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The Role of Bone Fracture at the Site of Carcinogen Exposure on Nickel Carcinogenesis in the Soft Tissue

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The influence upon nickel subsulfide (α-Ni_3S_2; Ni-SS)-induced carcinogenesis in the soft tissue of bone fracture at the site of Ni-SS exposure was studied using female Fischer rats. During the one year of the experiment, the group subjected to bone fracture exhibited the shortest tumor induction time and survival time, and a significantly higher metastatic rate. The present study may suggest a model for the metastasis of human soft tissue tumors.

Key words: Experimental carcinogenesis — Nickel subsulfide — Bone fracture — Sarcoma — Soft tissue tumor

Sarcomas of the bone and soft tissue may develop after trauma, crushing or bone fracture. The influence of such kinds of injury at the site of the tumor on the development, incidence, and prognosis of soft tissue tumors is still not clear. To our knowledge, there has been no study on the relationship between injury and sarcoma development.

There have been studies on experimental carcinogenesis in bone and soft tissue induced by chemicals,[1-4] heavy metals,[5-10] or irradiation.[11] Various histological types of soft tissue or bone sarcomas in different animal species have been reported: rhabdomyosarcoma in rats induced by dehydroretronecine,[1] 20-methylcholanthrene,[2] or nickel compounds[5-10]; malignant fibrous histiocytomas in rats induced by 4-(hydroxyamino)quinoline 1-oxide[3]; leiomyosarcomas in rabbits induced by Ni-SS[12]; osteosarcomas in mice induced by irradiation.[11] Regarding nickel carcinogenesis, Shibata *et al.*[13] observed rhabdomyosarcomas, malignant fibrous histiocytomas, fibrosarcomas, and unclassified sarcomas, depending on the injection route.

In this study, we examined the influence of bone fracture at the site of carcinogen exposure on nickel carcinogenesis in soft tissue, particularly on tumor induction time, tumor incidence, histological types of tumor, metastatic rate, and survival time or mortality rate of rats in which tumors were induced.

MATERIALS AND METHODS

Animals A total of sixty-seven female Fischer 344 rats (Charles River Japan Inc., Atsugi) were housed in plastic cages (3 or 4 rats/cage) in an air-conditioned room at 25 $\pm$2°C, under conditions of 12 h of light a day and given commercial rations (Oriental MF, Oriental Yeast Co. Ltd., Tokyo) and tap water *ad libitum*. For the comfort of the rats, sawdust was laid in the plastic cages, and was renewed every week.

Carcinogen Nickel subsulfide (α-Ni_3S_2, median particle diameter less than 2 μm) was obtained from INCO Ltd. (Toronto, Ontario).

Experimental procedure A total of 67 female Fischer rats, 10 weeks of age and weighing 153–166 g (mean 159 g), were divided into 4 groups and before the experimental procedure each received ether anesthesia: G I: 20 rats; The crural bones (tibia and fibula) were fractured and 10 mg of Ni-SS suspended in 0.2 ml of Chloromycetin solution (CM; 5 w/v%, 50 mg/ml, Sankyo Co. Ltd., Tokyo) was immediately injected into the site of the bone fracture. G II: 20 rats; 10 mg of Ni-SS was intramuscularly injected into the right thigh. G III: 20 rats; 10 mg of Ni-SS was intra-articularly injected into the right knee joint. G IV: control group; 3 rats underwent the same bone fracture as the G I group but CM only was injected, and the remaining 4 rats were injected with CM only, 2 intramuscularly and 2 intra-articularly.

The intra-articular injection is somewhat problematical compared to other injection routes as a small amount of the carcinogen and/or CM solution may leak outside the articular space. The nodule at the site of carcinogen exposure of each rat was palpated weekly or biweekly to monitor enlargement of the nodule or development of a tumor. The rats were weighed monthly. Throughout the one year of the experimental period, rats that became moribund were killed and all organs were examined. At the termination of the experiment, all surviving rats were killed and examined in the same way.

