

Effect of the militarily-relevant heavy metals, depleted uranium and heavy metal tungsten-alloy on gene expression in human liver carcinoma cells (HepG2)

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Abstract

Depleted uranium (DU) and heavy-metal tungsten alloys (HMTAs) are dense heavy-metals used primarily in military applications. Chemically similar to natural uranium, but depleted of the higher activity 235U and 234U isotopes, DU is a low specific activity, high-density heavy metal. In contrast, the non-radioactive HMTAs are composed of a mixture of tungsten (91–93%), nickel (3–5%), and cobalt (2–4%) particles. The use of DU and HMTAs in military munitions could result in their internalization in humans. Limited data exist however, regarding the long-term health effects of internalized DU and HMTAs in humans. Both DU and HMTAs possess a tumorigenic transforming potential and are genotoxic and mutagenic *in vitro*. Using insoluble DU-UO₂ and a reconstituted mixture of tungsten, nickel, cobalt (rWNiCo), we tested their ability to induce stress genes in thirteen different recombinant cell lines generated from human liver carcinoma cells (HepG2). The commercially available CAT-Tox (L) cellular assay consists of a panel of cell lines stably transfected with reporter genes consisting of a coding sequence for chloramphenicol acetyl transferase (CAT) under transcriptional control by mammalian stress gene regulatory sequences. DU, (5–50 µg/ml) produced a complex profile of activity demonstrating significant dose-dependent induction of the hMTIIA FOS, p53RE, Gadd153, Gadd45, NFκBRE, CRE, HSP70, RARE, and GRP78 promoters. The rWNiCo mixture (5–50 µg/ml) showed dose-related induction of the GSTYA, hMTIIA, p53RE, FOS, NFκBRE, HSP70, and CRE promoters. An examination of the pure metals, tungsten (W), nickel (Ni), and cobalt (Co), comprising the rWNiCo mixture, demonstrated that each metal exhibited a similar pattern of gene induction, but at a significantly decreased magnitude than that of the rWNiCo mixture. These data showed a synergistic activation of gene expression by the metals in the rWNiCo mixture. Our data show for the first time that DU and rWNiCo can activate gene expression through several signal transduction pathways that may be involved in the toxicity and tumorigenicity of both DU and HMTAs. (Mol Cell Biochem 255: 247–256, 2004)

Key words: depleted uranium, heavy-metals, tungsten alloy metals, HEPG2 cells, gene expression

Abbreviations: DU – depleted uranium; HMTA – heavy metal tungsten alloys; rWNiCo – reconstituted tungsten, nickel, cobalt mixture

Introduction

Heavy-metals such as depleted uranium (DU) and tungsten are used as kinetic energy penetrators in military applications. While the use of DU in these applications has been limited

to the United States, heavy-metal tungsten alloy (HMTA) penetrators (tungsten/nickel/cobalt) are manufactured and tested in numerous countries and are deployed worldwide. A friendly-fire accident that occurred during the 1991 Gulf War, resulting in US soldiers with retained large DU-frag-

ments (approximately 2–20 mm), has focused attention on the potential health effects of internalized heavy metals like tungsten and DU used in military applications. In future conflicts, the United States will have to deal with an increased number of casualties from the use of these weapons. Because both DU- and HMTA-based munitions are relatively recent additions to the list of militarily relevant metals, little is known about the health effects of these metals after internalization as embedded shrapnel.

Chemically similar to natural uranium [1], DU is a low specific activity radioactive heavy metal, with a density approximately 1.7 times that of lead (19 g/cm³ vs. 11.35 g/cm³). DU differs from natural uranium in that it has been depleted of ²³⁵U and ²³⁴U. As a result, the specific activity of DU is significantly less than natural uranium (0.4 µCi/g vs. 0.7 µCi/g, respectively) [2]. In contrast, HMTA-penetrators consist of a combination of tungsten, nickel, and either cobalt or iron (tungsten greater than 90%; nickel, 1–6%; iron 1–6%; or cobalt approximately 1–6%) [3]. Unlike DU, HMTA's are not radioactive.

Several recent studies have investigated the potential health effects of these militarily relevant heavy metals [1–2, 4–8]. These *in vitro* and *in vivo* investigations have not only demonstrated the transforming ability [1] and the mutagenicity [2] of DU, but also its neurotoxicity *in vivo* [6]. Studies using neoplastically-transformed human cells and the athymic nude mouse assay, demonstrate the carcinogenic potential of DU [1]. Similarly, we have shown that HMTA (tungsten/nickel/cobalt-rWNiCo) is genotoxic to human cells and causes malignant cell transformation *in vitro* [7]. A common mechanism by which both DU and rWNiCo induce cell transformation *in vitro* does appear to involve, at least partially, direct damage to the genetic material manifested as increased DNA breakage or chromosomal aberrations (i.e. micronuclei) [7]. Both militarily relevant heavy metals have been shown to induce genomic instability in a human cell model [8]. The carcinogenicity of both DU and the HMTA (tungsten/nickel/cobalt) is currently being evaluated in our laboratory using a rodent model.

