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Embedded Weapons-Grade Tungsten Alloy Shrapnel Rapidly Induces Metastatic High-Grade Rhabdomyosarcomas in F344 Rats

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ABBREVIATIONS: WA - Tungsten Alloy; DU – Depleted Uranium; Ta – Tantalum; Ni – nickel; Co – Cobalt.

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ABSTRACT

Continuing concern regarding the potential health and environmental effects of depleted uranium and lead has resulted in many countries adding tungsten alloy-based munitions to their battlefield arsenals as replacements for these metals. Because the alloys used in many munitions are relatively recent additions to the list of militarily relevant metals, very little is known about the health effects of these metals after internalization as embedded shrapnel. Previous work in this laboratory developed a rodent model system that mimicked shrapnel loads seen in wounded personnel from the 1991 Persian Gulf War. In this study, we used that system and male F344 rats, implanted intramuscularly with pellets (1 mm x 2 mm cylinders) of weapons-grade tungsten alloy, to simulate shrapnel wounds. Rats were implanted with 4 (low dose) or 20 pellets (high dose) of tungsten alloy. Tantalum (20 pellets) and nickel (20 pellets) served as negative and positive controls, respectively. The high-dose tungsten alloy-implanted rats (n=46) developed extremely aggressive tumors surrounding the pellets within 4-5 months after implantation. The low-dose tungsten alloy-implanted rats (n=46) and nickel-implanted rats (n=36) also developed tumors surrounding the pellets, but did so at a slower rate. Rats implanted with tantalum (n=46), an inert control metal, did not develop tumors. Tumor yield was 100% in both the low- and high-dose tungsten alloy groups. The tumors, characterized as high-grade pleomorphic rhabdomyosarcomas by histopathology and immunohistochemical examination, rapidly metastasized to the lung and necessitated euthanasia of the animal. Significant hematological changes, indicative of polycythemia, were also observed in the high-dose tungsten alloy-implanted rats. These changes were apparent as early as 1 month post-implantation in the high-dose tungsten alloy rats, well before any overt signs of tumor development. These results point out the need for further studies investigating the health effects of tungsten and tungsten-based alloys.

INTRODUCTION

Tungsten has been used for many years in a variety of applications. Combining the hard, brittle tungsten metal with various other metals, including nickel and cobalt, produces tungsten alloys (WA) with specific characteristics, some of which are of interest to the military. Recently, WAs have replaced lead in some small caliber ammunition (the “green bullet”) (ORNL 1998) and depleted uranium (DU) in kinetic-energy penetrators (ORNL 1996). Based upon a small number of studies, prevailing theory is that elemental tungsten or insoluble tungsten compounds have only limited toxicity (Leggett 1997). For example, tungsten coils implanted into the subclavian artery of rabbits rapidly degrade, leading to elevated serum tungsten levels as early as 15 minutes after implantation. However, after 4 months, no signs of local or systemic toxicity were observed (Peuster et al. 2003). Studies on health effects of nickel and cobalt are more numerous. Intramuscular injections (28 mg) of soluble metallic nickel or cobalt result in formation of rhabdomyosarcomas at the injection site. With nickel, 100% of injected rats develop a tumor within 41 weeks (Heath and Daniel 1964), while administration of cobalt results in tumor formation in 40% of the rats with a latency period of 71 weeks (Heath 1954, 1956). However, intramuscular implantation of rods or pellets composed of various nickel or cobalt alloys used in orthopedic prosthetics results in no excessive tumor formation (Gaechter et al. 1977; Sunderman 1989). A variety of other nickel compounds, including nickel subsulfide, nickel oxide, and nickel monosulfide, have been tested for carcinogenic potential via intramuscular administration (Gilman 1962; Sunderman and Maenza 1976; Sunderman et al. 1977). Tumors (rhabdomyosarcoma and fibrosarcoma) were found in many cases at the injection site, with tumor yield dependent on solubility and concentration of the administered compound. It has been postulated that the yield of localized tumors is inversely related to the

rate of solubilization of the nickel-containing compound (Kasprzak et al. 1983). This hypothesis does not appear to hold for cobalt compounds (Lison et al. 2001).

Metal alloys present additional problems when investigating health effects. The various metals comprising the alloy, as well as the method of production, can all factor into the overall health effect observed upon exposure. Investigations on hard-metal disease have shown that either tungsten carbide or cobalt alone has limited toxicity on lung tissue (Lasfargues et al. 1992). However, when combined, the tungsten carbide/cobalt mixture acts synergistically to increase the observed toxicity. It is not known whether this is due to the combined toxicity of the tungsten carbide/cobalt mixture or to an increase in the bioavailability of the known toxicant, cobalt (Lison and Lauwerys 1997). *In vitro* studies investigating malignant transformation of immortalized human cells by mixtures of tungsten, nickel, and cobalt suggest a synergistic effect that greatly exceeds the effects of the metals individually (Miller et al 2001, 2002).

Advancements in metallurgy have led the military of many nations to replace DU in some armor penetrating munitions and lead in small-caliber ammunition with various alloys of tungsten. One motivation for such a replacement is widespread public concern about the health and environmental impact of continued use of these metals. However, to our knowledge, none of these militarily relevant tungsten alloys have been tested for potential health effects, especially as embedded shrapnel. There is a growing list of health concerns related to tungsten exposure. Although a definitive link has not been established, several cancer clusters in the United States are associated with elevated levels of tungsten in the environment. Those findings, along with the results presented in this manuscript, raise questions about the possible consequences of tungsten exposure. More importantly, it raises extremely serious concerns over the potential health effects of tungsten alloy-based munitions currently being used as non-toxic alternatives to lead and DU.

MATERIALS AND METHODS

Rodents

Male F344 rats (6 weeks of age, Harlan, Frederick, Maryland, USA) were maintained in an Association of Assessment and Accreditation of Laboratory Animal Care (AAALAC)-accredited facility in accordance with the Guide for the Care and Use of Laboratory Animals (NAS 1996). All procedures, including euthanasia criteria (Tomasovic et al. 1988), were approved by the Institute's Animal Care and Use Committee. Upon arrival, animals were screened for common rodent pathogens. Rats were pair-housed in plastic microisolator cages with hardwood chips for bedding and fed a certified NTP-2000 (Quality Lab Products, Elkridge, Maryland, USA) diet (Rao 1996) with acidified water provided *ad libitum*. Animals were on a 12-hour light/dark cycle with no twilight and were weighed weekly.

