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Conference paper

The effect of bee venom on tumor growth and metastasis formation of mammary carcinoma in CBA mice

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Abstract

The effect of honeybee venom on the tumor growth and metastasis formation in mice was studied. Bee venom was injected into mice either subcutaneously *(s.c.)* or intravenously *(i.v.)* at different doses. The tumor was a transplantable mammary carcinoma (MCA) weakly immunogenic to the syngeneic CBA mouse. The tumor was generated by injecting 10^5 MCA cells *i.v.* When the tumor cells were injected *s.c.* into the footpad immediately after bee venom, the growth of the tumor was suppressed regardless of the dose of the venom. The survival of the mice treated with 0.30 mg of bee venom was prolonged as compared to the controls. The number of lung metastases in the mice treated *i.v.* with 0.15 or 0.075 mg of bee venom was significantly lower ($P < 0.001$) than that in nontreated mice.

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However, both doses of bee venom given *s.c.* did not reduce the number of lung metastases, indicating that the antitumor effect of the venom could be highly dependent on the route of injection.

Key words: murine mammary carcinoma, antitumor and antimetastatic activity, honeybee venom.

Ključne riječi: karcinom mliječne žlijezde miša, protutumorsko i protumetastatsko djelovanje, pčelinji otrov.

Introduction

The antitumor effects of royal jelly *(Townsend* et al., 1959; 1960) and other products of honey bee *(Apis melifera)* have already been reported *(Rode* et al., 1979; *Vrščaj,* 1988). Both, the whole royal jelly and its constituent 10-hydroxydecenoic acid prevent the development of a transplantable leukemia or ascitic tumors in AKR mice *(Townsend* et al., 1959). Similar results were obtained when slow growing tumors such as Ehrlich or Sarcoma-180 ascites tumors were treated with royal jelly *(Tamura* et al., 1985a). *Orlov* (1979) noted that nontoxic doses of honeybee venom could elicit the resistance of mice to X-rays. Recently, the radioprotective ability of honeybee venom was confirmed on Wistar rats irradiated with γ-rays at the dose of 300 cGy *(Varanda* et al., 1989).

The study reported herein was conducted to determine whether bee venom at different doses and routes of injection affects the tumor growth and metastasis formation in mice.

Material and methods

Bee venom was produced using a highly efficient technology under industrial beekeeping conditions in the "Medex" (Ljubljana, Slovenia), dissolved in 0.5 ml of distilled water and injected subcutaneously *(s.c.)* into the left footpad of CBA mice (of both sexes, 3-month-old, weighing 20 g on average) at different doses (0.15, 0.30, or 0.60 mg per mice). The tumor was a transplantable mammary carcinoma (MCA) syngeneic to CBA mouse. The tumor was weakly immunogenic for its syngeneic recipient *(Bašić* and *Varga,* 1979). To generate the tumor, 1×10^5 tumor cells were injected *s.c.* into the same footpad immediately after the bee venom injection. A group of mice receiving tumor cells only served as a control. The tumor growth and survival of mice were recorded for at least 5 weeks after the treatment (Experiment 1). In Experiment 2 the groups containing seven mice each were used. All mice, except controls were treated either *s.c.* into the axillary region or *i.v.* with 0.15 or 0.075 mg of bee venom. Immediately after bee venom treatment the mice received an *i.v.* injection containing 1×10^5 tumor cells. The mice were killed 18 days after the treatment and the number of tumor nodules in the lungs was determined.

Results and discussion

As shown in Table 1, the treatment with three different doses of bee venom resulted in the prolonged survival of CBA mice during the period of 50 days after the inoculation of MCA cells. Bee venom-treated mice survived for at least 33 days after inoculation while one nontreated mouse died before the 25 postinoculation (PI) day. A single dose of 0.30 mg of bee venom protected 40% of mice, whereas 100% of nontreated MCA-inoculated mice died on the 50 PI day. The tumor growth was suppressed during the observation period of 37 days in bee venom-treated mice but differences regarding controls were not significant. Even a fourth of growing tumors

Table 1. Survival of CBA mice inoculated with 1 x 10^5 mammary carcinoma (MCA) cells and treated with three different doses of bee venom *s.c.*

