Basic Science

The effect of surgically implanted metallic bullet fragments on the intervertebral disc using a canine model[☆]

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Abstract

PURPOSE: To characterize the gross, histologic, and systemic changes caused by implantation of metal fragments commonly used in commercial bullets into the intervertebral disc.

BACKGROUND CONTEXT: Long-term complications of retained bullet fragments in the spine have been documented in the literature; however, the impact of different metal projectiles on the intervertebral disc has not been described. This study was performed to assess the local effects of the metallic bullet fragments on the intervertebral disc and their systemic effects regarding metal ion concentrations in serum and solid organs.

STUDY DESIGN: Animal Model Study.

METHODS: Funding for this project was provided by the Cervical Spine Research Society in the amount of $10,000. Copper, lead, and aluminum alloys from commercially available bullets were surgically implanted into sequential intervertebral discs in the lumbar spine of six canines. Kirschner wire implantation and a sham operation were performed as controls. Radiographs were performed to confirm the location of the bullets. Animals were sacrificed at 4, 6, and 9 months postimplantation. Whole blood, plasma, cerebrospinal fluid, kidney tissue, and liver tissue samples were analyzed for copper and lead concentrations. Histologic and gross samples were examined at the time of sacrifice.

RESULTS: Significant tissue reactions were noted in the discs exposed to copper and lead. Copper resulted in significantly more severe disc degeneration than either the lead or aluminum alloy. In the short interval follow-up of this study, no statistically significant trend was observed in whole blood, plasma, cerebrospinal fluid, and tissue levels.

CONCLUSION: This study demonstrates that the canine intervertebral disc is differentially susceptible to metallic fragments depending on the composition. Trends were noted for increasing levels of lead and copper in liver tissue samples although statistical significance could not be reached due to short time interval and small sample size. The metallic composition of retained fragments can be a determining factor in deciding on surgical intervention.   © 2018 Elsevier Inc. All rights reserved.

Keywords: Aluminum; Copper; Gunshot wound; Intervertebral disc; Lead; Lead toxicity.

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Introduction

The long-term local effects of bullet fragments lodged within the intervertebral disc and the effect on distant organs and ionic blood levels have not been well described. The aim of this study was to characterize the effects of different metals commonly used in commercial bullets implanted within the intervertebral disc using a canine model. Previously, the effects of different bullet compositions on the spinal cord were reported. It was shown that implanted copper and, to a lesser degree, lead fragments led to significant destructive and inflammatory changes within the spinal cord as compared to aluminum bullet fragments [1]. In this current study, we examined the local and systemic effects of surgically implanted copper, lead, and aluminum bullet fragments within the intervertebral disc. Long-term clinical consequences of retained fragments have been described [2,3]; however, the specific effects relating to bullet composition have not. With considerable controversy remaining about the timing and indications for surgical removal of bullets or bullet fragments lodged within the intervertebral disc [4], investigation of the effects of the bullets on the local tissues, systemic serum as well as distant organs may help provide better prognostic information for patients.

Materials and methods

Canine model

The committee on animal research approved our protocol including all of the surgical procedures. Six purpose-bred Mongrel dogs, approximately 6 months of age and weighing an average of 23.5 kg (range 22−25.6 kg), underwent surgical implantation of bullet cores or casings at sequential intervertebral disc space levels in the lumbar spine. Stainless steel Kirschner wire implantation served as a placebo disc level. A sham operation, drilling a hole into the disc space without any implantation was a second control. The Mongrel dog model was chosen because a relatively large animal model was needed to minimize bullet fragment extrusion from the implantation sites. The procedures were done under general anesthesia using standard sterile conditions and surgical technique. Two dogs were sacrificed each at 4, 6, and 9 months. The animals were housed in the medical sciences building for the duration of the study and allowed unlimited activity on the grounds at our veterinary facility prior to sacrifice. Postoperatively, the animals were monitored for signs of neurologic compromise, lead toxicity, and infection. None of the animals showed any of the aforementioned side effects during the study period.

