Original article

Real-time analysis of impedance alterations by the effects of vanadium pentoxide on several carcinoma cell lines

Vanadium compounds have various pharmacological effects and all evidences reveal that the effects of vanadium compounds depend on many factors, mainly on the type of cells and dose. The proapoptotic or antiapoptotic effect of vanadium compounds depends strongly on the cell type. In this study, effects of the vanadium pentoxide (V_2O_5) were investigated using several tumor cell lines: Colorectal cancer cell line (Colo-205), human breast adenocarcinoma cell line (MCF-7) and normal human fibroblast cell line. Five different concentrations of V_2O_5 between 25-200 μM were applied on the cells and xCELLigence Real Time Cell Analysis was conducted to evaluate the impedance alterations. This study is the known first study to show V_2O_5's effects on Colo-205 and MCF-7 and human fibroblast cell lines in a real-time manner. In Colo-205 cell line, the CI alterations decreased slightly 25 μM and 50 μM, increased at the 100 μM, 150 μM and 200 μM concentrations. In MCF-7 cell line, the CI alterations increased at all the concentrations with the untreated control. However, in healthy fibroblast cell line, the CI alterations decreased at all the concentrations compared with the untreated control which limits the use of V_2O_5 for its cytotoxic effect in vivo. The combination with conventional anticancer drugs can be used to increase the effectiveness and reduce the side effects of these drugs that considering stages of cancer and cancer type. Our results suggest that V_2O_5 has disparate effects on several cancer cells at different concentrations.

Key words: xCELLigence, Colo-205, MCF-7, human fibroblast cell line, vanadium pentoxide.

Vanadyum pentoksidin çeşitli kanser hücre hatlarındaki etkilerinin empedans değişiklikleri ile gerçek zamanlı analizi

Vanadyum bileşiklerinin çeşitli farmakolojik etkileri vardır ve tüm kanıtlar, vanadyum bileşiklerinin etkilerinin, başta doz ve hücre tipi olmak üzere birçok faktöre bağlı olduğunu ortaya koymaktadır. Vanadyum bileşiklerinin proapoptotik veya antiapoptotik etkisi esas olarak hücre türüne bağlıdır. Bu çalışmada, çeşitli kanser hücre hatlarında; kolorektal kanser hücre hattı (Colo-205), insan meme adenokarsinoma hücre hattı (MCF-7) ve normal insan fibroblast hücre hattı kullanılarak vanadyum pentoksidin (V_2O_5) sitotoksik aktivitesi araştırıldı. Sitotoksik etkilerini incelemek için V_2O_5 25-200 μM arasında 5 farklı konsantrasyonda hücrelere uygulandı ve empedans değişikliklerini değerlendirmek için xCELLigence Gerçek Zamanlı Hücre Analizi yapıldı. Bu çalışma, V_2O_5'in Colo-205, MCF-7 ve insan fibroblast hücre hatlarındaki etkilerini gerçek zamanlı olarak gösteren ilk çalışmadır. V_2O_5, normal insan fibroblastında 12 saat sonuçlarına göre tüm konsantrasyonlarda Colo-205 ve MCF-7'den daha düşük sitotoksik etki gösterdi. Sağlıklı fibroblast hücre hattında, cell indeks (CI) kontrol ile karşılaştırıldığında tüm konsantrasyonlarda azaldı. Colo-205 hücre hattında CI, 25 μM ve 50 μM'de azaldı; 100 μM, 150 μM ve 200 μM konsantrasyonlarında arttı. MCF-7 hücre hattında, CI kontrole kıyasla tüm konsantrasyonlarda arttı. Konvansiyonel antikanser ilaçlar ile kombinasyonu, kanser türü ve evreleri dikkate alınarak bu ilaçların yan etkilerini azaltmak ve etkinliği arttırmak için kullanılabilir. Bulgularımız; V_2O_5'in farklı konsantrasyonlarda çeşitli kanser hücreleri üzerinde farklı etkilere sahip olduğunu düşündürmektedir.

Anahtar kelimeler: xCELLigence, Colo-205, MCF-7, insan fibroblast hücre hattı, vanadyum pentoksit.

