Inflammatory, Proliferative, and Neoplastic Lesions at the Site of Metallic Identification Ear Tags in Wistar [Crl:(WI)BR] Rats

Michael P. Waalkes,1 Sabine Rehm, Kazimierz S. Kasprzak, and Haleem J. Issaq

ABSTRACT

During a 2-yr study of carcinogenesis by CdCl₂ in male Wistar [Crl(WI)BR] rats, weekly clinical observations during the last 6 mo of the study revealed many cases of persistent tumor-like masses at the site of the metal identification tags in the ears of the animals. A total of 14 tumors (mostly compound osteosarcomas) was diagnosed in 168 rats. Histologically, almost 90% of the rats in this study (henceforth referred to as Study I) showed some significant lesion at the tag site including various degrees of chronic inflammation, chondroid hyperplasia, and osseous metaplasia of the pinna cartilage. In marked contrast, only two tumors were detected in 193 animals in a second study (Study II) in the same strain of rats, and only 56% of the rats had lesions at the tag site. A high incidence (>25%) of clinically severe inflammation at the tag site was seen early in Study I and persisted during the first 6 mo of the study, while the incidence of such reactions in Study II was never more than 1%. Elemental analysis of the tags provided no explanation for the differences between the two studies, as tags used in both studies were of the same composition, predominantly nickel and copper. Metallic internal prostheses have induced local malignancies in humans and animals, and the present observations provide further evidence of the hazard posed by such devices at the site of prolonged contact with tissues. These findings suggest that a persistent tissue reaction may be an important factor in tumor development.

INTRODUCTION

Bone-fixing plates and internal prostheses made of metal alloys have proven to be a valuable aid in the medical or dental management of a variety of human maladies. Bone pins and plates have also been widely used in veterinary medicine, generally as fracture fixation devices in small animals. Such implants have been reported to induce local malignancies in both humans and animals (1-15). In humans, at least 11 such cases have been described thus far (1-11). In veterinary medicine, numerous cases of tumor development at the site of implantation have been reported in dogs (12-15) and occasionally in cats (13). The tumors occur at various intervals following the surgical implantation, generally ranging in humans from 2 to over 25 yr (1-11). Tumor types arising at the site of metallic implantations vary widely but are usually soft tissue sarcomas. Hemangiopericytomas, histiocytomas, Ewing’s sarcomas, fibrosarcomas, and osteosarcomas have been reported in humans (1-5, 9-11), but osteosarcomas appear to predominate in dogs (12-15). Lymphomas have also been reported in association with bone plates (6, 7), while a single case of a squamous cell carcinoma has been found in conjunction with a mandibular staple bone plate (9). In rats, intraosseous administration of metallic orthopedic implant materials can result in sarcomata and hematological malignancies (i.e., lymphoma, myeloid leukemia) (16).

The metals used to manufacture implants commonly include chromium, cobalt, copper, iron, manganese, and nickel in different proportions. Nickel was early recognized as a human carcinogen following industrial exposure, and its insoluble salts have proven to be potent inorganic carcinogens in test animals (17-20). Likewise, chromium is a well-recognized carcinogen in humans (18-20) and animals (17-20). The carcinogenicity of the other metallic components of the implants is less well established, particularly in humans, although cobalt can induce local tumors in rats when injected i.m. (21), and iron and copper have induced tumors in animals under unusual circumstances (17, 19, 22, 23).

Several factors may be associated with the eventual development of the tumor at the site of metal implantations. Most often tumors in humans arise in association with indwelling implants (1-4, 6-11), although tumors have arisen in cases in which the plate had been removed as much as 6 yr earlier (5). Several malignancies have been noted at sites adjacent to spots of local corrosion of bone plates if fixing screws had been made of slightly different material (2, 8). Such corrosion could result in higher local release of carcinogenic metal or alternatively could cause a loosening of the implant resulting in persistent local injury. Corrosion is, however, not seen in all cases. Dodion et al. (7) have also pointed out the possibility that long-standing infections, as seen in their case of a lymphoma developing at an infected vitallium bone plate, could also be a factor in tumor development. Similar associations between local infections and tumor development at the site of intramedullary splinting have also been noted in dogs (13, 14). Given the great numbers of implantations performed yearly, however, the factors involved in predisposing a small percentage of the cases to tumor formation remain obscure.

