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Phosphodiesterase 3 inhibitor cilostazol induces migraine-like attacks via cyclic AMP increase

Song Guo, Jes Olesen and Messoud Ashina

Danish Headache Centre and Department of Neurology, Glostrup Hospital, Faculty of Health and Medical Sciences, University of Copenhagen, DK-2600 Glostrup, Copenhagen, Denmark

Correspondence to: Messoud Ashina, MD, PhD, D.Med.Sci,
Danish Headache Centre and Department of Neurology,
Glostrup Hospital,
Faculty of Health and Medical Sciences, University of Copenhagen,
Nordre Ringvej 57, DK-2600 Glostrup,
Copenhagen,
Denmark
E-mail: ashina@dadlnet.dk

The initiating mechanisms of migraine attacks are very complex but may involve the cyclic AMP signalling pathway. It is unknown whether intracellular cyclic AMP accumulation induces migraine attacks. We investigated whether administration of cilostazol, which causes cyclic AMP accumulation, may induce migraine attacks. We included 14 migraine patients without aura in a double-blind, placebo-controlled crossover study. All participants received oral cilostazol or placebo on two separate days. We recorded migraine headache characteristics, associated symptoms and time of rescue medication intake using a questionnaire. Cilostazol induced delayed migraine-like attacks in 12 patients (86%) compared with two (14%) patients after placebo ($P = 0.002$). The median time to onset for migraine-like attacks was 6 h (range 3–11 h). Patients reported that the attacks mimicked their usual migraine attacks and that cilostazol-induced attacks responded to their usual migraine treatment. Median time of medication intake was 6 h (range 4–11 h). The present study suggests that intracellular cyclic AMP accumulation plays a crucial role in migraine induction. This knowledge is a further step in our understanding of the intracellular pathway of migraine initiation.

Keywords: migraine; cilostazol; headache; cyclic adenosine monophosphate; phosphodiesterase inhibitor

Abbreviations: AUC = area under the curve; CGRP = calcitonin gene-related peptide; PACAP = pituitary adenylate cyclase-activating peptide; PDE = phosphodiesterase

Introduction

Migraine is a very prevalent neurological disorder (Lipton *et al.*, 2007). Its initiating mechanisms may involve the cyclic adenosine 3′,5′-monophosphate (AMP) signalling pathway (Lassen *et al.*, 2002). Second messenger cyclic AMP controls a diverse range of cellular processes and two types of enzymes, adenylate cyclases and cyclic nucleotide phosphodiesterases (PDEs) regulate the level of intracellular cyclic AMP (Birk *et al.*, 2004*a*; Kruuse *et al.*, 2004). Extracellular signalling molecules like neuropeptides activate receptors in the cell membrane that cause intracellular cyclic AMP increase (Kruuse *et al.*, 2004; Dickson and Finlayson, 2009).

Migraine provocation studies showed that intravenous infusion of calcitonin gene-related peptide (CGRP) and pituitary adenylate cyclase-activating peptide 38 (PACAP38) induced migraine attacks in migraine sufferers (Ho *et al.*, 2010; Schytz *et al.*, 2010). Both peptides activate adenylate cyclase by transmembrane receptors and cause increased formation of intracellular cyclic AMP in vascular smooth muscle cells of cerebral arteries (Jansen-Olesen *et al.*, 1996; Dickson *et al.*, 2006). However, it remains unclear whether the migraine-inducing effects of CGRP and PACAP are caused by upregulation of cyclic AMP as both peptides also activate other pathways (Gray and Marshall, 1992; Odum *et al.*, 1998; Hashimoto *et al.*, 2006; Li *et al.*, 2008).

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Cilostazol is a selective inhibitor of phosphodiesterase type 3 (PDE3) (Liu *et al.*, 2001), which is one of the two quantitatively most important cyclic AMP degrading enzymes in endothelium cells and vascular smooth muscle cells of cerebral arteries (Birk *et al.*, 2004*a*). In contrast to CGRP and PACAP38, which stimulate production of cyclic AMP, cilostazol inhibits the breakdown of cyclic AMP and causes accumulation of cyclic AMP via an intracellular pathway (Birk *et al.*, 2004*b*). Our previous headache provocation study of cilostazol in healthy volunteers reported moderate to severe headache and in some participants (18%) it was migraine-like (Birk *et al.*, 2006).

In the present study, we investigated whether intracellular cyclic AMP accumulation might induce migraine attacks. We hypothesized that cilostazol would provoke migraine attacks in migraine patients without aura and tested this in a double-blinded, randomized, crossover study.

Materials and methods

Design

We recruited 15 patients with migraine without aura (Road, 2013) and randomly allocated them to receive 200 mg cilostazol (Pletal®, Otsuka Pharmaceuticals) or placebo (lactose, potato starch, magnesium stearate and talc) in a non-transparent gelatine capsule on two experimental days separated by at least 5 days. The central pharmacy performed the randomization of the experimental drug in a balanced fashion. The randomization code remained in the hospital during the study and was not available to the investigators until the study was completed.

