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Letter to the Editor

Antitumor Effect of Calmodulin Antagonist against MH-134 Hepatoma, Ehrlich Ascites Carcinoma and B-16 Melanocarcinoma in Mice*

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RECENTLY we have demonstrated that calmodulin may play an important role in initiation of DNA synthesis [1]. Moreover, transformation of cells to malignancy was found to represent one general mechanism that results in a specific increase in the intracellular content of calmodulin [2–4]. All these findings suggest that calmodulin may be involved in cell proliferation and in transformation [5].

In this study the effects of calmodulin antagonists such as *N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide (W-7) or *N*-(6-aminohexyl)-1-naphthalenesulfonamide (W-5) against MH-134 hepatoma (MH-134), Ehrlich ascites carcinoma (EAC) and B-16 melanocarcinoma (B-16) were investigated. The correlation between the antitumor activity and the calmodulin content of the tumor cells is discussed.

EAC (5×10^6 cells) and MH-134 (2×10^6 cells) were inoculated intraperitoneally (i.p.) into male BALB/c and C3H/He mice respectively. B-16 (1×10^6 cells) was implanted subcutaneously in the right flank area of the BDF_1 mice. The tumors were initially supplied from the National Cancer Center Research Institute of Japan. W-7 and W-5 were dissolved in 0.9% sterile saline solution. Each animal was given an i.p. injection of the drug and the control animals were given saline only, by the same route and in the same volume. The antitumor activity was evaluated by comparing the mean survival time (± S.D.) of the treated animals with that of saline-treated control animals [6]. The calmodulin content in the tumor cells at the end of 14 days after the tumor inoculation was assayed according to the method previously reported [7]. Protein amounts were determined by the method of Lowry *et al.* [8] with bovine serum albumin as a standard.

We have previously reported that W-7 and its derivatives are putative calmodulin antagonists, are bound to calmodulin and inhibit Ca^{2+}/calmodulin-regulated enzyme activities [9]. W-5, an analogue of W-7 that interacts only weakly with calmodulin, has been proved to be a much weaker inhibitor of cell proliferation.

As shown in Table 1, W-7 was almost ineffective against MH-134. However, our results clearly show that W-7 has an antitumor activity against EAC and B-16. In particular, W-7 strongly inhibited the growth of B-16 in mice given at doses of 1–10 mg/kg for 10 consecutive days. The effect of W-7 on B-16 did not depend on the dose administered. Although this point is of interest with regard to the effectiveness of a low dose of W-7, definite conclusions were not reached. The calmodulin contents of B-16, EAC and MH-134 were 537, 375 and 45 ng/mg protein respectively. Therefore calmodulin may play some role in the growth of B-16 and EAC, whereas a factor other than calmodulin may be involved in the process of growth of MH-134. It is interesting to note that W-7 shows an antitumor effect against the tumor containing the highest concentration of calmodulin.

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Table 1. Effects of W-7 and W-5 on tumor-bearing mice

W-7 Treatment* (mg/kg × days)	W-7 Mean survival (days)	W-5 Treatment* (mg/kg × days)	W-5 Mean survival (days)
MH-134 (calmodulin content: 45 ng/mg protein)			
Control ($n = 10$)	15.3 ± 1.1	Control ($n = 7$)	15.4 ± 0.9
1 × 7 ($n = 10$)	19.0 ± 1.3 (24)†‡	1 × 7 ($n = 7$)	15.9 ± 1.3 (3)
3 × 7 ($n = 10$)	19.2 ± 1.0 (25)‡	3 × 7 ($n = 7$)	16.2 ± 1.0 (5)
10 × 7 ($n = 10$)	19.2 ± 1.2 (25)‡	10 × 7 ($n = 7$)	16.5 ± 1.3 (7)
EAC (calmodulin content: 375 ng/mg protein)			
Control ($n = 10$)	17.4 ± 0.4	Control ($n = 10$)	17.0 ± 0.7
1 × 7 ($n = 10$)	18.5 ± 0.7 (6)	1 × 7 ($n = 10$)	17.2 ± 1.5 (1)
3 × 7 ($n = 10$)	29.4 ± 3.2 (69)‡	3 × 7 ($n = 10$)	16.1 ± 1.0 (-5)
10 × 7 ($n = 10$)	22.6 ± 1.3 (30)‡	10 × 7 ($n = 10$)	18.4 ± 0.7 (8)
B-16 (calmodulin content: 537 ng/mg protein)			
Control ($n = 16$)	20.4 ± 1.3	Control ($n = 10$)	20.9 ± 1.9
1 × 10 ($n = 8$)	33.8 ± 2.8 (66)‡	1 × 10 ($n = 8$)	21.5 ± 2.2 (3)
3 × 10 ($n = 8$)	34.0 ± 3.2 (67)‡	3 × 10 ($n = 8$)	22.7 ± 3.1 (9)
10 × 10 ($n = 8$)	33.0 ± 3.8 (62)‡	10 × 10 ($n = 8$)	21.3 ± 2.7 (2)

*W-7 and W-5 were injected i.p. once daily for seven or ten consecutive days starting 24 hr after the tumor inoculation.

†Number in parentheses is increase in mean survival day above controls (%).

‡Significantly different ($P < 0.05$) compared to control group.

A chlorine-deficient analogue, W-5, which interacts weakly with calmodulin, had no antitumor activity against these tumors in a dose similar to that of W-7.

Although the antitumor effect of W-7 on MH-134 was not so potent, the antitumor spectrum of this drug does warrant further investigation.

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