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Comparison of World Trade Center dust with zinc acetate and lead oxide combinations to determine damage to human lung cells

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The exposures of the World Trade Center (WTC) dust of the September 11, 2001 tragedy are linked to increased respiratory problems and emergence of World Trade Center Cough. Zinc (Zn) and Lead (Pb) are among the many heavy metals found in WTC dust. Synergistic effects among several chemicals might be contributing to respiratory illness. The individual toxicity, and combination treatments of Pb and Zn at a particular ratio of concentration (1:10) typical to Market Streets were carried out on MRC-5 human lung cells. Cells were treated as both physiologically stressed (2.5% Fetal bovine serum, {FBS}) and unstressed (10% FBS) condition *in vitro*. Triplex assays indicated that Pb and Zn do show antagonistic effects with each other. The combination treatments of both Pb and Zn reduced high individual toxicity of either alone, in both stressed and unstressed cells. In summary, Pb, which is known to be highly damaging to human lung cells is modified by the presence of Zn. It can be possible that Zn is a reward in disguise to WTC victims. Without unusual levels of Zn, Pb might be much more damaging to those exposed to WTC dust.

Key words: World Trade Center, toxicity, synergistic, antagonistic, stressed cell, unstressed cell, heavy metals.

INTRODUCTION

The explosion and collapse of the World Trade Center (WTC) produced an aerosol plume affecting many workers, residents, and commuters. The inorganic analyses of three bulk samples indicated high amount of metals, ionic species, and asbestos. The organic analyses implied polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls, polychlorinated ibenzodioxins, pesticides, phthalate esters, brominated diphenyl ethers, and other hydrocarbons. The morphological analyses of the mass were fibrous, consisting of mineral wool, fiberglass, asbestos, wood, paper, and cotton (Lioy et al., 2002). The very high levels of exposure are linked to increased cardiovascular deaths, respiratory problems, and emergence of WTC cough.

"WTC cough" first emerged within 24 h after the buildings collapsed. Rescue workers showed symptoms which resulted in the diagnosis of Reactive airway dysfunction syndrome (RADS) that includes wheezing, difficulty breathing, bronchial constriction, reduced lung volume capacity, as well as possible abnormalities in the morphology of cells lining the air passages (Greenberger, 2008). Though research is ongoing, processes responsible for decreased lung function have not yet been understood on a cellular level. It has been found that workers and residents within a 1 mile radius of the WTC site are still affected by lower respiratory symptoms (Lin et al., 2010). The most recent study indicated that for average forced expiratory volume (FEV) decreased significantly

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significantly without recovery over a six year period (Aldrich et al., 2010).

Asthma prevalence among children less than 5 years of age was higher than National estimates, and new asthma diagnosis was associated with dust cloud exposure in all age groups of children (Thomas et al., 2010). WTC dust (at least the super coarse particle fraction) or cigarette smoke extract (CSE) alone has been demonstrated to direct adverse effects on airway epithelial and smooth muscle cells.

In addition, the combination of CSE and very small sized particulate matter from WTC exerted an interactive effect on cell toxicity (Xu et al., 2011). Studies of the exposure to WTC dust led to decreased cell proliferation and increased apoptosis levels in cell grown *in vitro* (Lambroussis et al., 2009). The components that make up the dust may have an additive effect, even if the components on their own are non reactive or non-toxic. One toxicological profile when combined with another can alter the properties of the original, making the combined profile more toxic than the individual effects alone (Donaldson et al., 1998; Ferin, 1994).

Occupational inhalation exposures to heavy metals are associated with lung cancers (Fatur et al., 2003). Materials within the WTC dust included lead, asbestos, glass, as well as numerous aromatic ring compounds (Greenberger, 2008). The International Agency for Research on Cancer (IARC) classified Pb as possible human carcinogen (group 2B) (IARC, 1987) and inorganic Pb compounds as probable human carcinogens (group 2A) (IARC, 2006). The WTC dust contains Pb that was produced from the electrical wiring, power cords, plumbing and general building material. About 50,000 personal computers were destroyed, with each containing approximately 4 pounds of Pb (Nordgrén et al., 2002). There has been found 1 to 3 g/kg of Zn present in WTC dust, and the concentration of Zn exceeds 1,000 ppm in all dust collections. The trace amount would normally be considered less than 10 ppm (Tahil, 2006).

