



THE EFFECTS OF PARICALCITOL ON HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS ARE DEPENDENT UPON VITAMIN D RECEPTOR GENE POLYMORPHISMS

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Introduction

All the components of the vitamin D axis are known to participate in the pathogenesis of chronic kidney disease (CKD), and this knowledge can be exploited to devise therapeutic strategies targeting specific component of the axis. The vitamin D axis includes vitamin D, the polymorphic vitamin D receptor (VDR), and the vitamin D-binding protein (also known as G α -globulin) that is the precursor of the vitamin D-binding protein-derived Macrophage Activating Factor termed GcMAF/ DBP-MAF probably the most potent macrophage activator. Vitamin D and the non-hypercalcemic VDR agonists (such as paricalcitol) exert their effects through interaction with VDR, a ligand-activated nuclear receptor coded for by a gene harbouring polymorphisms associated with bone turnover and density, and with several diseases, including diabetes, cancer, cardiovascular disease and AIDS as well as with conditions characterized by chronic inflammation. DBP-MAF eradicates advanced human cancer and HIV infection. In this study we investigated the effects of paricalcitol on basal and stimulated angiogenesis in the chick embryo chorioallantoic membrane (CAM) assay. We also evaluated the association between *Bsm1* polymorphism of the VDR gene and the response of PBMCs to paricalcitol. In addition, since the cAMP signal transduction pathway induced by vitamin D and GcMAF received little attention so far, and in order to establish the role of VDR genotypes in modulating cAMP formation, we investigated cAMP formation in human PBMCs from donors with different *Bsm1* polymorphisms.

Materials & Methods

The effects of paricalcitol (kindly donated by Abbot) and DBP-MAF (kindly donated by gcmf.eu) were studied on human peripheral blood mononuclear cells (PBMCs) collected from healthy volunteers, and on the human acute monocytic leukemia-derived monocytes/macrophages cell line Mono Mac 6 (MM6). Assessment of cell viability was determined by Calbiochem Rapid Cell Proliferation Kit. cAMP levels were measured by a competitive EIA assay. VDR polymorphisms were determined by the absence or presence of the *Bsm1* restriction site and were denominated **B** and **b** respectively. Angiogenesis was studied in the chick embryo chorioallantoic membrane (CAM) assay.

Results

Effects of paricalcitol and DBP-MAF on cAMP production in human PBMCs of subjects harbouring different *Bsm1* alleles of the VDR gene.

Intracellular cAMP formation in response to paricalcitol was highest in the homozygous *bb* genotype whereas it was not significant in the homozygous *BB* genotype. In the heterozygous *Bb* genotype, only the highest paricalcitol concentration (100 nM) elicited a statistically significant response in terms of cAMP formation (Fig. 1). DBP-MAF significantly stimulated cAMP formation in a dose-dependent manner. Intracellular cAMP formation was highest in the homozygous *bb* genotype whereas it was not significant in the homozygous *BB* genotype. In the heterozygous *Bb* genotype, the response was intermediate and the lowest concentration (0.01 ng/ml) did not elicit a statistically significant response in terms of cAMP formation.

Effects of paricalcitol and DBP-MAF on human PBMC viability in subjects harbouring different *Bsm1* alleles

Vitamin D inhibits macrophage function and viability and these effects are mediated by interaction with the VDR. DBP-MAF, on the contrary, has a potent mitogenic capacity to act on the macrophage progenitor cells (1). When we challenged PBMCs from subjects harbouring different *Bsm1* polymorphisms, we observed that the *b* allele of the VDR gene and the *bb* genotype were associated with the highest inhibition of PBMCs viability by paricalcitol and with the highest stimulation of proliferation by DBP-MAF (Fig. 2). The highest concentration of paricalcitol (100 nM) showed some (albeit non statistically significant) inhibitory effect on the heterozygous *Bb* genotype. Heterozygous subjects (*i.e.* *Bb*) also showed an intermediate response to DBP-MAF. In the homozygous *BB* genotype none of the concentrations of paricalcitol or DBP-MAF showed any significant effect. These data, together with those obtained measuring cAMP formation, demonstrate an association between the presence of *b* alleles and the degree of the response to paricalcitol and DBP-MAF.