The present report details the unexpected finding of a significant incidence of tumors at the site of metallic identification tags in rats that appear to have been predisposed to tumor formation by an early and persistent tissue reaction at the ear tag site. These data are further evidence of the carcinogenic hazard posed by metal alloys at the site of prolonged contact with tissues.

MATERIALS AND METHODS

The ear tumors and lesions described in this work were seen in two separate chronic studies concerning the carcinogenicity of cadmium salts in male Wistar rats [Crl(WI)BR] obtained from the Charles River Breeding Laboratories (Kingston, NY) and housed in the same facility. The first study (termed Study I) was started in September 1983 to determine the dose-response relationships for CdCl₂ injected s.c. at approximately 8 wk of age. This study involved a total of 315 rats and lasted for 104 wk. The second study (Study II) was begun 2 mo after Study I to investigate effects of zinc pretreatment on cadmium carcinogenesis. Study II involved 364 rats and continued for 104 wk. The animals were group housed, 3 per polycarbonate cage, in a temperature-controlled animal room with a 12-h light/dark cycle maintained via artificial illumination. Water and food (Purina No. 5001 rodent chow) were provided ad libitum. Two wk prior to treatment, when the animals were 6 wk of age, metallic identification tags (Self-Piercing, Style 4-1005, No. 3 size; National Band and Tag Co., Newport, KY) were
applied to the right ear with specifically designed application pliers, piercing the skin and cartilage at the base of the pinna.

Animals were inspected weekly for the duration of the study, and any clinically observed lesions were recorded for each individual animal. The first clinically visible mass at the ear tag site was detected at 75 wk in Study I, and from that time both pinnae from all animals in both studies were carefully examined clinically and at necropsy were processed for microscopic examination. Prior to that time, 147 of 315 rats in Study I and 171 of 364 rats in Study II died or were sacrificed in extremis for a variety of reasons unrelated to ear pathology. All animals alive at 104 wk were sacrificed. Tissues were fixed in 10% neutral buffered formalin, decalcified for 72 h in a solution of formic acid and sodium citrate, embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

For metal analysis, the ear tags were first placed in 3 N NaOH for 24 h to solubilize any tissue remnants, then rinsed in double-distilled water, dried, and weighed to the tenth of a mg. The tags were then dissolved in 2.0 ml of concentrated, ultrapure nitric acid (Baker Chemical Co.) for 72 h. The dissolved tags were then diluted to 50 ml with double-distilled water for metal determinations. Metals (arsenic, cadmium, chromium, copper, iron, lead, manganese, nickel, and zinc) were then determined by flame atomic absorption spectrophotometry using a Perkin Elmer Model 5000, and concentrations were calculated using certified standards.

Significance of differences in the incidences of the various lesions associated with the identification tags between the two studies was estimated by the χ² test with Yates' correction (24). Differences in the metal content of the identification tags between the two studies were compared by Student's t test. In all cases P ≤ 0.01 was considered significant.

RESULTS

A high incidence of what was termed "ear tag infection" of unknown etiology was seen during the first 6 mo (Fig. 1) of Study I. The hallmarks of such tissue reactions were swelling and redness that often involved the entire ear or were seen in streaks radiating from the identification tag. Occasionally a flaky purulent exudate with a noticeable odor and bleeding from the tag insertion site were noted. In marked contrast, the incidence of apparent "ear tag infection" in Study II was never more than 1%. After the first 6 mo, fewer acute exudative but more chronic, proliferative inflammatory reactions were observed in most animals at the tag site in Study I. Histological data from rats aged 18 to 24 mo showed that persistent inflammations were diagnosed 2.6 times more often in Study I than in Study II (Table 1).

