

The pharmacological effect of BGC20-1531, a novel prostanoid EP₄ receptor antagonist, in the Prostaglandin E₂ human model of headache

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Abstract Using a human Prostaglandin E₂ (PGE₂) model of headache, we examined whether a novel potent and selective EP₄ receptor antagonist, BGC20-1531, may prevent headache and dilatation of the middle cerebral (MCA) and superficial temporal artery (STA). In a three-way cross-over trial, eight healthy volunteers were randomly allocated to receive 200 and 400 mg BGC20-1531 and placebo, followed by a 25-min infusion of PGE₂. We recorded headache intensity on a verbal rating scale, MCA blood flow velocity and STA diameter. There was no difference in headache response or prevention of the dilation of the MCA or the STA ($P > 0.05$) with either dose of BGC20-1531 relative to placebo, although putative therapeutic exposures were not reached in all volunteers. In conclusion, these data suggest that the other EP receptors may be involved in PGE₂ induced headache and dilatation in normal subjects.

Keywords Prostaglandin E₂ · EP₄ receptor antagonist · BGC20-1531 · Vasodilatation · Headache

Introduction

The arachidonic acid metabolite prostaglandin E₂ (PGE₂) plays an important physiological role in the human body including the regulation of vascular tone [1] and modulation of pain [2]. PGE₂ acts via four different G-protein-coupled receptor subtypes: EP₁, EP₂, EP₃ and EP₄ [3]. Once activated by PGE₂, EP₁ and EP₃ receptors mediate Ca²⁺ mobilisation and decrease levels of cAMP, which leads to smooth muscle contraction [4]. In contrast, PGE₂ action on EP₂ and EP₄ stimulates adenylate cyclase and thereby causes relaxation of vascular smooth muscles [5]. It has been demonstrated that PGE₂-mediated vasodilatation of the human middle cerebral (MCA) and meningeal arteries (MMA) occurs primarily due to activation of the EP₄ receptors and the EP₄ receptor antagonist, AH 23848, is able to attenuate the PGE₂ vasodilating response [6]. PGE₂, as a principal pro-inflammatory prostanoid, plays a role in nociceptive processing [7]. It has both direct activating and sensitizing effects on sensory neurones [8]. Furthermore, increased levels of PGE₂ caused up-regulation of the EP₄ receptor subtype in rat sensory dorsal root ganglion (DRG) neurons, but not EP₁ and EP₃ receptor subtypes [9]. Given that sensitization of the sensory neurones mediated mainly through the EP₄ receptors [10] it has been suggested that the prostanoid EP₄ receptor may be a potential target for the treatment of pain [11].

A novel selective and potent EP₄ receptor antagonist, BGC20-1531, has been tested in an in vitro human study [12]. BGC20-1531 antagonized PGE₂-mediated dilatation of human middle cerebral and middle meningeal artery rings, pre-contracted with phenylephrine. It has therefore been suggested that BGC20-1531 has the potential to alleviate the symptoms of migraine pain caused by dilatation of cerebral arteries [12]. A human PGE₂ model of

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headache has been developed and has demonstrated that PGE₂ induces dilatation of cranial arteries and causes headache in healthy subjects [13]. Whether BGC20-1531 can block the PGE₂ induced responses in humans has previously not been studied.

The aim of the present study was to evaluate the effect of two different single oral doses of the EP₄ receptor antagonist BGC20-1531 on PGE₂-induced dilatation of cranial vessels and headache in a randomised, double blind, placebo-controlled, three-way intra-individual crossover study.

Methods

Design and subjects

The study was designed as a randomised, double blind, placebo-controlled, three-way intra-individual crossover study. Eight healthy volunteers (5 male and 3 female), mean age 24 years (range 21–30 years) and mean weight 75.7 kg (range 67–93.5 kg) completed the study. Subjects had no history or family history of migraine, or any other type of headache (except episodic tension-type headache less than once a month) and no previous serious somatic, psychiatric or infectious diseases. Physical and neurologic examination, electrocardiography (ECG), clinical-chemical and haematological screenings were done on the day of enrolment.

All subjects were randomly assigned to receive BGC20-1531 200 mg, BGC20-1531 400 mg or placebo, followed 75 min later by an infusion of PGE₂ at 0.40 µg/kg/min over 25 min on three different days at intervals of at least 1 week. The study drug BGC20-1531 and equivalent placebo were provided by BTG International Ltd, London, UK, and randomised and blinded by the central pharmacy, Herlev Hospital, Denmark. The randomization code was kept in the hospital during the study and the unblinding procedure was first performed after the study was completed. PGE₂ was purchased from Cayman Pharma, Neratovice, Czech Republic. The dose of PGE₂ (0.40 µg/kg/min) was selected based on a PGE₂-induced headache study in healthy volunteers [13].

The study protocol was approved by the Ethics Committee of the Country of Copenhagen (VEK H-D-2008-134), Danish Medical Agency (EudraCT 2008-008713-20) and Danish Data Protection Agency and performed in accordance with the Helsinki Declaration of 1964, as revised in Edinburgh in 2000. The study was registered on <http://www.clinicaltrials.gov>. All subjects gave written informed consent to participate in the study. The trial was conducted according to the protocol and Good Clinical Practice (GCP), and monitored externally by the GCP unit from the Copenhagen University Hospital.