Bullet composition

Three commercially available bullet cartridges supplied by a local gun shop were used. The metals examined in this study were copper, lead, and aluminum. The lead cores were not altered prior to implantation. The copper and aluminum jackets were peeled off of their cores and then rolled into a solid mass with a size and shape similar to the lead bullets. The lead core fragments were taken from American Eagle 0.38 Special 158 grain (7,000 grains/pound or 15.4 grains/gram) lead round nose pistol cartridges (Federal Cartridge Co., Anoka, MN). The chemical composition of the lead alloy was roughly 92.4% lead, 6.2% antimony, 0.45% tin, and trace impurities [5]. The copper jacket fragments were taken from American Eagle 9 mm Luger automatic pistol 124 grain metal case bullet cartridges (Federal Cartridge Co., Anoka, MN). The copper was nearly 99.9% pure with only trace impurities [5]. The Silvertip jacket fragments were taken from Winchester 44 S & W Special 200 grain Silvertip hollow point bullet cartridges (Olin Corp., East Alton, IL). Although the name Silvertip implies that silver is contained within the alloy, the content was as follows: 98.5% aluminum, 1.2% manganese, and 0.12% copper with other trace impurities [6].

Surgical technique

The dogs were anesthetized with intravenous ketamine (43 mg/mL), xylazine (8.6 mg/mL), and acepromazine (1.4 mg/mL). Cefazolin (30 mg/kg) was given intramuscularly preoperatively and on the first and second postoperative days. Once deeply anesthetized, the animals were placed into a lateral decubitus position and the left lateral abdomen and flank were prepped. Then, using a retroperitoneal exposure, the anterior aspect of the lumbar spine was exposed. A drill hole was made through the anterolateral aspect of the anterior longitudinal ligament of each of the lumbar disc spaces using a 1/8 inch drill bid. An entrance hole was made in the intervertebral disc by hand after piercing the annulus, and the respective bullet fragment was placed within the disc space. All fragments were shaped so that they easily fit within the space created. Postoperative pain was managed with buprenex intramuscular 0.02 to 0.05 mg/kg every 12 hours.

The lumbar region was chosen because the large size of the disc could accommodate a foreign body without compromising the spinal canal. Each disc level held one type of bullet or fragment and each animal underwent all five experimental protocols. From cephalad to caudal, the order of implantation was a whole copper jacket rolled into a bullet shape (L1−L2), a sham hole (L2−L3), a whole lead bullet (L3−L4), stainless steel wire control (L4−L5), and a Silvertip jacket rolled into a bullet shape (L5−L6). Prior to implantation, the fragments were thoroughly washed with betadine detergent and heat sterilized.

Cerebrospinal fluid (CSF) was obtained using a 22 gauge spinal needle with stylet and a short bevel via a posterior occiput-C1 approach. The fluid was placed directly into sterile royal blue stopper trace element blood collection tubes (Sherwood Medical, St. Louis, MO) prior to analysis. The animals were euthanized using sodium pentobarbital
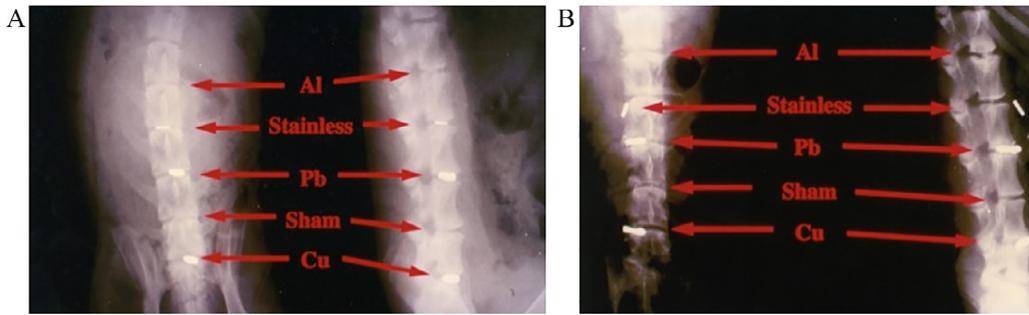
Fig. 1. (A) Anteroposterior and lateral radiographs one week postimplantation demonstrating metallic fragments within intervertebral discs. Al, aluminum; Stainless, stainless steel (K-wire); Pb, lead; Sham, no implant; Cu, copper. (B) Anteroposterior and lateral radiographs 6 months postimplantation demonstrating a cystic lesion adjacent to the lead bullet. Al, aluminum; Stainless, stainless steel (K-wire); Pb, lead; Sham, no implant; Cu, copper.