There is little available epidemiological evidence among humans with which to conclude that these metals are carcinogenic or not. While epidemiological studies have linked uranium mining and milling to human carcinogenesis [1], there are no published studies to permit an accurate assessment of risks for carcinogenesis from internalized DU. Similarly, there is no information regarding the health effects of imbedded HMTAs. Studies have shown that occupational exposure to hard metal dust, a mixture of cobalt- and tungsten carbide-containing particles, is associated with development of different pulmonary diseases including fibrosing alveolitis and lung cancer [9–12]. The HMTAs used in military applications however, are somewhat different from

conventional hard metal. HMTA-penetrators consist of a combination of tungsten, nickel, and either cobalt or iron, in contrast to hard metal dust, which is a mixture of cobalt metal and tungsten carbide particles [3]. The differences in metal composition and percentages of hard metal particles and tungsten alloys used in military applications preclude the assumption that the biological effects of hard-metal particles and tungsten-alloy particles would be the same.

Therefore, in view of carcinogenesis risk estimates and medical management questions relevant to possible future incidents of DU and HMTA internalization, an examination of molecular and cellular effects, including mechanistic studies of DU and tungsten alloys are necessary to understanding the potential carcinogenic effects of these metals. The use of cell culture models to investigate potential or known carcinogens can provide important insights into the cellular and molecular mechanisms of carcinogenesis. The Xenometrix CAT-Tox (L) Assay system measuring transcriptional activation has been used to study mechanisms of metal-induced effects [13–15]. In the current study our objective is to use this assay system to assess DU or HMTA effects on the transcriptional activation of a battery of 13 promoter-CAT stress genes in HepG2 liver cells. Several metal powders were chosen for this study. Depleted-uranium dioxide, along with tungsten powder (W), pure crystalline nickel, (Ni); and cobalt (Co) were used since they are the components of one of several possible tungsten alloys used in military applications. Crystalline nickel was previously been shown to transform cells *in vitro* [7] and was tested as a positive control. Tantalum oxide (Ta₂O₅) was also used for comparison since tantalum, which is widely used in prosthetic devices, is considered an inert metal with few reported toxic effects [2, 5].

Materials and methods

Cell lines and culture

The HepG2 cell line, a human hepatoma cell line, and the HepG2-derived cell lines (CAT-Tox [L] assay) were purchased from a commercial supplier (Xenometrix, Boulder, CO, USA). The cultures were incubated in minimum essential medium (MEM) supplemented with 10% fetal bovine serum, non-essential amino acids, 2 mM L-glutamine, 50 U/ml penicillin, 50 µg/ml streptomycin, and 50 µg/ml neomycin. All cell culture media were obtained from Gibco BRL/Life Technologies (Grand Island, NY, USA). The parental HepG2 cell line and thirteen different recombinant cell lines carrying specific promoter-chloramphenicol CAT fusion genes were studied. Table 1 describes each promoter/response element-fusion construct and their biological function.

Table 1. Stress gene promoter/response element – CAT fusion constructs and their biologic function

Promoter	Name/endogenous gene product	Biologic function
CYP1A1	Cytochrome P450 1A1	Phase I biotransformation enzyme
GST Ya	Glutathione-S-transferase Ya subunit	Phase II biotransformation enzyme
HMTIIA	MetallothioneinIIA	Sequestration of heavy metals
FOS	c-fos	Member of AP-1 transcription fact
HSP70	70 kDa heat shock protein	Sequesters damaged protein
GADD153	153 kDa growth arrest and DNA damage protein	Cell cycle regulation
GADD45	45 kDa growth arrest and DNA damage protein	Cell cycle regulation
GRP78	78 kDa glucose-regulated protein	Endoplasmic reticulum protein chaperone
XRE	Xenobiotic response element	Response to DNA damage/calcium ionophores
NFκBRE	Nuclear factor kappa (B site) response element	Binding site for AH-receptor-planar aromatic hydrocarbon complexes
CRE	cAMP response element	Binding site for NFκB transcription factor
P53RE	53 kDa protein tumor supp response element	Binding site for CREB protein
RARE	Retinoic acid response element	Binding site for p53 transcription factor
		Binding site for retinoic acid-ret acid receptor complexes

Metal powders

The aim of the study was to examine the capability of DU and an HMTA (tungsten/nickel/cobalt) to induce transcriptional activation of stress genes. The DU compound DU-uranium dioxide was obtained from Sigma (St. Louis, MO, USA). The powders, which are used to make the HMTA, were obtained from Alfa Aesar, Ward Hill, MA. The weight percentage compositions of these alloys when used for military applications is approximately, tungsten (91–93%), nickel (5–3%), and cobalt (2–4%) [3]. Since the HMTA's used by the military are not commercially available, we used a mixture of these metals, in the same percentages used by the military, to model the particles of the alloys. In this study we therefore tested the effects of a pure mixture of these materials. The following powders were used:

1. Extrafine cobalt metal (Alfa Aesar 10455, 99.5% of purity); median particles size (d_{50} 1–4 μM), called hereafter Co.
2. Extrafine nickel metal (Alfa Aesar 10256, 99% of purity); median particle size (d_{50} 3–5 μM), called hereafter Ni.
3. Extra fine tungsten metal (Alfa Aesar 10400, 99.9% of purity); median particle size (d_{50} 1–3 μM), called hereafter W.
4. A pure mixture of W (92%), Ni (5%), and Co (3%) particles made in the laboratory without extensive milling, called hereafter rWNiCo.

