# BIBN4096BS antagonizes human α-calcitonin gene related peptide–induced headache and extracerebral artery dilatation

*Background and Objective:* Calcitonin gene–related peptide (CGRP) plays a pivotal role in migraine pathogenesis. BIBN4096BS is the first CGRP receptor antagonist available for human studies, and its efficacy in the acute treatment of migraine has been demonstrated. We investigated the ability of BIBN4096BS to inhibit human αCGRP (h-αCGRP)–induced headache and cerebral hemodynamic changes in healthy volunteers.

*Methods:* Ten healthy volunteers completed this double-blind, placebo-controlled crossover study with 2.5 mg BIBN4096BS and placebo as pretreatments before a 20-minute intravenous infusion of h-αCGRP (1.5 μg/min). Transcranial Doppler ultrasonography was used to measure blood flow velocity in the middle cerebral artery (MCA); regional and global cerebral blood flow (CBF) was measured by xenon 133 inhalation single-photon emission computed tomography. The temporal and radial artery diameter was measured by high-frequency ultrasound. Systemic hemodynamics, end-tidal partial pressure of carbon dioxide ($P_{ETCO_2}$), and headache were monitored.

*Results:* Of the 10 volunteers, 6 had a CGRP-induced headache during the in-hospital phase after placebo pretreatment but none after BIBN4096BS ($P = .031$). BIBN4096BS did not affect changes in the diameter of the MCA or changes in CBF induced by h-αCGRP. Vasodilatation of the extracranial arteries was, however, significantly inhibited ($P < .001$ for temporal artery and $P = .001$ for radial artery).

*Conclusions:* These results show that BIBN4096BS effectively prevents CGRP-induced headache and extracerebral vasodilatation but does not significantly affect the induced cerebral hemodynamic changes. (Clin Pharmacol Ther 2005;77:202-13.)

**Kenneth A. Petersen, MD, Lisbeth H. Lassen, PhD, Steffen Birk, PhD, Lynna Lesko, PhD, and Jes Olesen, DMSc** *Glostrup, Denmark, and Ridgefield, Conn*

Calcitonin gene–related peptide (CGRP) is a neuropeptide found in the perivascular nerve terminals surrounding arteries.[1] A measurable concentration of CGRP is circulating in the blood at rest,[2] and CGRP receptors are localized throughout the body.[3] Cerebral and other cephalic arteries have a particularly rich innervation of CGRP-containing afferent trigeminal nerve fibers, and these arteries, as studied in tissue baths, are particularly sensitive to CGRP.[4] CGRP is found in an increased concentration in external jugular venous blood but not in blood from the cubital vein during a migraine attack.[5] After infusion in patients with migraine, CGRP caused a migraine-like headache and in some a genuine migraine attack with associated symptoms that were indistinguishable from the patients' normal migraine attacks.[6] Peptide antagonists of CGRP receptors have been available for experimental studies for several years, but previously available compounds have not been tested for safety and are, therefore, not suitable for human clinical studies. One CGRP receptor antagonist, BIBN4096BS, has been developed with the purpose of treating acute migraine, and a phase II study has provided proof of efficacy.[7] Although BIBN4096BS potently interacts with the human CGRP

From the Danish Headache Center, University of Copenhagen, and Department of Neurology, Glostrup University Hospital, Glostrup, and Boehringer Ingelheim Pharmaceuticals Inc, Ridgefield.

Boehringer Ingelheim sponsored the study and provided BIBN4096BS. The authors were independently responsible for the study design, data analysis, and manuscript. The technical equipment used was partly sponsored by the Villum Kann Rasmussen Foundation, Toyota Foundation, and Simon Fougner Hartmann Foundation. The Lundbeck Foundation funds the research of the Danish Headache Center.

Received for publication June 29, 2004; accepted Oct 6, 2004.