100 mg/kg or more as needed intravenously after intramuscular sedation with atropine and acepromazine. Immediately postmortem, the fragments were retrieved using the same approach described above for implantation.

Sample analysis

CSF samples were collected prior to implantation and after sacrifice. Whole blood and plasma were collected prior to implantation and at 8-week intervals until the time of sacrifice. Kidney and liver tissue samples were only collected at the time of sacrifice.

All blood and CSF samples were stored on dry ice and analyzed by the State of Florida Department of Agriculture and Consumer Services, Bureau of Diagnostic Laboratories (Kissimmee Diagnostic Laboratory Kissimmee, FL). A PerkinElmer model-3030 atomic absorption spectrometer with a deuterium arc background correction and a heated graphite furnace atomizer (HGA)-400 graphite furnace (PerkinElmer) were used for lead and copper analysis, respectively, according to the manufacturer's recommendation. The kidney and liver samples were also stored on dry ice. They were analyzed by National Medical Services (Willow Grove, PA) by flame atomic
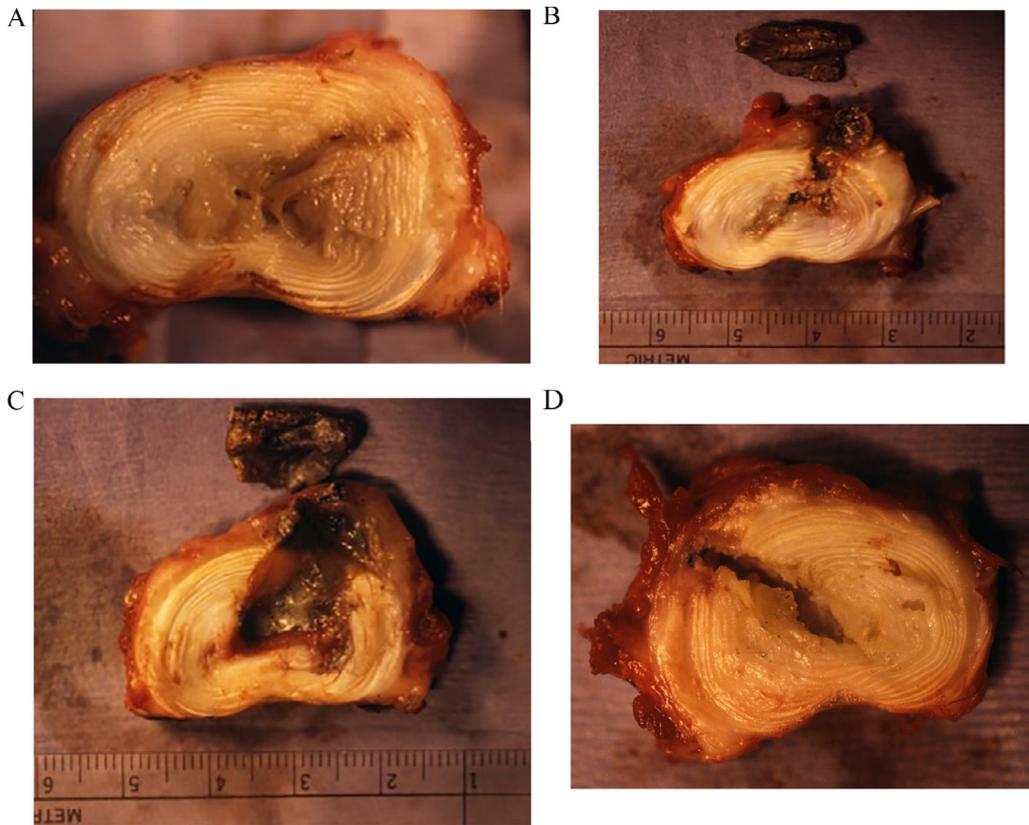