Effects of paricalcitol and DBP-MAF on the human acute monocytic leukemia-derived monocytes/macrophages cell line Mono Mac 6

Here we demonstrate for the first time that paricalcitol and DBP-MAF inhibited proliferation of the human acute monocytic leukemia-derived monocytes/macrophages cell line MM6. Determined MM6 VDR genotype was *Bb*. Fig. 3 shows dose-dependent inhibition of MM6 proliferation by DBP-MAF and paricalcitol. The minimal DBP-MAF concentration required to inhibit MM6 proliferation was 40 ng/ml, whereas paricalcitol was effective at 240 nM. When administered together, the effect of the two compounds was additive. It is worth noting that the inhibiting concentration of DBP-MAF was similar to that reported by Gregory *et al.* in LnCaP prostate cancer cells (2). Inhibition of MM6 proliferation is consistent with the known effects of DBP-MAF and VDR agonists on monocyte differentiation. It is known that monocytes/macrophages activated by GcMAF immediately block DNA synthesis and rapidly differentiated (1), and 1,25-dihydroxyvitamin D3 directs monocytic maturation of normal and leukemic cells (3). Inhibition of leukemic cell proliferation suggests that DBP-MAF might be effective in the treatment of human leukemia, and it is consistent with the dramatic effects of DBP-MAF in eradicating a variety of human advanced cancers (4-6). Considering that kidney involvement is frequent in hematologic malignancies and it is associated with adverse outcome and treatment difficulties (7), we believe that these effects of paricalcitol and DBP-MAF could be of particular interest for the nephrologist and the oncologist as well.

