RESEARCH PAPER

BGC20-1531, a novel, potent and selective prostanoid EP_4 receptor antagonist: a putative new treatment for migraine headache

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Background and purpose: Prostanoid EP_4 receptor antagonists may have therapeutic utility in the treatment of migraine since EP_4 receptors have been shown to be involved in prostaglandin (PG)E_2-induced cerebral vascular dilatation, which may be an important contributor to migraine pain. This study reports the pharmacological characterization of BGC20-1531, a novel EP_4 receptor antagonist.

Experimental approach: BGC20-1531 was characterized in radioligand binding and *in vitro* functional assays employing recombinant and native EP_4 receptors. Changes in canine carotid haemodynamics were used to assess the pharmacodynamic profile of BGC20-1531 *in vivo*.

Key results: BGC20-1531 exhibited high affinity at recombinant human EP_4 receptors expressed in cell lines (pK_B 7.6) and native EP_4 receptors in human cerebral and meningeal artery (pK_B 7.6–7.8) but showed no appreciable affinity at a wide range of other receptors (including other prostanoid receptors), channels, transporters and enzymes ($pKi < 5$). BGC20-1531 competitively antagonized PGE_2-induced vasodilatation of human middle cerebral (pK_B 7.8) and meningeal (pK_B 7.6) arteries *in vitro*, but had no effect on responses induced by PGE_2 on coronary, pulmonary or renal arteries *in vitro*. BGC20-1531 (1–10 mg·kg^{-1} i.v.) caused a dose-dependent antagonism of the PGE_2-induced increase in canine carotid blood flow *in vivo*.

Conclusions and implications: BGC20-1531 is a potent and selective antagonist at EP_4 receptors *in vitro* and *in vivo*, with the potential to alleviate the symptoms of migraine that result from cerebral vasodilatation. BGC20-1531 is currently in clinical development for the treatment of migraine headache.

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Introduction

Migraine is characterized by intermittent neurovascular headache with certain associated features, such as sensitivity to light, sound or movement, and often with nausea or vomiting (Goadsby and Olesen, 1996). Migraine affects 10–20% of the world population and is listed in the top 20 causes of disabling conditions, and in the top four neurological disabling conditions by the World Health Organization. This condition accounts for an estimated 30 million days of lost productivity, at a cost of up to $17 billion per annum in the USA alone (Hu *et al.*, 1999).

Migraine is a complex disorder and the mechanism underlying migraine attacks remains to be fully elucidated. While cerebrovascular dilatation has long been known to be associated with the aetiology of the disorder, its contribution to the

BGC20-1531: EP$_4$ receptor antagonist for migraine
KA Maubach *et al*
317

occurrence of head pain and associated aura remains to be established. Furthermore, extensive research in the field has led to additional theories as to the mechanistic basis of migraine, including the neurological (neuronal sensitization, particularly of the trigeminal nerve), and the neurogenic (dural inflammation resulting in the release of inflammatory neuropeptides) (Goadsby, 2007). There is now good reason to believe that cortical spreading depression is involved at least in the aura phase in migraine, although there is less evidence for its involvement in head pain (Richter and Lehmenkühler, 2008).

At present, the most commonly used therapies in the treatment of acute migraine are paracetamol, non-steroidal anti-inflammatory drugs (NSAIDs) and triptans (5-HT$_{1B/1D}$ receptor agonists) (Adelman and Adelman, 2001; Goadsby *et al.*, 2002). A recent survey assessing the level of satisfaction of patients with their current migraine therapy has revealed that a significant proportion of migraineurs do not experience sufficient relief of symptoms or are otherwise dissatisfied with current treatment (Bigal *et al.*, 2007). Thus, while triptans and NSAIDs are effective in some migraineurs, there remains a significant need for novel agents that can act via alternative mechanisms to (i) treat acute migraine attacks in patients for whom triptans and NSAIDs are unsuitable due to their adverse effect profile; (ii) treat acute migraine attacks in patients for whom triptans and NSAIDs are insufficiently effective; and (iii) complement current pharmacological therapy in patients who only experience partial relief with triptans and NSAIDs.

Prostaglandin E$_2$ (PGE$_2$) has long been recognized as an important mediator of pain and inflammation, and considerable clinical and experimental evidence implicates it in the pathogenesis of migraine. Elevated levels of PGE$_2$ occur in the jugular venous blood (Sarchielli *et al.*, 2000), plasma (Nattero *et al.*, 1989) and saliva (Vardi *et al.*, 1983; Tuca *et al.*, 1989) of migraineurs undergoing an attack. In peripheral blood monocytes isolated from migraineurs, nitric oxide, a potential migraine trigger, elevates PGE$_2$ release twofold over that observed in non-migraineurs (Stirparo *et al.*, 2000). Furthermore, intravenous infusion of prostaglandins can trigger migraine-like symptoms in migraineurs (Carlson *et al.*, 1968; Peatfield *et al.*, 1981). As previously indicated, the pain of migraine is associated with aberrant relaxation of cerebral and cranial blood vessels, and PGE$_2$ has been shown to be a potent and direct vasodilator of human cerebral and meningeal arteries (Davis *et al.*, 2004). In addition, *in vivo* and *in vitro* chemical stimulation of dura mater (Zimmermann *et al.*, 2002), trigeminal neurones (Jenkins *et al.*, 2001), afferent nerves (Hua *et al.*, 1994) and sensory afferents (Friese *et al.*, 1997; Boku *et al.*, 2001) cause PGE$_2$ release.

The actions of PGE$_2$ are mediated primarily via prostanoid EP receptors, of which there are four subtypes, EP$_1$, EP$_2$, EP$_3$ and EP$_4$, each of which exhibits nanomolar affinity for the endogenous prostanoid, PGE$_2$ (Coleman *et al.*, 1994a). While EP$_1$ and EP$_3$ receptors mediate smooth muscle contraction, EP$_2$ and EP$_4$ receptors have previously been shown to exert relaxant effects on vascular smooth muscle (Negishi *et al.*, 1995).

Rank orders of agonist potencies pointed to a role for EP$_4$ receptors in the cerebral vasodilatory response to PGE$_2$ in human middle cerebral and meningeal arteries (Davis *et al.*,
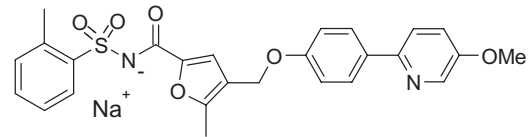

Figure 1 The chemical structure of BGC20-1531 (N-(4-[4-(5-methoxypyridin-2-yl)phenoxymethyl]-5-methylfuran-2-carbonyl)-2-methylbenzenesulphonamide sodium salt).

2004). Pre-treatment with EP$_4$ receptor antagonists (AH23848, EP$_4$A) caused concentration-related rightward, surmountable displacement of PGE$_2$ concentration-effect curves (Davis *et al.*, 2004). The antagonist potencies in blocking the effects of PGE$_2$ were consistent with their affinities for the human recombinant EP$_4$ receptor (Coleman *et al.*, 1994b).

In addition, although sensory neurons are capable of expressing multiple EP receptor subtypes, the use of reverse transcription polymerase chain reaction and antisense oligonucleotides of EP$_4$ mRNA, have shown that PGE$_2$-induced augmentation of peptide release and sensitization of sensory neurons are mediated by EP$_4$ receptors (Southall and Vasko, 2001). As PGE$_2$-induced cerebrovascular dilatation and neuronal sensitization may contribute to migraine pain, these data suggest that antagonists at EP$_4$ receptors may have therapeutic utility in the treatment of migraine.

Based on the hypothesis that EP$_4$ receptor blockade will prove useful in alleviating migraine pain, whether through its inhibition of cerebrovascular dilatation or its direct anti-hyperalgesic activity, a lead identification and optimization programme was initiated which resulted in the generation of a novel small molecule antagonist at EP$_4$ receptors, BGC20-1531 (N-(4-[4-(5-methoxypyridin-2-yl)phenoxymethyl]-5-methyl-furan-2-carbonyl)-2-methylbenzenesulphonamide sodium salt) (Figure 1). Here we describe, for the first time, the pharmacological characteristics of this molecule *in vitro* and *in vivo*.

