Methylcholanthrene-induced Sarcoma in Singly Fractured Bone of Mice

Tsutomu Watanuki and Masayuki Ogata

*The Second Department of Pathology (Emeritus Prof. K. Akazaki and
Prof. N. Sasano), Tohoku University School of Medicine, Sendai*

Minoru Yamada

*Department of Orthopedic Surgery (Prof. S. Iino),
Tohoku University School of Medicine, Sendai*

In order to find a positive correlation between the development of sarcoma of the bone and preceding local traumatic injury, the right femur of dd-strain mice was fractured at the diaphysis, and 0.2 mg of 20-methylcholanthrene (MC) was injected into the bone marrow or callus at the site of fracture. Various types of sarcoma of the bone developed after various latent periods (57-202 days). A positive sarcoma induction seemed to be related to the histological pattern of the callus at the time of MC-administration.

In the callus composed of already mature cells, no sarcoma developed, while the tumor was induced in 45.5% of animals with the callus of immature cells after a short latent period. It was assumed that this difference would be due to the different nature of cells composing callus: An active proliferating ability with evident susceptibility to certain carcinogens would be inherent to immature cells but not to mature cells. This would be one of the important factors, which must be taken into consideration in the interpretation of so-called traumatic sarcoma.

Much attention has been paid to the induction of sarcoma at the site of single preceding trauma. This type of sarcoma is well-known in the bone and is referred to as 'traumatic sarcoma'. There have been, however, many controversies on this concept, which do not reach an agreement. In the present paper a successful induction of malignant bone tumors of mice in the region of bone fracture is described.

MATERIALS AND METHODS

A single fracture was manually made at the diaphysis of the right femur of dd-strain mice. A single dose of 0.2 mg of 20-methylcholanthrene (MC) was injected into the bone marrow or into the callus of fractured bones.

The animals, 67 in total, were divided into 6 groups according to the length of the interval between fracture and administration of MC (Table 1). The animals of Group 1 were submitted to fracture of the femur immediately after MC-administra-

TABLE 1. *Grouping of experimental animals*

No. of group	Period from fracture to MC-administration	Number of experimental animals	Histological findings of callus at the time of MC-administration
	Fracture was made immediately after MC-administration into the bone marrow of the right femur	20	
2	5 days	11	
3	6 days	8	Usually composed of connective tissue
4	10 days	14	Concurrent appearance of connective and tumorous tissues.
5	11 days	5	The bone tissue is still recognizable.
6	27 days	9	Fracture is almost cured. Osteoblasts are sparsely seen arranged on the surface of the newly formed immature trabecle and cortex.

tion into the bone marrow *in situ*. In the other 5 groups, MC was given 5 to 27 days after fracture.

Rate of sarcoma development: The animals which were injected MC 11 days after fracture (Group 5) died without developing tumor. Furthermore, no tumor was observed in the animals, of which bones had been fractured immediately after MC-administration (Group 1) and in the animals, which had received MC-injection 27 days after the fracture (Group 6). On the contrary, in the animals with MC-administration 5, 6 and 10 days (in Groups 2, 3 and 4, respectively) after the fracture, malignant tumor was confirmed in 6, 2 and 7 cases, respectively. In these three groups, the incidence of the tumor in relation to the effective total number was very high and attained or exceeded 50%.

Latent period: The period between administration of MC and development of grossly detectable tumor ranged from 57 to 202 days. The shortest period was observed in three cases in Group 2 and one case in Group 3. The tumors measured 10×9×9 mm to 16×14×14 mm or more in size. Roentgenologically, the findings suggestive of destruction and resorption of the osseous cortex were confirmed in the fractured lesion almost in every case. Grossly, a majority of the tumor was soft in consistency and the cut surface was usually glistening, semitransparent and grayish white in color. Whirlpool structures were occasionally observed. Histologically, fibrosarcoma was most prevalent and followed by pleomorphic cell sarcoma. Osteo-

TABLE 2. *Relationship among fracture, MC-administration and occurrence of the tumor*

No. of group	Period from fracture to MC-administration	Number of experimental animals	Effective total number	Incidence of sarcoma	Rate of occurrence of sarcoma	Period from MC-administration to occurrence of sarcoma	Histological diagnosis of tumor
1	At the fracture	20	20	0	0		
2	5 days	11	11	6	54.5%	57 days 57 days 57 days 99 days 119 days 160 days	Pleomorphic cell sarcoma Pleomorphic cell sarcoma Pleomorphic cell sarcoma Pleomorphic cell sarcoma Fibrosarcoma Spindle cell sarcoma
3	6 days	8	3	2	66.6%	57 days 99 days	Fibrosarcoma Fibrosarcoma
4	10 days	14	14	7	50.0%	99 days 149 days 149 days 160 days 170 days 170 days 202 days	Fibrosarcoma Fibrosarcoma Fibrosarcoma Fibrosarcoma Fibrosarcoma Osteochondroplastic sarcoma Fibrosarcoma
5	11 days	5	0				
6	27 days	9	9	0	0		

chondroplastic sarcoma was observed in one case.

DISCUSSION AND CONCLUSION

Two opposing opinions have been known concerning the relationship between preceding trauma and occurrence of tumor. One opinion denies the possibility of trauma as a major factor of tumor induction based upon both clinical and experimental observations.¹⁻⁵ The other stresses that trauma directly activates either the latent proliferative ability of the embryonal stray germ⁶ or of separated tissue cells,⁷ or evokes tumor producing predisposition inherent to cells.^{8,9} In the latter mechanism secondary tissue changes such as circulatory disturbance, inflammation or tissue proliferation were regarded to play an important part in the tumor induction.¹⁰⁻¹⁵ Actually, not a few examples of sarcoma with a close relationship to preceding trauma have been reported. Furthermore, there are several

experimental studies indicating the probable influence of preceding trauma on the tumor induction.¹⁶⁻¹⁸

In our experiment, no occurrence of tumor was seen in the animals with MC-injection immediately before or 27 days after the fracture (Groups 1 and 6), whereas the animals with MC-administration 5, 6 or 10 days after the fracture (Groups 2, 3 and 4) had high tumor induction rates of 50% or more. In these three groups, the latent period was considerably short, the shortest period being only 57 days after MC-administration.

It is assumed that such differences of tumor incidence between Groups 1 and 6 on the one hand and Groups 2, 3 and 4 on the other are due to difference in the stage of histological repair of fracture at the time of MC-administration. In the groups without tumor development, MC was either administered into the bone marrow before immature cells proliferated (Group 1), or given at the time when newly proliferating cells in the callus had fairly completed their maturation (Group 6). On the contrary, numerous immature cells were present in actively proliferating tissues of the callus at the time of MC-administration in Groups 2, 3 and 4. Therefore, it is reasonable to assume that tumor induction of the callus by MC was associated with the presence of immature cells in the callus. The generally accepted concept that 'immature cells are very susceptible to some carcinogenic action than mature cells' appears to be applicable to the interpretation of our experimental results.

In conclusion, the predominance of tumor after trauma is attributed to appearance of actively proliferating immature mesenchymal cells in the course of wound healing. Traumatic injuries probably acts as one of the factors accelerating tumor development, rather than as a causative factor.

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