Headache intensity and adverse events

To record headache intensity, a 10-point verbal rating scale (VRS) was used, where 0 indicated no headache; 1 indicated a different sensation, pounding or throbbing, but not necessarily painful; 5 indicated moderate headache and 10 indicated worst imaginable headache [14]. Subjects were encouraged to self-report any changes in their well-being during the study. Subjects were questioned about the presence of adverse events (AEs), headache and accompanying symptoms according to the International Headache Classification (IHC) [15] at T_{-75} , T_{-30} , T_0 and then every 10th min until T_{90} . During the out-of-hospital period, defined as a period after discharge and until bedtime, all subjects were carefully instructed to make hourly recordings of headache and accompanying symptoms according to IHC [15] and any other AEs. All AEs were classified as related or not related to the study drug by the investigator. Subjects were allowed to take rescue medication of their own choice after consulting the study physician.

Transcranial Doppler and C-scan

Blood flow velocity was recorded in the middle cerebral artery (V_{MCA}) by a Transcranial Doppler (TCD) ultrasonography (2 MHz) with handheld probes (Multidop X; DWL, Sipplingen, Germany) [13]. The recordings were performed bilaterally and simultaneously with measurements of end-tidal partial pressure of pCO₂ ($P_{et}CO_2$), obtained with an open mask without any respiratory resistance (ProPac Encore[®]; Welch Allyn Protocol, Beaverton, OR, USA) as previously described [16]. A fixed point was used with the best possible signal along the MCA, as close as possible to the bifurcation of the anterior cerebral artery and MCA. The fix point was marked and noted and was reused in each participant for all recordings. All measurements were done by the same skilled laboratory technician.

A high resolution ultrasound scanner, C-scan (20 MHz, bandwidth 15 MHz; Dermascan C; Cortex Technology, Hadsund, Denmark) was used to measure the diameter of the frontal branch of the left superficial temporal artery (STA) and the left radial artery (RA). All C-scans were performed in the same place as ensured by markings drawn on the skin. The coordinates of the marks were kept for reuse in the following trial days. All measurements within the same study subject were done by the skilled laboratory technician.

Pharmacokinetics

Blood samples for the plasma concentration of BGC20-1531 were collected at T_{-75} , T_0 , T_{30} , T_{60} and T_{90} on each

study day in Vacuette® Lithium Heparin 4 ml tubes (Greiner Bio-one, Austria). Samples were immediately stored on ice and then separated by centrifugation at $1,500\times g$ and 4°C for 10 min. Two identical aliquots of plasma were transferred in to polypropylene tubes (Sarstedt, Germany) and stored at -25°C until analyzed at Simbec Research Ltd, UK.

BGC20-1531 analytical methods

Plasma concentration of BGC20-1531 was determined by liquid chromatography with tandem mass spectrometry detection (LC-MS-MS). The analyses was performed using atmospheric pressure ionization with turbo ion spray followed by multiple reaction monitoring (MRM) of the characteristic ion transitions for BGC20-1531 and internal standard.

Trial procedure

Subjects were required to limit alcohol intake to 2 units per day for 7 days before the first dose and until the trial period was finished and to avoid alcoholic beverages entirely for 2 days prior to and 2 days after each treatment session. Subjects had to abstain from caffeine intake 2 days before the first dosing and until the end of the study and cocoa and chocolate were not allowed 24 h before the dosing day. All subjects were non-smokers. Use of pharmacologic agents apart from oral contraceptives were not permitted. Subjects fasted overnight and reported to the laboratory at 8 a.m. and were confirmed to be headache-free. Subjects rested in the supine position throughout the study period from time -75 min (start of study period, 75 min prior to the infusion) to T_{90} (end of study period, 90 min post infusion). The procedures were performed in a quiet room at room temperature between 21 and 24.7°C . Two intravenous catheters Venflon® (Becton Dickton, Sweden) were inserted into the antecubital veins for the PGE₂ infusion and blood sample collection for BGC20-1531 plasma concentration analysis. The subjects rested for at least 30 min before T_{-75} values of flow velocity in the middle cerebral artery (V_{MCA}), diameters of STA and RA, mean arterial blood pressure (MAP), heart rate (HR), $P_{\text{et}}\text{CO}_2$, transcutaneous arterial oxygen saturation (SAT), ECG, headache score and AEs were recorded.

Following the baseline measurements, subjects were randomized to BGC20-1531 200 mg, BGC20-1531 400 mg or placebo. At T_0 , the infusion of PGE₂ ($0.40\ \mu\text{g}/\text{kg}/\text{min}$) was initiated by a time and volume controlled infusion pump (Braun Perfuser, Melsungen, Germany). The timing of the infusion ensured that a steady state of BGC20-1531 was reached, as the T_{max} was predicted to be approximately 60 min.

All measurements were recorded at T_{-75} , T_{-30} , T_0 and then every 10th min until T_{90} . MAP and HR were measured

by an auto-inflatable cuff (ProPac Encore®; Welch Allyn Protocol, Beaverton, OR, USA). ECG was obtained continually using Cardiofax V (Nihon-Cohden, Japan) and recorded on paper at time as described above.

Statistics

Vascular variables are presented as mean \pm SD and as mean percentage from baseline. Headache scores are presented as median and quartiles. As we did not record any vascular or headache responses after BGC20-1531 administration during T_{-75} to T_0 baseline was defined as T_0 before start of PGE₂ infusion. Immediate headache was defined as any headache during the in-hospital period (0–90 min) and delayed headache (1.5–11 h) was defined as any headache during the out-of-hospital period. The data were baseline-corrected and the area under the curve for the time period T_0 – T_{90} (AUC) for V_{MCA} , headache score, MAP, HR and PetCO_2 was calculated, using the trapezium rule [17].

