

# Prostaglandins and prostaglandin receptor antagonism in migraine

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## THE 3 ORIGINAL PAPERS ARE

1. Prostaglandin E<sub>2</sub> induces immediate migraine-like attack in migraine patients without aura. *Cephalalgia*. 2012 Aug; 32(11):822-33. Epub 2012 Jun 19.
2. The pharmacological effect of BGC20-1531, a novel prostanoid EP<sub>4</sub> receptor antagonist, in the prostaglandin E<sub>2</sub> human model of headache. *J Headache Pain*. 2011 Oct; 12(5):551-9.
3. Pro-inflammatory and vasoconstricting prostanoid PGF<sub>2</sub> alpha causes no headache in man. *Cephalalgia*. 2011 Nov; 31(15):1532-41.

## INTRODUCTION

Migraine is ranked 19 among top 20 disorders of disability worldwide in a World Health Organisation (WHO) report of 2000 [1]. The worldwide prevalence of current migraine is over 10% [2]. In Europe the prevalence of current migraine in adults is 14.7% (8% in men and 17.6% in women) [3] and the total cost of migraine disorder was € 18.5 billion in 2010 [4].

At present, the acute treatment of migraine includes triptans and non-steroidal anti-inflammatory substances (NSAID) [5]. Triptans were designed to constrict cranial blood vessels and first launched in 1991 as a revolutionary treatment for acute migraine [6]. However, triptans are not superior than NSAIDs [7], which act as non-selective inhibitors of the enzyme cyclooxygenase (COX); the latter catalyzes the formation of prostaglandins (PGs) [8;9]. The amelioration of migraine pain after the NSAIDs indicates that PGs are involved in the pathophysiology of migraine.

Human models of headache are a unique and powerful tool, which contributes greatly to understanding of migraine patho-

physiology [10]. During the past six years several human studies investigated prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), prostaglandin I<sub>2</sub> (PGI<sub>2</sub>) and prostaglandin D<sub>2</sub> (PGD<sub>2</sub>) in headache and migraine [11-14]. Studies in healthy volunteers reported that PGE<sub>2</sub> and PGI<sub>2</sub> induced headache and dilatation of both extra- and intra-cerebral arteries [11;12]. Migraine patients reported delayed migraine-like attacks after PGI<sub>2</sub> [13], and similar to healthy volunteers immediate dilatation of extra- and intra-cerebral arteries was observed [11;13]. A provocation study in healthy volunteers using intravenous PGD<sub>2</sub> reported that this prostaglandin, in spite of strong dilatation of cerebral arteries, induced a very mild headache [14]. Taken together, these data suggested that vasodilatation of the cerebral arteries alone cannot explain PG-induced headache and that the other mechanisms may play a role in the generation of head pain [15] and that there is a need for further investigation. The specific aims of this thesis were to investigate whether:

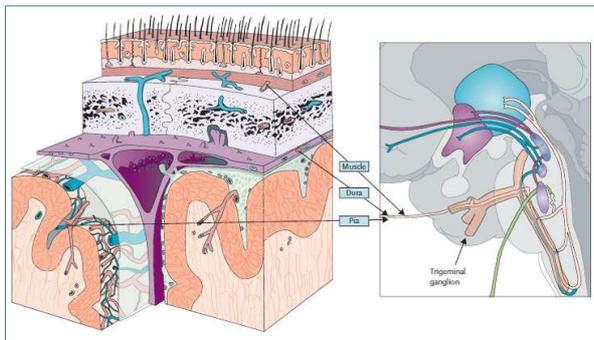
- PGE<sub>2</sub> induces migraine in patients with migraine without aura.
- Highly specific EP<sub>4</sub> receptor antagonist, BGC20-1531, prevents PGE<sub>2</sub>-induced headache and dilatation of cerebral arteries in healthy volunteers.
- Prostaglandin F<sub>2α</sub> (PGF<sub>2α</sub>) constricts cerebral arteries and induces headache in healthy volunteers.

## MIGRAINE PATHOPHYSIOLOGY

The pathophysiology of migraine is complex and the mechanisms are still unrevealed. To date, the role of vasodilatation, neurogenic inflammation and the central neuronal theory are the most discussed topics in migraine research [16]. The vascular theory is based on Ray and Wolff's observations in conscious patients during brain surgery, where the electrical stimulation of the dural and cerebral arteries was resulting in the experience of headache and nausea in those patients, whereas the brain substance itself was not pain producing [17]. The vascular theory suggests sensory neurones, which transfer pain signals from intra- and extracranial blood vessels, as the generators of migraine pain [18] (Fig. 1). The neurogenic inflammation theory is another theory, explaining the possible mechanisms of migraine pain. It is primarily based on animal models showing that activation of the trigeminal ganglion causes release of pro-inflammatory mediators from mast cells, plasma protein extravasation and vasodilatation [15;19;20]. Thus it has been suggested that the meningeal neurogenic inflammation induces sensitization of the trigeminal afferents and headache. The central theory is in general based on genetic findings, showing mutation in genes controlling brain excitability in migraineurs [21;22]. According to the central theory migraine pain develops due to overall impaired activity and modulation of the trigeminal circuit [16].

Most of the PGs are the primary vasodilators and the mediators of the inflammation. The role of the PGs in migraine may therefore both be explained by either neurovascular or neural theory.

**Figure 1:** Extra- and intra-cranial pain-sensitive structures.

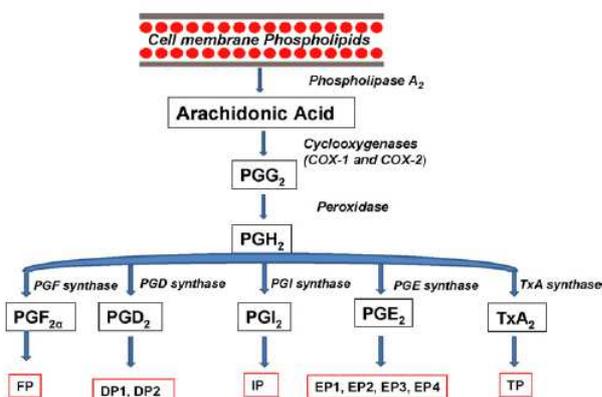


Input from blood vessels at three different locations: the pia mater, the dura mater, and the extra-cranial structures (primarily extra-cranial muscle). Afferents from these structures are trigeminal and project primarily, but not exclusively, through the first division of the trigeminal nerve to the brainstem and on to the thalamus and cortex. Reprinted from Olesen et al [18], with permission from Elsevier.

## PROSTAGLANDINS

PGs are synthesized from arachidonic acid by activated COX in response to various stimuli in various types of cells (Fig. 2). When synthesized, PGs are immediately released and exert their actions on cells in the vicinity of their synthesis [23;24]

**Figure 2:** Prostanoid biosynthesis and response pathway.



Arachidonic acid is metabolized by cyclooxygenases to the unstable endoperoxide  $PGH_2$ , the common precursor for the prostaglandins and tromboxane. Prostaglandins  $F_{2\alpha}$ ,  $D_2$ ,  $I_2$ ,  $E_2$  and tromboxane  $A_2$ , generated by individual prostaglandin synthase enzymes, elicit their biological effects by activating G protein-coupled receptors. Reprinted from Melatovic et al [25], with permission from Elsevier.

## PROSTAGLANDIN $E_2$

$PGE_2$  is the most widely synthesised principal pro-inflammatory PG in the human body [26] involved in various biological functions, including pain processing [27-30] and the regulation of vascular tone [31].  $PGE_2$  acts via four different G-protein-coupled receptor subtypes:  $EP_1$ ,  $EP_2$ ,  $EP_3$  and  $EP_4$  [32]. There is an evidence for the presence of  $PGE_2$  and its receptor subtypes  $EP_{1-4}$  in both peripheral [27;33-35] and central nervous systems [30;36;37] and

that  $PGE_2$  plays a prominent role in both peripheral [38;39] and central sensitization [29;30;40-43].  $PGE_2$  is also involved in regulation of cerebral haemodynamics [24]. However, the role of  $PGE_2$  in the regulation of the vascular tone is complex, as it mediates both vasoconstriction and vasodilatation [31], through activation of different receptor subtypes. Once activated by  $PGE_2$ ,  $EP_1$  and  $EP_3$  receptors cause  $Ca^{2+}$  mobilisation and decrease levels of cyclic adenosine monophosphate (cAMP), which leads to smooth muscle contractions [44]. In contrast,  $PGE_2$  action on  $EP_2$  and  $EP_4$  stimulates adenylatecyclase (AC) and thereby causes relaxation of vascular smooth muscles [45].

