Vanadate and Bone Metabolism: Effect on Proliferation and Mineralization of Fish Bone Derived Cells

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Summary

Vanadate is known for mimicking insulin action through activation of insulin and/or insulin like growth factor 1 (IGF 1) receptors. Vanadate insulin-like effect on bone-related metabolism has been previously investigated using mammalian *in vitro* cell systems but other vertebrate systems have rarely been used. We have recently demonstrated the suitability of a fish bone derived cell line (VSa13) to study anti-mineralogenic effects of vanadate. Here, we propose that vanadate stimulation of cell proliferation involves MAPK signalling pathway and IGF 1 receptor activation, while impairment of extracellular matrix (ECM) mineralization is likely to involve both MAPK and PI 3K pathways and insulin receptor activation.

Introduction

Vanadium is recognized as an important trace element in living organisms (1,2). In mammals, one of its key features is its ability to mimic insulin and IGF-1 (3,4), demonstrated *in vivo* using streptozocin-treated rats (5,6) and *in vitro* using adipocyte cultures (7,8). Insulin-like action of vanadium (in particular vanadate) has been associated with inhibition of protein tyrosine phosphatases (PTPases) and consequent activation of tyrosine kinase receptors (9) such as those for insulin and IGF 1. Deprivation of vanadium in goat diet induces severe bone malformation (10), suggesting its requirement for specific growth factor-dependent signalling. In the last decade, effects on bone metabolism were investigated using mouse bone derived cell lines (11) and more recently the fish bone derived cell line VSa13 (12,13) where vanadium compounds and salts have been shown to: a) down regulate alkaline

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phosphatase and PTPases activity, b) stimulate type I collagen synthesis and cell proliferation, c) impair ECM mineralization, and d) activate insulin and IGF-1 signalling pathways. Data related to vanadium effects on bone metabolism are however still controversial. In this paper, proliferative and anti-mineralogic effects of vanadate were investigated *in vitro* using VSa13 cells, then compared to those of insulin and IGF-1, and finally characterized in the presence of known inhibitors of mitogen activated protein kinase (MAPK) and phosphatidylinositol-3 kinase (PI 3K) pathways.

Materials and methods

VSa13 cells were maintained in DMEM as previously described (13,14); Vanadate solutions were prepared and characterized as described by Tiago and colleagues (13). Bovine insulin peptide (Sigma Aldrich) solution was prepared at 10 µM in pH 2.0 milliQ water. *Pagrus auratus* IGF 1 mature peptide (Novozymes Gropep) solution was prepared at 0.1 µg/µl in milliQ water. PD98059 and wortmannin solutions were prepared in dimethyl sulfoxide at 6 and 10 mg/ml, respectively; Proliferation of VSa13 cells was determined using CellTiter 96 non radioactive proliferation assay kit (13); Mineral deposition was determined by von Kossa staining and quantified using densitometric method (13).

Results and discussion

Dividing VSa13 cells were untreated or treated with 0.1 µM wortmannin (PI-3K pathway inhibitor), 100 µM PD98059 (MAPK pathway inhibitor), and/

