Three new vanadyl(IV) complexes with non-steroidal anti-inflammatory drugs (Ibuprofen, Naproxen and Tolmetin). Bioactivity on osteoblast-like cells in culture

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Abstract

The synthesis and spectral and magnetic characterization of VO^{2+} complexes with Ibuprofen (2-(4-isobutylphenyl)propionic acid), Naproxen (6-methoxy-α-methyl-2-naphthalene acetic acid) and Tolmetin (1-methyl-5-(4-methylbenzoyl)-1H-pyrrole-2-acetic acid) were studied. The complexes $[VO(Ibu)_2] \cdot 5CH_3OH$, $[VO(Nap)_2] \cdot 5CH_3OH$ and $[VO(Tol)_2]$ were obtained from methanolic solutions under nitrogen atmosphere. The biological activities of these complexes on the proliferation of two osteoblast-like cells in culture (MC3T3E1 and UMR106) were compared with that of the vanadyl(IV) cation. The complexes exhibited different effects depending on the concentration and the cellular type, while no effect was observed for their parent drugs. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Anti-inflammatory drugs; Vanadyl complexes; Spectroscopy; Osteoblast proliferation

1. Introduction

It is well known that most of the anti-inflammatory drugs are carboxylic acids in which the carboxylate group is available for metal–ligand interaction [1–3]. Ibuprofen (2-(4-isobutylphenyl)propionic acid), Naproxen (6-methoxy-α-methyl-2-naphthalene acetic acid) and Tolmetin (1-methyl-5-(4-methylbenzoyl)-1H-pyrrole-2-acetic acid) (see Fig. 1) are non-steroidal anti-inflammatory drugs (NSAIDs) that exhibit favourable anti-inflammatory, analgesic and antipyretic properties. The major clinical application of NSAIDs is their action as anti-inflammatory agents in muscle skeletal diseases [4]. There are a number of recently published papers on the effects of NSAIDs in bone-related tissues under different inflammatory conditions [5–7].

Several transition metal complexes with NSAIDs have been extensively studied since Sorenson [3] demonstrated that copper(II) complexes are more active than their parent

drugs and exhibit an anti-ulcer activity. These complexes show a lower toxicity than the inorganic forms of copper(II). On the other hand, some studies suggest that regular Ibuprofen intake may also be an effective chemopreventive agent against breast cancer and can decrease non-small cell lung cancer growth in vitro [8].

Vanadium is an ultramicrotrace bioelement with interesting biological properties [9–12]. In mammals, vanadium is
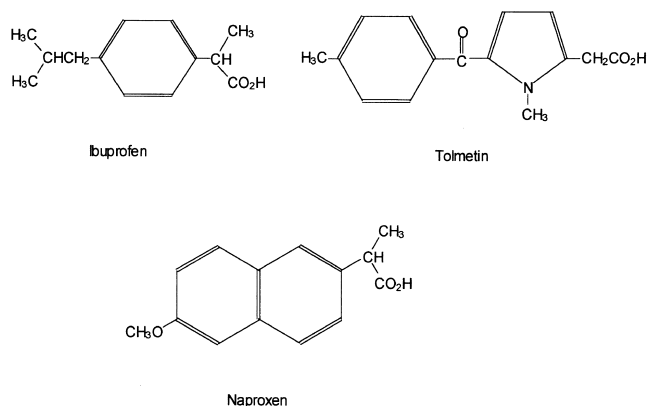

Fig. 1. Structures of Ibuprofen, Naproxen and Tolmetin.

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mainly stored in bones and kidneys [10]. For this reason it is very interesting to study the biological effects of vanadium compounds in bone-related cells. Using an in vitro model of osteoblast-like cells in culture, it has previously been shown that vanadium exerts a biphasic effect: while low concentrations stimulate, high concentrations induce inhibition of osteoblast growth and differentiation as well as morphological transformations [13–18].