Reprint requests: Kenneth A. Petersen, MD, Danish Headache Center, University of Copenhagen and Department of Neurology, Glostrup University Hospital, KAS Glostrup, DK-2600 Glostrup, Denmark.
E-mail: *kapetersen@dadlnet.dk*

0009-9236/$30.00



doi:10.1016/j.clpt.2004.10.001

receptor in vitro,[8,9] no information is available on its ability to inhibit CGRP changes in human volunteers at increased levels of the peptide as seen during a migraine attack.[5]

We, therefore, decided to conduct a placebo-controlled, double-blind crossover experiment, in which BIBN4096BS and placebo in random order were used as pretreatment followed by infusion of human αCGRP (h-αCGRP). The aims of this study were to validate the ability of h-αCGRP to provoke headache and to describe its effect on cerebral, extracerebral, and systemic circulatory parameters. Furthermore, we analyzed whether BIBN4096BS could partly or fully block the changes evoked by h-αCGRP. It was our hope that the study could thus contribute to a better understanding of the role of CGRP in neurovascular headache and the site of action of this new antimigraine compound.

## METHODS

***Design and participants.*** This was a placebo-controlled, double-blind crossover study that included 11 healthy subjects (7 men and 4 women). One female participant had claustrophobia that developed during baseline single-photon emission computed tomography (SPECT) scanning and was excluded before any trial medication was given. Ten participants completed both treatment days. The participants were aged 24 to 31 years (mean, 26.5 years) and weighed 68 to 89.4 kg (mean, 77.4 kg). The participants had no current or previous cardiovascular, cerebrovascular, endocrine, or neurologic disorder, including no migraine, hypotension, or hypertension. A frequency of tension-type headache of 4 d/mo or lower was accepted. On the day of enrollment, physical and neurologic examination, electrocardiography, and blood sampling were done.

The healthy volunteers were randomized to receive either 2.5 mg BIBN4096BS or placebo (xylitol 5%) as an intravenously administered pretreatment of 10 minutes' duration. After a free interval of 10 minutes, 1.5 μg/min h-αCGRP was administered continuously for 20 minutes on both trial days. The 2 trial days were separated by at least 1 week.

Boehringer Ingelheim GmbH supplied BIBN4096BS and performed the randomization and blinding, which was balanced (ClinPro, version 6; Clinical Systems, Inc, Garden City, NY). The dose effective in the treatment of acute migraine attacks was used.[7]

Human-αCGRP was purchased from Clinalfa AG, Läufelfingen, Switzerland. In a study performed previously, we used a dose of 2 μg/min.[6] This dose, however, induced pronounced hypotension that in 2 patients necessitated premature termination of the infusion. In the current study we, therefore, used the lower dose of 1.5 μg/min.

All participants gave written informed consent before randomization. The Ethical Committee of Copenhagen (KA00079gs) and the Danish Medicines Agency (2612-1376) approved the study, which was conducted in accordance with the Helsinki II Declaration and the Guidelines for Good Clinical Practice.[10]

***Recording of adverse events.*** Every 15th minute from time (T) zero ($T_0$) (baseline) to $T_{240}$ (end of study period), the volunteers were questioned regarding the presence of adverse events (AEs) and rated headache. Between questionings, the participants self-reported any changes that they might have. The intensity of the AEs was graded as mild, moderate, or severe, and their relationship to study medication was classified as related or not related by the investigator. Headache intensity was scored on an 11-point verbal rating scale with 0 indicating no headache; 1 indicating a feeling of the occurrence of something unusual inside the head, not necessarily actual pain; 5 indicating headache of medium severity; and 10 indicating worst imaginable headache. Accompanying symptoms were recorded according to the International Headache Classification.[11] During the study period, the investigator recorded the AEs. After discharge, the volunteers made an hourly recording of AEs up to 24 hours after the infusion of placebo or BIBN4096BS.

***Cerebral blood flow measurements.*** Global and regional cerebral blood flow (CBF) was measured with xenon 133 inhalation and SPECT with a brain-dedicated camera (Ceraspect; DSI, Waltham, Mass). The apparatus consisted of a stationary annular sodium iodide crystal and a fast-rotating collimator system. Each rotation took 10 seconds, thereby acquiring 1 frame in a 30-frame dynamic protocol of $^{133}Xe$ inhalation, with 3 background, 9 wash-in, and 18 wash-out frames by use of the Kanno-Lassen algorithm.[12] A photoelectric window of 70 to 100 keV was used.