Pellets

All metal pellets were cylinders 1 mm in diameter and 2 mm in length. Nickel (99.995% metallic Ni) and tantalum (99.95% Ta) pellets were purchased from Alfa Aesar (Ward Hill, Massachusetts, USA). WA pellets were fabricated by Aerojet Ordnance Tennessee (Jonesborough, Tennessee, USA) using standard kinetic energy penetrator production processes. The weight of an average WA pellet was 27.5 mg and consisted of 91.1% tungsten, 6.0% nickel, and 2.9% cobalt. Ni and Ta pellets weighed 14 mg and 27 mg, respectively. Before implantation surgery, all pellets were cleaned and chemically sterilized (Pellmar et al. 1999).

Pellet-implantation surgery

A rodent model system (AFRRI 1996), originally developed to mimic DU shrapnel loads seen in wounded personnel from the 1991 Persian Gulf War, was used to investigate the health effects of retained WA shrapnel. All rats were implanted with a total of 20 pellets split evenly between each hind leg. Experimental groups included Ta (negative control, 20 Ta pellets), low-dose WA

(four WA pellets / 16 Ta pellets), high-dose WA (20 WA pellets), and Ni (positive control, 20 Ni pellets). Tantalum was used as a negative implantation control since it is considered inert and has been used in human prostheses (Johansson et al. 1990; Hockley et al. 1990). Nickel, a known carcinogen, was used as a positive control (Kasprzak et al. 2003; Costa and Klein 1999). Rats were implanted at 9 weeks of age. For the pellet implantation procedure, anesthesia was induced by continuous administration of isoflurane using an open circuit system with a scavenger/recapture system. All surgery was done using aseptic techniques. After the surgical sites were clipped and cleansed with betadine, an incision was made through the skin to expose the gastrocnemius muscle. Pellets were implanted in the muscle, spaced approximately 1.5 mm apart on the lateral side of each leg. The incision was closed with sutures and tissue adhesive. Rats were closely monitored following surgery until they were ambulatory. An analgesic (buprenorphine hydrochloride, Reckitt and Colman, Hull, UK) was administered preoperatively and then as needed post-operatively. The surgical sites were examined daily for signs of inflammation, infection, and local metal toxicity.

Experimental groups

Our pellet implantation groups included Ta (negative control), WA (both a low- and high-dose group), and Ni (positive control). The original euthanasia timepoints were to be 1, 3, 6, 12, 18, and 24 months; however, because of the rapid tumor development, no WA- or Ni-implanted rat survived much past 6 months post-implantation. Final survival data therefore included rats originally assigned to the 12-, 18-, and 24-month experimental groups, whose animals died earlier than those designated time points. This resulted in group sizes of n=46 for the Ta and both WA groups, and n=36 for the Ni group. Hematological assessments were conducted on the separate 1-, 3-, and 6-month WA implantation groups.

Pathology

At various times post-implantation or when moribund, rats were euthanized by isoflurane overdose. A complete gross pathology examination was conducted, noting any abnormalities, and tissues were collected for analysis. Weights of representative tissues, including spleen, thymus, testes, kidney, and liver were determined and normalized to body weight. Tissues for histopathology were fixed in buffered formalin, processed and embedded in paraffin, cut at 5-6 μm , mounted, and stained with hematoxylin and eosin (H & E). Immunohistochemical analysis was conducted on 5- μm thick sections of formalin-fixed, paraffinized tissue. After deparaffination and rehydration, non-specific binding was blocked with Power Block® (Biogenex, San Ramon, California, USA). The tissue was then reacted with pre-diluted rabbit anti-desmin polyclonal antibody (Biogenex) and treated with biotinylated secondary anti-rabbit antibody (Biogenex). After blocking with hydrogen peroxide, the tissue sections were labeled with peroxidase-conjugated streptavidin (Biogenex) and AEC was used as a chromogen. Slides were then counter-stained with hematoxylin and mounted.

Hematology

At euthanasia, blood for hematological assessments was obtained from the abdominal aorta of isoflurane-anesthetized rats using a heparinized needle and sample tubes containing EDTA (Becton-Dickinson, Franklin Lakes, New Jersey, USA). White and red blood cell counts; hemoglobin; hematocrit; mean corpuscular volume, hemoglobin, and hemoglobin concentration; red cell distribution width; platelet counts and volume; as well as neutrophil, lymphocyte, monocyte, eosinophil, and basophil counts were determined with a Bayer Advia 120 Hematology Analyzer (Bayer Diagnostics, Terrytown, New York, USA).

RESULTS

All rats tolerated the pellet implantation procedure with no apparent adverse effects. The incision sites were examined daily. No rat showed any signs of infection from the surgery, or any discomfort post-operatively. Body weights were recorded weekly. Once they had recovered from the surgical procedure, all rats gained weight at equivalent rates. However, in the first week following the pellet implantation surgery, the rate of weight gain by the Ta and low-dose WA rats was slower than normal, and high-dose WA and Ni rats lost weight. This was followed by large weight gains in post-implantation week 2 in all experimental groups. There were no statistical differences in rate of body weight gain between any of the groups throughout the remaining experimental period. As previously reported, the implantation and retention of cylindrical metal pellets (1 mm x 2 mm) had no effect on locomotive abilities in rats (AFRRI 1996; Pellmar et al. 1999) nor did we observe any such difficulties in this study.

At approximately 16-20 weeks post-implantation, we began to observe tumors at the pellet implantation sites in the WA and Ni rats. In some high-dose WA animals, palpable tumors were apparent as early as 14 weeks post-implantation. Tumors developed rapidly in WA-implanted animals. The tumors were aggressive and fast growing, necessitating euthanasia of the animals several weeks later. Based on previously published literature, we expected the Ni-implanted positive control rats to develop tumors at the implantation site, but the speed at which the tumors developed was surprising – approximately 5 months after implantation. Figure 1 shows the percentage of surviving animals as a function of time after pellet implantation. Rats implanted with Ta pellets (n=46) survived well beyond 12 months with no apparent health problems. All rats in the high- and low-WA and the Ni groups developed tumors and were euthanized upon becoming moribund. Rats in the high-dose WA group (n=46) survived the least amount of time (mean survival time \pm standard deviation: 21.8 ± 2.1 weeks). Nickel-implanted

animals (n=36) and the low-dose WA group (n=46) survived slightly longer, with mean survival times of 25.4 ± 2.1 and 27.0 ± 4.6 weeks, respectively. The mean survival time of the high-dose WA animals was significantly shorter than that of the low-dose WA- or Ni-implanted animals (ANOVA followed by Dunnett's test, $P < 0.05$). The mean survival times of the low-dose WA- and the Ni-implanted animals were not statistically different from each other. We would like to note that the results reported here are part of a larger study that also investigated the health effect of embedded DU fragments. We did not observe tumor formation in the DU-implanted rats (manuscript in preparation).