We have recently reported the synthesis and bioactivity of a new vanadyl(IV)–Aspirin complex (Asp-VO) [19]. This complex seems to be more potent than uncomplexed vanadyl salt in inhibiting the growth of osteoblast-like cells and regulating cell proliferation and differentiation [19]. These effects are partially associated with the ability of vanadium derivatives to indirectly induce phosphorylation in tyrosine residues of proteins by inhibition of protein tyrosine phosphatases (PTPases). In addition, the antineoplastic effects of vanadium have been demonstrated both in vitro and in vivo models. For instance, organometallic complexes of vanadium(IV) linked to bis(cyclopentadienyl) moieties or vanadocenes [20] and oxovanadium compounds that included substituted phenanthroline and 2,2′-bipyridine ligands induce apoptosis in human cancer cells [21].

The aim of this study was the synthesis and characterization of new NSAIDs–vanadyl complexes. Moreover, the effects of these complexes on osteoblast proliferation were determined in two cell lines in culture: the non-transformed MC3T3E1 derived from mouse calvaria and the tumoral UMR106 from a rat osteosarcoma. The results were compared with those for the parent drugs.

2. Experimental

2.1. Materials and methods

Ibuprofen, Naproxen, Tolmetin (Sigma), and $VOSO_4$ (Merck) were used as supplied. Tissue culture materials were provided by Corning or Falcon. Dulbecco's modified Eagle's medium (DMEM), trypsin, fetal bovine serum (FBS) and ethylenediamine-tetraacetic acid (EDTA) were supplied by Gibco. All other chemicals used were of analytical grade.

Electronic UV–vis and diffuse reflectance spectra were recorded on a Hewlett-Packard 8453 diode-array spectrophotometer and a Shimadzu UV-300 spectrophotometer, respectively. Infrared spectra were recorded on a Perkin-Elmer 580B spectrophotometer using the KBr pellet technique. Room temperature magnetic susceptibility was determined with a Cahn-2000 balance, calibrated with $Hg[Co(SCN)_4]$ and at a magnetic field strength of 6 kG. Elemental analyses for carbon, hydrogen and nitrogen were performed using a Carlo Erba EA 1108 analyzer.

2.2. Syntheses

2.2.1. [VO(Ibu)₂]·5CH₃OH

A solution of 2 mmol of Ibuprofen in 6 ml MeOH was added to a methanolic $VOSO_4 \cdot 5H_2O$ solution (10 ml, 1 mmol) under nitrogen atmosphere. The generated precipitate Na_2SO_4 was separated by centrifugation and discarded. The violet solution was kept at $-20°C$ overnight. The violet crystals formed were not suitable for X-ray diffraction analysis and their powder diagram showed a highly amorphous character. Recrystallization procedures failed due to decomposition of the samples. Yield: 80%. Anal. Calcd. for $C_{31}H_{54}O_{10}V$: C, 58.4%; H, 8.5%. Found: C, 58.1%; H, 8.2%. Room temperature magnetic moment: 1.71 BM. $MW_{calcd.}$: 637.

2.2.2. [VO(Nap)₂]·5CH₃OH

Naproxen methanolic solution (2 mmol, 20 ml) was added to a solution of $VOSO_4 \cdot 5H_2O$ (1 mmol in 10 ml MeOH) under nitrogen atmosphere. Na_2SO_4 precipitated immediately and the suspension was maintained with constant stirring for 24 h under N_2 atmosphere. Precautions were taken to avoid alcohol evaporation. Sodium sulphate was then separated by centrifugation and the final violet solution was kept at $-20°C$ overnight. A grey powder was formed, separated by filtration and washed with cold MeOH. Yield: 60%. Anal. Calcd. for $C_{33}H_{46}O_{12}V$: C, 56.0%; H, 6.7%. Found: C, 55.6%; H, 6.4%. Room temperature magnetic moment: 1.78 BM. $MW_{calcd.}$: 685.

