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Dobesilate is an Angiogenesis Inhibitor

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Abstract: Aberrant angiogenesis is essential for the progression of solid tumors and hematological malignancies. Antiangiogenic therapy is one of the most promising approaches to treat such diseases. Dobesilate is an oral agent for treatment of vascular complications of diabetic retinopathy. We have examined the possibility that this compound could interfere with the process of angiogenesis in a mouse gelatine sponge assay using acidic fibroblast growth factor (aFGF) as an inducer of neovascularization. According to the results reported here, dobesilate remarkably reduced vessel ingrowth in aFGF-containing subcutaneous sponges in mice. These findings suggest that dobesilate could be an effective agent in the treatment of angiogenesis-dependent diseases involving FGFs.

Key words: Dobesilate, Fibroblast growth factor, Angiogenesis, Mouse gelatine sponge assay.

Introduction

Angiogenesis is a sequence of events that is essential for a broad array of physiological events, including embryogenesis, menstrual cycle and wound healing. Angiogenesis is also involved in pathological situations such as tumor growth, atherosclerosis, diabetic retinopathy, chronic inflammation, and endometriosis [1, 2]. The use of angiostatic agents seems a new therapeutic option for some of these pathological processes [3, 4]. Under both physiological and disease-associated conditions, angiogenesis is a complex multistep process involving an orchestrated interaction of endothelial cells and pericytes with both the extracellular matrix and soluble angiogenic factors. Angiogenesis is controlled by a counteracting balance between antiangiogenic and proangiogenic factors [3], which suggests that inhibition of even a single major angiogenic promoter may cause inhibition of the process. Fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF) are major proangiogenic promoters [5, 6]. The FGFs are a group of 23 closely related peptides [7] with a paramount of biological functions on a variety of cell types. It has been repeatedly shown that the two FGF prototypes, acidic FGF (aFGF, FGF-1) and basic FGF (bFGF, FGF-2), are potent angiogenic factors *in vivo* [5].

During the multiple stages of tumor development, FGFs exert highly pleiotropic functions. They sometimes promote tumor cell proliferation in an autocrine fashion; they can stimulate cell survival, and they very often participate in the induction of tumor angiogenesis [8, 9]. FGFs interact with certain specific tyrosine-kinase receptors (FGFRs) [10] and heparan sulfate proteoglycans (HSPGs) of the cell surface [11]. Assemblage of an HSPG/FGF/FGFR ternary complex triggers mitogenesis, an initial step of angiogenesis [12]. To date, much effort has been directed toward the discovery of antiangiogenic agents and the evaluation of their therapeutic applications [1]. Calcium dobesilate is a drug commonly used in the treatment of diabetic retinopathy and chronic venous insufficiency [13]. Although the mechanism of action of dobesilate is as yet unclear, the pharmacology of dobesilate reveals its ability to decrease capillary permeability, as well as platelet aggregation and blood viscosity [14]. Recent experimental data suggest that antiangiogenesis can be involved in the therapeutic benefit of calcium dobesilate in diabetic retinopathy [15, 16]. Furthermore, topical dobesilate treatment showed efficacy to clear basal cell carcinoma (BCC) [17], an epidermal angiogenesis-dependent disease characterized by an overexpression of FGF [18].The above findings led us to test calcium dobesilate for its effects on vascular proliferation using a subcutaneous sponge assay for angiogenesis [19].

Material and Methods

Chemicals: Calcium dobesilate (Doxium®; calcium 2,5-dihydroxybenzene sulfonate) was provided by Laboratorios Dr. Esteve (Barcelona, Spain). Its chemical structure is shown in Fig. 1. aFGF used in this study was synthesized and purified as described [20].
Animals: Pathogen-free C57/BL/6 mice (Charles River) weighing 30 ± 6g were used. The animals were housed in plastic cages under temperature- and humidity-controlled conditions; food and water were available ad libitum, and a 12-h light/dark schedule was maintained. The animal welfare guidelines of the National Institute of Health and the European Union were followed.
Angiogenesis assay: Sterile gelatine sponge cubes of 1mm^3 (Curaspon Dental, Clinimed Holding, Zwanenburg, The Netherlands) were implanted subcutaneously in the backs of the mice after induction of intraperitoneal anesthesia as described [21]. The animals were divided into four groups. In group A (n = 10) the im-

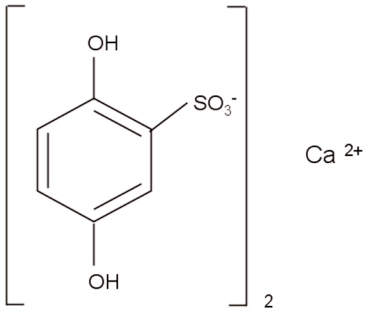

Fig. 1. Chemical structure of calcium dobesilate.