The respiratory system is a direct target of possible environmental carcinogens (Klein-Szanto et al., 1982). It is important that the metal ions should be maintained within strict limits to maintain human health (Bertini and Cavallaro, 2008). It has been proposed that Zn acts as an antioxidant and a chelator agent in lead toxicity (Dykeman et al., 2002; Lidsky and Schneider, 2003; Huang and Schneider, 2004; Shalan et al., 2005; Bellinger, 2006). Zinc co-administration might alleviate toxic effects of Pb on the male reproductive system, whereas it could enhance the toxicity on thyroid function. Divalent Pb and Zn have similar chemical properties and it is possible that Pb can influence the absorption and distribution of Zn in blood and other tissue. The double-edged effects of Zn on Pb toxicity on different organ systems should be evaluated. The protective effect of Zn on reproductive toxicity of Pb may be attributed to competition between Pb and Zn, or reduction of available Pb-binding sites in the testicular tissue (Fengyuan et al., 2007).

There is a power relationship between Pb and Zn in WTC dust. Also, in almost all WTC sample collected, the ratio of Pb and Zn varies from 1:7 to 1:12. In the market street samples, the ratio of concentration between Pb and Zn is around 1:10 (Tahil, 2006). In this study, Zn and Pb are used in ratios found in WTC dust. These heavy metals alone, and in conjunction with WTC dust, become indicators of damage to human lung cells shown by changes in cytotoxicity.

MATERIALS AND METHODS

MRC-5 human lung fibroblast cells were obtained from American Type Culture Collection. Cells lines were initially grown in Eagles Minimum Essential Medium (MEM) that had been supplemented with 1 ml of Penicillin-streptomycin, 1 ml of glutamine, 1 ml of kanamycin and 10 ml Fetal Bovine Serum (FBS) per 100 ml of media. The experiments utilized stressed MRC-5 cells that were exposed to 2.5% FBS and unstressed MRC-5 cells that were grown in 10% FBS. This study used lower percentages of FBS in the media to simulate the effects of physiological stress on the cells. Cells were allowed to grow to a confluent monolayer until needed.

Zinc acetate $\{Zn(O_2CCH_3)_2\}$ and Lead oxide (PbO) were used in these experiments, as these were completely soluble in media. One (1 g) PbO was dissolved in 100 ml of complete medium with 10 and 2.5% FBS, respectively. These solutions represent 10,000 ppm stock solution for PbO for both stressed and unstressed conditions. Appropriate dilutions were performed to make 1, 50, 100, 300, and 500 ppm. Similar procedure was followed to prepare 10,000 ppm stock solution of $\{Zn(O_2CCH_3)_2\}$, and 10, 100, 500, 1000, and 3000 ppm solutions for zinc acetate in both 10 and 2.5% FBS were prepared. All the procedures were carried out aseptically.

The combination treatments for the experiments are the combination of Pb and Zn in specific ratio (1:1, 1:5, 1:10 and 1:15) of their concentration in 10 and 2.5% FBS in complete medium. All ratios were followed to comply with the ratios of these heavy metals found in the analyzed dust from the WTC (Lioy et al., 2002). The preparation of the required combinations followed those shown in Table 1.

To prepare for assays, MRC-5 cell cultures were scraped, centrifuged, and seeded in fresh complete medium containing 10% FBS. A 100 µl of cells were plated into 3 black 300 µl 96-well clear flat bottom plates and incubated for 24 h at 37°C. After a 24 h incubation period, the old media was aseptically removed and various solutions of $\{Zn(O_2CCH_3)_2\}$, PbO and combination of $\{Zn(O_2CCH_3)_2\}$ and PbO solution were put in the designated well. In all assays performed in this type of 96-well plates, two wells with media were used for negative control.