Thirty-two slices were reconstructed in a 64 × 64 matrix with each pixel measuring 0.33 × 0.33 cm by use of a Butterworth one-dimensional filter (cutoff, 1.5; order, 6). The 32 slices were reduced to sets of 8 transaxial slices generated by adding 4 slices together to a total slice thickness of 1.32 cm. A correction by use of the Chang algorithm ($\mu m = 0.05$ cm) and nose artifact was performed. The output for each pixel was the inhibition constant ($K_i$) value, and flow values were estimated from these by use of the partition coefficient ($\lambda$) of 0.85 (gray matter).

A Datex Normocap 200 (Dameca, Roedovre, Denmark) was used for end-tidal partial pressure of carbon

dioxide ($P_{ETCO_2}$) measurements during the CBF acquisitions. A Ceratronic XAS SM 32C (Randers, Denmark) was used for the $^{133}Xe$ administration. Each measurement lasted 5 minutes.

Calculations of flow in the perfusion territories of the major cerebral arteries were performed by fitting of standard vascular regions of interest on the 5 rostral slices at 3.6, 5.0, 6.3, 7.6, and 9 cm above the orbitomeatal line. Flow in the territory of the middle cerebral artery (MCA) ($rCBF_{MCA}$) was calculated as a mean of the left and right side.

***Transcranial Doppler and C-scan.*** Transcranial Doppler (TCD) ultrasonography (2 MHz) (Multi-Dop X; DWL, Sipplingen, Germany) was used for the measurement of blood flow velocity. The recordings were done simultaneously and bilaterally as previously described but with handheld probes.[13] Along the MCA, a fixed point was found for the measurement. The fixed point was chosen as close as possible to the bifurcation of the anterior cerebral artery and MCA. The same fix point was used for each individual and for each recording, for which the signal was optimized. On the basis of the envelope curve (the spectral TCD curve), a time averaged mean ($V_{mean}$) over approximately 4 cardiac cycles or 4 seconds was calculated by the built-in software (version 7.40x of MDX TCD-7 software for Multi-Dop X hardware, DWL). The final measure used for each time point was an average of 4 cycles ($V_{MCA}$). Simultaneously with the TCD recording, a mask covering the subject's mouth and nose region was placed for the measurement of $P_{ETCO_2}$ (Datex Normocap 200; Dameca).

A high-resolution ultrasound scanner, C-scan (Dermascan C, 20 MHz; bandwidth, 15 MHz) (Cortex Technology, Hadsund, Denmark),[14] was used to measure the diameter of the left temporal and left radial artery. The diameter of the former was measured at the front branch of the superficial temporal artery and the latter at the wrist. To ensure that the repeated measurements with TCD and C-scan were performed in the same place, marks were drawn on the skin. After the last recording on the first trial day, the coordinates of the marks were kept for reuse on the following trial day.

***Pharmacokinetics.*** Plasma concentrations of BIBN4096BS were sampled at the following time points: $T_{-10}$ (baseline), $T_{9.5}$, $T_{30}$, $T_{60}$, and $T_{180}$ on each trial day in Vacutainer blood-collecting tubes with ethylenediaminetetraacetic acid (K3 10-mL glasses; Becton Dickinson, Rutherford, NJ). Samples were stored on ice for a maximum of 30 minutes before centrifuged for 10 minutes (2000 rpm) at 4°C. The plasma was stored at −20°C until analyzed at Boehringer Ingelheim Pharma GmbH & Co KG (Biberach an der Riis, Germany). The plasma concentration of CGRP was determined twice, at baseline ($T_{-10}$) and at the end of the h-αCGRP infusion ($T_{40}$).

***BIBN4096BS antibodies.*** BIBN4096BS was modified with succinic acid anhydride. This hapten was covalently coupled to human serum albumin. Polyclonal antibodies were produced by immunization of 3-month-old female New Zealand rabbits with the immunogen in complete Freund's adjuvant. After several booster immunizations, the antibodies were purified from rabbit serum by use of protein A–Sepharose (Sepharose is a registered trademark of Amersham Biosciences).