Methods

Selectivity and specificity

Broad spectrum specificity screening of BGC20-1531 was carried out by Cerep SA (http://www.cerep.com/) against a range of seven transmembrane (7TM) receptors, transmitter-gated channels, ion channels, nuclear receptors, transporters and enzymes (nomenclature utilized conforms to the BJP's Guide to Receptors and Channels; Alexander *et al.*, 2008). The IC$_{50}$ values (concentration causing a half-maximal inhibition of control values), EC$_{50}$ values (concentration causing a half-maximal stimulation of control values) and Hill coefficients (nH) were determined by non-linear regression analysis of the concentration-response curves using Hill equation curve fitting. In each experiment, the respective reference compound was tested concurrently with BGC20-1531 in order to assess the assay suitability and the data were compared with historical values determined at Cerep SA.

Human recombinant EP$_4$ receptors – radioligand displacement binding

Membranes were prepared from cells stably transfected with human EP receptor cDNA. In brief, human embryonic kidney-

293 Epstein Barr nuclear antigen (HEK-293 EBNA) cells were cultured to confluency, scraped from culture flasks, and centrifuged (800× g, 8 min, 4°C). Cells were twice washed in ice-cold homogenization buffer containing (mmol·L⁻¹) 10 Tris-HCl, 1 ethylenediaminetetraacetic acid, 2 Na (EDTA), 250 sucrose, 1 phenylmethylsulphonyl fluoride, 0.3 indomethacin, pH 7.4, then homogenized and re-centrifuged as before. The supernatant was stored on ice and pellets re-homogenized and re-spun. Supernatants were pooled and centrifuged at 40 000× g for 10 min at 4°C. Resultant membrane pellets were stored at –80°C until use.

Membranes expressing human EP₄, EP₃ or EP₂ receptors were incubated in Millipore (MHVBN45) plates containing assay buffer [10 mmol·L⁻¹ 2-(N-morpholino)ethanesulphonic acid (MES) pH 6.0; 10 mmol·L⁻¹ MgCl₂; 1 mmol·L⁻¹ EDTA, 3 µmol·L⁻¹ indomethacin], radiolabelled [³H] PGE₂ and 0.1–10 000 nmol·L⁻¹ concentrations of compounds. Incubations were performed at 30°C for 60 min to allow equilibrium to be reached. Non-specific binding was determined in the presence of 10 µmol·L⁻¹ PGE₂. Bound and free radiolabel was separated by vacuum manifold filtration using appropriate wash buffers (10 mmol·L⁻¹ MES pH 6.0; 10 mmol·L⁻¹ MgCl₂), and bound radiolabel was determined by scintillation counting. The affinity or pK_i of each compound for each receptor was calculated from the concentration causing 50% radioligand displacement (IC₅₀) using the Cheng-Prusoff equation: $K_i = IC_{50}/(1 + ([[^3H] \ PGE_2]/KD)$.

Saturation analysis was also performed using 3 nmol·L⁻¹ PGE₂ or 10 nmol·L⁻¹ BGC20-1531 in the presence of increasing concentrations of radiolabelled [³H] PGE₂. Non-specific binding was determined in the presence of 10 µmol·L⁻¹ PGE₂. Saturation data were transformed in the form of a Scatchard plot.

Human recombinant EP₄ receptors – functional studies
The ability of BGC20-1531 to antagonize PGE₂-mediated functional responses mediated via the human EP₄ receptor was determined by measuring concentration-dependent PGE₂-stimulated cAMP accumulation.

Transfected HEK-293 cells were cultured at 37°C in a 5% CO₂ incubator, in 96-well poly-L-lysine coated plates at a density of 50 000 cells per well. Culture medium was Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% foetal bovine serum, 100 U·mL⁻¹ penicillin, 100 ng·mL⁻¹ streptomycin, 2.5 µg·mL⁻¹ fungizone, 2 mmol·L⁻¹ glutamine, together with an appropriate selection antibiotic, Zeocin (200 µg·mL⁻¹), hygromycin (200 µg·mL⁻¹) and geneticin (G418, 250 µg·mL⁻¹).

Cells were cultured to confluency (3–4 days) prior to use. Medium was removed and replaced with DMEM, containing 1 mmol·L⁻¹ 3-isobutyl-1-methylxanthine and BGC20-1531 (concentration range 0.1, 1.0 and 10 µmol·L⁻¹) or vehicle (0.1% DMSO) for 60 min, 37°C. PGE₂ (concentration range 0.0001 to 10 µmol·L⁻¹) was subsequently added for 15 min, 37°C, before the reaction was terminated by the addition of 1 mol·L⁻¹ HCl. Plates were stored at –80°C until assay, then rapidly thawed and samples neutralized by the addition of 1 mol·L⁻¹ NaOH. Plates were centrifuged (1000× g, 5 min) before the supernatant was transferred to a fresh 96 well plate,

containing assay buffer (in mmol·L⁻¹) 50 Tris, 10 EDTA, 0.1% bovine serum albumin, pH 7; 8 µg cAMP binding protein and ≈2 nmol·L⁻¹ [³H]cAMP, then incubated on ice for 2 h. Bound and free radiolabel were separated by rapid vacuum filtration and scintillation counting was used to determine bound radioligand. The amount of cAMP generated was determined from a standard curve. Comparison of the rightward displacement of the PGE₂-stimulated cAMP accumulation by BGC20-1531 was used to estimate the antagonist affinity (pK_B). The mean concentration ratio (CR) was plotted as log (CR-1) against log antagonist molar concentration, according to the method of Arunlakshana and Schild (1959). If the slope of the plot did not differ significantly from unity, it was constrained to unity to calculate an apparent pK_B value. Where only one concentration of BGC20-1531 was tested, a slope of unity was assumed and pA_2 estimated using the Gaddum-Schild equation (where $pA_2 = \log[CR – 1] – \log[antagonist]$).

EP₄ receptors in human cerebral and meningeal arteries in vitro
All samples of human tissue were obtained through medically qualified intermediaries at the Netherlands Brain Bank with the informed consent of the donor or donor's next of kin, and with approval of the Local Research Ethics Committee (LREC). Tissues were rapidly removed at autopsy, transported on wet ice in phosphate-buffered saline (PBS), and stored in Krebs' buffer at 4°C until the experiment, which was carried out within 72 h of tissue removal from the patient. Viable cerebral arteries were obtained from 19 donors (11 male, 8 female) with an age range of 59–88 years.

Sections of middle cerebral artery were carefully removed from samples of human cerebral vasculature containing an intact circle of Willis using sharp dissection scissors. Regions of tissue which appeared to be atherosclerotic on visual inspection were not used. Intact rings of middle cerebral arteries, 2–3 mm in length and 1–2 mm internal diameter, were suspended between stainless steel hooks in 10 mL organ baths containing oxygenated (95% O₂/5% CO₂) Krebs' buffer containing (in mmol·L⁻¹): NaCl 118.2, KCl 4.69, MgSO₄.7H₂O 1.18, K₂HPO₄ 1.19, glucose 11.1, NaHCO₃ 25.0, CaCl₂.6H₂O 2.5, indomethacin 0.003, pH 7.4 at 37°C. Tissues were placed under a tension equivalent to 5 mN and left to equilibrate for a period of at least 60 min. Responses were recorded using isometric transducers coupled to an Apple Macintosh computer via a MacLab interface. Following the equilibration period, a cumulative contractile concentration-response curve to phenylephrine (minimum of 5 min contact time of each concentration of agonist) was constructed in all tissues. After washout, an approximate EC₅₀ concentration of phenylephrine (1 µmol·L⁻¹) was added to obtain a stable contraction. Tissues were subsequently exposed to increasing concentrations of PGE₂, in the absence or presence of BGC20-1531 (incubated for 60 min prior to exposure to PGE₂). Untreated tissues were run in parallel to agonist-treated tissue, to serve as time-matched controls. Experiments on the relaxant effects of prostanoids were performed in the presence of the thromboxane (TP) receptor antagonist [1R-[1(Z),2,3,5]]-(+)-7-[5-([1,1'-biphenyl]-4-ylmethoxy)-3-hydroxy-2-(1-piperidinyl)cyclopentyl]-4-heptanoic acid (GR32191) (1 µmol·L⁻¹) (Lumley et al., 1989); as in the

BGC20-1531: EP$_4$ receptor antagonist for migraine
KA Maubach *et al*
319

absence of GR32191, PGE$_2$ caused vasodilatation at low concentrations but vasoconstriction at concentrations greater than 0.1 µmol·L^{-1} ($n = 3$, data not shown). The maximal relaxation of tissues was determined at the end of each experiment by the addition of prostacyclin (1 µmol·L^{-1}).