The study was approved by the Ethics Committee of Copenhagen (H-2-2013-066) and the Danish Data Protection Agency, and was conducted according to the Declaration of Helsinki of 1964, as revised in 2008. The study was also registered at ClinicalTrials.gov (NCT01841827).

All patients were enrolled from the Danish Headache Centre and through the website (www.forsøgsperson.dk). All participants gave their written informed consent before inclusion. The female patients were requested to have sufficient contraception. Exclusion criteria were any other type of headache (except episodic tension-type headache $\leqslant$3 days per month), previous serious somatic or psychiatric diseases, or intake of daily medication including prophylactic migraine treatment, except oral contraceptives. A full medical examination and ECG were performed on the day of recruitment. The patients were informed that cilostazol might induce headache or migraine in some individuals, but the timing or the characteristics of headache were not discussed.

Experimental protocol

All participants arrived non-fasting at the clinic between 8:30 a.m. and 10:30 a.m. Patients were requested to have been without any kind of headache 48 h before the study day. Intake of coffee, tea, cocoa, smoking or other methylxanthine-containing foods or beverages was not allowed at least 8 h before the start of the study, and use of pharmacological agents apart from oral contraceptives was not permitted. A pregnancy test was taken at the beginning of each study day on all female patients.

The patients were then kept in the supine position in quiet surroundings. After 15 min of rest we measured the baseline values (T = 0). Right after, the patients received either cilostazol (200 mg) or placebo by mouth. After the administration of the experimental drug the patient was observed for 1 h and then discharged.

Headache intensity and questionnaire

Headache intensity was recorded at baseline (T = 0) and every 10 min up to 1 h on a verbal rating scale from 0 to 10. Zero is no headache, 1 represented a very mild headache (including a sensation of pressing or throbbing or otherwise altered sensation in the head not associated with pain), 5 is headache of moderate intensity and 10 is the worst headache imaginable (Iversen *et al.*, 1989). Headache localization, characteristics and associated symptoms were also recorded. After discharge from the hospital, the patients were carefully instructed to continue recording their headache by a self-administered questionnaire every hour until 13 h post-administration or until they had gone to bed. The questionnaire recorded headache characteristics and associated symptoms according to the International Headache Society (IHS) criteria (Road, 2013) but also included questions concerning adverse events, premonitory symptoms (unusual fatigue, yawning, neck stiffness, mood swings) and if the reported headache mimicked the spontaneous migraine attacks. The patients were allowed to take over-the-counter rescue medication or their usual migraine treatment at any time.

Migraine-like attack criteria

Experimentally provoked migraine by pharmacological substances is not spontaneous and therefore cannot fulfil strict IHS criteria for migraine without aura (Road, 2013). The following facts are important in defining criteria for an induced migraine-like attack. First, the majority of patients report them as attacks that mimic spontaneous migraine attacks (Olesen *et al.*, 1994; Lassen *et al.*, 2002). Second, 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 before the headache gets worse, becomes unilateral and has the associated symptoms required for migraine. Third, most patients can often predict the development of migraine in the early stage and in a research study cannot be denied treatment. Accordingly, the induced migraine attacks are often treated before all migraine criteria are fulfilled.

Based on these circumstances the following two criteria were used for a migraine-like attack induced 0–13 h after administration of cilostazol:

Migraine-like attack fulfilling either (i) or (ii):

(i) Headache fulfilling criteria C and D for migraine without aura according to the IHS criteria (Road, 2013): C. Headache has at least two of the following characteristics: unilateral location; pulsating quality; moderate or severe pain intensity (moderate to severe pain intensity is considered $\geqslant$4 on verbal rating scale); aggravation by cough (in-hospital phase) or causing avoidance of routine physical activity (out-hospital phase); D. During headache at least one of the following: nausea and/or vomiting; photophobia and phonophobia; and

(ii) Headache described as mimicking the patient's usual migraine attack and treated with acute migraine medication (rescue medication).

Vital signs

Heart rate and mean arterial blood pressure were measured at baseline and every 10 min during the observation period of 1 h after drug administration using an auto-inflatable cuff (Omega 1400).

Statistical analysis

Headache intensity scores are presented as median (range). Heart rate and mean arterial blood pressure data are presented as mean values ± standard deviation (SD). We calculated median peak headache intensity and median time to onset of migraine-like attacks after cilostazol and placebo.

Sample size was chosen on the basis of previous similar studies performed by our group showing induction of migraine in 60–70% of migraine patients after administration of CGRP, PACAP and sildenafil (Lassen *et al.*, 2002; Kruuse *et al.*, 2003; Schytz *et al.*, 2009).