INTRODUCTION

Cancer is a disease in which the control of growth is lost in one or more cells, leading either to a solid mass of cells known as a tumor or to a liquid cancer. It is one of the leading causes of death throughout the world (1). Colorectal carcinoma is the most common gastrointestinal neoplasm and the second cause of death from cancer in the western world. In spite of recent advances in neoadjuvant therapeutic modalities, treatment success is limited in advanced stages of colorectal carcinoma (2). Breast cancer has become the most prevalent cancer and the leading cause of death among females worldwide. Despite the fact that available therapeutics has successfully controlled breast cancer mortality, particularly in advanced countries (3).

Vanadium is a trace element that is present in mammalian body. Recent studies have shown that both inorganic compounds of vanadium(IV) or vanadium(V) and their complexes with organic ligands, in which vanadium valence may vary from III to V, do exhibit cytostatic activity and suppress tumor cell growth *in vitro* and *in vivo* (4). Studies on various cell lines reveal that vanadium exerts its antitumor effects by means of inhibition of cellular tyrosine phosphatases and/or activation of tyrosine phosphorylases. Both effects activate signal transduction pathways leading either to apoptosis and/or to activation of tumor suppressor genes. Moreover, vanadium compounds (VCs) may induce cell-cycle arrest and/or cytotoxic effects through DNA cleavage and fragmentation and plasma membrane lipoperoxidation. Vanadium may also exert inhibitory effects on cancer cell metastatic potential via modulation of cellular adhesive molecules and reverse antineoplastic drug resistance (5).

In different cancer cell lines, some VCs act as inhibitors of cell proliferation in the all range of tested concentrations. These VCs were then evaluated as potentially antitumor agents (6). Vanadium pentoxide (V_2O_5) was appointed to be studied by the National Cancer Institute as a representative of the metals class study (7). It is obvious that tumor cells could be treated with various metal oxide nanoparticles, and specifically V_2O_5 nanoparticles have an admirable potential due to the high cytotoxicity and antitumor effects of vanadium. Mostly, it is shown that vanadium oxides could be more toxic than vanadium salts. Additionally, it is showed that the same vanadium compounds could possess selective cytotoxicity to various cell lines (8).

The Roche xCELLigence System provides incessant, quantitative and real-time monitoring of cells and based on impedance measurements for analyzing the status of

adherent cells *in vitro*. Measurement of the electrical impedance gives an idea about adheration, proliferation and migration of cells. When the changes observed in impedance due to the cell attachment and spreading, it is expressed as the Cell Index (CI). The CI reflects the cell viability thus cell number, attachment quality and cell morphology (9, 10). Monitoring of cell viability is critical and the xCELLigence system enables continuous measurement and quantification of cells (11). Also, time-dependent physiological IC_{50} values can be calculated. It is a more reliable test because classical toxicity tests measure single IC_{50} end-points at one time point (10).

The present study was designed to detect impedance alterations reflecting the cytotoxicity of V_2O_5 on different cell lines. The xCELLigence technology was chosen, as it has been used previously for monitoring cell viability in real time (9). In this study we have investigated the effect of the compound on growth of colorectal cancer cell line (Colo-205), human breast adenocarcinoma cell line (MCF-7) and also normal human fibroblast cell line at different doses *in vitro*. This study is the known first study to show V_2O_5's effects on Colo-205, MCF-7 and human fibroblast cell lines in a real-time manner.

EXPERIMENTAL

Chemicals

Trypsin-EDTA (T3924), fetal bovine serum (FBS, F2442), penicillin-streptomycin (P4333), vanadium pentoxide (V_2O_5) (CAS Number:1314-62-1), Dulbecco's Modified Eagle's Medium (DMEM, D5546) purchased from Sigma Aldrich.