The sample size was calculated based on proven difference between treatments, measured as reduced pain intensity on the VRS at 5% significance (one-sided) with 90% power. We assumed 20% deviation on the VRS for each study subject and 70% reduction of pain intensity was considered to be clinically significant, therefore 8 subjects were included [18].

The primary end-points were differences in the AUC for headache score ($\text{AUC}_{\text{headache score}}$) between active and placebo arm, placebo versus BGC20-1531 200 mg and placebo versus BGC20-1531 400 mg. The secondary end-points were differences in the AUC for VMCA (AUC_{VMCA}), STA (AUC_{STA}), RA (AUC_{RA}), PetCO_2 ($\text{AUC}_{\text{PetCO}_2}$), MAP (AUC_{MAP}) and HR (AUC_{HR}) between placebo and two active treatment arms. To test the statistical difference between the variables we applied a paired, two-way *t* test for vascular data, the Wilcoxon signed ranks test for headache score, and the McNemar test for AEs. To explore possible changes over time for vascular variables we conducted post hoc analysis by repeated measures one-way ANOVA (including the Dunnett post hoc test).

Five percent ($P < 0.05$) was accepted as the level of significance. All analyses were performed with PASW Statistics 18 for Windows (SPSS Inc., Chicago, IL, USA). Post hoc exploratory analyses were performed using GraphPad Prism® (GraphPad Software Inc., CA, USA).

Results

Eight healthy volunteers completed the study. 11 subjects were enrolled with 3 participants being withdrawn after the first day of dosing. One was withdrawn due to severe chills and shivering during PGE₂ infusion, another due to an

unspecific T-wave inversion in the pre-cordial leads on ECG and the third due to a drop in diastolic blood pressure below 40 mmHg, which was a safety limit according to the study protocol.

Baseline values

There were no differences in baseline recordings for any variables between placebo and active days. There were no differences in baseline velocity in the middle cerebral artery (V_{MCA}) between the left and the right side on all three study days (data not shown).

Effect of BGC20-1531 on PGE₂-induced headache

The incidence of immediate and delayed headache is shown in Table 1. There was a large variation in the severity of headache between the subjects on placebo day and we found no difference in area under the curve (AUC) for headache between both pretreatment days and placebo day (BGC20-1531 200 mg: $P = 0.14$; BGC20-1531 400 mg: $P = 0.173$) (Fig. 1).

Effect of BGC20-1531 on velocity of middle cerebral artery

We found no difference in the AUC_{VMCA} between placebo and BGC20-1531 200 mg ($P = 0.849$) and 400 mg ($P = 0.529$) (Fig. 2). There was no difference in the AUC for end-tidal partial pressure of pCO₂ (PetCO₂) between both pretreatment days and placebo day (BGC20-1531 200 mg: $P = 0.700$; BGC20-1531 400 mg: $P = 0.712$). Explorative ANOVA analysis revealed significant changes over time in V_{MCA} after placebo ($P < 0.05$) but not after BGC20-1531 200 and 400 mg ($P > 0.05$). As expected, post hoc Dunnetts test showed a significant drop in V_{MCA} at T_{20} after PGE₂ infusion on placebo day compared to baseline ($P < 0.05$).

Table 1 Incidence of Prostaglandin E₂ (PGE₂)-induced immediate and delayed headache in eight healthy subjects

| | Placebo plus PGE ₂ | BGC20-1531 200 mg plus PGE ₂ | BGC20-1531 400 mg plus PGE ₂ |
|---------------------------------|-------------------------------|---|---|
| Incidence of immediate headache | 6 | 6 | 7 |
| Incidence of delayed headache | 1 | 1 | 1 |

McNemar test showed no difference in incidence of immediate and delayed headache between placebo and BGC20-1531 200 and 400 mg ($P > 0.05$)

Effect of BGC20-1531 on diameter of superficial temporal and radial arteries

The superficial temporal artery AUC (AUC_{STA}) on BGC20-1531 200 mg day was significantly larger than on placebo day ($P = 0.033$). We found no difference in the AUC_{STA} between placebo and BGC20-1531 400 mg day ($P = 0.451$) (Fig. 3). There was no difference in the radial artery AUC (AUC_{RA}) between each pretreatment day compared with placebo day ($P = 0.678$ and $P = 0.575$ on BGC20-1531 200 mg and BGC20-1531 400 mg pretreatment day respectively).

Peripheral hemodynamics

We found no difference in the AUC for mean arterial blood pressure (AUC_{MAP}) between placebo and BGC20-1531 200 mg day ($P = 0.267$) and placebo and BGC20-1531 400 mg day ($P = 0.450$). There was also no difference in the AUC_{HR} on placebo day compared with the AUC_{HR} on BGC20-1531 200 mg ($P = 0.799$) day and 400 mg day ($P = 0.074$).

Pharmacokinetic profile of BGC20-1531

The highest plasma concentration of BGC20-1531 in our study was detected 75 min after oral administration of BGC20-1531 200 and 400 mg at T_0 (Fig. 4). No plasma BGC20-1531 was detected in samples taken on placebo day. The AUC plasma concentration on pretreatment with BGC20-1531 400 mg was significantly larger compared to the AUC plasma concentration on BGC20-1531 200 mg ($P = 0.036$) (Fig. 4). Putative therapeutic concentrations of $\geq 10,000$ ng.hr/ml were only reached in 5 out of 8 subjects.

