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Original experimental

The nitric oxide synthase inhibitor and serotonin-receptor agonist NXN-188 during the aura phase of migraine with aura: A randomized, double-blind, placebo-controlled cross-over study

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ABSTRACT

Background and aims: NXN-188 is a combined neuronal nitric oxide synthase (nNOS) inhibitor and 5HT-1B/1D receptor agonist which has previously shown efficacy in the acute treatment of migraine. Nitric oxide (NO) is involved in the pathogenesis of migraine pain and is formed after cortical spreading depression. Therefore NXN-188 could perhaps prevent the development of the headache phase in migraine with aura if taken during the aura. The aims of the present study were to evaluate the efficacy and safety of 600 mg NXN-188 in the acute treatment of migraine when dosed during the aura.
Methods: A single-centre, randomized, double-blind, placebo-controlled, two-way crossover trial. The study medication was taken during the aura and the patients kept a study diary for 48 h post-dose.
Results: Of 615 patients screened, 50 patients were included in the study and randomized. Only 18 patients completed both treatments in compliance with the study procedures. 22% of patients reported freedom of headache at 2 h after intake of NXN-188 compared with only 11% of patients after placebo.
Conclusion: The dual-action drug NXN-188 with 5HT-1B/1D agonism and nNOS inhibition, taken orally during the aura phase did not have a statistically substantial effect on migraine headache in this study. This study was limited by a high drop-out rate and small sample of included patients who were able to complete the cross-over protocol. Therefore, efficacy of the treatment cannot be refuted with certainty.
Implications: This study illustrates the difficulties of doing well controlled studies in migraine patients with aura. nNOS inhibition is expected to be effective mostly in the aura phase, i.e. the oral administration may have had too slow pharmacokinetics to have effect. Parenteral administration may overcome this obstacle. 5HT-1B/1D agonism is not effective when dosed during migraine aura. Repeated dosing of the NXN-188 during and immediately following the aura may have exploited more the dual-action. The high drop-out rate may be reduced in future studies by having the patients familiarize themselves with the study procedure by filling out the attack report form while treating one attack with their own medication before entering the trial. A parallel group comparison may be a more effective trial design for treatment during an aura.
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1. Introduction

NXN-188 is a new oral therapeutic principle with dual-action 5HT1B/1D agonist activity and an inhibition of the neuronal nitric oxide synthase enzyme (nNOS). Nitric oxide (NO) is a very important molecule in the pathogenesis of migraine and other primary headaches [1]. Intravenous infusion of the NO donor glyceryl trinitrate (GTN) induces a migraine attack in 80% of patients with migraine without aura [2] and in 50% of patients with migraine with aura [3]. Platelet nitrates (a signal for increased NO) increase before and during a migraine attack [4–6]. Inhibition of endogenous NO-production by a non-selective NO synthase (NOS) inhibitor (L-NMMA) relieves migraine and chronic tension type headaches in human studies [7,8]. There are three different enzyme isoforms of NOS: endothelial NOS (eNOS), neuronal NOS (nNOS) and inducible NOS (iNOS) [9]. nNOS is found in both central and peripheral neurones. In several models of neuropathic and inflammatory pain, nNOS inhibition reduces central sensitization [10,11]. In addition, increasing NO levels can enhance pain responses in animals, including allodynia in rats [10]. The development of cutaneous allodynia [12], and thus central sensitization, in the course of a migraine attack suggests a role for the neuronal isoform of the NOS enzyme.

NXN-188 selectively binds to nNOS with a level of nNOS inhibition similar to L-NMMA and it can also bind to both 5-HT_{1D} and

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5-HT_{1B} receptors with potency similar to sumatriptan [13]. The first phase 2 trial of NXN-188 included patients suffering from migraine with and without aura [13]. In that trial NXN-188 showed no statistically significant superiority over placebo for the primary endpoint of headache relief at 2 h after treatment. Exploratory analysis stratifying subjects in the NXN-188 cohorts by a migraine history with and without aura showed that more subjects who had a migraine history with aura experienced headache relief than subjects who had a migraine history without aura. The reason for this could be that NO is formed during cortical spreading depression (CSD) [14], which probably underlies migraine aura [15]. According to this hypothesis nNOS activation is the link between the aura and the headache phase of migraine with aura. Thus, an nNOS-inhibitor such as NXN-188 could prevent the development of migraine headache if taken during the aura phase. This would provide an advantage over the triptans, which have been shown to be ineffective if given during migraine aura [16,17]. The objectives of the present trial were therefore to compare effects of NXN-188 with placebo when administered during the aura of migraine attacks.

2. Methods

2.1. Trial design

This was a single-centre randomized, double-blind, placebo-controlled two-way crossover study.