The toxicity level of Zn, Pb and their interaction were analyzed by using ApoTox-Glo Triplex Assay obtained from Promega, Inc. Fluorescence was measured at 485 nm emission and 528 nm excitation to determine cytotoxicty, using a Bio-Tech Synergy 2 multi plate reader.

Statistical analysis

All data are expressed as mean ± standard deviation (SD). For statistical analyses, One-way Analysis of variance (ANOVA) with Student Newman Keul post-hoc analysis was used to determine significant difference between exposure and control.

Table 1. Different concentrations and volume of PbO and {$Zn(O_2CCH_3)_2$} taken to prepare required WTC dust ratios of combination.

Ratio of PbO/{Zn ($O_2CCH_3)_2$}	Concentration of PbO (ppm)	Concentration of {Zn ($O_2CCH_3)_2$} (ppm)	Volume of PbO (µl)	Volume of {$Zn(O_2CCH_3)_2$} (µl)	Final concentration of PbO (ppm)	Final Concentration of {$Zn(O_2CCH_3)_2$} (ppm)
1:1	100	100	50	50	50	50
1:5	200	1000	50	50	100	500
1:10	100	1000	50	50	50	500
1:15	200	3000	50	50	100	1500

RESULTS

Cytotoxicity of combination treatment

Toxicity of cells stressed by reduction in serum level is consistently higher than unstressed cells. This is visualized in Figures 2, 3a and b. The combination treatment of 1:10 ratio of Pb and Zn represent the established market street Pb and Zn ratio for these heavy metals (Lioy, 2002).

The analysis of Figure 5 indicates that Pb at 50 ppm and Zn at 500 ppm generate an expected high toxicity in unstressed condition. Combination of these two treatments producing a ratio of their concentration of (1:10) create a toxicity considerably lower as compared to individual toxicity of Pb or Zn. Combination treatments were able to reduce the high amount of toxicity produced by Pb in unstressed condition. Figure 4 demonstrates that the combination treatments of Pb and Zn produce a toxicity which is very low in comparison to the individual toxicity of Pb and Zn in stressed condition. Thus, combination treatment is able to reduce the individual toxicity of Pb or Zn in stressed condition.

DISCUSSION

The results of these experiments have shown that toxicity is always highest in cells stressed by serum level reduction. Surprisingly, the high amount of Zn reduced the damaging effects of Pb both in stressed and unstressed cell. It indicates Zn and Pb appear to show some antagonistic interaction with each other in human lungs cells *in vitro*. The issue of chemical additivity and synergism is particularly important in regards to the unique mixture created by the unprecedented circumstances of the WTC event (Goad et al., 2004). Chemicals are said to exert an antagonistic effect when combination of both metals have an overall effect that is less than the sum of their individual effects (Peyrat-Maillard et al., 2003). From Figures 4 and 5, it is seen that Zn administration could reduce 92% of Pb toxicity in unstressed condition whereas it could reduce 72% of Pb toxicity in stressed condition.

By comparing Figures 4 and 5, it can be concluded that antagonistic effects of Zn and Pb is lower in stressed cell in comparison with unstressed cells. It can be concluded that Pb and Zn had antagonistic effects which reduced the high amount of individual toxicity of Pb or Zn both in stressed and unstressed condition. Divalent Pb and Zn have similar chemical properties and it is possible that Pb can influence the absorption and distribution of Zn in blood and other tissue by interfering with the transport system of Zn. The previously published evidence for a protective effect of Zn on the reproductive toxicity of Pb may be attributed to competition between Pb and Zn, or reduction of available Pb-binding sites in the testicular tissue (Fengyuan et al., 2007).