Prior to each experiment, the insoluble metal particles were washed once in sterile H_2O and again in acetone. They were then suspended in acetone, agitated with a magnetic stirring bar, and dispensed into cell cultures. The suspensions were carefully mixed and dispersed before being added to the cells. Dose-response experiments were conducted by altering the amounts of each metal powder, based upon its percentage of 100% of the total amount of powders. For example, a 100 μg

rWNiCo powder/ml consists of 92 μg W; 5 μg Ni, and 3 μg Co. A 50 μg rWNiCo powder/ml consists of 46 μg W, 2.5 μg Ni, and 1.5 μg Co. In this manner the total; amount (weight) of metal powder-mixture was varied while the ratios of the component metals were held constant.

Gene profile and cytotoxicity assays

Exponentially growing cells were seeded (1×10^5 cells/well) in the wells of a 96-well microtiter plate. The plates were incubated for 24 h before metal exposure at 37°C in complete media in the presence of 5% CO_2 /95% air. The particulate suspensions were added to duplicate culture wells at 0–10 μg /well (0.20 ml total volume of culture medium; 0.3 cm^2 culture surface area per well). The culture plates were incubated at 37°C for 48 h. The amount of test metal added to each well ranged from 0, 5, 10, 25, 50 $\mu\text{g}/\text{ml}$ using acetone as a solvent. For quality assurance, positive control plates were also made using known inducers including 3-methyl cholantrene (3-MC-10 μM), methyl methane sulphonate (MMS-100 $\mu\text{g}/\text{ml}$) and all-trans retinoic acid (RA-10 μM). Each metal was tested in triplicate.

Following metal exposure, the cells were re-incubated for 48 h at 37°C and 5% CO_2 . The total protein was measured at 600 nm using a microtiter plate reader (Bio-Tek Instruments). A standard sandwich ELISA was performed using a biotinylated primary anti-CAT antibody followed by streptavidin-conjugated alkaline phosphatase. In the final step the horse radish peroxidase catalyzed a color change reaction that was measured at 405 nm. Viability was assessed in separate wild-type HepG2 cell culture wells by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] reduction; plates were read at 550 nm. The ELISA absorbance values were divided by protein absorbance values to provide an estimate of CAT expression. For each cell line and test metal,

expression of CAT in test cultures was normalized to that in the control wells (dose 0) giving a fold-induction value for the fusion genes in the metal-exposed cells.

Statistical analysis

The transcriptional fold inductions for each recombinant cell line exposed to each metal or metal mixture were calculated using the CAT-Tox computer software based on the optical density readings at 600 and 405 nm. The software also converted the 550 nm readings to cell viability percentages. Standard deviations were determined and the Student's *t*-test values were computed to determine if there were significant differences in cell viability and gene induction in metal-exposed cells.

Results

Gene profile assay

Since the metal powders were suspended in acetone, an assessment of the effect of acetone on cell viability and reporter gene induction was done. The data shown in Fig. 1 demonstrate that acetone had no significant effect on any of the

thirteen promoters represented in this assay. Any changes in gene transcription were approximately, or less than 2 fold and were not statistically significant. The highest dose of acetone produced a slight decrease in cell viability as expected. These results indicate that acetone can be used as a suspension agent for these metals. Tantalum oxide, a non-transforming metal, had no effect on gene expression in this assay (data not shown).

DU produced a complex profile of inductions among the 13 promoters examined in this assay as shown in Fig. 2. With the exception of the CYP1A1, GSTYA, and XRE promoters, DU induced a consistently strong dose-dependent induction of all the other promoters tested. The most dramatic dose-dependent induction occurred with the HMTAII, FOS, p53RE, Gadd153, Gadd45, and NFκBRE; promoters ranging from 55–93 fold induction. There was a moderate but dose-dependent induction of the CRE, HSP70, RARE, and GRP78 promoters ranging from 7–43 fold induction. The profile of stress gene inductions was observed under conditions where DU produced a nearly linear decrease in cell viability over the range of doses tested with viability decreasing to 51% at the highest dose. Cell viability data is shown in the inset panel.

