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Development of anti-migraine therapeutics using the capsaicin-induced dermal blood flow model

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The efficacy of calcitonin gene-related peptide (receptor) (CGRP-(R)) blocking therapeutics in the treatment of acute migraine headache provided proof-of-concept for the involvement of CGRP in the pathophysiology of this disorder. One of the major hurdles for the development of any class of drugs, including CGRP blocking therapeutics, is the early clinical development process during which toxic and inefficacious compounds need to be eliminated as early as possible in order to focus on the most promising molecules. At this stage, human models providing proof of target engagement, combined with safety and tolerability studies, are extremely valuable in focusing on those therapeutics that have the highest engagement from the lowest exposure. They guide the go/no-go decision making, establish confidence in the candidate molecule by de-risking toxicity and safety issues and thereby speed up the early clinical development.

In this review the focus is on the so called 'capsaicin model' as a typical example of a target engagement biomarker used as a human model for the development of CGRP blocking therapeutics. By applying capsaicin onto the skin, TRPV1 channels are activated and a CGRP-mediated increase in dermal blood flow can be quantified with laser Doppler perfusion imaging. Effective CGRP blocking therapeutics in turn, display blockade of this response. The translation of this biomarker model from animals to humans is discussed as well as the limitations of the assay in predicting the efficacy of anti-migraine drugs.

Introduction

Migraine is a chronic, incapacitating neurovascular disorder. According to the World Health Organization, 324 million people worldwide suffer from migraine [1]. It is characterized by attacks of moderate to severe headache and autonomic nervous system dysfunction. In approximately one-third of migraine patients, the headache attacks are accompanied by an aura involving neurological symptoms such as transient visual, sensory, language or motor disturbances [2].

For many years the aetiology of migraine has been under investigation. The current general consensus is unified in the so called 'trigeminovascular theory'. This theory argues that the trigeminovascular system (TGVS) is the main anatomic substrate for the pathophysiology of migraine. The TGVS encompasses the meninges and blood vessels as pain sensitive intracranial structures as well as the major afferent pathways (i.e. the trigeminal nerve) for transmitting pain to the central nervous system [3, 4]. Activation of the TGVS results in cranial vasodilatation mediated by the release of vasoactive neuropeptides, including calcitonin gene-related peptide (CGRP) [5]. It is primarily located in small, unmyelinated sensory C fibres and myelinated Aδ fibres in the periphery. CGRP is usually found in nerves that are closely associated with other peptides in C fibres, in particular tachykinins, substance P and neurokinin A [6].

CGRP is a 37 amino acid neuropeptide which is widely distributed in the central and peripheral nervous system [7–9]. It binds to the CGRP receptor, which is known as a calcitonin-like receptor (CLR) and belongs to the family of G-protein-coupled receptors (GPCRs) [10]. The CGRP receptor is located on target cells in the surrounding tissue such as mast cells, immune cells and vascular smooth muscle cells (VSMCs) [11, 12]. By interaction with VSMCs, CGRP is known to be a very potent vasodilator. The resulting response, in peripheral tissues, is mainly characterized by local redness and warmth (secondary to vasodilatation), limited swelling and allodynia (i.e. hypersensitivity to heat and touch secondary to alterations in the excitability of

primary sensory neurons). Collectively, these changes are referred to as 'neurogenic inflammation', because of the inflammatory symptoms resulting from the release of substances from the afferent fibres of primary sensory neurons [13, 14].

The role of vasodilatation and neurogenic inflammation in migraine is supported by the fact that triptans, which are currently the most effective class of acute anti-migraine drugs, cause relative selective cranial vasoconstriction by 5-HT_{1B} activation on VSMCs while they also inhibit presynaptic neuropeptide release by 5-HT_{1D} activation [15–18]. The release of CGRP from afferent fibres is considered pivotal in the pathogenesis of migraine. This is supported by the observation that intravenous infusions of CGRP induce dilatation of the middle meningeal artery and induce migraine-like headache in migraine patients [19, 20]. The involvement of CGRP was first confirmed by small molecule CGRP-R antagonists including olcegepant (i.e. BIBN4096BS), telcagepant (i.e. MK-0974) and MK-3207 which showed clear efficacy in phase II and/or phase III clinical trials in the treatment of acute migraine. Later on, the humanized monoclonal antibodies (mAbs) of Alder Biopharmaceuticals (ALD-403) and Eli Lily and Co. (LY2951742), which target the CGRP ligand itself, showed positive proof-of-concept (PoC) study results with a single dose (Phase 2a). Currently, these antibodies are in Phase 2b (dose-ranging study) of clinical development. The same accounts for AMG 334 (a fully human mAb targeting the CGRP receptor) and TEV-48125 (fully humanized antibody targeting the CGRP ligand) [21–24]. Further on in this review, we will refer to these drugs collectively as CGRP blocking therapeutics irrespective of the fact whether they target the CGRP receptor or CGRP as ligand.