The ability of BGC20-1531 (1 µmol·L^{-1}) to reverse an established PGE$_2$-mediated relaxation in cerebral arteries was also determined. Tissues were pre-contracted with 1 µmol·L^{-1} phenylephrine before relaxation was induced by the addition of 100 nmol·L^{-1} PGE$_2$. BGC20-1531 (1 µmol·L^{-1}) was subsequently added to observe possible reversal of the PGE$_2$ – induced vasorelaxation.

Meningeal arteries were dissected from the dura mater and intact rings (internal diameter <1 mm) were mounted as previously described. Following the equilibration period, a cumulative concentration-response curve to phenylephrine (0.01–100 µmol·L^{-1}) was generated in all tissues. After washout, tissues were pre-incubated (60 min) with either BGC20-1531 (0.03, 0.1 or 1 µmol·L^{-1}) or vehicle (0.1% DMSO) and then pre-contracted with an ≈EC$_{70}$ concentration of phenylephrine (10 µmol·L^{-1}). Once a stable phenylephrine contraction was obtained, tissues were exposed to increasing concentrations of PGE$_2$ (0.001–100 µmol·L^{-1}, log units). Prostacyclin (1 µmol·L^{-1}) was added at the end of the PGE$_2$ concentration-effect curve and the responses to PGE$_2$ were normalized relative to the prostacyclin response.

Human coronary, pulmonary and renal arteries in vitro

All samples of human tissue were obtained through medically qualified intermediaries with the informed consent of the donor or the donor's next of kin, and with approval of the LREC. Tissues were transported on wet ice in PBS and stored in Krebs' buffer at 4°C until the experiment could be performed within 25 h of tissue removal from the patient. Coronary artery was obtained from five donors (four male, one female; age range 32–59), pulmonary artery was from four donors (two male, two female; age range 23–66) and renal arteries were obtained from four donors (four male; age range 37–63).

Sections of right proximal, distal and left coronary artery, pulmonary and renal artery were dissected from whole heart, lungs or kidneys, respectively, which were obtained from multi-organ donors. Sections of all arteries were cut into intact rings of approximately 2–3 mm in length (internal diameter 3–5 mm) and suspended between stainless steel hooks in 10 mL organ baths containing oxygenated (95% O$_2$/5% CO$_2$) Krebs' buffer, containing (in mmol·L^{-1}): 118.2 NaCl, 4.69 KCl, 1.18 MgSO$_4$·7H$_2$O, 1.19 K$_2$HPO$_4$, 11.1 glucose, 25.0 NaHCO$_3$, 2.5 CaCl$_2$·6H$_2$O, 0.003 indomethacin, pH 7.4 at 37°C. Responses were recorded using isometric transducers (Pioden, UK) coupled to a computer via a MacLab interface.

Tissues were placed under a tension equivalent to 10 mN and left to equilibrate for a period of at least 60 min. Tissue viability was subsequently assessed by challenge with 120 mmol·L^{-1} KCl and the generation of a cumulative contractile concentration-response curve to phenylephrine (0.001–100 µmol·L^{-1}). In coronary artery experiments, isoprenaline (1 µmol·L^{-1}) was also added at the start of each equilibration to reduce spontaneous activity. Tissues were

subsequently washed with Krebs' solution and pre-treated for 30 min with a TP receptor antagonist (either SQ29548 or GR32191; 1 µmol·L^{-1}). Tissues were either pre-contracted with a sub-maximal concentration of phenylephrine (1 µmol·L^{-1}) or left untreated prior to incubation with BGC20-1531 (1 µmol·L^{-1}) or DMSO (0.1%) for 30 min. A PGE$_2$ concentration-response curve (0.001–10 µmol·L^{-1}, log units) was subsequently constructed, followed by the addition of 1 µmol·L^{-1} PGI$_2$ (prostacyclin) and 120 mmol·L^{-1} KCl, to allow normalization of the response.

Canine native EP$_4$ receptors in vitro

All animal studies were conducted in accordance with Animals (Scientific Procedures) Act 1986, with UK Home Office Guidance on the implementation of the Act and with all applicable Codes of Practice for the care and housing of laboratory animals.

Organs were obtained from three male and three female beagle dogs (mean ± SEM ages 17.7 ± 0.6, 16.5 ± 0.9 months respectively). Brains and dura were transported in PBS and stored in Krebs' buffer at 4°C until the experiment could be performed, within 72 h of tissue removal.

Meningeal arteries were dissected from the dura mater and intact rings (outer diameter ≈300–500 µm) were mounted on 40 µm tungsten wires in Mulvany-Halpern wire myographs at a tension equivalent to that generated at 0.9 times the diameter of the vessel at 100 mm Hg. Arterial rings were maintained in a static 5 mL bath at 37°C in oxygenated (95% O$_2$/5% CO$_2$) Krebs' buffer containing (in mmol·L^{-1}): 118.2 NaCl, 4.69 KCl, 1.18 MgSO$_4$·7H$_2$O, 1.19 K$_2$HPO$_4$, 11.1 glucose, 25.0 NaHCO$_3$, 2.5 CaCl$_2$·6H$_2$O, 0.003 indomethacin, pH 7.4. Following several washes and an equilibration period, tissues were incubated with GR32191 (1 µmol·L^{-1}), as in its absence PGE$_2$ caused vasoconstriction at high concentrations. Viability was assessed by challenge with a single concentration of phenylephrine (10 µmol·L^{-1}). Following washout, a cumulative concentration-response curve to phenylephrine (0.01–100 µmol·L^{-1}, log units) was generated in all tissues. After a further washout and equilibration stage, tissues were pre-incubated for 60 min with either BGC20-1531 (0.03, 0.1 or 1 µmol·L^{-1}) or its vehicle (0.1% DMSO) and then pre-contracted with an ≈EC$_{50-70}$ concentration of phenylephrine (1–3 µmol·L^{-1}). Once a stable phenylephrine contraction was maintained, tissues were exposed to increasing concentrations of PGE$_2$ (0.01–10 µmol·L^{-1}). Prostacyclin (1 µmol·L^{-1}) was added at the end of the concentration-response curve. Responses to PGE$_2$ were subsequently normalized relative to the response to prostacyclin. A pA$_2$ estimate for BGC20-1531 was calculated using the Gaddum-Schild equation, where pA$_2$ = log [CR − 1] − log[antagonist].

Canine native EP$_4$ receptors in vivo – effects on PGE$_2$-mediated carotid vasodilator responses

Four beagle dogs (Marshall BioResources, North Rose, NY; 6.5 kg) were anaesthetized, intubated and artificially ventilated. For induction of anaesthesia, inhalant anaesthetic (sevoflurane) was delivered through a facemask. An i.v. catheter was placed and the animals were anaesthetized with a

320
BGC20-1531: EP₄ receptor antagonist for migraine
KA Maubach *et al*

bolus dose of pentobarbital (20 mg·kg^{-1} i.v.) followed by an intravenous infusion at a rate between 2 and 20 mg·kg^{-1}·h^{-1} (adjusted to maintain a proper plane of anaesthesia). Total volume flow was approximately 5 mL·kg^{-1}·h^{-1}. A midline incision was made on the ventral neck. The left common carotid artery exposed and a flow probe (Transonic) placed around the vessel to measure carotid artery blood flow. A side branch of the vessel (e.g. cranial thyroid) was isolated and a needle or cannula inserted for the injection of PGE$_2$ into the carotid artery. An incision was made to expose the femoral artery which was cannulated for measurement of arterial pressure and heart rate (HR). The femoral vein was cannulated for injection of vehicle and BGC20-1531. The animal was then allowed to stabilize for at least 30 min after instrumentation.

Blood pressure [systolic, diastolic and mean arterial pressure (MAP)], HR, carotid blood flow (CBF) and carotid vascular resistance were continuously recorded on a computerized data acquisition system.

After stabilization, PGE$_2$ was administered by bolus dosing at 0.02 µg·kg^{-1} directly into the carotid artery via the needle or catheter previously placed in a side branch. This dose was increased to 0.04 µg·kg^{-1} to achieve a measurable and reproducible response. Changes in MAP, HR and CBF response were recorded for 15 min. This process was repeated three times to establish the baseline response of each animal to intra-arterial PGE$_2$ injections. After the baseline response was established, the dose-response profile to BGC20-1531 or vehicle was determined. For each dose level, the dose of test drug was administered intravenously (femoral vein catheter) as a slow bolus over approximately 1 min. Within 10 min after dosing, a 3 mL blood sample was obtained. Following the removal of the blood sample, and 15 min after the previous PGE$_2$ challenge, the PGE$_2$ challenge was repeated. After each dosing episode and PGE$_2$ challenge, haemodynamics were allowed to stabilize and the process was repeated for each of the five dosing levels (vehicle and 1, 3, 10 and 10 mg·kg^{-1} i.v.). Following the final dosing episode, additional plasma samples were obtained at 25 and 35 min after the last dose was administered. The PGE$_2$ challenge was repeated immediately after obtaining the plasma samples.