The chronic inflammatory, proliferative, and neoplastic lesions discovered at the tag insertion site are shown in Tables 1 and 2 and Fig. 2. All such lesions occurred with varying degrees of severity, but were, in general, much more frequently observed in Study I than in Study II. None of the lesions at the identification tag site described herein was associated with treatment. Lesions were equally distributed among the treatment groups within each study. Rats died or were sacrificed due to a variety of causes (tumors, etc.), which were not associated with pinna lesions; results of the CdCl₂ studies will be described in later papers.

Skin lesions adjacent to the tags ranged from mild dermatitis to deep ulceration and epidermal hyperplasia. Frequently, the tissue surrounding the tag was covered by dried mixtures of purulent exudate and keratinized masses (Fig. 2, A and C). From foci adjacent to the point where the tag had been inserted, chondroid hyperplasia could be observed ranging from small single lesions with occasional mineralizations to larger multifocal cartilaginous nodules up to 5 mm in diameter (Fig. 2, C and D). Osseous metaplasia of hyperplastic cartilage readily occurred, and bony nodules at the ear tag site measured up to 1 cm in diameter. These bone formations often exhibited neighboring zones of osteoblastic and osteoclastic activity (Fig. 2B) next to areas of no particular cellular proliferations, i.e., lamellar bone being surrounded and interwoven by fibrous connective tissue stroma (Fig. 2D). Occasionally, bone marrow formation was observed within the metaplastic bone (Fig. 2E). Granulation tissue growing adjacent to the metallic tag consisted of a delicate fibroblastic stroma with many newly formed vessels and was infiltrated by neutrophilic granulocytes, lymphocytes, plasma cells, and histiocytes (Fig. 2F). In cases in which previous tag loss had been reported, no skin lesions were observed, the inflammatory response and osteoblastic activity had ceased, and the granulation tissue had been replaced by fibrous connective tissue. In such cases occasional osteo- and chondroblastic cells were indicative of further tissue restitution. The right

### Table 1 Incidence of lesions at the identification tag site

<table>
<thead>
<tr>
<th>Lesion</th>
<th>Study I</th>
<th>Study II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animals analyzed</td>
<td>168 (100)*</td>
<td>193 (100)</td>
</tr>
<tr>
<td>Tumors</td>
<td>14 (8.3)</td>
<td>2 (1.0)</td>
</tr>
<tr>
<td>Bone formation</td>
<td>90 (53.6)</td>
<td>75 (38.8)</td>
</tr>
<tr>
<td>Mild to moderate</td>
<td>35 (20.8)</td>
<td>17 (8.8)</td>
</tr>
<tr>
<td>Severe</td>
<td>21 (12.5)</td>
<td>87 (42.5)</td>
</tr>
<tr>
<td>Chondroid hyperplasia</td>
<td>45 (28.0)</td>
<td>13 (6.7)</td>
</tr>
<tr>
<td>Granulation tissue</td>
<td>64 (40.5)</td>
<td>30 (15.5)</td>
</tr>
<tr>
<td>Chronic inflammation</td>
<td>19 (10.7)</td>
<td>85 (44.0)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses. percentage.

### Table 2 Classification of tumors found at the identification tag site

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Study I</th>
<th>Study II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total tumors</td>
<td>14</td>
<td>2</td>
</tr>
<tr>
<td>Compound osteosarcoma</td>
<td>7*</td>
<td>0</td>
</tr>
<tr>
<td>Simple osteosarcoma</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Fibrosarcoma</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Giant cell tumor</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Histiocytic sarcoma</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Skin papilloma</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

* Includes 5 osteo/fibrosarcomas, 1 osteo/chondro/fibrosarcoma, and 1 osteo/fibrosarcoma/giant cell tumor.
maxillary lymph nodes of those rats with an inflammatory process of the corresponding pinna regularly exhibited plasmacytosis and lymphoid hyperplasia, were often cystic, and occasionally contained an abscess. All nonneoplastic lesions occurred at a much higher incidence in Study I than Study II, with the exception of chondrous hyperplasia (Table 1). Indeed, only 9% of those animals examined in Study I were without a lesion at the tag insertion site. In contrast, 44% of the rats in Study II had no significant tag-associated lesion.