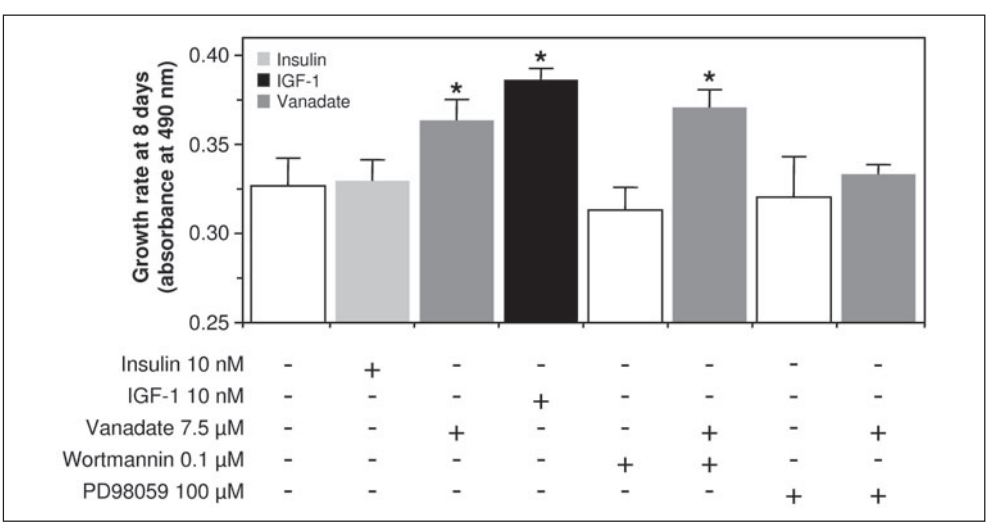

Figure 1: Effect of vanadate, insulin, IGF 1, wortmannin and PD98059 on VSa13 cell proliferation (data from at least 3 independent experiments; asterisk = statistically different from controls, P*<0.05, one-way ANOVA).*

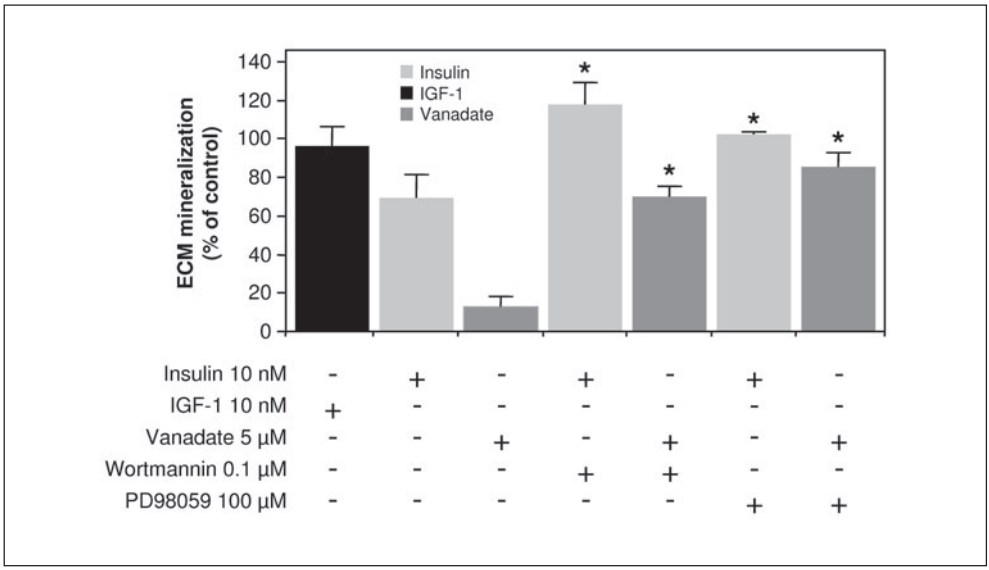

Figure 2: Effect of vanadate, insulin, IGF 1, wortmannin and PD98059 on VSa13 ECM mineralization (data from at least 3 independent experiments; asterisk = statistically different from controls, P*<0.05, one-way ANOVA).*

or 7.5 µM metavanadate. In separated experiments, cells were also treated with 10 nM insulin or IGF 1. Vanadate stimulated VSa13 cell proliferation in a PD98059-dependent manner, suggesting the involvement of MAPK pathway (Figure 1). Comparable effects were observed in mammals using TreVO-treated cells (15), suggesting similar mechanisms in fish and mammals. Vanadate proliferative effect was comparable to that induced by IGF 1, but not by insulin, in agreement with the higher capacity of IGF 1 receptor (compared to IR) to activate MAPK pathway in mammals (16).

Mineralizing VSa13 cells were treated as above but for a longer time (4 weeks). Insulin and vanadate, but not IGF 1, inhibited ECM mineralization by 30 and 90%, respectively (Figure 2). These anti-mineralogic effects were totally or partially (60-70%) reverted, respectively, in the presence of PD98059 or wortmannin. Involvement of both MAPK and PI 3K pathways in mediating vanadate and insulin effects in fish cells is consistent with recent observation of strong anti mineralogenic effects in MC3T3 E1 cells through PI 3K\ras\ERK pathway activation (17). IGF 1 mitogenic effects are consistent with previous observations in mammalian systems (18), but to date there is no clear association with increased *in vitro* mineral deposition, although increased bone turnover *in vivo* has been suggested (19)

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