Additional investigations should be continued to study the cytotoxicity of Zn in unstressed and stressed condition. The *in vitro* stressed conditions brought on by serum lowering or deprivation, are a representation of the actual physiological stress conditions of some of the individuals exposed to the particulate matter of the WTC tragedy. Zinc, in the form of zinc acetate, is highly basic and needs an acidic environment to dissolve. Even if concentration is very high, it may not be dissolved in lung cells enough to demonstrate its toxicity. Thus, toxicity of Zn should be tested in other cells such as nerve cells and stomach cells. Stomach acid contains hydrochloric acid, in which metallic zinc dissolves readily to give corrosive zinc chloride (Bothwell et al., 2003).

Comparing the graphs of Figures 2, 3a and b, it is concluded that the toxicity in stressed cells is at considerably higher levels than unstressed cells. In Figure 2, Pb has higher toxicity in all the treatment in stressed cell than unstressed cell. Comparing the graphs of Figure 3a and b, it is seen that the toxicity of stressed cell is unpredictably higher than the unstressed cell in all the treatments, including 1:5, 1:10 and 1:15 ratios of treatment. The ratio of 1:10 (Pb: Zn) represents market street Pb and Zn ratio, and the toxicity of stressed cell is 40.79% higher than toxicity of unstressed cell. This amount of increased toxicity of

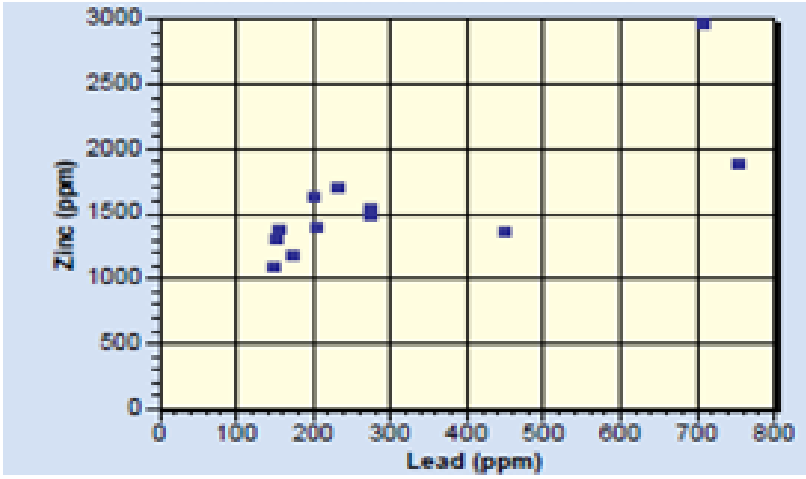

Figure 1. The ratio of concentration of Pb and Zn in WTC; not including Girder Coating Samples (Tahil, 2006).
It shows that the ratios of Pb to Zn in every collected sample are between 1:7 and 1:12.

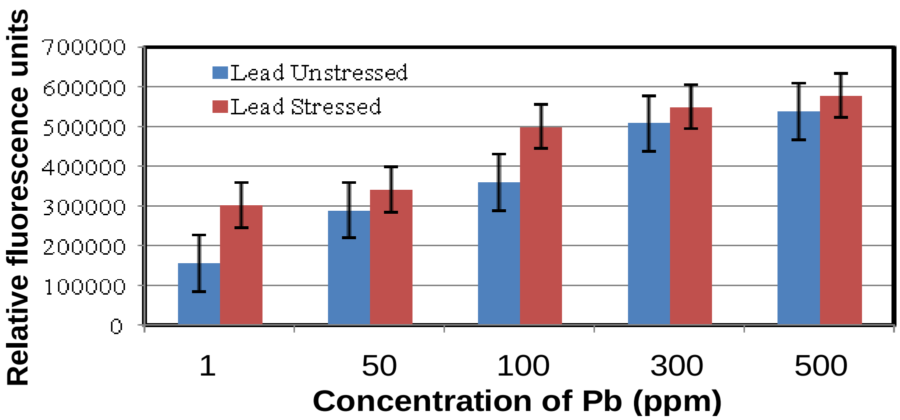

Figure 2. Cytotoxicity of Pb in unstressed and stressed condition. The toxicity increases as the concentration of Pb increases.

stressed cell is unpredictably high.