## $PGE_2$ AND $EP_4$ RECEPTOR SUBTYPE IN RELATION TO MIGRAINE

Studies in migraineurs have reported increased levels of  $PGE_2$  in saliva [46] and blood [47] during migraine attacks. The electrical stimulation of trigeminal ganglion (TG) or the application of inflammatory mediators on rat dura mater causes  $PGE_2$  release [48] most likely from branches of the middle meningeal artery (MMA), perivascular nerve endings and mast cells, where COX isoforms were found [49]. In healthy volunteers  $PGE_2$  causes cephalic dilatation and headache [12]. However,  $PGE_2$  has previously never been studied in migraine patients.

*In vitro* studies using human [50;51] and animal tissues [51-53] found  $PGE_2$ -induced dilatation of the middle cerebral artery (MCA) and the MMA. Interestingly, the  $PGE_2$ -mediated vasodilatation of the human MCA occurs primarily due to activation of the  $EP_4$  receptors and the  $EP_4$  receptor antagonist, AH 23848, was able to attenuate this response [50]. A novel selective and potent  $EP_4$  receptor antagonist, BGC20-1531, was developed for clinical trials and Phase I investigations showed a favourable adverse events profile (unpublished data from BTG Ltd). An *in vitro* study demonstrated that BGC20-1531 antagonized  $PGE_2$ -mediated dilatation of human MCA and MMA rings, pre-contracted with phenylephrine [51] (Fig. 3). Therefore it has been suggested that BGC20-1531 may have the potential to alleviate the symptoms of migraine that result from cerebral vasodilatation [51]. High levels of  $PGE_2$  caused up-regulation of the  $EP_4$  receptor subtype in rat sensory dorsal root ganglion (DRG) neurons, but not  $EP_{1-3}$  receptor subtypes [35]. Given that sensitization of the sensory neurons was mediated through the  $EP_4$  receptors [54] the  $EP_4$  receptor was proposed as a new target for the treatment of pain [55]. Thus, the *in vitro* findings indicate that BGC20-1531 may alleviate vasodilatation and headache in a  $PGE_2$  model of headache in healthy volunteers.

## PROSTAGLANDIN $F_{2A}$

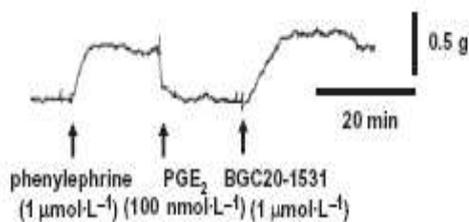
In different animal studies  $PGF_{2\alpha}$  was shown to constrict isolated canine basilar, MCA [56] and posterior communicating arteries [57] as well as rhesus monkey basilar artery [58] and bovine MCA [59]. *In vitro* studies on human tissues also reported  $PGF_{2\alpha}$ -induced contraction of the MCA and basilar arteries of infant (gestational age 30-40 weeks) [60], adult pial [61], temporal [62] and radial arteries [63;64]. *In vivo* animal studies in rats and cats using the *open* cranial window model found constriction of the basilar [65] and the pial arteries [66;67] after extra-luminal application of  $PGF_{2\alpha}$ . Aside  $PGF_{2\alpha}$ 's ability to constrict cephalic arteries, it is one of the most abundant PGs in the brain [68]. Prostaglandin F synthase (PGFS) and FP receptor have been found in the gray matter of all segmental levels in rat spinal cord and in neuronal dendrites [69]. PGFS has also been detected in the white matter

of the brain and the spinal cord of mice [70]. Furthermore, functional FP receptors were reported in mice spinal cord [71] and mRNA for FP receptor was expressed in cultured rat astrocytes and oligodendrocytes [72].

### PGF<sub>2A</sub> IN RELATION TO MIGRAINE

*In vivo* animal models showed that intrathecal administration of PGF<sub>2α</sub> caused touch-evoked allodynia in mice [73-75]. Electrophysiological experiments reported a dose-dependent increases in mechanically evoked discharges of spinal nociceptive specific neurones during the PGF<sub>2α</sub>-induced mechanical hyperalgesia in rats [29]. PGF<sub>2α</sub>-induced mechanical allodynia was attenuated in mice lacking FP receptors (FP<sup>-/-</sup>) [76]. Thus, PGF<sub>2α</sub> is involved in pain processing and furthermore elevated levels of urinary PGF<sub>2α</sub> metabolite were found in children with migraine [77] and recently an increased level of PGF<sub>2α</sub> during attacks in saliva in migraineurs have been reported [78]. A development of migraine in patient without any previous history of migraine has been reported during the treatment for glaucoma with Latanoprost (PGF<sub>2α</sub> analogist) [79]. However, PGF<sub>2α</sub>-induced headache characteristics and vascular changes have never been described in a randomized double-blind experiment.

**Figure 3:** Reversal of an established PGE<sub>2</sub>-mediated relaxation in rings of human middle cerebral artery, by BGC20-1531 (1 mmol·L<sup>-1</sup>).



The tissue was pre-contracted with phenylephrine (1 mmol·L<sup>-1</sup>), before vasodilatation was induced by the addition of PGE<sub>2</sub> (100 nmol·L<sup>-1</sup>). BGC20-1531 induced a rapid and complete reversal of the PGE<sub>2</sub> response (by 133%). The response to BGC20-1531 shown here is representative of data from three independent experiments. Reprinted from Maubach et al [51], with permission from John Wiley and Sons.

## METHODS

### Study subjects

Healthy volunteers (age of 18-40) for study II and III were recruited via the web site [www.forsoeegsperson.dk](http://www.forsoeegsperson.dk), via the MOK journal for medical students at the University of Copenhagen and on bulletin boards at the hospitals and institutions of higher education. The healthy volunteers, participated in study II 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. The results from physical, neurological examinations, electrocardiography (ECG), broad clinical-chemical, haematological, serum screenings for HIV, Hepatitis B and C, and urine screenings for drug abuse of the participated subjects, done on the day of enrolment, were either negative or normal. The exclusions criteria for the healthy volunteers participated in study III were: history of migraine or any other type of headache (except epi-

sodic tension-type headache less than once a month) and previous serious somatic, psychiatric or infectious diseases. All healthy volunteers, participated in study III had a normal ECG as well as physical and neurological examination.

Migraine patients (age of 18-55) for study I, were recruited as described for study II and III, and additionally via the Danish Headache Center. The patients were diagnosed with migraine without aura according to the International Headache Society (IHS) [80] and had no other type of headache (except episodic tension-type headache less than three a month). The other exclusion criteria were as in study III.

No daily medication apart from oral contraceptives was permitted during the studies. It was not allowed to smoke or consume any alcoholic beverages, caffeine, cocoa and chocolate for at least 8 h before the infusion in study I and III and for the whole study period in study II.

All healthy volunteers and migraine patients gave their written informed consent and all three studies were performed in accordance with the Helsinki Declaration of 1964, as revised in 2008, and registered on [www.clinicaltrials.gov](http://www.clinicaltrials.gov).

### Experimental design

All studies were designed as randomised, double-blind, placebo-controlled, cross-over experiments. In study I, the migraine patients were randomly assigned to receive 0.4 μg/kg/min of PGE<sub>2</sub> or placebo (saline) intravenously over 25 min on 2 days separated by at least one week. In study II the healthy volunteers were randomly assigned to receive BGC20-1531 200 mg, BGC20-1531 400 mg or placebo, followed 75 min later (in order to reach the maximum plasma concentration of BGC20-1531) by an infusion of PGE<sub>2</sub> at 0.40 μg/kg/min over 25 min on three different days at intervals of at least one week. In study III the healthy volunteers were randomly assigned to receive 3.5 μg/kg/min of PGF<sub>2α</sub> or placebo intravenously over 20 min on two days separated by at least one week.

