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REVIEW

Calcium Dobesilate: Pharmacology and Future Approaches

T. Tejerina and E. Ruiz*
Department of Pharmacology, School of Medicine, Complutense University
28040 Madrid, Spain [Tel: 34-911-3941476; Fax: 34-911-3941463; E-mail: teje@eucmax.sim.ucm.es]

ABSTRACT. 1. Calcium dobesilate (2,5-dihydroxybenzene sulfonate) is a drug commonly used in the treatment of diabetic retinopathy and chronic venous insufficiency.

2. The pharmacology of calcium dobesilate reveals its ability to decrease capillary permeability, as well as platelet aggregation and blood viscosity.

3. Furthermore, recent data show that calcium dobesilate increases endothelium-dependent relaxation owing to an increase in nitric oxide synthesis. GEN PHARMAC 31;3:357–360, 1998. **© 1998 Elsevier Science Inc.**

KEY WORDS. Calcium dobesilate, rabbit, rat, cat, venous insufficiency, diabetic vascular reactivity

INTRODUCTION

The pharmacology of capillary vessels is scanty and references only to flavonoids and some natural products can be found in old textbooks. An interesting exception is calcium dobesilate (calcium 2,5-dihydroxybenzene sulfonate; Fig. 1), a drug currently used in the treatment of chronic venous insufficiency (Hachen and Lorenz, 1982) and diabetic retinopathy (Leite *et al.*, 1990; Salama *et al.*, 1985). The objective of this minireview is to discuss the pharmacology of calcium dobesilate, including recent findings, and to evaluate the future prospects of this drug.

PHARMACOKINETICS

Studies on the metabolism and pharmacokinetics of calcium dobesilate were carried out by Benakis *et al.* (1974). They reported the results of calcium dobesilate ^{35}S blood levels, protein binding and urinary excretion in humans. The studies in humans were carried out by the administration of the drug by the oral or intravenous (IV) route. After IV medication, 500 mg, the maximum value is obtained 5 min after administration and is about 65 μg/ml. This value decreases rapidly, the plasma half-life being 1 hr.

After administration by the oral route, 500 mg in a capsule, the maximum value was obtained at the 6th hour after medication and is about 8μg/ml, and a plateau was obtained between the 3rd and the 10th hour. Levels decreased slowly and were undetectable 24 hr after administration. Intestinal absorption was 15% per hour during the first 7 hr and then decreased to 5% per hour. More than 80% of the drug is absorbed in the first 8 hr.

The drug is 20–25% protein bound. The specific affinity of the drug to aggregated and nonaggregated platelets has been demonstrated. The drug does not cross the hematoencephalic barrier.

Urinary elimination in the first 24 hr reaches 75% after IV medication and 50% after oral medication.

*To whom correspondence should be addressed.
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ANIMAL TOXICOLOGY OF CALCIUM DOBESILATE

Single oral or parenteral doses of calcium dobesilate are well tolerated in rats and dogs. Even in cases of repeated oral and intravenous administration of high doses of the drug, it did not present teratogenous effects in mice, rats or rabbits (Thomas *et al.*, 1972). It had no effect on electrocardiograms and the respiration of cats, rats and dogs after oral or IV administration of the drug (Marmo, 1971). In cats and guinea pigs, calcium dobesilate did not shown any cardiovascular effect for a broad range of doses and acted as a cardiovascular depressant only when administered in massive intravenous doses (Marmo, 1971). Thomas *et al.* (1972) showed that the oral LD_{50} of calcium dobesilate (by gastric probe) was 7.6–7.7 (7.0–8.4 g/kg in mice and 7.1–9.4 (8.0–11.0)g/kg in the rat, and the intravenous doses were 775 (684–860) mg/kg in mice.