Fig. 2. (A−D) Gross samples from the animals sacrificed at 4 months. (A) The sham operation produced mild fibrosis of the disc without destruction. (B) Lead implantation resulted in erosion of the area of the disc surrounding the bullet. (C) Copper fragment implantation caused destructive changes which are subjectively more intense than lead, sham, and aluminum. (D): Aluminum fragment implantation produced minimal changes to the disc.
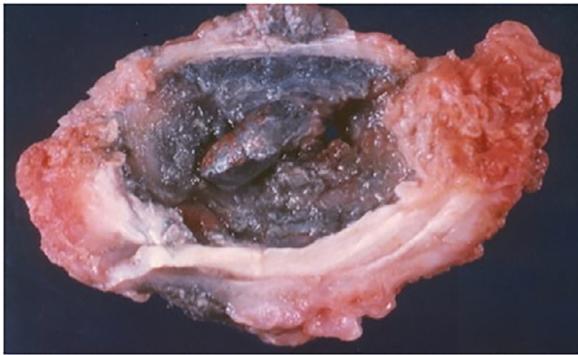
Fig. 3. Gross sample 6 months postimplantation with copper fragment demonstrating extensive destruction of the entire disc.

absorption spectroscopy, for the copper values, and graphite furnace atomic absorption spectroscopy, for the lead values.

Radiographic evaluation

Anteroposterior and lateral radiographs of the spine were obtained 1 week after implantation of the fragments and repeated immediately prior to sacrifice.

Histology

After gross observation, histologic analysis of the intervertebral discs using a subjective grading scale was performed. The tissues were stained with hematoxylin and eosin.

Statistical methods

Lead and copper metal analyses of whole blood, serum, and CSF fluid, as well as levels from the kidney and liver were performed on samples isolated at the time of spine and intervertebral disc harvest. Aluminum, although a large component of the Silvertip alloy, was not assayed in this study. The Pearson correlation coefficient and an analysis of variance were used to test the statistical significance of changes in tissue samples and fluid samples, respectively.

Results

Radiographic results

Radiographs performed 1 week after fragment implantation were evaluated to confirm the location of the metal fragments (Fig. 1A). The aluminum fragments are radiolucent. Radiographs at 6 months postimplantation demonstrated a cystic lesion adjacent to the lead bullet in one case (Fig. 1B).

Gross examination

The implanted fragments produced a varied response depending on the type of metal. Compared to the mild fibrosis produced by the sham procedure (Fig. 2A), copper and to a lesser degree lead resulted in destructive changes to the intervertebral disc. Lead produced a moderate reaction with localized tissue destruction (Fig. 2B). The copper jackets were noted to cause severe destruction of the nucleus pulposus which was replaced with necrotic tissue
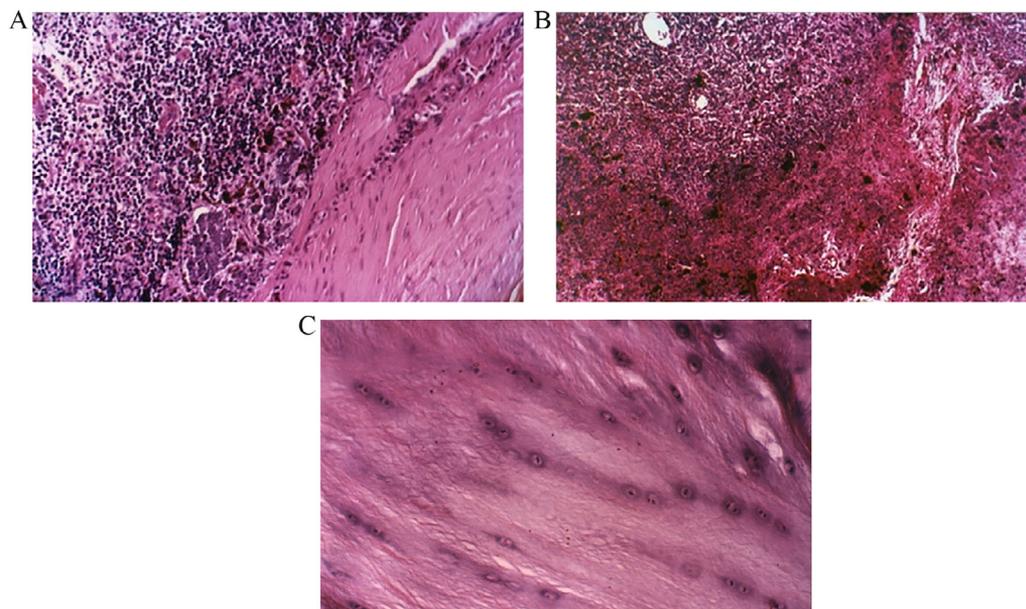
Fig. 4. (A−C) Histologic sections of the intervertebral disc 4 months postimplantation stained with hematoxylin and eosin (100× magnification). (A) Lead implantation resulted in an inflammatory reaction as well as microparticle deposition represented by the areas of darker discoloration. (B) Copper produced a more intense inflammatory reaction with similar areas of microparticle deposition throughout the disc. (C) Aluminum did not produce an inflammatory response.