In study I and III the randomization and preparation of the study drug was done by medical staff not involved in the study (<http://www.randomization.com/>). BGC20-1531 and equivalent placebo were randomised and blinded by a central pharmacy. The randomization codes for all three studies were kept in the hospital during the studies and the unblinding procedure was first performed after the single study was completed.

All participants reported to the laboratory at 8 AM and were confirmed to have been headache-free. In study I and II the infusion was initiated after the baseline measurements by a time and volume controlled infusion pump. Headache score and accompanying symptoms, blood flow velocity in the MCA (V<sub>MCA</sub>), diameters of the superficial temporal artery (STA) and the radial artery (RA), mean arterial blood pressure (MAP), heart rate (HR), end-tidal partial pressure of CO<sub>2</sub> (P<sub>et</sub>CO<sub>2</sub>), transcutaneous arterial oxygen saturation (SAT), ECG and any adverse events (AEs) were recorded at baseline and then every 10 min until time 90 min from the infusion start.

Study II was conducted in the exactly same way as study I and III. However, there were two additional measurements of the parameters, described above, at time -75 min and time -30 min. Furthermore, blood samples for the plasma concentration of BGC20-1531 were collected at T<sub>-75</sub>, T<sub>0</sub>, T<sub>30</sub>, T<sub>60</sub> and T<sub>90</sub> on each study day.

All participants were carefully instructed to make hourly recordings of headache and accompanying symptoms according IHS [80] and any other AEs on study days after the discharge. The healthy volunteers were allowed to take rescue medication of

their own choice after consulting the study physician and the migraine patients were encouraged to take their usual rescue migraine medication in case of the development of the delayed migraine after the infusion.

#### Headache intensity

A 10-point verbal rating scale (VRS) was used to record headache intensity, where 0 indicated no headache; 1, a very mild headache (including pre-pain – a pressing or throbbing feeling); 5 indicated moderate headache and 10 indicated worst imaginable headache [81]. Together with the headache intensity the accompanying symptoms, the quality and localisation of the headache have been recorded.

#### Migraine-like attacks definition

Experimental migraine is not spontaneous and therefore cannot fulfil strict IHS criteria for migraine without aura [80]. The majority of patients report them as attacks that mimic spontaneous migraine attacks [13;82]. However, it is well known that many spontaneous migraine attacks develop in a matter of hours and in the early stage phenomenologically only fulfil the criteria for tension-type headache and associate symptoms, unilateral localisation and increase in pain severity occur later. Furthermore, the majority of migraineurs can often predict development of migraine at the very early stage and cannot be denied treatment of experimentally induced headache. Based on these circumstances the following criteria were used to characterize migraine-like attack induced 0-12 h after the infusion of PGE<sub>2</sub> in study I: Migraine-like attack fulfilling either 1 or 2:

1. Headache fulfilling criteria for migraine without aura C and D [80]:
  - C. Headache has at least 2 of the following characteristics:
    - Unilateral location
    - Pulsating quality
    - Moderate or severe pain intensity (moderate to severe pain  $\geq 4$  on VRS)
    - Aggravation by cough (in-hospital phase) or causing avoidance of routine physical activity (out-hospital phase) (e.g., walking or climbing stairs)
  - D. During headache at least 1 of the following:
    - Nausea and/or vomiting
    - Photophobia and phonophobia
2. Headache described as mimicking usual migraine attack and treated with a triptan.

#### Transcranial Doppler

Blood flow velocity was recorded in the middle cerebral artery ( $V_{MCA}$ ) by a Transcranial Doppler (TCD) ultrasonography (2MHz) with hand-held probes [12;83]. The day-to-day coefficient variation (CV) is 16% and the CV for 5 min is 7% [83]. The recordings were performed bilaterally and simultaneously with measurements of  $P_{et}CO_2$ , obtained with an open mask without any respiratory resistance as previously described [83]. 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 fixed point was marked and noted, and reused in each

subject for all recordings. An average of 4 cycles (every single cycle lasting for 4 sec and comprising approximately one cardiac cycle) [83] was used for the statistical analysis. All measurements were done by the same skilled laboratory technician (LE).

#### Dermascan C

A high resolution ultrasound scanner, C scan (20 MHz, bandwidth 15 MHz; Dermascan C) was used to measure the diameter of the frontal branch of the left STA and the left RA [84;85]. All C-scans were performed in the same place, as ensured by markings drawn on the skin, and at time points described in the experimental design section after the blood pressure was measured. The angle and the distance to the orbito-meatal line (OM) (STA) and the distal volar crest of the wrist (RA) were obtained for each mark at the first study day and the same coordinates were used for the recordings during the second and third study day. For the statistical analysis a mean of four measurements performed within approximately 1 min and at arbitrary time point in the cardiac cycle was used. When the measuring points are marked the day-to-day CV is 12% [84;85]. All measurements within the same study subject were done by the skilled laboratory technician (WG or LE) or by Dr. Maria Antonova (MA).

#### Vital signs

MAP and HR were measured by an auto-inflatable cuff. ECG was obtained and monitoring continually and recorded on paper at the time points as described in the experimental design section.

#### Adverse events

All participants were asked to report any AEs during the in-hospital and post-hospital phase on the study days. AEs were classified as related or not related to the study drug by the investigator. Furthermore, the headache diary that had to be fulfilled during the out-hospital phase were also containing questions about the premonitory symptoms (e.g. tired, yawning, stiff neck etc.) [86] and allodynia symptoms (e.g. abnormal sensation while wearing glasses, to cold or heat etc.) [87].

#### Blood samples collection and analysis for BGC20-1531

Blood samples were collected and immediately after stored on ice and then separated by centrifugation at 1500 x g and 4°C for 10 min. Two identical aliquots of plasma were transferred in to polypropylene tubes and stored at -25°C until analysed (blinded). Plasma concentration of BGC20-1531 was determined by liquid chromatography with tandem mass spectrometry detection (LC-MS-MS) at Simbec Research Ltd.

#### Statistical analysis

Baseline values and peak vascular values are presented as mean  $\pm$  SD. Headache scores are presented as median and quartiles. The baseline was defined as  $T_0$  (also for study II as no vascular or headache responses after BGC20-1531 administration during  $T_{.75}$  to  $T_0$  were recorded) before the start of the infusion. The in-hospital phase was defined as time 0-90 min and the out-hospital phase as time 1.5 -12 h (study I and III) and 1.5-11h (study II). The in-hospital phase was additionally divided into the infusion phase time 0-30 min, due to a short half-life (approximately 1-5 min for ProstinE<sub>2</sub><sup>™</sup> and ProstinF<sub>2 $\alpha$</sub> <sup>™</sup> according to the manufacture instructions, Pfizer Ltd) in plasma, and post-infusion phase 30-90 min. The immediate headache was defined as any headache during the in-hospital phase and the delayed headache as any headache during the out-hospital phase.

All data were baseline-corrected to reduce variation between sessions within subject and the area under the curve (AUC) for the time period  $T_0$ - $T_{90}$  for  $V_{MCA}$ , headache score, MAP, HR and  $P_{et}CO_2$  (study I-III) and  $T_{1.5-12h}$  for headache (study I) was calculated, using the trapezium rule [88]. The AUC mean difference (MD) between the two experimental days is presented with 95% confidence intervals (CI). Furthermore hypocapnia decreases  $V_{MCA}$  due to changes in arteriolar tone. The  $V_{MCA}$  data were corrected by  $e^{0.034}$  for each mmHg decrease in  $P_{et}CO_2$  [89] in study III, as there was a significant difference in  $AUC_{P_{et}CO_2}$  between two experimental days.