The capsaicin model for neurogenic inflammation

One way of investigating the role of CGRP in the pathophysiology of migraine and evaluating target engagement of CGRP blocking therapeutics, is by making use of what is commonly referred to as the 'capsaicin model'.

Capsaicin is the pungent ingredient in hot chilli peppers activating the transient receptor potential vanilloid type 1 receptor (TRPV1). The TRPV1 receptor belongs to the TRP cation channel subfamily and is expressed on a subpopulation of primary sensory neurons consisting of Aδ and C fibre nociceptors. Although several putative endogenous ligands of the TRPV1 receptor have been identified, their physiological and pathophysiological effects in the sensory nervous system remain unclear. In contrast, the effect of binding of the exogenous ligand capsaicin with the TRPV1 receptor is well known to provoke the release of a number of bioactive substances including CGRP (Figure 1) [25, 26]. Activation of the TRPV1 receptor leads to the non-selective influx of cations, nerve fibre depolarization and the subsequent release of neuropeptides by exocytosis. If induced in peripheral tissues, such as the skin, the resulting vasodilation can be clearly observed. Over time, a repetitive release can lead to depletion of neuropeptides and desensitization of sensory nerves. Indeed, long term treatment with capsaicin in order to deplete the sensory neurogenic component has been exploited in a beneficial manner to treat hyperalgesia and pain conditions in humans, for example neuropathic pain after a herpes zoster infection [27]. Apart from chemical stimuli, also physical stimuli such as low pH and heat can activate the TRPV1 receptor and induce the release of CGRP [28, 29].

Capsaicin-induced vasodilatation is blocked with the TRPV1 antagonist capsazepine as well as by CGRP blocking therapeutics [30]. This confirms that vasodilatation, induced by capsaicin, is mediated by the release of CGRP. TRPV1 activation might play a major role in the release of CGRP from trigeminal nerves [31]. TRPV1 receptors are found on neurons in the trigeminal and dorsal root ganglia [32]. The peripheral terminals of capsaicin-sensitive neurons are sites of release for various pro-inflammatory neuropeptides, including CGRP. Hence, it has been suggested that activation of TRPV1 possibly initiates a migraine attack by causing CGRP release.

Investigating CGRP and CGRP blocking therapeutics with the capsaicin model

The complexity of migraine as a disease, the lack of translational models and the increasing drug development costs hamper the development of successful anti-migraine drugs. In order to tackle these challenges, the use of biomarkers to evaluate the potential of new targets at the earliest possible clinical stage of drug development has gained more interest. With the use of biomarkers one attempts to demonstrate target engagement at an early stage in order to obtain confidence in a drug candidate and to facilitate the further clinical drug development process.

Already in 1985, Helme *et al.* studied the dermal neurogenic inflammation that occurs after the topical application of capsaicin on the human skin. They assessed both the size of the flare response, induced by an increase in blood flow, and allodynia [14]. Several years later, Brain *et al.* investigated the nature of the vasodilatating mediator(s) involved in this response. Capsaicin topically applied to the mouse ear induced significant increases in blood flow and oedema. Blood flow was assessed by laser Doppler flowmetry and oedema formation by ^{125}I-albumin accumulation. The CGRP-R antagonist $CGRP_{8–37}$, a CGRP fragment, abolished