Figure 3 shows the results observed with rWNIco in the CAT-TOX(L) assay. Similar to DU, rWNIco produced a complex profile of inductions among the 13 promoters examined in this assay. The rWNIco mixture induced a con-

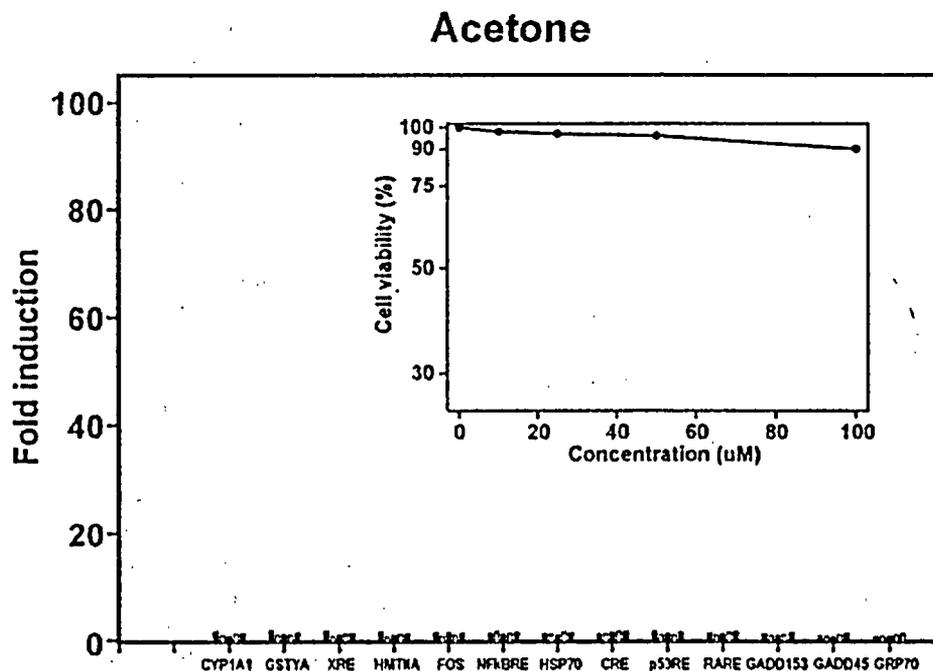


Fig. 1. Profile of transcriptional and cell viability for transgenic cells exposed to acetone. HepG2 cells were incubated with 0, 10, 25, 50 or 100 µM acetone for 48 h. Three replicates from three independent experiments were averaged. Each bar gives the mean fold induction and each error bar gives the standard error of the average. An asterisk marks any difference in the transcriptional activity between a metal-treated group and the control group that is significant at the 0.05 level. The inset panel shows the cell viability of the parental HepG2 cell line as a function of acetone concentration.

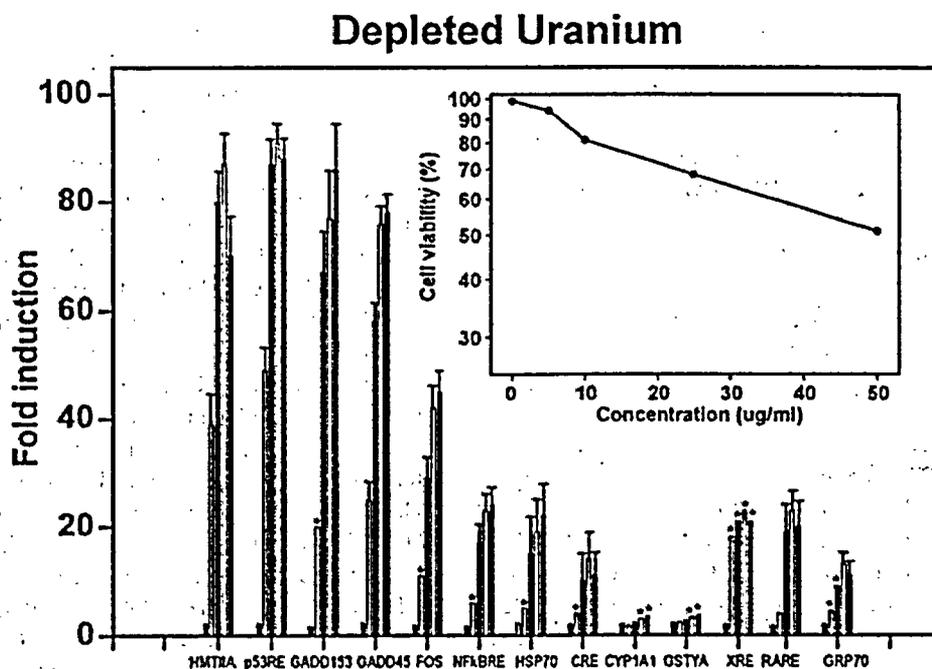


Fig. 2. Profile of transcriptional and cell viability for transgenic cells exposed to depleted uranium-dioxide. HepG2 cells were incubated with 0, 10, 25, 50, or 100 μM acetone for 48 h. Three replicates from three independent experiments were averaged. Each bar gives the mean fold induction and each error bar gives the standard error of the average. An asterisk marks any difference in the transcriptional activity between a metal-treated group and the control group that is significant at the 0.05 level. The inset panel shows the cell viability of the parental HepG2 cell line as a function of acetone concentration.