Cell Culture

MCF-7 (ATCC, HTB-22) human breast cancer cell line, Colo-205 (CCL-222) human colorectal cancer cell line purchased from American Type Culture Collection (ATCC). Human fibroblast cell line gifted to us from GENKOK (Kayseri/Turkey). MCF-7 (12500 cells/well) and Colo-205 (12500 cells/well) cells cultured with DMEM containing FBS 10%, L-glutamin 1%, 100 U/ml penicillin and 100 µg/ml streptomycin. Human fibroblast cells (3000 cells/well) cultured with DMEM containing FBS 20%, L-glutamin 1%, 100 U/ml penicillin and 100 µg/ml streptomycin. The cells were grown to 80% confluence at 37^0C and humidified atmosphere with 5% CO_2. When cells reached approximately 80% confluence, we detached cells with 0.25% trypsin-EDTA. The cells were

centrifuged with the Universal 320R (Hettich, Zentrifugen, 1406 Germany) at 1000 rpm for 5 min at 25ºC and seeded on 96 wells E-plate xCELLigence analysis. When cells reached log growth phase approximately 24 h later from seeding to E-plate, we treated cells with V_2O_5 for 25, 50, 100, 150, 200 µM concentrations.

xCELLigence Real Time Cell Analysis

Cytotoxic effect of V_2O_5 was monitored with xCELLigence Real-Time Cell Analyser (RTCA) as described by manufacturer's instructions (Roche Applied Science and ACEA Biosciences) with slight modifications. Firstly, optimal seeding concentration of MCF-7, Colo-205 and fibroblast cells were determined and then the cells were seeded in E-plate 96 wells. Cell proliferation, attachment and spreading were monitored every 15 min via the impedance of E-plate wells. Approximately 24 h post-seeding when the cells were in the log growth phase, we treated cells with V_2O_5 and controls received only medium and replicated 4-times and the experiments were run for about 72 h.

Figure 1. xCELLigence real time cell analyser single plate (10)

Cell Growth and Proliferation Assay Using xCELLigence System

At the end of experiment all calculations were done with the RTCA-integrated software of the xCELLigence system (Figure 1). An unit-less parameter termed CI is derived to represent cell status based on the measured relative change in electrical impedance that occurs in the presence and absence of cells in the wells, which is calculated based on the following formula: $CI = (Zi-Z_0)/15$, where Zi is the impedance at an individual point of time during the experiment and Z_0 is the impedance at the start of the experiment (Figure 2). RTCA software performs the curve-fitting of selected

"sigmoidal dose-response equation" and calculated logarithmic half maximum effect of concentration [log (IC_{50})] values at a given time point based on log concentration producing 50% reduction of CI value relative to the control CI value (100%).

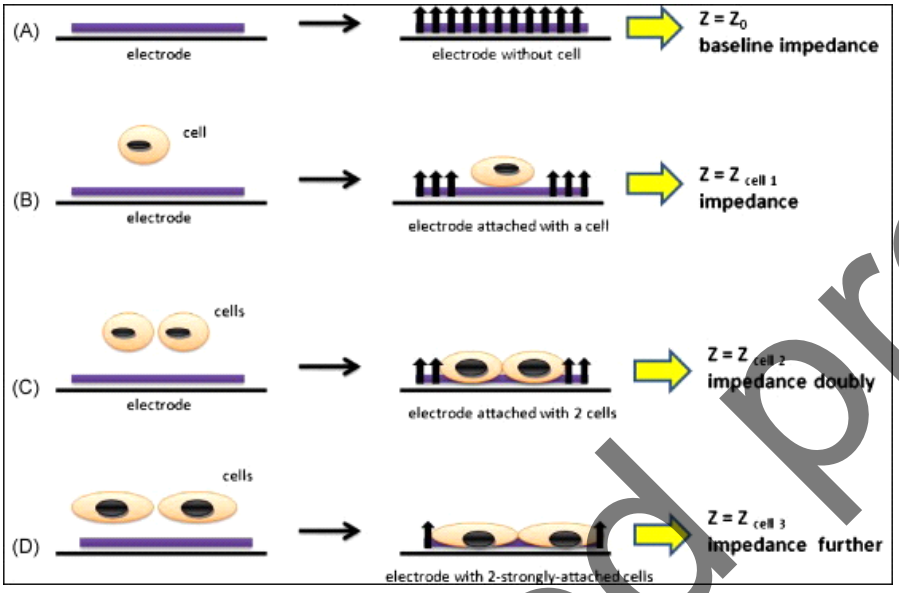

Figure 2. Scheme of xCELLigence impedance alteration (10)

Baseline impedance: There are no cells on an electrode surface (A). *Impedance*: A cell labels to the electrode surface and blocks partially the electrical current in the circuit, inducing an increase in the electrode impedance (B). *Impedance doubly*: Two cell labels to the electrode surface and reduce even further the electrical current, as compared with B inducing to doubly increased impedance (C). *Impedance further*: Two cell labels to the electrode surface with more extension, which induce much more impedance in comparison with C (D).