The primary end-points were the difference in incidence of migraine-like attacks and the difference in area under the curve (AUC) for headache intensity scores (0–13 h) between cilostazol and placebo. Secondary end-points were differences in incidence of head pain, in AUC for mean blood pressure and in heart rate during the in-hospital phase (0–1 h).

The incidence of migraine-like attacks, head pain, associated symptoms, premonitory symptoms and adverse events were analysed as binary categorical data with McNemar's test. Difference in AUC for headache intensity scores were tested using the Wilcoxon signed ranks test, while differences in AUC for heart rate and mean arterial blood pressure values were tested with a paired two-way *t*-test. We calculated AUC according to the trapezium rule (Matthews *et al.*, 1990) to obtain a summary measure to analyse the differences in response between cilostazol and placebo. Baseline values were subtracted before calculating AUC to reduce variation between sessions within patient. We also tested for period and carry-over effects for baseline values with Mann-Whitney test and independent *t*-test.

All analyses were performed with SPSS Statistics version 19 for Windows. $P < 0.05$ was considered as the level of significance.

Results

Fourteen patients (13 female/one male) completed the study. The mean age was 36.1 years (range 20–51 years) and mean weight was 61.4 kg (range 47–77 kg). The migraine attack frequency ranged between one and six per month. None of the patients has previously participated in similar provocation studies. One patient was excluded due to personal issues after completing the first study day (placebo). The excluded patient was not included in the statistical analysis.

There was no carry-over or period effect for baseline values of headache, heart rate and mean arterial blood pressure.

Migraine-like attacks and headache

Headache characteristics and associated symptoms for all patients on both study days are presented in Table 1.

Twelve of 14 patients (86%) developed migraine-like attacks after cilostazol compared with two patients (14%) after placebo ($P = 0.002$). The median time to onset for migraine-like attacks was 6 h (range 3–11 h). The head pain localization of the migraine-like attacks is mostly in the frontal and temporal regions (Fig. 1).

Nausea was the only associated symptom that was significantly different between the two experimental days ($P = 0.027$) while photophobia ($P = 0.371$) and phonophobia ($P = 1.000$) were not. Median time of nausea onset was 5.5 h (2–9 h), which corresponded to the onset of migraine (6 h), and median duration of nausea was 3 h (1–7 h). Symptoms like stiff neck ($P = 0.074$), unusual tiredness ($P = 0.371$), mood swings ($P = 1.000$) and yawning ($P = 1.000$) were reported but were insignificantly different from the placebo day (Fig. 2). Only one patient fulfilled the IHS definition for a premonitory symptom: 'Symptoms preceding and forewarning of a migraine attack by 2–48 hours, occurring before the aura in migraine with aura and before the onset of pain in migraine without aura' (Road, 2013). Adverse events were quite few including dizziness, palpitations and flushing and none were significantly different from the placebo day (Table 2).

On the cilostazol day, 11 of 14 (79%) patients took rescue medication and on the placebo Day 2 (14%) did so ($P = 0.003$). The median time of medication intake was 6 h (range 4–11). All patients except two responded well to their usual migraine treatment, which was mostly a triptan (Fig. 3).

The incidence of head pain over the 13 h observation period was significantly higher on cilostazol day ($n = 13$) than on placebo day ($n = 4$) ($P = 0.004$). One of the patients who developed headache on placebo had a headache intensity of only 1 for 1 h. The AUC for headache intensity was significantly larger after cilostazol compared to placebo ($AUC_{0–13\ h}$, $P = 0.003$) (Fig. 4). Three patients on cilostazol and two patients on placebo had missing values in their headache intensity scores, because they went to bed or tried to sleep through the attack. Data at these time points were excluded from the AUC analysis.

The median peak headache intensity was 6 (0–9) and the median time to peak headache occurred 6.5 h (0–11 h) after administration of cilostazol.

Heart rate and mean arterial blood pressure

No difference in heart rate and mean arterial blood pressure was found between cilostazol and placebo during the in-hospital phase (heart rate $AUC_{0–1\ h}$, $P = 0.812$ and mean arterial blood pressure $AUC_{0–1\ h}$, $P = 0.229$).

Discussion

The major finding of the present study was that cilostazol induced delayed migraine-like attacks in 86% of migraine patients without aura, which makes cilostazol one of the most powerful migraine-inducing substances (Table 3). Moreover, patients reported that attacks mimicked their usual migraine attacks and responded to their usual migraine treatment.