2.2.3. [VO(Tol)₂]

The same procedure as for Naproxen-VO was performed. The blue solution was kept at $-20°C$. A grey powder was obtained in a few days with poor yield (ca. 10%). The solid was filtered and washed with cold MeOH. Anal. Calcd. for $C_{30}H_{28}N_2O_7V$: C, 62.2%; H, 4.8%; N, 4.8%. Found: C, 62.5%; H, 5.0%; N, 4.8%. Room temperature magnetic moment: 1.99 BM. $MW_{calcd.}$: 579.

2.3. Cell culture

Rat osteosarcoma UMR106 and osteoblastic non-transformed mouse calvaria derived MC3T3E1 cells were grown in DMEM supplemented with 10% (v/v) FBS and antibiotics (100 U/ml penicillin and 100 mg/ml streptomycin) in a humidified atmosphere of 95% air/5% CO_2. Cells were grown at near-confluence (70–80%) and were subcultured using 0.1% trypsin–1 mM EDTA in Ca^{2+}–Mg^{2+}-free phosphate-buffered saline (PBS). For experiments, about 5.5×10^4 cells/well (UMR 106) and 3.3×10^4 cells/well (MC3T3E1) were plated onto 24-well plates. After the culture reached 70% confluence, the cells were washed twice with DMEM. Cells were incubated overnight with vanadium compounds at different doses in serum-free DMEM.

2.4. Cell proliferation assay

A mitogenic bioassay was carried out as described by Okajima et al. [22] with some modifications. Briefly, cells in 24-well plates were washed with PBS and fixed with 5% glutaraldehyde/PBS at room temperature for 10 min. Cells were then stained with 0.5% crystal violet/25% methanol for 10 min. The dye solution was then discarded and the plate was washed with water and dried. The dye in the cells was extracted using 0.5 ml/well 0.1 M glycine/HCl buffer, pH 3.0/30% methanol and transferred to test tubes. Absorbance was read at 540 nm after a convenient sample dilution. The correlation between cell number/well and the absorbance at 540 nm of diluted extraction sample after crystal violet staining has been established previously [14]. Data are expressed as the mean±S.E.M. Statistical differences were analyzed using Student's t-test. t-Tests were performed to compare treated cultures with the untreated cultures. The recently prepared vanadyl(IV) complexes were used to obtain fresh solutions to be added to the culture dishes at the following concentrations: 0, 2.5, 5, 10, 25, 50, 75 and 100 μM. The studies were performed in this concentration range because we and other authors have demonstrated that higher concentrations of vanadium compounds are toxic and cause osteoblast death [18].

3. Results and discussion

3.1. UV–vis spectra

The UV–vis spectra were recorded in methanolic solutions (see Table 1, L/M=2:1). Using the well-known Ballhausen and Gray MO scheme for C_{4v} symmetry [23], the ligand field transitions in $[VO(H_2O)_5]^{2+}$ can be assigned as follows: the band at lower energies corresponds to the $b_2 \rightarrow e$ transition and the second band to the $b_2 \rightarrow b_1$ transition. The shift of the $b_2 \rightarrow e$ and $b_2 \rightarrow b_1$ bands to the red and blue, respectively, compared with that of $[VO(H_2O)_5]^{2+}$, agrees with carboxylate coordination to the metal center [24,25]. For the bioactivity studies the solid complexes were dissolved in culture medium and added in different concentrations to the cell monolayer. For this reason, the UV–vis spectra in DMEM medium for the three complexes were measured. Table 1 shows the electronic absorption bands of the complexes dissolved in the cell culture medium. The solutions were prepared 10^3 times more concentrated than those used for biological assays, in a L/M molar ratio of 5:1. These conditions were selected to allow detection by UV–visible electronic spectroscopy and to reduce hydrolysis effects. These spectra remain unchanged for 2 h. This period was chosen because it is sufficient for cellular uptake of the complexes. Inside the cells, the compounds are metabolised and the nature of the biologically active species is not fully understood [15]. The diffuse reflectance spectra of the three solid complexes show the same pattern (Table 1). The differences observed in the positions of the band maxima can be attributed to solvatochromic effects. Nevertheless, the above shifts are a general pattern observed in both types of solutions and in the solid state, suggesting the same structure for the complexes under the three experimental conditions.