planted sponges were embedded with 200µl of phosphate-buffered saline (PBS) containing 29µg/ml heparin. In group B (n = 12), the solution embedding the sponges contained, in addition, 10µg/ml aFGF. In group C (n = 12), the sponges were embedded with the same solution as that in Group B plus 50µM calcium dobesilate. The concentration of dobesilate used in these studies is the same which elicits the highest inhibitory effect of the proliferation of glioma cell cultures [22]. After implantation of the sponges into the subcutaneous pouch, the skin was sutured. All procedures were performed under sterile conditions. For angiogenesis evaluation, the mice were re-anesthetized as described 7 days after the implants, and the sponges were surgically extracted and fixed in situ. Neovascularization was assessed in deparaffined microtome sections stained with hematoxylin and eosin and quantified with computerized morphometric software connected to a microscope. Ingrowth of neovessels into sponges was assessed by measuring the surface area with erythrocyte content. Neovascularization was analyzed in four predetermined visual fields in three different sections at 10-fold magnification.

Statistical analysis: Results were expressed as mean ± standard error of the mean (S.E.M.) of n determination. A Student's t-test for unpaired samples was used for statistical analysis. Significant differences were accepted for $p<0.05$.

Results

An evaluation of the *in vivo* relevance of dobesilate as an angiogenesis inhibitor was carried out using a standard mouse angiogenesis assay [19]. Figure 2 illustrates the inhibitory effect of dobesilate on neovascularization induced by aFGF in gelatine sponges subcutaneously implanted in mice. No new capillaries were