The headache intensity curve after cilostazol in migraine patients was remarkably similar to the headache intensity reported in healthy volunteers (Birk *et al.*, 2006) (Fig. 5). Both studies used

Table 1 Clinical characteristics of headache and associated symptoms in 14 migraine patients after cilostazol and placebo (0–13 h observation period)

Patient	Peak headache (duration)	Headache characteristics[a]	Associated symptoms[b]	Mimics usual migraine	Migraine-like attack (onset)[c]	Treatment (time)/efficacy[d]
1 Spontaneous		Right/5/throb/+	+/+/+			
Cilostazol	7 h (1 h)	Right/7/pres/+	+/+/+	Yes	Yes (4 h)	Acetylsalicylic acid (4 h) / No Rizatriptan 10 mg (7 h) / Yes
Placebo	None					
2 Spontaneous		Right/5/throb/+	+/+/–			
Cilostazol	4 h (1 h)	Right/2/pres/–	–/+/–	Yes	Yes (4 h)	Eletriptan 40 mg (4 h) / Yes
Placebo	None					
3 Spontaneous		Left/7/throb/+	+/+/+			
Cilostazol	9 h (1 h)	Left/6/throb/+	+/–/–	Yes	Yes (9 h)	Sumatriptan 50 mg (9 h) / Yes
Placebo	None					
4 Spontaneous		Bilat/6/throb/+	+/+/+			
Cilostazol	10 h (1 h)	Bilat/8/throb/+	+/+/+	Yes	Yes (10 h)	Rizatriptan 10 mg (10 h) / Yes
Placebo	None					
5 Spontaneous		Right/6/throb/+	+/+/+			
Cilostazol	7 h (1 h)	Bilat/6/throb/+	+/–/–	No	Yes (3 h)	Rizatriptan 10 mg (6 h) / Yes
Placebo	7 h (1 h)	Right/4/throb/+	+/+/–	Yes	Yes (7 h)	Sumatriptan 50 mg (7 h) / Yes
6 Spontaneous		Right/7/pres/+	+/+/+			
Cilostazol	6 h (5 h)	Right/7/pres/+	+/–/–	Yes	Yes (5 h)	Rizatriptan 10 mg (5 h) / No
Placebo	6 h (4 h)	Right/2/pres/+	–/–/–	Yes	No	None
7 Spontaneous		Left/8/throb/+	+/–/–			
Cilostazol	5 h (1 h)	Bilat/6/pres/+	+/–/–	Yes	Yes (4 h)	Paracetamol 1 g (4 h) / No Ibuprofen 400 mg (7 h) / No
Placebo	None					
8 Spontaneous		Right/5/throb/+	+/+/+			
Cilostazol	None					
Placebo	None					
9 Spontaneous		Left/5/throb/+	+/–/–			
Cilostazol	6 h (1 h)	Left/7/throb/+	+/–/–	No	Yes (5 h)	Zolmitriptan 2.5 (5 h) / No Eletriptan 40 mg (6 h) / Yes
Placebo	None					
10 Spontaneous		Right/6/throb/+	+/+/+			
Cilostazol	6 h (5 h)	Right/2/pres/–	+/–/–	Yes	Yes (11 h)	Sumatriptan 100 mg (11 h) / Yes
Placebo	None					
11 Spontaneous		Bilat/8/throb/+	–/+/+			
Cilostazol	6 h (4 h)	Bilat/7/pres/+	–/–/–	No	No	None
Placebo	4 h (2 h)	Bilat/5/pres/+	–/–/–	No	No	None
12 Spontaneous		Right/7/pres/+	+/+/+			
Cilostazol	9 h (1 h)	Bilat/6/pres/+	+/+/–	Yes	Yes (9 h)	Sumatriptan 50 mg (9 h) / Yes
Placebo	None					
13 Spontaneous		Left/6/throb/+	–/+/+			
Cilostazol	9 h (1 h)	Left/6/throb/+	–/+/–	Yes	Yes (7 h)	Zolmitriptan 2.5 mg (7 h) / No Zolmitriptan 2.5 mg (9 h) / Yes
Placebo	2 h (2 h)	Left/8/throb/+	+/+/+	Yes	Yes (2 h)	Zolmitriptan 2.5 mg (2 h) / No Zolmitriptan 2.5 mg (6 h) / Yes
14 Spontaneous		Left/6/throb/+	+/+/–			
Cilostazol	11 h (1 h)	Left/9/pres/+	+/–/–	Yes	Yes (9 h)	None
Placebo	None					

[a]Localization/intensity/quality (throb = throbbing; pres = pressing)/aggravation (by cough during in-hospital phase and by movement during out-hospital phase).
[b]Nausea / photophobia / phonophobia.
[c]Migraine-like attacks are defined according to criteria described in methods.
[d]Pain freedom or pain relief (⩾50% decrease of intensity) within 2 h.

