Migraine is a common episodic neurovascular brain disorder associated with increased risk of cardio- and cerebrovascular ischemia. Migraine headache is likely caused by activation of the trigeminovascular system and release of calcitonin gene-related peptide (CGRP). Monoclonal antibodies against CGRP or its receptor are currently being evaluated for the prevention of migraine attacks. Preliminary efficacy data are promising. However, because CGRP may act as a vasodilatory safeguard during cerebral and cardiac ischemia, CGRP blockade could transform transient mild ischemic events into full-blown infarcts. Here, we review the cerebro- and cardiovascular risks that might be associated with CGRP blockade and which clinical and preclinical studies should be conducted to better assess the potential safety issues of this new promising class of drug.

CGRP and Its Putative Role in Migraine
Migraine is a highly prevalent, episodic, neurovascular brain disorder associated with increased risk of stroke and myocardial infarction [1]. Migraine headache is believed to be caused by activation of the trigeminovascular system [2] and associated release from activated trigeminal sensory nerves of CGRP, a potent vasodilator and modulator of cerebrovascular nociception [3,4]. Infusion of CGRP in patients with migraine may provoke migraine attacks [5]. For all these reasons, it has been proposed that CGRP might have an important role in the pathophysiology of migraine and that blockade of CGRP or its receptors might abort or even prevent migraine attacks (reviewed in [3]). Unlike triptans (5-HT1B/1D/1F receptor agonists, e.g., sumatriptan), the current mainstay of acute antimigraine treatment (for references, see [6]), blocking CGRP or its receptors would theoretically not be associated with potentially harmful vasoconstriction of cerebral and coronary blood vessels [3,6]. Moreover, triptans are fully effective in less than 50% of patients with migraine [7], do not appear to be useful as prophylactic agents, and are formally contraindicated in patients with cerebro- or cardiovascular disease [8]. For clarity, throughout this review, we use the term ‘cardiovascular’ to refer to both cerebrovascular and cardiovascular.

Thus, the concern arises that, after CGRP blockade, mild and usually transient ischemic events might be transformed into full-blown infarcts.

CGRP, a 37-amino acid peptide that exists in two homolog isoforms in humans. The first, αCGRP, is formed by alternate splicing of the calcitonin gene, in particular in the nervous system. The second form, βCGRP, is encoded by a second CGRP gene and is predominantly expressed in the enteric sensory system. Human αCGRP and βCGRP differ by three amino acids and appear to have similar biological effects (reviewed in [8,9]). The CGRP receptor comprises three parts, namely: receptor activity-modifying protein 1 (RAMP1); calcitonin-like receptor (CLR), which has seven transmembrane domains; and receptor component protein (RCP) [10]. RCP links the receptor to an intracellular G protein-mediated signaling pathway that mainly increases cAMP levels [11].
Blocking the CGRP System

Pharmacological inhibition of the CGRP-binding site formed by RAMP1 and CLR using small-molecule competitive CGRP receptor antagonists (gepants) proved effective in the acute and prophylactic treatment of migraine attacks [3,8]. However, further clinical development of the gepants was halted because of adverse events (elevated liver transaminases) [12] and formulation issues [13]. This led to the development of novel classes of drug acting as CGRP scavengers. These include CGRP-binding RNA-Spiegelmers (e.g., NOX-L41 [14]) and monoclonal humanized antibodies directed against CGRP (‘nezumabs’, for the nomenclature of antibodies, see [15]: ALD403; TEV-48125; LY2951742–galcanezumab) [16–19]. In addition, a fully human monoclonal antibody (AMG 334, erenumab, a ‘numab’ [15]) has been developed that is targeted against the CGRP receptor [20].

Whereas TEV-48125 binds equally to both α- and βCGRP [18], it is unknown whether this is also the case for ALD403 and LY2951742. However, the fact that LY2951742 blocked most of the CGRP-induced peripheral vasodilation [21] suggests that antibodies against CGRP or its receptor would wipe out the entire endogenous CGRP system for at least several weeks. The question we discuss here is, although abolishing the effects of CGRP might be beneficial by preventing migraine attacks, could this also have harmful effects? As discussed below, there is ample evidence that the CGRP system has an important role in maintaining cardiovascular homeostasis under pathophysiological conditions [22]. As such, it may act as a vasodilatory safeguard mechanism during cerebral and cardiac ischemia. The concern thus arises that, in subjects whose CGRP system has been blocked for a long time, transient mild ischemic events, such as cerebral transient ischemic attacks or cardiac angina, might be transformed into full-blown infarcts. In this review, we focus on the cardiovascular risks that might be associated with CGRP blockade and which clinical and preclinical studies should be done to assess these risks and to better understand the underlying mechanisms. The present review is by no means intended to be overly critical; we only want to improve the clinical program for this new generation of antimigraine drugs.