Data analysis
All values shown in the text and figures are mean ± SEM. Differences between groups were tested by one-way ANOVA and unpaired two-tailed Student's *t*-test. *P* values less than 0.05 at 95% confidence level were considered significant.

Materials
PGE$_2$, prostacyclin, PGD$_2$, PGE$_1$-OH, 11-deoxy PGE$_1$ and PGF$_{2}$ were obtained from Alexis Corp, UK and GR32191 was a kind gift from GlaxoSmithKline, UK.

Results

Affinity, selectivity and specificity for human EP₄ prostanoid receptors
In membrane binding studies, BGC20-1531 displaced [^{3}H]-PGE$_2$ from human recombinant EP$_4$ prostanoid receptors
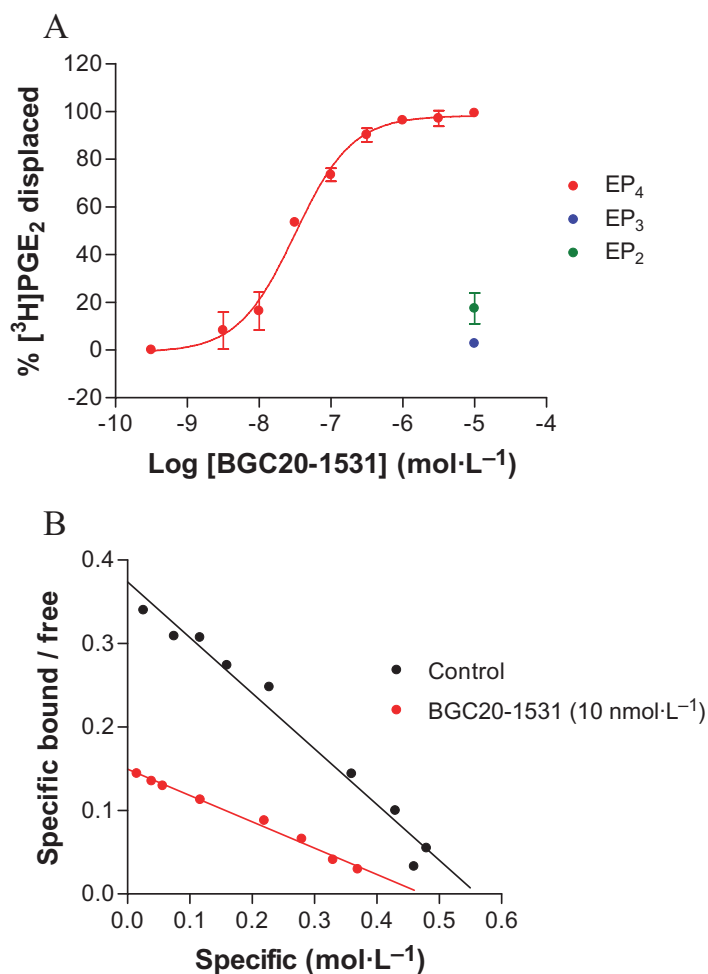

Figure 2 Radioligand displacement binding by BGC20-1531 at recombinant human EP$_4$, EP$_3$ and EP$_2$ receptors stably expressed in HEK-293 EBNA cells (A). Data are representative of mean ± SEM derived from three independent experiments (EP$_2$, EP$_3$, EP$_4$). Scatchard plot generated from [^{3}H]-PGE$_2$ saturation binding data, in the presence and absence of BGC20-1531 (10 nmol·L^{-1}; $n = 2$) (B).

(stably expressed in HEK-293 EBNA cells) with a pK$_i$ of 7.9 ± 0.1 (11.7 nmol·L^{-1}; $n = 3$, Figure 2A). BGC20-1531 exhibited negligible affinity for both EP$_2$ and EP$_3$ receptors, displacing 17.4 ± 6.5% and 2.7 ± 1.9% of [^{3}H]-PGE$_2$ at concentrations of 10 µmol·L^{-1} respectively (Figure 2A). Furthermore, BGC20-1531 displayed negligible affinity for other members of the prostanoid receptor family (mean pK$_i$ values derived from radioligand binding studies using human recombinant receptors as follows: EP$_1$ < 5, EP$_2$ < 5, EP$_3$ < 5, EP$_4$ 7.9, TP < 5, DP 5.6 and IP < 5). Scatchard analysis (Figure 2B) of the saturation binding of BGC20-1531 reveals a profile consistent with simple competitive binding with [^{3}H]-PGE$_2$ at the human recombinant EP$_4$ receptor.

In addition to the above human recombinant EP receptors, the affinity of BGC20-1531 for a range of 42 7TM receptors, nine transmitter-gated channels, eight ion channels, four nuclear receptors, five cell-surface transporters and 16 enzymes has also been tested.

Binding assays comprised those for the 7TM receptors adenosine A$_1$ (h), A$_{2A}$ (h) and A$_3$ (h), adrenoceptor α$_1$ (non-

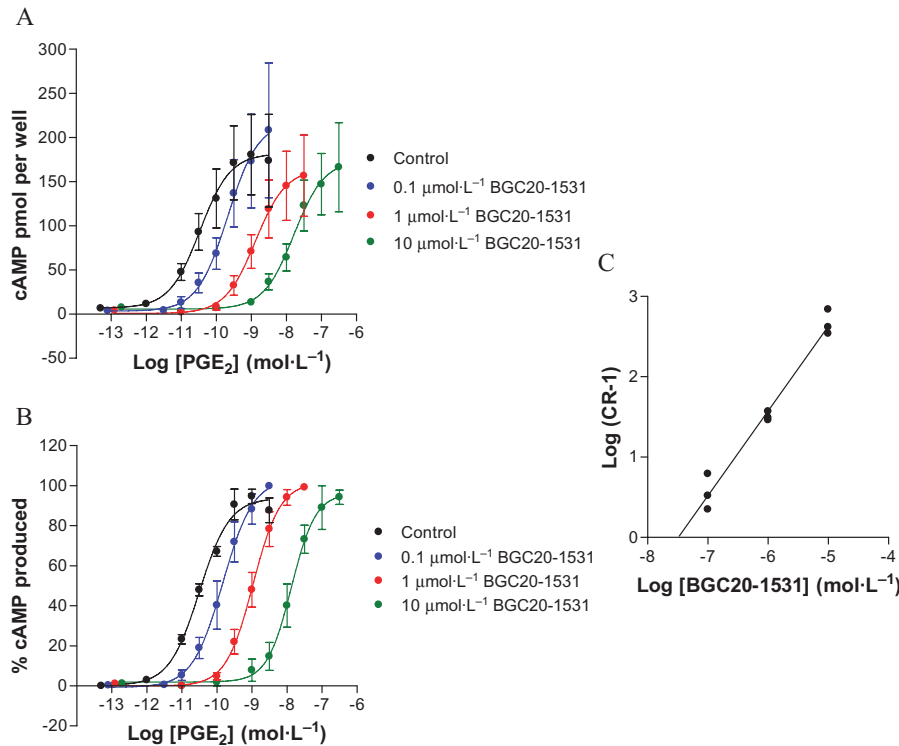**Figure 3** PGE$_2$-stimulated cAMP accumulation in HEK-293 cells stably expressing the EP$_4$ prostanoid receptor in the absence or presence of BGC20-1531. BGC20-1531 was pre-incubated with cells for 1 h prior to 15 min stimulation with PGE$_2$. Data are representative of mean $\pm$ SEM derived from three independent experiments at each concentration and are expressed as cAMP pmol per well (A) and percentage normalized to that obtained in response to maximal PGE$_2$ stimulation (B). Both these sets of data show a surmountable competitive antagonism of cAMP accumulation. In (C) is shown a Schild analysis of the antagonism of PGE$_2$-stimulated cAMP accumulation in transfected HEK-293 cells by increasing concentrations of BGC20-1531 (C). A best fit linear regression of unity is shown.