In Study I, a total of 14 tumors was microscopically diagnosed in 168 rats (Table 1). In contrast, only 2 tumors were found at the site of the identification tag in Study II. Tumors arising at the tag site were described grossly as masses between 1 and 3 cm in diameter that enveloped the pinna and occasionally grew into the external ear canal. The consistency was usually described as firm with hard, bony inclusions. The giant cell tumors, however, were described as soft fleshy growths bleeding intermittently. Microscopically, all neoplasms appeared to have originated from the previously described tag associated chronic nonneoplastic lesions of the pinna. With the exception of the skin papilloma, all tumors were diagnosed according to the histogenetic classification system for bone tumors in humans (25) and animals (26). The characteristic features of these tumors are depicted in Fig. 3, and their incidence is given in Table 2. Frequently, remnants of chondrous hyperplasia or metaplastic bone formations were seen within the neoplastic tissue or at the edges of normal pinnal cartilage. The most frequently observed tumors at the identification tag site were osteosarcomas (Table 2), of which only three consisted exclusively of osteoblastic cells, osteoid, and bone of different stages of maturation (Fig. 3, A and B). The compound osteosarcomas exhibited a highly pleomorphic nature with a mixture of osteoblastic, fibroblastic, or chondroblastic differentiation (Fig. 3, B to D), interspersed by occasional giant cells (osteoclasts). All neoplasms invaded the pinnal cartilage and skin giving rise to peripheral ulceration and local inflammation. Three osteosarcomas metastasized to the maxillary lymph node. The giant cell tumors (osteoclastomas; Fig. 3C) were very well vascularized and consisted of many multinucleated giant cells containing up to 20 nuclei and smaller round to ovoid cells with a single, eccentrically located nucleus. The cytoplasm of both the giant and the mononuclear cells stained deeply eosinophilic and was slightly vacuolated. The histiocytic sarcoma (Fig. 3F) consisted of cells with pleomorphic nuclei and possessed a lightly basophilic cytoplasm. Numerous mitotic figures were present, and giant cells were seen particularly in the extensive metastases to the maxillary lymph nodes. The single papilloma (not shown) originated from the skin overlying a larger metaplastic bony nodule at the tag insertion site.

It was initially suspected that the marked difference in the tumor incidence between Studies I and II might be accounted
for by a difference in the metal content of the tags, as the carcinogenic potency for different metals is known to vary considerably (17-19). This, however, was not the case, as the elemental compositions of the tags used in the two studies were essentially identical (Table 3) and consisted primarily of nickel and copper. The tags also contained significant amounts of iron, manganese, and chromium, while cadmium and zinc were detectable. There was, however, no detectable content of lead or arsenic.

DISCUSSION

Previous observations have shown that metallic implants are occasionally associated with the local formation of tumors (1-14), and the observations detailed in the present study provide further evidence for the carcinogenic hazard posed by the prolonged contact of metallic devices with tissues. Furthermore, the fortuitous finding of two studies using identical conditions and animals but showing highly different tumor frequencies at the site of the metallic device provides a unique situation for analysis of this phenomenon.

The exact stimulus for the formation of the malignancies seen at the site of tissue contact with metallic devices is as yet unknown, although several possibilities have been previously proposed (1, 3, 7, 12-14). In the context of tumors in domestic animals associated with plated fractures, Stevenson et al. (14) discuss several hypotheses, including some which suggest that the implants are the causative agent while others implicate a deranged healing process of the host tissue. The specific hypotheses that could apply to the present case include as initiating factors the metallic components of the implant along with factors that enhance surface release of the metal. Conversely, initial tissue damage with altered cellular activity during subsequent healing, with or without local infection (e.g., osteomyelitis), has been mentioned as a possible initiating event independent of the metal content of the device. So-called solid-state carcinogenesis (also known as foreign body or smooth surface carcinogenesis) may also play a role as a mechanism of tumor induction independent of the nature of the device in contact with the tissue. In the present case, initial tissue damage can be

