Letters in
General Microbiology

The Role of Vanadium Pentoxide in Insulin Mediated Genotoxicity

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Abstract

Vanadium is an essential micronutrient with a proposed role in diabetes mellitus of substituting insulin or increasing the body's sensitivity to insulin by increasing glucose transport and metabolism, reducing blood glucose levels. It has been speculated that vanadium pentoxide is genotoxic and can cause cancer. Using a wall-less mutant of *Neurospora crassa* that contains an insulin receptor in its cell membrane and human embryonic fibroblast cells, cells were subject to varying treatments of vanadium pentoxide and insulin to test the hypothesis that vanadium pentoxide is damaging to DNA and can be mediated by insulin. Unexpectedly, vanadium treatments showed a positive effect on cell viability, which was more apparent in the old cells than the younger cells. The results in this study are not conclusive and require more established data.

Introduction

Diabetes Mellitus is illustrated as a chronic metabolic disorder that clinically presents with elevated blood glucose levels due to failure of the pancreas and its ability to produce enough insulin. Diabetes can be further divided into two categories: type 1 and type 2. Type 1 diabetes is the result of an autoimmune obliteration of β cells; the factory of insulin for the body. Type 2 diabetes; once called adult-onset diabetes is a result of insulin resistance and failure to generate insulin. Insulin, being the primary vehicle needed for glucose to enter cells, needs to be provided synthetically to meet the body's needs for energy. Diabetes can have major implications on one's long-term health with complications including; heart disease and increased risk of infection. The prevalence of diabetes has increased to almost five percent in the U.S (Permutt 2005). The primary treatment for most types of diabetes is the injections of insulin typically for the rest of one's life. Other treatments, including chromium and vanadium, are frequently being used to help minimize the effects of insulin resistance.

Vanadium is a hard transition metal and essential micronutrient that is present at very low concentrations in most plant and animal cells (Liu 2012). Vanadium pentoxide (V_2O_5) has lately been used to help treat chronic diseases, like diabetes, showing effects on glycemic metabolism by reducing blood glucose levels (Liu 2012). Its' purposed role in diabetes 1 and 2 is to substitute insulin or to increase the body's sensitivity to insulin by increasing glucose transport and metabolism (Iglesias-Gonzalez 2012). Vanadium pentoxide has lately come under scrutiny for causing serious side effects, such as damage to DNA and over time this damage could to lead to cancer.

Neurospora crassa (FGSC stock number 4761) possess an insulin receptor in their cell membranes, and wall-less mutants make the cell much more susceptible to treatment (Gaddameedi 2011). This makes *Neurospora crassa* a good model for studying the toxicity of V_2O_5 in combination with insulin. The U.S National Toxicology Program did an inhalation study with V_2O_5 in rats and mice and determined there was *some evidence of carcinogenic activity* of V_2O_5 in male and female rats and *clear evidence of carcinogenic activity* of V_2O_5 in male and female mice (2002). To our knowledge the genotoxic effects of V_2O_5 have yet to be determined at the cellular level. This study will explore the genotoxic effect of vanadium pentoxide in the wall-less mutant of *N. crassa* and human embryonic fibroblast cells and whether its effect can be mediated by insulin.

Materials & Methods

Organisms:

The wall-less mutant of *Neurospora crassa* (FGSC 4761) was obtained from Fungal Genetic Stock Center. The cells were grown on a slant for 24 hours at 28°C in Vogel's supplemented medium (VSM) with 2% (w/v) glucose. 50ml of *N. crassa* cells were added to six flasks containing 2% (w/v) high glucose media at 28°C with shaking for 21 days.

The human embryonic fibroblast cells (cell line GM00011, passage 13) were obtained from Coriell Institute, Camden, NJ. Cells were added to 15 wells in a 24 well plate with 500µl solution containing Eagle's

Minimum Essential Medium with Earle's salts, 10% FBS, 0.8% L-glutamine, 100(IU/ug)/ml penicillin/streptomycin grown at 37°C, 5% CO_2, 95% humidity for five days prior to treatment.

Treatments:

Six flasks containing 50µl *Neurospora crassa* cells were incubated in high glucose media with varying amounts of insulin bovine and V_2O_5 for varying time periods in a 50ml solution. Flask one served as the negative control and contained *N. crassa* cells in high glucose media, flask two contained *N.crassa* with 1x V_2O_5 solution, flask three contained *N. crassa* with 1000x V_2O_5 solution, flask four contained *N. crassa* with 1x V_2O_5 and 100x insulin solution, flask five contained *N. crassa* with 1000x V_2O_5 and 100x insulin solution, and flask six served as the positive control and contained *N. crassa* with 5x MMS solution as a positive control. All flasks were incubated at 28°C with shaking. Cell viability tests were done at 0 hour, 24 hours, and 48 hours. Experiment was repeated and tested three times.