selective), α_2 (non-selective), β_1 (h) and β_2 (h), imidazoline I$_1$ and I$_2$, angiotensin AT$_1$ (h) and AT$_2$ (h), bradykinin B$_1$ (h) and B$_2$ (h), cannabinoid CB$_1$ (h) and CB$_2$ (h), cholecystokinin CCK$_1$ and CCK$_2$, corticotrophin-releasing factor CRF$_1$, dopamine D$_1$ (h), D$_2$ (h), D$_3$ (h) and D$_4$ (h), endothelin ET$_A$ (h) and ET$_B$ (h), histamine H$_1$ (h), H$_2$ (h) and H$_3$, leukotriene CysLT$_1$ (h), melanocortin MC$_4$ (h), acetylcholine muscarinic M (non-selective), tachykinin NK$_1$ (h), NK$_2$ (h) and NK$_3$ (h), neuropeptide Y (non-selective), opioid (non-selective) and N/OFQ (h), prostanoid TP and IP, P2Y, 5-HT (non-selective), thyrotrophin-releasing hormone TRH, and vasopressin V$_{1a}$ (h) and V$_2$ (h); the transmitter-gated channels, acetylcholine nicotinic $\alpha4$, benzodiazepine, GABA$_A$, glutamate (ionotropic) AMPA, kainate, NMDA and PCP, P2X; the ion channels, calcium Ca$_v$1.1, Ca$_v$1.2 and Ca$_v$1 potassium K$_{IR}$, K$_V$ and K$_{Ca}$, sodium Na$_v$1.2 and chloride CIC; the nuclear receptors, glucocorticoid GR (h), oestrogen ER (h), progesterone PR (h) and androgen AR (h); and the cell-surface transporters, monoamine NA (h), DA (h), and SERT (h), GABA GAT, and choline CHT.

Enzyme assays comprised those for the enzymes 3′,5′-cyclic nucleotide phosphodiesterases 1 (h), 2 (h), 3 (h), 4 (h) and 5 (h), adenylyl cyclase, guanylyl cyclase, protein kinase C, L-tyrosine hydroxylase, acetylcholinesterase (h), catechol-O-methyl transferase, GABA transaminase, monoamine oxidase(MAO)-A (h), MAO-B (h), phenylethanolamine-N-methyl transferase, and ATPase (Na$^+$/K$^+$).

Based on screening data at a concentration of 10 μmol·L^{-1}, BGC20-1531 caused less than 50% inhibition of binding (corresponding to a pK$_i$ < 5) at all targets described above (data not shown).

Antagonism of responses mediated by human recombinant EP$_4$ receptors in cell-based assays

In adherent HEK-293 EBNA cells that stably express the human EP$_4$ receptor, increasing concentrations of BGC20-1531 caused surmountable, competitive antagonism of PGE$_2$-mediated cAMP accumulation (Figure 3A and 3B). Schild analysis (slope of 0.95) resulted in an estimated mean pK$_B$ for BGC20-1531 of 7.6 (*n* = 3 at each concentration; Figure 3C). PGE$_2$ was also shown not to stimulate cAMP accumulation in non-transfected HEK-293 cells, demonstrating that PGE$_2$-induced cAMP accumulation is mediated via EP$_4$ receptor stimulation (*n* = 3, data not shown). Furthermore, at concentrations of 1 and 10 μmol·L^{-1}, BGC20-1531 had no effect on forskolin-stimulated cAMP accumulation in these cells, suggesting that BGC20-1531 acts as a specific antagonist of PGE$_2$ (*n* = 3, data not shown).

Antagonism of the effects of PGE$_2$ in human cerebral and meningeal arteries in vitro

Addition of phenylephrine (10 μmol·L^{-1}) caused reproducible contractions of isolated preparations of the human middle cerebral artery. Contractions observed at all phenylephrine
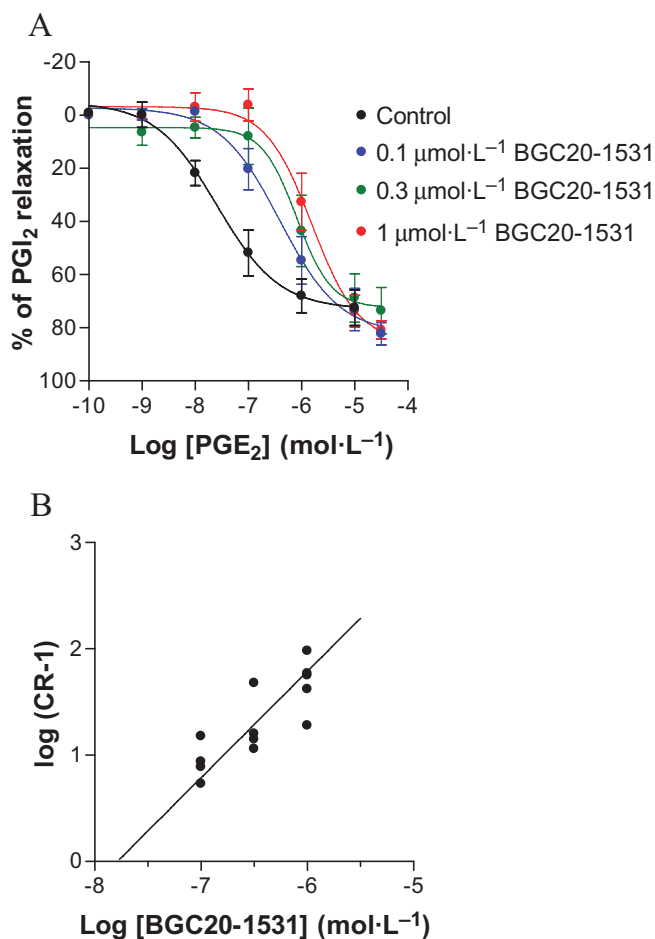
A

B

Figure 4 PGE$_2$-stimulated vasodilatation in human cerebral artery rings in the absence or presence of BGC20-1531. In (A), cerebral artery rings were pre-incubated with BGC20-1531 30 min prior to stimulation with PGE$_2$, and demonstrated surmountable competitive antagonism. Data are representative of mean $\pm$ SEM derived from 4–5 independent experiments at each concentration, and are normalized to the maximal relaxation caused by PGI$_2$. In (B), Schild analysis of the antagonism of EP$_4$ receptor mediated PGE$_2$-stimulated vasodilatation in human middle cerebral artery by increasing concentrations of BGC20-1531 is shown. Data are from 4–5 independent experiments at each concentration and a linear regression slope of unity is shown.

A

B

Figure 5 Effects of BGC20-1531 on PGE$_2$-stimulated relaxation of human middle meningeal artery rings. In (A), BGC20-1531 (1 or 10 μmol·L^{-1}) was pre-incubated with tissues 30 min prior to PGE$_2$ stimulation, and demonstrated surmountable competitive antagonism. Data obtained in the presence of 0.1 μmol·L^{-1} BGC20-1531 have been omitted for ease of graphical interpretation. Data are representative of mean $\pm$ SEM derived from 3–5 independent experiments at each concentration, and are normalized to the maximal relaxation to PGI$_2$. In (B), a Schild analysis of the antagonism of EP$_4$ receptor mediated PGE$_2$-stimulated vasodilatation in human middle meningeal artery by increasing concentrations of BGC20-1531 is shown. Data are from 3–5 independent experiments at each concentration and a linear regression slope of unity is shown.

concentrations were well maintained. Application of increasing concentrations of PGE$_2$ to cerebral artery rings pre-contracted with phenylephrine produced concentration-dependent relaxations to basal levels (Figure 4A).

In human middle cerebral arterial rings pre-contracted with phenylephrine (10 μmol·L^{-1}), BGC20-1531 antagonized PGE$_2$-mediated vasorelaxation with a pK$_B$ of 7.8 ($n = 4$–5 at each concentration). The antagonism appeared to be competitive (Figure 4A), and Schild analysis of the antagonism showed a slope not significantly different from unity (Figure 4B).