In study I assuming that no changes in cerebral blood flow (CBF) occurred during the  $PGE_2$  infusion [12] the maximum percent change in diameter ( $\Delta d$ ) of the MCA was calculated as:

$$\Delta d = (V_{MCA_{time0}} / V_{MCA_{time20}} - 1) \times 100 \text{ [90].}$$

#### Primary end-points (study I-III)

The primary end-points were differences in the AUC for headache score ( $AUC_{\text{headache score}}$ ) between active and placebo arm and the incidence of headache and migraine-like attacks (study I).

#### Secondary end-points (study I-III)

The secondary end-points were differences in the AUC for  $V_{MCA}$  ( $AUC_{V_{MCA}}$ ), STA ( $AUC_{STA}$ ), RA ( $AUC_{RA}$ ),  $P_{et}CO_2$  ( $AUC_{P_{et}CO_2}$ ), MAP ( $AUC_{MAP}$ ) and HR ( $AUC_{HR}$ ) between placebo and active treatment.

To test the statistical difference between the continuous variables (vascular data) the paired, two-way *t*-test was applied, the Wilcoxon signed ranks test for the categorical data (headache score), and the McNemar test for the binary categorical (AEs, headache incidence). In study II and III a post hoc analysis to explore possible changes over time for vascular variables was done 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, Illinois, USA). Post hoc exploratory analyses were performed with GraphPad Prism® (GraphPad Software Inc., CA, USA).

## RESULTS

### Study 1

In total nine migraine patients without aura (75%) experienced migraine-like attacks after  $PGE_2$  compared to none after placebo ( $P = 0.004$ ). Of those nine patients, seven reported the migraine-like attacks during the immediate phase (0-90 min) and two during the delayed (1.5-12h) (Table 1).

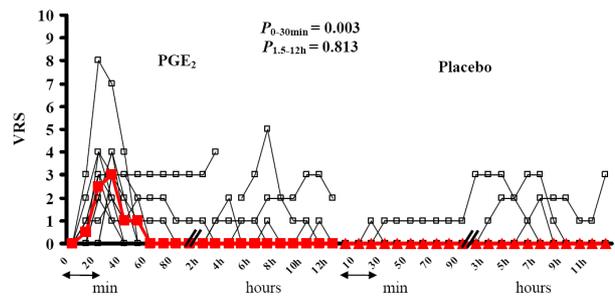
**Table 1:** Incidence of  $PGE_2$ -induced headache and migraine-like attack in 12 migraine patients without aura (McNemar test).

| Incidence                      | $PGE_2$ day  | Placebo day | <i>P</i> -value |
|--------------------------------|--------------|-------------|-----------------|
| Headache (0-12h)               | 12/12 (100%) | 5/12 (42%)  | 0.016           |
| Immediate headache             | 12/12 (100%) | 2/12 (17%)  | 0.002           |
| Delayed headache               | 6/12 (50%)   | 4/12 (33%)  | 0.727           |
| Migraine-like attack (0-12h)   | 9/12(75%)    | 0/12(0%)    | 0.004           |
| Immediate migraine-like attack | 7/12(58%)    | 0/12(0%)    | 0.016           |
| Delayed migraine-like attack   | 2/12(17%)    | 0/12(0%)    | 0.500           |

The AUC for immediate headache (0 to 90 min) was significantly larger after  $PGE_2$ , 8 (7-16.1) than after placebo, 0 (0-0) ( $P = 0.003$ ). There was no difference in the AUC for delayed headache (1.5 to

12 h) between  $PGE_2$ , 0.25 (0-3.8) and placebo, 0 (0-8.4) ( $P = 0.813$ ). The median peak headache occurred at  $T_{30}$  and the score was 3 (1-7) after  $PGE_2$  infusion and 0 (0-1) after placebo (Fig. 4).

**Figure 4:** Median (■,▲) and individual (□) headache intensity on a VRS on  $PGE_2$  and placebo days in 12 migraine patients without aura. The median peak immediate headache intensity after  $PGE_2$  was 3 (0-10 on VRS) and occurred at  $T_{30}$  after start of infusion.



### Vascular responses after $PGE_2$

The MD between the  $AUC_{V_{MCA}0-30min}$  on  $PGE_2$  and placebo day was 17.2 (95% CI: 6.3 – 28,  $P = 0.005$ ). The peak mean vascular changes from baseline on the  $PGE_2$  day are presented in Table 2. There was no difference in the  $AUC_{STA}$  between  $PGE_2$  and placebo, MD = 0.04 (95% CI: -0.4 – 0.5,  $P = 0.850$ ). A significant difference was found in the  $AUC_{RA}$ , MD = -1.9 (95% CI: -2.7 – -1.1,  $P = 0.0001$ ) and the  $AUC_{HR}$ , MD = 132.3 (95% CI: 111.6 – 153,  $P = 0.0001$ ) between  $PGE_2$  and placebo. There was no difference in the  $AUC_{MAP}$  between two experimental days, MD = -7.3 (95% CI: -29.4 – 14.8,  $P = 0.482$ ) and the  $AUC_{P_{et}CO_2}$ , MD = 12 (95% CI: -0.9 – 25,  $P = 0.065$ ).

### Adverse events

There was an increased incidence of stiff muscles, increased saliva production, flushing, palpitations, heart sensation and paraesthesia on the  $PGE_2$  day compared with placebo ( $P > 0.05$ , McNemar test).

**Table 2:** Peak mean vascular changes from baseline (%) in 12 patients with migraine without aura on  $PGE_2$  infusion day.

| Variable     | Peak time (min) | Peak mean (±SD) |
|--------------|-----------------|-----------------|
| $V_{MCA}$    | 10              | -12 ± 10.8%     |
| MCA diameter | 10              | 7.2 ± 7.1%      |
| STA diameter | 20              | 7.2 ± 11.3%     |
| RA diameter  | 10              | 0.9 ± 7.1%      |
| HR           | 10              | 55.2 ± 24%      |
| MAP          | 20              | -2.4 ± 8.4%     |

### Study 2

There was no decrease in the incidence of the  $PGE_2$ -induced headache on BGC20-1531 200 mg or BGC20-1531 400 mg day compared with placebo day ( $P > 0.05$ ). The incidence of the immediate and the delayed headache is shown in Table 3. There was no difference in the AUC for headache between both pre-treatment days and placebo day (BGC20-1531 200 mg 2 (0.25 – 4.8) and placebo 3 (0.25 – 6.8),  $P = 0.14$ ; BGC20-1531 400 mg 2 (0.75 – 6.9) and placebo 3 (0.25 – 6.8),  $P = 0.173$ ) (Fig. 5).

**Secondary end points: vascular parameters, vital signs and  $P_{et}CO_2$**   
 There were no significant changes in the vascular parameters between both pre-treatment days and placebo, apart from STA response on BGC20-1531 200 mg day. Explorative ANOVA analysis revealed a significant drop in  $V_{MCA}$  at  $T_{20}$  after  $PGE_2$  infusion on placebo day compared to baseline ( $P < 0.05$ ) but no changes in  $V_{MCA}$  during BGC20-1531 200 and 400 mg day. The differences of the AUC means for the secondary end points between placebo and both pre-treatment days with BGC20-1531 are shown in Table 4.