PHARMACOLOGICAL EFFECTS OF CALCIUM DOBESILATE

Effect of calcium dobesilate on platelet aggregation

Platelet-active drugs are of potential benefit in the prevention of diabetic microangiopathy as well as other thromboembolic complications. Calcium dobesilate reduced aggregation and the release reaction induced by thrombin and collagen in rabbit platelets (Michal and Gotti, 1988). Calcium dobesilate also increased platelet cAMP levels *in vitro* and *ex vivo* probably through activation of adenylate cyclase (Michal and Gotti, 1988). In addition, this drug reduced platelet electrophoretic mobility (Heidrich *et al.*, 1983), and it inhibited, in a time- and concentration-dependent manner, platelet-activating factor (PAF) production by the EA926 endothelial cell line stimulated by thrombin (Bussolino *et al.*, 1986). The effects of PAF on the microvasculature bed of glomerular or pulmonary microcirculation suggest that PAF might play a role in microcirculation disease.

Effect of calcium dobesilate on capillary permeability

Endothelial cells constitute the major structural backbone of capillary permeability and capillary resistance. Several endogenous fac-

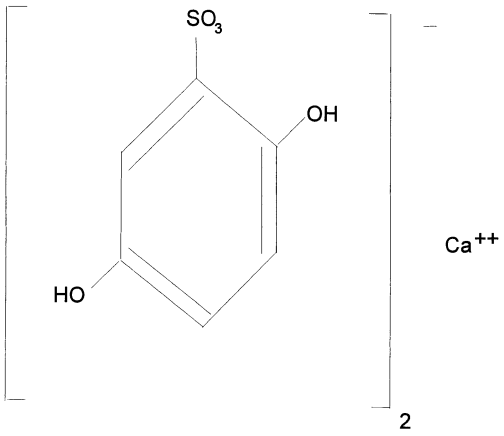

FIGURE 1. Chemical structure of calcium dobesilate.

tors, such as histamine or bradykinin, are capable of disturbing capillary function. The increase in capillary permeability would be the result of separation of endothelial cells at their boundaries. According to Beyer *et al.* (1980), the increased capillary permeability found in diabetics is inhibited by calcium dobesilate; in particular, in diabetics with or without retinopathy, calcium dobesilate increases the intravascular retention of [^{131}I]albumin, manifesting a decrease in the extravasation of this protein. Moreover, Kappert (1974) showed that calcium dobesilate blocks histamine- and bradykinin-induced hyperpermeability. Effects on capillary permeability at the retinal level have been claimed in diabetes mellitus (Binkhorst and van Bijsterveld, 1976; Vinnazer and Hachen, 1987). In diabetic patients, calcium dobesilate has been shown to stabilize blood–retinal barrier permeability (Leite *et al.*, 1990).

Effect of calcium dobesilate on blood hyperviscosity

The rheological properties of blood in diabetes mellitus have received increasing attention since Skovborg *et al.* (1966) demonstrated elevation of whole-blood viscosity in diabetics. In particular, evidence has accumulated that abnormal blood viscosity plays a role in the pathogenesis of diabetic retinopathy. Increased blood viscosity in diabetes mellitus has been attributed to changes in plasma protein composition (Barnes *et al.*, 1977; Hoare *et al.*, 1976; Hudomel *et al.*, 1977; McMillan, 1975; Skovborg *et al.*, 1966); and to increased aggregation of red blood cells (Little, 1976; McMillan, 1976; Dintenfass and Davis, 1977). The mechanism by which hyperviscosity contributes to the deterioration of the microcirculation in the retina and other organs in diabetics is that, by increasing the resistance to blood flow, it causes blood stasis, especially in the capillaries and postcapillary venules, sites where the very early lesions of diabetes microangiopathy characteristically appear. In some studies, calcium dobesilate has been shown to decrease hyperviscosity in blood (Barras and Graf, 1980; Koltringer *et al.*, 1988; Vojnikovic, 1984, 1991), perhaps through a fibrinogen-lowering effect (Barras and Graf, 1980; Salama *et al.*, 1985; Vinnazzer and Hachen, 1987).