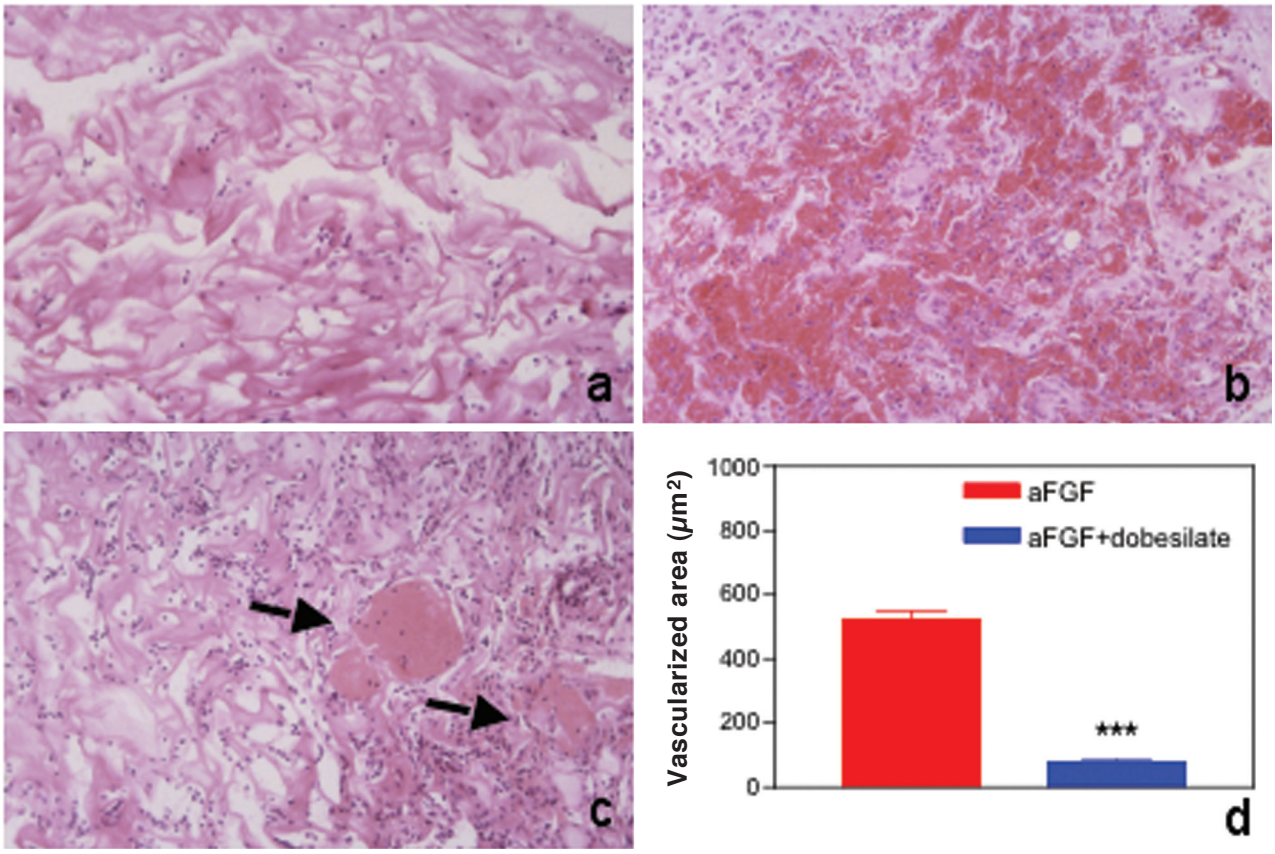

Fig. 2. In vivo antiangiogenic effect of dobesilate in the mouse gelatine sponge assay. Representative hematoxylin and eosin histological sections, at day 7, of sponge implants embedded in PBS (a), in PBS plus aFGF (b) and in PBS plus aFGF and dobesilate (c). Note the absence of neovessels in sponges embedded with PBS (a) and the decreased angiogenesis in sponges containing dobesilate (c) in comparison to sponges embedded with aFGF alone (b). The arrows in c indicate vessels plugged with erythrocytes. The exuberant cell infiltration depicted in (b) decreased significantly in sponge implants embedded with aFGF and dobesilate. Original magnification x 50. Quantification of the angiogenesis was performed by calculating the area covered by erythrocytes (d).

found in sponges loaded with PBS alone (Fig 2a), whereas sponges containing aFGF were infiltrated by many new blood vessels and inflammatory cells (Fig 2b). In sponges embedded with aFGF plus dobesilate, neovessels were scattered and slightly more inflammatory cells were present (Fig. 2c). Equivalent results were obtained when aFGF was substituted by bFGF and also when calcium dobesilate was substituted by magnesium or potassium dobesilate (not shown). A quantitative analysis, based on a computation of the areas containing erythrocytes in animals of groups B and C, respectively (Fig. 2d), shows that dobesilate decreases angiogenesis by 85.28% [$p<0.0001$; unpaired Student' t-test]. The angiostatic agent dobesilate did not appear to affect the overall health of the mice, as the body weights of the mice in the various treatment groups were not significantly different. The behavior of the mice during treatment was also normal.

Discussion

New blood vessel formation (angiogenesis) is a fundamental event in the process of tumor growth and metastatic dissemination. FGF, which has the potential to induce other angiogenic factors including VEGF [for review see, ref. 23] and hepatocyte growth factor (HGF) [24-26] that also play an important role in tumorigenesis [27], is likely to be a main member of the "angiogenic orchestra" [23]. Furthermore, since FGF exhibits a synergistic effect with VEGF it is conceivable that both FGF and VEGF are required for the switch to the angiogenic phenotype during tumorigenesis [28, 29]. Thus, therapeutic approaches that are based on the inhibition of FGF function may allow, indirectly, the simultaneous targeting of different angiogenesis factors. Moreover, such treatment may boost the therapeutic inhibition of other growth factors in cases in which these growth factors are expressed and act in a synergistic manner. This well-established role of FGF in promoting tumor angiogenesis, and in the pathogenesis of human cancers, has led to the rational design and development of low molecular mass agents that selectively inhibit FGF activity. A recent list of compounds showing such inhibitory activity includes some naphthalenesulfonate derivatives [21, 30], a short peptide domain of platelet factor 4 (PF-4$^{47-70}$) [31] and semisynthetic heparins [32]. The aim of the study reported here was to evaluate in a mouse sponge model of angiogenesis whether dobesilate could be included in that list of low molecular mass inhibitors of FGFs. Our data demonstrate that dobesilate effectively interferes with FGF-induced angiogenesis, in agreement with previous reports which could suggest an antiangiogenic effect of this compound [15, 16]. Furthermore, a previous report also shows a proapoptotic activity of dobesilate towards glioma cells, whose viability autocrinely depends on FGF [22]. Recently it has been reported that dobesilate inhibits rat choroidal angiogenesis in vitro through, at least in part, inhibition of VEGF via antioxidant properties of dobesilate [16]. Our study suggests that the antiangiogenic effects of dobesilate could be mediated, at least in part, by the inhibition of FGF. One of the most serious clinical side effects associated with the prolonged use of antiangiogenic therapy is the frequency of thrombotic events observed in several clinical trials [27, 33]. In this context, dobesilate, by its antithrombotic activity could be a safe and advantageous angiostatic agent.

Since, as demonstrated in this study, dobesilate interferes with the process of angiogenesis, this compound seems a very promising lead for further developments in the treatment of cancer and other angiogenesis-dependent diseases. Work is in progress to analyze the structural relationship between dobesilate and FGF and FGFR.

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