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METHODOLOGY

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Revisiting substance P in migraine: a methodological approach inspired by anti-CGRP and anti-PACAP success

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Abstract

Substance P, previously dismissed as a therapeutic target for migraine due to the failure of neurokinin-1 receptor antagonists, warrants renewed attention. Building on the success of therapies targeting the calcitonin gene-related peptide (CGRP) system and pituitary adenylate cyclase-activating peptide (PACAP) in migraine prevention, which highlight the importance of targeting peptides, this proposal reexamines substance P as a mediator in migraine pathophysiology. Using an established methodological framework, migraine-inducing properties of substance P can be evaluated through randomized, double-blind, placebo-controlled crossover studies involving healthy volunteers and individuals with a history of migraine. This approach aims to establish proof of concept for substance P's role in migraine, laying the groundwork for investigations with animal and cell-based models and advancing the development of innovative treatments for patients refractory to current therapies.

Keywords CGRP, Headache, Substance P, Neurokinins, Pain

Background

Recent advancements in migraine have underscored the critical role of neuropeptides in disease pathophysiology, exemplified by the clinical success of therapies targeting the calcitonin gene-related peptide (CGRP) [1]. Pituitary adenylate cyclase-activating polypeptide-38 (PACAP-38) is another multifunctional peptide expressed in the trigeminovascular system [2]. It modulates nociceptive signaling, vasodilation, and neurogenic inflammation, processes central to migraine pathogenesis [3, 4]. The ability of PACAP-38 to induce migraine-like attacks in individuals with a history of migraine has validated its role as a therapeutic target, leading to the development of monoclonal antibodies targeting the PACAP pathway [5]. PACAP-38's effects are mediated through multiple receptors, including PAC_1, $VPAC_1$, and $VPAC_2$. Despite initial setbacks with a PAC1 receptor antagonist, such as AMG 301, a new therapy which directly inhibits PACAP-38 has demonstrated promising results in reducing migraine days [6]. These findings emphasize the importance of targeting peptides rather than individual receptors and suggest to revisit other neuropeptides previously dismissed as therapeutic targets, such as substance P. Substance P, a neuropeptide co-expressed with CGRP in trigeminal neurons [7–9], is known to contribute to neurogenic inflammation, plasma extravasation, and vascular changes during migraine [10, 11]. While early clinical trials with neurokinin-1 (NK_1) receptor antagonists failed to show efficacy, these studies focused exclusively on a single receptor pathway [12, 13]. Recent evidence

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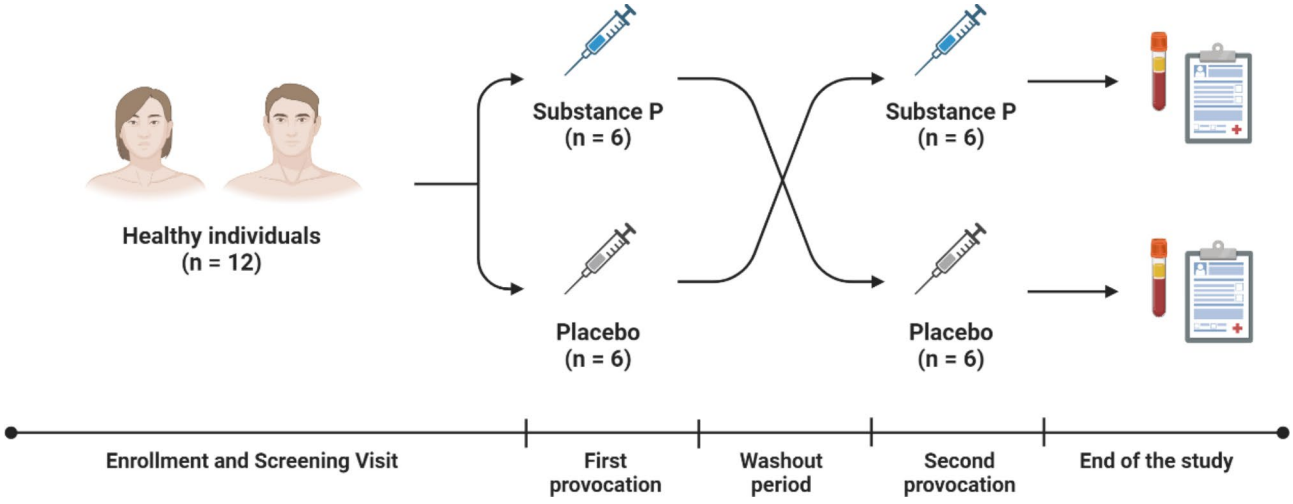