the same dosage of cilostazol and showed gradual onset of headache ~3–4 h after administration, which corresponds to the occurrence of peak plasma concentrations at 2.8 h (range 1.5–4.0 h) (Bramer *et al.*, 1999). The headache intensity in patients probably did not become maximal because patients took rescue migraine medication while headache was still rising (Fig. 4). Seventy-nine per cent of migraine patients took their usual migraine rescue medication, whereas 67% of the healthy volunteers took over-the-counter rescue medication (Birk *et al.*, 2006). Many of the healthy volunteers developed migraine-like features as aggravation by physical activity (92%), pulsating pain (75%), unilateral pain (33%) and nausea (17%) after cilostazol. Interestingly, two of the healthy volunteers, and two volunteers who withdrew from the study, developed a headache that fulfilled the The International Headache Classification edition 2 criteria of migraine without aura (Birk *et al.*, 2006). None of the healthy volunteers had any history of migraine or any first-degree relatives with migraine.

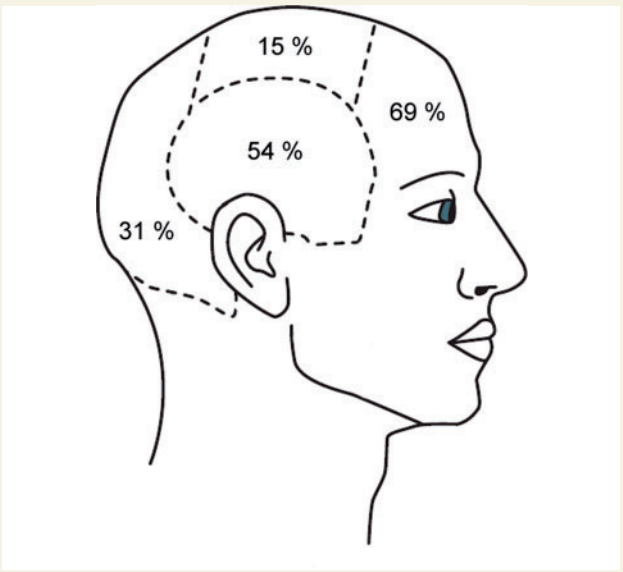

Figure 1 Localization of head pain during the migraine-like attacks induced by cilostazol in percentage (frontal, vertex, temporal or occipital).

Cilostazol treatment is associated with possible side effects including diarrhoea, abnormal stools, fast/pounding heartbeats and dizziness (FDA, 1999). In the present study, all participants were informed about possible adverse events. However, few patients reported them and we found no statistical difference between the two experimental days. This could be explained by the fact that cilostazol was taken as a single dose (200 mg) and not as the recommended daily dose of 100 mg over longer periods for intermittent claudication. Pulsating pain, nausea, photo- or phonophobia has not previously been described as side effects of cilostazol (FDA, 1999) and therefore we consider these symptoms related to migraine. Furthermore, unlike