Histology and histochemistry Deparaffinized tissue sections obtained from primary tumors and metastatic tumors were cut to 4 μm thickness and stained with hematoxylin and eosin (H-E). Sections were also stained with periodic acid-Schiff (PAS) with or without dia-

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stase, Azan-Mallory, phosphotungstic acid-hematoxylin (PTAH), alcian blue, elastica van Gieson, and silver impregnation for reticulin fibers.

RESULTS

The rats that underwent bone fracture recovered rapidly and became well after a few days. Two weeks later, all injured rats had healed completely, with a slight to moderate deformity of the lower limb; the rats limped but moved about no differently from the other rats.

Tumor induction time and the cumulative number of tumor-bearing rats for each group are shown in Fig. 1. Tumors were induced in the shortest period in G I, from week 15 to week 39; in G II, from week 23 to week 48; in G III, from week 29 to week 48. Survival time and cumulative number of tumor-caused mortalities are shown in Fig. 2. The survival period was the shortest in G I, from week 17 to week 47; in G II, from week 28 to week 50; in G III, from week 35 to week 50.

The number and incidence of rats with tumor, average size of the primary tumor, and blood-borne or lymph-borne metastatic rate are shown in Table I. The tumor induction rate was 100% in G II, 85% in G I and 80% in G III. Average tumor size was 5.05 cm in G I, 5.77 cm in G II and 5.16 cm in G III. These differences were not statistically significant.

However, the very high metastatic rate in G I was statistically significant. The number of rats in G I with retroperitoneal lymph-nodal metastasis was 16 out of 17,

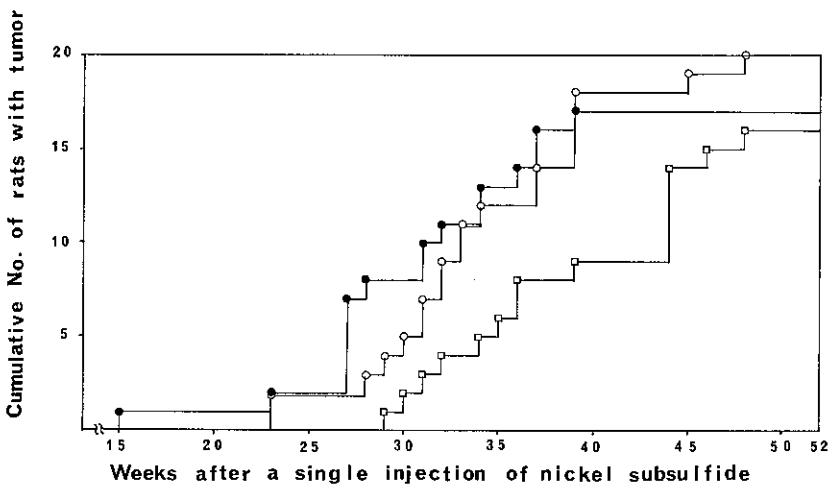

Fig. 1. Tumor induction time and cumulative number of rats with tumor after a single injection of nickel subsulfide (10 mg/rat). ●, G I bone fracture; ○, G II intra-muscular; □, G III intra-articular.