Upon euthanasia, the animals underwent necropsy, and tissue samples were taken for various analyses. Figure 2 shows the appearance of the hind limb of rats implanted with Ta (Fig. 2a) or WA (Fig. 2c) for 26 and 23 weeks, respectively, before surgical removal of the implanted pellets. The gross anatomy of the Ta-implanted leg is normal, while, in the WA leg, the tumor is clearly visible. Upon dissection, no obvious abnormalities were observed in the Ta-implanted animals, and the pellets could be easily removed (Fig. 2b). However, in the WA-implanted animals, the pellets were surrounded by tumor (Fig. 2d). In many cases, the interior of the tumor had become necrotic and/or hemorrhagic. Similar tumors were found for both WA- and Ni-implanted animals. In low-dose WA animals, tumors were found surrounding the WA pellets only. No tumors were found surrounding implanted Ta pellets. Implanted WA pellets rapidly oxidized and had a slightly eroded appearance. Ta pellets did not have an eroded appearance even after implantation for 6 months. However, despite their appearance, the WA pellets lost less than 5% of their mass over this time.

Tumor tissue was histopathologically examined and characterized. Figure 3a shows the neoplastic cells surrounding the site of the implanted WA pellet. These cells infiltrated preexisting skeletal muscle fibers. Fibers that became isolated by this process degenerated and

demonstrated a loss of cross-striations and internalization of nuclei (Fig. 3b and 3c). As seen in Fig. 3d, neoplastic cells are pleomorphic with marked anisocytosis and anisokaryosis. In addition, there is an extremely high mitotic rate observed in these cells, and bizarre mitoses are present. Immunohistochemical staining was used to determine the origin of these neoplastic cells. The cells were strongly positive for desmin (Fig. 3e and 3f), suggesting a skeletal muscle origin.

In the WA-implanted animals, the tumors had metastasized to the lung. None of the Ni-implanted animals showed signs of lung metastases, although some exhibited endogenous histiocytic lipid pneumonia not seen in the WA animals. Figure 4a shows numerous metastatic foci in the lungs of a high-dose WA rat. These multiple masses obscure over 50% of the lung surface and up to 90% in the latter stages of development. Figure 4b shows a photomicrograph of these pulmonary metastases. Apparent is the multifocal, vascular orientation of these neoplasms. There are neoplastic cells surrounding the arterioles and bronchioles, expanding the alveolar septae, and replacing alveolar spaces. These neoplastic cells have a high mitotic rate and are often seen surrounding or occluding arterioles (Fig. 4c). Figure 4d shows that the metastatic neoplastic cells, as well as vascular and airway smooth muscle, are strongly positive for the muscle marker desmin.

Selected hematological and organ weight parameters for euthanized rats are shown in Table 1. The Ta data were obtained from rats implanted with Ta pellets for 6 months. The data for the remaining groups were obtained at the time the rats became moribund because of tumor development. No significant differences in organ/body weight ratios were seen for the low-dose WA- or Ni-implanted animals when compared to Ta-implanted control rats. However, high-dose WA-implanted rats showed significantly higher spleen/body weight ratios when compared to control rats. In addition, thymus/body weight ratios were decreased in the high-dose WA rats.

Since the spleen and thymus are integral components of the immune system, these changes suggest that embedded WA, at certain levels, may be immunotoxic. The kidney/body weight ratio for high-dose WA rats was also significantly higher than that of Ta-implanted rats. High-dose WA rats euthanized 1 and 3 months after pellet implantation also exhibited significantly elevated spleen/body weight ratios when compared to the appropriate Ta-implanted control rats (Tables 2 and 3). Thymus/body weight ratios, however, were not significantly different. At 3 months post-implantation, the kidney/body weight ratio in high-dose WA rats was significantly higher than in Ta rats, but it was significantly lower at 1 month post-implantation. There were no 1 and 3 month Ni-implanted groups.

WA-implanted animals had significant changes in a number of hematological parameters. Rats implanted with 20 WA pellets exhibited significant increases in white blood cell counts, red blood cell counts, hemoglobin, and hematocrit levels compared to Ta control rats, while rats implanted with 20 Ni pellets had significant decreases in red blood cell counts, hemoglobin, and hematocrit levels (Table 1). Hematological parameters from low-dose WA rats were not statistically different from controls. Statistically significant increases in red blood counts, hemoglobin, and hematocrit levels were observed in high-dose WA animals as early as 1 month after pellet implantation and persisted throughout the experimental period (Tables 2 and 3). In addition, there were statistically significant increases in the numbers of neutrophils, lymphocytes, monocytes, and eosinophils present in high-dose WA animals. Low-dose WA animals had elevated neutrophil, lymphocyte, and monocyte numbers at 3 months post-implantation, but only the neutrophil numbers were statistically different from the controls at the 5-6 month euthanasia point. The Ni-implanted animals had significantly lower lymphocyte counts than the controls. All other parameters were statistically identical to the controls. These

results suggest there is a dose-dependent perturbation in many hematology parameters as a result of an increasing WA pellet number.

DISCUSSION

Tungsten-based alloys are currently being used as replacements for DU in kinetic-energy penetrators and for lead in small-caliber ammunition. However, the health effects of these unique alloys have not been investigated, especially in the case of embedded fragments such as shrapnel wounds. In this study, using male F344 rats and a system designed to investigate the effects of embedded metal fragments (AFRRI 1996), we have shown the embedded weapons-grade WA (91.1% W/6.0% Ni/2.9% Co) results in rapid tumor formation at the implantation site in 100% of the rats. The rate of tumor formation correlates with pellet number. Ni-implanted rats also develop tumors at the implantation site, although not as rapidly as seen with WA. Histopathologic and immunohistochemical data support a diagnosis of a pleomorphic rhabdomyosarcoma for both the WA- and Ni-induced leg tumors (Altmannsberger et al. 1985).