Effect of BGC20-1531 on PGE₂ related AEs

We found no difference in incidence of the AEs between the trial days (Table 2). No adverse events were reported during the pre-infusion period $T_{-75}-T_0$ except one participant who had an asymptomatic T-wave inversion on ECG during pre-infusion period. The finding was defined by cardiologist as non-specific, but a decision was taken to exclude the participant from further experiments.

Discussion

To our knowledge, this is the first study where a potent and selective EP₄ receptor antagonist, BGC20-1531, has been tested in a human model of headache. The main result was that the specific EP₄ receptor antagonist did not prevent PGE₂ induced headache in normal volunteers in this study.

Fig. 1 Median (filled square) and individual headache scores on a verbal rating scale (VRS) on placebo compared to either pretreatment day with BGC20-1531. The median peak immediate headache score was 2 at T_{20} on placebo day, 1.5 at T_{30} on BGC20-1531 200 mg day, and 1 at T_{20} on BGC20-1531 400 mg day. No difference between AUC headache score on placebo and either BGC20-1531 pretreatment was found (Wilcoxon signed ranks test)

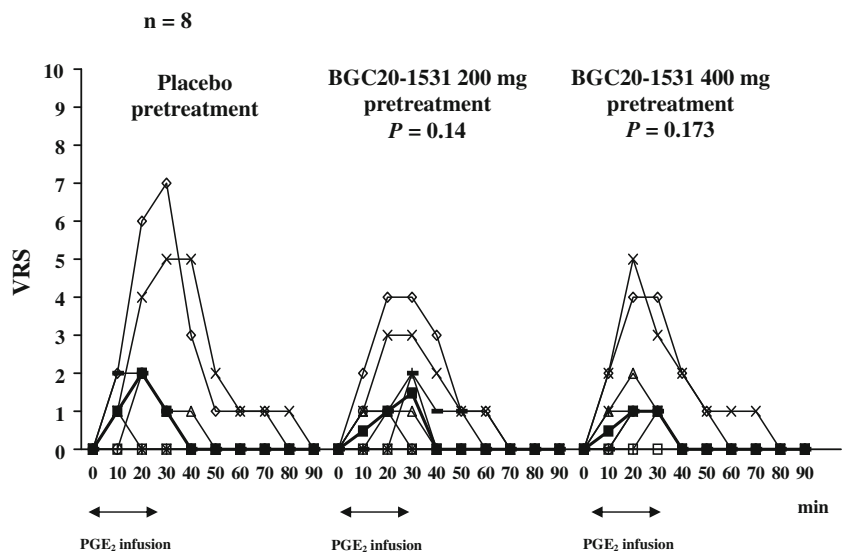
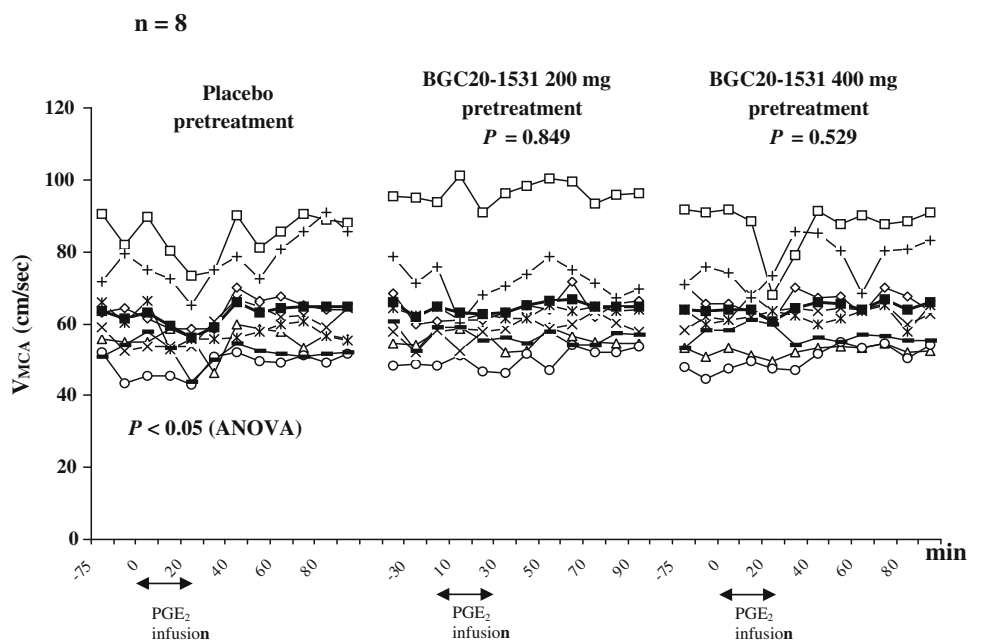


Fig. 2 Individual and mean (filled square) flow velocities (V_{MCA}) in the middle cerebral arteries (cm/s) in the middle cerebral arteries (V_{MCA}) on placebo day compared with BGC20-1531 before and after Prostaglandin E_2 (PGE_2) infusion. There was no difference between $AUC_{V_{MCA}}$ on placebo and BGC20-1531 200 or 400 mg pretreatment (paired t test)



PGE_2 plays an important role in the regulation of cerebral haemodynamics [19]. In vitro studies have shown that PGE_2 induces dilatation of human MCA and MMA [6, 12]. Similar results have also been obtained in cerebral and cranial arteries of animals [12, 20, 21]. However, in vivo animal studies have yielded conflicting results. An open cranial window model demonstrated that topical application of PGE_2 caused dilatation of small and large pial arterioles in cat [22]. In a closed cranial window model, it caused dilation of the pial arteries in newborn pigs [23]. PGE_2 -induced dilatation of canine common carotid artery was also reported [12]. In the closed cranial window model, intracarotid administration of PGE_2 caused dilatation of dural arteries but not pial arteries [20]. The

conflicting data on whether or not PGE_2 causes dilatation of arteries in different animal's models may critically depend on the availability of PGE_2 directly at the smooth muscle receptors, which is certainly different between luminal and abluminal administration.