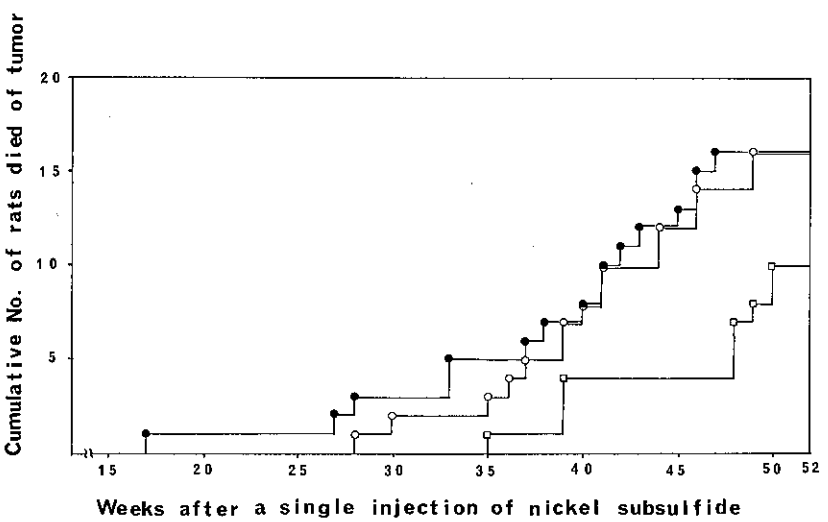

Fig. 2. Survival time and cumulative number of rats that died of tumor after a single injection of nickel subsulfide (10 mg/rat). ●, G I bone fracture; ○, G II intra-muscular; □, G III intra-articular.

Table I. Number and Incidence of Rats with Tumors and Metastasis, and Size of Tumors Induced by a Single Injection of Nickel Subsulfide (10 mg/rat)

Group		Total No. of rats	No. of rats with tumor (%)	No. of rats with metastasis (%)		Average size of primary tumor (cm)
				Lymph nodes	Lungs	
I	bone fracture	20	17 (85)	16 (94.1)[a]	9 (52.9)[b]	5.05±1.6
II	intramuscular	20	20 (100)	5 (25.0)	3 (15.0)	5.77±1.7
III	intra-articular	20	16 (80)	3 (18.8)	2 (12.5)	5.16±2.1
IV	control	7	0	0	0	

a) Significant by chi-square test at the 5% level relative to group II, and at the 2.5% level relative to group III.
b) Significant by chi-square test after Yates' correction at 5% relative to groups II and III.

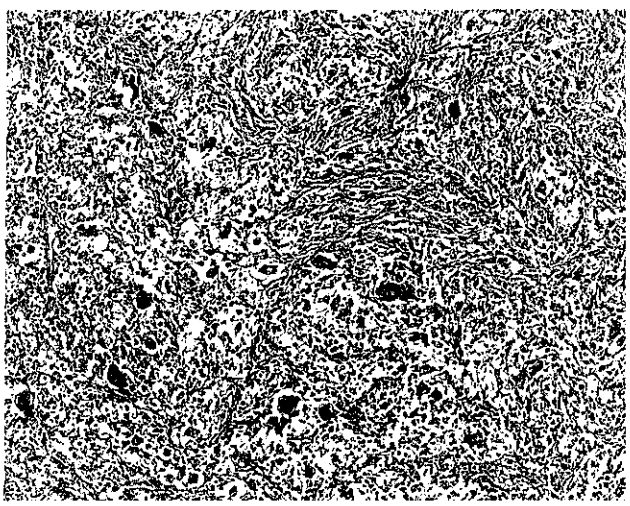

Fig. 3. Malignant fibrous histiocytoma composed of round histiocytic cells, bizarre giant cells and fibroblastic cells, forming an occasional storiform pattern, and having inflammatory infiltration (H-E, ×13).

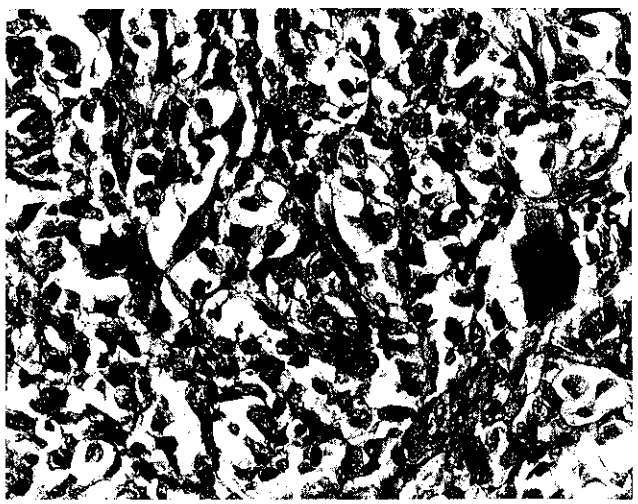

Fig. 4. Malignant fibrous histiocytoma composed of fibroblastic cells, bizarre cells, multinucleated giant cells and round histiocytic cells with a few inflammatory cells (H-E, ×66).