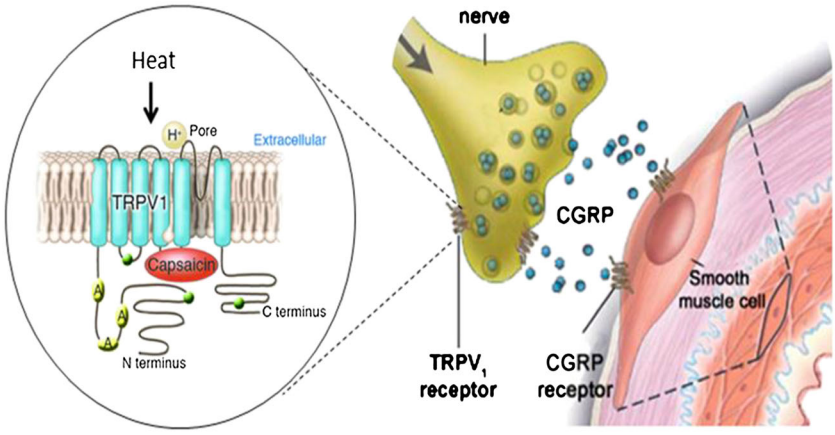

Figure 1

Activation of the TRPV1 receptor by capsaicin causes CGRP release. Figure 1 is adapted, with permission, from Hershey *et al*. [34]

the increased blood flow to capsaicin in wild-type mice, which indicates that the capsaicin-induced increased blood flow involves activation of, and possible interactions with, CGRP receptors [33]. Hereafter, Hershey *et al*. [34] developed a non-invasive pharmacodynamic capsaicin model in monkeys (Figure 2A). Topical application of capsaicin was utilized to induce the release of endogenous CGRP and a vasodilatatory response which can be measured using laser Doppler imaging. Using the potent and selective CGRP antagonist compound 3, which is an analogue of the well-characterized compound BIBN4096BS, they demonstrated 62% inhibition in rat with 300 $\mu g\,kg^{-1}$ i.v. and complete inhibition with only 30 $\mu g\,kg^{-1}$ i.v.in the rhesus monkey. At the doses studied, compound 3 was equally effective on both the acute and prophylactic inhibition of CGRP-mediated vasodilation. This was the first non-invasive model in non-human primates that allowed rapid evaluation of CGRP-R antagonist activity against endogenous CGRP [34].

The capsaicin-induced dermal blood flow (CIDBF) model was translated into a human *in vivo* pharmacodynamic model by de Hoon *et al*. (Figures 2B and 3) [35, 36]. The human CIDBF model involves the topical application of capsaicin on the human forearm, which induces CGRP release and thereby vasodilatation and increased dermal blood flow (DBF). This DBF is measured using laser Doppler scanning techniques. First, a capsaicin dose producing a robust and reproducible dermal blood flow response was established. Second, the influence of the forearm location on the capsaicin response was assessed. Third, the within-subject arm-to-arm and period-to-period reproducibility of the CIDBF model were confirmed (Table 1 and Figure 3). Finally, it was shown that there was no within-subject diurnal variation in the capsaicin response [35].