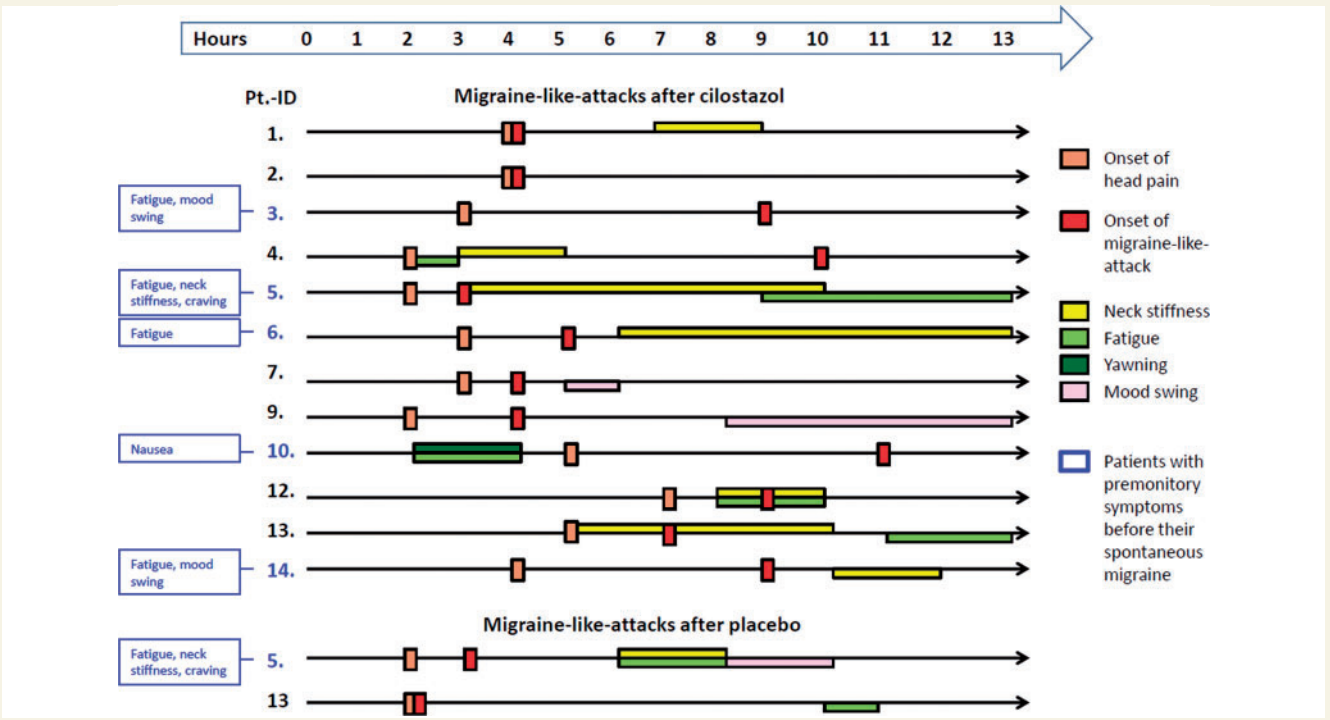

Figure 2 Premonitory symptoms reported by patients after administration of cilostazol and placebo during 0–13 h observation period. Median onset time of these symptoms was 5 h (range 1–9 h) and median duration was 2 h (range 1–12 h). The relationship of reported premonitory symptoms to onset of head pain and onset of migraine attacks are shown. Only one patient (Patient 10) fulfilled the definition of premonitory symptoms according to IHS criteria (Road, 2013). Patients who reported premonitory symptoms during their spontaneous attacks are shown.

non-steroidal anti-inflammatory drugs that block platelet aggregation and reduce peripheral pain, cilostazol has no anti-prostaglandin effect and therefore does not display anti-nociceptive properties (Bjorkman, 1998).

Table 2 Reported symptoms after administration of cilostazol and placebo from 0-13 h

Adverse events	Cilostazol	Placebo	*P*-value*
Stiff neck	7	2	0.074
Dizziness	3	0	0.248
Unusual tiredness	5	2	0.371
Mood swings	2	1	1.000
Palpitations	1	0	1.000
Flushing	1	1	1.000

**P*-value: McNemar's test.

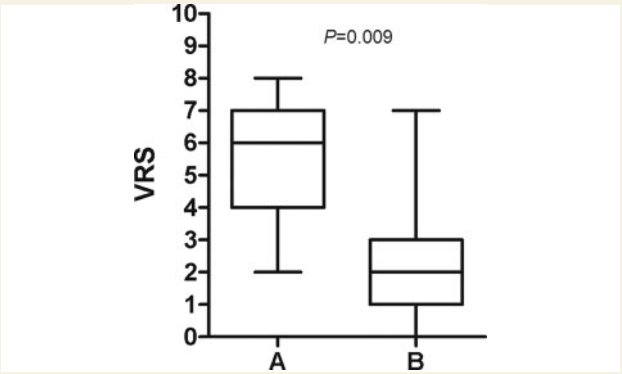

Figure 3 Box-and whisker plot representing medians and quartiles of headache intensity score at the time of rescue medication intake (**A**) and 2 h after intake of rescue medication (**B**) in 11 patients. There was significant reduction in headache intensity after treatment ($P = 0.009$, Wilcoxon signed ranks test). Median time of rescue medication intake for the 11 patients was 6 h (range 4–11) that corresponded to the onset of migraine (median 6 h, range 3–11 h).

Possible mechanisms responsible for migraine-inducing effect

Cilostazol induced more migraine-like attacks than CGRP and PACAP (Table 3), which suggests that cilostazol might activate a mechanism more downstream from CGRP and PACAP. CGRP and PACAP activate adenylate cyclise-coupled transmembrane receptors in cerebral vascular cells leading to intracellular formation of cyclic AMP (Jansen-Olesen *et al.*, 1996; Dickson *et al.*, 2006), whereas cilostazol causes accumulation of intracellular cyclic AMP by inhibition of both isoforms of PDE3, PDE3A and PDE3B (Birk *et al.*, 2004*a*). PDE3A is primarily located in vascular smooth muscle cells and endothelium cells while PDE3B is predominantly located in fat tissues but also in the brain (Degerman *et al.*, 1997). It has been shown that trigeminal neurons can be sensitized through elevation of cyclic AMP (Ingram and Williams, 1996) and a recent study demonstrated that PDE3A and PDE3B are also present and active in the neuronal part of the trigeminovascular system and are co-localized with CGRP in the trigeminal ganglia (Nordgaard *et al.*, 2013). This augments the role of PDE3 in the migraine-relevant pain pathway. The study also showed that another phosphodiesterase-inhibitor (PDE5), sildenafil, which was thought to induce migraine-like attacks via cyclic GMP-relevant mechanism, apparently has an ability to increase intracellular levels of cyclic AMP as well (Nordgaard *et al.*, 2013). Thus, it supports the result of the present study suggesting that the key relay molecule in migraine could be cyclic AMP acting as a common pathway for cilostazol, sildenafil, CGRP and PACAP.