Table 1
UV–vis and diffuse reflectance spectra of $[VO(Ibu)_2]\cdot5CH_3OH$, $[VO(Nap)_2]\cdot5CH_3OH$ and $[VO(Tol)_2]$

	$b_2 \rightarrow e$	$b_2 \rightarrow b_1$
Ibu-VO	845 (25)[a,b]	595 (14.5)[a,b]
	840[c]	565[c]
	[820]	[570]
Nap-VO	840 (17)[b]	620 (10.2)[b]
	850[c]	565[c]
	[810]	[560]
Tol-VO	835 (20.5)[b]	610 (16)[b]
	850[c]	570[c]
	[840]	[550]

Electronic absorption bands in nm; absorptivity (M^{-1} cm^{-1}) in parentheses; diffuse reflectance in brackets.
[a] Ref. [25].
[b] Methanolic solutions.
[c] Cell culture medium solutions.

Table 2
Infrared spectra of Ibuprofen, its sodium salt and the complex with VO(IV)

HIbu	NaIbu	Ibu-VO	Assignments
	3497 sh		$\nu(OH)$, H_2O
	3356 vs		$\nu(OH)$, H_2O
		3435 s	$\nu(OH)$, CH_3OH
1719 vs			$\nu(C=O)$–COOH
1697 sh	1694 w		
	1670 w		
		1588 vs	$\nu_{as}(COO^-)$
	1584 sh		
	1572 sh		
	1552 vs		$\nu_{as}(COO^-)$
		1548 sh	
1506 m	1521 sh	1509 m	Ring mode
1461 m	1470 m	1458 m	$\delta^{as}CH_3$
1451 w			
1419 s			
	1411 vs	1415 vs	$\nu_s(COO^-)$
1379 m	1390 sh	1383 w	
1364 m	1364 m	1368 m	δ^sCH_3 (isopropyl)
1329 m	1333 w	1333 w	
1321 s			$\nu(C-O)$–COOH
	1290 m	1290 m	
1266 m	1258 w	1266 sh	ωphenyl, p-substituted
1231 vs			$\delta_{ip}CH$
1183 s	1188 w	1188 w	νCH isopropyl
...	...	...	
		933 s	$\nu(V=O)$
...	...	...	
		572 (br,s)	$\nu(V-O)$

vs, very strong; s, strong; m, medium; w, weak; sh, shoulder; br, broad.

3.2. Infrared spectra

The most important features of the infrared spectra of the three complexes and the corresponding free ligands are shown in Tables 2–4.

The O–H stretchings due to water or methanol can be observed in the 3600–3300 cm^{-1} range.

The ν(C=O) of the carboxylic acid group appeared at ca. 1730–1720 cm^{-1} for the free ligands. The assignments of IR frequencies for the antisymmetric ν_{as}(COO$^-$) and symmetric ν_s(COO$^-$) stretching vibrations for the sodium salts and the complexes are given in the tables. The positions of these bands for the sodium salts and the separation between them (Δ ca. 146 cm^{-1}) are consistent with an anionic carboxylate group. In the complexes, ν_{as}(COO$^-$) occurs at ~1590 cm^{-1} and ν_s(COO$^-$) at ~1412 cm^{-1} with an average $\Delta\nu$ value of ~178 cm^{-1}. The correlations between the $\Delta\nu$ values and the carboxylate mode of bonding are essentially empirical [26]. For comparative purposes, in the case of metal complexes with carboxylate ligands other than acetates, it is important to consider the $\Delta\nu$ values of similar metallic complexes with known crystalline structures [27–31].