Effects of paricalcitol and DBP-MAF on angiogenesis

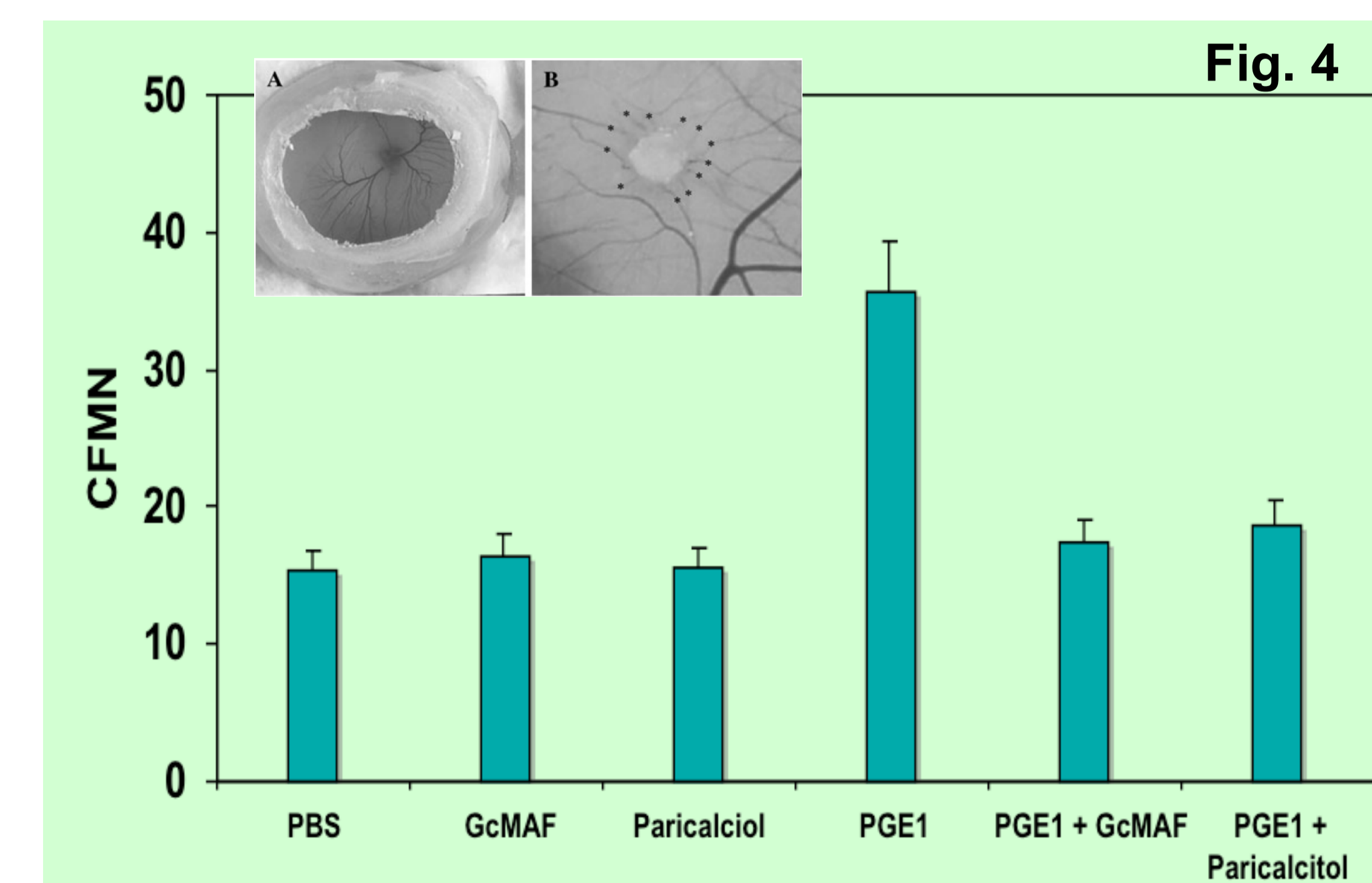
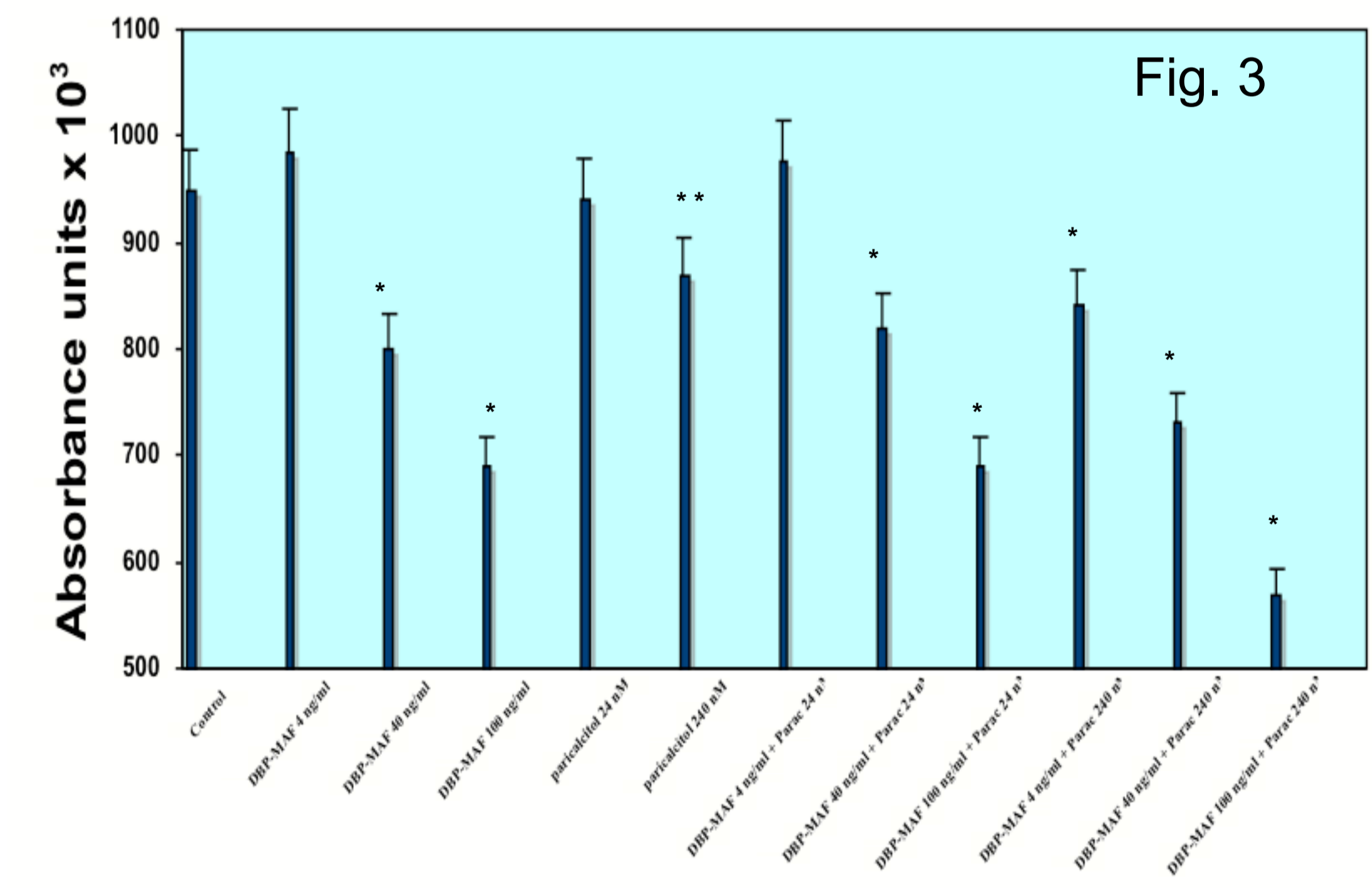
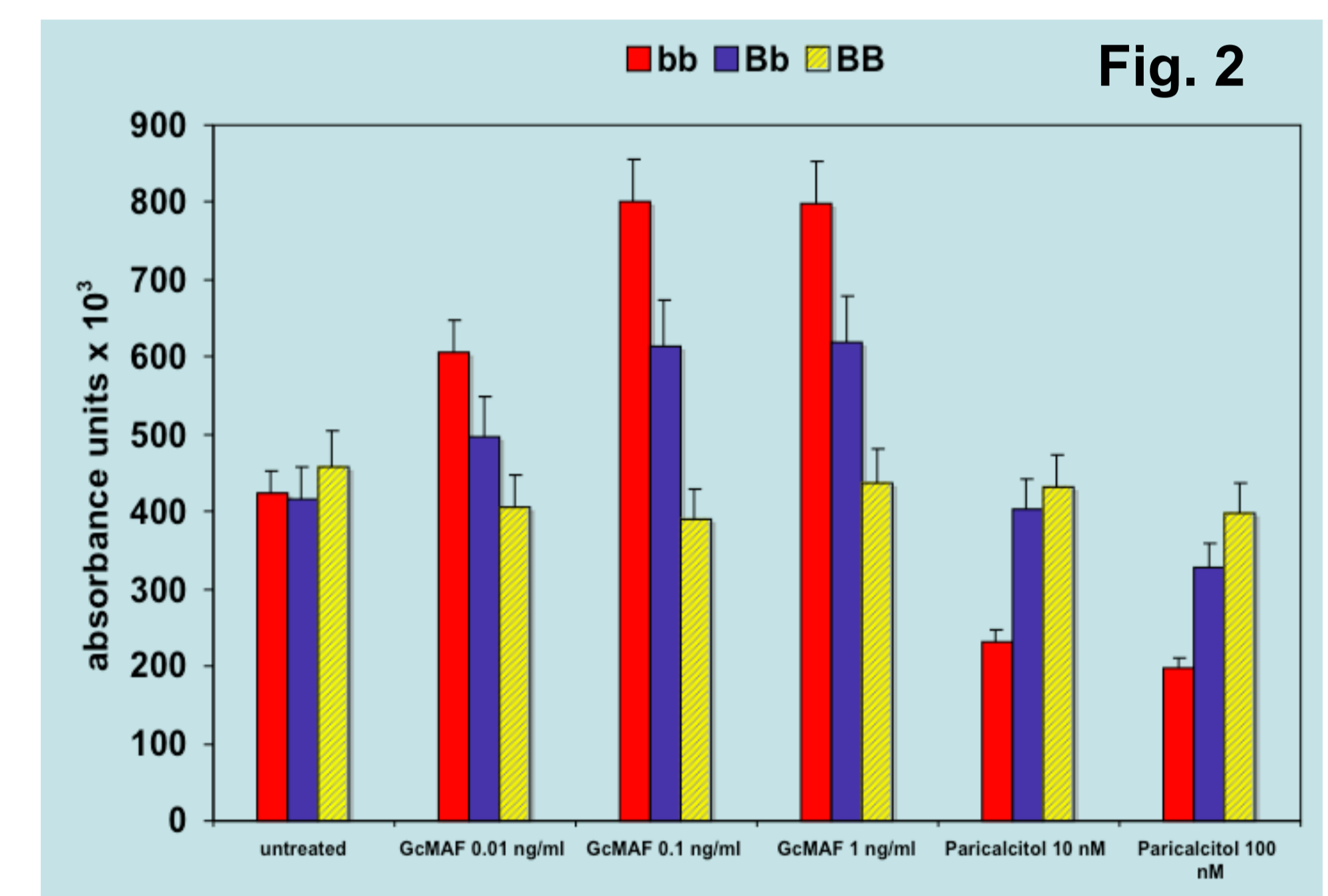
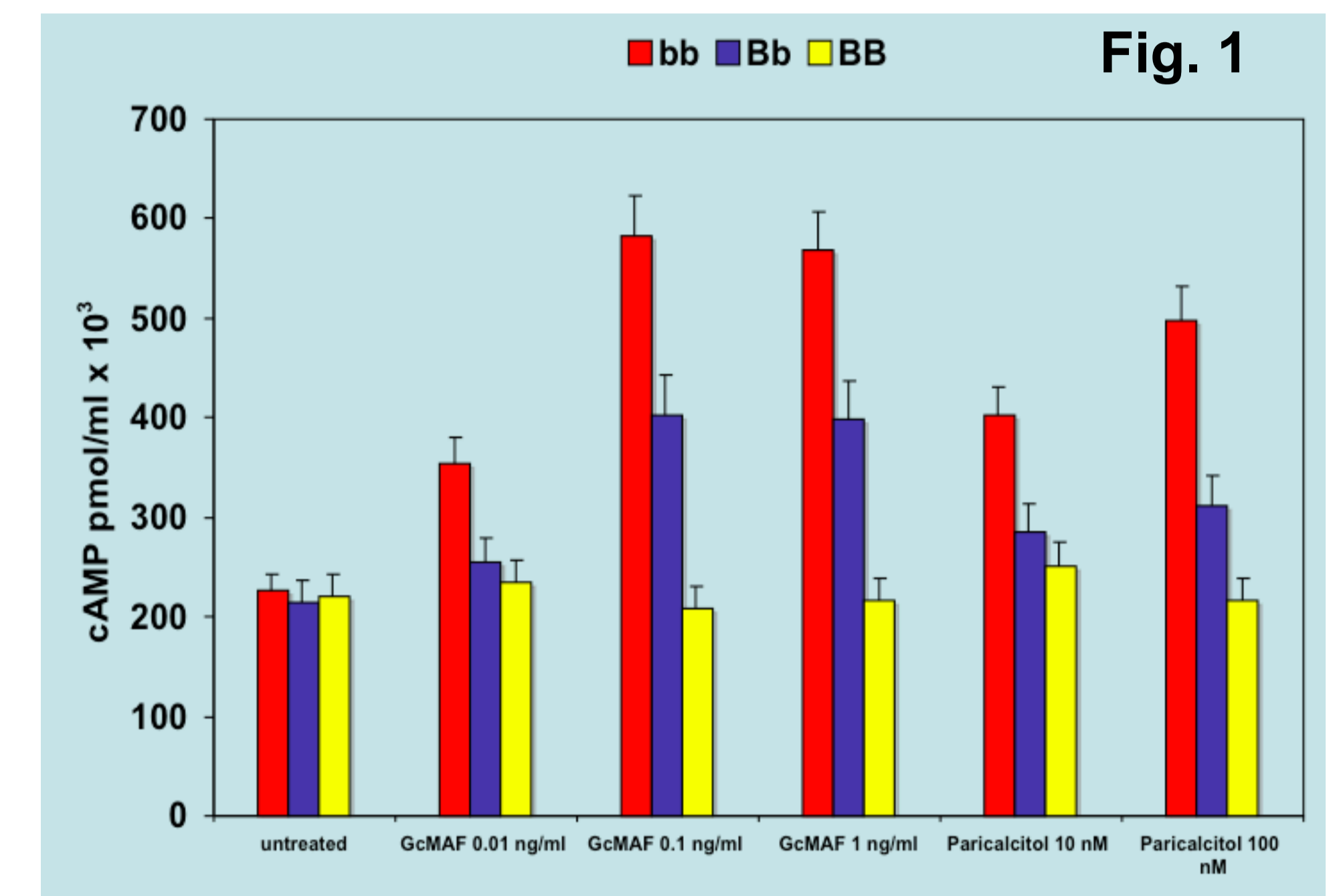
The relationship between angiogenesis and CKD is a complex one. On one side, damage of the renal microcirculation and deterioration of the angiogenic response are considered early steps in the pathway leading to progressive renal injury and there is evidence that stimulation of angiogenesis may stabilize renal function and slow the progression of CKD (8). On the other side, there is considerable evidence to suggest that angiogenesis and chronic inflammation are co-dependent (9), and it is well assessed that inflammation is a significant