Rats implanted with 20 WA pellets (high-dose WA) showed significantly increased spleen/body weight ratios compared to tantalum control rats. Low-dose WA rats (4 WA pellets) also exhibited increased spleen/body weight ratios, but these increases were not statistically significant (ANOVA followed by Dunnett's test). Values for Ni-implanted rats were identical to control rats. The spleen changes observed in the high-dose WA rats were apparent as early as 1 month after pellet implantation. Once again, low-dose WA rats showed increased, but not statistically significant, spleen/body weight ratios. With the exception of the spleen, the only other organ/body weight perturbations were seen in high-dose WA rats and included a decrease in thymus/body weight ratio at approximately 5 months and changes in kidney/body weight ratios. The 1-month kidney/body weight ratio for high-dose WA rats was significantly lower than control. However, from 3 months on, these ratios were significantly higher than control. It

is possible that the lower kidney weights at 1 month post-implantation represent a toxic response to the heavy metals from the implanted pellets, but by 3 months and later, the kidney has begun to respond in a different manner. Although there were no gross abnormalities of the kidney at necropsy, we continue to investigate this observation.

A variety of hematological changes were observed in WA- and Ni-implanted rats. Ni-implanted rats showed a significant decrease in red blood cells, hemoglobin, and hematocrit at the time of morbidity, indicating possible Ni-induced anemia. For low-dose WA rats the hematological changes, including significant increases in red blood cells, white blood cells, hemoglobin, hematocrit, neutrophils, lymphocytes, and monocytes, peaked at 3 months post-implantation and returned to normal by 5-6 months. High-dose WA rats demonstrated the same changes observed in low-dose WA rats, but they occurred much more rapidly (as early as 1 month post-implantation) and persisted throughout the life of the animal. The splenomegaly and hematological changes observed in these rats are suggestive of polycythemia. Cobalt has been used experimentally to induce polycythemia in rats (Rakusan et al. 2001; Endoh et al. 2000), although the concentration required is far greater than found in the WA pellets. In addition, the speed at which these hematological changes occurred in the high-dose WA rats was also surprising. These results suggest a dose-dependent perturbation in many hematology parameters as a result of an increasing WA pellet number.

The search for munitions that are considered environmentally friendly yet still retain their military effectiveness has led to the appearance of many unique alloys on the modern battlefield. Often, decisions on the health consequences of exposure (inhalation, ingestion, wound contamination, etc.) to these specific alloys are based on studies that investigated only one specific metal of the alloy rather than the particular alloy in question. Tungsten-based munitions are a recent addition to many countries' arsenals primarily in response to the continuing concerns

regarding the potential environmental and health effects of DU in kinetic-energy penetrators and of lead in small-caliber ammunition. For years, exposure to tungsten was thought to be of little consequence to health. In fact, tungsten is occasionally found as a minor component in some of the various alloys used to produce medical implant devices such as artificial hips and knees. The tungsten concentration in these alloys ranges from 5% to 15%. Since the alloy used in WA munitions usually contains greater than 90% tungsten, along with smaller amounts of other metals, it was also assumed that exposure to these alloys would present little or no health risk. As we have shown here, this is not the case in our rodent model. Embedded WA pellets not only resulted in aggressive, metastatic, pleomorphic rhabdomyosarcomas, but also caused significant hematopoietic changes well before the carcinogenic effect was observed. It seems unlikely that these adverse health effects can be attributed solely to the small amounts of nickel and/or cobalt present in the alloy. The tumors induced by the 100% Ni implants occurred later than those induced by the alloys containing 6% Ni. However, recent *in vitro* studies have demonstrated a synergistic effect in terms of damage when tungsten is present with these metals (Miller et al. 2001, 2002).

The mechanism of the effects reported here with embedded WA pellets remains unclear. Despite the fact that the smooth and impermeable surface of the pellets represent characteristics known capable of inducing foreign-body or solid-state carcinogenesis (Bates and Klein, 1966; Brand et al. 1975), this process is unlikely to have occurred in our experiments because implanted tantalum pellets of an identical geometry and surface resulted in no tumor formation. One possibility is that free-radical reactions at the interface of the pellet and tissue could result in damage leading to carcinogenesis. Recently, the role of tungsten in human health and disease has come under increased scrutiny. Environmental testing of the leukemia cluster around Fallon, Nevada, in the United States showed slightly elevated levels of several heavy metals including

uranium and cobalt, but significantly elevated levels of tungsten (CDC 2003). Although no definitive link between elevated tungsten levels and cancer has been established, because of the uncertainty surrounding this issue, the U.S. National Toxicology Program recently added tungsten to their list of compounds to be assessed for adverse health effects. Further study of the health effect of tungsten and tungsten alloys is clearly indicated.

REFERENCES

- AFRRI. 1996. Establishment of an animal model to evaluate the biological effects of intramuscularly embedded depleted uranium fragments. Technical Report 96-3. Bethesda, MD: Armed Forces Radiobiology Research Institute.
- Altmannsberger M, Weber K, Droste R and Osborn M. 1985. Desmin is a specific marker for rhabdomyosarcomas of human and rat origin. *Am J Pathol* 118: 85-95.
- Bates RR and Klein M. 1966. Importance of smooth surface in carcinogenesis by plastic film. *J Natl Cancer Inst* 37: 145-151.
- Brand KG, Buoen LC, Johnson KH and Brand T. 1975. Etiological factors, stages, and the role of the foreign body in foreign-body tumorigenesis: a review. *Cancer Res* 35: 279-286.
- CDC. 2003. Cross-sectional exposure assessment of environmental contaminants in Churchill County, Nevada. Atlanta, GA: Centers for Disease Control and Prevention.
- Costa M and Klein CB. 1999. Nickel carcinogenesis, mutation, epigenetics, or selection. *Environ Health Persp* 107: 1-4.
- Endoh H, Kaneko T, Nakamura H, Doi K and Takahashi E. 2000. Improved cardiac contractile functions in hypoxia-reoxygenation in rats treated with low concentration Co^{2+} . *Am J Physiol Heart Circ Physiol* 279: H2713-H2719.
- Gaechter A, Alroy J, Andersson GBJ, Galante J, Rostoker W and Schajowicz F. 1977. Metal carcinogenesis: a study of the carcinogenic activity of solid metal alloys in rats. *J Bone Joint Surg* 59(A): 622-624.
- Gilman JPW. 1962. Metal carcinogenesis. II. A study on the carcinogenic activity of cobalt, copper, iron, and nickel compounds. *Cancer Res* 22: 158-165.
- Heath JC. 1954. Cobalt as a carcinogen. *Nature* 173: 822-823.