Cilostazol is a selective inhibitor of PDE3. Although, PDE5 can also be inhibited by cilostazol, it only happens at concentrations much higher than in the present study (Sudo *et al.*, 2000). Even at high pharmacological doses, cilostazol only increases cyclic AMP levels and not cyclic GMP levels (Nordgaard *et al.*, 2013). Thus, an inhibitory effect on PDE5 in the present study seems unlikely. Moreover, cilostazol seems to have no relevant effect on PDE1, PDE2, PDE4 and PDE7 (Sudo *et al.*, 2000).

Cilostazol dilates cerebral arteries in healthy volunteers without affecting regional cerebral blood flow (Birk *et al.*, 2004*b*).

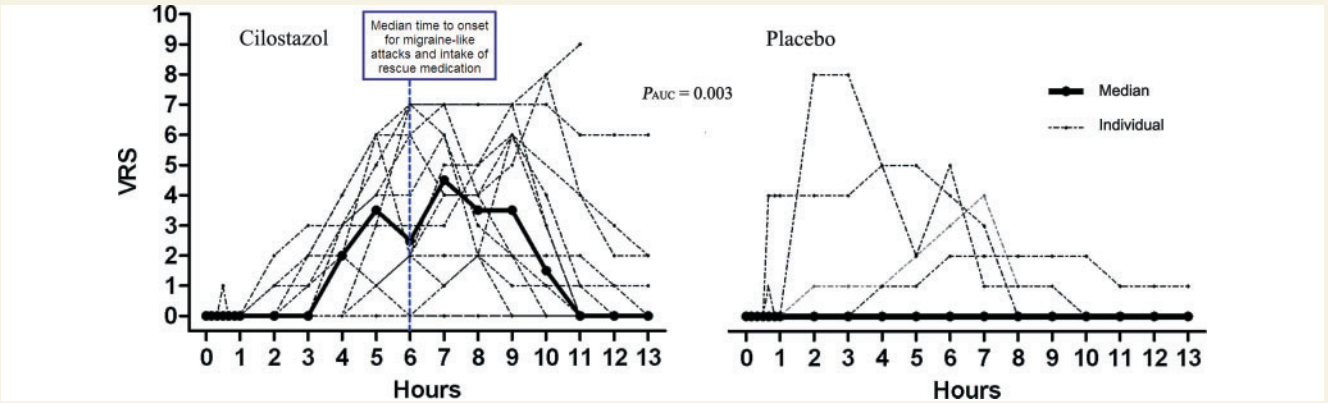

Figure 4 Median (thick line) and individual (thin lines) headache intensity on a verbal rating scale (VRS) on cilostazol and placebo days in 14 migraine patients. There was a significant difference in the $AUC_{0–13\ h}$ between cilostazol and placebo ($P = 0.003$). Headache intensity progressed slowly over time and the median time to onset for migraine-like attacks was 6 h (range 3–11 h). Rescue medication was taken by 11 patients before reaching a plateau of headache intensity.

Table 3 Incidence of migraine-like attacks, median peak headache intensity (0–10 verbal rating scale), median time to peak and median time to onset (when fulfilling criteria for migraine-like attack) after cilostazol and previous studies of PACAP38, CGRP, glyceryl trinitrate and sildenafil in migraine patients without aura (MO)

	Migraine-like attacks in migraine patients without aura patients	Number of individuals	Median peak headache intensity	Median time to peak headache intensity (h)	Median time to onset of migraine (h)	Reference
Cilostazol (oral)	86%	14	6 (0–9)	6.5 (0–11)	6 (3–11)	
Sildenafil (oral)	83%	12	6.5 (0–10)	4.5 (0–9)	Not reported	Kruuse *et al.*, 2003
Glyceryl trinitrate (iv)	80%	10	5.5 (0–10)	5.5 (3–10)	Not reported	Thomsen *et al.*, 1994
CGRP (iv)	67%	9	4 (1–6)	5 (1–12)	Not reported	Lassen *et al.*, 2002
PACAP38 (iv)	66%	12	2.5 (0–10)	4 (0–12)	6.2 (0.3–11)	Schytz *et al.*, 2009

All the previous studies were performed under similar conditions and design.