PGE_2 -induced headache and EP_4 receptor antagonist

We have previously demonstrated in a double blind randomized crossover experiment, that intravenous PGE_2 induces headache in healthy subjects [13]. Mean headache data recorded on the placebo day in the present study were in agreement with our previous study [13]. Given that the EP_4 receptor antagonist, BGC20-1531 is highly selective

Fig. 3 Individual and mean (filled square) diameter (mm) in the superficial temporal artery (STA) on placebo day compared with BGC20-1531 before and after Prostaglandin E₂ (PGE₂) infusion. There was a difference between AUC_{STA} on BGC20-1531 200 mg pretreatment and placebo, and no difference between AUC_{STA} on placebo and 400 mg pretreatment (paired *t* test)

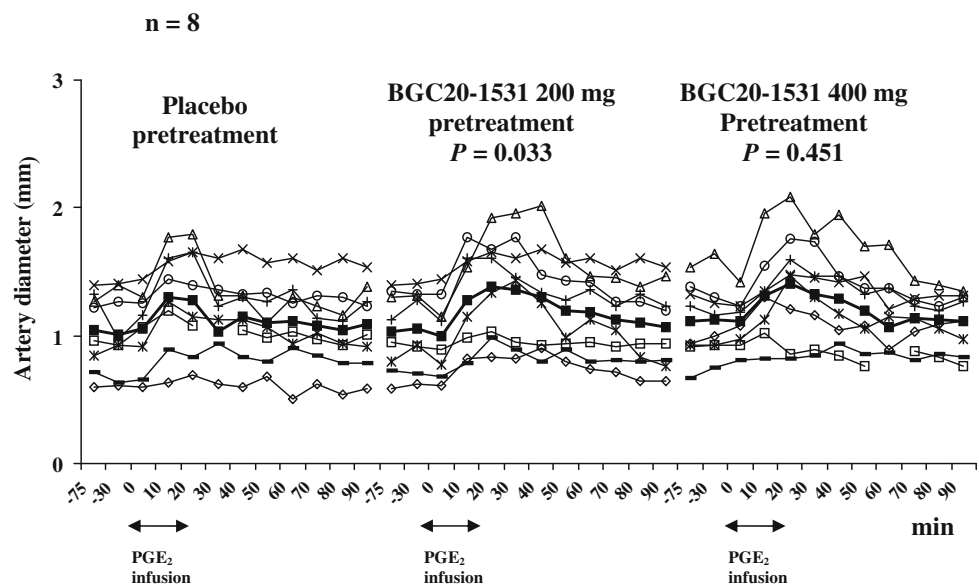
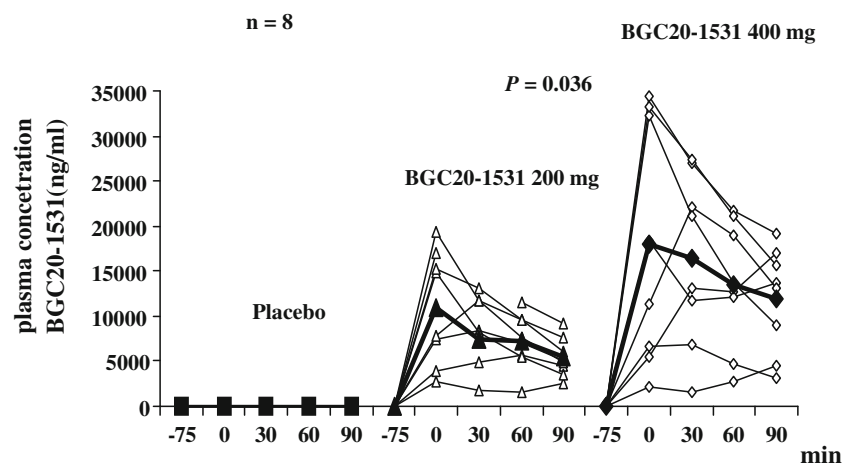


Fig. 4 Individual (open geometric shapes) and mean (filled geometric shapes) plasma concentration of BGC20-1531 on placebo and either active day. Significant difference between AUC_{PC} on BGC20-1531 400 mg pretreatment and BGC20-1531 200 mg pretreatment ($P = 0.036$) (paired *t* test)



[12], doses used in the present study should almost completely block EP₄ receptors [12]. We therefore expected amelioration of PGE₂-induced headache. However, two single doses of BGC20-1531 did not prevent the PGE₂-induced headache. Oral dosing with BGC20-1531 (200 and 400 mg) in healthy volunteers has resulted in consistent plasma exposure in previous clinical studies with relatively low inter-subject variability (unpublished observations; single ascending dose study C_{max} $9,850 \pm 2,900$ and $22,700 \pm 5,500$ ng/ml at the 200 and 400 mg dose, respectively). The pharmacokinetic profile in the current study showed that exposure to BGC20-1531 was more variable (C_{max} $11,850 \pm 5,800$ and $21,100 \pm 11,600$ ng/ml at the 200 and 400 mg dose, respectively) and reached putative therapeutic concentrations in 5 out of 8 subjects ($<10,000$ ng hr/ml). However, results from the five subjects with sufficient plasma exposure did not indicate an effect of BGC20-1531.