- Heath JC. 1956. The production of malignant tumors by cobalt in the rat. *Brit J Cancer* 10: 668-673.
- Heath JC and Daniel MR. 1964. The production of malignant tumors by nickel in the rat. *Brit J Cancer* 18: 261-264.
- Hockley AD, Goldin JH, Wake MJC and Iqbal J. 1990. Skull repair in children. *Pediatr Neurosurg* 16: 271-275.
- Johansson CB, Hansson HA and Albrektsson T. 1990. Qualitative interfacial study between bone and tantalum, niobium or commercially pure titanium. *Biomaterials* 11: 277-280.
- Kasprzak KS, Gabryel P and Jarczewska K. 1983. Carcinogenicity of nickel (II) hydroxides and nickel (II) sulfate in Wistar rats and its relation to the in vitro dissolution rates. *Carcinogenesis* 4: 275-279.
- Kasprzak KS, Sunderman Jr FW and Salnikow K. 2003. Nickel carcinogenesis. *Mutat Res* 533: 67-97.
- Lasfargues G, Lison D, Maldague P and Lauwerys R. 1992. Comparative study of the acute lung toxicity of pure cobalt powder and cobalt-tungsten carbide mixture in rat. *Toxicol Appl Pharmacol* 112: 41-50.
- Leggett RW. 1997. A model of the distribution and retention of tungsten in the human body. *Sci Tot Environ* 206: 147-165.
- Lison D, DeBoeck M, Verougstraete V and Kirsch-Volders M. 2001. Update on the genotoxicity and carcinogenicity of cobalt compounds. *Occup Environ Med* 58: 619-625.
- Lison D and Lauwerys R. 1997. Study of the mechanism responsible for the selective toxicity of tungsten-carbide-cobalt powder toward macrophages. *Toxicol Lett* 60: 203-210.

- Miller AC, Mog S, McKinney L, Luo L, Allen J, Xu J, et al. 2001. Neoplastic transformation of human osteoblast cells to the tumorigenic phenotype by heavy-metal tungsten-alloy metals: induction of genotoxic effects. *Carcinogenesis* 22: 115-125.
- Miller AC, Xu J, Prasanna PGS and Page N. 2002. Potential late health effects of the heavy metals, depleted uranium and tungsten, used in armor piercing munitions: comparison of neoplastic transformation and genotoxicity using the known carcinogen nickel. *Military Med* 167: 120-122.
- National Academy of Sciences. 1996. *Guide for the Care and Use of Laboratory Animals*. Washington, DC: National Academy Press.
- ORNL. 1996. Environmental acceptability of high-performance alternatives for depleted uranium penetrators. ORNL/TM-13286. Oak Ridge, TN: Oak Ridge National Laboratory.
- ORNL. 1998. Application of life cycle analysis: the case of green bullets. ORNL/CP-98264. Oak Ridge, TN: Oak Ridge National Laboratory.
- Pellmar TC, Fuciarelli AF, Ejniak JW, Hamilton M, Hogan J, Strocko S, et al. 1999. Distribution of uranium in rats implanted with depleted uranium pellets. *Toxicol Sci* 49: 29-39.
- Peuster M, Fink C, Wohlstein P, Brueggemann M, Gunther A, Kaese V, et al. 2003. Degradation of tungsten coils implanted into the subclavian artery of New Zealand white rabbits is not associated with local or systemic toxicity. *Biomaterials* 24: 393-399.
- Rakusan K, Cicutti N and Kolar F. 2001. Cardiac function, microvascular structure, and capillary hematocrit in hearts of polycythemic rats. *Am J Physiol Heart Circ Physiol* 281: H2425-H2431.
- Rao GN. 1996. New diet (NTP-2000) for rats in the National Toxicology Program toxicity and carcinogenicity studies. *Fund Appl Toxicol* 32: 102-108.

- Sunderman Jr FW. 1989. Carcinogenicity of metal alloys in orthopedic prostheses: clinical and experimental studies. *Fund Appl Toxicol* 13: 205-216.
- Sunderman Jr FW and Maenza RM. 1976. Comparisons of carcinogenicities of nickel compounds in rats. *Res Commun Chem Pathol Pharmacol* 14: 319-330.
- Sunderman Jr FW, Maenza RM, Alpass PR, Mitchell JM, Damjanov I and Goldbalatt PJ. 1977. Carcinogenicity of nickel subsulfide in Fischer rats and Syrian hamsters after administration by various routes. *Adv Exp Med Biol* 91: 57-67.
- Tomasovic SP, Coghlan LG, Gray KN, Mastromarion AJ and Travis EL. 1988. IACUC evaluation of experiments requiring death as an end point: a cancer center's recommendations. *Lab Animal* 17: 31-34.