***BIBN4096BS analytic methods.*** The procedures were conducted in accordance with current international guidelines.[15] In this competitive enzyme-linked immunosorbent assay, the biotinylated anti-BIBN4096BS antibodies (immunoglobulin G fraction) were bound to microtiter plates that were adsorptive-coated with avidin. BIBN4096BS in the plasma sample competed with added horseradish peroxidase–labeled BIBN4096BS reagent for binding sites on the solid-phase antibodies. After incubation, unbound BIBN4096BS and plasma components were removed by washing. Antibody-bound enzyme activity was detected with a chromogenic substrate. The amount of colored product formed was measured photometrically and decreased with the increasing concentration of BIBN4096BS in the plasma sample. The BIBN4096BS concentration corresponding to the measured optical absorbance was calculated via data fitting of the nonlinear standard curve.

To compensate for slight variations in immunochemical reaction parameters (such as temperature and antibody binding capacity) between microplates, a standard curve was included on each plate. All steps of the enzyme-linked immunosorbent assay were performed at 22°C ± 1°C, which corresponded to room temperature of the air-conditioned laboratory.

Assay precision as assessed from 886 triplicate determinations by construction of a precision profile was 9.1% coefficient of variance (CV) at the lower limit of quantification, 2.7% CV at the upper limit of quantification, and 1.6% CV in the middle of the working range (0.5 ng/mL).

***Human-αCGRP analysis.*** The analysis of h-αCGRP plasma concentrations was performed at the Department of Clinical Physiology and Nuclear Medicine, Glostrup Hospital (Glostrup, Denmark). The method of analysis has been described in detail else-

where.[2] In this study only 100 μL of serum was used. The normal values were 85 ± 35.4 pmol/L for women and 88 ± 36.2 pmol/L for men (Schifter S, oral communication, November 2002).

***Trial procedure.*** The healthy volunteers began the study at 8 AM, headache-free. For the preceding 8 hours, they had abstained from drinking coffee, tea, and caffeine-containing beverages and smoking tobacco and they had not taken any medication, except oral contraception. They rested in the supine position throughout the study period ($T_{-20}$ to $T_{180}$) Two intravenous catheters (Optiva*2 [18 gauge]; Johnson & Johnson, Ethicon SpA, Pomezia, Italy) were inserted into the cubital veins, one for the administration of human αCGRP and BIBN4096BS and the other for blood sampling. The volunteers rested for at least 30 minutes before baseline values of CBF, $V_{MCA}$, temporal and radial diameter, blood pressure (BP), heart rate (HR), and electrocardiogram were recorded. The start of infusion of 2.5 mg BIBN4096BS or placebo was designated as time zero ($T_0$). The infusion lasted 10 minutes. At $T_{20}$, a 20-minute infusion of h-αCGRP (1.5 μg/min) was initiated. Infusions were administered by a time- and volume-controlled infusion pump (Braun perfusor; B. Braun Melsungen AG, Melsungen, Germany).

All measurements, except the CBF measurements, were recorded quarterly for 3 hours (study period), and BP, HR, electrocardiogram (Cardiofax; Nihon Kohden Corporation, Tokyo, Japan), AEs, and headache were recorded for an additional hour. BP and HR were measured every 5 minutes for the first hour and thereafter every 15th minute with an automatically inflating cuff (Omega 1400, In Vivo Research Laboratories Inc, Copiague, NY). In the observation period from $T_{180}$ to $T_{240}$, the participants were allowed to sit upright. Three SPECT scans were done as follows: at baseline, at $T_{60}$, and at $T_{90}$. $V_{MCA}$ was measured immediately after each SPECT scan.