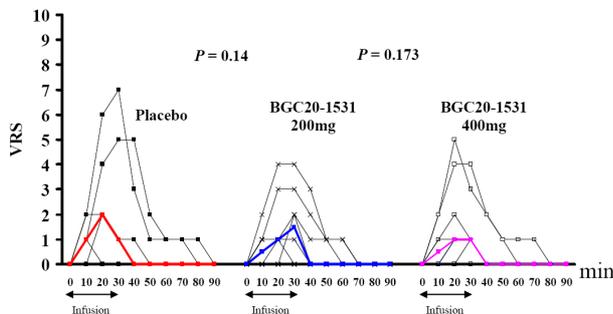
**Table 3:** Incidence of  $PGE_2$ -induced immediate and delayed headache in eight healthy subjects (McNemar test).

|                                 | Placebo plus $PGE_2$ | BGC20-1531 200 mg plus $PGE_2$ | BGC20-1531 400mg plus $PGE_2$ |
|---------------------------------|----------------------|--------------------------------|-------------------------------|
| Incidence of immediate headache | 6                    | 6                              | 7                             |
| Incidence of delayed headache   | 1                    | 1                              | 1                             |

**Table 4:** Mean difference between AUC on BGC20-1531 200 mg and BGC201531400 mg, and placebo day for the secondary end points. There was a significant difference between the AUC of the STA on BGC20-1531 200 mg and placebo day (paired t-test).

| Parameters   | Mean difference between AUC BGC20-1531 200 mg plus $PGE_2$ versus AUC placebo plus $PGE_2$ (95% CI and P value) | Mean difference between AUC BGC20-1531 400 mg plus $PGE_2$ versus AUC placebo plus $PGE_2$ (95% CI and P value) |
|--------------|---|---|
| $V_{MCA}$    | -3.8 (-49.3 - 41.7, $P = 0.9$ )   | -9.9 (-45.2 - 25.4, $P = 0.5$ )   |
| STA          | -1.1 (-2.1 - -0.1, $P = 0.03$ )   | -0.27(-1.1 - 0.5, $P = 0.5$ )   |
| RA           | -0.1 (-1.1 - 0.8, $P = 0.7$ )   | -0.2(-1.1 - 0.7, $P = 0.6$ )  |
| HR           | 8.2 (-65.2 - 81.6, $P = 0.8$ )  | 45.5(-5.7 - 96.7, $P = 0.08$ )  |
| MAP          | -17.4 (-51.6 - 16.8, $P = 0.3$ )  | -10.5 (-41.6 - 20.6, $P = 0.5$ )  |
| $P_{et}CO_2$ | 1.8 (-8.9 - 12.5, $P = 0.7$ )   | 1.7 (-8.7 - 12.1, $P = 0.7$ )   |

**Figure 5:** Median (■;▲;◆) and individual (■;□;△;×) headache scores on a VRS on placebo compared to either pre-treatment day with BGC20-1531. The median peak immediate headache score was 2 (0-6) at  $T_{20}$  on placebo day, 1.5 (0-4) at  $T_{30}$  on BGC20-1531 200 mg day, and 1(0-5) at  $T_{20}$  on BGC20-1531 400 mg day (Wilcoxon signed ranks test).

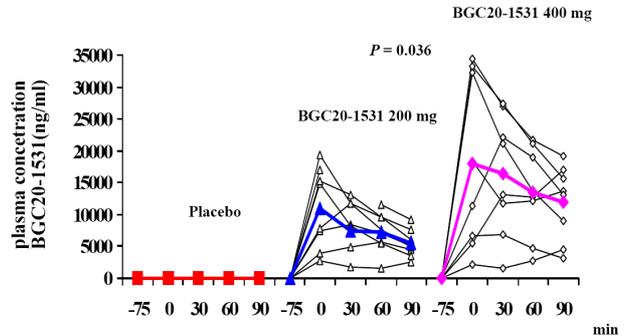


**Pharmacokinetic of BGC20-1531**

The highest plasma concentration of BGC20-1531 was detected 75 minutes after oral administration of BGC20-1531 200 mg and 400 mg at  $T_0$  (Fig. 6). The plasma concentration on pre-treatment with BGC20-1531 400 mg was significantly larger compared to

plasma concentration on BGC20-1531 200 mg (AUC, MD = -23461.6 (95%CI: -44899 - -2024,  $P = 0.036$ ). Putative therapeutic concentrations of  $\geq 10,000$  ng/ml were only reached in 5 out of 8 subjects.

**Figure 6:** Individual (□;△;◇) and mean (■;▲;◆) plasma concentrations of BGC20-1531 on placebo and either active day. BGC20-1531 plasma concentration was higher on BGC20-1531 400 mg pre-treatment day (paired t test).



**Adverse events**

There was no difference in the incidence of the AEs between the trial days. The most frequent AEs were: flushing, heat sensation, palpitation, tightness in chest, increased mucus production in throat and thirst. No AEs were reported during the pre-infusion period  $T_{-75} - T_0$  except one participant who had an asymptomatic, non specific T-wave inversion on ECG during pre-infusion period. The subject was excluded from further experiments.

**Study 3**

Infusion of  $PGF_{2\alpha}$  induced headache in 2 out of 12 healthy subjects. On placebo day 4 out of 12 subjects reported headache during the in-hospital phase. The incidence of immediate and delayed headache is shown in Table 5.

**Table 5:** Incidence of  $PGF_{2\alpha}$ -induced immediate and delayed headache in 12 healthy subjects.

|                                 | Placebo | $PGF_{2\alpha}$ | P-value |
|---------------------------------|---------|-----------------|---------|
| Incidence of immediate headache | 4       | 2               | 0.500   |
| Incidence of delayed headache   | 2       | 3               | 1.000   |

There was no difference in the AUC for immediate headache (0-90 min) between  $PGF_{2\alpha}$  0.5 (0-0) and placebo 1.1 (0-2.4) ( $P = 0.144$ ) (Fig. 7).

**Vascular parameters**

There was a difference in the AUC  $P_{et}CO_2$  between placebo and  $PGF_{2\alpha}$  day (MD = -8.6 (95% CI: -16.6 - -0.6,  $P = 0.039$ ). The MCA data were therefore corrected as described in the statistical analysis section. There was no difference in the AUC for any vascular parameter between  $PGF_{2\alpha}$  and placebo day (Table 6). Explorative ANOVA analysis revealed no changes over the time in  $V_{MCA}$  on either study day ( $P > 0.05$ ).

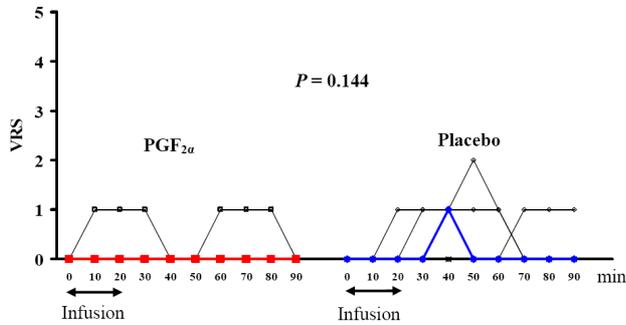
**Vital signs**

The AUC  $MAP$  and the AUC  $HR$  were significantly larger on  $PGF_{2\alpha}$  day than on placebo day ( $P < 0.0001$ ) (Table 6). Mean percentage changes of the vascular parameters and the median headache on  $PGF_{2\alpha}$  and placebo day are shown in Figure 8.

### Adverse events

There was an increased incidence of heat sensation on PGF<sub>2α</sub> day compared with placebo ( $P = 0.004$ ).

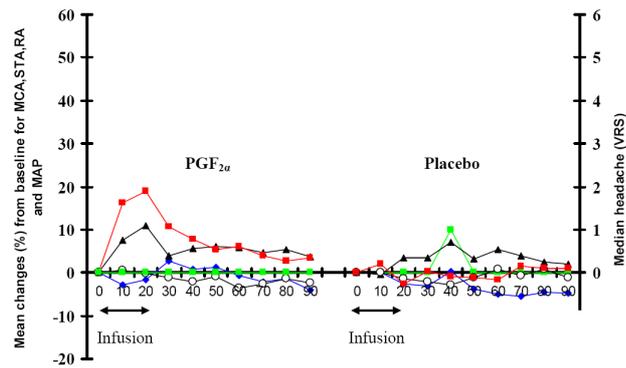
**Figure 7:** Individual (□;○) and median (■;◆) headache scores on a VRS after intravenous infusion of PGF<sub>2α</sub> and placebo in 12 healthy volunteers (Wilcoxon signed ranks test).