Statistical Analysis

For each study group, data were derived from at least three independent experiments. Statistical analysis was performed GraphPad Prism Software Version 5.03 using a Sidak's multiple comparisons test to compare differences in values between the control and experimental group. Data were expressed as mean ± standard deviation. Values of all significant correlations ($p<0.05$) are given with degree of significance indicated (*$p<0.01$, **$p<0.001$, *** $p<0.0001$, **** $p<0.00001$).

RESULTS

To analysis of impedance alterations by the cytotoxic effect of V_2O_5 on normal human fibroblast, Colo-205 and MCF-7 cell lines, xCELLigence System was performed. Normal human fibroblast, Colo-205 and MCF-7 cell lines were exposed to V_2O_5 25 µM, 50 µM, 100 µM, 150 µM and 200µM for 72 h. V_2O_5 exhibited cytotoxic effect on normal human fibroblast at 25 µM, 50 µM, 100 µM, 150 µM and 200µM concentrations (Figure 3). In healthy fibroblast cell line, the CI alterations decreased at all the concentrations compared with the untreated control (Figure 6). In Colo-205 cell line, the CI alterations decreased slightly at 25 µM and 50 µM, increased at the 100 µM, 150 µM and 200 µM concentrations (Figure 4 and Figure 7). In MCF-7 cell line, the CI alterations increased at all the concentrations with the untreated control (Figure 5 and Figure 8). The IC_{50} values obtained in the studied concentrations for 12 h and 24 h incubations in these cell lines are shown in Table 1.

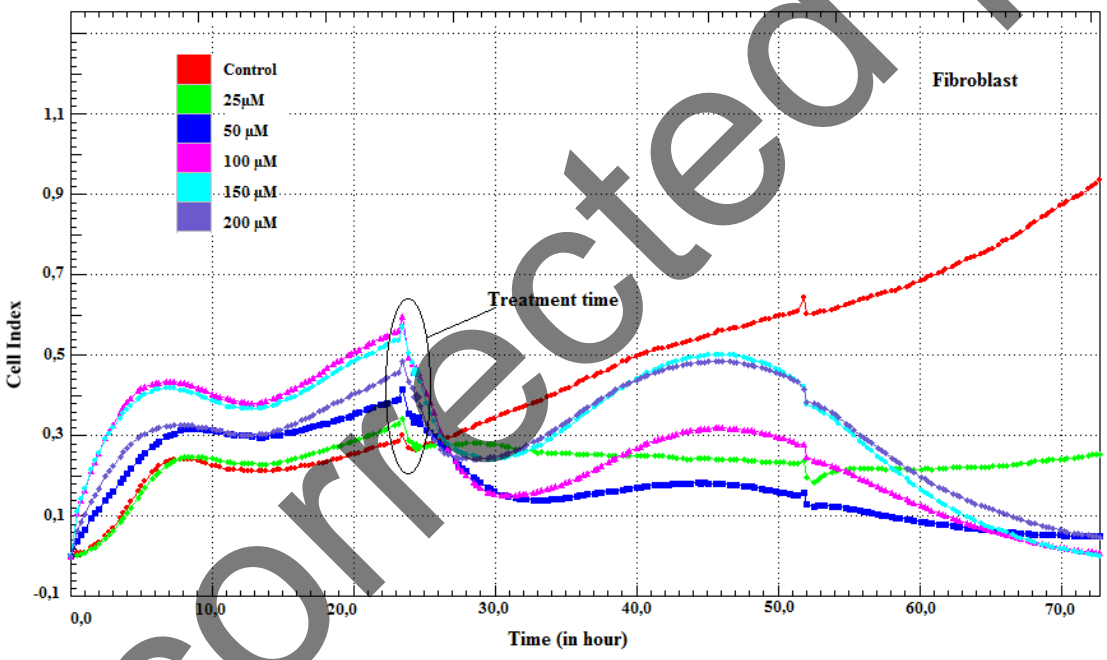

Figure 3. Dynamic monitoring of fibroblast cell adhesion and proliferation using the xCELLigence system. Fibroblast at a density of 3000 cells/well per well in E-Plates 96 were observed during 72 h.