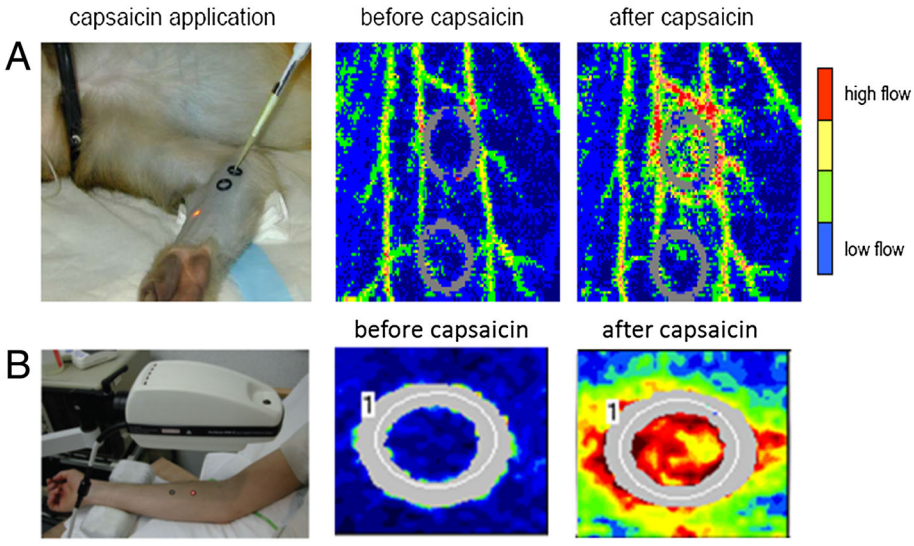

Figure 2

Non-invasive preclinical capsaicin model in primates (A) *vs*. humans (B). Capsaicin application clearly induces an increase in dermal blood flow, measured with laser Doppler imaging. Figure 2 is adapted, with permission, from (A) Hershey *et al*. [34] and (B) Sinclair *et al*. [38]

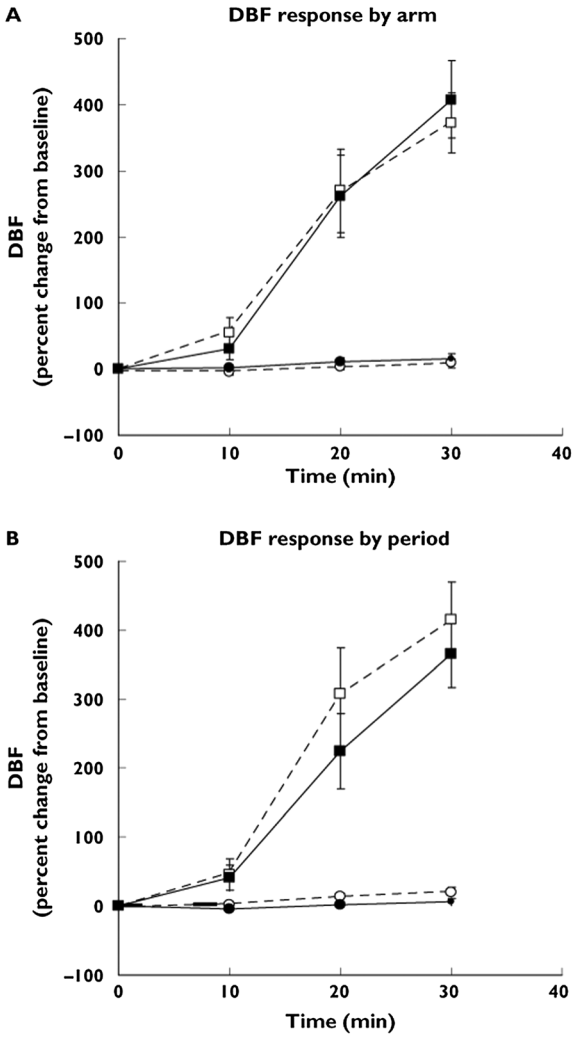

Figure 3

Dermal blood flow (DBF) response by arm and by period. (A) DBF response (percent change from baseline) after application of placebo and 1000 mg capsaicin. The mean of the DBF responses at the capsaicin or placebo application sites during both study periods was calculated. Number of subjects = 1, number of observations = 44. D, dominant arm; ND, non-dominant arm. Data are mean of observations and 90% confidence interval (CI). Placebo D (white circle); placebo ND (black circle); 1000 mg capsaicin D (white square); 1000 mg capsaicin ND (black square). (B) DBF response (percent change from baseline) after application of placebo and 1000 mg capsaicin. The mean of the DBF responses at the capsaicin or placebo application sites in both arms was calculated. Number of subjects = 1, number of observations = 44. P1, period 1; P2, period 2. Data are mean of observations and 90% of CI. Placebo P1 (white circle); placebo P2 (black circle); 1000 mg capsaicin P1 (white square); 1000 mg capsaicin P2 (black square). Figure 3 is adapted, with permission, from Van der Schueren *et al.* [35]