It has previously been shown that other EP receptor subtypes such as EP₁ [24, 25], EP₂ [24, 25], EP₃ [25], EP_{3A/α} [24], EP_{3B} [24], EP_{3β} [24], EP_{3C} [10] are expressed in sensory neurons and are involved in PGE₂-induced sensitization [10, 24, 26, 27] and hyperalgesia [28]. Moreover, EP₁ receptor antagonist GSK345931A attenuated hypersensitivity in a dose related manner in a preclinical model of inflammatory pain [29] and EP₃ receptor knockout mice had reduced licking responses in the second phase of the formalin assay [30]. Aside from the possible activation of the other EP receptor subtypes in the presence of blocked EP₄ receptors, PGE₂ may also stimulate release of other vasoactive substances. It has been shown that EP₂ receptor selective agonist, butaprost, as well as EP₃ > EP₂ receptor agonist, misoprostol, stimulate release of the vasodilator neuropeptide, calcitonin-gene related peptide (CGRP), which is well-known to be involved in the pathogenesis of neurovascular headaches

Table 2 Adverse events reported and recorded during the in-hospital period

| | Placebo plus PGE ₂ | BGC20-1531 200 mg plus PGE ₂ | BGC20-1531 400 mg plus PGE ₂ |
|--|-------------------------------|---|---|
| Headache | 6 | 6 | 7 |
| Nausea | 2 | 0 | 2 |
| Photophobia | 1 | 2 | 3 |
| Phonophobia | 0 | 0 | 0 |
| Flushing | 8 | 8 | 7 |
| Heat sensation | 6 | 5 | 5 |
| Palpitation | 4 | 7 | 4 |
| Low back pain | 1 | 0 | 1 |
| Tightness in chest | 4 | 3 | 2 |
| Stiff muscles | 2 | 1 | 2 |
| Chills | 1 | 1 | 0 |
| Increased mucus production in throat | 3 | 2 | 2 |
| Parasthesia lips/fingers/arm | 1 | 1 | 2 |
| Local irritation around injection place | 1 | 3 | 1 |
| Low abdominal pain which mimics menstruation | 1 | 1 | 1 |
| Urge to void | 3 | 1 | 2 |
| Thirst | 3 | 2 | 2 |
| Face heat sensation | 0 | 0 | 1 |

There was no difference between occurrence of AEs on placebo and BGC20-1531 200 mg pretreatment and BGC20-1531 400 mg pretreatment ($P > 0.05$, McNemar test)

[31]. Thus, we believe that involvement of the other PGE₂ receptors subtypes is the most likely explanation of our headache results. Although due to the PK variability and low exposures noted with BGC20-1531, this does not preclude an involvement of the EP₄ receptor subtype.

EP₄ receptor expression and EP₄ receptor mediated dilatation of the intra- and extracerebral vessels

In our previous study on PGE₂ induced headache in healthy subjects, we showed dilatation of MCA (13.9%, mean change from baseline) and STA (23.5%). The current vascular data, recorded on the placebo day were in agreement with this study [13]. It is well reported that PGE₂-induced dilatation of cerebral blood vessels is mediated via EP₂ and EP₄ receptors [5, 32]. To our knowledge, very few immunohistochemical studies have reported the distribution of EP₄ receptors in human vasculature. EP₄ receptors, but not EP₂ receptors are highly expressed in human pulmonary vein [33] and human renal artery [34] whereas EP₄ and EP₂ receptors show low expression in human

pulmonary artery [33]. No data are available at present to show the distribution of EP₄ and EP₂ receptors in human intra- or extracranial arteries or in radial artery. Previous *in vitro* studies have reported that PGE₂-induced dilatation of both human and animal isolated MCA [6, 12, 20] and MMA [12, 20] can be abolished by BGC20-1531 [6, 12, 20]. Furthermore, a specific EP₂ receptor agonist causes no dilatation during stimulation [6, 20]. This suggests that PGE₂-induced dilatation of those vessels is mediated through EP₄ receptors.

In the present study, EP₄ receptor antagonist BGC20-1531 did *not* prevent PGE₂-induced velocity drop of MCA velocity and thereby dilatation of MCA. Interestingly, exploratory ANOVA analysis revealed statistical changes over time in V_{MCA} on the placebo day, but not on the active treatment day. Although there was no statistical effect of BGC20-1531 on PGE₂ responses, we found a modest trend of less velocity drop (Fig. 2). A weak antagonist effect could be due to low permeability of BGC20-1531 through the blood brain barrier and/or by the activation of EP₂ receptors by PGE₂. The prolonged and increased dilatation of STA after 200 mg BGC20-1531 is difficult to explain. It is possible that EP₂ receptors are responsible for the dilating effect of PGE₂ in the STA. The EP₂ receptor has a shorter cytoplasmatic carboxyl terminus [35–37] and therefore, undergoes less internalization [38] and desensitization [39] after exposure to PGE₂ compared to the EP₄ receptor. In contrast to EP₄, EP₂ remains sensitive to metabolites of PGE₂ [39, 40]. Hence, the activation of EP₂ receptors could both prolong and intensify dilatation of STA. Future studies on EP₂/EP₄ receptors distribution in human STA, MCA and MMA may clarify these issues.