Table 1
Mean measurements of copper and lead content in liver and kidney tissue samples

	4 months (N=2)	6 months (N=2)	9 months (N=2)
Liver-Copper (micrograms/gram)	91.0	74.1	126.0
Kidney-Copper (micrograms/gram)	5.4	4.9	5.6
Liver-Lead (micrograms/gram)	3.1	2.8	9.5
Kidney-Lead (micrograms/gram)	1.4	1.8	2.5

(Fig. 2C). The tissue changes were noted to extend radially from the fragments. The degree of tissue destruction increased with time (Figs. 2C and 3). Aluminum did not stain or damage the intervertebral disc tissue (Fig. 2D). Additionally, copper was noted to most consistently remain in the intervertebral disc, while aluminum and lead shifted slightly from the site of implantation in one and two cases, respectively.

Histologic results

Subjectively, tissue changes observed in the intervertebral discs paralleled the gross findings. Lead implants resulted in scattered areas of necrosis as well as a dense infiltrate of chronic inflammatory cells and pigment laden macrophages (Fig. 4A). Comparatively, a more intense response was seen in the discs implanted with copper. Numerous areas of necrosis and a dense infiltrate of chronic inflammatory cells were visualized in the discs implanted with copper fragments (Fig. 4B). For both copper and lead, the severity of the inflammatory reaction was directly proportional to the time of exposure. In contrast, the aluminum fragments produced no inflammatory reaction (Fig. 4C). This was the case for the sham and stainless steel control, as well.

Whole blood, CSF, and liver analysis

The average copper and lead metal concentrations in liver and kidney tissue obtained via biopsy immediately prior to sacrifice are shown in Table 1. Average plasma and whole blood concentrations of lead and copper at 2-month intervals are shown in Table 2. Whole blood and plasma were used to assay lead and copper, respectively, as per the recommendations of the testing laboratory. Preoperative

whole blood lead values were not available due to improper collection. CSF lead and copper levels were only obtained prior to fragment implantation and harvest and are also reported in Table 2. Whole blood, plasma, and CSF samples showed no statistically significant elevation in copper or lead levels with time compared to preoperative values.

Liver analyzed for lead content at the time of harvest at 4, 6, and 9 months did show a threefold increase with time (Table 1). Liver analyzed for copper content for similar harvest periods also trended upward. However, neither pattern was statistically significant. Kidney copper and lead values showed no pattern of elevation with time.

Discussion

Copper, lead, and aluminum are common components of bullets. The results of this study show that these different metals can produce profoundly different effects on the intervertebral disc tissue. These data are similar to a previous observation on the effect of retained metallic fragments on the spinal cord [1].

The reaction of tissues to metallic fragments is determined by the rate of metallic corrosion as well as the toxicity of the products of corrosion [7,8]. In this study, copper was most corrosive. A less intense response was seen with lead as the area of destruction was localized to the perimeter of the bullet. In contrast, aluminum invoked little to no reaction and the reaction was similar to the stainless steel wire and the sham procedure. These destructive changes with copper and lead fragments were noted to be time dependent. Our findings are consistent with those of similar investigators of the experimental effects of metal foreign bodies on brain tissue [9], spinal cord [1], and bone [10].