| Table 3: Metal content (percentage) of identification tags |
|---------------------------------|------------------|
| Study I                        | Study II         |
| Nickel                         | 65.3 ± 3.0<sup>a</sup> | 68.3 ± 0.7 |
| Copper                         | 32.4 ± 3.7       | 28.9 ± 0.9 |
| Iron                           | 1.27 ± 0.36      | 1.76 ± 0.20 |
| Manganese                      | 0.85 ± 0.14      | 1.03 ± 0.09 |
| Chromium                       | 0.20 ± 0.03      | 0.24 ± 0.03 |
| Zinc                           | <0.01            | <0.01      |
| Cadmium                        | <0.001           | <0.001     |

<sup>a</sup> Mean ± SE of 3 to 6 identification tags as determined by flame atomic absorption spectrometry after dissolution with concentrated nitric acid. There was no detectable lead or arsenic. In no case were significant differences found between the two studies.
eliminated from consideration as the same devices were inserted in the same locale and fashion in both studies, indicating that differences in initial tissue damage could probably not have varied to such an extent as to have accounted for the different frequencies of neoplasms observed in the two studies. Similarly, solid-state carcinogenesis, which is thought to be due to the host’s reaction when chronically presented with a smooth surface and which appears to occur during the encapsulation of such a surface (14), would seem unlikely as a complete explanation for the current observations. Although solid-state carcinogenesis could possibly account for a base-line occurrence of tumors at the site of the identification tag, this mechanism cannot fully or solely explain the marked differences in frequency of neoplastic or nonneoplastic lesions observed in the two studies.

The metal content of the devices in contact with tissue was the same in both the studies, indicating that the metal content alone cannot account for the differences in frequency of lesions at the tag site. Factors that would presumably enhance the surface release of metal, such as electrolytic release at contact points of fixing screws and plates made of even slightly dissimilar metal content, have also been noted in conjunction with tumors developing around implants in both humans and animals (1, 3, 13). This increased release of metal locally would provide greater stimuli for malignant transformation, presuming that one or more of the metallic components were carcinogenic. In the present study, if the metallic components of the device in contact with the tissue are of importance in the carcinogenic events, it would appear that nickel, a well-documented and potent carcinogenic metal (17–20), was the causative agent. A role of the other metallic constituents, either singly or in concert with nickel, cannot, however, be fully eliminated. Regardless of the exact carcinogenic metal or combination of metals that is involved, the stimulus for enhanced surface release of metal could have been provided by the initial marked inflammatory reactions and their persistence detected in the study in which there was a high incidence of malignancies. In this case there could be enhancement of metal ion release from the tag surface by a locally acidic environment resulting from the cellular response at the site, as inflammation is known to result in a substantial lowering of local pH (27). When the tag remained in place, clinical findings and later histological analysis showed that the inflammations, although less severe, persisted. In contrast, when the tag was lost, rapid restitution of damaged tissue appeared to occur. These findings indicate that, even though an initial bacterial infection might have played an important role in the severity of the inflammation at the tag site, the presence of the metal tag prevented healing and accounted for the formation of granulation tissue. Another important point is that the early onset of the inflammatory response seen in the study with the high incidence of tumors would have allowed sufficient time for the complete carcinogenic process to have occurred. In this regard the incidence of chondroid hyperplasia at the tag site, an event preceding metaplastic bone formation, was higher in the study without the tag-related tissue alterations seemed to lag behind those of the first study, indicating that conditions at the time of tag application might have been more favorable for preventing an initial severe tissue response. Hence, it would appear that the difference in the early local tissue response to the metallic device is the most probable explanation for the remarkable difference in the incidence of tumors between the two studies.