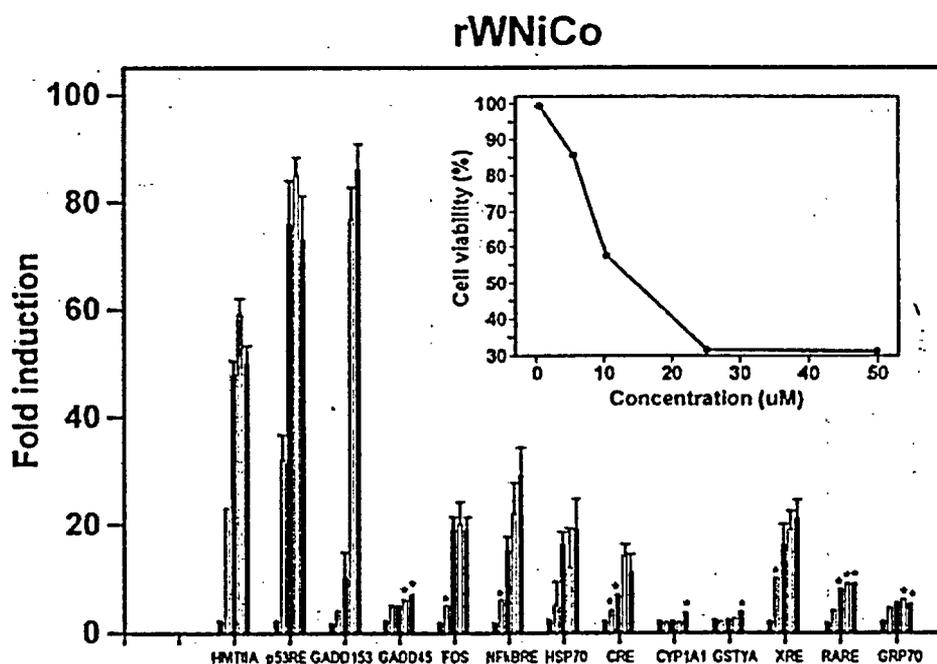


Fig. 3. Profile of transcriptional and cell viability for transgenic cells exposed to rWNiCo. HepG2 cells were incubated with 0, 10, 25, 50, 100 μg rWNiCo for 48 h. Three replicates from 3 independent experiments were averaged. Each bar gives the mean fold induction and each error bar gives the standard error of the average. An asterisk marks any difference in the transcriptional activity between a metal-treated group and the control group that is significant at the 0.05 level. The inset panel shows the cell viability of the parental HepG2 cell line as a function of acetone.

sistently strong dose-dependent induction of HMTIIA, and p53RE promoters ranging from 20.0–83 fold induction. There was a moderate but dose-dependent induction of the FOS, NFκBRE, HSP70, and CRE promoters ranging from 5–32 fold induction. There were small fold inductions (2 fold) of the GSTYA, CYP1A1, XRE, RARE, GADD153, GADD45, and GRP78 promoters that were only statistically significant at the highest doses. The profile of stress gene inductions was observed under conditions where the rWNiCo mixture produced a nearly linear decrease in cell viability over the range of doses tested with viability decreasing to 66% at the highest dose. Cell viability data is shown in the inset panel.

To test the contribution of each of the individual metals in the rWNiCo mixture, each metal was tested in the assay separately. The data are shown in Figs 4–6. As shown in Fig. 4, W induced a moderate dose-dependent induction of HMTIIA, and p53RE promoters ranging from 3–12 fold induction. There were small fold inductions (2–6 fold) of the FOS, NFκBRE, HSP70, CRE, CYP1A1, XRE, RARE, GADD153, GADD45, and GRP78 promoters that were only statistically significant at the highest doses. The profile of stress gene inductions was observed under conditions where the W powder produced a nearly linear decrease in cell viability over the range of doses tested with viability decreasing to

78% at the highest dose. Cell viability data is shown in the inset panel.

Data observed with Ni powder demonstrated similar results to those obtained with W as seen in Fig. 5. There was a moderate dose-dependent induction of HMTIIA, and p53RE promoters ranging from 2–18 fold induction. As was seen with W, there were small fold inductions (2–7 fold) of the FOS, NFκBRE, HSP70, CRE, CYP1A1, XRE, RARE, GADD153, GADD45, and GRP78 promoters that were only statistically significant at the highest doses. Similar to the other metals tested, there was a decrease in cell viability over the range of doses tested with the viability decreasing to 69% at the highest dose. The inset panel shows the cell viability data.

Cellular exposure to Co powder induced a stress gene pattern similar to that observed with either W or Ni powder alone. Data show a moderate dose-dependent induction of HMTIIA, and p53RE promoters ranging from 2–22 fold induction. Co exposure only induce a small fold induction (2–8 fold) of the FOS, NFκBRE, HSP70, CRE, CYP1A1, XRE, RARE, GADD153, GADD45, and GRP78 promoters that were only statistically significant at the highest doses. Similar to the other metals tested, there was a decrease in cell viability over the range of doses tested with the viability decreasing to 71% at the highest dose. Cell viability data is shown in the inset panel.

