouse CGEN-855A is comparable with that obtained by administration of Ac2-26 at 200 μg/mouse.

To validate that CGEN-855A does not elicit proinflammatory activity, we also tested its direct effect upon administration into the air pouch in the absence of zymosan A. As shown in Fig. 3B, intrapouch administration of 100 μg of CGEN-855A did not induce neutrophil recruitment into the air pouch when used alone.

**CGEN-855A Displays Cardioprotection in Animal Models of I/R-Induced Myocardial Infarction.** The inhibitory activity on neutrophil migration demonstrated by CGEN-855A in the air pouch model, prompted us to study its effect on I/R-induced myocardial injury. When administered intravenously at 30 or 60 μg/mouse (corresponding to 1 or 2 mg/kg, respectively) immediately before reperfusion, CGEN-855A afforded significant and dose-dependent cardioprotection, as illustrated by the reduction in infarct size (36% reduction at the highest dose, Fig. 4A). As expected, the AAR was similar in all groups, with AAR/LV values ranging between 50 and 52% (data not shown). In addition, plasma levels of troponin I, an established marker of myocardial damage, were also reduced in a dose-dependent manner (50% reduction at the highest dose; Fig. 4B), with a pattern mirroring that observed for reduction of infarct size.

In addition, a rat model of I/R was used to compare the cardioprotective effect of CGEN-855A to that of postconditioning, a mechanical maneuver defined as a series of brief (i.e., seconds) interruptions of reperfusion following a specific prescribed algorithm, applied at the very onset of reperfusion, that was shown to trigger cardioprotective responses to reperfusion injury in animal models and in clinical studies (Vinten-Johansen et al., 2007). Administration of CGEN-855A at 2 mg/kg reduced infarct size to a similar extent as postconditioning (Fig. 5A; 43.6 ± 2.9 and 41.2 ± 2.7%, respectively, compared with 57.0 ± 2.3% in the control group). Interestingly, the combination of CGEN-855A with postconditioning did not further reduce infarct size (44.6 ± 1.3%).

Finally, PMN accumulation in the AAR was analyzed to confirm that the cardioprotective activity provided by CGEN-855A is due to inhibition of PMN recruitment. CGEN-855A significantly attenuated PMN accumulation to the AAR compared with vehicle (30.1 ± 0.6 versus 43.2 ± 0.7 PMNs/high-power field) (Fig. 5, B and C). This attenuation was comparable with that achieved by postconditioning (34.8 ± 1.5).

**CGEN-855A Does Not Affect Cytokine Secretion by Human PBMCs.** The human and murine families of FPRs are diverse and might be differently affected by certain compounds. This is of special importance due to the apparent inconsistency in the effects mediated by FPRL1 agonists. Thus, we studied the effect of CGEN-855A on the secretion of inflammatory cytokines by human cells. Incubation of PBMCs with CGEN-855A at 0.25, 2.5, or 25 μg/ml (corresponding to 0.1, 1, and 10 μM) did not affect secretion of IL-6, IL-1β, or TNF-α (Fig. 6, A–C). A moderate elevation in IL-8 levels (up to 2-fold) was observed upon cells’ treatment with the highest dose CGEN-855A (Fig. 6D). IL-1β (100 ng/ml), which was used as positive control, induced high levels of cytokine secretion.

![Fig. 3.](image-url) CGEN-855A inhibits PMN migration into mouse air pouch inflamed with zymosan A. A, zymosan A (1 mg) was injected intrapouch immediately following intravenous treatment with either CGEN-855A, Ac2-26, or vehicle as indicated. Lavage fluid was collected after 4 h, stained with anti-Gr-1 antibody, and analyzed by FACScan (BD Biosciences). Irrelevant rat IgG2b antibody was used as isotype control. Shown is the number of Gr-1+ cells recovered in the lavage fluids (mean ± S.E.M.; n = 8). *P < 0.05 versus vehicle group. B, CGEN-855A (0.1 mg), zymosan A (1 mg), or vehicle was injected intrapouch. Lavage fluids were collected and analyzed as described in A.
Fig. 4. CGEN-855A reduces I/R-mediated myocardial injury in mice. Mice were subjected to 25-min ischemia followed by 120-min reperfusion, by LCA occlusion. Vehicle (PBS) or CGEN-855A were administered at indicated doses immediately after reperfusion. A, myocardial infarct was determined as described under Materials and Methods and expressed as percentage of AAR. Data presented as mean ± S.E.M.; n = 6. *, P < 0.05 and **, P < 0.01 versus vehicle group. B, plasma samples were tested for troponin I using ELISA. Values were extrapolated from a calibration curve and presented as mean ± S.D. of duplicates. *, P < 0.05 and **, P < 0.01 versus vehicle group.