In human middle meningeal artery rings, again pre-contracted with phenylephrine (10 μmol·L^{-1}), BGC20-1531 antagonized PGE$_2$-mediated vasorelaxation with a pK$_B$ of 7.6 $\pm$ 0.1 ($n = 3$–5 at each concentration; Figure 5A). The antagonism was apparently competitive in nature, and Schild analysis of the antagonism showed a slope not significantly different from unity (Figure 5B).
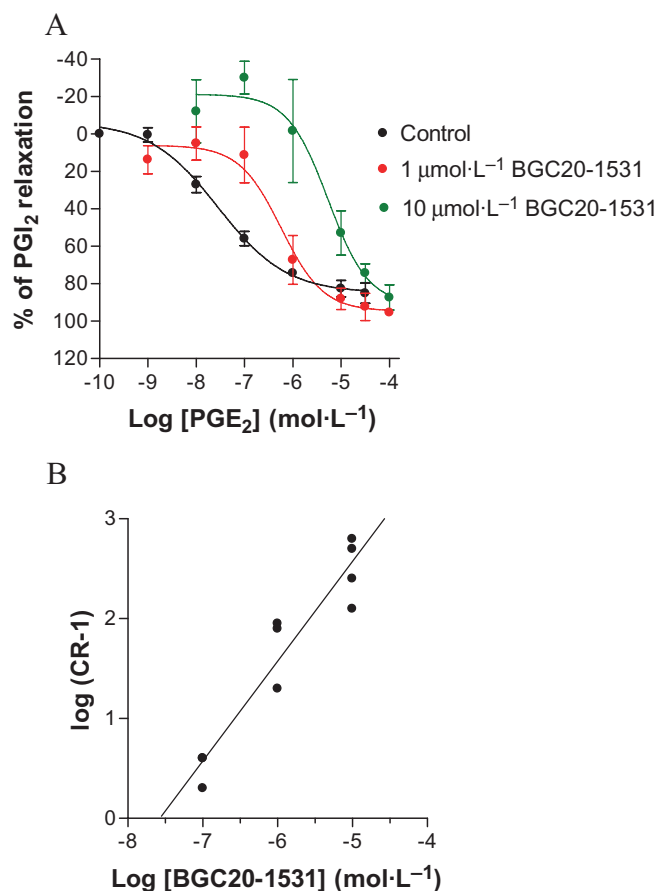
For BGC20-1531 to be useful in providing acute pain relief during an episode of migraine, it would be given after the point at which PGE$_2$ has begun to evoke vasodilatation of pain-sensitive cerebral vessels. Attempts were made to model this situation *in vitro* under conditions where PGE$_2$-induced relaxation of cerebral vessels was already established (more relevant to migraine). Under these conditions and as illustrated in Figure 6, BGC20-1531 (1 μmol·L^{-1}) demonstrated a rapid and complete reversal of an established vasorelaxant response to PGE$_2$ (100 nmol·L^{-1}) in human middle cerebral arterial rings (by 169 $\pm$ 34%, $n = 3$). A similar rapid reversal of an established PGE$_2$ response (100 nmol·L^{-1}) was observed with BGC20-1531 (1 μmol·L^{-1}) in human middle meningeal artery (by 95 $\pm$ 21%, $n = 3$). These data suggest that BGC20-1531 will offer pain relief after a migraine attack has commenced, if the pain is caused by PGE$_2$- induced cerebral vasodilatation.
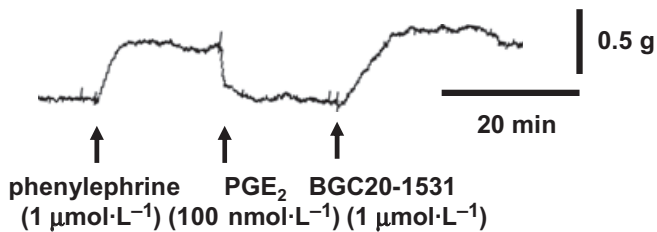
Figure 6 Reversal of an established PGE$_2$-mediated relaxation in rings of human middle cerebral artery, by BGC20-1531 (1 μmol·L^{-1}). The tissue was pre-contracted with phenylephrine (1 μmol·L^{-1}), before vasodilatation was induced by the addition of PGE$_2$ (100 nmol·L^{-1}). BGC20-1531 induced a rapid and complete reversal of the PGE$_2$ response (by 133%). The response to BGC20-1531 shown here is representative of data from three independent experiments.

In summary, BGC20-1531 is a potent antagonist of the human prostanoid EP$_4$ receptor. The affinity and potency of the antagonist demonstrated in cell lines expressing recombinant human EP$_4$ receptors (pK$_i$ = 7.9 and pK$_B$ = 7.6) are in line with the antagonist potency estimates at native human EP$_4$ receptors expressed in middle cerebral and meningeal arteries (pK$_B$ 7.6–7.8).

Potential for cardiovascular, pulmonary or renal side effects of BGC20-1531

In human coronary, pulmonary and renal arteries *in vitro*, PGE$_2$ was found to cause concentration-dependent vasoconstriction [pEC$_{50}$ (mol·L^{-1}) 7.2 ± 0.1, 7.8 ± 0.2, 6.4 ± 0.2 respectively], and not vasodilatation as observed in the cerebral and meningeal arteries. The magnitude of the response mediated by PGE$_2$ was greater in coronary than in pulmonary than in renal arteries. In all three vessel types, BGC20-1531 alone was shown to have no effect on basal or PGE$_2$-induced elevated tone (data not shown, n = 3–4 each preparation). BGC20-1531 also failed to exhibit any significant antagonism of PGE$_2$-induced vasoconstriction in coronary, pulmonary or renal arteries respectively (Figure 7).

Antagonism of the effects of PGE$_2$ in canine middle meningeal arteries in vitro

In preparations from each of four animals, PGE$_2$ caused concentration-dependent relaxation of phenylephrine pre-contracted (1–3 μmol·L^{-1}) canine middle meningeal arterial rings, exhibiting a mean pEC$_{50}$ of 7.6 ± 0.2 (EC$_{50}$ = 25 nmol·L^{-1}, Figure 8A,B). BGC20-1531 (0.1, 0.3 or 1 μmol·L^{-1}, 60 min incubation) showed competitive antagonism of PGE$_2$-induced relaxation of canine meningeal artery (Figure 8B), exhibiting a mean pA$_2$ of 7.7 (n = 8 tissues from four animals at three BGC20-1531 concentrations). Hence, the functional effects of BGC20-1531 at canine EP$_4$ receptors expressed in the middle meningeal artery are comparable to those observed in human vessels (pK$_B$ 7.6–7.8).

Antagonism of the effects of PGE$_2$ on canine carotid vascular bed in vivo

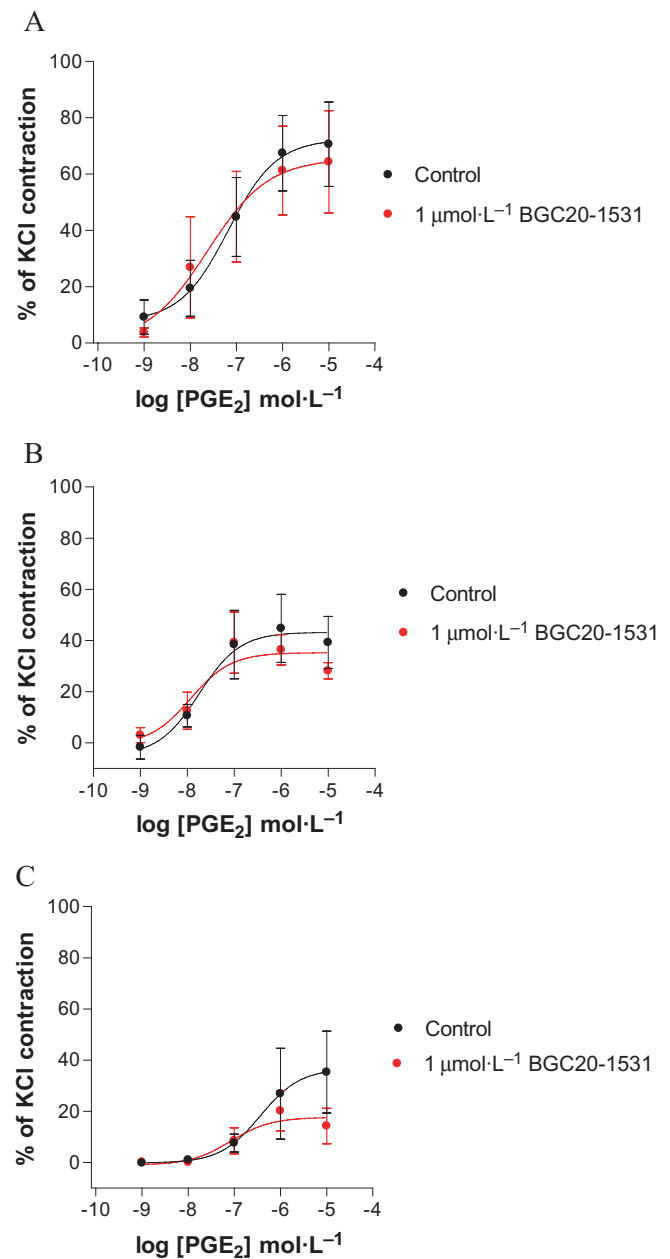Inhibition of PGE$_2$-induced haemodynamic responses in the canine carotid vascular bed was investigated as a suitable

Figure 7 PGE$_2$-stimulated contraction of rings from human (A) coronary, (B) pulmonary and (C) renal arteries in the absence or presence of BGC20-1531 (1 μmol·L^{-1}). Either vehicle (DMSO 0.1%) or BGC20-1531 were incubated with tissues 30 min prior to PGE$_2$ stimulation. BGC20-1531 had no effect on the PGE$_2$-mediated contraction of human coronary, pulmonary or renal arteries. Data are representative of mean ± SEM derived from four independent experiments and are normalized to the maximal contractile response observed to KCl (120 mmol·L^{-1}).

pharmacodynamic model and to establish an *in vivo* dose–response relationship of this novel EP$_4$ receptor antagonist.