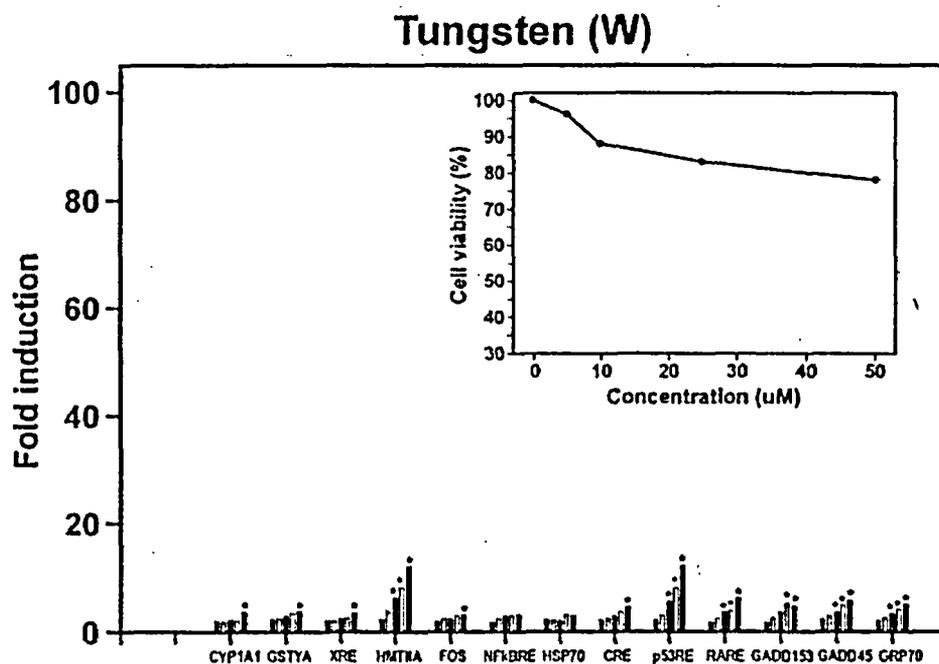


Fig. 4: Profile of transcriptional and cell viability for transgenic cells exposed to W powder. HepG2 cells were incubated with 0, 9.2, 23, 46, or 92 µg W for 48 h. Three replicates from 3 independent experiments were averaged. Each bar gives the mean fold induction and each error bar gives the standard error of the average. An asterisk marks any difference in the transcriptional activity between a metal-treated group and the control group that is significant at the 0.05 level. The inset panel shows the cell viability of the parental HepG2 cell line as a function of acetone.

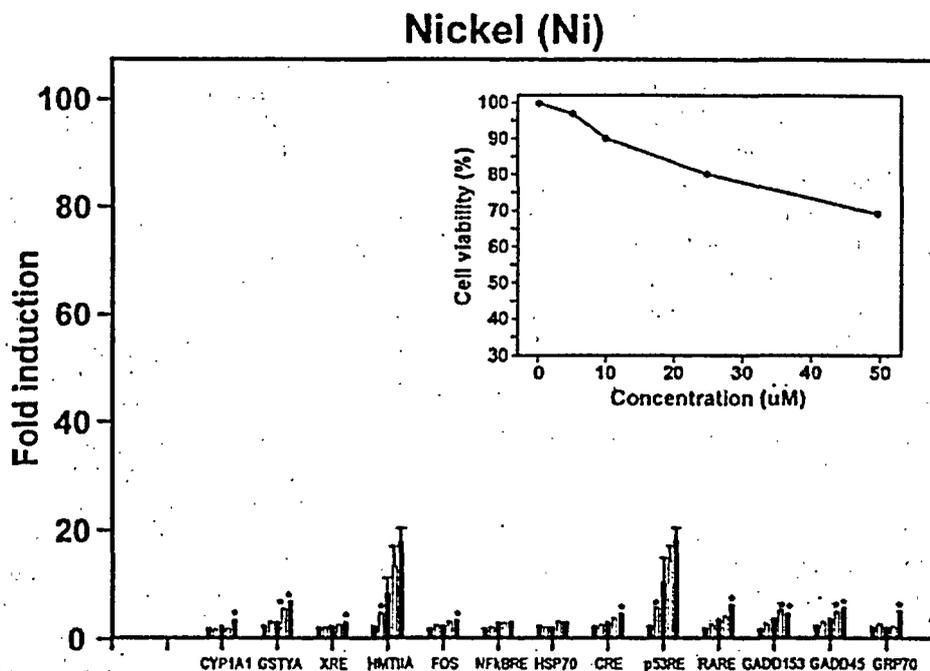


Fig. 5. Profile of transcriptional and cell viability for transgenic cells exposed to Ni powder. HepG2 cells were incubated with 0, 0.5, 1.25, 2.5, 5 μ g Ni for 48 h. Three replicates from 3 independent experiments were averaged. Each bar gives the mean fold induction and each error bar gives the standard error of the average. An asterisk marks any difference in the transcriptional activity between a metal-treated group and the control group that is significant at the 0.05 level. The inset panel shows the cell viability of the parental HepG2 cell line as a function of acetone.

