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Antitumor Effect of the Organogermanium Compound Ge-132 on the Lewis Lung Carcinoma (3LL) in C57BL/6 (B6) Mice*

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Kumano, N., Ishikawa, T., Koinumaru, S., Kikumoto, T., Suzuki, S., Nakai, Y. and Konno, K. *Antitumor Effect of the Organogermanium Compound Ge-132 on the Lewis Lung Carcinoma (3LL) in C57BL/6 (B6) Mice.* Tohoku J. exp. Med., 1985, **146** (1), 97-104 —— Effects of the organogermanium compound Ge-132 (i.p.) were examined on the 3LL local tumor (1×10^5/mouse, s.c.) and its pulmonary metastases in B6 mice. A characteristic feature of its action was the preferential antimetastatic effect under strictly defined conditions. Either inhibition or facilitation was observed depending on the treatment schedules; 7 daily doses of 100 mg/kg yielded the inhibition ratio 49% when started from day 1, whereas the treatment from day 8 resulted in the ratio −99%. The maximum inhibition was obtained at 100 mg/kg. The postsurgical-adjuvant treatment with Ge-132 was of no beneficial effect. The local tumor growth was affected only marginally and temporarily. When inoculum size was minimized (1×10^4), a single dose of 300 mg/kg on day 1, but not on day 8, was effective in prolonging the latency before tumor take. The antitumor action of Ge-132 was discussed with reference to its interferon (IFN)-inducing activity. ———— Lewis lung carcinoma; antimetastatic effect; organogermanium compound Ge-132; IFN-inducer

Ge-132 [carboxyethylgermanium sesquioxide, O_3 $(GeCH_2CH_2COO)_2$] is one of the water-soluble organogermanium compounds synthesized in Asai Germanium Institute, Tokyo, in 1967 (Oikawa and Kakimoto 1968). Its peculiar chemical structure and physicochemical characteristics were elucidated (Tsutsui et al. 1976), suggesting possible unique physiological activities of this compound. In fact, an antihypertensive effect (Sato and Ishikawa 1973) and protective effect

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against the senile amyloidosis (Kuga et al. 1976) were found subsequently. Ge-132 has also been comfirmed for its extremely low toxicity by the toxicological and pharmacokinetic studies in rats and dogs (Tomizawa et al. 1978; Nagata et al. 1978).

During the past years, this compound has been calling a particular attention with respect to its antitumor activities lately disclosed. Thus, Sato and Iwaguchi (1979) initially reported prolongation of the survival period with Ge-132 in rat ascites hepatomas AH44 and AH66 as well as in a syngeneic bladder cancer BC47 in ACI/N rats. Meantime, we found the prophylaxis of 3-methylcholanthrene-induced tumor production in mice (Kumano et al. 1978).

In the present study, we evaluated further the antitumor effect of Ge-132 in the 3LL in B6 mice (Sugiura and Stock 1955; Mayo 1972), a well-known model of pulmonary metastases from a distant tumor inoculum.

Materials and Methods

Mice

Male B6 mice of 8- to 12-week-old were purchased from Funabashi Farm, Ltd., Chiba. They were kept in metal cages, fed commercial pellet FM (Oriental Yeast Co., Tokyo) and tap water *ad libitum*.

Tumor

The 3LL was originally supplied by N.C.I., Bethesda, Md., U.S.A., and has been kept by biweekly passages in the syngeneic hosts subcutaneously (s.c.). The local tumors became palpable around 5 days after inoculation of 1 to 3×10^5 viable cells/mouse, and grew progressively until death of the host animals within 25 to 40 days. Metastatic nodules in the lungs became macroscopically observable from 10 to 14 days after inoculation.

For the tests, cell suspension was prepared from the 10- to 12-day-old tumors as described in a previous paper (Kumano et al. 1985). Each specified number of viable cells was contained in a 0.2-ml-portion of the final cell suspension.

Ge-132

Crystaline Ge-132 was generously provided by Asai Germanium Institute, Tokyo. It was dissolved in saline and administered intraperitoneally (i.p., 0.2 ml per dose) according to the treatment schedules listed in Tables 1 and 2.

Experimental design

B6 mice were inoculated with the 3LL cells into the right axilla s.c. on day 0 (1×10^5/mouse unless otherwise stated), and randomly divided into each experimental group of 7 to 10 mice.