The estimated perfusion ($rCBF_x$) in the area of a given artery (x) is dependent on the mean blood flow velocity [$V_{mean(x)}$] and the cross-sectional area ($\pi \times r^2$) of the artery. The following equation is valid for the regional CBF:

$$rCBF_{(x)} = Vmean_{(x)} \cdot \pi \cdot r^2$$

Hence,

$$\Delta \text{ Diameter} = \left(\left[\sqrt{rCBF_{2(x)}/V_{mean_{2(x)}} \times V_{mean_{1(x)}}/rCBF_{1(x)}}\right] - 1\right) \times 100$$

Δ Diameter is the relative percentage change in diameter, $V_{mean1(x)}$ is the mean blood velocity before infusion of drugs, and $V_{mean2(x)}$ is the velocity at a relevant time point after the infusion; the same designation is applied for rCBF.[16,17]

***Statistics.*** Baseline was calculated as a mean of the measurements at time points $T_{-20}$ and $T_{-10}$ in the analysis. Values are presented as means ± SD. $P < .05$ was considered significant. All analyses were performed by use of SPSS statistical software, version 10.0 (SPSS Inc, Chicago, Ill).

For changes over time on each trial day, $V_{MCA}$, global CBF, $rCBF_{MCA}$, diameter of the temporal and radial artery, BP, and $PETCO_2$ were analyzed by a univariate ANOVA for the factors time and subject. If a significant change was found, a post hoc analysis (Dunnett multiple comparisons test) was performed to localize the change. To eliminate the risk of mass significance of measurements with numerous repeated measurements, 4 points of interest were chosen as follows: baseline, 45 minutes, 105 minutes, and 165 minutes. Absolute values were used for the statistical analysis. For the comparison between BIBN4096BS and placebo, a paired *t* test was performed for the following measurements: $V_{MCA}$, global CBF, $rCBF_{MCA}$, diameter of the temporal and radial arteries, BP, and $PETCO_2$. The summary measure for the *t* test was the area under the curve (AUC) calculated on percentage changes from baseline.

Immediate headache was defined as any headache during the first 60 minutes after the start of the h-αCGRP infusion. Any headache occurring thereafter was referred to as delayed headache. Peak values and area under the curve for headache ($AUC_{headache}$) were compared between the 2 trial days by use of the Wilcoxon signed rank test. The occurrence of headache and AEs on the 2 trial days was compared by use of the McNemar test.

## RESULTS

***Baseline values.*** All baseline measurements of the hemodynamic responses are summarized in Table I. Only the baseline $PETCO_2$ measured simultaneously with the TCD recordings showed a significant difference between study days ($P = .03$). The finding was interpreted as incidental and was not taken into account in the processing of data.

***Effect of BIBN4096BS on CGRP-induced headache and other AEs.*** On placebo days, 5 participants had an immediate headache and 3 had a delayed headache; the maximum immediate headache score was 2 and the maximum delayed headache score was 1. No participants had an immediate headache but 1 had a

**Table I.** Baseline values of measured variables

| *Measured variable* | *Placebo plus h-αCGRP* | *BIBN4096BS (2.5 mg) plus h-αCGRP* | *P value* |
|---|---|---|---|
| Global CBF (mL · 100 g brain tissue$^{-1}$ · min$^{-1}$) | 46.7 ± 10.8 | 45.6 ± 10.5 | .5 |
| $rCBF_{MCA}$ (mL · 100 g brain tissue$^{-1}$ · min$^{-1}$) | 45.9 ± 10.5 | 44.7 ± 10.6 | .3 |
| $P_{ETCO_2}$ (mm Hg) | | | |
| CBF | 39 ± 3.5 | 39 ± 4.1 | .9 |
| TCD | 41 ± 3.0 | 39 ± 3.6 | .03* |
| $V_{MCA}$ (cm/s) | 78 ± 17.0 | 74 ± 15.3 | .1 |
| C-scan | | | |
| Temporal (mm) | 1.26 ± 0.4 | 1.29 ± 0.3 | .8 |
| Radial (mm) | 2.76 ± 0.5 | 2.63 ± 0.4 | .2 |
| Systolic blood pressure (mm Hg) | 113 ± 8 | 113 ± 6 | .9 |
| Diastolic blood pressure (mm Hg) | 64 ± 8 | 64 ± 5 | .8 |
| Mean arterial blood pressure (mm Hg) | 79 ± 6 | 80 ± 7 | .5 |
| Heart rate (beats/min) | 54 ± 8 | 53 ± 4 | .7 |
| Plasma CGRP (pmol/L) | 89 ± 20.3 | 91 ± 20.4 | .5 |