Table 3
Infrared spectra of Naproxen, its sodium salt and the complex with VO(IV)

HNap	NaNap	Nap-VO	Assignments
	3465 br		ν(OH), H$_2$O
	3371 br		ν(OH), H$_2$O
		3452 br	ν(OH), CH$_3$OH
1729 vs			ν(C=O)–COOH
	1700 w	1709 w	
1687 m			
1631 w	1631 w	1633 w	
1606 m	1606 m	1605 sh	
	1562 s		
	1550 vs	1592 vs	ν_{as}(COO$^-$)
1507 w	1502 w	1506 w	Ring mode
1482 w	1485 w	1484 w	
	1471 w		
1453 w	1453 w	1461 m	δ^{as}CH$_3$
1419 w			
	1404 m	1412 s	ν_s(COO$^-$)
1395 m	1393 m	1392 m	δ^sCH$_3$ (isopropyl)
	1364 m	1369 w	δ^sCH$_3$ (isopropyl)
1347 m	1341 w	1346 w	ν(C–O)–COOH
	1304 w		
1268 m	1265 m	1268 m	
	1248 m		
1229 m	1231 w	1230 m	δ_{ip}CH
	1215 s	1214 m	
1196 m		1197 w	νCH isopropyl
1178 s		1175 m	
1161 s	1162 m	1158 m	
. . .	. . .	. . .	
		942 m	ν(V=O)
. . .	. . .	. . .	
		572 m	ν(V–O)

vs, very strong; s, strong; m, medium; w, weak; sh, shoulder; br, broad.

Table 4
Infrared spectra of Tolmetin, its sodium salt and the complex with VO(IV)

HTol	NaTol	Tol-VO	Assignments
	3514 br		ν(OH), H$_2$O
1732 s			ν(C=O)–COOH
1701 vs			ν(C=O)
	1678 m	1665 sh	ν(C=O)
1630 sh	1640 w		
1617 vs	1612 m	1610 sh	
1605 sh			
	1597 s		
	1565 m	1590 vs	ν_{as}(COO$^-$)
1488 m	1489 m	1490 m	Ring mode
1459 m	1452 m	1455 s	δ^{as}CH$_3$
1428 w		1430 m	
	1414 m	1410 s	ν_s(COO$^-$)
1407 w			
1377 m	1379 sh	1375 s	ν(C–O)–COOH, δ^sCH$_3$
	1369 s		
	1341 m		
	1306 w		
	1281 s	1291 m	
1268 m		1266 s	ωphenyl, p-substituted
1237 m	1228 w	1237 s	δ_{ip}CH
1196 w	1191 m	1194 m	
1178 m	1179 m	1181 m	νCH
1155 m	1155 m	1155 m	
. . .	. . .	. . .	
		943 s	ν(V=O)
. . .	. . .	. . .	
		545 m	ν(V–O)

vs, very strong; s, strong; m, medium; w, weak; sh, shoulder; br, broad.

The stretching frequencies ν(V=O) are located at ca. 940 cm^{-1} for the three complexes. Complexes of vanadyl(IV) cation with monodentate carboxylate ligands exhibit this frequency at higher values [31]. On the other hand, for polymeric chains in which the V=O moieties are held together by double acetate bridges, ν(V=O) is <900 cm^{-1}. In these complexes, there is an interaction involving the V–O bond itself and the neighbouring oxometal units [32–36]. In the case of a coordination sphere around the vanadium atom consisting of two chelate-sugar moieties ligated through deprotonated hydroxyl atoms, the position of the stretching frequencies are ca. 940–930 cm^{-1} [37,38]. This suggests that the vanadyl complexes reported here present the same type of chelate environment around the metal center.

The V–O stretchings are located between 570 and 545 cm^{-1}.