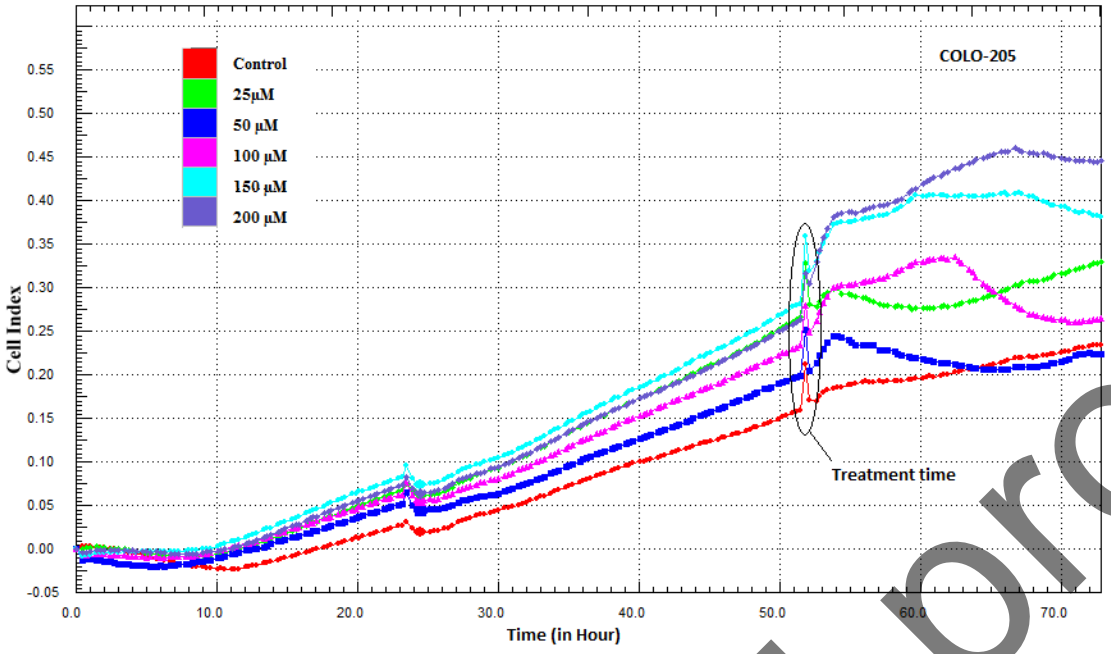

Figure 4. Dynamic monitoring of Colo-205 cell adhesion and proliferation using the xCELLigence system. Colo-205 at a density of 12500cells/well per well in E-Plates 96 were observed during 72 h.