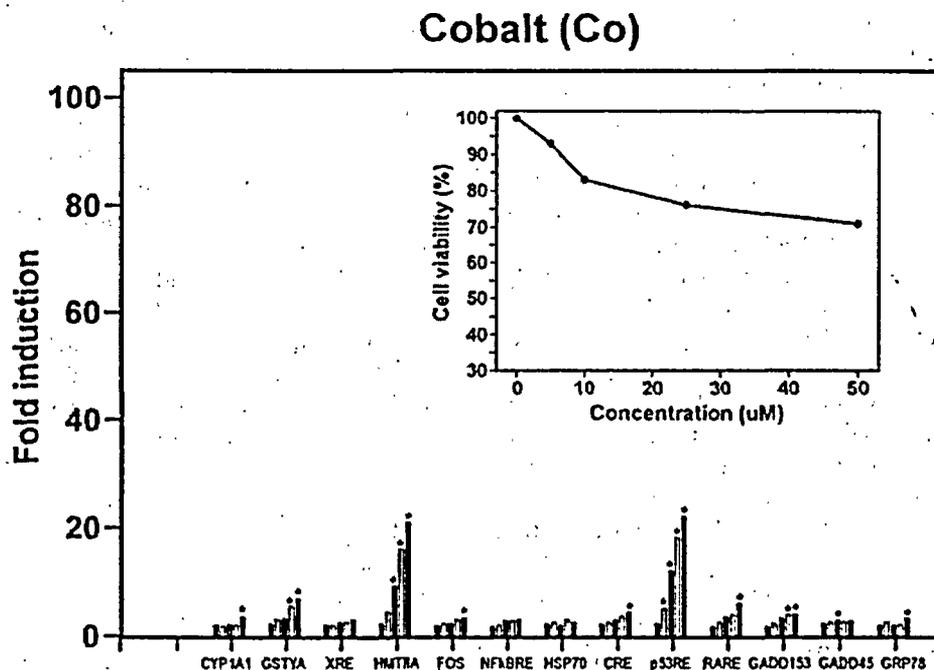


Fig. 6. Profile of transcriptional and cell viability for transgenic cells exposed to Co powder. HepG2 cells were incubated with 0, 0.3, 0.75, 1.5, or 3 μ g Co for 48 h. Three replicates from 3 independent experiments were averaged. Each bar gives the mean fold induction and each error bar gives the standard error of the average. An asterisk marks any difference in the transcriptional activity between a metal-treated group and the control group that is significant at the 0.05 level. The inset panel shows the cell viability of the parental HepG2 cell line as a function of acetone.

Discussion

This study was undertaken to assess the ability of two militarily relevant heavy metals, DU and the reconstituted rWNiCo mixture (a HMTA) to induce gene transcription in a panel of cell lines stably transfected with reporter genes consisting of a coding sequence for chloramphenicol acetyl transferase (CAT) under transcriptional control by mammalian stress gene regulatory sequences. The low-specific activity compound, DU-UO₂ was used to assess the effects of DU. Since these tungsten alloys are not commercially available, we used a mixture of the pure metals that compose each heavy-metal tungsten-alloy. This metal mixture, which mimics one of the several mixtures used in military applications, was composed of tungsten, nickel, and cobalt.

Our studies demonstrate for the first time that the activation of gene transcription in hepatoma cells can be achieved by exposure to DU or a mixture of W, Ni, and Co. DU, (5–50 µg/ml) produced a complex profile of activity demonstrating significant dose-dependent induction of the hMTIIA FOS, p53RE, Gadd153, Gadd45, NFκBRE, CRE, HSP70, RARE, and GRP78 promoters. The rWNiCo mixture (5–50 µg/ml) showed dose-related induction of the GSTYA, hMTIIA, p53RE, FOS, NFκBRE, HSP70, and CRE promoters. An examination of the individual metals, tungsten (W), nickel (Ni), and cobalt (Co), comprising the rWNiCo mixture demonstrated that each individual metal exhibited a similar pattern of gene induction, but at a significantly decreased magnitude than that of the rWNiCo mixture.

Our results confirm previous findings by others that mutagenic and/or genotoxic heavy metals like arsenic, cadmium, and chromium, cause transcriptional activation of a variety of the 13 promoters in HepG2 cells in the CAT-Tox assay [13–15]. As with those other metals (arsenic, cadmium, chromium, and lead) [13–15], DU and rWNiCo induced transcriptional activation of several gene promoters, but similar and significant dose-dependent effects were particularly observed with the hMTIIA, FOS, GADD153, GADD45 and p53RE promoters.

DU produced a complex profile of inductions among the 13 promoters examined in this assay inducing a consistently strong dose-dependent induction of all the other promoters tested with the exception of the Cyp1A1, GSTYA, and XRE promoters. The most dramatic dose-dependent induction occurred with the HMTIIA, FOS, p53RE, Gadd153, Gadd45, and NFκBRE promoters ranging from 55–93 fold induction. There was a moderate but dose-dependent induction of the CRE, HSP70, RARE, and GRP78 promoters ranging from 7–43 fold induction.