Table 1. Selected hematological and organ weight parameters for euthanized rats

	Ta	WA (low)	WA (high)	Ni
White Blood Cells ($10^3/\mu\text{l}$)	3.19 ± 0.24	3.95 ± 0.43	4.56 ± 0.29 *	2.56 ± 0.20
Red Blood Cells ($10^6/\mu\text{l}$)	8.32 ± 0.09	8.03 ± 0.19	10.10 ± 0.07 #	7.46 ± 0.13 #
Hemoglobin (g/dL)	14.50 ± 0.13	13.90 ± 0.36	16.46 ± 0.30 #	12.95 ± 0.23 #
Hematocrit (%)	41.77 ± 0.53	40.38 ± 0.96	50.18 ± 0.39 #	38.12 ± 0.77 #
MCV (fL)	50.22 ± 0.16	50.26 ± 0.28	49.71 ± 0.16	51.08 ± 0.66
MCH (pg)	17.46 ± 0.15	17.31 ± 0.13	16.30 ± 0.28 #	17.35 ± 0.08
MCHC (g/dL)	34.77 ± 0.36	34.46 ± 0.32	32.81 ± 0.62 #	34.05 ± 0.50
RDW (%)	12.54 ± 0.09	13.07 ± 0.11 #	13.77 ± 0.09 #	13.04 ± 0.16 *
Platelets ($10^3/\mu\text{l}$)	562.00 ± 14.72	542.05 ± 14.27	467.50 ± 17.57 #	487.18 ± 26.10 *
Mean Platelet Vol. (fL)	9.93 ± 0.69	8.64 ± 0.52	10.13 ± 0.62	8.97 ± 0.52
Neutrophils ($10^3/\mu\text{l}$)	0.79 ± 0.05	1.03 ± 0.09 *	1.31 ± 0.12 #	0.78 ± 0.09
Lymphocytes ($10^3/\mu\text{l}$)	2.21 ± 0.18	2.42 ± 0.17	2.95 ± 0.23 *	1.63 ± 0.12 *
Monocytes ($10^3/\mu\text{l}$)	0.07 ± 0.01	0.09 ± 0.02	0.13 ± 0.02 *	0.05 ± 0.01
Eosinophils ($10^3/\mu\text{l}$)	0.08 ± 0.01	0.08 ± 0.01	0.12 ± 0.01 #	0.06 ± 0.01
Basophils ($10^3/\mu\text{l}$)	0.02 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.02 ± 0.00
Spleen (mg) / BW (g)	2.18 ± 0.10	2.30 ± 0.08	2.60 ± 0.06 #	2.17 ± 0.05
Thymus (mg) / BW (g)	0.86 ± 0.03	0.76 ± 0.04	0.70 ± 0.04 *	0.74 ± 0.07
Liver (mg) / BW (g)	29.21 ± 0.28	29.39 ± 0.24	28.77 ± 0.35	29.52 ± 0.39
Kidney (mg) / BW (g)	5.13 ± 0.06	5.13 ± 0.06	5.36 ± 0.05 *	5.15 ± 0.08
Testes (mg) / BW (g)	7.31 ± 0.07	7.20 ± 0.08	7.40 ± 0.10	7.21 ± 0.14

Data represent mean of 20 observations (10 for Ni group). Errors are standard error of the mean. Symbols * and # indicate significant difference from Ta control group at $P < 0.05$ and $P < 0.01$, respectively, using one-way analysis of variance followed by Dunnett's test for group mean comparisons. Abbreviations: MCV (mean corpuscular volume); MCH (mean corpuscular hemoglobin); MCHC (mean corpuscular hemoglobin concentration); RDW (red blood cell distribution width); BW (body weight).

Table 2. Selected hematological and organ weight parameters for rats implanted with metal pellets for 3 months.

	Ta	WA (low)	WA (high)
White Blood Cells ($10^3/\mu\text{l}$)	2.88 ± 0.20	4.06 ± 0.14 #	4.01 ± 0.21 #
Red Blood Cells ($10^6/\mu\text{l}$)	7.48 ± 0.06	8.48 ± 0.15 *	9.10 ± 0.70 #
Hemoglobin (g/dL)	12.90 ± 0.09	15.48 ± 0.35 *	17.29 ± 0.15 #
Hematocrit (%)	38.10 ± 0.27	42.14 ± 0.73 *	44.79 ± 0.62 #
MCV (fL)	50.96 ± 0.45	49.70 ± 0.09	48.87 ± 0.39
MCH (pg)	17.26 ± 0.12	18.27 ± 0.17	17.65 ± 0.12
MCHC (g/dL)	33.84 ± 0.35	36.71 ± 0.31 #	35.89 ± 0.31 #
RDW (%)	12.82 ± 0.33	12.68 ± 0.12	13.61 ± 0.09 #
Platelets ($10^3/\mu\text{l}$)	513.20 ± 38.36	585.11 ± 35.87	568.29 ± 8.82
MPV (fL)	9.58 ± 1.13	9.14 ± 0.59	11.74 ± 0.51
Neutrophils ($10^3/\mu\text{l}$)	0.62 ± 0.04	0.79 ± 0.03 *	0.91 ± 0.08 *
Lymphocytes ($10^3/\mu\text{l}$)	2.10 ± 0.16	3.06 ± 0.14 *	2.82 ± 0.17 *
Monocytes ($10^3/\mu\text{l}$)	0.04 ± 0.01	0.07 ± 0.01 *	0.08 ± 0.01 *
Eosinophils ($10^3/\mu\text{l}$)	0.09 ± 0.01	0.09 ± 0.01	0.09 ± 0.01
Basophils ($10^3/\mu\text{l}$)	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.00
Spleen (mg) / BW (g)	2.07 ± 0.03	2.16 ± 0.03	2.50 ± 0.03 #
Thymus (mg) / BW (g)	0.73 ± 0.03	0.84 ± 0.03	0.70 ± 0.04
Liver (mg) / BW (g)	30.58 ± 0.33	31.00 ± 0.33	30.27 ± 0.31
Kidney (mg) / BW (g)	5.43 ± 0.06	5.73 ± 0.23	5.76 ± 0.04 #
Testes (mg) / BW (g)	8.34 ± 0.12	8.21 ± 0.46	8.42 ± 0.18

Data represent mean of 15 observations. Errors are standard error of the mean. Symbols * and # indicate significant difference from age-matched Ta control group at $P < 0.05$ and $P < 0.01$, respectively, using one-way analysis of variance followed by Dunnett's test for group mean comparisons. Abbreviations: MCV (mean corpuscular volume); MCH (mean corpuscular hemoglobin); MCHC (mean corpuscular hemoglobin concentration); RDW (red blood cell distribution width); MPV (mean platelet volume); BW (body weight).

Table 3. Selected hematological and organ weight parameters for rats implanted with metal pellets for 1 month.