Ge-132 was given in 7 daily doses of 10, 100, or 500 mg/kg i.p. from day 1. Seven daily doses of 100 mg/kg were administered from day -7, 1, or 8. A single dose of 300 mg/kg was also given on day -1, 1 or 8.

Evaluation of the antitumor effect

The effect on the local tumor and its metastatic spread in the lungs were evaluated as described in the foregoing paper (Kumano et al. 1985). In the experiment with a smaller inoculum size (1×10^4 or 10^3/mouse), latency before tumor appearance was recorded individually.

Postsurgical-adjuvant therapy

B6 mice were inoculated with the 3LL into the left footpad, and the tumor-bearing legs were surgically removed on day 20. From the next day of surgery, 1 of 4 groups of randomized mice received 10 daily i.p. injections of Ge-132 at 100 mg/kg. Survival period was compared to those of the surgery- and the untreated control groups (see also the accompanying paper for more detail (Kumano et al. 1985).

Results

Temporary and marginal inhibition of the local tumor growth

As summarized in Table 1, effects of Ge-132 on the 3LL local tumor growth in B6 mice were compared among 8 different treatment schedules. As evaluated on the basis of mean tumor volume on day 16, the maximum inhibition was achieved with 7 daily doses of 100 mg/kg from day 1 (Group V, inhibition ratio 63%). A single dose of 300 mg/kg on day 1 (Group II) or day 8 (Group III) yielded a moderate inhibition (the ratio around 40%). The prior treatment (7 daily doses at 100 mg/kg, Group IV) also gave a moderate inhibition. However, hardly any difference was found in the weight of those tumors extirpated on day 21, reaching around 6 gm in all the experimental groups. Thus, the inhibition of the local tumor growth with Ge-132 was only marginal and was temporary as well.

Schedule-dependent dual effects on the pulmonary metastases

Metastatic spread in the lungs were compared among the above 9 groups on day 21. Fig. 1 shows the representative lung specimens of Ge-132 treated (1 of

Table 1. *Effect of Ge-132 on the 3LL local tumor in B6 mice*

Group	Ge-132 dose (mg/kg, i.p.)	Treatment schedule	Tumor vol. on day 16 (cm^3)	Tumor wt. on day 21 (gm)
I	300	Day −1	1.43±0.75 (11.7)	6.6±1.21
II	300	Day 1	1.01±0.64 (37.7)	6.3±1.69
III	300	Day 8	0.95±0.69 (41.4)	5.8±2.88
IV	100	Days −7 to −1	0.95±0.40 (41.4)	5.7±0.89
V	100	Days 1 to 7	0.60±0.23 (63.0)*	5.4±1.00
VI	100	Days 8 to 14	1.27±0.68 (21.6)	6.1±1.36
VII	500	Days 1 to 7	1.20±0.82 (25.9)	6.4±0.91
VIII	10	Days 1 to 7	1.25±0.69 (22.8)	6.2±2.04
IX†	0	Untreated control	1.62±0.77 (0)	6.0±1.68

B6 mice (10/group) were inoculated with 3LL on day 0 (1×10^5 cells, s.c.).
Each value represents mean±S.D. (inhibition ratio %).
* $p<0.05$ (Student's t-test against Group IX).
† Same as Group VI in the foregoing paper.

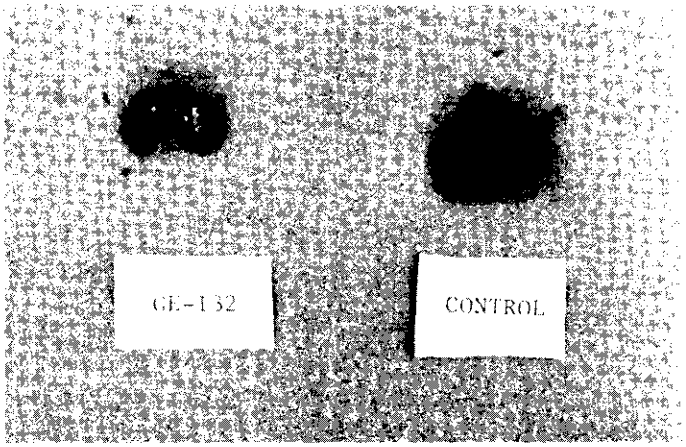

Fig. 1. Lungs of B6 mice inoculated with 3LL
The lungs were excised before insufflated with India ink. Left: Ge-132 treated (1 of Group V), right: untreated control (1 of Group IX).