Fig. 5. CGEN-855A reduces I/R-mediated myocardial injury and recruitment of PMNs in rats. Rats were subjected to 30-min ischemia followed by 180-min reperfusion, by LAD occlusion. Vehicle (saline) or CGEN-855A was administered 5 min before reperfusion, whereas postconditioning was applied immediately before terminal reperfusion. A, myocardial infarct was determined as described under Materials and Methods and expressed as percentage of AAR. Data presented as mean ± S.E.M.; n = 9. *, P < 0.05 versus vehicle group. B, accumulation of PMNs in the AAR tissue presented as mean ± S.E.M.; n = 5. *, P < 0.05 versus vehicle group. C, representative sections of AAR stained for accumulation of PMNs by immunohistochemistry using anti-CD11 and anti-CD18 antibodies are presented for each study group as indicated. Magnification, 200×.
Discussion

FPRL1 is a promiscuous receptor, activated in vitro by a variety of ligands that greatly vary in their biological features, including origin, nature, size, and specificity (Le et al., 2002). The biological activities induced by interaction of an individual ligand with FPRL1 are inconsistent, whereas some induce proinflammatory responses, others, namely, annexin 1 and Ac2-26 as well as LXA4, promote resolution of inflammation; the latter findings have underpinned current clinical programs aiming at discovering novel FPRL1 agonists for treatment of acute and chronic inflammatory conditions. We demonstrated here that CGEN-855A activates FPRL1 and displays important anti-inflammatory properties by reducing PMNs recruitment to inflamed sites. CGEN-855A produced a similar efficacy to that of Ac2-26 in the air pouch model but a lower dose was required to achieve similar degrees of inhibition (50 versus 200 μg/mouse corresponding to 20 and 80 nmol, respectively). The smaller reduction in PMN accumulation obtained with administration of higher doses of CGEN-855A might result from receptor desensitization and might indicate that even lower doses would be sufficient to exert important checkpoint functions on the experimental inflammatory response. Nevertheless, these results implied on the effective therapeutic range of CGEN-855A, and when administered at lower doses in the disease-related model, the I/R-induced myocardial infarction (Fig. 4; 10, 30, and 60 μg/mouse), a clear dose-dependent effect was observed both in infarct size and troponin levels in the plasma.

Due to the perplexing effect mediated via FPRL1, the possibility that CGEN-855A might elicit proinflammatory responses was eliminated because PMNs were not recruited to the air pouch upon intrapouch administration of CGEN-855A alone. Furthermore, although a moderate elevation in IL-8 secretion was observed upon treating human cells with the highest tested concentration of CGEN-855A, it did not induce any prominent elevation in secretion of the other proinflammatory cytokines tested, supporting a lack of proinflammatory, or activating downstream effects, upon human FPRL1 agonism by this compound. In addition, the biological implications of FPRL2 activation by CGEN-855A are difficult to foresee because the biological role of FPRL2 is unknown, and

Fig. 6. CGEN-855A does not affect cytokine secretion by PBMCs. PBMCs were incubated for 24 h with CGEN-855A at 0.25, 2.5, and 25 μg/ml (corresponding to 0.1, 1, and 10 μM). The levels of IL-6 (A), IL-1β (B), TNF-α (C), and IL-8 (D) in the supernatants were evaluated by ELISA. Presented are means ± S.E.M. of duplicate ELISA from duplicate assay samples of two donors. *, P < 0.05 and **, P < 0.01 versus untreated cells.
an endogenous agonist for FPRL2 was only recently reported (Gao et al., 2007). Although controversial, previous findings substantiate a role for PMNs and specifically neutrophils in the early stage of reperfusion-injury (for review, see Vinten-Johansen, 2004), and several studies describe experimental interventions aimed at inhibiting PMN recruitment at the time of reperfusion or shortly before. PMN inhibition was achieved by leukodepletion using neutrophil antiserum (Kin et al., 2006), or by antibodies targeting adhesion molecules such as P- and E-selectins (Lefer et al., 1994), CD11/CD18 (Ma et al., 1991), intercellular adhesion molecule-1 (Ma et al., 1992; Ioculano et al., 1994; Zhao et al., 1997, 2003), or platelet/endothelial cell adhesion molecule-1 (Gumin et al., 1996). These strategies provided up to ~50% reduction in infarct size. However, none of these anti-PMN approaches have been shown to consistently be effective in the clinic (Vinten-Johansen, 2004; Frangogiannis, 2006; Yelon and Hausenloy, 2007). In contrast, postconditioning has shown significant protection when applied to patients (Tissier et al., 2007; Thibault et al., 2008).

When tested in mouse and rat models of I/R, CGEN-855A afforded a significant cardioprotective effect manifested by reduced infarct size (by 36 and 25% in the mouse and rat models, respectively) that was further confirmed by reduced levels of troponin I in plasma (shown in mice). In accordance with the inhibitory effect of CGEN-855A on PMN recruitment to the inflamed site attained by the air pouch model, the cardioprotective activity of CGEN-855A in rats was accompanied by reduced recruitment of PMN to the AAR. The extent of cardioprotection obtained by CGEN-855A treatment is comparable with that observed by other groups using FPRL1 agonists in similar models (Gavins et al., 2005) and comparable with that observed with postconditioning. It is noteworthy that no additive protection was elicited upon combination of the two treatments. It is possible that both FPRL1 and postconditioning exert their effect through similar pathways, i.e., PMN-mediated injury. Indeed, studies report that postconditioning is associated with a reduction in PMN accumulation, adherence to coronary vascular endothelium, endothelial dysfunction, and cytokines relevant to the PMN recruitment process (Zhao et al., 2003; Halkos et al., 2004). Overall, these data might suggest that the apparently partial cardioprotective effect observed in these models of I/R is the maximal effect that can be achieved via inhibition of PMNs recruitment and that processes mediated by other cells, such as T cells (Varda-Bloom et al., 2000; Spagnoli et al., 2004), also play a role in the myocardial damage resulting from I/R.

Collectively, these data provide strong evidence that activation of the FPRL1 pathway could be beneficial for the treatment of acute and chronic inflammation. The results presented herein support further development of CGEN-855A as a potential candidate for therapeutic control of inflammatory diseases, in general, and for the treatment of reperfusion-related cardiovascular damage, in particular.

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