**Table 6:** Mean difference between AUC on placebo and PGF<sub>2α</sub> day for vascular parameters and vital signs. There was a difference in the AUC for MAP and HR (paired t-test).

| Parameters | Mean difference between AUC <sub>PGF<sub>2α</sub></sub> and AUC <sub>placebo</sub> (95% CI and P value) |
|------------|---|
| VMCA       | 3.3 (-22.3 – 29, $P = 0.8$ )  |
| STA        | -0.2 (-0.9 – 0.4, $P = 0.5$ )   |
| RA         | -0.1 (-1 – 0.8, $P = 0.8$ )   |
| MAP        | -53 (-73 – -34, $P = 0.0001$ )  |
| HR         | 46 (27 – 65), $P = 0.0001$ )  |

**Figure 8:** Median headache (■) and mean percentage changes from baseline for MAP (■), V<sub>MCA</sub> (◆), STA (▲) and diameter of the RA (○). MAP increased by 18.9% from baseline at T<sub>20</sub> indicating the presence of the active PGF<sub>2α</sub> in the blood.



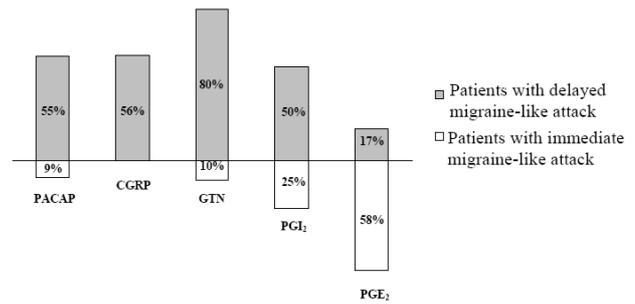
### DISCUSSION

The main findings of the current thesis were that PGE<sub>2</sub> induced *immediate* migraine-like attacks in migraine sufferers. The blockade of a single EP<sub>4</sub> receptor was not sufficient to prevent PGE<sub>2</sub>-induced headache and vascular changes. Finally, pro-inflammatory vasoconstrictor, PGF<sub>2α</sub>, failed to trigger headache in healthy subjects.

### PGE<sub>2</sub>-induced migraine-like attacks

Study I showed that PGE<sub>2</sub> induced *immediate* migraine-like attacks in 58 % of migraine patients. This is in contrast to the previous pharmacological provocation experiments, where the majority of migraineurs reported *delayed* migraine-like attacks [13;82;91;92] (Fig. 9).

**Figure 9:** Percentage of patients reporting migraine-like attacks after GTN [92], PACAP [82], PGI<sub>2</sub>[13], CGRP [91] and PGE<sub>2</sub> (study I) infusion.



How can the immediate migraine response after PGE<sub>2</sub> be explained?

*In vivo* experiments in animals reported that PGE<sub>2</sub> dilates both extra- and intra-cerebral arteries. The topical application of PGE<sub>2</sub> induced dilatation of pial arteries in the *open* and the *closed* cranial window models [93;94]. In contrast, intra-carotid injection of PGE<sub>2</sub> dilated dural, but not pial arteries [52]. Study II in healthy volunteers demonstrated that infusion of PGE<sub>2</sub> caused dilatation of the STA and the MCA, which is in agreement with Wienecke et al findings [12]. However, in migraineurs the STA response during PGE<sub>2</sub> infusion was not statistically different from placebo, whereas the MCA dilated by 7.2 % as it was observed in controls [12;95]. Interestingly, non-significant dilatation of the STA compared to placebo was also reported after infusion of carbachol in a provocation experiment in migraineurs [96]. Thus, both carbachol [97] and PGE<sub>2</sub> [12] induced significant dilatation of the STA in healthy volunteers, but not in migraineurs. These findings are difficult to explain. A difference between migraine patients and controls seems most likely in view of the high accuracy of measurement of the STA [85]. However, unexpectedly large intra-observer variability may explain this discrepancy. Given that the mechanical dilatation of the cranial arteries per se cannot explain head pain it is possible that PGE<sub>2</sub> induced migraine headache via activation and sensitization of the sensory neurones. Intradermal injections of PGE<sub>2</sub> in humans cause dose-related hyperalgesia [98]. Furthermore, an “inflammatory soup” used in animal experiments to sensitize trigeminal sensory afferents contains several inflammatory mediators, including PGE<sub>2</sub> [99-101]. The “inflammatory soup”- induced peripheral and central sensitization of trigeminal neurons is reversed by COX-inhibitors [100;101]. Interestingly, the topical application of pure PGE<sub>2</sub> on dura mater did not cause excitation of the second order neurones in the trigeminal nucleus caudalis (TNC) [102]. To date there have been no electrophysiological studies on direct effect of PGE<sub>2</sub> on meningeal sensory neurones. Whether PGE<sub>2</sub> sensitizes meningeal nociceptors without the presence of the other mediators should be clarified.

The puzzling difference, observed between PGE<sub>2</sub> and PGI<sub>2</sub> in migraineurs is interesting because both are products of the arachidonic acid cascade and one would expect similar migraine responses. PGI<sub>2</sub> sensitizes meningeal nociceptors [103] and hyperalgesia induced by PGI<sub>2</sub> and PGE<sub>2</sub> occur due to activation of EP<sub>1</sub>/IP – PKC cascade and activation of TRPV<sub>1</sub> receptors of sensory neurones [104]. TRPV<sub>1</sub> receptors mediate transmission and modulation of pain [105]. Furthermore, PGI<sub>2</sub> is considered to be a full agonist of EP<sub>1</sub> and EP<sub>4</sub> receptors [106]. Therefore, it is likely that PGI<sub>2</sub> might have induced immediate migraine-like attacks in the majority of migraineurs if given in a higher concentration, but it was not possible due to AEs [13].

The results of PGE<sub>2</sub>-induced immediate migraine attacks are also in contrast to provocation experiments using other pharmacological triggers such as glyceryl trinitrate (GTN) and calcitonin gene-related peptide (CGRP). Nitric oxide (NO) can in different populations of dural nociceptors either increase or decrease the mechanical responsiveness [107]. A recent study reported an increase of c-Fos expression in the TG and the TNC 4 hours after the intraperitoneal stimulation with GTN in mice [108]. NO stimulates COX synthesis and PG production [109]. GTN injected intraperitoneal in rats caused increase of COX-2, followed by PGE<sub>2</sub> increase in hypothalamus and lower brain stem [110]. Thus, GTN-induced delayed migraine headache might be due to increase of PGE<sub>2</sub> production. CGRP does not sensitize meningeal nociceptors [111], but CGRP receptor antagonist is effective in acute treatment of migraine [112], indicating CGRP's role during migraine attack. PGE<sub>2</sub> induces CGRP release in rat TG [113]. Opposite, CGRP was found to induce a secondary liberation of PGE<sub>2</sub> [114]. Based on these data, it would be plausible to suggest that PGE<sub>2</sub> might be one of the final products involved in spontaneous and experimentally induced migraine attacks. A specific blockade of PGE<sub>2</sub> receptors is therefore of great interest as a possible new anti-migraine drug target.

#### **EP<sub>4</sub> receptor antagonist and PGE<sub>2</sub> in a human model of headache**

BGC20-1531 is highly selective, therefore the doses used in study II should almost completely block EP<sub>4</sub> receptors [51]. The pharmacokinetic profile of BGC20-1531 in study II was more variable ( $C_{max}$  11850 ± 5800 ng/ml and 21100 ± 11600 ng/ml at the 200 mg and 400 mg dose respectively) than in the previously conducted human study (unpublished observations from BTG:  $C_{max}$  9850 ± 2900 ng/ml and 22700 ± 5500 ng/ml at the 200 mg and 400 mg dose respectively). The putative therapeutic concentrations of BGC20-1531 were reached in 5 out of 8 subjects (>10,000 ng/ml). However, the results from the 5 subjects with sufficient plasma exposure did not indicate any effect of BGC20-1531, as it failed to prevent PGE<sub>2</sub>-induced vasodilatation and headache.