the metastatic rate being 94.1%, whereas in G II and G III the numbers were 5 out of 20 (25.0%) and 3 out 16 (18.8%), respectively. The very high lymph-nodal metastatic rate of G I was statistically significantly greater than that of G II at the 5% level and than that of G III at the 2.5% level by the chi-square test. In addition, the rate of blood-borne pulmonary metastasis in G I was also the highest; in G I it was 52.9% (9 out of 17) and in G II and G III 15.0% (3 out of 20) and 12.5% (2 out of 16), respectively. The very high rate of pulmonary metastasis of G I was also statistically significant by the chi-square test after Yates' correction at the 5% level, as compared with G II and G III.

The tumors induced in each group were classified microscopically into 4 types (Figs. 3–8) as shown in Table II. In every group, malignant fibrous histiocytoma was most frequent: 10 out of 17 tumors (58.8%) in G I, 14 out of 20 tumors (70.0%) in G II, and 11 out of 16 tumors (68.8%) in G III. A total of 18 tumors of other types were induced: 7 rhabdomyosarcomas (4 tumors in G I and 3 in G II), 6 fibrosarcomas (4 tumors in G III, and 1 each in G I and G II), and 5 leiomyosarcomas (2 tumors in G I, 2 in G II, and 1 in G III); no tumors with characteristics suggesting osteosarcoma or synovial sarcoma were induced in any group. However, the histological and cytological characteristics of each histological type of tumor were not consistent: there was much histological and cytological variation among tumors, with undifferentiated round cells or rhabdomyoblastic round cells in a somewhat alveolar pattern, elongated spindle cells or fibroblastic cells in a storiform pattern or herringbone pattern with much or little collagenous matrix, and occasional histiocytic or vacuolated round cells. Therefore, the histological classification of each

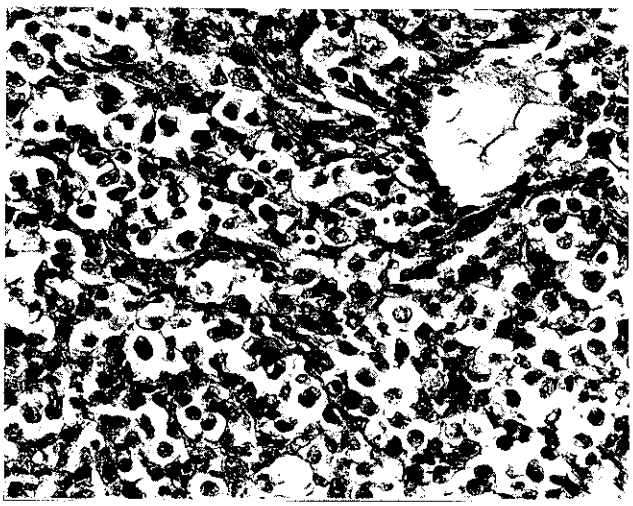

Fig. 5. Poorly differentiated rhabdomyosarcoma chiefly composed of undifferentiated round cells and a few scattered rhabdomyoblasts (H-E, ×100).