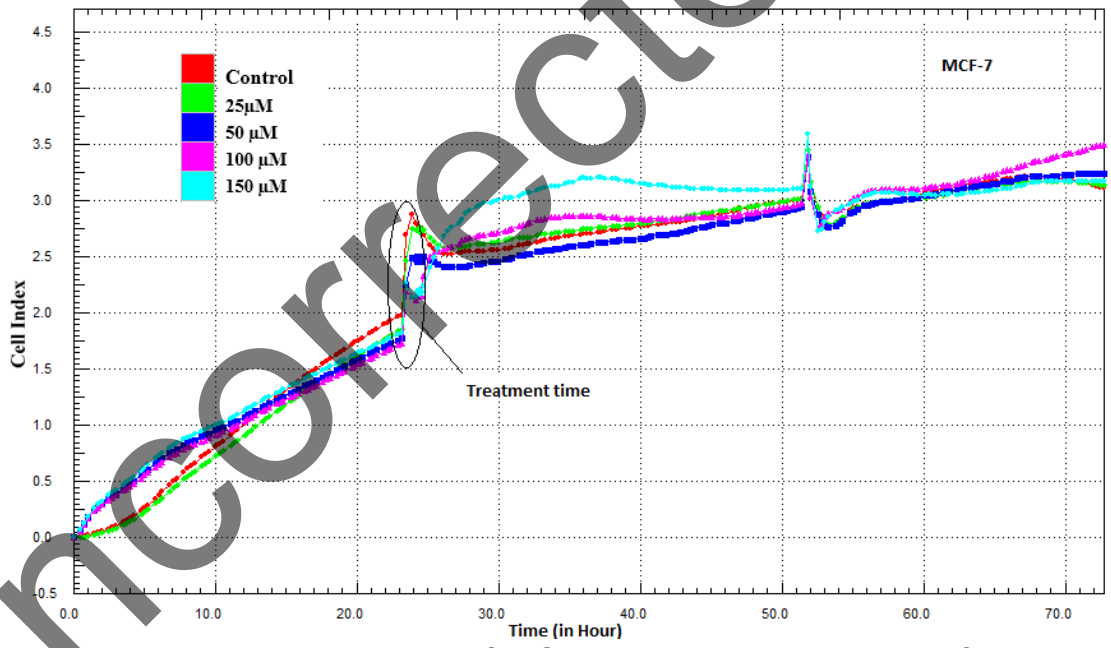

Figure 5. Dynamic monitoring of MCF-7 cell adhesion and proliferation using the xCELLigence system. MCF-7 at a density of 12500cells/well per well in E-Plates 96 were observed during 72 h.

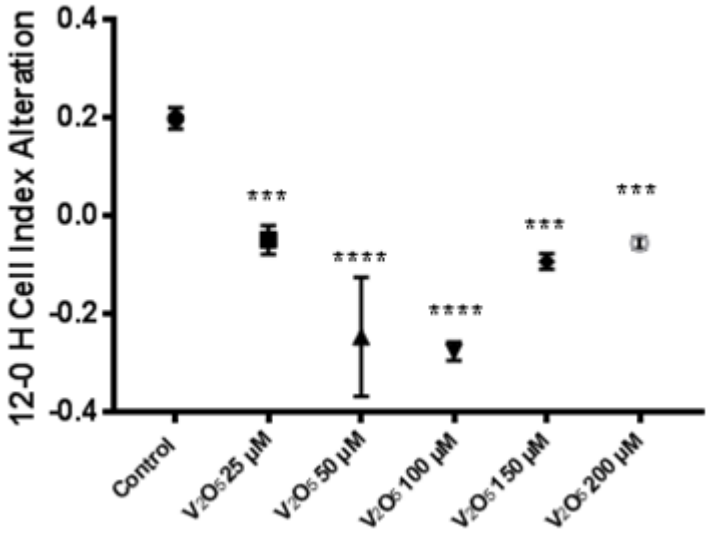

Figure 6: CI alterations after 12 h incubation of the healthy fibroblast cell line by V_2O_5 treatment. Data were calculated from three independent experiments. Data were presented as mean ±S.D., *** p<0.0001 and **** p<0.00001 compared with the untreated control.