Capsaicin application is well tolerated and provokes only a local flare and stinging sensation which disappears within 2–6 h after application. A gradual increase in the DBF after both 300 μg and 1000 μg capsaicin application was quantified over a period of 30 to 45 min after capsaicin application. The lower 100 μg dose of capsaicin failed to increase DBF significantly. The most proximal sites of the human forearm skin showed the most reproducible and robust responses to capsaicin [35]. Subsequently, the involvement of NO, CGRP, substance P (SP) and prostaglandins as mediators in the CIDBF model was tested. Capsaicin was topically applied on the forearm and the antagonist $CGRP_{8-37}$ was administered intra-arterially via the brachial artery. $CGRP_{8-37}$ significantly decreased the capsaicin-induced DBF increase, in contrast to indomethacin (cyclo-oxygenase inhibitor), L-NMMA (non-selective NOS inhibitor) and aprepitant (NK1-receptor antagonist) which did not affect the DBF response to capsaicin. These findings identified CGRP as the key mediator in CIDBF in humans. Consequently, these studies confirmed that CIDBF could be used as a pharmacodynamic model to assess target engagement of the CGRP receptor or 'scavenging' of CGRP by CGRP blocking therapeutics *in vivo* in early clinical trials [36].

In a subsequent study, long term repeatability of the model was further investigated in healthy male volunteers. No desensitization occurred after weekly applications for 4 weeks in healthy male volunteers [37]. This opened the door for using the CIDBF model to evaluate CGRP blocking therapeutics in the early development of mAbs targeting CGRP as a ligand or its receptor. Indeed, because of the long half-life of these biologicals, long term reproducibility needed to be confirmed.

Because migraine prevalence is higher in women compared with men, during phase II clinical trials female migraineurs will also be included, Vermeersch *et al.* [37] went on to investigate the influence of female hormones during the menstrual cycle on the CIDBF response. In healthy women, the DBF response to capsaicin is increased during menstruation compared with the last week of the secretory phase of the menstrual cycle. This could be the result of (1) increased neuronal/TRPV1 sensitivity to capsaicin, (2) increased release of CGRP or (3) increased sensitivity to CGRP during the menstruation period. These results clearly indicate that the CIDBF response is influenced by female hormone changes during the menstrual cycle. In women suffering from migraine, the CIDBF response was consistently high, irrespective of the menstruation period compared with non-migraineurs. No difference was found between healthy men and men with migraine. This supports the general hypothesis that migraine headache is associated with female hormonal changes and primarily affects females [37]. Taken together, these data give a potential explanation for the higher frequency of migraine in women compared with men and its relationship to menstruation.

The capsaicin model has not only been used to investigate the physiopathology of migraine but has mostly been used as a target engagement biomarker for several small molecule CGRP-R antagonists including telcagepant (MK-0974) [38, 39] and MK-3207 [40, 41] as well as mAbs binding CGRP as a ligand (e.g. LY2951742 [42, 43], ALD403 [44, 45] and TEV-48125, formerly known as PF-04427429 or LBR-101 [46]) or the CGRP receptor (e.g. AMG 334).

Table 1

Test–retest reproducibility of CIDBF response and sample size calculations. Number of subjects = 11, number of observations = 44, the mean dermal blood flow (DBF) response in the two proximal rings was used in the test–retest analysis. Test–retest period-to-period and arm-to-arm reproducibility data for DBF response expressed as percent change from baseline at 30 min (t_{30}) and as the area under the curve of the percent change from baseline (AUC(0,30 min)). RC repeatability coefficient; WCV within-subject coefficient of variation; CCC concordance correlation coefficient; 95% CI 95% two-sided confidence interval. Table 1 is adapted, with permission, from Van der Schueren *et al*. [35]

DBF response	Test–retest reproducibility	Mean difference (95% CI)	RC	CCC	Sample size 20% shift	Sample size 50% shift
t_{30} (%)	Period-to-period	49 (−19.6, 118.0)	307	0.40	34	7
	Arm-to-arm	36 (−85.4, 14.4)	223	0.68	19	5
AUC(0,30 min) (% min^{-1})	Period-to-period	1119 (−239, 2476)	6058	0.33	76	14
	Arm-to-arm	139 (−340, 619)	2140	0.91	11	4