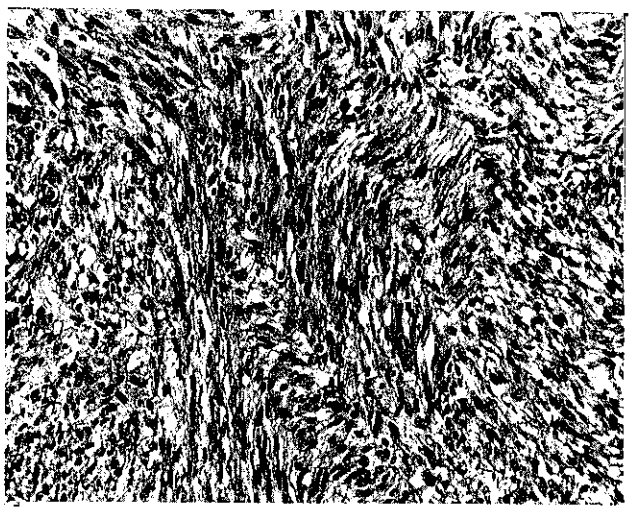

Fig. 7. Fibrosarcoma composed of uniform fibroblastic cells arranged in intersecting ("herringbone") and fascicular patterns (H-E, ×66).

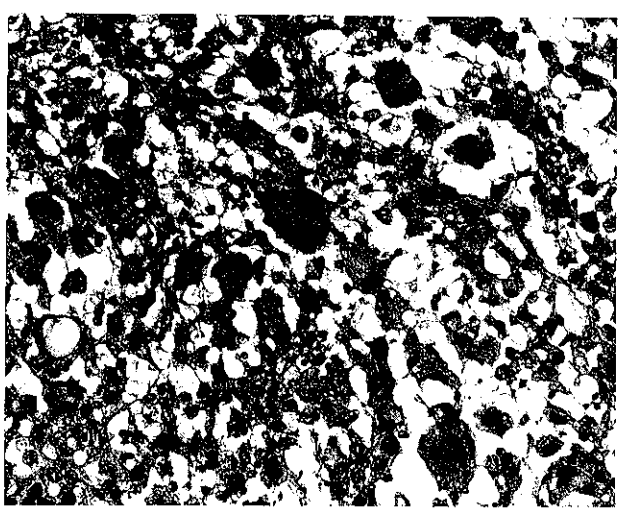

Fig. 6. Pleomorphic rhabdomyosarcoma composed of multinucleated giant cells with somewhat peripherally placed "wreath-like" nuclei, some rhabdomyoblasts and small undifferentiated round cells (H-E, ×66).

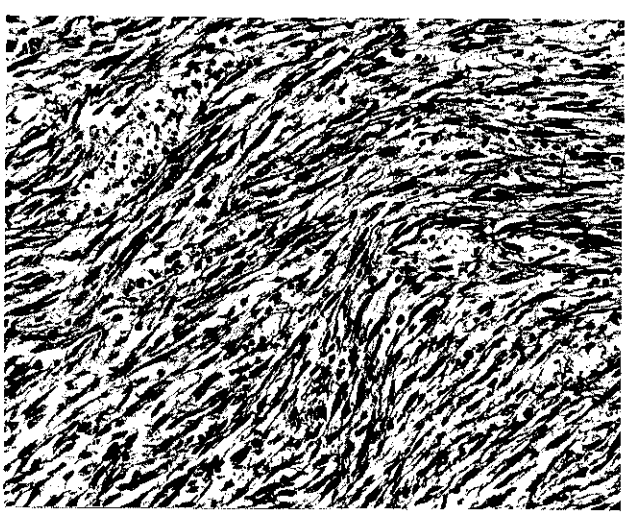

Fig. 8. Leiomyosarcoma composed of uniform smooth muscular cells showing a streaming pattern in a somewhat myxoid matrix (H-E, ×66).

tumor was based on its predominant histological and cytological characteristics in the histochemical staining reaction. Furthermore, within these 4 types of tumor, particularly the malignant fibrous histiocytomas, no distinct differences of histological pattern were revealed, so that it was very difficult to classify them into subtypes.

