

● PERSPECTIVE

## Mechanisms of retinal neuroprotection of calcium dobesilate: therapeutic implications

Current treatments for diabetic retinopathy (DR) are based on laser photocoagulation and intravitreal injections of corticosteroids or anti-vascular endothelial growth factor (VEGF) agents. These treatments are applicable only at advanced stages of the disease. In addition, they are expensive, require a vitreo-retinal specialist and are associated with significant adverse effects. Therefore, new pharmacological treatments for the early stages of the disease are needed.

Although DR is still often considered a microcirculatory disease of the retina, growing evidence suggests that retinal neurodegeneration is an early event in the pathogenesis of DR which participates in the microcirculatory abnormalities. For this reason, therapeutic strategies based on neuroprotection should be effective not only in preventing or arresting retinal neurodegeneration, but also in preventing the development and progression of the microvascular abnormalities that exist in the early stages of DR (Simó and Hernández, 2015).

However, when the early stages of DR are the therapeutic target, it would be inconceivable to recommend an aggressive treatment such as intravitreal injections. Therefore, systemic treatments addressed to block the main pathways involved in the pathogenesis of DR are needed. However, most of these treatments can barely reach the retina at pharmacological concentrations and, in addition, could have serious adverse effects. Nevertheless, three classes of oral drugs have emerged as potential systemic treatments for DR: renin-angiotensin (RAS) system blockers, fenofibrate, and calcium dobesilate monohydrate (CaD).

Regarding RAS, the current clinical evidence does not support the concept that they possess an extra value in preventing or arresting the progression of DR in hypertensive patients when compared with other anti-hypertensive agents. In the case of fenofibrate, several sub-studies have demonstrated its usefulness in arresting the progression of DR but not in preventing its development. Therefore, it seems reasonable to propose its use for patients with preexisting DR. However, fenofibrate for DR treatment has been approved only in Australia, Singapore, Philippines and Malaysia. By contrast CaD has been approved for the treatment of DR in numerous countries and clinical evidence supports its use in early stages of DR.

Although CaD has been approved for the treatment of DR for many years, it has not been widely used in clinical practice. A poor understanding of its mechanisms of action has been one of the reasons for this. However, recent experimental evidence showing the neuroprotective action of CaD makes it a very attractive drug for early stages of DR. In fact, the American Diabetes Association in its more recent position statement defines DR as a “highly specific neurovascular complication” (Solomon et al., 2017). Therefore, drugs such as CaD which exert a multifaceted action on neurovascular unit impairment can be contemplated as excellent candidates for targeting the early stages of DR.

**Systemic actions of CaD:** The antioxidant and anti-inflammatory properties of CaD are among the systemic actions of CaD that could exert beneficial effects in DR. In addition, the beneficial hemorrheologic effects based on the evidence of CaD in reducing blood hyperviscosity and platelet aggregation facilitate capillary perfusion and reduce the inflammatory status.

**Mechanisms of retinal neuroprotection:** As previously mentioned, neurodegeneration is an early event in the pathogenesis of DR. In recent years, emerging evidence has supported the concept that the impairment of the neurovascular unit plays a key role in the pathogenesis of DR. In this regard, the hallmarks of neurodegeneration such as glial activation and neural apoptosis coexist with early features of microvascular impairment such as altered hemodynamics, loss of capillaries and vascular leakage. In addition, whereas neurodegeneration has a deleterious effect on retinal microvasculature, early microvascular impairment is also harmful for the neuroretina. Therefore, a bidirectional link between neurodegeneration and microvascular impairment exists in the pathogenesis of DR.

CaD exerts a multifaceted action that can be divided into vasculotropic and neurotropic actions (Figure 1). However, considering that main underlying mechanisms are involved in both the neurodegenerative and the microangiopathic processes, this classification is not fully realistic.

**Vasculotropic actions:** CaD prevented the retinal increase of nuclear factor kappaB (NF- $\kappa$ B), interleukin (IL)-6, IL-8, tumor necrosis factor-alpha (TNF- $\alpha$ ), monocyte chemoattractant protein 1 (MCP-1) and oxidative stress induced by diabetes in experimental models (Leal et al., 2010; Bogdanov et al., 2017). These effects were obtained using 100 mg/kg per day for 10 days or 200 mg/kg per day for 15 days, and were associated with a significant reduction of vascular leakage. Moreover, it should be emphasized that these anti-inflammatory and antioxidant actions have beneficial effects not only on microvessels but also in glial cells and neurons. In fact, neuroinflammation is one of the primary events of the neurodegenerative process. In addition, as previously mentioned, the vasculotropic actions have a beneficial impact on the neurovascular unit and, consequently, in the neuroretina.