PGE<sub>2</sub> was shown to up-regulate EP<sub>4</sub> receptors in sensory neurones [35] and to sensitize sensory neurons mainly through the EP<sub>4</sub> receptors [54]. Interestingly, other EP receptor subtypes, such as EP<sub>1</sub> [33;115], EP<sub>2</sub> [33;115], EP<sub>3</sub> [115], EP<sub>3A/α</sub> [33], EP<sub>3B</sub> [33], EP<sub>3β</sub> [33], EP<sub>3C</sub> [54] are also presented in sensory neurons and involved in PGE<sub>2</sub>-induced sensitization [33;54;104;116] and hyperalgesia [42]. Trigeminal nociceptors express EP<sub>2</sub> and EP<sub>3</sub> receptors co-expressed with TRPV<sub>1</sub> receptors [117]. Blockade of EP<sub>1</sub> receptor by EP<sub>1</sub> receptor antagonist, ONO-NT-012, alone could attenuate allodynia, but not hyperalgesia [118]. Another EP<sub>1</sub> receptor antagonist, GSK345931A, was able to decrease hypersensitivity in a dose-related manner in a preclinical model of inflammatory pain [119] and EP<sub>3</sub> receptor knockout mice had reduced licking responses in the second phase of the formalin assay [120]. Thus, a

blockade of several EP receptor subtypes may be necessary to prevent the PGE<sub>2</sub>-induced sensitization.

Pre-treatment with two different dosages of BGC20-1531 could not prevent PGE<sub>2</sub>-induced dilatation of the MCA and the STA. However, during the PGE<sub>2</sub> infusion a significant decrease in  $V_{MCA}$  compared with baseline was observed on the placebo day, but not on BGC20-1531 treatment days, indicating a weak antagonist effect of BGC20-1531. The weak antagonist effect might be due to a low permeability of BGC20-1531 through the blood-brain barrier (BBB) and/or a simultaneous activation of EP<sub>2</sub> receptors by PGE<sub>2</sub>. At present no data are available to show the distribution of EP<sub>4</sub> and EP<sub>2</sub> receptors in human intra- and extra-cranial arteries. The unexpected prolongation and increase of the STA dilatation after 200 mg BGC20-1531 may also be explained by the activation of EP<sub>2</sub> receptors. The EP<sub>2</sub> receptor has a shorter cytoplasmic carboxyl terminus [121-123] and therefore, undergoes less internalization [124] and desensitization [125] after exposure to PGE<sub>2</sub> compared to the EP<sub>4</sub> receptor. In contrast to EP<sub>4</sub>, EP<sub>2</sub> remains sensitive to metabolites of PGE<sub>2</sub> [125;126]. Hence the activation of EP<sub>2</sub> receptors could both prolong and intensify dilatation of the STA. Another possible explanation is that a blockade of a specific vasodilating receptor might have induced a down regulation of the vasoconstricting receptors such as EP<sub>1</sub> and EP<sub>3</sub>. The decreased ability of the artery to constrict might have resulted in a prolonged dilatation of the artery in the presence of PGE<sub>2</sub>. Collectively, BGC20-1531 failed to prevent PGE<sub>2</sub>-induced headache more likely due to its inability to abolish sensitization, however the cephalic dilatation has been observed during both pre-treatment days, therefore the role of the dilatation in the induction of pain in study II as well as in study I can not be excluded.

#### **PGF<sub>2α</sub> in a human model of headache in healthy subjects**

Study III was the first study where a vasoconstricting prostaglandin was applied in a human model of headache. PGF<sub>2α</sub> failed to trigger headache in healthy volunteers in contrast to other vasodilating prostaglandins [11;12;14].

The pro-nociceptive features of PGF<sub>2α</sub> are well described [29;73;74]. PGF<sub>2α</sub> significantly enhances responses from nociceptors to submaximal thermal stimulation, whereas PGD<sub>2</sub> does not [127]. PGF<sub>2α</sub> has also been reported as being less potent to sensitize sensory neurones *in vitro* compared to PGD<sub>2</sub>, PGI<sub>2</sub> and PGE<sub>2</sub> [128]. Thus, both PGD<sub>2</sub> and PGF<sub>2α</sub> were found in some studies to be less potent to sensitize sensory neurones [127;128]. However, PGD<sub>2</sub> induced a mild headache in healthy subjects [14], whereas PGF<sub>2α</sub> failed to induce any headache. Table 7 shows headache and vascular responses observed across the PG studies. During PGE<sub>2</sub>, PGI<sub>2</sub> and PGD<sub>2</sub> infusions the maximum velocity drop in the MCA compared to baseline was 14.3% (-4.6%- 10.5%) at T<sub>10</sub> and T<sub>20</sub> [12-14]. The STA dilated by 37.6% (23.5%-55.7%) and the RA dilated by 9.15% (4.4% - 16.3%) [12-14]. No vascular changes in either intra- or extra-cranial arteries were observed after the infusion of PGF<sub>2α</sub>, suggesting that the pro-nociceptive features of prostaglandins alone are *not* sufficient to induce headache and that prostaglandin-induced vasodilatation may contribute to generation of headache.

The missing constriction of the investigated cephalic arteries during the intravenous PGF<sub>2α</sub> infusion in healthy subjects is in contrast to the *in vitro* studies on human tissues [61-64]. However, an *in vivo* study in cats demonstrated dilatation of small and large feline pial arterioles during topical application of PGF<sub>2α</sub> [94] and another study reported no response from the same vessels [129]. Since a significant increase in blood pressure during the PGF<sub>2α</sub> infusion was observed a specific immediate constriction

of arterioles might have been induced by PGF<sub>2α</sub>. This is in agreement with a previous study in FP (-/-) mice that showed abrogation of the immediate dose-dependent increase in blood pressure during the PGF<sub>2α</sub> infusion [130]. Due to the fact that little is known about FP receptor distribution in human intra- and extra-cranial arteries, the missing constriction of the cerebral arteries after PGF<sub>2α</sub> administration might be explained by species specific responses to PGF<sub>2α</sub> exposure *in vivo* or possibly to a simultaneous activation of other dilating prostaglandin receptors. Thus, a cross-talk between FP and EP<sub>2</sub> receptors could result in an increase of cAMP [131] and attenuation or decrease of vasoconstriction caused by the FP receptor.

In conclusion, the absence of the intra- and extra-cerebral vascular changes during the PGF<sub>2α</sub> infusion is the best possible explanation of the insignificant incidence of headache. The data from study III suggest that the vasodilating abilities as well as the ability to sensitize nociceptors of PGs are necessary to provoke headache in healthy volunteers.

**Table 7:** Peak vascular responses (percents from base line) of different PGs in humans.

\* Significant mean difference for AUC between placebo and active day or significant incidence of headache.

NS non significant mean difference for AUC or incidence of headache between placebo and active day.

|                   | Immediate headache   | V <sub>MCA</sub>    | STA                 |
|-------------------|----------------------|---------------------|---------------------|
| PGE <sub>2</sub>  | 11 (11)*             | -13.9% *            | 23.5% *             |
| PGI <sub>2</sub>  | 11 (12)*             | -10.5% *            | 32.9% *             |
| PGD <sub>2</sub>  | 10 (12)*             | -28.3%*             | 55.7% *             |
| PGF <sub>2α</sub> | 2 (12) <sup>NS</sup> | -2.7% <sup>NS</sup> | 10.9% <sup>NS</sup> |

## METHODOLOGICAL CONSIDERATIONS

PGs are synthesized in almost all body cells and are involved in the regulation of various normal physiological processes in humans. The infusion of different PGs is always accompanied by AEs as well as changes in MAP and HR. The physiological reaction induced by PGs might have compromised the blindness of study I and III (in study II all participants received PGE<sub>2</sub> on all three study days). However, the data agreement between the two independent human studies in healthy volunteers [12;95] indicates the reproducibility of the PGE<sub>2</sub> model of headache. The current double-blind, cross-over design is the best possible way to avoid the methodological biases. One might argue that the dose given in the main experiment in study III was not sufficient to induce headache. Before the main experiment (study III) a pilot study was conducted. Four different doses of PGF<sub>2α</sub> (2 µg/kg/min, 2.5 µg/kg/min, 3 µg/kg/min, 3.5 µg/kg/min) were tested to determine the highest well tolerated dose. The average max MAP increase during 20 min injection of 2 µg/kg/min PGF<sub>2α</sub> was 80 (60-89 safety limits, defined in the protocol), 2.5 µg/kg/min PGF<sub>2α</sub> 82 (62-94), 3 µg/kg/min PGF<sub>2α</sub> 88 (60-90) and 3.5 µg/kg/min PGF<sub>2α</sub> 89 (61-91). The maximal dose of 3.5 µg/kg/min PGF<sub>2α</sub> was well tolerated and therefore used in the main experiment. Due to safety reasons it was not possible to use a higher concentration of PGF<sub>2α</sub>, as it could result in MAP increase over 20%. Furthermore, the previously conducted human experiments with PGE<sub>2</sub>, PGD<sub>2</sub> and PGI<sub>2</sub> indicate that the highest dose within the safety limits is sufficient to induce headache if any. It should also be noted that the low exposures of BGC20-1531 in three out of eight volunteers

might have contributed to the negative outcome in study II. However, the power calculations in study II were based on the expected significant attenuation of the PGE<sub>2</sub>-induced headache. If BGC20-1531 would have prevented the headache, the statistical difference could still be detected even in five volunteers, as it was seen in a previous study, using CGRP antagonist [132].