Treatment	Died by PI* day				Survived until
	25	29	33	37	PI day 50
None	1/5**	1/5	1/5	1/5	0/5
Bee venom					
0.15 mg	0/5	0/5	0/5	1/5	1/5
0.30 mg	0/5	0/5	0/5	1/5	2/5
0.60 mg	0/5	0/5	0/5	0/5	1/5

* PI = postinoculation;

**No. of dead mice/no. of MCA inoculated mice.

regressed, which probably influenced the survival of bee venom-treated mice. The number of tumor nodules in the lugns of the mice treated *i.v.* with either 0.15 mg or 0.075 mg of bee venom was significantly lower ($P < 0.001$) than that recorded in nontreated mice (Table 2). In contrast, the *s.c.* treatment of either dose of bee venom did not influence the number of lung metastases, indicating that the antimetastatic effect of apitoxin could be highly dependent on the route of injection. The latter is in agreement with the observations by others *(Townsend* et al., 1959; 1960; *Tamura* et al., 1985a) who proposed that the tumor cells and antitumor agent, i.e. royal jelly, should be given by the same route. However, they used the other tranplantable tumors and bee product by intraperitoneal route which is a quite differen model incomparable with ours.

The mechanism(s) by which the tumor growth was suppressed and the number of metastases lowered may have a few possible explanations. Enhanced humoral and

cellular immune responses against bee venom *(Ćurić* et al., 1992) should be taken into consideration regarding the mode of action of apitoxin as an antitumor agent. Secondly, a simple contact of tumor cells with bee venom might directly change the

Table 2. Effect of bee venom on the number of MCA nodules in CBA mice*

Treatment**	Route of bee venom injection	No. of lung metastases (Mean ± SD)
None		14.7 ± 3.5
Bee venom		
0.15 mg	*s.c.*	21.4 ± 6.0
0.15 mg	*i.v.*	5.1 ± 1.2***
0.075 mg	*s.c.*	33.0 ± 20.0
0.075 mg	*i.v.*	5.1 ± 3.1***

* groups comprised 7 mice each;

** 1×10^5 MCA cells per mouse were given *i.v.* immediately after the bee venom injection; mice were killed 18 days after the treatment;

*** significantly different ($P < 0.001$) comparing to nontreated mice.

composition of some membrane receptors on tumor cells making them more sensitive to antitumor factors produced by the host. Finally, bee venom could affect the DNA of tumor cells, as suggested by *Tamura* et al. (1985b) for rayal jelly, and in this way exhibit antitumor activity.

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Djelovanje pčelinjeg otrova na rast i stvaranje metastaza karcinoma mliječne žlijezde u miševa soja CBA

Sažetak

Istraživali smo djelovanje pčelinjeg otrova (Medex, Ljubljana, Slovenija) na rast i stvaranje metastaza karcinoma mliječne žlijezde (mammary carcinoma = MCA) u miševa soja CBA. Pčelinji smo otrov uštrcali ili potkožno *(s.c.)* ili u venu *(i.v.)* u različitim dozama. MCA je slabo imunogen za singenične CBA miševe, te je izazvan uštrcavanjem 10^5 tumorskih stanica u repnu venu. Kada su tumorske stanice bile uštrcane *s.c.* u lijevu šapicu odmah nakon davanja pčelinjeg otrova, rast MCA bio je zaustavljen bez obzira na primijenjenu dozu pčelinjeg otrova (0,15, 0,30 ili 0,60 mg). Preživljavanje miševa obrađenih dozom od 0,30 mg pčelinjeg otrova bilo je produženo u usporedbi s kontrolom. Broj plućnih metastaza u miševa obrađenih *i.v.* dozama od 0,15 ili 0,075 mg pčelinjeg otrova bio je značajno manji ($P < 0,001$) u odnosu na broj metastaza u plućima neobrađenih miševa. Međutim, te doze pčelinjeg otrova dane *s.c.* nisu smanjile broj plućnih metastaza, pokazujući da bi protutumorsko djelovanje pčelinjeg otrova moglo biti ovisno o putu njegova davanja.