TABLE 2. *Schedule-dependent dual effect of Ge-132 on the pulmonary metastases of 3LL in B6 mice*

Group	Ge-132 dose (mg/kg, i.p.)	Treatment schedule	Mean No. of nodules/mouse (range)			Inhibition ratio (%)
			Large[a]	Small	Total	
I	300	Day −1	4.5 (0-17)	10.9 (3-32)	15.4 (4-41)	40.5
II	300	Day 1	4.1 (0-12)	17.9 (3-95)	22.0 (4-107)	15.1[c]
III	300	Day 8	9.1 (2-27)	13.8 (6-32)	22.9 (8-59)	11.6
IV	100	Days −7 to −1	5.7 (0-11)	22.2 (6-39)	27.8 (6-43)	−7.3
V	100	Days 1 to 7	2.5 (0-8)	10.8 (1-21)	13.3[b] (1-26)	48.6
VI	100	Days 8 to 14	13.0 (4-32)	38.4 (11-75)	51.4[b] (17-107)	−98.5
VII	500	Days 1 to 7	4.8 (1-14)	12.3 (3-20)	17.2 (4-27)	33.6
VIII	10	Days 1 to 7	4.3 (0-13)	22.7 (7-55)	27.0 (8-64)	−4.2
IX	0	Untreated control	7.1 (1-21)	18.8 (7-47)	25.9 (11-59)	0

B6 mice (10/group) were inoculated with 3LL on day 0 (1×10^5 cells, s.c.).

a) Diameter > 1 mm (most of those in Group III were > 3 mm).

b) Statistically not significant (Student's t-test against Group IX).

c) 61.8% was obtained excluding 1 unusual case with 107 nodules (12 large, 95 small).

Group V, left) and the untreated control (1 of Group IX, right). Whereas a numerous metastatic foci are visible on the surface of the untreated one which is larger in size, the macroscopical view of the treated one is substantially normal. All these identifiable foci were counted individually after the lungs being insufflated with dilute India ink (Wexler 1966). The results are summarized in Table 2.

A dual action of Ge-132 was revealed here depending on the treatment schedules. Thus, 10 daily doses of 100 mg/kg were inhibitory when started from day 1 (13 nodules in Group V compared to 26 in Group IX), whereas the same treatment delayed until day 8 resulted in a marked facilitation (51 nodules in Group VI). The prior treatment was of negative effect (Group IV). Ge-132 at the dosage of 500 mg/kg was only slightly inhibitory (Group VII), while no effect was observed at 10 mg/kg (Group VIII). A single dose of 300 mg/kg was moderately inhibitory when administered on day -1 (Group I, inhibition ratio 41%). The one given on day 1 (Group II) was also inhibitory with 1 exception of marked facilitation, yielding the inhibition ratio 62% instead of 15% altogether. A single dose of 300 mg/kg on day 8 (Group III) was of no effect in terms of the number of foci. It was notable, however, that nearly one-half of those nodules were extremely large in size (diameter >3 mm), suggesting an adverse effect of the treatment. The antimetastatic effect of Ge-132 was thus observed in strictly defined conditions. Alternatively, Ge-132 was revealed for its schedule-dependent dual action.

Effect of Ge-132 against the minimized tumor inoculum

In another set of experiment, B6 mice were inoculated s.c. with each 10^4 or 10^3 3LL cells, and received a single i.p. dose of Ge-132 at 300 mg/kg on either day 1 or 8. The day of tumor appearance was recorded individually. As shown in

TABLE 3. *Delayed tumor take in Ge-132-treated B6 mice after inoculation of a minimized number of 3LL cells*

Group	Inoculum size (s.c.)	Ge-132 dose (mg/kg, i.p.)	Time of treatment	Cumulative No. of tumor take/Total (%)			
				Day 13	Day 16	Day 20	Day 26
I	1×10^4	300	Day 1	2/8 (25)	4/8 (50)*	5/8 (63)	6/8 (75)
II	1×10^4	300	Day 8	4/8 (50)	6/8 (75)	6/8 (75)	6/8 (75)
III	1×10^4	0	Untreated control	5/8 (63)	8/8 (100)	8/8 (100)	8/8 (100)
IV	1×10^3	300	Day 1	0/7	0/7	1/7	1/7
V	1×10^3	300	Day 8	0/6	1/6	2/6	2/6
VI	1×10^3	0	Untreated control	0/7	0/7	0/7	0/7

* $p<0.05$ (χ^2-test against Group III).