For Tolmetin, ν(C=O) of the carbonyl group is located at 1701 cm^{-1} for the free acid. This band is very sensitive when electron donor or electron acceptor groups are attached to the ligand [39]. As expected, when the ligand is deprotonated (as in the sodium salt) or coordinated to vanadium, this band shifts to 1678 and 1665 cm^{-1}, respectively.

3.3. Magnetic behaviour

Measurements of the room temperature magnetic moments are good criteria to determine whether the carboxylate anion is acting as a bridge or chelate ligand. Subnormal μ_{eff} values are indicative of the coupling between metal ions as in the case of copper(II)-carboxylate adducts of the type $[Cu(RCOO)_2L]_2$ [30,40]. For NSAIDs–vanadyl complexes, the measured μ_{eff} are in the range 1.71–1.99 BM, in accordance with the values expected for a d^1 ($s = 1/2$) system. When coupling between oxovanadium(IV) centers occurs, the room temperature μ_{eff} values are lower [32–36]. Based on these results, carboxylate bridging coordination has to be discarded.

3.4. Effect of NSAIDs–vanadyl(IV) complexes on osteoblast growth

The free ligands, Ibuprofen, Naproxen and Tolmetin were tested on both osteoblast-like cell lines in the concentration range 0–100 µM. These drugs did not inhibit or promote cell proliferation in the tested concentration range. No statistical differences were observed in comparison with the basal condition, without vanadium compounds (data not shown).

On the other hand, the effects of the NSAIDs–VO(IV) complexes on non-transformed mouse calvaria MC3T3E1 and UMR106 rat osteosarcoma-derived cells are shown in Fig. 2(A)–(C).
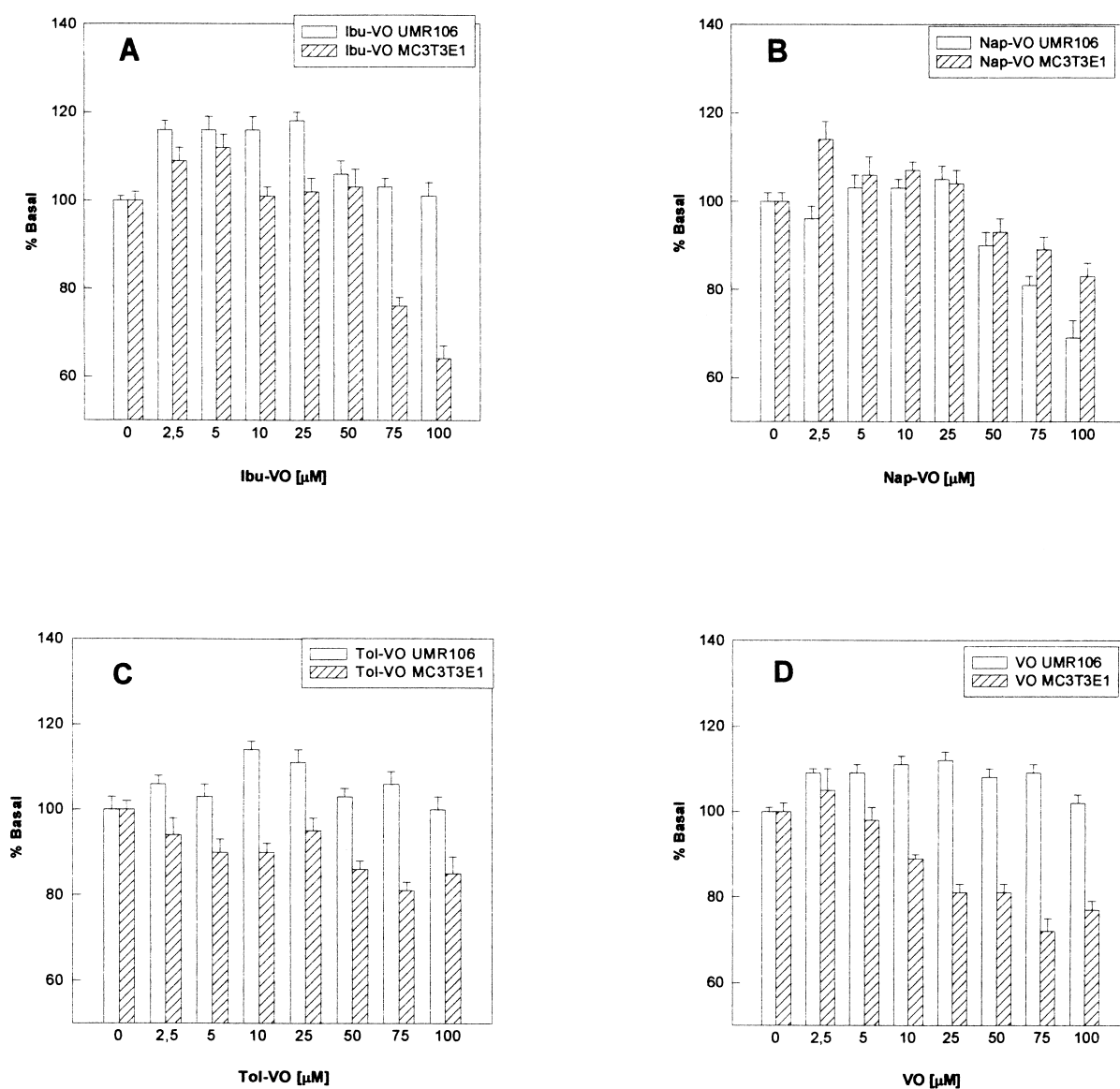