Naturally arising bone tumors in rats are rare, and none has been reported to develop from the pinna (28, 29). Morphologically, induced and spontaneously occurring osteosarcomas in rats are usually well differentiated, forming large portions of mature bone. In most cases these tumors are highly malignant and readily metastasize to various organs but most frequently to the lung (28, 30). We are unaware of any previous descriptions of pure giant cell tumors in rats. This difference in tumor range could possibly be related to the nature of the tissues at risk, i.e., normal mature tissue versus newly formed, metaplastic tissue. Although the spectrum of bone tumors found in rats in the present study is not typical of this species, it is quite similar to those found in dogs and in humans (25, 26). Histogenetically, all tumors arising at the tag site were of mesenchymal origin with the exception of a single epithelial papilloma. Chondroblasts, osteoblasts, and fibroblasts are derived from osteogenic precursor cells, while macrophages and osteoclasts are derived from monocytes (26, 31). All five cell types participated in the development of the tumors found in association with metallic ear tags. In this regard a wide variety of tumor types are also found in association with implanted metallic devices in dogs and humans.

The results of the present study may provide a basis for the development of a model system for the study of tumor induction by metallic implantation devices. Tissue alterations developing at this site have the advantages of being detectable both easily and early. Furthermore, biopsy specimens can be readily obtained. Local manipulations, such as infections with different bacteria, could be artificially introduced and tested for a contributing role in this carcinogenic process. A wide variety of metallic components could also be tested for potency in this system. Further research, however, is required before the utility of such a system can be fully analyzed.

In summary, the observations detailed in the present study indicate that metal identification tags of a nickel-copper alloy can produce tumors at the site of attachment. Furthermore, although the pathogenesis of the neoplastic lesions is as yet unclear, an enhanced metal ion release from the tag surface by a local acidic pH may play a major role in the final tumor incidence. Lastly, the possibility for the development of a system for study of metal implantation device carcinogenesis using an identification tag-like system deserves further exploration.

REFERENCES

8. Friedman, K. E., and Vernon, S. E. Squamous cell carcinoma developing in a local acidic pH environment resulting from the cellular response at the site, as inflammation is known to result in a substantial lowering of local pH (27). When the tag remained in place, clinical findings and later histological analysis showed that the inflammations, although less severe, persisted. In contrast, when the tag was lost, rapid restitution of damaged tissue appeared to occur. These findings indicate that, even though an initial bacterial infection might have played an important role in the severity of the inflammation at the tag site, the presence of the metal tag prevented healing and accounted for the formation of granulation tissue. Another important point is that the early onset of the inflammatory response seen in the study with the high incidence of malignancies. In this case there could be enhancement of metal ion release from the tag surface by a locally acidic environment resulting from the cellular response at the site, as inflammation is known to result in a substantial lowering of local pH (27). When the tag remained in place, clinical findings and later histological analysis showed that the inflammations, although less severe, persisted. In contrast, when the tag was lost, rapid restitution of damaged tissue appeared to occur. These findings indicate that, even though an initial bacterial infection might have played an important role in the severity of the inflammation at the tag site, the presence of the metal tag prevented healing and accounted for the formation of granulation tissue. Another important point is that the early onset of the inflammatory response seen in the study with the high incidence of malignancies. In this case there could be enhancement of metal ion release from the tag surface by a locally acidic environment resulting from the cellular response at the site, as inflammation is known to result in a substantial lowering of local pH (27). When the tag remained in place, clinical findings and later histological analysis showed that the inflammations, although less severe, persisted. In contrast, when the tag was lost, rapid restitution of damaged tissue appeared to occur. These findings indicate that, even though an initial bacterial infection might have played an important role in the severity of the inflammation at the tag site, the presence of the metal tag prevented healing and accounted for the formation of granulation tissue. Another important point is that the early onset of the inflammatory response seen in the study with the high incidence of malignancies.
Inflammatory, Proliferative, and Neoplastic Lesions at the Site of Metallic Identification Ear Tags in Wistar [Crl:(WI)BR] Rats

Michael P. Waalkes, Sabine Rehm, Kazimierz S. Kasprzak, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/47/9/2445

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.