2.2. Participants

Eligible patients were between 18 and 65 years old, inclusive, and suffered from migraine with aura as defined by the second edition of The International Classification of Headache Disorders with at least one attack of aura every 8 weeks followed within 2 h by moderate to severe migraine headache. Patients were required to be in good general health as determined by the medical history, physical exam, clinical laboratory tests, vital signs and ECG. Patient body mass index (BMI) was required to be within the range of 18–32 kg/m^2. Use of a reliable form of contraception was required for women of childbearing potential. Patients with basilar or hemiplegic migraine, any history of significant disease or active drug or alcohol abuse were excluded.

2.3. Study settings

The study was conducted at The Danish Headache Center, Glostrup Hospital, Denmark from June 2009 to April 2011.

2.4. Study procedures

Current and former patients of The Danish Headache Center, diagnosed with migraine with aura, and who had given prior consent to being asked about clinical research participation, were screened for eligibility and invited to participate in the study. Advertising on the Internet, in the magazine of a migraine patients' association and in waiting rooms of practicing neurologists was applied to increase recruitment. Patients who fulfilled the inclusion and exclusion criteria at the screening visit received oral capsules of 600 mg NXN-188 or placebo and were instructed to treat an attack of migraine with aura within 30 min of aura onset provided that they were awake when the aura started and that they had not taken NSAIDs, ergotamine, triptans, analgesics or anti-emetics within 24 h prior to intake of the study drug. After treating one attack the patient would contact the study site and receive the second dose. Rescue medication of any kind was allowed at all times but patients were encouraged to await the study drug effect for 2 h before taking rescue medication. The patients were instructed to return to the study site within about 7 days of the second treatment. Patients who did not take the study drug within 16 weeks were excluded. The patients were contacted by telephone every three weeks throughout the study.

2.5. Data collection

Patients recorded headache severity in a paper diary using a four-grade scale (no pain, mild pain, moderate pain, severe pain) at baseline (time of taking study drug) and at 1, 2, 4, 8 and 24 h after. The presence of functional disability (four-grade scale: normal, mildly impaired, severely impaired, requires bed rest) was recorded at the same time points as the headache severity ratings. Patients also recorded their overall evaluation of the study medication on a four-grade scale (poor, moderate, good, excellent) as well as information about any adverse events that occurred during the study. Use of rescue medication within 24 h was also recorded.

Tolerability and safety were assessed with reports of adverse events and routine pre-study and post-study physical and laboratory testing, including electrocardiography.

2.6. Effect and tolerability measures

The primary efficacy measures were the percentage of patients pain-free 2 h after intake of study medication and headache intensity at 2 h after dosing. The secondary efficacy measures were: headache intensity scores at the remaining time points (1, 4, 8, 24 h), area under the headache curve for 24 h, clinical disability scores at all time points, percentage of patients using rescue medication, the time to rescue medication and the overall evaluation of the study medication. Tolerability measures were the occurrence and severity (mild, moderate, severe) of adverse events (AEs).

2.7. Sample size

Based on our knowledge from studies with patients with migraine without aura we estimated that the effect of placebo would be 25% and the effect of the active substance (NXN-188) would be 60%. In order to detect this difference with a two-sided 5% significance level and at least 80% power using McNemar's test 33 participants were needed.

2.8. Randomization and blinding

Subjects that met the eligibility requirements at the screening visit would receive each of the study treatments according to a computer-generated random code. NXN-188 and placebo were identical in appearance. All of the study personnel were kept blinded to the treatment sequence until the last patient completed the study. Data analysts were kept blinded until the statistical analyses were complete.

2.9. Statistical methods

Statistical analyses of the proportion of patients pain-free were done with McNemar's test for paired proportions (patients who completed both treatments) and Fisher's exact test for independent proportions (parallel group comparison of patients who dosed once). $P < 0.05$ was chosen as the significance level. Area under the curve (AUC) was calculated according to the trapezium rule. Missing data were replaced by last value carried forward. The efficacy population included patients who took both doses of study medication and returned to the study site for paired analyses and patients who dosed once and returned their study diary for parallel group analyses. The intention-to-treat (ITT) populations were all patients