Fig. 2. Effects of Ibu-VO (A), Nap-VO (B), Tol-VO (C) and VO (D) on MC3T3E1 and UMR106 osteoblast-like cell proliferation. Values are expressed as a percentage of the basal value and represent the mean±S.E.M. ($n=9$). Basal values (0 µM vanadyl complexes) are 3.3×10^4 cells/well for MC3T3E1 and 5.5×10^4 cells/well for UMR106 cells. For statistically significant differences, see text.

MC3T3E1 cells: as can be seen, Ibu-VO (Fig. 2(A)) caused a biphasic effect in cell proliferation, being stimulatory at lower concentrations (2.5 and 5 μM, $P < 0.01$) and cytotoxic at higher concentrations (75 and 100 μM, $P < 0.01$). Nap-VO (Fig. 2(B)) also behaved in a similar way, producing stimulation and inhibition of cell proliferation at concentrations of 2.5 μM ($P < 0.01$) and 75–100 μM ($P < 0.01$), respectively. On the contrary, the complex Tol-VO (Fig. 2(C)) significantly inhibited the growth of MC3T3E1 cells at all the tested concentrations ($P < 0.01$) in a dose–response manner.

UMR106 cells: Ibu-VO (Fig. 2(A)) significantly stimulated cell growth in a narrow range of concentrations (2.5–25 μM, $P < 0.01$) and showed no significant effects at higher values. Nap-VO (Fig. 2(B)) did not stimulate cell growth at low doses, but it was the more potent cytotoxic agent ($P < 0.01$) at 50–100 μM concentrations. This effect was even stronger than in MC3T3E1 non-transformed cells. On the other hand, Tol-VO (Fig. 2(C)) did not show any statistically significant effect in the whole range of concentrations in the UMR106 cell line.

In order to investigate further the Nap-VO-induced cytotoxic effect in both osteoblast cell lines, additional experiments were performed in the 50–100 μM concentration range. As can be seen from Fig. 3, this vanadium(IV) derivative was the most potent inhibitor of cell growth in osteosarcoma cells. This effect contrasts with the cytotoxic effect of most of the vanadium(IV) complexes that we have previously reported in these two cell lines, such as VO, BMOV and Asp-VO [16–19]. In those studies the non-transformed cell line was always more sensitive to vanadium derivatives than the osteosarcoma UMR106 cells.