Apart from its anti-inflammatory and antioxidant action, CaD prevented the diabetes-induced upregulation of endothelin-1 (ET-1) and its cognate receptors (ETA-R and ETB-R) in the retina of db/db mouse (Solà-Adell et al., 2017). These effects were associated with a significant reduction of vascular leakage. ET-1 through ETA-R exerts a potent vasoconstrictive action and participates in the vasodilation impairment and vasoconstriction that occurs in DR (Chou et al., 2014). In addition, this pathway has also been involved in neuroretinal pathology (Chou et al., 2014). By contrast, the activation of ETB-R mainly contributes to neurodegeneration (Minton et al., 2012). Therefore, ET-1 has a dual deleterious action in the diabetic retina which is inhibited by CaD. The molecular mechanisms involved in the inhibitory effect of CaD on ET-1 remain to be elucidated. However, recent evidences suggest the anti-inflammatory activity of CaD plays an important role (Solà-Adell et al., 2017).

The intraretinal downregulation of VEGF and PKC-delta-P38 MAPK are also significant mechanisms by which CaD prevents the disruption of the BRB (Leal et al., 2010; Solà-Adell et al., 2017). Furthermore, the inhibition of PKC-delta by CaD

might also be involved in the downregulation of ET-1 expression (Park et al., 2000).

**Neurotrophic actions:** Oral treatment with CaD (200 mg/kg per day for 14 days) prevents both glial activation and apoptosis in db/db mice in comparison with diabetic mice treated with vehicle (Solà-Adell et al., 2017). This morphological improvement was associated with a significant improvement of ERG parameters, thus revealing a clear impact on global retinal function. Notably, the neuroprotective action of CaD was associated with a preventive effect on the development of early microvascular abnormalities such as albumin leakage due to the disruption of the BRB (Solà-Adell et al., 2017). The mechanisms involved in the neuroprotective effect of CaD include the following:

**Reduction of excitotoxicity induced by glutamate:** Glutamate is the major excitatory neurotransmitter in the retina and it is elevated in the extracellular space in the diabetic retina. This extracellular and synaptic excess of glutamate levels leads to overactivation of ionotropic glutamate receptors, mainly alpha-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA) and N-methyl-D-aspartate (NMDA) receptors, which results in an uncontrolled intracellular calcium response in postsynaptic neurons and cell death. This deleterious effect of glutamate on retinal neurons is known as “excitotoxicity”. The reason why CaD inhibits the extracellular accumulation of glutamate is unknown. However, the anti-inflammatory activity of CaD seems to play a critical role in this action. The most dominant glutamate transporter is glutamate/l-aspartate transporter (GLAST), which permits glutamate uptake by the Müller cells and is downregulated in the diabetic retina due to glial activation (Simó and Hernández, 2015). Therefore, it can be reasonably be postulated that any drug with significant anti-inflammatory activity within the retina, and in particular on Müller cells, would result in an inhibition of GLAST downregulation induced by diabetes. In fact, CaD prevents glutamate accumulation by inhibiting the downregulation of GLAST induced by diabetes (Solà-Adell et al., 2017). In addition, as ET-1 enhances glutamate-induced neurotoxicity in retinal neural cells (Kobayashi et al., 2005), the inhibitory effect of CaD on ET-1 could also contribute to neuroprotection.

**Inhibition of the ET-1 and ETB-R upregulation induced by diabetes:** The recent demonstration that CaD prevents the diabetes induced upregulation of ET-1 and its receptors opens up new insights into the mechanisms of action of this drug in the setting of DR (Solà-Adell et al., 2017). In particular, the inhibition of the ET-1/ETB-R pathway could contribute to its neuroprotective effect in a significant manner. Moreover, it has been recently demonstrated that the blockade of ETA-R apart from ameliorating retinal vascular pathology by reducing pericyte loss and acellular capillaries, prevents retinal thinning in both the optic nerve and retinal periphery in db/db mice (Chou et al., 2014). Therefore, ET-1 seems to be a key player involved in the cross-talk between neurodegeneration and vascular abnormalities in the setting of DR.

The main proposed mechanisms by which CaD participates in the improvement of the neurovascular unit are summarized in **Figure 1**. However, further research is needed to fully understand not only the link between the different molecular pathways, but also the sequential pathogenic events that take place in the diabetic retina after CaD administration.

There is no reason to think that the neuroprotective effects of CaD should be restricted to DR and, therefore, other neuro-

degenerative retinal diseases could be treated with CaD. However, no experimental evidence has been published so far. In addition, since the retina is ontogenetically derived from the brain, it could be postulated that CaD also exerts neuroprotective effects in brain neurons. This could open up a fascinating area of research with important clinical repercussions.

**Therapeutic implications:** Tight control of blood glucose levels and hypertension is essential in preventing DR development or arresting its progression. However, there are other factors that play an important role in the pathogenesis of DR. Among these components of the diabetic milieu, oxidative stress and pro-inflammatory cytokines, are two of the most relevant factors. Therefore, treatments such as CaD targeting these two pathogenic pathways can be envisaged as a new approach which goes beyond the current standard of care.

