

INFLUENCE OF ASCORBATE ON ANTI-HELA EFFECT OF Fe(III)-HETEROPOLYANION: CONCENTRATION RATIO 1:5

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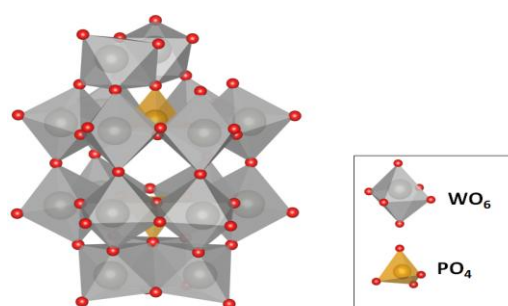
Abstract: This study aims to assess the effect of L(+)-ascorbic acid sodium salt (ascorbate) on the cytotoxic activity of Fe(III)-substituted monolacunary Wells-Dawson polyoxometalate (Fe-WD), $K_7[Fe^{III}(\alpha_2-P_2W_{17}O_{61})(H_2O)]$ (Fe-WD/ascorbate ratio 1:5) against cervical carcinoma HeLa cells. HeLa cells were treated *in vitro* with Fe-WD, ascorbate, and Fe-WD/ascorbate mixtures (concentration ratio 1:5) for 48 and 72 hours at 37 °C. Single and simultaneous treatments with Fe-WD/ascorbate resulted in concentration- and time-dependent HeLa cell cytotoxicity with different potencies. Fe-WD showed remarkably more potent cytotoxic action compared with ascorbate, inducing approximately 10-15 times lower IC_{50} values for both exposure times. The results obtained for the simultaneous exposure to Fe-WD/ascorbate mixtures (1:5) demonstrated that the presence of ascorbate does not significantly affect Fe-WD-induced anti-HeLa action. This effect might be associated with the fact that the concentration ratio of Fe-WD and ascorbate 1:5 causes ascorbate consumption due to its autoxidation in the presence of Fe(III) ions.

Key words: anti-tumor action, ascorbate, cell viability, concentration ratio 1:5, Fe(III)-substituted Wells-Dawson, HeLa cell line, *in vitro* cytotoxicity

1. INTRODUCTION

Metal-based compounds such as cisplatin have been the most commonly employed anti-cancer drugs in clinical practice and a gold standard in chemotherapy [1]. Based on this fact, new transition metal-containing such as polyoxometalates (POMs) have been designed and their anti-tumor potential has been widely studied using various model systems [2]. This versatile class of metal-oxygen polyanions exhibited different biological actions in *in vitro* and *in vivo* studies and thus could be explored as promising next-generation metallodrug candidates [3-6].

The purpose of this research is to investigate the effect of L(+)-ascorbic acid sodium salt (ascorbate) on the cytotoxic activity of Fe(III)-substituted monolacunary Wells-Dawson POM (Fe-WD), $K_7[Fe^{III}(\alpha_2-P_2W_{17}O_{61})(H_2O)]$ (Fe-WD/ascorbate ratio 1:5) against cervical carcinoma HeLa cells as a frequently used tumor model system *in vitro*. Since ascorbate and Fe-WD can generate reactive oxygen species (ROS) inducing cell structure damage and death [7], this study is the continuation of searching for an optimal Fe-WD/ascorbate ratio resulting in the strongest anti-tumor effect. The structure of monolacunary Wells-Dawson polyoxotungstate is presented in Scheme 1.



Scheme 1. Monolacunary Wells-Dawson polyoxotungstate (oxygen – red balls).

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2. EXPERIMENTAL

2.1. Synthesis of Fe-WD

Fe(III)-containing monolacunary Wells-Dawson heteropolytungstate (Fe-WD), $K_7[Fe^{III}(\alpha_2-P_2W_{17}O_{61})(H_2O)]$ was synthesized according to the reported method [8]. All chemicals were purchased from Sigma-Aldrich Chemical Co. (Steinheim, Germany). First, stock aqueous solutions of Fe-WD (20 mM) and ascorbate (1 M) were made and kept frozen (-20 °C) until used. Working solutions were made by diluting the stock solutions immediately before use and diluted ten times in incubation mixtures to achieve final concentrations.

2.2. Cell Culture and Experimental Conditions

Cervical carcinoma HeLa cells were purchased from the American Tissue Culture Collection (ATCC, Manassas, VA, USA) and cultured in high-glucose DMEM medium (Sigma-Aldrich, Steinheim, Germany) supplemented with 10% fetal bovine serum (Sigma-Aldrich, Steinheim, Germany) and penicillin/streptomycin (Sigma-Aldrich, Steinheim, Germany), in humidified atmosphere of 5% CO₂ at 37 °C.

2.3. Cell Viability Assay

HeLa cells (2×10^3 cells/well) were seeded into flat-bottom 96-well plates and allowed to adhere overnight. Exponentially growing cells were treated with the selected concentrations of Fe-WD/ascorbate for 48 and 72 hours, at 37 °C. Cell viability was determined using the sulforhodamine B (SRB) assay measuring cellular protein concentration [9]. Briefly, cell monolayers are fixed with 10% trichloroacetic acid for 1 h at 4 °C and stained with 0.4% SRB. The unbound color is removed and the plates are washed with 1% acetic acid and air-dried. Protein-bound dye is dissolved in 10 mM Tris buffer pH 7.4 and absorbance is read at 550 nm and a reference wavelength of 690 nm using a microplate reader (Wallac, VICTOR2 1420 Multilabel counter, PerkinElmer, Turku, Finland). Results are expressed as relative cell viability (a percentage of control).