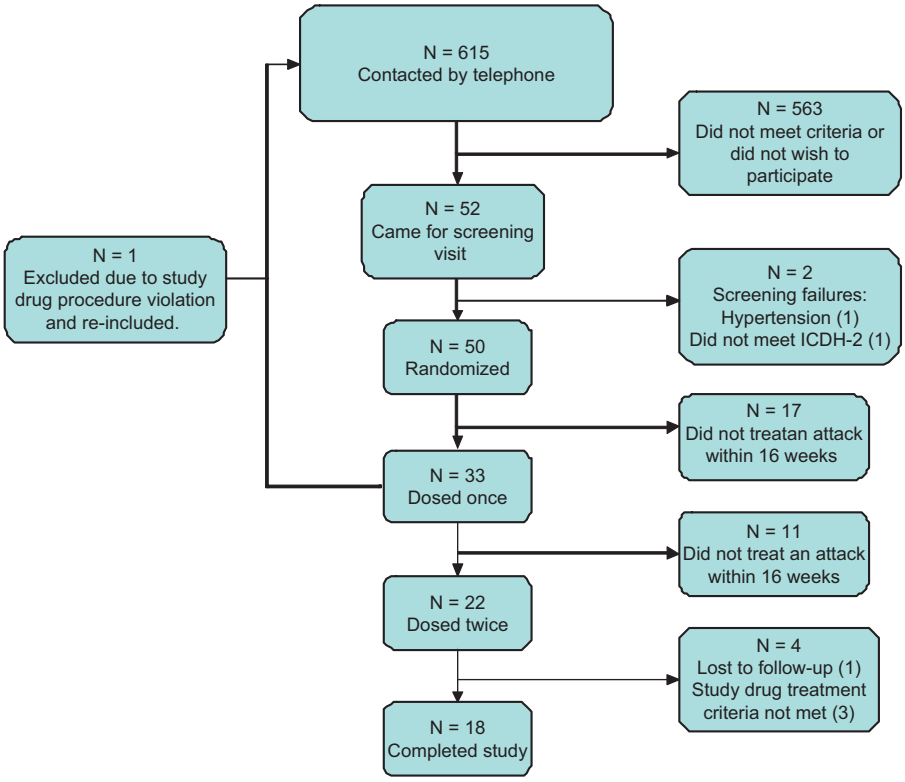

Fig. 1. Disposition of study subjects.

who dosed twice and all patients who dosed once, respectively. The safety population included all patients who took study medication.

2.10. Ethical approval

The study protocol was approved by the local ethics committees. Written, informed consent was obtained from the patients, prior to inclusion.

2.11. Registration

The trial was registered with clinicaltrials.gov, ID no. NCT00877838

3. Results

3.1. Patients' characteristics and recruitment

Disposition of the study subjects is shown in Fig. 1. Baseline characteristics are shown in Table 1. 50 patients were included in the study. Most patients reported an attack frequency of less than 10 attacks per month at the time of inclusion but three patients had 10, 14 and 30 monthly attacks respectively. Disregarding these three patients the average attack frequency was 1.7 attacks per month in the entire group of included patients and 1.8 in the group of patients who completed the study. One subject took only one of three capsules during the 30 min treatment window and was therefore excluded from the study. This subject was included in the study again and subsequently completed both treatments. 28 out of the 49 randomized individuals were excluded because they did not treat an attack within the 16 week limit. 4 patients took both doses of study medication but did not successfully complete the study as one was lost to follow-up, one did not report the time of aura onset, one took a dose 40 min after aura onset and one took study medication even though the aura started when the patient was asleep. Of the 33 patients who took the first dose, 24 returned their study diary (placebo: $N = 12$; NXN-188: $N = 12$).

Table 1
Demographics and baseline characteristics.

	Randomized	Completed
Total no. of subjects	49	18
Males	12	7
Females	37	11
Mean age [years] (SD)	39 (11)	38 (12)
Median attack frequency per month [range]	1 [0.5–30]	1 [0.5–5]

3.2. Efficacy

The difference in headache scores between NXN-188 and placebo at the 6 different time points are shown for each patient in Fig. 2. Four of 18 patients were pain free at 2 h after NXN-188 compared to 2 of 18 patients after placebo ($P = 0.68$, McNemar). Of the 24 patients who dosed once and returned their study diary 1 of 11 reported pain freedom at 2 h both in the NXN-188 and in the placebo group.

In the following, only results from patients who completed the study are mentioned: Mean headache intensity was 1.6 after NXN-188 and 1.9 after placebo. The percentage of patients who took rescue medication was 33% (6/18) after NXN-188 and 28% (5/18) after placebo. The average time to rescue medication was 4.8 h after NXN-188 and 4.2 h after placebo. Average AUC for headache scores was 25 after NXN-188 and 31 after placebo. No significant differences were seen for disability scores at any time point. Patient overall evaluation of the study treatments is shown in Table 2.