The proliferative effect of vanadium(IV) complexes was also compared with the effect of the vanadium(IV) cation on both cell lines (Fig. 2(D)). The addition of VO(IV) to the culture media induced a significant stimulation of UMR106 cell proliferation at 2.5–75 μM ($P < 0.01$). On the contrary, in MC3T3E1 non-transformed cells, VO(IV) inhibited cell growth in a dose–response manner (Fig. 2(D)). Another NSAID-VO derivative, the Asp-VO complex [19], also exerted a biphasic effect on UMR106 cells. This complex stimulated cell growth at low doses (2.5–25 μM) and inhibited at over 50 μM concentration. In addition, Asp-VO, like VO(IV), significantly inhibited MC3T3E1 cells at all the tested concentrations.

4. Conclusions

Three new vanadyl(IV) complexes with non-steroidal anti-inflammatory drugs, Ibuprofen, Naproxen, and Tolmetin, were synthesized and characterized by means of elemental analysis, UV–vis, diffuse reflectance and IR spectroscopies, as well as their magnetic behavior. The biological activity of these vanadium compounds was tested on two osteoblast-like cells in culture through a proliferation bioassay. Neither Tolmetin nor Tol-VO affected the UMR106 osteosarcoma cell growth. In non-transformed cells, the complex inhibits cell proliferation in a manner dependent on the concentration. This effect was similar to that induced by VO(IV). Ibu-VO was a stimulatory agent at low concentrations for both cell lines. However, this complex was cytotoxic at high doses for MC3T3E1 cells. On the other hand, Nap-VO only stimulated MC3T3E1 growth at the lower doses tested without induction of proliferation in UMR106 cells. Above 50 μM, this complex was cytotoxic to both cell lines. Surprisingly, Nap-VO-induced cell inhibition was more pronounced in UMR106 than in MC3T3E1 cells. The results of the biological assays show that NSAIDs moderate vanadyl(IV) action on osteoblast-like cells. Further experiments are in progress in order to investigate the anti-carcinogenic potential of Nap-VO in other tumoral cell lines.
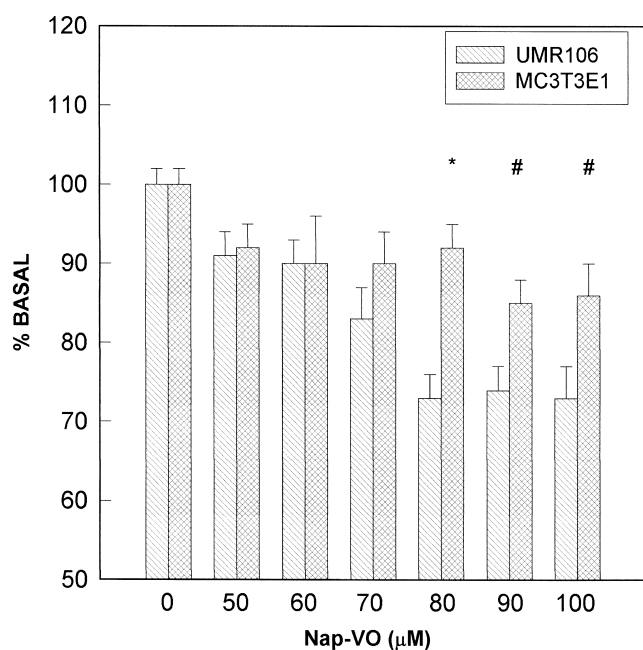
Fig. 3. Effects of Nap-VO on UMR106 and MC3T3E1 cell proliferation. Values are expressed as a percentage of the basal value and represent the mean$\pm$S.E.M. ($n = 6$). *$P < 0.001$, #$P < 0.05$.

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