Effect of calcium dobesilate on chronic venous insufficiency

Chronic venous insufficiency (CVI) of the lower limbs is often due to weakness of the venous wall, which, together with the accompanying valvular insufficiency, is responsible for changes in venous hemodynamics (Widmer *et al.*, 1990). These changes are characterized by an increase in hydrostatic pressure and by venous stasis and dilation, which, in turn, affects the microcirculation (Widmer *et al.*, 1990), leading to ankle and calf edemas and symptoms such as pain, day or night cramps or both, discomfort, paresthesia and heavy or restless legs or both. Royle (1988) showed, in a double-blind trial, that calcium dobesilate resulted in significant improvements in the measured volume of the foot and lower limb, in clinical tenderness and in most of the symptoms (including feelings of heaviness, swelling, tiredness and aching in the lower strumitis) in patients with CVI. Even though the CVI had existed for an average of 15 years, a relatively short course of the drug caused considerable and significant improvements in a number of signs and symptoms of the disease. In another double-blind placebo-controlled multicenter study (Widmer *et al.*, 1990), the results showed that, at the end of the trial, all the examined parameters (leg edema, pain, day and night cramps, discomfort, heavy legs, paresthesia and restless legs) were significantly more improved in the calcium dobesilate-treated group than in the placebo group. Similar results were reported by Hachen and Lorenz (1982).

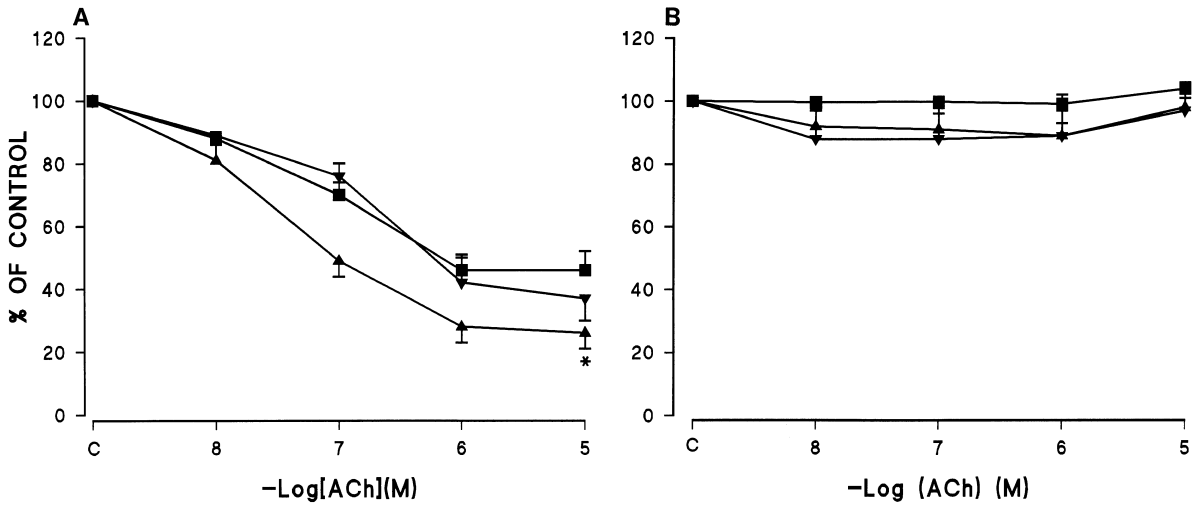

FIGURE 2. Effect of calcium dobesilate (DOBE) on endothelium-dependent relaxation induced by acetylcholine (ACh; 10^{-8}–10^{-5} M) in (A) arteries plus endothelium or (B) arteries minus endothelium. (■) control, (▲) DOBE (10^{-5} M), (▼) DOBE (10^{-4} M). Each point shows the mean±SEM of seven to nine experiments. *$P<0.05$.