Prior to treatment 15 wells in cell well plate contained $5x10^4$ cells. Three wells were committed to five different treatments. Wells one to three contained 1000x V_2O_5, wells four to six contained 1000x V_2O_5 and 100x insulin, wells seven to nine contained 1x V_2O_5, wells ten to twelve contained 1x V_2O_5 and 100x insulin, wells thirteen to fifteen contained just cells and minimal media as the negative control. Well plate incubated at 37°C, 5% CO_2, 95% humidity for 48 hours before cell viability tests were done.

Cell Viability:

To test cell viability of the *Neurospora crassa* cells at 0 hours, 24 hours, and 48 hours after exposure, a 20µl aliquot was extracted from each flask. Samples were centrifuged for four min at 3500xg. Supernatant was discarded. Cell pellet was resuspended in 1x PBS and 0.4% trypan blue in a 1:1 ratio. Cells were counted using a hemocytometer.

To test cell viability of human embryonic fibroblast cells, cells were vacuumed to remove media from the top of solution and remaining attached cells were washed with 1x PBS. A detaching solution containing 200µl 0.25% trypsin and 0.1% EDTA, heated to 37°C was added to each well; then incubated for five minutes. 300µl of Eagle's media was added bringing final well volume to 500µl. Cell viability was tested using 25µl of cells from each well with a 1:1 ratio of 0.4% trypan blue. Four squares of hemocytomer were counted and averaged to find total living cells in each well.

Results

To test the genotoxic effect of vanadium *N. crassa* cells and human fibloblast cells were treated with different amounts of vanadium with and without insulin (Figure 1).

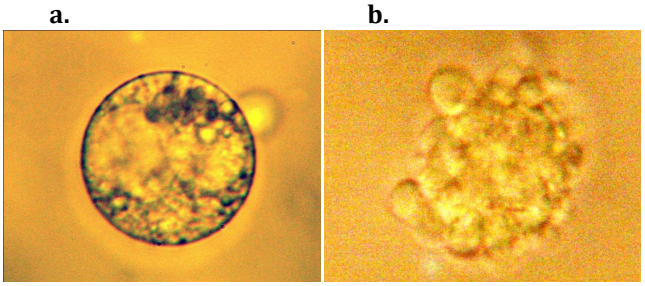

Figure 1 a. Wall-less mutant of *Neurospora crassa* cell after incubation with 1000x V_2O_5 during trypan blue exclusion test. Cell is alive and membrane is still intact. b. Single, dividing human embryonic fibroblast cell also after incubation with 1000x V_2O_5 and during a trypan blue exclusion test.

Three independent experiments using *Neurospora crassa* show experimental variation when considering cell viability results of different vanadium treatments. Figures 2a, 2b, and 2c show the cell viability of *Neurospora crassa* cells after incubation with V_2O_5 and insulin over a 48 hour time period. In experiments one and two MMS, known to be damaging to DNA, was used as a positive control.