Sinclair *et al*. investigated whether MK-0974, the first orally bioavailable CGRP antagonist, inhibited the capsaicin-induced increase in dermal microvascular blood flow in humans (Figure 4) [38]. MK-0974 caused a robust inhibition of CIDBF, 1 and 4 h after oral drug administration. Surprisingly, although MK-0974 has a high affinity for the CGRP receptor, relatively high plasma concentrations were required to reduce CGRP-mediated skin vasodilatation in healthy volunteers and alleviate headache in migraine patients. This is most likely due to the fact that access to peripheral CGRP receptors is restricted owing to high protein binding of the compound. However, it cannot be completely excluded that, in the case of a central mode of action being required for efficacy, restricted access to CGRP receptors located in the central nervous system, because of unfavourable brain penetration properties of the compound, might also have contributed [38]. Unfortunately, the clinical development of telcagepant had to be terminated because of drug-induced hepatotoxicity [39]. One might speculate that the high levels of drug required in order to achieve a sufficiently high free fraction of telcagepant, and consequently an efficacious anti-migraine dose, might have promoted hepatotoxicity liability.

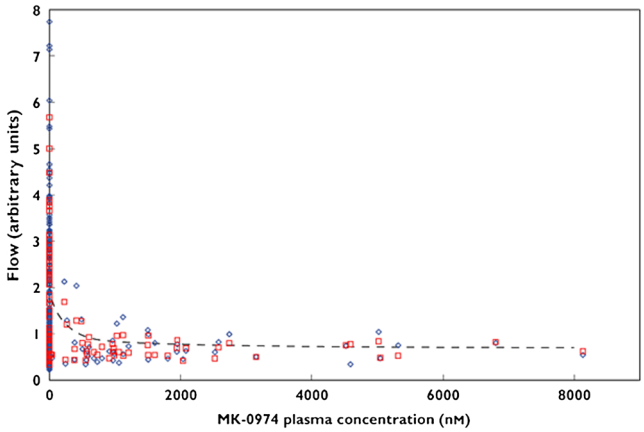

Figure 4

Capsaicin induced dermal blood flow (CIDBF) *vs*. telcagepant (MK-0974) plasma concentration. The blue diamonds show the measured DBF, red squares show the individual predicted values and the dashed lines indicate the population predicted means values. Figure 4 is adapted, with permission, from Sinclair *et al*. [38]

MK-3207 is a potent and orally bioavailable CGRP receptor antagonist of the human and rhesus monkey CGRP receptors *in vitro*. The *in vivo* potency was assessed in a rhesus monkey pharmacodynamic assay measuring capsaicin-induced changes in forearm DBF as well as in humans. MK-3207 produced a concentration-dependent inhibition of dermal vasodilation [40, 41, 47]. In a randomized controlled trial in 574 migraine patients, MK3207 was shown to be well tolerated and effective in acute migraine treatment [22].

As migraine is a chronic disease, prophylactic treatment is an approach which can be considered to be complementary to acute treatment. Given the typically long half-life of mAbs, the development of CGRP (receptor) binding antibodies is an attractive alternative approach for migraine treatment. Recently, the first clinical phase II data with CGRP binding mAbs (i.e. LY2951742 [42, 43, 48, 49], ALD403 [45, 50] and TEV-48125 [46]) and CGRP-R binding monoclonal antibody AMG 334 [51] have been completed and presented. During the development of these antibodies, as well as for previous CGRP-R targeting small molecules (i.e. telcagepant and olcegepant), the capsaicin challenge was used to prove target engagement. It was shown that the small molecule antagonists and AMG 334 targeting the CGRP-R were able to inhibit the capsaicin response by 90% or more whereas with mAbs targeting the CGRP ligand only partial inhibition was established [51]. Although one might expect that the differences observed in the ability to inhibit the capsaicin response should translate into a difference in clinical efficacy, this has not been shown so far. All antibodies, regardless of their mode of action, showed a small, but significant reduction of 1 to 2 migraine days per months compared with placebo in phase II trials [48–52]. In these trials, the 50% responder rate ranged from 46.5 to 61% for active treatment *vs*. 29.9% to 33% for placebo [48–53]. Although these data show no difference in clinical efficacy between the receptor targeting antibody (AMG 334) and the ligand

targeting antibodies (LY2951742, ALD403 and TEV-48125), it should be pointed out that the results of the different phase II trials are difficult to compare due to large differences in dosing ranging from 70 mg subcutaneous injection of AMG 334 up to 1000 mg [52] intravenously for ALD403 [53]. Therefore, phase III data need to be awaited to make a fair comparison between these compounds and to draw any firm conclusions.

