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Preclinical pharmacology, ocular tolerability and ocular hypotensive efficacy of a novel non-peptide bradykinin mimetic small molecule

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ABSTRACT

We sought to characterize the ocular pharmacology, tolerability and intraocular pressure (IOP)-lowering efficacy of FR-190997, a non-peptidic bradykinin (BK) B_2-receptor agonist. FR-190997 possessed a relatively high receptor binding affinity (K_i = 27 nM) and a high in vitro potency (EC_{50} = 18.3 ± 4.4 nM) for inositol-1-phosphate generation via human cloned B_2-receptors expressed in host cells with mimimal activity at B_1-receptors. It also mobilized intracellular Ca^{2+} in isolated human trabecular meshwork (h-TM), ciliary muscle (h-CM), and in immortalized non-pigmented ciliary epithelial (h-iNPE) cells (EC_{50}s = 167–384 nM; E_{max} = 32–86% of BK-induced response). HOE-140, a selective B_2-receptor antagonist, potently blocked the latter effects of FR-190997 (e.g. IC_{50} = 7.3 ± 0.6 nM in h-CM cells). FR-190997 also stimulated the release of prostaglandins (PGs) from h-TM and h-CM cells (EC_{50}s = 60–84 nM; E_{max} = 29–44% relative to max. BK-induced effects). FR-190997 (0.3–300 μg t.o.) did not activate cat corneal polymodal nociceptors and did not cause ocular discomfort in Dutch-Belted rabbits, but it was not well tolerated in New Zealand albino rabbits and Hartley guinea pigs. A single topical ocular (t.o.) dose of 1% FR-190997 in Dutch-Belted rabbits and mixed breed cats did not lower IOP. However, FR-190997 efficaciously lowered IOP of conscious ocular hypertensive cynomolgus monkey eyes (e.g. 34.5 ± 7.5% decrease; 6 h post-dose of 30 μg t.o.; n = 8). Thus, FR-190997 is an unexampled efficacious ocular hypotensive B_2-receptor non-peptide BK agonist that activates multiple signaling pathways to cause IOP reduction.

1. Introduction

The ocular hypertension associated with primary open angle glaucoma (POAG) is a well known risk factor, and permanent blindness results if the elevated intraocular pressure (IOP) is left untreated. POAG afflicts millions of people around the globe (Quigley, 1996; Congdon et al., 2004; Quigley and Broman, 2006), and even though various classes of drugs and surgical treatments exist that provide some level of clinical benefit (Sharif and Klimko, 2007), suitable new options to lower IOP are still sought that may provide appropriate efficacy with less local ocular and systemic side-effects. In this regard, a recent drug discovery search for new pharmacological compounds to lower IOP revealed that bradykinin (BK), a nine amino-acid peptide, lowered IOP in Dutch-belted rabbits when delivered to the inside of the eye by intravitreal (ivt) injection but not when dosed topically to the eye (Sharif et al., 2013a,b, 2014a,b). These studies helped eliminate the confusion that existed in the literature regarding the role of BK in causing or preventing ocular hypertension (Zeller et al., 1971; Chiang, 1974; Cole and Unger, 1974; Green and Elijah, 1981; Kaufman et al., 1982; Bynke et al., 1983; Llobet et al., 1999; Webb et al., 2006). Furthermore, since BK activates two known receptor sub-types (B_1 and B_2; Leeb-Lundberg et al., 2005) the identity of the receptor sub-type mediating the IOP-lowering effects of ivt BK in the rabbits needed to be determined. Indeed, the lack of ocular hypotensive activity of two different B_1-receptor-selective agonists (Des-Arg^9-BK; Sar-[De-Phe^9]-Des-Arg^9-BK), also injected via the ivt route in Dutch-belted rabbits, strongly suggested that B_2-receptor activation was responsible for the IOP reduction observed with BK (Sharif et al., 2013b, 2014a). However, BK being a peptide does not possess the necessary stability and ocular permeability for topical delivery agents needed for a drug candidate to be used as a therapeutically useful agent in the treatment of elevated IOP associated with

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glaucoma. Therefore, we sought and found a small molecule, organic non-peptidic BK receptor agonist, FR-190997 (Aramori et al., 1997; Sawada et al., 2004; Fig. 1) to conduct further studies. In the current report, we describe the various ocular pharmacological characteristics of FR-190997 and present data on its ocular irritation potential and ocular hypotensive properties in conscious cynomolgus monkey eyes rendered glaucomatous by laser-induced trabecular meshwork ablation. Some of this work was presented as an abstract at Association for Research in Vision and Ophthalmology (ARVO) (Sharif et al., 2013a).

2. Materials and methods

2.1. [^{3}H]-BK and [^{3}H]-des-Arg^{10}-kallidin receptor binding assays

Cell membranes of Chinese hamster ovary cells transfected with human cloned BK B_1-receptors (CHO-B1 cells) or B_2-receptors (CHO-B2 cells) (Euroscreen [Gosselies, Belgium]; Cerep Inc. [Le Bois L'Evegue, France]; Chantest Corp., Bethesda, MD, for custom expansion of cell cultures and cell homogenates) were incubated with either 0.35 nM [^{3}H]-des-Arg^{10}-kallidin or 0.1–0.2 nM [^{3}H]-BK (Perkin Elmer Corp., Cambridge, MA) in the absence or presence of unlabeled Des-Arg^9[Leu^8]bradykinin or BK (1 μM) in 96-well microtiter plates at 23 °C to label B_1-receptors and B_2-receptors, respectively. Aliquots of test agents or incubation buffer (50 μl) were added to various wells containing a total volume of 0.5 mL. The incubation was continued for 60 min and then the contents of the wells harvested over GF/B glass fiber filters previously soaked in 0.5% polyethyleneimine under rapid vacuum (Tomtec Inc.; Gaithersburg, MD). The filter-bound radioactivity was determined by liquid scintillation spectrometry and the data analyzed using a sigmoidal-fit, iterative curve-fitting computer program (Activity-Base®; IDBS, Surrey, UK) (Sharif et al., 2013b, 2014a,b). All cumulative data were represented as mean ± SEM. Origin Software® (Microcal Inc; Northampton, MD) was utilized to graph the data.