Prostaglandin E$_2$ (0.04 μg·kg^{-1}, dose volume 0.5 mL·kg^{-1}) injected into the carotid artery caused an increase in CBF, with a corresponding decrease in carotid resistance (CR). Reproducible responses to repeated PGE$_2$ challenges were seen with mean reductions in CBF of 52%, 49% and 41% seen on the first, second and third applications, respectively; corre-
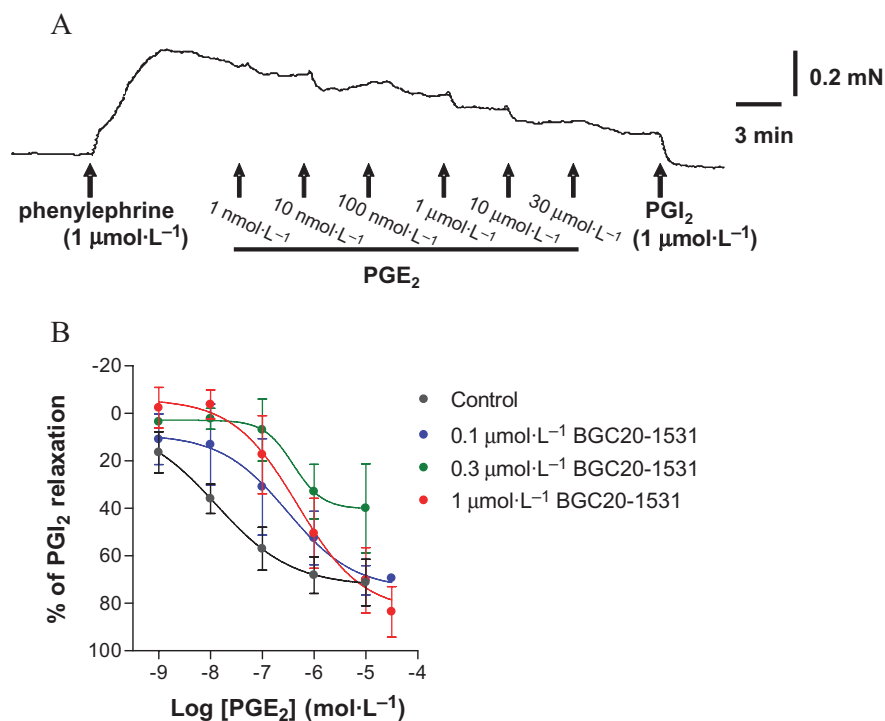
Figure 8 PGE$_2$ induced relaxation of rings of canine meningeal artery, pre-contracted with phenylephrine and antagonism by the EP$_4$ receptor antagonist, BGC20-1531. (A) Typical concentration-dependent relaxant responses to PGE$_2$ (1 nmol·L^{-1} to 30 µmol·L^{-1}) in phenylephrine pre-contracted canine middle meningeal artery rings *in vitro*. Representative data are shown from one of four independent experiments. (B) Concentration-dependent rightward shift of relaxant concentration-effect curves to PGE$_2$. Rings of meningeal artery were pre-incubated for 60 min with vehicle, 0.1 µmol·L^{-1}, 0.3 µmol·L^{-1} or 1 µmol·L^{-1} BGC20-1531, then cumulatively concentration-dependently relaxed with PGE$_2$. Data are expressed as percentage of the relaxation produced by prostacyclin (1 µmol·L^{-1}) and are expressed as mean ± SEM derived from four dogs.

sponding mean decreases in CR were measured to be 34, 31 and 28% respectively. These doses of PGE$_2$ had no effect on MAP, indicating that the dose and route of administration used in the study induced effects restricted to the carotid vascular bed.

There was no effect of BGC20-1531 on either MAP or HR. However, BGC20-1531 (1–10 mg·kg^{-1} i.v.) caused a dose-dependent antagonism of the PGE$_2$-induced increase in CBF and a corresponding antagonism of the PGE$_2$-mediated decrease in CR, with an ID$_{50}$ ≈ 5 mg·kg^{-1} (Figure 9). In contrast, there was no effect of the vehicle on either CBF or CR. Blood samples were taken at regular intervals throughout the study, each blood sample being taken prior to PGE$_2$ injection, and analysed for BGC20-1531 concentration by LC-MS. There was an apparent correlation between the plasma concentration of BGC20-1531 and the degree of antagonism of PGE$_2$-induced carotid vasodilatation. Therefore, BGC20-1531 displayed dose-dependent efficacy in this pharmacodynamic model.

Discussion and conclusions

The novel small molecule BGC20-1531 was identified through a lead identification and medicinal chemistry optimization programme. Emphasis was placed on the use of human recombinant receptors and disease relevant, ethically
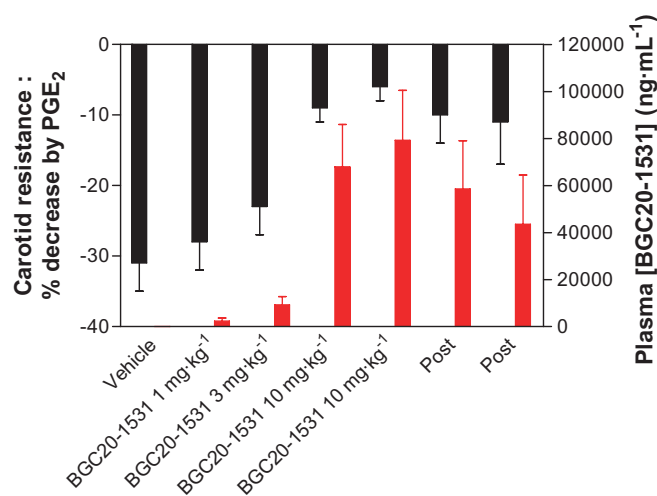

Figure 9 Effects of BGC20-1531 on PGE$_2$-induced changes in common carotid resistance in anaesthetized dogs. Resistance in the vascular bed served by the common carotid artery was decreased by close intra-arterial injection of PGE$_2$ (0.04 µg·kg^{-1}, 15 min apart), as shown on the left hand axis. Ten minutes after a dose of vehicle or BGC20-1531 (1, 3, 10 and 10 mg·kg^{-1} i.v.) and immediately before a test dose of PGE$_2$, blood samples were taken for measurement of BGC20-1531 levels in plasma (right hand axis). The effects of PGE$_2$ (decreased carotid resistance) were markedly reduced by 10 mg·kg^{-1} of BGC20-1531. As the plasma levels of BGC20-1531 fell (values marked Post), the effects of the test doses of PGE$_2$ returned. Carotid resistance is presented as % change from baseline and the plasma level of BGC20-1531 as ng·mL^{-1}; data are mean ± SEM ($n = 4$).

BGC20-1531: EP$_4$ receptor antagonist for migraine
KA Maubach *et al*
325

donated human tissues to investigate the pharmacological profile of BGC20-1531. Thus, radioligand binding studies confirmed nanomolar affinity for the human EP$_4$ receptor. Furthermore, at the recombinant human EP$_4$ receptor, BGC20-1531 was devoid of agonist activity and competitively antagonised PGE$_2$-mediated cAMP accumulation.

