

Indomethacin influences Moloney's sarcoma and associated periosteal osteogenesis in the mouse

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The effect of indomethacin on periosteal osteogenesis mediated by the Moloney sarcoma virus was studied using a mouse model. In the indomethacin-treated animals, the development of sarcoma was inhibited, as evaluated by the tumor incidence, tumor size, and maximal tumor duration. Periosteal osteogenesis mediated by this sarcoma was lower than in saline-treated control mice.

A decade ago, we demonstrated that inoculation of Moloney's murine sarcoma virus (Mu-MSV) into shank muscles resulted in sarcoma development and periosteal membrane proliferation with vigorous bone formation (Wlodarski et al. 1979, 1981). Recently, it was reported that in mice transplanted tumor cells of various origin induce periosteal osteogenesis (Wlodarski and Reddi 1987). Some authors suggest that Mu-MSV-induced tumor is not a true neoplasm, but merely an inflammatory reaction against virus-infected cells (Becker and Haskill 1981). However, this suggestion is negated by the *in vitro* establishment of a murine Moloney sarcoma cell line MSVC (Wlodarski et al. 1986), which produces tumors that upon transplantation into host animals regress slower than virus-induced tumors. Nevertheless, the inflammatory component of tumor-mediated periosteal osteogenesis should be considered when the mechanism of periosteal membrane activation is discussed.

Prostaglandins are potent stimulators of bone resorption *in vitro* and *in vivo* (Goodson et al. 1974, Katz et al. 1983, Dewhirst 1984, Hoffmann et al. 1985). Indomethacin and other nonsteroid anti-inflammatory agents are potent inhibitors of prostaglandin synthesis (Vane 1971).

In this paper the effect of systemic administration of indomethacin on the Mu-MSV-induced periosteal osteogenesis was examined.

Material and methods

All the experiments were performed on 2-4-month-old male mice. The animals received 0.2 mL of standard Moloney's murine sarcoma virus (Mu-MSV) injected in the right shank muscles; saline was injected into the left shank muscles. The standard Mu-MSV was prepared as described previously (Wlodarski et al. 1981).

Indomethacin (Metindol, Polfa) was dissolved in saline and injected subcutaneously according to several dose regimens, as indicated in Table 1. Indomethacin was given in doses ranging from 0.03 to 0.25 mg (1.5-12 mg per kg body weight) at time intervals ranging from 2 to 6 days. The doses are close to the therapeutic ones in human beings. Control animals were given the same volume of saline.

All the animals were inspected daily; and the time of tumor appearance, duration of the tumor, and the time of tumor disappearance were recorded. The kinetics of the Mu-MSV-induced tumors was characterized by (1) the tumor incidence (percentage of animals in which tumor developed), (2) mean time when the tumor incidence reached a plateau, and (3) the maximal duration of the tumor (Figure 1).

The animals were killed 15-32 days after virus inoculation. Both hind legs, *i.e.*, Mu-MSV-treated and contralateral saline-treated control were excised and hydrolyzed in 0.2 N KOH at 64 °C overnight. During such a procedure, the soft tissues are dissolved and bones are easily removed. Isolated tibiae and fibulae free from adjacent tissues were washed and dried overnight at 64 °C, weighed with an accuracy of ± 0.1 mg, and the yield of dry-bone mass, resulting from activation of periosteal osteogenesis, was

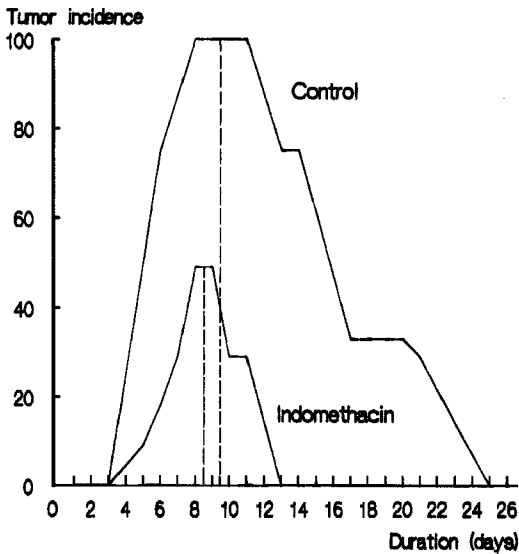


Figure 1. The kinetics of Mu-MSV-tumor development in controls and indomethacin-treated CFW/LI mice. The broken lines indicate an average time of the maximum of tumor development—a mean time of the tumor plateau.

expressed as a percentage of the contralateral bone mass for each mouse. The means of bone-mass yield

and standard deviation were calculated for the whole group.

The significance of the differences was analyzed using the Student's *t*-test at $P < 0.01$.

Results

In the saline-treated control mice, Mu-MSV sarcoma developed in 49 out of 52 animals (Table 1). Tumors appeared on Days 4–6 after virus inoculation, reached a plateau on Days 7–15, and then gradually disappeared (Figure 1). In some cases the tumor was observed even 28 days after virus inoculation.