	Ta	WA (low)	WA (high)
White Blood Cells ($10^3/\mu\text{l}$)	3.86 ± 0.20	3.81 ± 0.14	3.86 ± 0.21
Red Blood Cells ($10^6/\mu\text{l}$)	7.84 ± 0.08	7.74 ± 0.07	8.50 ± 0.07 #
Hemoglobin (g/dL)	13.65 ± 0.15	14.81 ± 0.16	15.84 ± 0.14 #
Hematocrit (%)	40.15 ± 0.42	39.66 ± 0.50	43.29 ± 0.35 #
MCV (fL)	51.20 ± 0.14	51.22 ± 0.31	50.98 ± 0.19
MCH (pg)	17.41 ± 0.05	19.12 ± 0.09	18.64 ± 0.19 #
MCHC (g/dL)	34.01 ± 0.12	37.37 ± 0.29	36.56 ± 0.41 #
RDW (%)	12.21 ± 0.11	12.69 ± 0.11	14.18 ± 0.18 #
Platelets ($10^3/\mu\text{l}$)	646.50 ± 18.76	641.00 ± 17.97	756.20 ± 43.48 *
MPV (fL)	7.91 ± 0.40	8.56 ± 0.39	9.90 ± 0.55 *
Neutrophils ($10^3/\mu\text{l}$)	0.65 ± 0.04	0.79 ± 0.05	0.81 ± 0.04 #
Lymphocytes ($10^3/\mu\text{l}$)	3.04 ± 0.18	2.85 ± 0.13	2.90 ± 0.18
Monocytes ($10^3/\mu\text{l}$)	0.06 ± 0.00	0.06 ± 0.01	0.07 ± 0.00
Eosinophils ($10^3/\mu\text{l}$)	0.07 ± 0.01	0.08 ± 0.01	0.05 ± 0.00 *
Basophils ($10^3/\mu\text{l}$)	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.00
Spleen (mg) / BW (g)	2.37 ± 0.06	2.42 ± 0.05	2.73 ± 0.04 #
Thymus (mg) / BW (g)	1.07 ± 0.03	1.14 ± 0.04	1.06 ± 0.03
Liver (mg) / BW (g)	34.47 ± 0.26	34.31 ± 0.22	34.18 ± 0.61
Kidney (mg) / BW (g)	6.17 ± 0.08	6.06 ± 0.06	5.91 ± 0.05 *
Testes (mg) / BW (g)	10.10 ± 0.16	9.86 ± 0.13	9.98 ± 0.11

Data represents mean of 15 observations. Errors are standard error of the mean. Symbols * and # indicate significant difference from age-matched Ta control group at $P < 0.05$ and $P < 0.01$, respectively, using one-way analysis of variance followed by Dunnett's test for group mean comparisons. Abbreviations: MCV (mean corpuscular volume); MCH (mean corpuscular hemoglobin); MCHC (mean corpuscular hemoglobin concentration); RDW (red blood cell distribution width); MPV (mean platelet volume); BW (body weight).

FIGURE LEGENDS

Figure 1. Survival times of pellet-implanted rats.

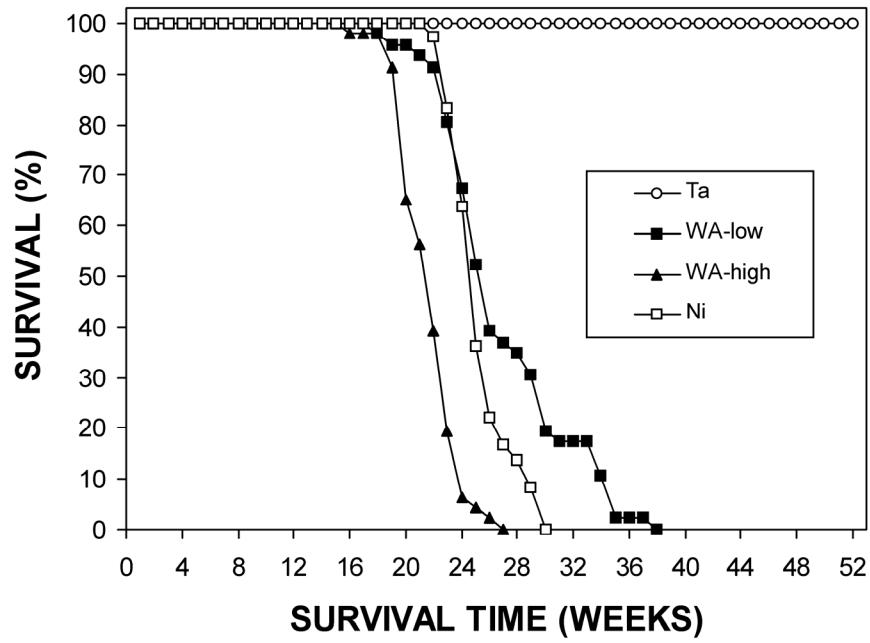
Figure 2. Effect of implanted WA pellets on F344 rats. (a) Gross appearance of Ta-implanted hind leg. (b) Dissected area around implanted Ta pellet (arrow indicates pellet).

(c) Gross appearance of WA-implanted hind leg with tumor(s). (d) Dissected area around implanted WA pellet with tumor surrounding pellet (arrow indicates pellet).

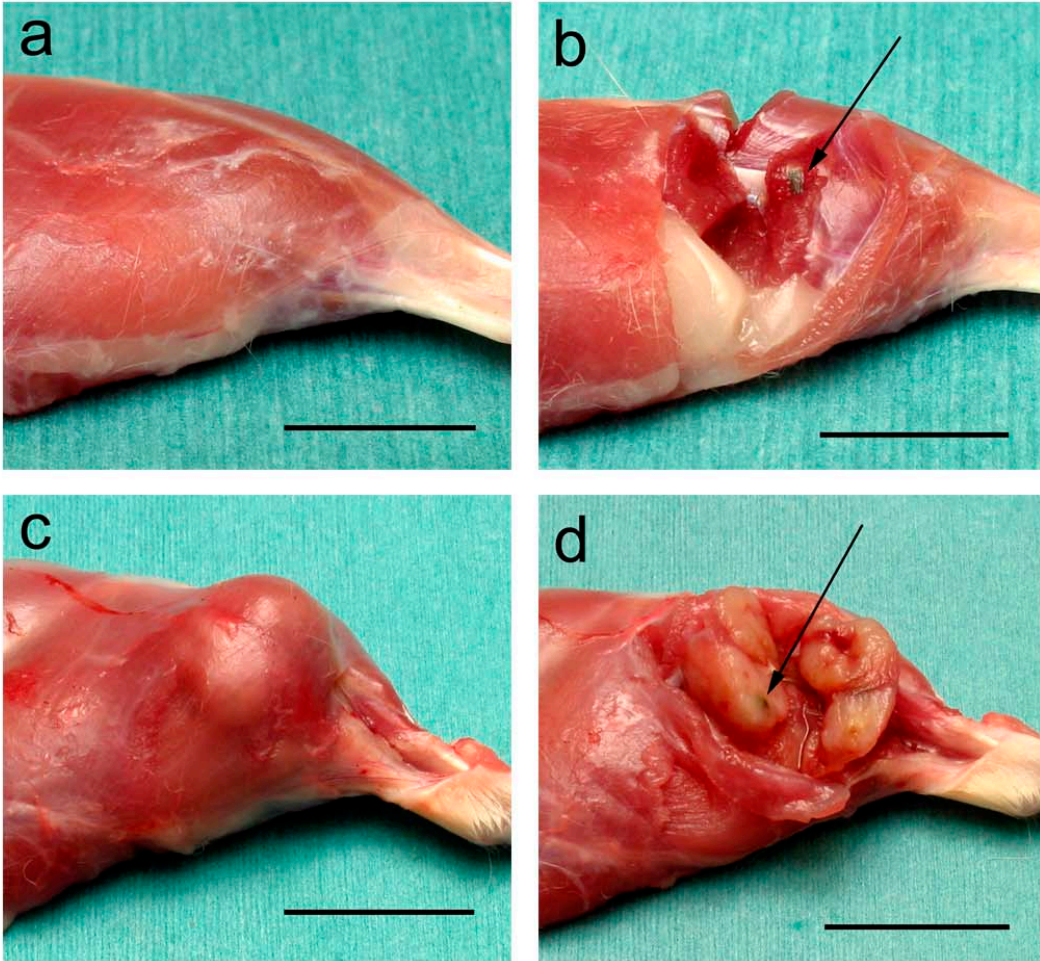
Figure 3. Histopathological examination of leg tumor surrounding WA pellet. (a) Hematoxylin and eosin (H&E) stained section of leg tumor from F344 rat showing WA pellet hole (P), scale bar = 500 μm . (b) H&E stained tumor section showing neoplastic infiltration of preexisting muscle fibers (MF), scale bar = 200 μm . (c) H&E stained tumor section showing neoplastic cells with numerous mitoses (arrows) and bizarre mitotic figures (BZ), scale bar = 100 μm . (d) H&E stained tumor section showing pleomorphic cell (arrow), scale bar = 100 μm . (e) Desmin staining of leg tumor showing neoplastic cells (NC) and muscle fibers (MF), scale bar = 500 μm . (f) Desmin staining of neoplastic cells, scale bar = 50 μm .