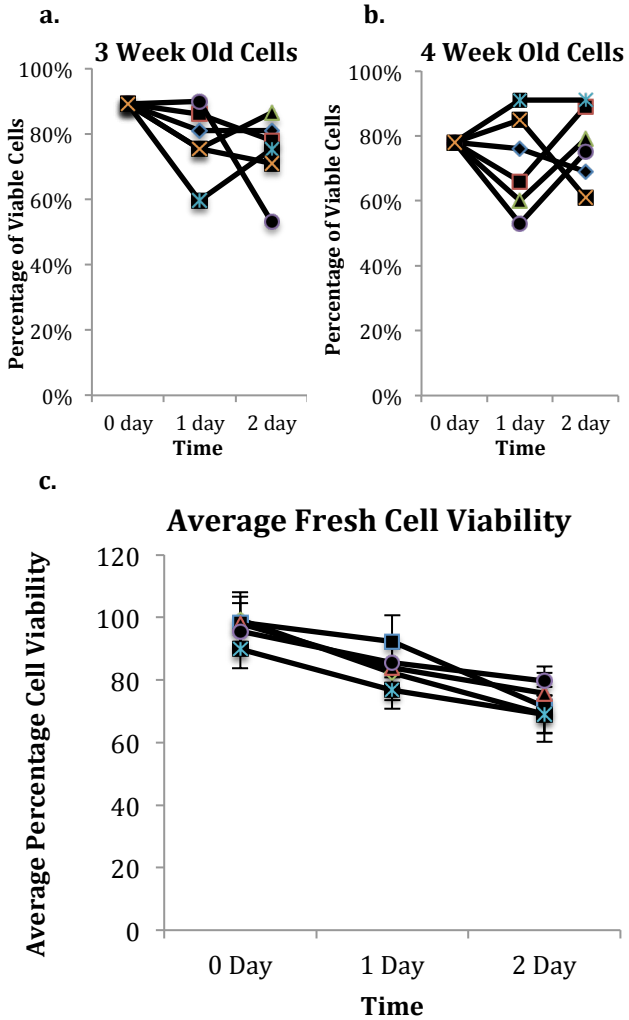

Figure 2a. Cell viability after vanadium treatments. Graph shows cell viability of *Neurospora crassa* cells after incubating with

various concentrations of V_2O_5 and insulin after being analyzed using trypan blue exclusion over a 48-hour time span. Results are of the first individual experiment. b. Displays cell viability of *Neurospora crassa* cells after incubating with various concentrations of V_2O_5 and insulin after being analyzed using trypan blue exclusion over a 48 hour time span. Results are of the second individual experiment. c. Graph shows cell viability of *Neurospora crassa* cells after incubating with various concentrations of V_2O_5 and insulin after being analyzed using trypan blue exclusion over a 48 hour time span. Three samples were tested from each flask. In each graph, the negative control is represented by a diamond, 1x vanadium by a square, 1000x vanadium by a triangle, 1x vanadium with insulin by a circle, 1000x vanadium with insulin by an asterisk, and the positive control by an X.

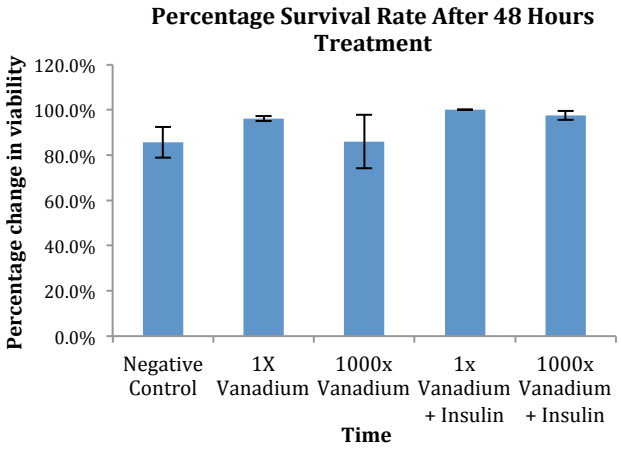

Figure 3. Assuming cell viability prior to treatment with various concentrations of V_2O_5 and insulin was 100%, these results display the average change in cell viability for the treatment of human embryonic fibroblast cells. Error bars represent standard error. Results are of one independent experiment,

Figure 2a portrays a steady decline in cell viability in the negative control and treatment with 1x V_2O_5 and a prominent, yet steady decline in the positive control. 1x V_2O_5 and insulin treatment resulted in a slight increase in cell viability, followed by a significant decline. Both the 1000x V_2O_5 treatments with and without insulin resulted in a gradual decline in cell viability, followed by a considerable decline. Figure 2b graphs results of experiment two and shows similar results with the negative and positive controls as well as treatment with 1000x V_2O_5. The 1x V_2O_5 treatment varied, as it showed a significant decline, then increase in cell viability. Treatment with 1x V_2O_5 and insulin had an inverse relationship with the results of the first experiment, with a decline then increase in cell viability. Treatment with 1000x V_2O_5 and insulin caused a steady increase in cell viability over two days. Figure 2c portrays the results of experimental three, which is an average of three tests performed on each treatment sample. No positive control was added in this experiment. The negative control gave similar results with a steady decrease in cell viability. Treatment with 1x V_2O_5 showed a gradual, then prominent decrease in cell viability, whereas treatment with 1000x V_2O_5 with and without insulin as well as 1x V_2O_5 with insulin resulted in a gradual reduction in cell viability. Ttests comparing cell viability in *Neurospora crassa* results after 48 hour incubation revealed p-values of 0.719 comparing the negative control and treatment of 1x V_2O_5, 0.106 comparing 1x V_2O_5 treatments and 1x V_2O_5 and insulin treatments, 0.496 comparing 1000x V_2O_5 and 1000x V_2O_5 with insulin, 0.306 comparing 1x V_2O_5 and 1000x V_2O_5, 0.317 comparing 1x V_2O_5 with insulin and 1000x V_2O_5 with insulin, and 0.126 comparing negative control and 1000x V_2O_5. Figure 3 displays cell viability results 48 hours after treatment with 1x V_2O_5, 1000x V_2O_5, and insulin. Assuming cell viability was 100% prior to treatment negative control experienced 14%, 1x V_2O_5 a 4%, 1000x V_2O_5 a 14% reduction, 1x V_2O_5 +Insulin a 0% change, and 1000x V_2O_5+Insulin a 3% change all experienced a decrease in cell viability. Ttests comparing cell viability in human embryonic fibroblast cell results after 48 hour incubation incubation revealed p-values of 0.258 comparing the negative control and treatment of 1x vanadium, 0.078 comparing 1x vanadium treatments and 1x vanadium and insulin treatments, 0.433 comparing 1000x vanadium and 1000x vanadium with insulin, 0.479 comparing 1x vanadium and 1000x vanadium, 0.319 comparing 1x vanadium with insulin and 1000x vanadium with insulin, and 0.977 comparing negative control and 1000x V_2O_5.

