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Short Communication

Synthesis, characterization and bioactivity of a new VO^{2+}/Aspirin complex

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Abstract

A new VO^{2+} complex with salicylic acid acetate (Aspirin) of formula $C_{18}H_{18}Cl_2O_{12}V_2$ was synthesized and characterized. Its biological effects upon cell proliferation, differentiation and promotion of tyrosine protein phosphorylation have been tested in two lines of osteoblast-like cells in culture.

Keywords: VO^{2+}/Aspirin complex; Aspirin; Bioactivity

1. Introduction

Vanadium is a trace transition metal with relevant biological properties [1]. For instance, it can pharmacologically regulate the glucose level in the blood of diabetic animals [2,3] and patients [4,5]. It can interact with different biomolecules in its anionic and cationic forms [6]. Although the precise mechanisms of action are not completely known, it has been suggested that vanadium inhibits protein tyrosine phosphatases (PTPases) and thus regulates the level of protein tyrosine phosphorylation [7]. In this study we report the synthesis and physicochemical characterization of a new complex between VO^{2+} cation and Aspirin (Fig. 1), a pharmacological drug used worldwide. The bioactivity of the VO^{2+}/Aspirin complex has been tested on bone-related cells in culture.

2. Results and discussion

2.1. Synthesis and physicochemical characterization of VO^{2+}/Aspirin complex

When a mixture of Aspirin and $VOCl_2$ (50% aqueous solution) in a molar ratio of 2/1 in 96% ethanol was stirred under nitrogen atmosphere (pH 3.5, 0 °C), green oil was formed. Successive washings with bidistilled water produced a light green solid. It was filtered and immediately dried in a vacuum line to avoid decomposition. Yield: 52%. *Anal*. Calc. for $C_{18}H_{18}Cl_2O_{12}V_2$ (599): C, 36.00; H, 3.00; V, 17.03; Cl, 11.85. Found: C, 36.26; H, 3.16; V, 16.95; Cl, 12.05%.

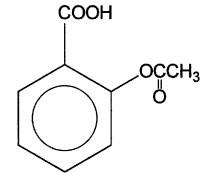

Fig. 1. Salicylic acid acetate (Aspirin).

The electronic spectrum of the $[VO(Aspirin)ClH_2O]_2$ complex in a mixture of ethanol/water (1/1) under nitrogen atmosphere shows two typical absorption bands [8] at 778 nm ($\varepsilon = 36\ M^{-1}\ cm^{-1}$) and 608 nm ($\varepsilon = 38\ M^{-1}\ cm^{-1}$). IR (KBr disk spectrum): Aspirin: 1690 (vs) cm^{-1} (carboxylic acid). VO^{2+}/Aspirin complex: 1537 (s), 1403 (vs) cm^{-1} ($\nu_{as}\ COO^-$ and $\nu_s\ COO^-$, respectively) and 988 (s) cm^{-1} (V=O stretching). The difference between the antisymmetric and symmetric stretching modes of the carboxylate group is indicative of the formation of a binuclear complex with carboxylate bridges [9–11] (see Fig. 2). The carbonyl (acetyl) stretching frequency (1754 cm^{-1}) remains unchanged upon coordination. The strong and broad

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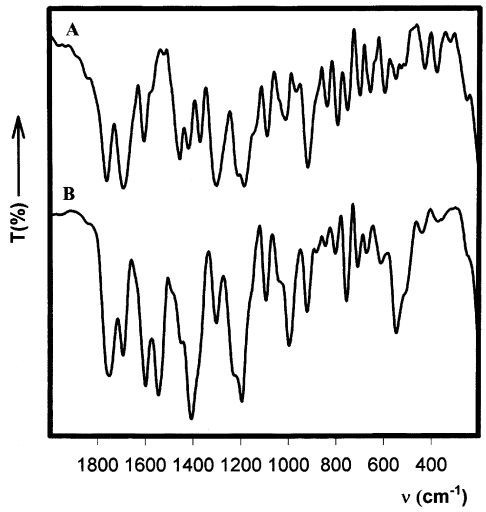

Fig. 2. IR spectra of Aspirin (A) and VO^{2+}/Aspirin complex (B). KBr pellet technique.

band at about 3500 cm^{-1} (νOH) as well as the band at 1592 cm^{-1} (δ OH_2) indicate the presence of water in the coordination sphere of the vanadium centers [11].

2.2. Bioactivity of VO^{2+}/Aspirin complex

In order to test the biological effects of vanadium compounds, UMR106 and MC3T3E1 osteoblast-like cells were cultured in DMEM without serum and with the addition of different concentrations of VO^{2+} cation or VO^{2+}/Aspirin complex (0, 10, 50, 100 μM) for 24 h at 37 °C. Then, the cells were fixed and stained with crystal violet and the dye extracted was quantitated at 540 nm [12]. As seen in Table 1, VO^{2+}/Aspirin complex showed a biphasic effect upon UMR106 cell proliferation: a stronger stimulatory effect of VO^{2+}/Aspirin complex than VO^{2+} could be observed at low concentrations (10 μM; $^{*}p < 0.02$), the complex being more cytotoxic for these cells at higher doses (50–100 μM, $^{**}p < 0.001$). These results suggest that the complex might generate a stronger mitogenic signal than vanadyl at low doses in these cells. However, at higher concentrations the cytotoxic effect predominates, probably through the induction of reactive oxygen species [13]. On the other hand, both vanadium(IV) compounds were inhibitory upon the proliferation of MC3T3E1 cells in the whole range of tested concentrations ($^{**}p < 0.002$) (Table 2).