Importantly, *in vitro*, BGC20-1531 was also shown to be a potent antagonist of the vasodilator effects of PGE$_2$ on the vessels implicated in migraine pain. Thus, BGC20-1531 acts as a competitive antagonist of PGE$_2$ on middle cerebral and meningeal arteries. Moreover, BGC20-1531 rapidly reversed a pre-existing PGE$_2$-induced dilator response in such vessels, and hence is likely to provide pain relief during an episode of migraine that has already commenced, that is, where the PGE$_2$-induced cerebral dilatation and associated pain are already established. The possibility that antagonists at EP$_4$ receptors may have therapeutic utility in the treatment of migraine is strengthened by reports that both administration of an EP$_4$ receptor antagonist (AH23848) and EP$_4$ receptor knockdown in mutant mice result in attenuated inflammation-induced thermal and mechanical behavioural hypersensitivity (Lin *et al.*, 2006). More recently, another EP$_4$ receptor antagonist (CJ-023,423) has been shown to display anti-hyperalgesic effects in animal models of acute (mechanical hyperalgesia in the carrageenan model) and chronic inflammatory pain, induced by complete Freund's adjuvant (Nakao *et al.*, 2007). These observations suggest that blockade of EP$_4$ receptors may prove a valid target for the pharmacological treatment of inflammatory pain.

Inhibition of haemodynamic responses in the carotid vascular bed was utilized to investigate the pharmacodynamic profile of BGC20-1531. Previous work (Lumley *et al.*, 1982) indicated that PGE$_2$ induced increases in CBF in dogs, possibly reflecting dilatation within the cranial vasculature. The validity of this model to predict responses in human cranial vasculature was achieved through demonstrations both that PGE$_2$ relaxed canine cranial vessels, and that BGC20-1531 inhibited this relaxant effect in the same way as in human vessels. Meningeal arteries only were used in this study, as middle cerebral arteries from the dog proved too small to prepare in the same way as their human counterpart. Studies were then conducted in anaesthetized dogs to evaluate the pharmacodynamic properties of BGC20-1531, where external CBF was measured following intra-arterial administration of PGE$_2$ in the absence and presence of BGC20-1531. This study revealed that BGC20-1531 also behaves as a selective EP$_4$ receptor antagonist *in vivo*, causing dose- and plasma drug concentration-dependent antagonism of the increase in carotid artery blood flow seen in response to intra-arterial administration of PGE$_2$.

While no current experimental animal model of migraine completely reflects the pathophysiology observed in man, various models have been developed which reflect distinct facets of this clinically heterogeneous disorder (Eikermann-Haerter and Moskowitz, 2008). While these animal models have significant shortcomings, their use in order to profile existing treatments has contributed to promising new drugs that are now being developed and brought to the clinic. One such model is the measurement of drug-induced changes in

carotid haemodynamics (as described above), which has previously been used to characterize drugs believed to act in part via a vascular mechanism (Connor *et al.*, 1997; Kapoor *et al.*, 2003). It is encouraging therefore that BGC20-1531 has been shown to be effective in this model.

Triptans are generally recognized as the gold standard for pharmacological intervention in acute migraine, but while they remain a therapeutic and commercial success story, they are not without limitations. First, while they are relatively selective constrictors of the cerebral vasculature, which may contribute at least in part to their anti-migraine activity, they can also constrict coronary and pulmonary vasculature (Dodick, 2004), which may be responsible for the incidence in some patients of chest symptoms similar to angina. Indeed, in rare instances, triptans have been associated with myocardial infarction. For this reason, triptans are contraindicated in patients with ischaemic heart disease, uncontrolled hypertension and cerebrovascular disease (Papademetriou, 2004). The fact that BGC20-1531 did not cause constriction of human coronary, pulmonary and renal arteries, and that it had no effect on vasoconstriction mediated by PGE$_2$ suggests that BGC20-1531 will not have any effects on the vascular tone of coronary, pulmonary or renal arteries. These data are consistent with the view that, unlike the triptans, BGC20-1531 would also be acceptable for the treatment of migraine in patients with co-existing cardiovascular disease.

In migraineurs who are suitable for treatment with a triptan, efficacy is initially reasonably good, with approximately 60% of patients showing an improvement in headache at 2 h post-dose, and approximately 20% patients being pain free at the same time point (Ferrari *et al.*, 2001). However, studies measuring the effectiveness of the triptans in providing sustained pain relief have revealed relatively low results, ranging from 20.9% to 42.6% of the cases (Diener *et al.*, 2005; Loder *et al.*, 2005; Winner *et al.*, 2005; Cady *et al.*, 2006). It is interesting that a number of independent, double-blind, placebo-controlled clinical studies have demonstrated that NSAIDs (inhibitors of prostanoid synthesis) are as effective as triptans in alleviating migraine and tension headache (Diener, 1999; Kellstein *et al.*, 2000; Codispoti *et al.*, 2001; Dib *et al.*, 2002; Prior *et al.*, 2002). This not only suggests that prostanoids play a significant role in the aetiology of the migraine syndrome, but it also raises the possibility that EP$_4$ receptor antagonists which selectively block the action of prostanoids involved in the migraine cascade should exhibit efficacy as anti-migraine agents at least equal to that of triptans and NSAIDs.

Non-steroidal anti-inflammatory drugs act through inhibiting PG synthesis by inhibiting the activities of cyclooxygenase (COX) enzymes. Two isoforms of COX have been identified, COX-1 and COX-2. In the main, COX-2 is induced in the presence of inflammation, whereas COX-1 is generally constitutively expressed, and is thought to play an essential role for example in normal gastrointestinal and renal function. NSAIDs inhibit both isoforms; inhibition of COX-2 is primarily associated with the therapeutic effects of NSAIDs, while inhibition of COX-1 is thought to cause the adverse gastrointestinal effects such as gastric erosion, ulceration and haemorrhage which limit their use (Cryer, 2000). BGC20-

1531 is a highly selective antagonist at prostanoid EP_4 receptors, displaying negligible affinity for other prostanoid receptors (EP_1, EP_2, EP_3, TP, IP and DP), or a broad range of other receptors, ion channels, enzymes or transporters. This suggests that BGC20-1531 is likely to block the EP_4-mediated vasodilatation of cerebral vessels, as well as EP_4-mediated hyperalgesia, but is not likely to influence functions mediated by other prostanoid receptor subtypes. More specifically, BGC20-1531 is unlikely to antagonize the beneficial effects of PGs in the gastrointestinal tract, inhibition of which is known to result in gastric erosion, ulceration and haemorrhage (Araki *et al.*, 2000). Furthermore, there is a wealth of evidence for the anti-inflammatory effects of PGE_2 acting via EP_2 in a number of systems (Meja *et al.*, 1997; Aronoff *et al.*, 2004; Burelout *et al.*, 2004; Ahmad *et al.*, 2006), which would not be affected by BGC20-1531. Hence, BGC20-1531 is expected to show an improved adverse effect and efficacy profile compared with NSAIDs. Consistent with this hypothesis, an EP_4 receptor antagonist (CJ-42794), given orally, did not produce any damage in the rat gastrointestinal mucosa, while an NSAID (indomethacin) caused gross lesions (Takeuchi *et al.*, 2007).

One of the most promising additional approaches to migraine therapy is the development of calcitonin gene related peptide (CGRP) receptor antagonists. It is well established that there is a close relationship between CGRP and PGE_2. The release of CGRP from sensory nerve fibres results in the subsequent appearance of PGE_2 from the dura mater (Rich *et al.*, 1996; Ebersberger *et al.*, 1999). Conversely, PGE_2 can itself cause the release of CGRP, not only from cultured trigeminal neurones *in vitro* (Jenkins *et al.*, 2001), but also from sensory afferents *in vivo* (Friese *et al.*, 1997; Boku *et al.*, 2001). It is very interesting, therefore, that positive results in clinical trials with the CGRP receptor antagonists BIBN4096 (olcegepant) (Rudolf *et al.*, 2005; Doods *et al.*, 2007) and, more recently, MK-0974 (Ho *et al.*, 2008), have provided proof of a role for CGRP in migraine. The development of CGRP receptor antagonists for migraine is still in its infancy, but these early clinical results are encouraging.

In conclusion, BGC20-1531 is a potent and selective EP_4 receptor antagonist that has the potential to alleviate the symptoms of migraine caused by dilatation of cerebral blood vessels, with an improved side-effect profile in comparison with existing therapies. These data suggest that BGC20-1531 is suitable for clinical development as a 'first in class' approach for the treatment of migraine headache.

Acknowledgements

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