3.3. Tolerability

In total the participants reported 17 adverse events (AEs). The number of AEs was higher after NXN-188 (12/21, 52%) than

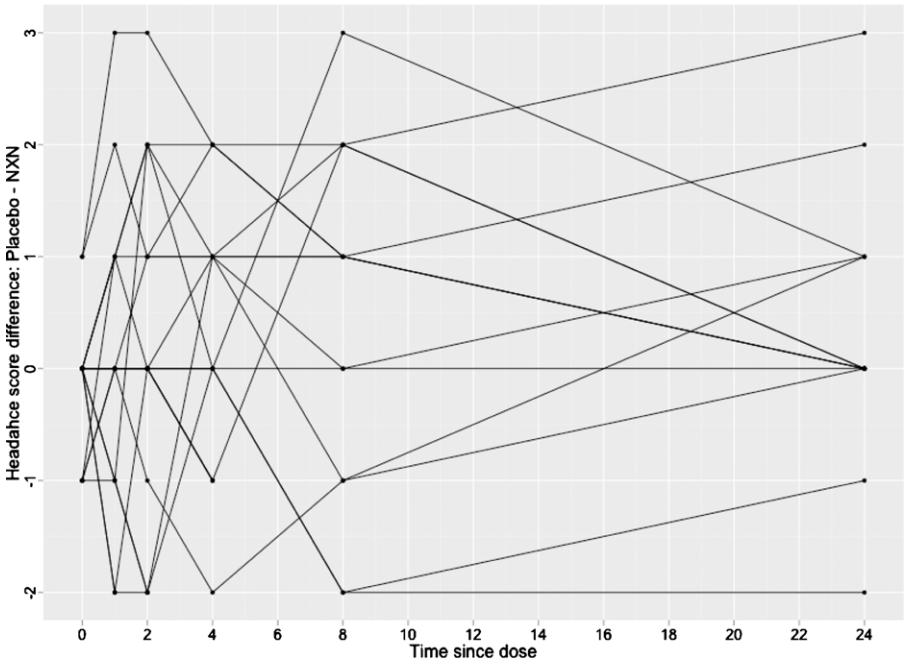

Fig. 2. Headache score differences between NXN-188 and placebo (headache score after placebo minus headache score after NXN-188) over time for each of the patients who completed both treatments.

Table 2
Overall rating of treatment.

NXN-188					
	Poor	Moderate	Good	Excellent	Total
Placebo					
Poor	4	2	3	1	10
Moderate		3	2	1	6
Good	1				1
Excellent					
Total	5	5	5	2	17[a]

[a] 1 missing value.

after placebo (5/21, 24%). AEs after intake of NXN-188 were nausea ($N = 3$), abdominal pain ($N = 2$), minor nosebleed ($N = 1$), nasal congestion ($N = 1$), pyrosis ($N = 1$), blurred vision ($N = 1$), and dizziness ($N = 1$). One event was considered a serious AE as it led to patient hospitalization. This event was a severe migraine attack that occurred 10 days after drug intake and was regarded as being unrelated to the study medication. Most other AEs were mild to moderate and of short duration.

4. Discussion

4.1. Methodological considerations

Based on experience from previous trials of acute migraine treatment [16,17] we expected that by including 50 patients into this study at least 30 would complete both treatments. The conduct of the study was, however, challenged by a surprisingly large exclusion rate of 64% (32/50). Patients were excluded if they did not treat an attack within 16 weeks, and 28 (56%) patients were excluded because of this criterion. The majority of these patients reported that they did not have any attacks during the 16-week period. The reason for this could be that most of the patients had a low attack frequency. The difference between baseline attack frequency in the entire group of included patients and in the group who completed the study was, however, not remarkable and the completion rate in the groups of patients with attack frequencies of 0.5, 1 and 2 were all the same, 33%. This suggests that general patient compliance to the study procedures is more important for the success of this kind of study than the patients having very frequent attacks. In future similar studies it could be useful to have the patients familiarize with the study procedures by filling out the attack report form while treating one attack with their own medication before entering the trial [18].

4.2. Results of this study

Because of the low completion rate, no firm conclusions about the effects of NXN-188 can be drawn from this study. Using the limited number of completed patients in statistical analyses, the study showed no significant differences in efficacy between NXN-188 and placebo for relieving migraine headache, when dosed during migraine aura.

A shortcoming of the study was the oral formulation. Without parenteral application there was no way to ensure that the drug reached biologically active plasma levels during a migraine attack with its known slowing of absorption. Also, the time of drug intake could be too late to abort the progression to the migraine headache phase but it would be practically impossible to administer the drug earlier in the aura phase than was done in this study.

The 5HT-1B/1D receptor agonist effect of the drug was not expected to relieve migraine headache in this study because triptans are not effective when taken during the aura phase [16,17]. Since NXN-188 has potency similar to that of sumatriptan, the results of this study support these previous findings. Repeated dosing of the NXN-188 during and immediately following the aura may have exploited more the dual-action.

5. Conclusion

In conclusion, the dual-action drug NXN-188 with 5HT-1B/1D agonism and nNOS inhibition, taken orally during the aura phase did not have a substantial effect on migraine headache in this study. This study was limited by a high drop-out rate and small sample

of included patients. Therefore, efficacy of the treatment cannot be refuted with certainty.

Conflict of interest

The authors declare that they do not have any financial or other relationship with other people or organizations or other conflicts of interest that may inappropriately influence the authors' work.

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