Osteoblast cell differentiation was evaluated using alkaline phosphatase specific activity as a marker of mature osteoblastic phenotype [12]. VO^{2+}/Aspirin complex and VO^{2+} both produced a similar inhibitory effect on UMR106 cell differentiation up to 50 μM of vanadium compounds. At higher doses there was a significant difference in the inhibitory effect of the two vanadium derivatives, VO^{2+}/Aspirin being more potent than VO^{2+} (75 μM, $p < 0.001$; 100 μM, $p < 0.01$, $n = 9$). Basal activity corresponded to 540 ± 30 nmol/min/mg protein.

In addition, 10 μM VO^{2+}/Aspirin complex induced evident morphological changes in MC3T3E1 cells similar to those previously found for other vanadium compounds [14]. In general, after staining with Giemsa, the cultures displayed cytoplasm condensation, loss of processes and the cells became fusiform in shape.

To investigate the mechanism of action of vanadium compounds, the tyrosine phosphorylation pattern induced by vanadium derivatives was assessed by the Western blot method (Fig. 3). MC3T3E1 cells were incubated overnight with 50 μM of vanadium derivatives in serum-free medium. As can be seen, VO^{2+} and VO^{2+}/Aspirin induced phosphorylation in tyrosine residues of several proteins, especially of those of 26–45 kDa molecular weight, over the basal control culture. Moreover, the complex showed a characteristic pattern of four phosphorylated bands in the range of 36–45 kDa. VO^{2+} cation presented three bands in the 26–45 molecular range but with a stronger intensity, while the basal culture exhibited only two bands. Different vanadium compounds induced tyrosine phosphorylation with different patterns, as previously reported [14].

In conclusion, the present study has demonstrated that the new VO^{2+}/Aspirin complex produces biological effects on osteoblast-like cells. This compound regulates cell proliferation and differentiation in a different way than VO^{2+}. These effects are partially associated with the ability of vanadium derivatives to induce phosphorylation in tyrosine residues of proteins by inhibition of PTPases. Like other vanadium deriv-

Table 1
Effect of vanadyl (VO^{2+}) and VO^{2+}/Aspirin complex on UMR106 cell proliferation [a]

Concentration (μM)	% Basal (VO^{2+})	% Basal (VO^{2+}/Aspirin)
0	100 ± 1	100 ± 1
10	111 ± 2	118 ± 2
50	108 ± 2	98 ± 3
100	102 ± 2	87 ± 3

[a] UMR106 osteoblast-like cells were grown in media alone or with the addition of different concentrations of vanadium for 24 h. After this period, cells were fixed and stained with crystal violet and the cell proliferation evaluated by the absorbance at 540 nm. Results are expressed as mean ± SEM ($n = 9$).

Table 2
Effect of vanadyl (VO^{2+}) and VO^{2+}/Aspirin complex on MC3T3E1 cell proliferation [a]

Concentration (μM)	% Basal (VO^{2+})	% Basal (VO^{2+}/Aspirin)
0	100 ± 2	100 ± 1
10	89 ± 1	76 ± 3
50	81 ± 2	66 ± 3
100	77 ± 2	61 ± 2

[a] MC3T3E1 osteoblast-like cells were grown in media alone or with the addition of different concentrations of vanadium for 24 h. After this period, cells were fixed and stained with crystal violet and the cell proliferation evaluated by the absorbance at 540 nm. Results are expressed as mean ± SEM ($n = 9$).

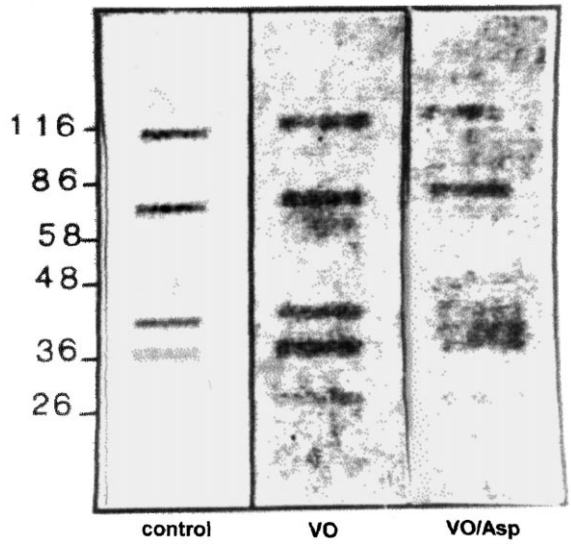

Fig. 3. Western blot. Effect of vanadium(IV) compounds on the phosphotyrosin protein pattern of MC3T3E1 cells. Osteoblasts were incubated with medium alone (lane 1) (control); vanadyl (lane 2); VO^{2+}/Aspirin complex (lane 3), at 50 μM for 24 h. After this time, proteins were separated by electrophoresis and analyzed by the Western blot method using a monoclonal anti-phosphotyrosine antibody. This figure is representative of three independent experiments.

atives, VO^{2+}/Aspirin induces morphological changes in osteoblast-like cells.

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