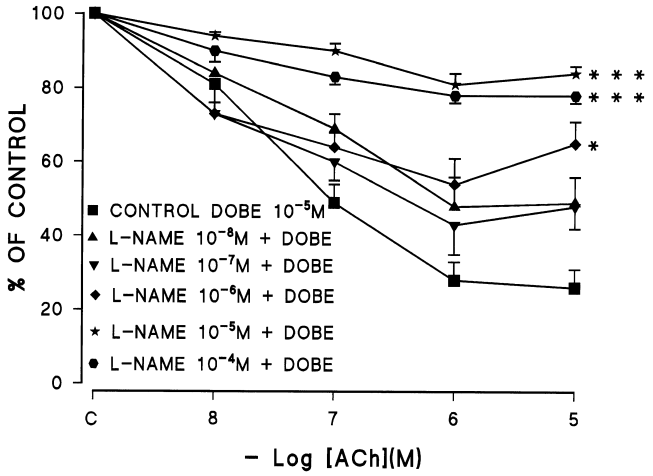

FIGURE 3. Effect of addition of increasing concentration of L-NAME (10^{-8}–10^{-4} M) on endothelium-dependent relaxation induced by acetylcholine [in the presence of calcium dobesilate (DOBE), 10^{-5} M]. (■) control DOBE (10^{-5} M), (▲) L-NAME (10^{-8} M), (▼) L-NAME (10^{-7} M), (◆) L-NAME (10^{-6} M), (★) L-NAME (10^{-5} M), (●) L-NAME (10^{-4} M). Each point shows the mean±SEM of seven to nine experiments. *P<0.05; ***P<0.001.

Effect of calcium dobesilate on vascular reactivity

It has been suggested that some of the cardiovascular disturbances that are known to occur in patients with diabetes mellitus are a consequence of alterations in the reactivity of blood vessels to neurotransmitters and circulating hormones (Weidman *et al.*, 1979). Thus, MacLeod and McNeill (1985) demonstrated that contractile responses of the aorta and mesenteric arteries from rats with streptozotocin-induced diabetes to norepinephrine were enhanced, possibly owing to a decrease in the spontaneous release of endothelium-derived relaxing factor (EDRF) resulting from damage to the endothelial cells (Harris and MacLeod, 1988). On the other hand, there are controversies about endothelium-dependent relaxation in arteries from diabetic animals. Wakabayashi *et al.* (1987) reported that endothelium-dependent relaxation of aortas in response to acetylcholine was unaffected by the diabetic state. In contrast, Oyama *et al.* (1986) reported that endothelium-dependent relaxation of diabetic aortas was attenuated in relation to control. Furthermore, McVeigh *et al.* (1992) demonstrated an abnormal relaxation to acetylcholine in the forearm of subjects with non-insulin-dependent (type II) diabetes mellitus. A reduction in basal and stimulated nitric oxide (NO) synthesis in patients with insulin-dependent diabetes associated with microalbuminuria also has been demonstrated (Elliot *et al.*, 1992).

Previous reports showed that endothelium-dependent relaxation of diabetic vasculature is more sensitive to free radical-induced injury (Pieper and Gross, 1988). The abnormal endothelium-dependent relaxation in aorta from diabetic rabbit was restored to normal by superoxide dismutase, suggesting a role for superoxide anions in the endothelial cell abnormality caused by diabetes mellitus (Tesfamarian and Cohen, 1992).

In a previous study carried out in our laboratory (Ruiz *et al.*, 1997), we found that calcium dobesilate enhanced the endothelium-dependent relaxation induced by acetylcholine in rabbit isolated aorta artery (Fig. 2A). This effect was clearly endothelium dependent because, when the endothelium is removed, the effect disappears (Fig. 2B). It seems to be likely that calcium dobesilate acts on EDRF (NO).

As shown in Figure 3, the effect of calcium dobesilate was inhibited when the rings were incubated with increasing concentrations of the NO-synthase inhibitor L-NAME, and this effect was reversed with L-Arg, the substrate in NO synthesis (Fig. 4).

Supporting these data, Kold-Bachofen and Suschek (1995) showed that, in endothelial cells in culture, obtained from normal Wistar rats as well as from BB rats (an animal model of spontaneous diabetes type I), magnesium dobesilate led to a concentration-dependent and significant increase in NO synthase (eNOS) activity; however, magnesium dobesilate treatment revealed no significant increase in cellular NOS-specific mRNA contents. The study also showed that dobesilate-medicated enhancement of NO synthesis in resting endothelial cells was not due to iNOS induction.