Figure 1 indicates the Pb and Zn ratio of WTC which is in between 1:7 and 1:12. It is predicted that in the areas of WTC containing these ratios, there must have been considerably higher toxicity in stressed cell than unstressed cell. Higher concentrations of FBS provide cells with a variety of proteins needed for survival and growth. The cells exposed to 10% FBS have access to higher levels of proteins that allow for optimal growth. Cells grown in 2.5% FBS are grown in more physiological stressful environments than cells grown in 10% FBS. The increase in toxicity noted in cells grown in lower FBS concentrations indicate that lower concentrations of FBS show a possible negative impact on cell growth at the various concentrations and ratios of Pb and/or Zn. This data implies that a person having lower resistance due to mental or physiological stress might actually be more susceptible to toxicity and cellular damage.

CONCLUSION AND RECOMMENDATION

The repeated experimental uses of assays to measure cytotoxicity demonstrate that Pb is capable of damage to cultured human lung cells in both stressed and unstressed condition. This study evolved out of a concern for the potential long-term lung problems faced by victims of exposure to the dust and other particulate matter from the WTC tragedy. Our research demonstrated that the high level of Zn, contrary to our expectation, displays an antagonistic effect with Pb which reduced the highly toxic effects of Pb. The very high concentration of Zn, ranging

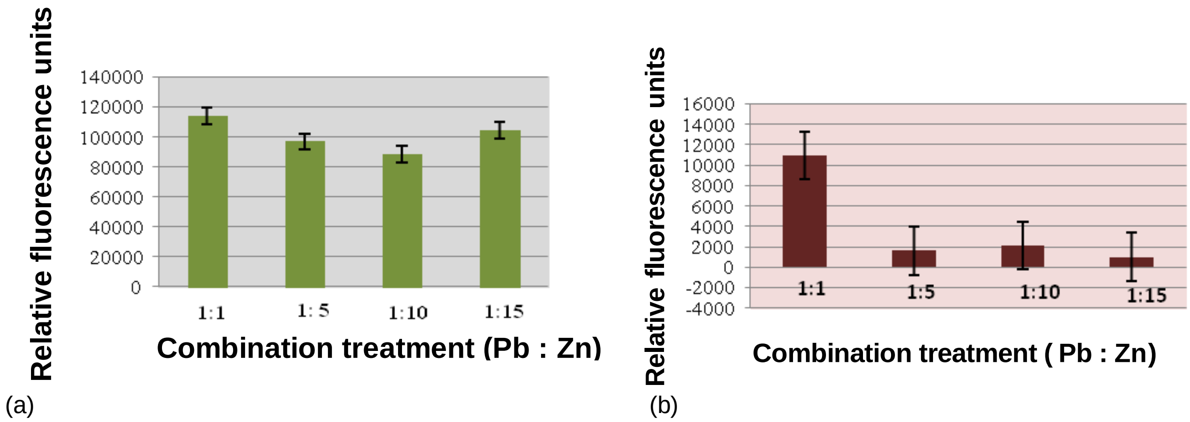

Figure 3. Graphs showing cytotoxicity of combination treatment of different ratio of Pb : Zn in (a) stressed and (b) unstressed condition. The Pb : Zn ratio of market street dust as 1:10.

