Short Communication

Effect of \(1\alpha\)-hydroxyvitamin \(D_3\) on metastasis of rat ascites hepatoma K-231

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\(1\alpha,25\)-Dihydroxyvitamin \(D_3(1\alpha,25(\text{OH})_2D_3)\), converted in the liver from \(1\alpha\)-hydroxyvitamin \(D_3(1\alpha(\text{OH})D_3)\) (Fukushima et al., 1975) has been known to function in target tissues in binding to a specific cytosol receptor and transportation to the nucleus (Brumbaugh & Haussler, 1975). Although it has been reported that the growth of some tumour cells is inhibited in vitro by \(1\alpha,25(\text{OH})D_3\) (Colston et al., 1981; Abe et al., 1981), its antitumour effect has been more recently demonstrated in vivo (Sato et al., 1982; Honma et al., 1983). \(1\alpha(\text{OH})D_3\) might be used as a new anticancer agent with a mode of action different from those currently used.

The purpose of this experiment was to examine the effect of \(1\alpha(\text{OH})D_3\) in vivo on metastases and survival time of tumour-bearing rats.

Animals used were 9- to 12-week old male ACI/N rats. The neoplasm was the ascites hepatoma K-231 which was poorly differentiated and rapidly growing (Sato & Sato, 1983). Animals were given water and standard rat chow ad libitum. After \(10^8\) tumour cells were transplanted s.c. into the right posterior dorsum of rats, they were divided at random into two groups which were administered vehicle and \(1\alpha(\text{OH})D_3\), respectively. \(1\alpha(\text{OH})D_3\) (Chugai Pharmaceutical Co., Ltd., Tokyo) dissolved in medium chain triglyceride (ODO\(^6\)) (Nishin Oil Mills, Ltd., Tokyo) was administered at a dose of 0.5 \(\mu\)g kg\(^{-1}\) into the stomach by a stomach tube daily from a day after inoculation to a day before killing. The animals were killed on Day 13, and primary tumour and right inguinal lymph nodes were weighed and the number of macroscopically visible metastases on the pulmonary surfaces counted. The survival times of rats bearing tumour treated consecutively for 22 days from a day after inoculation were also determined.

In a separate experiment the animals were inoculated i.m. with \(2 \times 10^4\) cells into the right hind leg and were given the agents daily to a day before killing as above-mentioned. The animals were killed on Day 20 and metastases of lymph nodes and lungs were also examined.

The tumour tissues were fixed in 10% formalin solution and paraffin sections were stained with H & E.

For testing the significance of the differences between two groups the U-test, which is a ranking test used for comparison when distribution is not normal and variances are large, was used.

The primary tumour weight of rats treated with \(1\alpha(\text{OH})D_3\) was slightly reduced, but metastases of lungs and right inguinal lymph nodes were significantly inhibited (Table I & Figure 1). Moreover, the prolongation of the life span of rats bearing tumour treated with \(1\alpha(\text{OH})D_3\) was slight, but significantly different from the ODO group (Figure 2).

In the i.m. implantation experiment because bilateral lumbar lymph nodes were enlarged and fused into one and invaded the retroperitoneum, on

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Figure 1 Metastases of right inguinal lymph nodes 13 days after s.c. inoculation. Lymph nodes of \(1\alpha(\text{OH})D_3\) group were evidently smaller than those of the control ODO group. Bar = 10 mm.
Table I: Effect of treatment with 1α(OH)D₃ on ascites hepatoma K-231 after s.c. implantation

| Group          | Effective number of rats | Primary tumour wt (g) | Inguinal lymph node wt (g) | No. of pulmonary metastases |
|----------------|--------------------------|-----------------------|---------------------------|-----------------------------|
| ODO treatment  | 6                        | 5.52±0.67             | 0.76±0.31                 | 8.7±1.8                     |
| 1α(OH)D₃ treatment | 5                      | 4.93±0.81             | 0.15±0.04b               | 3.2±1.9b                   |

Values are the mean ± s.e.

a Although the initial number of rats was 6 in each group, one animal died inadvertently.

b Significantly different, P<0.05.

Table II: Effect of treatment with 1α(OH)D₃ on ascites hepatoma K-231 after i.m. implantation

| Group          | Effective number of rats | Inguinal lymph node wt (g) | No. of pulmonary metastases |
|----------------|--------------------------|---------------------------|-----------------------------|
| ODO treatment  | 5                        | 0.30±0.07                 | 45.2±17.5                   |
| 1α(OH)D₃ treatment | 6                      | 0.25±0.09                 | 14.8±3.5c                   |

Values are the mean ± s.e.

a Although the initial number of rats was 6 in each group, one animal died inadvertently.

b The difference in lymph node wts between control groups (Tables I and II) might be due to differences in the site of injection and the number of tumour cells used.

c Significantly different, P<0.05.

Figure 2: Percent survival of rats bearing tumour. Seven rats in each group were inoculated s.c. with 10⁶ K-231 ascites hepatoma cells and were given 1α(OH)D₃ (0.5 μg kg⁻¹) or vehicle (ODO) daily for 22 days from one day after tumour inoculation. The mean survival times were 27.1±0.8 days in the 1α(OH)D₃ group (---) and 24.8±0.8 days in the ODO group (----), respectively (P<0.05).

Figure 3: Histology of inguinal lymph node metastasis in the 1α(OH)D₃ group. No morphological difference was found between this and the ODO groups. H & E. × 320.
1α(OH)D₃ might be useful in adjuvant therapy for prevention of metastases of human tumours.

It has been reported that 1α(OH)D₃ suppresses proliferation and induces differentiation of M1 myeloid leukaemia cells in vitro (Abe et al., 1981), and prolongs the life span of animals inoculated with them regardless of T-lymphocyte-mediated immune responses (Honma et al., 1983). However, no information is available on the effect of 1α(OH)D₃ following oral administration on the growth of metastases except for our previous report (Sato et al., 1982) and the mechanism of inhibition of metastasis is not yet evident. Further work will be necessary to examine whether metastases are suppressed because 1α(OH)D₃ arrests the release of tumour cells from the primary neoplasm or because their secondary implantation and development are impaired.

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