TABLE 4. *Negative effect of postsurgical-adjuvant therapy with Ge-132*

Group	Treatment		Survival rate (%)	
	Surgery	Ge-132	Day 50	Day 200
I	(+)	(+)	37.5 (3/ 8)	37.5 (3/ 8)
II	(+)	(−)	57.1 (4/ 7)	28.6 (2/ 7)
III	(−)	(−)	0 (0/10)	0 (0/10)

B6 mice were inoculated with 3LL into the left footpad on day 0 (2.5×10^4/mouse, s.c.).

Radical amputation of the tumor-bearing leg was performed on day 20.

Ge-132 (100 mg/kg daily i.p.) was administered for 10 days from the next day of surgery.

Table 3, the treatment given on the next day of tumor inoculation was effective in prolonging the latency before tumor take (10^4). Thus, the cumulative tumor incidence in Group I was 50% on day 16 ($p < 0.05$), 63% on day 20, and 75% on day 26, while that in Group III was already 100% by day 16. However, the delayed treatment was of negative effect, no significant difference being found among Groups II and III. The inoculum size of 10^3 seemed to be insufficient, since no tumor take was found in any one of the untreated controls during the observation period.

Negative effect of the postsurgical-adjuvant therapy

The possible beneficial effect of Ge-132 in the postsurgical-adjuvant model was examined in 1 of 4 groups of mice described in the previous paper (Kumano et al. 1985). Ge-132 at a daily dose of 100 mg/kg i.p. was administered for 10 days from the next day of surgery which was performed on day 20, and the survival rate was compared among the experimental groups. As shown in Table 4, Ge-132 gave no further improvement of the surgical 'cure' as evaluated on either day 50 or 200.

DISCUSSION

In the present study, Ge-132 was examined for its possible antitumor effect in a syngeneic transplantable tumor model, the 3LL in B6 mice. Preferential inhibition of the pulmonary metastases was found as the characteristic feature of its action. The antimetastatic effect of Ge-132 was observable only under limited conditions; either inhibition or facilitation was found depending on the treatment schedules. In this respect, Ge-132 was not exceptional of a variety of immune response modifiers in the literature (Proctor et al. 1977; Seshadri and Poduval 1980). A notable discrepancy was occasionally found in the therapeutic outcome even with the same treatment schedule; for instance, a marked facilita-

tion in one exceptional case of Group II (Table 2). The host status seemed to be an important variable of the efficacy of this compound, in addition to the treatment schedules described above. The number of tumor cells present at the time of treatment was also suggested as a relevant factor, since Ge-132 was only capable of inhibiting the metastasis formation at an earlier stage, allowing the local tumor growth substantially unaffected unless the inoculum size was minimized. Ge-132 showed no curative effect against those preexisting metastases in the present model of postsurgical-adjuvant therapy.

Evidence is now available suggesting this compound to be a biological response modifier. Thus, IFN-inducing activity has recently been revealed in association with augmentation of natural killer (NK) activity and activation of macrophages in mice (Aso et al. 1982), although not exactly in the present tumor-host system. IFN-inducing activity was also found in humans after oral administration of Ge-132 (Miyao et al. 1980). A group of 'immunopotentiators' of various origins with known antitumor and/or antiviral activities were also demonstrated for their IFN-inducing activity and NK augmentation (Ebina et al. 1981 ; Suzuki 1983). On the other hand, the antitumor action of IFN (Gresser and Bourali-Maury 1972) or IFN-inducers (Morahan et al. 1974 ; Snodgrass et al. 1975) was observed in the 3LL/B6 model. Despite our above findings with Ge-132, IFN was reported to be equally inhibitory against the local and metastatic tumor growth, even when treatment was delayed until 6 or 8 days after tumor inoculation. All these data taken together may still suggest the possible contribution of IFN and the above cellular components to the antimetastatic effect of Ge-132, probably being involved in the complexities of self-regulatory mechanisms (Evans 1983). The dual effects of Ge-132 revealed in the present study seems to be reflective of such a complicated aspect of its action. A better understanding of the relevant mechanisms is essential before one can expect its beneficial effect, while any possible adverse effect(s) being avoided.

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