In the indomethacin-treated animals, sarcoma developed in 38 out of 62 animals. Although the tumor appeared approximately at the same time as in the control animals, the plateau of maximal tumor incidence was 1–1.5 days shorter, and tumors disappeared a few days earlier. The indomethacin treatment also had profound effects on the periosteal bone formation mediated by the Moloney sarcoma development. The yield of new bone formation, as measured by the increase in dry mass, was much lower in the indomethacin-treated mice than in the control animals, and in many cases was not observed at all (Table 1).

Table 1. The effect of indomethacin administration on Mu-MSV-induced tumor development and tumor-mediated periosteal osteogenesis in mice

Schedule of indomethacin treatment (day and single dose)	Duration (days)	n	No. of animals developing		Dynamics of tumor developments	
			Tumor	Periosteal osteogenesis ^a	Mean time of plateau (days)	Maximal duration of tumor (days)
Control 0, 2, 5, 7, 9, 12 days (0.12 mg)	15	6	6	4 (8 6)	10.5	> 15
		9	3	2 (4 15)	9.0	12
Control 0, 2, 5, 7, 9 days (0.2 mg)	17	6	6	5 (16 7)	10.0	> 17
		6	1	0	8.5	10
Control 0, 5, 7, 11, 13, 15 days 0.25 mg)	20	9	8	6 (23 36)	10.5	20
		11	5	4 (21 15)	10.0	17
Control 5, 7, 8, 9, 12, 13, 14 days (0.03 mg)	22	10	8	7 (28 25)	11.0	> 22
		9	9	7 (5 4) ^b	10.0	16
Control 0, 3, 4, 7, 8, 10, 11, 14, 17 days (0.1 mg)	28	7	7	7 (72 43)	11.0	28
		8	8	5 (17 23) ^b	9.5	18
Control 1, 3, 6, 8, 13, 17, 20 days (0.2 mg)	29	8	8	4 (16 14)	10.0	23
		10	5	0	9.0	12
Control 0, 4, 6, 8, 9, 15 days (0.06 mg)	32	6	6	2 (34 46)	9.0	20
		9	7	2 (5 19)	8.0	18

^a In parentheses the mean yield of bone mass as the percentage of the contralateral, untreated shank bone weight, *SD*.

^b $P < 0.01$.

Discussion

It has been demonstrated that antitumor response can be inhibited specifically by suppressor T lymphocytes and their products (Mukherji et al. 1987) and unspecifically by macrophages and monocytes (Gabizon et al. 1980, Ting and Hargrove 1982, Fuji et al. 1987). Hence, the activity of macrophages and monocytes can enhance tumor growth. Stimulation of tumor growth is mediated by prostaglandins (Kort et al. 1986, Liu et al. 1986). Moreover, prostaglandins themselves are immunosuppressive agents. It has been reported that indomethacin blocks immunosuppression and retards tumor growth (Powels et al. 1973).

Presumably, inhibition of prostaglandin synthesis was responsible for the antitumoral effect and reduced osteogenesis observed in our experiment.

Our results indirectly confirm data of Strausser and Humes (1975), who reported an increase in prostaglandin E in MSV-induced tumors. The level of prostaglandin has been shown to increase with tumor diameter and to decrease with tumor regression. These authors, however, erroneously identified the observed bone changes as the result of increased osteoclastic activity. Apparently in mice treated with indomethacin, tumors fail to develop, the prostaglandin levels are markedly lowered, and no bone changes are observed. My results suggest that the reduction of periosteal bone formation in the indomethacin-treated mice is caused by decreased osteoblastic rather than increased osteoclastic activity. The reason for this osteoblastic inhibition remains unclear, but it may be suggested that production of putative osteoblast-activating factors by tumor and/or by tumor-rejecting cells (Wlodarski and Reddi 1987) is impaired.

It is commonly accepted that local inflammation results in osteopenia due to a transient inhibition of bone formation (Pfeilschifter et al. 1987). The bone resorption in inflammatory reactions can be mediated by lymphokines that activate osteoclasts (Chen et al. 1976) or by release of proteins that directly resorb bone (Gray et al. 1986). Prostaglandins, potent mediators of osteolysis, play a role in inflammatory reaction, and their synthesis is required for lymphokine-mediated bone resorption (Bockman and Repo 1981). On the other hand, there are reports that in some instances the inflammatory reaction could stimulate bone formation (Fukawa et al. 1985, Tomoda et al. 1986).

It has been established that tumor cells (Rubin 1970, Todars et al. 1980, Harada et al. 1982, Bessho et al. 1984) or macrophages involved in antitumor reactions (Rifas et al. 1984) produce growth factors

that stimulate osteoblasts and chondrocytes (Rifas et al. 1984, Fine et al. 1989). The indomethacin-mediated inhibition of inflammatory reactions most likely affects the production of growth factors by macrophages and thus diminished periosteal osteogenesis. There are reports on an inhibitory effect of indomethacin on bone regeneration (Ro et al. 1976, Keller et al. 1989). Indomethacin also inhibits ectopic osteogenesis (Nilsson et al. 1985). This inhibition is explained by reduced inflammatory response.

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