The profile of gene inductions resulting from DU exposure is consistent with previously shown cellular and toxic effects of DU [1–2, 6–8]. The hMTIIA promoter, which has

been shown to be associated with metallothionein production, was induced by DU exposure. This result, demonstrating that DU induced a strong dose-dependent expression of the CAT reporter regulated by hMTIIA, is also consistent with previous reports indicating that this promoter is induced by metals including arsenic, cadmium, and zinc [13–15]. Intracellular storage of heavy metals is hypothesized to be a function of metallothionein, so it is possible that a metal like DU which has been shown *in vitro* to be actively taken up and stored intracellularly [1], could be associated with transcriptional activation of hMTIIA. Similarly, we have shown that cellular DU exposure causes DNA damage [16–18] and therefore it is not surprising that DU induces expression of the promoters associated with DNA damage i.e., FOS, p53RE, Gadd153, Gadd45: Activation of GADD153 and GADD45 indicate a potential damage at the genomic level. Data published recently from our laboratory demonstrated that DU induced genomic instability [8] and DNA strand breaks [16]. Activation of the GRP78 promoter is also consistent with DU-induced cellular effects. Up-regulation of GRP78 is associated with proteins that are incorrectly folded because of mutagenesis. DU exposure has been shown to be mutagenic [18] and it is consistent that the GRP78 promoter would be induced. A coordinate dose-dependent induction of the FOS and NFκBRE promoters is strongly suggestive of cellular responses to oxidative stress. Recently we have demonstrated that DU can cause oxidative DNA damage, i.e. production of 8-hydroxy-guanone [18], which is consistent with the gene induction data. It is not surprising that DU exposure did not induce significant levels of transcriptional activation of CYP1A1, GSTYA and the XRE promoters. They have been associated with the presence of aryl hydrocarbons and there is no formation of these compounds associated with DU exposure.

Similar to DU, rWNiCo produced a complex profile of inductions among the 13 promoters examined in this assay. The rWNiCo mixture induced a consistently strong dose-dependent induction of GSTYA, HMTIIA, and p53RE promoters ranging from 20.0–83 fold induction. There was a moderate but dose-dependent induction of the FOS, NFκBRE, HSP70, and CRE promoters ranging from 5–32 fold induction. There were small fold inductions (2 fold) of the CYP1A1, XRE, RARE, GADD153, GADD45, and GRP78 promoters that were only statistically significant at the highest doses.

The profile of gene inductions resulting from rWNiCo exposure is also consistent with previously shown cellular and toxic effects of this reconstituted heavy metal tungsten-alloy mixture. Similar to what was observed with DU, rWNiCo caused a strong dose-dependent induction of hMTIIA. Considering the role of metallothionein in metal sequestration, it is not unusual that the HMTA mixture and the other previously studied heavy metals examined, have demonstrated effects of the induction of hMTIIA. The rWNiCo exposure

also exhibited a significant dose-dependent effect of p53RE. Our studies have shown that rWNIco exposure causes significant DNA and chromosomal damage [6] and since the p53 protein is associated with DNA damage, it is not unexpected that rWNIco could affect the p53RE. Similar to what was observed with DU, rWNIco exposure caused a coordinate dose-dependent induction of the FOS and NFκBRE promoters that is strongly suggestive of cellular responses to oxidative stress. Ongoing studies are being conducted to assess the potential induction of oxidative DNA and cellular damage by rWNIco.

Cellular exposure to the W, Ni, or Co powder induced a stress gene pattern similar to the rWNIco mixture, albeit to a lesser magnitude. Data show a moderate dose-dependent induction of hMTIIA, and p53RE promoters and a small fold induction (2–8 fold) of the FOS, NFκBRE, HSP70, CRE, CYP1A1, XRE, RARE, GADD153, GADD45, and GRP78 promoters that were only statistically significant at the highest doses. As with DU and the rWNIco mixture, these metals induced genes that are associated with DNA damage. This is consistent with our findings that these metals induce direct DNA damage [1, 6]. Minor induction of the other promoters listed above, also implicate cellular responses to oxidative stress. In contrast to the individual exposures of W, Ni, or Co, it appears that the mixture of W, Ni, and Co caused a synergistic increase in the induction of several genes, including hMTIIA, p53RE, FOS, NFκBRE, HSP70, CRE, CYP1A1, XRE, RARE, GADD153, GADD45, and GRP78.

Considering our previously reported results demonstrating that DU and the rWNIco mixture can transform human cells to the malignant phenotype [1, 7, 18], it is not surprising that these metals could activate gene expression through signal transduction pathways that can become aberrant during the carcinogenic pathway. Since there are little data regarding the potential tumorigenic and genotoxic effects of internalized DU, W and tungsten-alloys, these results are important to the understanding of the mechanism of the potential late health effects, i.e. carcinogenicity, of DU and tungsten alloys used in military applications.

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