Site and Duration of Action of CGRP (Receptor) Antibodies

Normally, only lipophilic drugs with a molecular weight ≤ ~400 Da may cross the blood–brain barrier (BBB). Given the much greater molecular weight of antibodies (around 150 000 Da), they are unlikely to cross the BBB to a major extent. In general, only 0.1–0.2% of circulating antibodies enters the brain or reaches the cerebrospinal fluid (CSF) [23,24]. Although CGRP may also have modulatory effects on motor, sensory, and integrative systems within the brain [3], the low brain penetrance of CGRP (receptor) antibodies clearly suggests a site of action outside the brain. Similar discussions are ongoing for the triptans and gepants. There is no evidence that triptans may enter the human brain in sufficient quantities to exert central pharmacological actions [25,26] and binding of telcagepant within the brain could only be demonstrated after administration of supratherapeutic doses [27]. Thus, structures that are located outside the BBB appear to be more likely therapeutic targets for the CGRP antibodies. Such structures include not only cranial blood vessels [28], but also neuronal structures that are not fully protected by the BBB, such as the trigeminal ganglion and the paraventricular structures within the brain stem [29–31].

Little is known about the pharmacokinetic and pharmacodynamic properties of the CGRP (receptor) antibodies. Given that CGRP plasma concentrations and half-life are known approximately and by assuming (i) linear efficacy during the whole dosing period and (ii) that a given dose of CGRP antibody will completely enter the circulation and will only disappear from the circulation after having bound to a molecule of CGRP, we may estimate that CGRP antibodies might scavenge CGRP for up to 1.5 months (Box 1). This estimation, although inevitably imprecise due to the various assumptions, corresponds remarkably well with the
commonly used dosing interval of 1 month. For the CGRP receptor antibody, we cannot make such a calculation because precise information about its half-life and the estimated number of CGRP receptors in the human body is lacking.

Cardiovascular Safety of Blocking the CGRP System

Cardiovascular safety of small-molecule CGRP receptor antagonists (gepants) has been primarily investigated in single-dose, acute treatment studies in which the CGRP system was blocked for only a few hours (reviewed in [32,33]). The cardiovascular effects of long-term blockade of the CGRP receptor with gepants were assessed in only two, relatively small and short-lasting, prophylactic treatment studies [34,35]. Little is known about the cardiovascular safety of the long-term blockade of the CGRP system with CGRP (receptor) antibodies. In animal models, CGRP antibodies were devoid of effects on heart rate or arterial blood pressure [36,37]. However, these studies were performed in normal, anesthetized animals without pre-existing or induced ischemia. In patients with migraine, no cardiovascular adverse events were observed in five, relatively small and short-lasting, Phase II clinical trials with four different antibodies directed against CGRP or its receptor [16,17,38–40]. The issue of cardiovascular safety was specifically addressed in two small studies, one in 31 healthy women [41] and another in 56 cynomolgus monkeys [42]. Although no cardiovascular events were noted, these studies were evidently too small and did not address the real issue, namely: what happens in (cardiovascularly compromised) subjects in whom the CGRP system is blocked and who were accidentally struck by mild cardiac or cerebral ischemia?

Cardiovascular Protective Role of CGRP

As discussed below, CGRP has been shown to prevent the onset of hypertension [43], focal cerebral ischemia (by increasing blood flow [44,45]), and myocardial infarction and heart failure after cardiac ischemia [46,47].

CGRP and Hypertension

Hypertension is an important risk factor for heart and brain infarcts and other vascular diseases. Despite the considerable number of antihypertensive drugs currently available, the mechanisms involved in the onset of hypertension remain unclear and not every patient can be treated satisfactorily. The renin-angiotensin-aldosterone system is a key system in the development of hypertension [48]. It is clear that the vascular smooth muscle is important in retaining vasodilator tone and, thus, a normal blood pressure. Many factors deriving from the endothelium have been identified, such as nitric oxide (NO), endothelium-derived hyperpolarizing factors (EDHF),
and prostacyclin [49]. CGRP is becoming increasingly recognized as another important factor in retaining vasodilator tone. CGRP does not primarily act at the endothelium, but exerts its action mainly directly at smooth muscle cells in the vascular wall [50], mostly in the microvasculature, which is responsible for the majority of the peripheral vascular resistance and, thus, the blood pressure [51]. While CGRP does not seem to be involved in the physiological regulation of blood pressure [52], evidence is now growing suggesting that CGRP has a protective role in the generation of hypertension, which is most likely mediated via effects at peripheral receptors [43]. There might also be a role for the RAMP1 component of the CGRP receptor within the brain stem [43]. While the central mechanisms are unlikely to be affected by CGRP (receptor) antibodies, the peripheral effects of CGRP will most likely be abolished or, at least, markedly reduced.