Lead is a toxic metal that has been shown to compete with calcium and thereby interfere with the regulation of

Table 2
Mean measurements of copper content in serum and CSF, and of lead content in whole blood and CSF

	Pre op (N=6)	8 weeks (N=6)	16 weeks (N=6)	24 weeks (N=4)	36 weeks (N=2)
Plasma-Copper (ppm)	0.370	0.290	0.400	0.360	0.380
CSF-Copper (ppm)	0.140	†	0.080	0.100	0.040
Whole blood-Lead (ppm)	*	0.320	0.340	0.412	0.340
CSF-Lead (ppm)	0.021	†	0.015	0.033	0.008

CSF, cerebrospinal fluid; ppm, parts per million.

  * Value unavailable due to an error at the time of collection.

  † CSF samples not obtained at this time point.

cell metabolism [11,12]. Local and systemic toxicity may occur from lead when it leaches out of a bullet located in other locations in the human body. This has been described with bullets bathed in synovial fluid [13−19] and with bullets in the intervertebral disc [20,21]. In the short interval of follow-up of this study, we did not detect statistically significant increases of lead in blood, CSF, kidney tissue, or liver tissue in our model although the concentration of lead in liver was notably elevated at 9 months. Onset of symptoms of lead toxicity in patients with retained bullet fragments in the spine and elsewhere whose symptoms improved after extraction of the metal was as early as 3 days or as late as 40 years after the initial injury [2,20−25]. Despite a trend in levels of lead in the liver tissue, the data are limited to interpretation due to the brief study duration period which limited statistical evaluation. Moreover, the small sample size limits the power of the study. None of the animals in this experiment displayed any signs of neurologic compromise or acute lead toxicity. Our findings on plain films of a pseudocyst adjacent to the intervertebral disc containing the lead fragment are consistent with other reports described elsewhere [4,20,23,26].

Copper has repeatedly been shown to exert a toxic and immunogenic effect on brain tissue resulting in necrosis [8,10,27−31]. Examining the histologic and gross specimens retrieved in this study suggests that copper exerts similar toxic effects on the intervertebral disc. Sights and Bye described marked inflammatory changes consisting of closely packed mononuclear cells and many pigment-laden macrophages in brain tissue exposed to copper pellets [9]. Similarly, our copper-exposed tissue samples showed a dense inflammatory infiltrate. The effect of copper to inhibit fibroblasts has previously been described and may be related to the results observed here [32]. In our model, elevated copper levels were seen with time in the liver, but not for the kidney. The changes were notable but were not statistically significant. Venable et al. implanted screws of various metallic compositions into the radius in 24 dogs. Among the dogs in which copper was part of the alloy used, excess copper was found in the liver and kidney [10]. Many commercially available bullets are jacketed with copper and radiological evidence of metallic foreign bodies of different densities can aid in determining the presence of copper [8]. Due to its caustic effects on neural tissue, some authors recommend removal of bullets containing copper from the spinal canal [33−35]. To our knowledge, no literature exists on the destructive effects of copper on the intervertebral disc.

Based on histologic and gross examination, aluminum appears to exert little to no effect on the intervertebral disc. We did not test for blood, CSF, kidney tissue, or liver tissue for aluminum in this study. To our knowledge, no report of systemic aluminum toxicity from bullets has been published.

Surgery to remove a bullet from the spine is not without risk. Infection, CSF leaks, and postoperative instability are documented complications [36−44]. The effects of copper and lead on the intervertebral disc observed in this study will definitely contribute to premature degenerative changes in the disc and may eventually lead to pain and instability.

The results of our study show that the intervertebral disc can have profoundly different reactions to metal fragments from commercially available bullet cartridges depending on the type of metal fragment. The metallic composition of the retained fragments may be a determining factor in deciding on surgical intervention [45]. Copper bullet fragments and, to a lesser degree, lead cause intense destruction which can be clearly seen in both gross and histologic samples. Our results show that the metallic composition is an important factor to be considered when deciding whether to remove bullet fragments retained within the intervertebral disc following a gunshot wound [46].

Funding

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