Fig. 1 Study design of the clinical trial in healthy volunteers

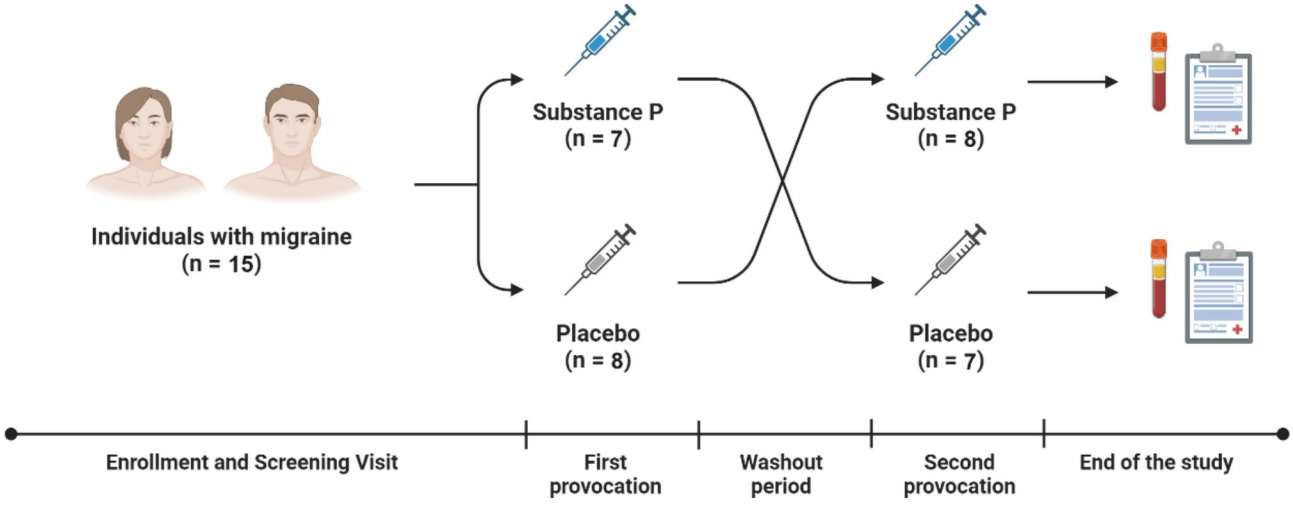

Fig. 2 Study design of the clinical trial in individuals with a history of migraine

suggests that substance P acts through additional receptors [9], which may play a critical role in its migraine-inducing effects. Moreover, its involvement in peripheral and central sensitization aligns with mechanisms implicated in migraine pathophysiology. This methodological approach builds on the lessons from anti-CGRP and anti-PACAP therapies to systematically evaluate the role of substance P in migraine. By focusing on the peptide's overall contribution to migraine pathogenesis, independent of specific receptor targets, these studies aim to provide a better understanding of its mechanisms. Using human provocation models, this study seeks to establish substance P as a clinically relevant mediator of migraine, paving the way for novel therapeutic approaches targeting refractory patients.