Indeed, basal blood pressure was increased in CGRP-knockout mouse models [53,54] (but not in all [55]). These animals also had renal damage that was independent from the increase in blood pressure [35]. Related with these findings, acute intravenous (i.v.) bolus injections of olcegepant have been shown to enhance peripherally the vasopressor sympathetic outflow in pithed rats [56]. Apart from the effects in hypertension per se, it is noteworthy that CGRP is also important in maintaining cerebral blood flow via autoregulation in chronic hypertension [57].

CGRP and Cerebral Ischemia

Almost 30 years ago, trigeminovascular fibers were demonstrated to increase cerebral blood flow after occlusion [58], acute severe hypertension, and seizures [59]. One of the trigemino-vascular neuropeptides is CGRP [60]. Later, this peptide was shown to have a neuroprotective effect against focal cerebral ischemia, partly via leptin, by increasing blood flow [45,61]. In rats, CGRP administration at the onset of the reperfusion period after experimental cerebral artery occlusion reduced postischemic increase of brain edema, likely mediated, at least partly, via a decrease in postischemic BBB disruption [62].

It has been suggested that CGRP prevents, at least partly, delayed vasospasm and subsequent ischemia after subarachnoid haemorrhage [63,64]. Indeed, CSF CGRP levels were higher in patients without vasospasm than in those with vasospasm [44]. Moreover, in an experimental rat model, CGRP levels in the basilar artery were reduced after subarachnoid haemorrhage [65]. Finally, CSF administration of slow-release CGRP tablets in monkeys prevented vasospasm after experimentally induced subarachnoid hemorrhage [66] and, in patients with subarachnoid hemorrhage, i.v. administration of CGRP reduced vasospasm as measured with transcranial Doppler [67].

CGRP and the Heart

Multiple studies in rats and mice strongly suggest that CGRP also acts in the heart as a protective safeguard against ischemia, probably by inducing vasodilatation [46,68–71]. In human studies, CGRP lowered blood pressure (see above) and protected against heart failure via positive chronotropic and inotropic effects [72,73]. Additional evidence for a cardioprotective role of CGRP in humans comes from studies showing: (i) decreased CGRP serum levels in coronary artery disease [74]; (ii) improved myocardial contractility after intravenous infusions of CGRP in congestive heart failure [75]; (iii) involvement of CGRP in the response to nitroglycerine in chronic heart failure [76]; and finally (iv) the finding that CGRP might be the effector molecule of nitroxyl (HNO) in the HNO-TRPA1-CGRP axis [77].

CGRP and Risk of Pre-Eclampsia

Systemic CGRP levels rise during pregnancy, peaking during the last trimester and dropping again after delivery [78]. CGRP expression and functional responses of the CGRP receptor are
increased in omental arteries of pregnant women [79], likely contributing to the vascular adaptations during pregnancy. In women with pre-eclampsia, CGRP levels are lower than in women with a normotensive pregnancy [78,80]. While it is unknown whether the low CGRP levels during pre-eclampsia are cause or a consequence, it is reasonable to assume that abolishing the effects of CGRP during pregnancy may have negative effects on both the mother and the fetus. This might be relevant for migraine because most patients with migraine are females of child-bearing age.

**Paradoxical Effects of CGRP on Aging**

CGRP expression and amount reduce with increasing age in human cerebral arteries [81]. By contrast, in rodent models, systemic CGRP levels rise with age, suggesting a role for CGRP in aging [82,83]. Several studies suggest that elimination of the effects of CGRP delay aging. CGRP was absent in the naked mole rat, a rodent model of exceptional longevity [84,85]. Inhibition in mice of CGRP release by genetic inhibition of pain-sensitive TRPV1 channels was associated with preserved metabolic health, extended longevity, reduction of cancer, and improved cognitive performance in old age [86]. Thus, long-term blockade of CGRP or its receptors might be associated with fewer age-related diseases [86] including, paradoxically, cardiovascular disease [87]. However, because migraine tends to disappear in older age [88], CGRP (receptor) antibodies are unlikely to be widely prescribed in the older population.

**Are Cardiovascular Risks of CGRP Blockade Higher in Migraineurs and Women?**

Patients with migraine, in particular females, are at increased risk of stroke [89–93] and, although less consistently demonstrated [91], coronary heart disease [94–97]. Although various mechanisms, including generalized vascular dysfunction, genetic factors, and patent foramen ovale, have been proposed to explain these increased risks, the true underlying mechanism is unknown. Therefore, it is difficult to assess whether CGRP (receptor) blockade in migraineurs might be associated with even higher cardiovascular risks.