In conclusion, the selective blockade of EP₄ receptors did not prevent PGE₂ induced headache or vasodilatation. It should be noted that the present study was sufficiently powered to demonstrate effect based on previous study [41], however the low exposures of BGC20-1531 in 3 out of 8 volunteers may have contributed to the negative outcome in this study. Furthermore, we cannot exclude the PGE₂-induced activation of the other EP receptors as well as possible low BBB permeability of the EP₄ receptor antagonist. Therefore, further investigations of both PGE₂ and EP₄ receptors role in the pathogenesis of the neurovascular headache are hence worthy.

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Conflict of interest Jes Olesen has received grants and/or research support from, has been a consultant and/or scientific adviser for, and has been on the speaker's bureau of Allergan Inc, AstraZeneca Pharmaceuticals LP, Boehringer Ingelheim, Eli Lilly, GlaxoSmithKline, Janssen Pharmaceutical Products, Lundbeck, Merck, and Pfizer.

Messoud Ashina has received grant support and honoraria for lecturing from Merck, and honoraria for lecturing from Pfizer, GlaxoSmithKline and AstraZeneca, and he is a consultant and/or scientific adviser for Merck and BTG International Ltd. Karen Mauback and Emma Thomas are employees of BTG International Ltd.

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References

- Norel X (2007) Prostanoid receptors in the human vascular wall. *Sci World J* 7:1359–1374
- Bianchi M, Martucci C, Ferrario P, Franchi S, Sacerdote P (2007) Increased tumor necrosis factor- α and prostaglandin E2 concentrations in the cerebrospinal fluid of rats with inflammatory hyperalgesia: the effects of analgesic drugs. *Anesth Analg* 104:949–954
- Coleman RA, Smith WL, Narumiya S (1994) International Union of Pharmacology classification of prostanoid receptors: properties, distribution, and structure of the receptors and their subtypes. *Pharmacol Rev* 46:205–229
- Jadhav V, Jabre A, Lin SZ, Lee TJ (2004) EP1- and EP3-receptors mediate prostaglandin E2-induced constriction of porcine large cerebral arteries. *J Cereb Blood Flow Metab* 24:1305–1316
- Negishi M, Sugimoto Y, Ichikawa A (1995) Molecular mechanisms of diverse actions of prostanoid receptors. *Biochim Biophys Acta* 1259:109–119
- Davis RJ, Murdoch CE, Ali M, Purbrick S, Ravid R, Baxter GS et al (2004) EP4 prostanoid receptor-mediated vasodilatation of human middle cerebral arteries. *Br J Pharmacol* 141:580–585
- Kassuya CA, Ferreira J, Claudino RF, Calixto JB (2007) Intraplantar PGE2 causes nociceptive behaviour and mechanical allodynia: the role of prostanoid E receptors and protein kinases. *Br J Pharmacol* 150:727–737
- Smith JA, Davis CL, Burgess GM (2000) Prostaglandin E2-induced sensitization of bradykinin-evoked responses in rat dorsal root ganglion neurons is mediated by cAMP-dependent protein kinase. *A Eur J Neurosci* 12:3250–3258
- Lin CR, Amaya F, Barrett L, Wang H, Takada J, Samad TA et al (2006) Prostaglandin E2 receptor EP4 contributes to inflammatory pain hypersensitivity. *J Pharmacol Exp Ther* 319:1096–2103
- Southall MD, Vasko MR (2001) Prostaglandin receptor subtypes, EP3C and EP4, mediate the prostaglandin E2-induced cAMP production and sensitization of sensory neurons. *J Biol Chem* 276:16083–16091
- Nakao K, Murase A, Ohshiro H, Okumura T, Taniguchi K, Murata Y et al (2007) CJ-023, 423, a novel, potent and selective prostaglandin EP4 receptor antagonist with antihyperalgesic properties. *J Pharmacol Exp Ther* 322:686–694
- Maubach KA, Davis RJ, Clark DE, Fenton G, Lockey PM, Clark KL et al (2009) BGC20-1531, a novel, potent and selective prostanoid EP receptor antagonist: a putative new treatment for migraine headache. *Br J Pharmacol* 156:316–327
- Wienecke T, Olesen J, Oturai PS, Ashina M (2009) Prostaglandin E2(PGE2) induces headache in healthy subjects. *Cephalalgia* 29:509–519
- Iversen HK, Olesen J, Tfelt-Hansen P (1989) Intravenous nitroglycerin as an experimental model of vascular headache. Basic characteristics. *Pain* 38:17–24
- Olesen J, Steiner TJ (2004) The International classification of headache disorders, 2nd edn (ICDH-II). *J Neurol Neurosurg Psychiatry* 75:808–811
- Thomsen LL, Iversen HK (1993) Experimental and biological variation of three-dimensional transcranial Doppler measurements. *J Appl Physiol* 75:2805–2810
- Matthews JN, Altman DG, Campbell MJ, Royston P (1990) Analysis of serial measurements in medical research. *BMJ* 300:230–235
- Altman DG (1999) Practical statistics for medical research. Chapman & Hall, London, pp 455–458
- Busija D (2002) Prostaglandins and other Eicosanoids 2. In: Edvinsson L, Krause DN (eds) *Cerebral blood flow and metabolism*, 2nd edn. Lippincott Williams & Wilkins, Philadelphia, pp 325–338
- Myren M, Baun M, Ploug BK, Jansen-Olesen I, Gupta S (2010) Functional and molecular characterization of prostaglandin E2 dilatory receptors in the rat craniovascular system in relevance to migraine. *Cephalalgia* 30:1110–1122
- Whalley ET, Schilling L, Wahl M (1989) Cerebrovascular effects of prostanoids: in-vitro studies in feline middle cerebral and basilar artery. *Prostaglandins* 38:625–634
- Wahl M, Schilling L, Whalley ET (1989) Cerebrovascular effects of prostanoids. In-situ studies in pial arteries of the cat. *Naunyn Schmiedebergs Arch Pharmacol* 340:314–320
- Armstead WM (1995) Role of nitric oxide and cAMP in prostaglandin-induced pial arterial vasodilation. *Am J Physiol* 268:H1436–H1440
- Donaldson LF, Humphrey PS, Oldfield S, Giblett S, Grubb BD (2001) Expression and regulation of prostaglandin E receptor subtype mRNAs in rat sensory ganglia and spinal cord in response to peripheral inflammation. *Prostaglandins Other Lipid Mediat* 63:109–122
- Oida H, Namba T, Sugimoto Y, Ushikubi F, Ohishi H, Ichikawa A et al (1995) In situ hybridization studies of prostacyclin receptor mRNA expression in various mouse organs. *Br J Pharmacol* 116:2828–2837
- Kumazawa T, Mizumura K, Koda H (1993) Involvement of EP3 subtype of prostaglandin E receptors in PGE2-induced enhancement of the bradykinin response of nociceptors. *Brain Res* 632:321–324
- Moriyama T, Higashi T, Togashi K, Iida T, Segi E, Sugimoto Y et al (2005) Sensitization of TRPV1 by EP1 and IP reveals peripheral nociceptive mechanism of prostaglandins. *Mol Pain* 1:3
- Oka T, Aou S, Hori T (1994) Intracerebroventricular injection of prostaglandin E2 induces thermal hyperalgesia in rats: the possible involvement of EP3 receptors. *Brain Res* 663:287–292
- Hall A, Brown SH, Budd C, Clayton NM, Giblin GM, Goldsmith P et al (2009) Discovery of GSK345931A: an EP(1) receptor antagonist with efficacy in preclinical models of inflammatory pain. *Bioorg Med Chem Lett* 19:497–501
- Popp L, Haussler A, Olliges A, Nusing R, Narumiya S, Geisslinger G et al (2009) Comparison of nociceptive behavior in prostaglandin E, F, D, prostacyclin and thromboxane receptor knockout mice. *Eur J Pain* 13:691–703
- Olesen J, Diener HC, Husstedt IW, Goadsby PJ, Hall D, Meier U et al (2004) Calcitonin gene-related peptide receptor antagonist BIBN 4096 BS for the acute treatment of migraine. *N Engl J Med* 350:1104–1110
- Narumiya S, Sugimoto Y, Ushikubi F (1999) Prostanoid receptors: structures, properties, and functions. *Physiol Rev* 79:1193–1226
- Foudi N, Kotelevets L, Louedec L, Leseche G, Henin D, Chastre E et al (2008) Vasorelaxation induced by prostaglandin E2 in human pulmonary vein: role of the EP4 receptor subtype. *Br J Pharmacol* 154:1631–1639