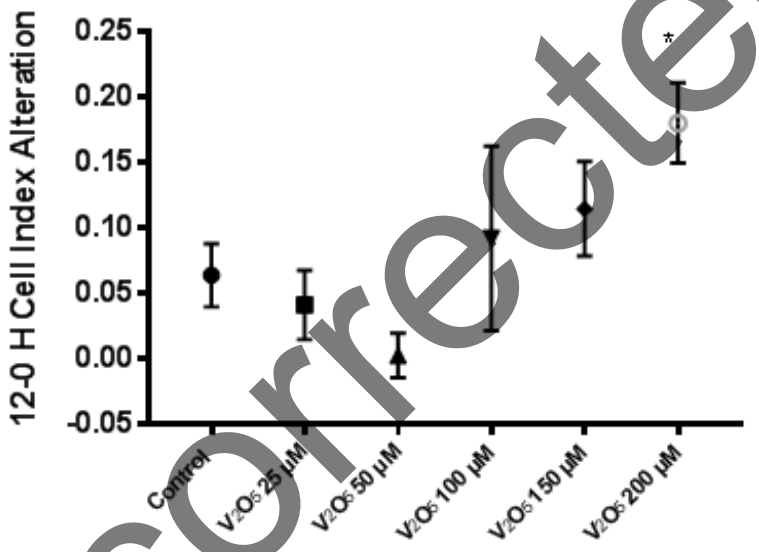

Figure 7: CI alterations after 12 h incubation of the Colo-205 cell line by V_2O_5 treatment. Data were presented as mean ±S.D., * p<0.01 compared with the untreated control.

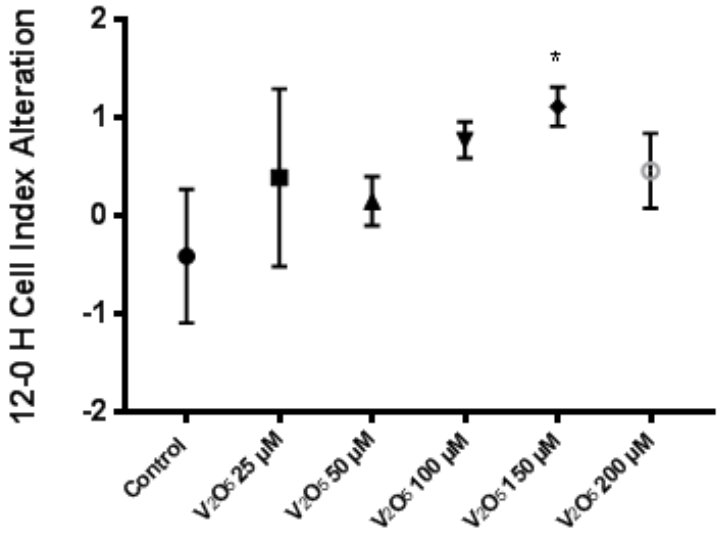

Figure 8: CI alterations after 12 h incubation of the MCF-7 cell line by V_2O_5 treatment. Data were presented as mean ±S.D., * p<0.01, compared with the untreated control.

Table 1. IC_{50} values for V_2O_5

Cell line	**IC_{50} 12 h**	**IC_{50} 24 h**
Human fibroblast cells	136.741 µM	101.18 µM
COLO-205	118.58 µM	145.29 µM
MCF-7	64.14 µM	219.82 µM

DISCUSSION

Especially, metallodrugs represent constitute new and powerful tools for diverse therapeutic applications. Until today, various metallodrugs show interesting biological activities for chemotherapy. In this field, cisplatin was the first inorganic compound with high relevance in cancer treatment. This compound was a leader agent in clinical use. Toxicity and resistance problems trigger the development of other platinum drugs with better clinical perspective and also raise the scientific interest for the putative antitumor properties of VCs. Various scientific articles reviewed by Leon et al. (12), show that complexes of these metals are the new metal-based drugs used in the treatment of several cancers, such as, lung, colon, breast, bladder, etc. Vanadate is a transition element that present in nature and was shown to be a nonspecific inhibitor of protein tyrosine phosphatases. VCs exhibit antitumor actions in several cancer cell lines (12, 13).

The xCELLigence RTCA equipment uses specific microtiter E-plates coated with gold-microelectrodes that detect the attachment of adherent cells, thus modifying the impedance signal. The xCELLigence RTCA is a non-invasive, impedance-based biosensor system that can measure cell viability, migration, growth, spreading and proliferation. Alterations in cell morphology and behavior are continuously monitored in real time using microelectronics located in the wells of RTCA E-plates. As shown previously, the xCELLigence system is well-suited to analyze drug effects on cell proliferation, cytostasis and cytotoxicity in real-time. In comparison with single end-point assays, the availability of drug effect profiles over the whole experimental period allows building of dynamic growth curves. To evaluate the utility of this system for analysis of targeted therapies, Ruiz et al. subjected two established non-small cell lung cancer cell lines H522 and H3122 to crizotinib treatment. The real-time monitoring system allowed them to determine the latency time of the drug effect on cell growth (14).