risk factor in CKD (10). In order to evaluate the effects of paricalcitol and those of DBP-MAF on angiogenic signaling pathways, we tested their effects in basal conditions and when administered together with powerful stimulator of angiogenesis *i.e.* PGE1, (1 mg/ml). None of the compounds altered basal angiogenesis (Fig. 4) or chick embryo development (not shown). As expected, PGE1, (1 mg/ml) strongly stimulated angiogenesis as previously described (11). Blood vessels with an irregular course and frequent branching were present at day 12; the gelatine sponges were surrounded by allantoic vessels that developed radially towards the implant in a *spoked-wheel* pattern. Tortuous vessels infiltrated the sponges and often modified their shape. Fig. 4, shows that paricalcitol (100 nM) and DBP-MAF (1 ng/ml) completely inhibited the angiogenesis induced by PGE1. These results suggest that paricalcitol and DBP-MAF could be used to control deregulated angiogenesis in CKD.

Conclusions

Our data demonstrate that paricalcitol and DBP-MAF exerts multiple effects on normal and transformed blood mono-nucleated cells; these effects are associated with VDR polymorphisms and determination of VDR polymorphisms could be used to preventively assess individual responsiveness to paricalcitol or DBP-MAF. While the association between VDR polymorphisms and responsiveness to VDR agonists such as paricalcitol is easily conceivable, the association between VDR polymorphisms and responsiveness to DBP-MAF is not straightforward. Since vitamin D binds to domain I of VDBP (*i.e.* the precursor of DBP-MAF), an interaction between the VDR localized on the cell membrane (12), and the DBP-MAF receptor cannot be ruled out. Alternatively, VDR polymorphisms could be simply associated (*i.e.* without a cause and effect relationship) with the response to DBP-MAF as it occurs in a variety of conditions, from cancer (13) to AIDS (14) and CKD (15). Finally, our data together with those presented in *J Med Virol* (16), provide experimental evidence for the words *Our immune system will get rid of the virus within a few weeks, if you have a good immune system*, thus reversing the long-assumed cause-effect relationship between HIV and AIDS.

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