34. Therland KL, Stubbe J, Thiesson HC, Ottosen PD, Walter S, Sorensen GL et al (2004) Cyclooxygenase-2 is expressed in vasculature of normal and ischemic adult human kidney and is colocalized with vascular prostaglandin E2 EP4 receptors. *J Am Soc Nephrol* 15:1189–1198
35. Bastepe M, Ashby B (1997) The long cytoplasmic carboxyl terminus of the prostaglandin E2 receptor EP4 subtype is essential for agonist-induced desensitization. *Mol Pharmacol* 51:343–349
36. Ichikawa A, Sugimoto Y, Negishi M (1996) Molecular aspects of the structures and functions of the prostaglandin E receptors. *J Lipid Mediat Cell Signal* 14:83–87
37. Regan JW (2003) EP2 and EP4 prostanoid receptor signaling. *Life Sci* 74:143–153
38. Desai S, April H, Nwaneshiudu C, Ashby B (2000) Comparison of agonist-induced internalization of the human EP2 and EP4 prostaglandin receptors: role of the carboxyl terminus in EP4 receptor sequestration. *Mol Pharmacol* 58:1279–1286
39. Nishigaki N, Negishi M, Ichikawa A (1996) Two Gs-coupled prostaglandin E receptor subtypes, EP2 and EP4, differ in desensitization and sensitivity to the metabolic inactivation of the agonist. *Mol Pharmacol* 50:1031–1037
40. Tai HH, Ensor CM, Tong M, Zhou H, Yan F (2002) Prostaglandin catabolizing enzymes. *Prostaglandins Other Lipid Mediat* 68–69:483–493
41. Petersen KA, Lassen LH, Birk S, Lesko L, Olesen J (2005) BIBN4096BS antagonizes human alpha-calcitonin gene related peptide-induced headache and extracerebral artery dilatation. *Clin Pharmacol Ther* 77:202–213