h-αCGRP, Human α-calcitonin gene–related peptide: CBF, cerebral blood flow; $rCBF_{MCA}$, cerebral blood flow in territory of middle cerebral artery; $P_{ETCO_2}$, end-tidal partial pressure of carbon dioxide; TCD, transcranial Doppler; $V_{MCA}$, middle cerebral artery blood flow velocity (average of 4 cycles); CGRP, calcitonin gene–related peptide.
*Significant difference between baseline on placebo and BIBN4096BS pretreatment days (paired *t* test).

**Table II.** Effect of BIBN4096BS pretreatment on h-αCGRP–induced symptoms

| *Symptom* | *Placebo plus h-αCGRP (No.)* | *BIBN4096BS (2.5 mg) plus h-αCGRP (No.)* | *P value (McNemar test)* |
|---|---|---|---|
| Flushing | 10 | 0 | $P = .002$ |
| Heat sensation | 8 | 0 | $P = .008$ |
| Palpitations | 5 | 0 | $P = .063$ |
| Conjunctival injection | 9 | 0 | $P = .004$ |
| Headache | 6 | 0 | $P = .031$ |

The flushing and conjunctival injection was based on the investigators' observations. Heat sensation and palpitation were reported and headache was systematically scored. The data shown are from the entire in-hospital study period.

delayed headache after BIBN4096BS pretreatment. The delayed headache occurred 6 hours after the infusion of BIBN4096BS, lasted 3 hours, and was scored 1. The effect of BIBN4096BS in preventing immediate headache was significant ($P = .034$ for peak headache and $P = .04$ for $AUC_{headache}$) and in preventing the occurrence of any headache during the in-hospital phase ($P = .031$, McNemar test).

After placebo pretreatment, h-αCGRP caused flushing in all participants and all but 1 had bilateral conjunctival injection. Eight experienced a sensation of heat. Five reported palpitations. None of these CGRP-induced changes were seen on days when BIBN4096BS was administered as pretreatment (Table II). AEs that could possibly be assigned to the CGRP receptor antagonist were located to the infusion site.

***CBF.*** Global CBF increased significantly after h-αCGRP on both study days ($P = .007$ after placebo and $P = .009$ after BIBN4096BS pretreatment). The increase was measured 20 minutes after the h-αCGRP infusion was stopped. No difference was found between the 2 days ($P = .42$).

After h-αCGRP, $rCBF_{MCA}$ increased significantly on both trial days ($P = .003$ and $P = .01$), again 20 minutes after the h-αCGRP infusion. No significant difference was observed between the 2 study days ($P = .38$). Data were not corrected for $P_{ETCO_2}$, because no significant changes were found on either day ($P = .2$ and $P = .6$) or between treatment days ($P = .1$).

***TCD.*** $V_{MCA}$ did not vary significantly over time ($P = .3$ for placebo and $P = .7$ for BIBN4096BS), and between the 2 trial days, no difference was seen ($P = .74$). On the basis of the $rCBF_{MCA}$ and $V_{MCA}$ measurements, the effect on the relative percentage diameter change of MCA can be estimated.[16,18] As seen in Table III, a dilation of the MCA was found on both study days. Compared with baseline, the dilation occurring on placebo days reached significance at $T_{60}$ ($P = .005$). This corresponded to a diameter increase of 9.3% ± 8.1%. When BIBN4096BS was given as pretreatment,

DOCKET ALARM

# Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

## Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

## Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

## Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

## API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

**LAW FIRMS**
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

**FINANCIAL INSTITUTIONS**
Litigation and bankruptcy checks for companies and debtors.

**E-DISCOVERY AND LEGAL VENDORS**
Sync your system to PACER to automate legal marketing.