DISCUSSION

To our knowledge, there has been no report dealing specifically with the relationship between injury and tumor development, or that between injury and mortality or the metastatic rate of sarcomas in experimental carcinogenesis in the soft tissue or bone. In the present

Table II. Number and Incidence of Histological Types of Primary Tumor Induced by a Single Injection of Nickel Subsulfide (10 mg/rat)

Group		Total No. of tumors	Malignant fibrous histiocytoma (%)	Rhabdomyosarcoma	Fibrosarcoma	Leiomyosarcoma
I	bone fracture	17	10 (58.8)	4	1	2
II	intramuscular	20	14 (70.0)	3	1	2
III	intra-articular	16	11 (68.8)	0	4	1
IV	control	0	0	0	0	0

study, we have demonstrated that injury, i.e., bone fracture at the site of carcinogen exposure, enhanced nickel carcinogenesis in the soft tissue and exacerbated the metastasis of the sarcoma and the prognosis of rats in which tumors were induced.

At the termination of the experiment, there was no particular difference of tumor induction rate. However, there were differences in tumor induction time and survival time. Throughout the one year of the experimental period, the induction time in G I was 3 weeks shorter than in G II and 9 weeks shorter than in G III on average. Concerning survival time, in G I it was also shorter than in G II and G III. Both these facts correspond to the higher incidence of metastatic rate in G I. These differences between G I and the other groups must be due to the effect of the bone fracture.

The pathogenesis or etiology of the shortened tumor induction time and the enhanced metastasis was not clarified. However, it may be that large amounts of granulation tissue develop during the healing process after bone fracture at the site of carcinogen exposure in G I. In previous literature, it has been demonstrated that proliferating tissue is more susceptible to carcinogens, i.e., chemical hepatocarcinogenesis after partial hepatectomy,[14, 15] or chemical renal carcinogenesis with unilateral hydronephrosis.[16, 17] At the site of the bone fracture, numbers of myofibroblastic cells, hemangioblastic cells and osteoblastic cells or unknown immature mesenchymal cells may be present and exposed to high levels of the carcinogen. The unknown target cell in nickel carcinogenesis may be among those in the stem cell line in the granulation tissue. For example, histiocytic cells as progenitor cells for malignant fibrous histiocytomas or myofibroblastic cells for fibrosarcomas and leiomyosarcomas or rhabdomyoblasts for rhabdomyosarcomas.

In the present study, no osteogenic sarcomas were induced in the bone-fracture group, and so osteoblastic cells developing after bone fracture may be excluded as a target cell of nickel carcinogenesis. Furthermore, in the intra-articular-injection group, no synovial cell sarcomas were found, as previously demonstrated by Shibata *et al.*[13] for nickel carcinogenesis and by Homma and Wuñsch[4] and Sakamoto[18] for DMBA (9,10-dimethyl-1,2-benzanthracene) carcinogenesis. Thus, synovial cells may be excluded as a target cell of this experimental carcinogenesis. Myofibroblastic cells have been observed as cells altered from histiocytes in long-term cultivation or through transplanting.[19, 20] In this study, every induced tumor in every group exhibited broad variations of malignant fibrous histiocytoma-like, or fibrosarcomatous, or leiomyosarcomatous, or rhabdomyosarcomatous, histologic and cytologic characteristics. These microscopic findings in tumors induced by nickel carcinogenesis in soft tissue suggest that myofibroblastic and histiocytic cells are progenitor cells for such types of induced tumor. In order to study the mechanism of nickel carcinogenesis, the metabolism and effects of Ni-SS in the target cell must be examined. The higher metastatic rate of the bone-fracture group is also of interest. Individual tumors for each group were not particularly different in histological type. Metastatic tumors were not different in the grade of malignancy. Therefore, we believe that the pathogenesis of the higher metastatic rate in the bone fracture group is dependent partially on the shorter tumor induction time but mostly on the exacerbated or higher vascularization of blood vessels and lymph vessels caused by the bone fracture injury at the site of carcinogen exposure, rather than on biological characteristics of individual tumors. To clarify this point, we hope to perform cytological and histological investigations of granulation tissue during the healing process around the site of a bone fracture injury in forthcoming studies under the same experimental conditions.

In conclusion, although there have been some earlier studies seeking a human tumor metastasis model using nude mice or rats,[21, 22] we would like to propose the present experimental system as a model for the metastasis of human soft tissue tumors using commercial rats.

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