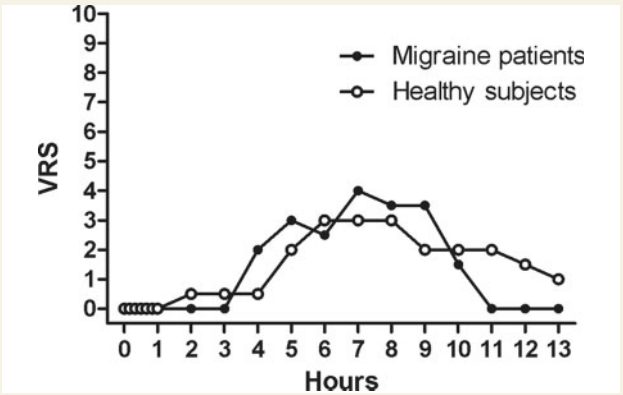

Figure 5 Median headache intensity on verbal rating scale (VRS) after administration of cilostazol in 14 migraine patients in the present study and in 12 healthy volunteers reported in a previous study (Birk *et al.*, 2006). Modified with permission.

In healthy volunteers cilostazol dilated the superficial temporal artery, radial artery and middle cerebral artery progressively and dilatation did not reach a plateau within the observation period of 4 h (Birk *et al.*, 2004*b*). A significant dilatation of ~15% of middle cerebral artery was measured at the end, which is at least equivalent to the immediate (20 min after start of infusion) vasodilative responses of glyceryl trinitrate and histamine (Iversen *et al.*, 1989; Lassen *et al.*, 1995).

Sildenafil caused migraine-like attacks in migraine patients without dilating the middle cerebral artery (Kruuse *et al.*, 2003) indicating that cilostazol acts via other mechanisms than PDE5, but it was at least as effective as sildenafil in causing migraine-like attacks. A recent study showed that sildenafil *in vivo* dose-dependently dilated middle meningeal artery concomitant with a reduction in blood pressure in rats (Kruuse *et al.*, 2012), which means that so far all known migraine-eliciting compounds have vasodilator properties and might have a common pathway.

In rats, PDE3 and cyclic nucleotide-gated ion channel (CNG) subunits are expressed in several components of the trigeminovascular system including middle cerebral artery, basilar artery, trigeminal ganglion and dura mater (Kruse *et al.*, 2006). Hypothetically, modulation of cyclic AMP levels by PDE3 and activation of cyclic nucleotide-gated ion channels may be a possible mechanism leading to migraine headache.

Furthermore, cilostazol is a lipophilic compound like glyceryl trinitrate that effectuates its effect intracellularly. It can therefore cross the blood–brain barrier. Expression of PDE3 has been identified in the CNS (neurons, astrocytes and oligodendrocytes) including hippocampus and hypothalamus (Sahu *et al.*, 2011; Mitome-Mishima *et al.*, 2013). Cerebral ischaemia suppresses the conversion of ATP to cyclic AMP (Moskowitz *et al.*, 2010), and inhibition of PDE3 by cilostazol is effective in experimental cerebral ischaemia (Choi *et al.*, 2002; Hong *et al.*, 2006; Watanabe *et al.*, 2006; Lee *et al.*, 2009). Cilostazol also strengthens barrier integrity in brain endotelial cells (Horai *et al.*, 2013). Despite the neuroprotective properties of cilostazol, we cannot rule out that central mechanisms may play a role in its migraine-inducing effects.

Based on the selectivity of cilostazol, we suggest that its migraine-inducing effect is mediated by cyclic AMP-dependent pathways via mainly PDE3.

Implications for future research

The present study shows that cilostazol is one of the most powerful migraine-inducing compounds tested so far and that intracellular cyclic AMP accumulation plays a crucial role in migraine induction. Our study has two major future prospects: (i) Given that intracellular cyclic AMP accumulation seems to be important in migraine induction, compounds that either inhibit the production or increase the degradation of cyclic AMP may be new anti-migraine drug targets such as activators of PDE3 or inhibitors of the membrane-associated or cytosolic site of adenylate cyclase (Nordgaard *et al.*, 2013); and (ii) cilostazol may be used to study migraine in humans and animals. Its powerful migraine response, response to triptans and practical administration are great advantages in observational imaging or biochemical studies investigating the signalling pathways in migraine initiation or testing of anti-migraine drugs. Furthermore, the headache-eliciting effects of PDEs have not been tested in migraine with aura patients, which could be interesting as tadalafil was associated with visual aura symptoms in a case report (Dinn and Wall, 2006).

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Conflicts of interest

Jes Olesen has received grants and/or research support from, has been a consultant and/or scientific adviser for, and has been in the speakers' bureau of Allergan Inc, AstraZeneca Pharmaceuticals LP, Boehringer Ingelheim, Eli Lilly, GlaxoSmithKline, Janssen Pharmaceutical Products, Lundbeck, Merck, Amgen, Alder and Pfizer. Messoud Ashina has received honoraria for lecturing from Allergan and Pfizer, and is a consultant and/or scientific adviser for the ATI, Allergan, Amgen and Alder. Song Guo has received a travel grant from Autonomic Technologies Inc.

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