Tumor cell lines with various proliferative rates were equally sensitive to orthovanadate cytotoxicity. Orthovanadate (Na_3VO_4) at concentrations greater than 5 μM has antineoplastic properties *in vitro* and *in vivo* (15). Within the concentration range of 1-20 μM, Na_3VO_4 demonstrated a time and dose-dependent inhibition of autocrine growth of the human carcinoma cell lines A549 (lung), HTB44 (kidney) and DU145 (prostate), as compared to appropriate controls (without Na_3VO_4). Klein et al. has also revealed that HTB44, A549 and DU15 cell lines have different sensitivity to Na_3VO_4(16). Kordowiak et al. has revealed progressive growth inhibition of rat hepatoma H35-19 cells within the range 0,5-20,0 μM concentrations of three vanadium salts. The most effective (and/or toxic) was $NaVO_3$, whereas $VOSO_4$ showed a relatively mild action. As compared with metavanadate or vanadyl sulphate and especially organic vanadium derivatives, previously studied by the same authors under similar experimental conditions, Na_3VO_4 showed an intermediate effect (17). Yang et al. have shown that sodium vanadate has cytotoxic effects on esophageal squamous carcinoma cell line EC109 at 50 μM (13). The antiproliferative effect of bis(acetylacetonato)-oxidovanadium(IV) and sodium metavanadate and the underlying mechanisms were investigated in human pancreatic cancer cell line AsPC-1. VCs can be regarded as a novel type of anticancer drugs through the prolonged activation of MAPK/ERK pathway but retained AKT activity (18). Vanadium has no carcinogen effect but its presence in cancer cells and its interactions with many key enzymatic

processes results in modified expression of p53 and Bax and in down regulation of Bcl2 proteins and in antiproliferative activity. Anticancer effects of vanadium in various forms have been demonstrated using *in vitro* and *in vivo* experiments (19). VCs introduced into the therapy due to their low IC_{50}, antiproliferative and proapoptotic effects. Additionally, VCs stimulate the cell cycle, so inhibiting apoptosis, as both processes are mutually related. The above processes promote tumor cell growth at early stages of the disease and have an antitumor effect in the advanced stages of cancer. VCs used at low concentrations have selective effects on the tumor cells *in vivo* and *in vitro* studies. The effects of VCs depend on many factors, mainly on the type of cells, the type of VC and its dose. It appears that the proapoptotic or antiapoptotic effect of VCs depends strongly on the cell type (20).

In our study we have investigated the effects of V_2O_5 on three different cell lines, including human fibroblast cells, MCF7 and Colo-205. In Colo-205 cell line, the CI alterations decreased slightly 25 µM and 50 µM, increased at the 100 µM, 150 µM and 200 µM concentrations. In MCF-7 cell line, the CI alterations increased at all the concentrations with the untreated control. In human fibroblast cell line the CI alterations decreased at all the concentrations. Our results suggest the idea that while V_2O_5 causes toxicity on human fibroblast cells, it also show antiproliferative effect at 25 and 50 µM concentrations and proliferative effect at the high concentrations on Colo-205; proliferative effect on MCF-7 cell lines at all the concentrations used.

CONCLUSION

VCs have various pharmacological effects and affect various biochemical processes, and all evidences reveal that the effects of VCs depend on many factors, mainly on the type of cells and their doses. It appears that the proapoptotic or antiapoptotic effect of VCs depends largely on the cell type. Our results are considerable for further mechanism of action studies and trace amounts of V_2O_5 compound with conventional therapies might strengthen or weaken the impact of the treatment. Additional mechanism of action studies should be performed to confirm beneficial and toxic effects of V_2O_5 in different experiments and cancer cell types at high or low doses. With the combination of conventional anticancer drugs can be used to increase the effectiveness and reduce the side effects of these drugs that

considering stages of cancer and cancer type. Our results suggest that V_2O_5 has disparate effects on several cancer cells at different concentrations. This study is the known first study to show V_2O_5's effects on Colo-205, MCF-7 and human fibroblast cell lines in a real-time manner.

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Declaration of interest

The authors declare that there are no conflicts of interest.

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