## CONCLUDING REMARKS AND FUTURE PERSPECTIVES

The current thesis contributes further to our understanding of the role of PGs in the pathogenesis of migraine. PGE<sub>2</sub> was the first vasoactive substance which provoked the immediate migraine-like attacks, accompanied by the MCA dilatation. Surprisingly, no statistical changes of the STA were observed. However, the insignificant dilatation of the STA does not indicate that the other extra-cerebral arteries were not dilated as well. Using the current methodological approach it was not possible to measure meningeal arteries, for example MMA. A recent study found dilatation of the MMA in migraine patients during the CGRP-induced migraine attacks [133]. It would be interesting to investigate if PGE<sub>2</sub> dilates the MMA and if the dilatation of the MMA occurs simultaneously with the migraine attack. The results from PGE<sub>2</sub> study in migraineurs indicate that a specific blockade of PGE<sub>2</sub> is interesting as a new potential drug target for the acute treatment of migraine. The study with BGC20-1531 revealed that a specific blockade of a single EP<sub>4</sub> receptor was not sufficient to prevent PGE<sub>2</sub>-induced headache and vasodilatation. This outcome could be explained by a possible low BGC20-1531 permeability through the BBB and by the activation of the other EP receptors. A pre-treatment with BGC20-1531 during a longer period could ensure its permeability through the BBB. If a longer pre-treatment with BGC20-1531 attenuates the PGE<sub>2</sub>-induced headache and vasodilatation then BGC20-1531 should be considered as a prophylactic drug for the treatment of migraine. Another possibility is to combine a specific EP<sub>4</sub> receptor blockade with a blockade of the other vasodilating receptor, EP<sub>2</sub> receptor. Recently, the EP receptors' distribution in the rat trigeminal-vascular system as well as other brain structures involved in the pathogenesis of migraine pain was reported. EP<sub>1</sub> and EP<sub>2</sub> were highest expressed in the TNC, and EP<sub>3</sub> and EP<sub>4</sub> in the TG [134]. The findings should be supported by the *in vitro* studies on human tissues. More knowledge about the EP receptor distribution within the human trigeminal-vascular system is necessary for the development of a specific PGE<sub>2</sub> antagonist.

Finally, the results from the PGF<sub>2α</sub> study suggest that the vasodilating properties as well as the ability to sensitize nociceptors of PGs are necessary to provoke headache in healthy volunteers. Interestingly, intravenous administration of norepinephrine, well known for its vasoconstrictor effect did not cause headache or vascular responses in healthy volunteers as well [135]. The findings from both studies indicate that the vasoconstricting compounds do not induce headache and therefore unlikely to provoke migraine.

## SUMMARY

Human models of headache may contribute to understanding of prostaglandins' role in migraine pathogenesis. The current thesis investigated the migraine triggering effect of prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) in migraine patients without aura, the efficacy of a novel EP<sub>4</sub> receptor antagonist, BGC20-1531, in prevention of PGE<sub>2</sub>-induced headache and the ability of prostaglandin F<sub>2α</sub> (PGF<sub>2α</sub>) to

trigger headache without any vasodilatation in healthy volunteers.

All studies were designed as a double-blind, placebo-controlled, cross-over experiments, where PGE<sub>2</sub> /PGF<sub>2α</sub> or saline were infused over 20-25 min. In study with EP<sub>4</sub> receptor antagonist healthy volunteers were pre-treated with two different doses of BGC20-1531 or placebo followed by PGE<sub>2</sub> infusion over 25 min. The headache data were collected during the whole study day, whereas the possible vascular changes were measured during the in-hospital phase of 1.5 h.

The infusion of PGE<sub>2</sub> caused the immediate migraine-like attacks and vasodilatation of the middle cerebral artery in migraine patients without aura. The highly specific and potent EP<sub>4</sub> receptor antagonist, BGC20-1531, was not able to attenuate PGE<sub>2</sub>-induced headache and vasodilatation of both intra- and extra-cerebral arteries. The intravenous infusion of PGF<sub>2α</sub> did not induce headache or statistically significant vasoconstriction of cerebral arteries in healthy volunteers.

Novel data on PGE<sub>2</sub>-provoked immediate migraine-like attacks suggest that PGE<sub>2</sub> may be one of the important final products in the pathogenesis of migraine. The lack of efficacy of EP<sub>4</sub> receptor antagonist suggests that a single receptor blockade is not sufficient to block PGE<sub>2</sub> responses, hence EP<sub>2</sub> receptor should be investigated as a potential drug target for the treatment of migraine. The absence of headache during the PGF<sub>2α</sub> infusion demonstrates that vasodilating properties are necessary for the induction of headache and migraine.

## ABBREVIATIONS

|          |   |                                 |  |
|----------|---|---------------------------------|--|
| AC       | adenylate cyclase   | OM                              | orbito-meatal  |
| AEs      | adverse events  | PACAP                           | pituitary adenylylate cyclase activating peptide                 |
| ANOVA    | analysis of variance  | P <sub>50</sub> CO <sub>2</sub> | end-tidal pressure of CO <sub>2</sub>                            |
| AUC      | area under the curve  | PGI <sub>2</sub>                | prostaglandin I <sub>2</sub>                                     |
| cAMP     | cyclic adenosine monophosphate                                | PGD <sub>2</sub>                | prostaglandin D <sub>2</sub>                                     |
| CBF      | cerebral blood flow   | PGE <sub>2</sub>                | prostaglandin E <sub>2</sub>                                     |
| CGRP     | calcitonin gene-related peptide                               | PGF <sub>2α</sub>               | prostaglandin F <sub>2α</sub>                                    |
| CI       | confidence interval   | PGFS                            | prostaglandin F synthase   |
| COX      | cyclooxygenase  | PGI <sub>2</sub>                | prostaglandin I <sub>2</sub>                                     |
| CV       | coefficient of variation                                      | PKC                             | protein kinase C   |
| DRG      | dorsal root ganglion  | SAT                             | transcutaneous arterial oxygen saturation                        |
| ECG      | electrocardiogram   | SD                              | standard deviation   |
| GTN      | glyceryl trinitrate   | STA                             | superficial temporal artery                                      |
| HIV      | human immunodeficiency virus                                  | TCD                             | transcranial doppler   |
| HR       | heart rate  | TG                              | trigeminal ganglion  |
| IHS      | International Headache Society                                | TNC                             | trigeminal nucleus caudalis                                      |
| LC-MS-MS | liquid chromatography with tandem mass spectrometry detection | TRPV <sub>1</sub>               | transient receptor potential cation channel subfamily V member 1 |
| MAP      | mean arterial blood pressure                                  | TX                              | thromboxanes   |
| MCA      | middle cerebral artery  | V <sub>MCA</sub>                | blood flow velocity in the middle cerebral artery                |
| MD       | mean difference   | VRS                             | verbal rating scale  |
| MMA      | middle meningeal artery                                       | WHO                             | World Health Organisation  |
| mRNA     | messenger ribonucleic acid                                    |                                 |  |
| NO       | nitric oxide  |                                 |  |
| NSAID    | non-steroidal anti-inflammatory substances                    |                                 |  |

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