On the other hand, calcium dobesilate showed an *in vitro* scavenger effect on oxygen-derived free radicals (Garay *et al.*, 1995; Ruiz *et al.*, 1997) (Fig. 5). This effect could be an additional mechanism

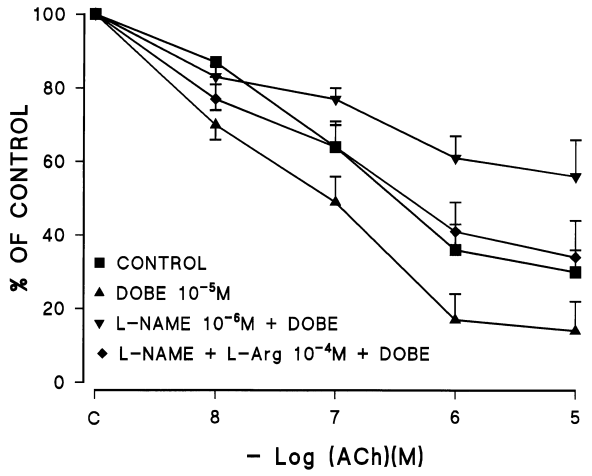

FIGURE 4. Effect of addition of L-NAME or L-NAME plus L-arginine on endothelium-dependent relaxation induced by acetylcholine [in the presence of calcium dobesilate (DOBE), 10^{-5} M]. (■) control, (▲) DOBE (10^{-5} M), (▼) L-NAME (10^{-6} M)+DOBE (10^{-5} M), (◆) L-NAME (10^{-6} M)+DOBE (10^{-5} M)+L-Arg (10^{-4} M). Each point shows the mean±SEM of seven to nine experiments.

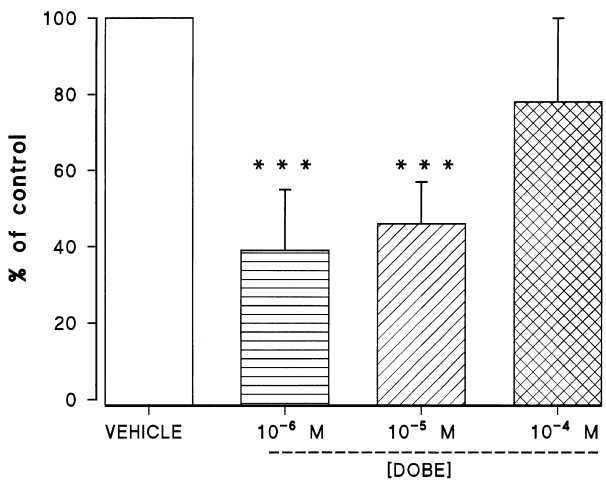

FIGURE 5. Scavenger effect of calcium dobesilate (DOBE). Each bar shows the percentage of inhibition of the nitro blue tetrazolium reduction (triplicated experiments). ***P<0.001.

implicated in the action of calcium dobesilate on the vascular complication that arises in diabetes mellitus. Previous reports have shown that endothelium-dependent relaxation of diabetic vasculature is more sensitive to free radical-induced injury (Pieper and Gross, 1988). The abnormal endothelium-dependent relaxation in aorta from diabetic rabbits was restored to normal by superoxide dismutase (Ruiz *et al*., 1997), which suggests a role for superoxide anions in the endothelial cell abnormality caused by diabetes mellitus (Tesfamariam and Cohen, 1992).

FUTURE APPROACHES

Calcium dobesilate exhibits an interesting pharmacological profile, even if only its effects on capillary permeability, platelet aggregation and blood viscosity are considered. More recent data, by us and others, showing that this drug is able to influence the vasoregulatory endothelial function through an effect on the NO system and that it may even decrease endothelial cell proliferation, open new avenues of basic and clinical research and give further value to an already quite interesting drug.

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