3. RESULTS AND DISCUSSION

HeLa cells were *in vitro* exposed to the mixtures of Fe-WD and ascorbate at the concentration ratios 1:5 for 48 and 72 hours. Induced values of cytotoxicity, expressed as relative cell viability (% of control), were determined and compared with the results of separate treatments with Fe-WD (0.005 – 0.5 mM) and ascorbate (0.025 – 2.5 mM) (Figure 1).

Single and simultaneous treatments with Fe-WD/ascorbate resulted in concentration-dependent HeLa cell cytotoxicity with different strengths observed for 48- and 72-hour exposure. After 48-hour exposure, IC₅₀ values (in mM) for Fe-WD, ascorbate, and Fe-WD/ascorbate mixture were 0.015, 0.135, and 0.019 (and 0.095 ascorbate), respectively. All investigated compounds induced an 80% decrease in cell viability for concentrations higher than 0.05 mM. Then, HeLa cells were found as more sensitive to Fe-WD/ascorbate after 72-hour treatment. Accordingly, 72 hours-induced IC₅₀ values for Fe-WD, ascorbate, and Fe-WD/ascorbate mixture were significantly lower compared with 48-hour treatment - 0.0075, 0.125, and 0.007 mM (and 0.035 mM ascorbate), respectively. Higher concentrations (≥ 0.05 mM) of all studied compounds resulted in almost complete inhibition of HeLa viability ($\geq 90\%$).

The results of single exposure demonstrate that Fe-WD exhibits significantly more potent cytotoxic action anti HeLa cells related to ascorbate, inducing approximately 10-15 times lower values of IC₅₀ for both exposure times. The results obtained for the simultaneous exposure to Fe-WD/ascorbate mixtures (1:5) show that the presence of ascorbate does not significantly affect Fe-WD-induced anti-HeLa action. Indeed, IC₅₀ values obtained for Fe-WD and Fe-WD/ascorbate mixture are very similar for the same exposure time and inhibition curves resulting from Fe-WD and Fe-WD/ascorbate mixture are almost overlapped. The observed effect might be associated with the fact that the concentration ratio of Fe-WD and ascorbate 1:5 results in ascorbate consumption due to its

autoxidation in the presence of Fe(III) ions. In addition, it seems this reaction does not generate ROS that could affect HeLa cell structure.

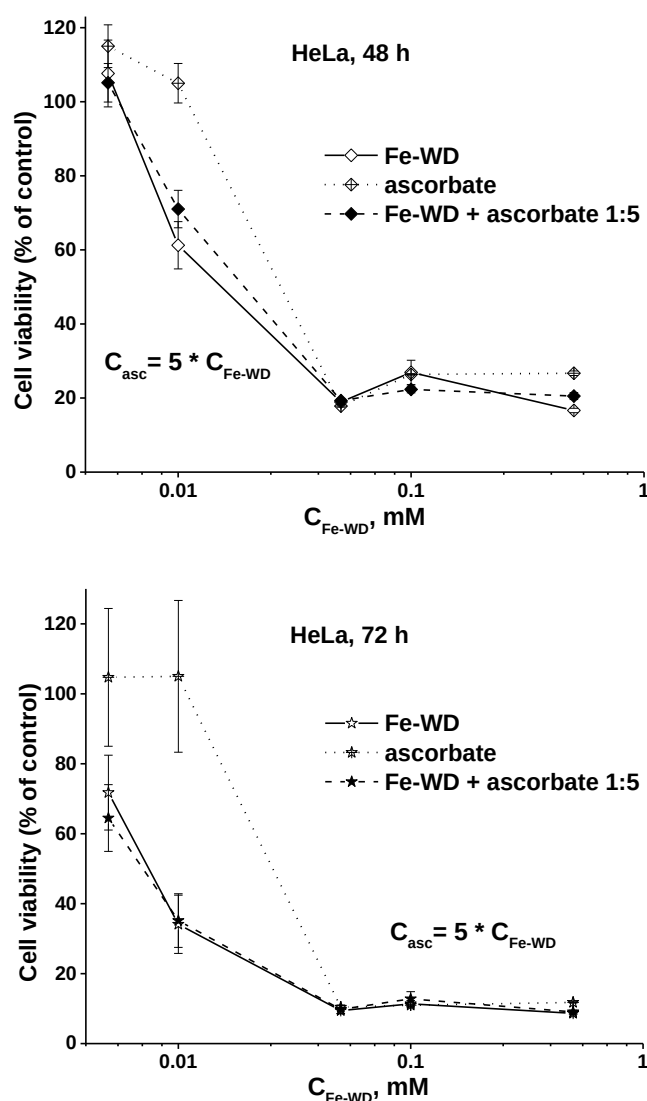


Figure 1. The influence of Fe-WD, ascorbate, and Fe-WD/ascorbate mixture (concentration ratio 1:5) on HeLa cell viability after 48- and 72-hour exposure. Cell viability is expressed as % of untreated (control) cell viability (100%). Results are presented as mean $\pm$ S.D. from at least 2 independent experiments done in triplicates.

4. CONCLUSION

Fe(III)-containing monolacunary WD POM showed significantly stronger concentration- and time-dependent anti-HeLa effects compared with ascorbate. The results of simultaneous exposure to Fe-WD/ascorbate mixture (ratio 1:5) indicate that the presence of ascorbate does not remarkably modulate Fe-WD-induced cytotoxicity. This effect might be explained by ascorbate consumption occurring in this concentration ratio of Fe-WD and ascorbate due to the autooxidation by oxygen and Fe(III) ions. Furthermore, the obtained cytotoxic effect of Fe-WD/ascorbate mixture suggests that this reaction does not generate reactive species resulting in cell structure and function damage.

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