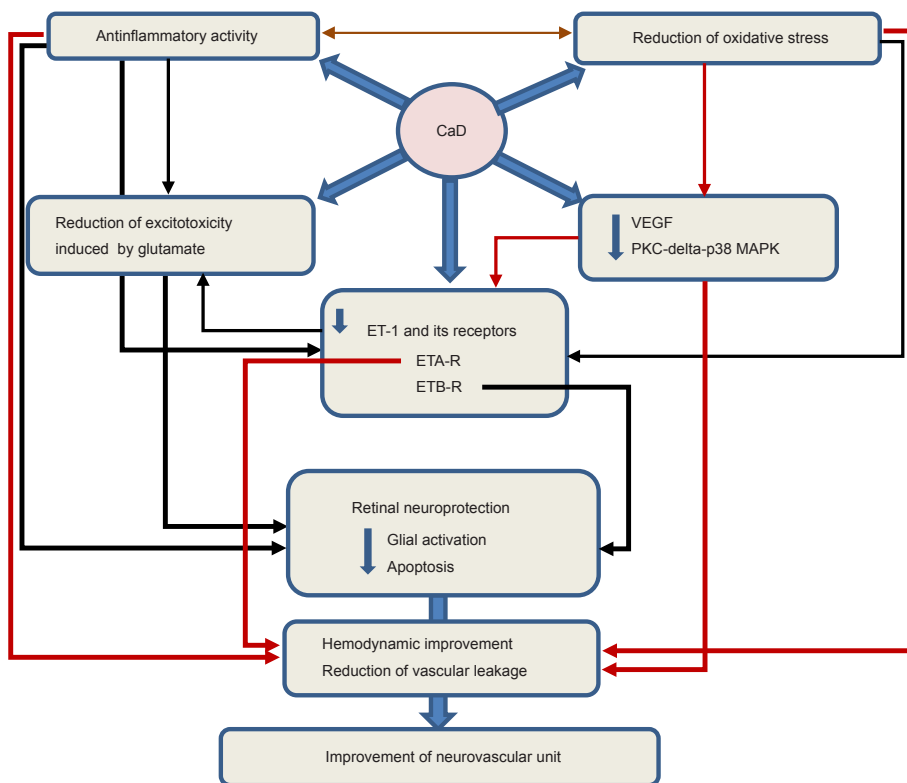
Two randomized placebo-controlled trials demonstrated the effectiveness of CaD in preventing the progression of early stages of DR (Leite et al., 1990; Ribeiro et al., 2006). In both studies the same dose of oral CaD (1,000 mg twice daily) was used. However, another randomized, placebo-controlled study (the CALDIRET study) conducted in 635 type 2 diabetic patients with mild-to-moderate NPDR presenting at the first visit with microalbuminuria and with a follow-up period of five years, showed that CaD did not reduce the risk of the development of clinically significant diabetic macular edema (CSDME) (Haritoglou et al., 2009). The main differences in the characteristics of the patients included in this study in comparison with the two previously mentioned were the inclusion of patients with more advanced stages of DR and microalbuminuria as per inclusion criteria. In addition, a lower dose of CaD was used (1.5 g/d vs. 2 g/d). Taken together these results suggest that CaD is beneficial in the very early stages of DR but its effectiveness in more advanced stages remains to be determined.

The experimental evidence showing the beneficial effects of CaD in avoiding neurodegeneration and the breakdown of the BRB pave the way to clinical trials addressed to testing whether CaD could be useful in preventing or arresting neurodegeneration or early stages of diabetic macular edema (DME) such as macular subedema. A pilot double blinded clinical trial (CADODIAME; EudraCT Number: 2017-000250-19) aimed at determining whether CaD is able to prevent or reduce the increase of retinal thickness and the progression of subclinical to clinically significant DME, as well as arresting the progression of neurodegenerative abnormalities is ongoing. In this 12 months follow-up study, 30 type 2 diabetic patients (40–70 years old) with diabetes duration  $\geq$  5 years and with subclinical DME will be randomly allocated to placebo vs. CaD (1,000 g twice daily). Apart from DR, studies aimed at exploring the neuroprotective effect of CaD in other ocular and brain neurodegenerative diseases are needed.

In summary, CaD has shown multifaceted pharmacological effects which abrogate multiple pathogenic pathways involved in DR, including neurodegeneration. Treatments such as CaD targeting multiple pathways could be more effective than those blocking a single pathogenic mechanism. However, further research to better understand the mechanisms of action and the clinical outcomes associated with its use is needed.

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**Figure 1** Mechanisms involved in the beneficial effects of CaD exerts in DR. The red arrows represents the vasculotropic actions and the black arrows the neuroprotective actions. CaD: Calcium dobesilate monohydrate; DR: diabetic retinopathy; ET-1: endothelin-1; VEGF: vascular endothelial growth factor.

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**Open peer review report:**

Reviewer: Yun-zheng Le, University of Oklahoma, USA.

Comments to authors: This is a nice perspective discussing the potential mechanisms of CaD in the treatment of DR. The subject matter is at the center of therapeutic strategy for DR, which will have an educational value for the general readership of this journal.

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