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Cytotoxicity of nanosize V_2O_5 particles to selected fibroblast and tumor cells

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Abstract

Two kinds of nanosize V_2O_5 particles were synthesized in our own laboratory and concomitantly applied to V79 and L929 fibroblasts and SCCVII, B16F10 and FsaR tumor cells. The morphologies of the cells were monitored using an inverted inverse microscope equipped with digital camera, while quantitative determination of the cytotoxicity of nanosize V_2O_5 particles was measured using crystal violet bioassay. Twenty four hours after the addition of nanosize V_2O_5 particles (20 μM), noticeable changes in the morphology and density of fibroblast and cancer cells were observed. Reculturing in a freshly prepared medium for the next 24 h showed a high recovery effect on V79, SCCVII and B16F10 cells, while FsaR and L929 cells were seriously damaged and unable to recover. At a higher concentration of nanosize V_2O_5 particles (100 μM), the cytotoxicity of V_2O_5 prevailed against the recovery effect in all cell types. Quantitative measurements have shown that the resistance of investigated cell cultures to the cytotoxicity of nanosize V_2O_5 particles decreases in the order V79 > SCCVII > B16F10 > FsaR > L929. The high cytotoxic effect found on FsaR cells suggests that nanosize V_2O_5 particles could be regarded as poisoning material in the treatment of FsaR fibrosarcoma cells. Possible mechanisms involved in the cytotoxicity of nanosize V_2O_5 particles were discussed.

Keywords: V_2O_5; Cytotoxicity; Nanoparticles; Glucose; Fibroblasts; Tumor cells; V79; SCCVII; B16F10; FsaR; L929

1. Introduction

Nanoparticles have been extensively studied for their possible application in various fields in biomedicine, such as drug delivery, imaging, diagnostics, cell separation and purification, as well as in cancer treatment. Roughly, nanoparticles may be categorized into polymeric nanoparticles, polymeric micelles, dendrimers (macromolecular compounds that comprise a series of branches around the inner core), liposomes (small artificial vesicles of spherical shape that can be produced from natural non-toxic phospholipids and cholesterol), quantum dots (particles that are generally composed from group II–IV or III–V of the periodic table with physical dimensions smaller than the excitation Bohr radius) and metal oxide nanoparticles that could be further categorized into ceramic, semiconducting or magnetic nanoparticles (Sahoo and Labhasetwar, 2003). Liposomes, polymeric nanoparticles and dendrimers are mainly used as drug carriers to overcome resistance phenomena (Vauthier et al., 2003) or to deliver and increase the efficacy of anticancer agents such as doxorubicin (Soma et al., 2000), mitoxantrone (Reszka et al., 1997), camptothecin (Miura et al., 2004) and others. On the other hand, metal oxide particle are active antitumor substances. In comparison with polymer and organic nanoparticles, metal oxide nanoparticles possess several advantages, such as compatibility with biological systems, possible tailoring of their size, shape and porosity or surface modification by different functional groups. These particles are also stable against

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the change in pH and/or temperature. In addition, in the metal oxide nanoparticles there are no swelling or porosity changes.

For example, magnetic metal oxide nanoparticles (magnetite and maghemite) have found application in cancer therapy (Jordan et al., 1999). These magnetic nanoparticles can be incorporated into malignant cells to generate heat under an alternating magnetic field by remagnetization losses. The heating so obtained at about 43 °C (hyperthermia) destroys cancer cells, because their oxygen supply via the blood vessels is not sufficient (Kawashita et al., 2005). The advantage of this technique is in its potential of treating embedded tumors in vital regions where surgical resection is not feasible. TiO_2 and Fe–TiO_2 nanoparticles have also been used in the treatment of tumor cells. Fujishima et al. (1986, 2000) and Cai et al. (1991, 1992) were the first to show that illuminated TiO_2 colloidal particles could be effective in killing tumor cells cultured in vitro and in vivo. In our previous work (Ivanković et al., 2003) it was shown that squamous carcinoma cells SCCVII cultured in vitro could be effectively killed by nanosize iron-doped TiO_2 particles in the presence of UV irradiation. Two different roles of iron were found: (a) the presence of iron dopant in nanosize TiO_2 particles stimulated the photokilling of cancer cells under UV irradiation and (b) iron provided the recovery of cell proliferation after reculturing in the dark. The advantage of the technique is its high selectivity, because the photocatalytic reactions only occur under illumination. The disadvantage of the photokilling effect is the difficulty of illuminating the tumor cell growth deep inside the body.