The clinical characteristics, etiologies, and timing over the lifespan of cardiovascular events in women are remarkably different to those in men. While women appear to be protected from cardiovascular events before menopause, thereafter the incidence of cardiovascular events, in particular stroke, increases dramatically [98,99]. Moreover, while in men myocardial infarctions are usually caused by occlusion of proximal conducting parts of the coronary circulation, vasospasm of the small intramyocardial parts of the coronary arteries is the most common cause in women [100,101]. Women are also more often misdiagnosed with noncardiac chest pain because blood vessels appear normal on angiographic investigation [100,102].

The pathophysiological differences between men and women might have important implications for the risk of cardiovascular events during CGRP (receptor) blockade. CGRP has differential effects on coronary arteries: small relaxation of the proximal portions and large vasodilation of the distal portions, which also are more densely innervated with CGRP-ergic fibers (Figure 1) [50,51,103]). In addition, plasma CGRP levels are higher in women than in men [104] and the vasodilator and hypotensive effects of CGRP are amplified in the presence of sex steroid hormones, such as 17ß-estradiol and progesterone [105]. Finally, independent from their vascular effects, 17ß-estradiol and progesterone may increase the positive inotropic effects of CGRP in cardiomyocytes [106]. All in all, one might speculate that women might be at particularly increased risk of myocardial infarction during CGRP (receptor) blockade. In this context and given that migraine is more prevalent among women, it is remarkable that studies on the safety of CGRP (receptor) blockade in cardiovascularly compromised patients have primarily been performed specifically in men (see, for example, [107]).
Which Studies Must Be Done?

In many of the above-mentioned studies providing evidence for a role of CGRP as a cardio-vascularly protective peptide, exogenously administered CGRP was administered to animals or patients with cardiovascular disease. Such an experimental condition differs from the situation that would occur after use of the antibodies (i.e., blockade of endogenous CGRP). Given that, for obvious ethical reasons, most studies cannot be performed in humans, we must fall back on experiments in (rodent) animal models. Important risks potentially associated with CGRP (receptor) blockade that need to be carefully assessed are: (i) augmentation of pre-existing hypertension or induction of hypertension de novo (e.g., [108]); (ii) transformation of transient mild ischaemia into full-blown brain (e.g., [109]) and myocardial infarcts (e.g., [110]); and (iii) development of heart failure (e.g., [111]). In such studies, CGRP should chronically be blocked by administration of antibodies (when necessary, an equivalent of the clinically investigated antibody with sufficient affinity for the animal CGRP or CGRP receptor should be used). Subsequently, animals should be subjected to the induction of hypertension, transient mild brain, or cardiac ischemia, and consequences should be compared between the groups with and without antibody. Moreover, these studies must take into account gender differences and hormonal status of the animal models: are the risks higher in female animals? The possible beneficial effects of CGRP in aging could also be studied, such as in animal models with accelerated aging (e.g., [87]).

Putative Differences between Antibodies against CGRP or the CGRP Receptor

It is fascinating to speculate about the potential clinically relevant differences between antibodies directed against the CGRP peptide and antibodies directed against the CGRP receptor. The latter, for example, are likely to reach binding equilibrium earlier than the former because CGRP receptors are constitutively present and CGRP is released instantaneously. Moreover, receptors other than the CGRP receptor, to which CGRP may still bind, might compensate for blockade of the CGRP receptor, and peptides other than CGRP that may also bind to the CGRP receptor,
may exert compensatory effects for antibodies against CGRP (Figure 2). As an example, CGRP may also stimulate amylin receptors within the trigeminovascular system[112]. Theoretically, this might provide a therapeutic advantage of antibodies against the CGRP peptide because these would also prevent activation of the amylin receptor. By contrast, it was recently suggested that the amylin receptor, as a ‘second’ CGRP receptor, might also be present in the human coronary artery [113]. If true, this might predict a cardiovascular safety benefit for antibodies directed against the CGRP receptor.

Concluding Remarks

Thus far, the results from the Phase II clinical trials evaluating the efficacy and tolerability of antibodies directed against CGRP or the CGRP receptor are promising [16,17,38–40]. This is potentially good news for patients with migraine or (possibly) cluster headache because specifically designed, effective, and well-tolerated prophylactic agents against these diseases are currently not available. However, we must await the results from the larger Phase III trials (see Outstanding Questions) and probably even more important from studies specifically testing the cardiovascular safety of these antibodies especially during ischemic events and in particular in women.

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Outstanding Questions

Can long-term CGRP (receptor) blockade augment or even induce hypertension?

Can (long-term) CGRP (receptor) blockade transform transient mild cardiac ischemia into a full-blown myocardial infarction?

Can (long-term) CGRP (receptor) blockade transform transient mild cerebral ischemia into a full-blown brain infarct?

Are these potential risks even higher in patients with migraine and women?

Are there clinically relevant differences with respect to these cardiovascular risks between antibodies directed against the CGRP peptide and antibodies directed against the CGRP receptor?


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