Discussion

Cells used in experiment one were approximately three weeks old before incubation treatments began, cells used in experiment two were approximately four weeks added to fresh media before treatments, and cells from experiment three were frozen cells that thawed overnight and added to fresh media. The age of the cells used in each experiment could explain the variation in the results.

The results of experiments one and two were more similar in consideration with experiment three. Comparing treatments with 1x V_2O_5 in all experiments show an increase in cell viability in experiment two and a decrease in cell viability with experiments one and three. Treatments of 1x V_2O_5 and insulin show similar results in experiment one and three with an eventual deterioration of living cells, whereas experiment two caused an increase. Similar results occurred again in experiment one and two with treatments containing 1000x V_2O_5 with and without insulin causing an accession of cell viability. Insulin alone does not seem to affect cell viability or in combination with any concentration of V_2O_5. 1000x V_2O_5 with and without insulin appear to have a compelling response causing an exaggeration in cell viability in older cells.

Ttests done comparing p-values of various vanadium and insulin treatments in *Neurospora crassa* and human embryonic fibroblast cells were >0.05. Based on these results, it is concluded that with 95% certainty the results

are consistent with the null hypothesis that V_2O_5 is not genotoxic.

Vanadium is an essential micronutrient available in very low concentrations in cells (Liu 2012). We conclude that based on V_2O_5 role in the body that older, nutrient deprived cells will rely on V_2O_5 for nutrients and in turn, less cell death will occur. Younger cells, as seen in experiment three, rely less on the additional nutrients that V_2O_5 provides and more on the available glucose media and experience gradual cell death similar to what is seen in the negative control. The results disprove the original hypothesis that V_2O_5 is gentoxic, instead it appears to improve cell viability by providing them with an essential micronutrient that extends their lives. Repeated experiments will give more insight into possible trends in varying treatments and how they affect cells depending on their age and also on the effects of long term exposure to V_2O_5.

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References:

Gaddameedi, R. R., S. Burgula, et al. (2011). "Role of insulin in Cr(VI)-mediated genotoxicity in Neurospora crassa." Letters in applied microbiology **53**(1): 14-21.

Iglesias-Gonzalez, T., C. Sanchez-Gonzalez, et al. (2012). "Absorption, transport and insulin-mimetic properties of bis(maltolato)oxovanadium (IV) in streptozotocin-induced hyperglycemic rats by integrated mass spectrometric techniques." Analytical and bioanalytical chemistry **402**(1): 277-285.

Liu, Z., P. Li, et al. (2012). "Protection by Vanadium, a Contemporary Treatment Approach to Both Diabetes and Focal Cerebral Ischemia in Rats." Biological Trace Element Research **145**(1): 66-70.

National_Toxicology_Program (2002). US National Toxicology Program Technical report on the studies of vanadium pentoxide (CAS no. 1314-62-1) in F344/N rats and B6C3F1 mice (inhalation studies) P. h. S. U.S Department of Health and Human Services, National Institutes of Health. Research Triangle Park.

Permutt, M. A., J. Wasson, et al. (2005). "Genetic epidemiology of diabetes." The Journal of clinical investigation **115**(6): 1431-1439.