Figure 4. Lung metastases from WA-implanted F344 rats. (a) Gross appearance of pulmonary metastases from WA-implanted rat (arrows indicate metastatic foci). (b) H&E stained section of pulmonary metastases (arrows), scale bar = 1 mm. (c) H&E stained section of an occluded pulmonary arteriole (arrow indicates vascular smooth muscle wall (AW)) showing neoplastic cells with numerous mitoses (arrows), scale bar = 50 μm . (d) Desmin staining of pulmonary metastases (arrows), scale bar = 500 μm .

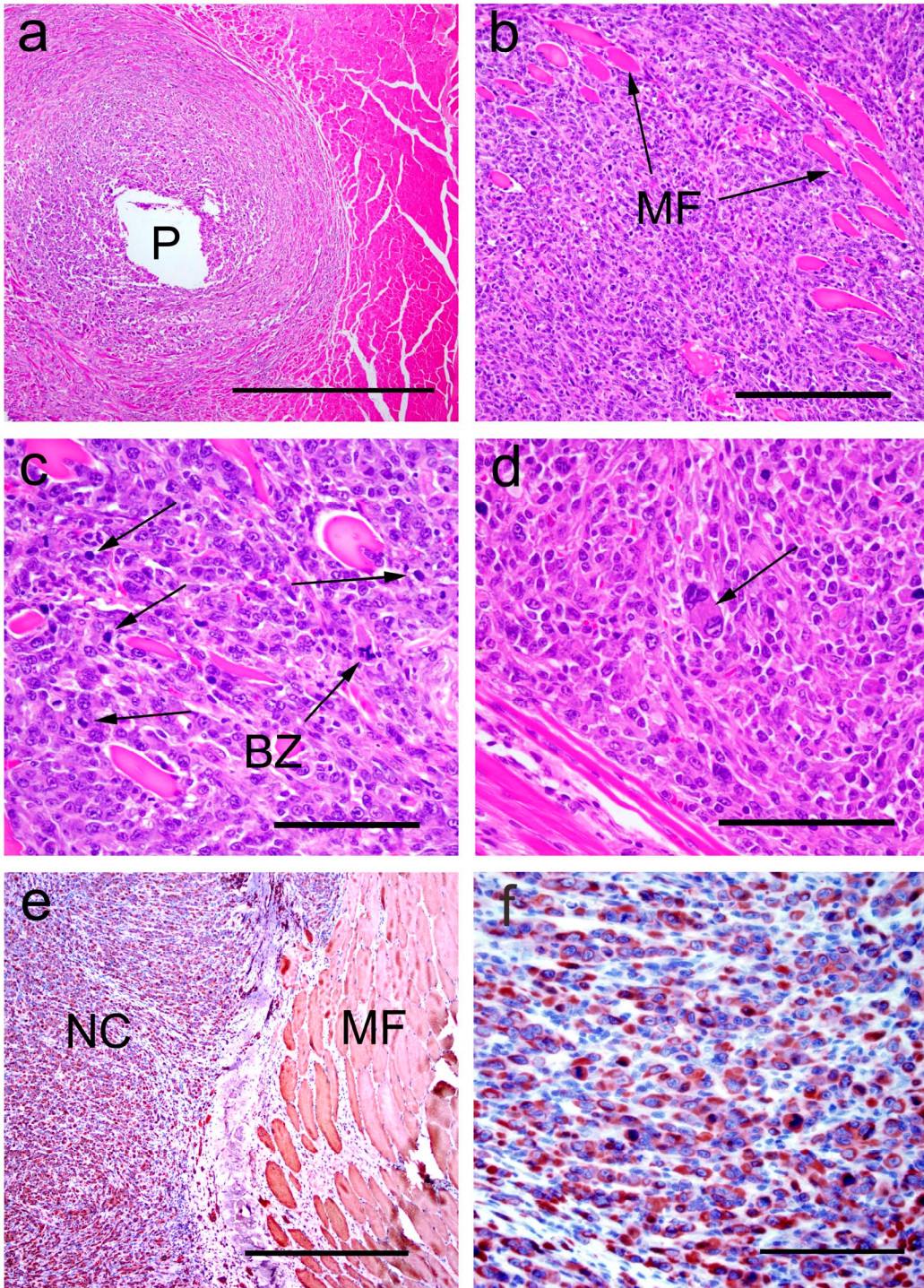
Kalinich, Figure 1



Kalinich, Figure 2



Kalinich, Figure 3



Kalinich, Figure 4