2.2. Ligand binding profiling assays

In order to ascertain the side-effect potential of FR-190997 it was profiled for its ability to bind to a battery of non-BK receptors (cell surface and intracellular), non-BK-related ligand binding sites, such as transmitter uptake sites, enzymes, ion-channels and immunological factors using 1 nM, 100 nM and 10 μM final concentrations. These studies were performed at Caliper LifeSciences [NovaScreen] (Hanover, MD) employing well document procedures as previously described (Sweetnam et al., 1993, 1995) and as can be found on the following websites for Perkinelmer, Euroscreen and Cerep: http://www.perkinelmer.com/services/contractresearch/default.xhtml; http://www.euroscreen.com; http://www.cerep.fr/Cerep/Users/index.asp.

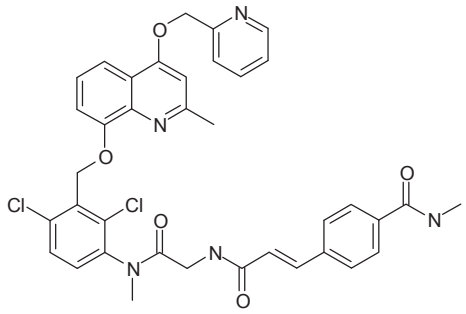

Fig. 1. FR-190997 chemical structure.

FR-190997 (Fig. 1), that originated from Fugisawa pharmaceutical company, was generously provided by Astellas Pharma (Tokodia, Tsukuba, Ibaraki, Japan).

2.3. Inositol-1-phosphate (IP_1) accumulation assay

An homogeneous time-resolved fluorescence (HTRF) assay was used in conjunction with cultured CHO-B1 and CHO-B2 cells expressing human cloned B_1- or B_2-receptors (Euroscreen Inc.; Cerep Inc. [Le Bois L'Eveque, France]; Chantest Corp. Cleveland, Ohia [http://www.chantest.com]). Some studies were conducted at Cerep Inc. (Le Bois L'Eveque, France) under a contract, while additional confirmatory studies were conducted at Alcon using cells produced by Chantest Corp. (Cleveland Ohio) under a contract. Cells were suspended in IP_1 stimulation buffer (containing HEPES 10 mM, $CaCl_2$ 1 mM, $MgCl_2$ 0.5 mM, KCl 4.2 mM, NaCl 146 mM, Glucose 5.5 mM, LiCl 50 mM pH 7.4) and then distributed in microtiter plates at a density of 40–80k cells/well (5k–20k cells/well yielded too small a signal). Assay buffer or compounds of interest were added to the cells at various concentrations as 50 μL aliquots and the incubation continued for 20 min at 37 °C. Following this incubation, the cells were lysed and the fluorescence acceptor (D2-labeled IP_1) and fluorescence donor (anti-IP_1 antibody labeled with europium cryptate) were added. After 60 min at room temperature, the fluorescence transfer was measured at λex = 337 nm and λem = 620 and 665 nm using a microplate reader. The results were expressed as a percent of the control response to 1 μM BK and appropriate concentration-response curves constructed to derive the respective agonist potency (EC_{50}; concentration needed to produce half-maximal response) values using Origin computer software (Microcal Software Inc., Northampton, MA) in an iterative, sigmoidal-fit mode. Mean ± SEM of the data were calculated thereafter.

2.4. Intracellular Ca^{2+} ($[Ca^{2+}]_i$) mobilization assay

$[Ca^{2+}]_i$ mobilization assays were performed as previously described (Kelly et al., 2003; Sharif et al., 2013b) using a Fluorescence Imaging Plate Reader (FLIPR-Tetra) in conjunction with a proprietary Ca^{2+}-sensitive dye (FLIPR Calcium Assay Kit) (Molecular Devices, Menlo Park, CA; http://www.moleculardevices.com/Products/Assay-Kits/GPCRs/FLIPR-Calcium.html). Isolated primary human ciliary muscle (h-CM; Husain et al., 2005), trabecular meshwork (h-TM, both normal and glaucomatous; Liu et al., 2001), and human immortalized non-pigmented ciliary epithelial (h-iNPE; Coca-Prados and Wax, 1986) cells of low passage number (2–8; Sharif et al., 2013b, 2014a,b) were grown to 80–90% confluency in 96-well microtiter plates (with bottom surface black). When human primary NPE cells were used they were purchased from ScienCell Research Laboratories, San Diego, CA) (http://www.sciencellonline.com/site/productInformation.php?keyword=6580) which characterized the cells using immunocytochemical techniques which were further supported by pharmacological techniques (Sharif et al., 2014b). All data were collated and mean ± SEM determined for each compound EC_{50} (concentration needed to achieve 50% of the maximum response), E_{max} (% max response relative to that elicited by BK [100%]) or IC_{50} (concentration needed to inhibit the maximum response by 50%).

2.5. Measurement of secreted prostaglandins (PGs)

Isolated cultured primary normal h-TM and h-CM cells of low passages (up to 6), as well as CHO-B2 cells, were distributed in 96-well microtiter plates, grown to ~95% confluency and then exposed to various concentrations of BK or FR-190997. The incubation was