Methods

The proposed approach utilizes a well-established human provocation model to investigate headache and migraine-like attacks in healthy individuals and those with a history of migraine. This model has been ethically accepted and widely applied for over 30 years due to its self-limiting, treatable nature, and lack of lasting harm to participants [14]. Two randomized, double-blind, placebo-controlled crossover trials can assess the effects of substance P in two cohorts: 12 healthy individuals (Figs. 1) and 15 individuals with episodic migraine without aura (Fig. 2), diagnosed according to international criteria [15]. The sample size of 12 healthy individuals reflects previous provocation studies conducted with other peptides [16], while the second study assume that 70% of participants with a history of migraine would develop migraine after substance P, compared to 10% after placebo. By using the asymptotic approximation for McNemar´s test, calculation of

sample size consists of 15 participants [17]. Intravenous infusions of substance P or isotonic saline (placebo) will be administered on separate study days, with a minimum washout period of two weeks between sessions to prevent carryover effects. Infusions of substance P have been previously performed in healthy volunteers up to 16 pmol/kg/min, without relevant side effects [18]. The primary outcomes include the induction of headache and migraine-like attacks, evaluated using validated criteria during and after the infusion [19]. Secondary outcomes focus on physiological markers such as superficial temporal artery vasodilation, measured using Dermascan ultrasound imaging. Real-time diaries and clinician evaluations will capture headache characteristics, associated symptoms, and participant-reported experiences throughout the study. Data analysis will employ a mixed-model approach to compare the effects of substance P and placebo while accounting for within-subject variability inherent to the crossover design. Ethical approval must be secured before study initiation, and rigorous safety protocols should be followed to ensure participant well-being. This includes continuous monitoring during and after the infusions to promptly address any adverse events. Importantly, blood pressure (both systolic and diastolic) and heart rate will be continuously recorded during and after the infusion for up to 2 h to add further aspects on the effect of substance P on the circulation in general.

Discussion

Despite significant interest in non-CGRP drug targets for migraine, many of these approaches have yet to yield the development of efficacious therapies [20]. Substance P is released from sensory fibers originating from the trigeminal ganglion, and their modulation has already demonstrated promise as a therapeutic strategy for treating migraine [21, 22]. The recent success of PACAP-targeted therapies [6] highlights the importance of revisiting dismissed mechanisms to uncover transformative treatments [23]. Substance P, with its multifaceted receptor interactions, represents an unexploited avenue for migraine therapy that could address the limitations of past research focused solely on the NK_1 receptor. This proposal's findings have implications beyond migraine, potentially advancing our understanding of other pain conditions where substance P has been implicated but not adequately explored. For example, trials testing NK_1 antagonists for acute postoperative pain and diabetic neuropathy yielded negative outcomes, likely due to an overemphasis on a single receptor pathway [24, 25]. Cluster headache, a condition sharing pathophysiological mechanisms with migraine, represents another opportunity for innovation [26]. Moreover, chronic conditions such as neuropathic itch– where substance P and sensory neurons play a role– may also benefit from insights gained through these studies [27]. Human provocation models have inherent limitations that should be recognized. Despite their value for hypothesis testing, their findings must be complemented by cellular and animal studies to identify the specific receptors mediating substance P's effects on migraine. The lesson from the PACAP pathway– where the precise mechanism remain unresolved despite a successful phase 2 trial– emphasize the need for integrated translational research.

A call for peer recognition and collaboration

Despite its scientific merit, this proposal faced rejection from several grant committees in 2024. Research proposals that aim to challenge established paradigms and re-evaluate previously explored topics often struggle to gain traction, particularly when evaluators lack expertise in the specific field. Publishing this methodology seeks to address these challenges by providing peer-reviewed validation of the proposal, offering a credible reference for future funding applications and highlighting the scientific value of the approach to evaluators. This is particularly significant for early-career researchers, who often operate under tight funding timelines and lack a proven track record of securing and managing grants. By sharing this methodology, we enable the broader research community to assess, critique, and refine the approach, fostering collaboration and innovation in headache research. Publishing in a respected journal ensures that this important topic remains accessible and encourages further exploration by other investigators.

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Author contributions
L.P. wrote the first draft of the manuscript. L.E. critically reviewed and provided substantial revisions to improve the manuscript. Both authors approved the final version for submission.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
Lanfranco Pellesi serves as member of Editorial Board of The Journal of Headache and Pain, BMC Neurology and European Journal of Medical Research. In addition, Lanfranco Pellesi is editor-in-training for Clinical and Translational Science. Lars Edvinsson has received research grants and received speaker support (fees and travel) from Amgen Inc, Novartis,

Lundbeck, and AbbVie regarding basic research on monoclonal antibodies and gepants to understand their sites of action.

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