It is obvious that tumor cells could be treated with various metal oxide nanoparticles, and specifically V_2O_5 nanoparticles have an excellent potential due to the high cytotoxicity and antitumor effects of vanadium. However, while the cytotoxicity effects of various soluble vanadium salts (sodium metavanadate, vanadyl sulfate, ammonium vanadate, peroxovanadates, etc.) have been extensively investigated in the literature (Evangelou, 2002; Morinville et al., 1998; Mukherjee et al., 2004) the cytotoxicity effects of nanosize V_2O_5 particles were a new approach to this subject. The toxicity of vanadium salts differed among salt speciation and in the reference literature we did not find the standard vanadium salt to compare with the cytotoxicity of nanosize V_2O_5 particles. Generally, it is known that vanadium oxides could be more toxic than vanadium salts. In addition, Capella et al. (2002) showed that the same vanadium compounds could possess selective cytotoxicity to different cell lines; among two epithelial kidney cell lines, MDCK cells were resistant to vanadate whereas Ma104 cells, which expressed the multidrug-resistant phenotype, were highly sensitive to the same vanadium compound. These findings motivate us to apply nanosize V_2O_5 particles to five cell lines in order to determine (i) the cytotoxic effect of nanosize V_2O_5 particles (in vitro) and (ii) to determine if the nanosize V_2O_5 particle showed selective cytotoxicity among the selected cell lines.

Besides the cytotoxic effect of vanadium, there are numerous reports on the biological activities of specific vanadium compounds due to their insulin-mimetic effect (Morinville et al., 1998; Rehder, 2003; Tsiani and Fantus, 1997). These compounds encompass (a) the stimulation of glucose intake into living cells, thus lowering the blood glucose level, (b) the inhibition of glyconeogenesis and glycogenolysis, and (c) the inhibition of lipolysis. The therapeutic effects of vanadium were demonstrated in insulin tolerance, type II diabetic rodents which did not respond to exogeneously administered insulin (Goldwaser et al., 2000). However, the possibility of explaining the cytotoxic effect of vanadium on the basis of its insulin-mimetic effect has never been used in the reference literature.

In an earlier paper (Ivanković et al., 2003) we showed that well-dispersed WO_3, TiO_2 and Fe–TiO_2 nanoparticles, synthesized and characterized in our own laboratory (Šijaković-Vujičić et al., 2004), were almost completely non-toxic to SCCVII cells. In contrast, some of these nanoparticles were highly cytotoxic to SCCVII cells, but only in the presence of UV irradiation. In the present work we report new results on the cytotoxicity of nanosize V_2O_5 particles, synthesized and characterized in our own laboratory, to selected fibroblast and cancer cell lines. Vanadium oxide nanoparticles applied to cancer cells showed quite a different cytotoxicity behavior in comparison with TiO_2 and Fe–TiO_2 nanoparticles. We have shown that nanosize V_2O_5 particles are cytotoxic to all cell lines in a dose-dependent manner per se and that there is no need for the UV irradiation assistance. Nanosize V_2O_5 particles killed most effectively the L929 and FsaR cells, whereas the fibroblast V79 cells and the squamous carcinoma cells SCCVII showed highest resistance to the treatment with nanosize V_2O_5 particles.

2. Materials and methods

2.1. Materials

Dowex® 50W-X (20–50 mesh) cation exchange resin with large effective pore size, (Bio-Rad Laboratories), vanadium(V)-triisopropoxide oxide (95–99%), $VO[CHO(CH_3)_2]_3$, supplied by Alfa Aesar®, isopropanol, $(CH_3)_2CHOH$, by Aldrich, HCl (p.a.), $NaVO_3$ (p.a.) and NaOH (p.a.), by Kemika, were used as received. Water obtained from the Milli-Q purified system was used.

2.2. Synthesis of V_2O_5 nanoparticles

Nanosize V_2O_5 particles were synthesized using two different procedures. Sample V1 was synthesized by the sol–gel procedure using 187 ml of isopropanol, 25 g of vanadium(V)-triisopropoxide oxide and 25 ml of water. Synthesis was performed in an oil bath at 70 °C in a specially designed all-glass assembly with reflux and a bubbling extra dry N_2. Sample was dried in a Petri dish at 60 °C for 48 h. Calcination was performed at 240 °C.