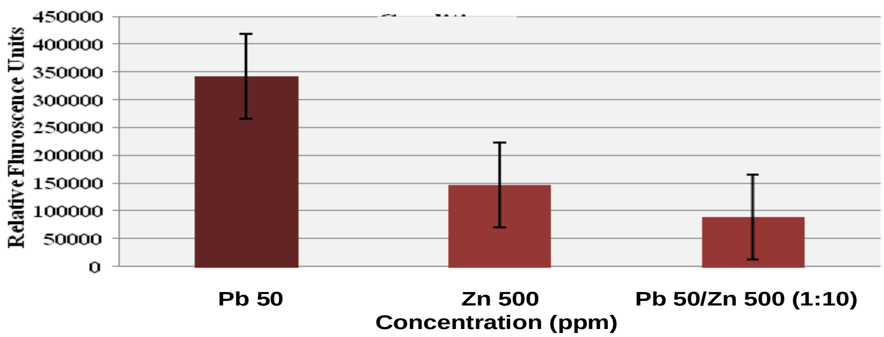

Figure 4. Comparison of individual toxicity of Pb and Zn with combination toxicity of Pb and Zn at 1:10 ratio in stressed condition. The graph shows that individual toxicity of Pb at 50 ppm is 342069.5 Relative Fluorescence Units (RFU) and individual toxicity of Zn at 500 ppm is 146372.5 RFU. Combination treatment of Pb at 50 ppm and Zn at 500 ppm gives a toxicity of 88383.5 RFU.

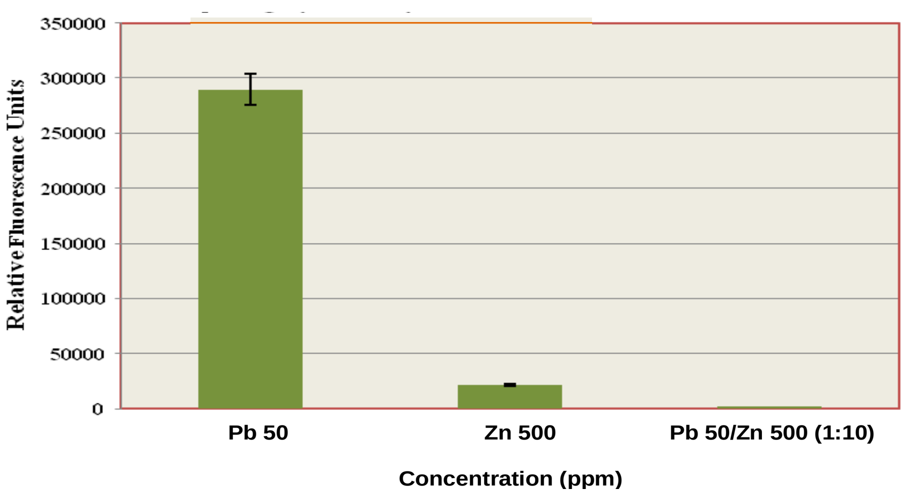

Figure 5. Comparison of individual toxicity of Pb and Zn with combination toxicity of Pb and Zn at 1:10 ratio of both metal’s concentrations in unstressed condition. Graph shows that individual toxicity of Pb at 50 ppm is 289,690 Relative Fluorescence Units (RFU) and individual toxicity of Zn at 500 ppm is 21,144.5 RFU. Combination treatment of Pb at 50 ppm and Zn at 500 ppm gives a toxicity of 2117.5 RFU.

from 1,000 to 3,000 ppm, is a “reward in disguise” to WTC victims, without which Pb could be much more damaging. The toxicity of Pb may not be damaging to healthy people because of the protective role of Zn but it can be troublesome to infants, diseased, sick, old and those with low resistance due to stress. Though more research is needed to properly identify the potential risk of heavy metals from the WTC dust, the results in this study suggest that Pb is more damaging to low resistant people, and the protective role of Zn may be able to hide the damaging effect of Pb to normal healthy people.

Additive and synergistic effects on toxicity are possible from the interaction of other hazardous substances found in the WTC dust. Synergistic and additive effects can occur with asbestos, PAHs and polychlorinated biphenyls (PCBs), with particulate matter and PAHs, with Pb and Hg(mercury), and also with dioxin and PCBs (Goad et al., 2004). Future research should be carried out for possible interaction between Pb and mercury. The individual toxicity, viability, and apoptosis experiments of Zn should be designed to study effects in both stressed and unstressed condition. In addition to lung cells, neuronal cells and in particular, stomach cells, should be tested to demonstrate the possible toxicity levels of Zn in these organs.

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