Advantages and limitations of the capsaicin model

The capsaicin model is an *in vivo* pharmacodynamic model in animals and humans, which is non-invasive, technically uncomplicated and has a rapid and objective endpoint. This pharmacodynamic model allows repeated measurements which are adequately reproducible and repeatable over time. This model therefore facilitates early clinical evaluation of antagonists of mediators involved in neurogenic inflammation, including CGRP blocking therapeutics.

Like all pharmacodynamic biomarker models, this model also has its limitations. The capsaicin model remains a simulation of a naturally occurring pathophysiological process one wants to study. The effect of a drug on capsaicin-induced DBF can provide us with an index of anti-CGRP activity but is only indicative for its efficacy for inhibiting peripheral DBF. In that perspective, it proves peripheral target engagement but not necessarily guarantees therapeutic efficacy. Indeed, changes measured in the peripheral vasculature such as superficial dermal capillaries of the forearm, are not necessarily predictive of changes in the cranial circulation such as the trigeminovascular system. One way to overcome this limitation could be the application of capsaicin to the forehead skin [39].

Also, there is a possibility that therapeutic efficacy in the treatment of migraine, a CNS disorder, might require penetration in the central nervous system and drug concentrations needed for a peripheral inhibition of the response might not be representative for concentrations needed to achieve central target engagement. However, a strong argument in favour of our peripheral CIDBF model is the increasing evidence that central penetration to obtain anti-migraine efficacy is not necessarily needed. Indeed, recent PET data with telcagepant convincingly showed that, at therapeutic doses, no meaningful occupancy of CGRP-receptors was observed in the central nervous system [54]. This is a very important observation supporting the use of the 'peripheral' CIDBF model as a relevant model to test potential CGRP blocking therapeutics for the treatment of migraine as a disorder of the CNS. In addition, this observation also supports the concept that, in order to relieve migraine, it is sufficient for CGRP blocking therapeutics to act peripherally and thus provides confidence in the development of mAbs as anti-migraine therapeutics despite their limited access to the brain.

Finally, when using the capsaicin model for testing target engagement of CGRP blocking therapeutics, it should be kept in mind that it is an indirect measurement, depending on TRPV1 receptor functionality. One way to avoid this is the direct application of CGRP. This can be achieved by topical application of CGRP in combination with iontophoresis [55], intravenous or intra-arterial infusion [20, 56]. Edvinsson *et al*. used laser Doppler flowmetry combined with iontophoresis of CGRP, acetylcholine and sodium nitroprusside in migraine patients and healthy controls [55]. Olesen's group used intravenous CGRP infusions in an attempt to develop a headache model [20, 57, 58]. These CGRP infusions caused migraine-like attacks in patients with migraine with aura whereas no migraine attacks were induced in patients with familial hemiplegic migraine. de Hoon *et al*. [56] used intra-arterial infusions of CGRP in the forearm blood flow (FBF) model. In this model bilateral venous occlusion plethysmography is used to measure changes in forearm perfusion induced by the intra-arterial infusion of vasoactive compounds into the brachial artery. By using this approach, the reproducibility of CGRP-induced vasodilatation of forearm blood flow was demonstrated, the vasodilatory mechanism of action of CGRP was further investigated in humans and potential differences between migraineurs and healthy subjects were explored. However, no differences between migraineurs and healthy volunteers were observed [59–61].

Conclusion

Development of the capsaicin-induced dermal blood flow model in humans has proven to be a very useful platform for evaluating target engagement and dose selection of CGRP blocking therapeutics in the early clinical development of anti-migraine drugs. The CIDBF sets an example that target engagement biomarkers should be more generally applied to improve the confidence in new compounds at the earliest possible stage of drug development. With the use of these biomarkers not only dose selection can be guided in early efficacy trials but also the go/no go decision making. By selecting the winners and killing the losers early, drug development costs and timelines can be significantly reduced.

Competing Interests

All authors have completed the Unified Competing Interest Form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare no support from any organization for the submitted work. JdH was principal investigator for clinical studies

commissioned by Amgen Inc., Eli Lilly and Company and Merck Sharp & Dohme in the previous 3 years. There are no other relationships or activities that could appear to have influenced the submitted work.

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