0.50 nm. Hence, one would not see such contacts in NOESY spectra.

Thus, we conclude that prostatic inhibin acquires a predominantly anti-parallel β-sheet structure and possibly the molecule is locked into several such sheets through disulphide linkages.


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ACKNOWLEDGEMENTS Use of the facilities provided by 500 MHz FT NMR National Facility supported by the Department of Science and Technology, Government of India and located at T. I. F R , Bombay, and the preliminary experiments done by Priya Chandran under Visiting Students Research Programme of T I F. R., are gratefully acknowledged

Received 3 May 1993; revised accepted 24 June 1993

# Cisplatin and ascorbic acid: Synergistic antitumour effect against Dalton's lymphoma in mice

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**Therapeutical potential of low dose of cisplatin alone or in combination with ascorbic acid was studied in $C_3H$/He mice bearing Dalton's lymphoma. Subtherapeutical dose of cisplatin (3 mg/kg) was able to increase the survival time of tumour-bearing mice without any tumour-free survivor. Ascorbic acid enhances the antitumour effect of cisplatin, resulting in increased life span of tumour-bearing mice as well as tumour-free survivors. The enhancement of cisplatin-induced tumour growth inhibition by ascorbic acid is probably due to the modulation of permeability of tumour cell membrane which increases the uptake of cisplatin into the tumour cells.**

A number of side effects of cisplatin like nephrotoxicity, ototoxicity, neuropathy and myelosuppression are widely described in various animals as well as in humans[1–7]. Cisplatin-induced renal toxicity is the main limiting factor for its clinical use and can be partially reduced by pretreatment hydration and administration of furosemide and/or mannitol[8], sodium 2-mercaptoethane sulfonate[9, 10], nicotinamide or 3-amino-benzamide[11] and selenium[12]. It is often not possible to successfully treat tumours by increasing the dose level of cisplatin because such therapy may be fatally toxic though it reduces the tumour burden significantly[12, 13]. Thus combination therapy with an agent which enhances the antitumour effect of cisplatin with little or no enhancement of cisplatin's toxicity is of value in the treatment of various tumours that fail to respond to treatment with cisplatin alone. It has been reported that nifedipine enhances the antitumour effect of cisplatin (4 mg/kg) against the cisplatin-sensitive murine amelanotic melanoma as well as Lewis lung carcinoma[13, 14]. Recently it has been reported that therapeutical potential of low dose of cisplatin (3 mg/kg) against Dalton's lymphoma is greatly enhanced when it is used in combination with glucose[15, 16]. The present studies were undertaken to determine the combined effect of ascorbic acid and cisplatin on the growth of Dalton's lymphoma in mice in order to find out the effect of ascorbic acid on enhancement of tumour growth inhibition induced by a low dose of cisplatin.

$C_3H$/He strain of mice-bearing Dalton's lymphoma originally procured from the Chittaranjan National Cancer Research Institute, Calcutta and maintained in the laboratory by regular serial transplantations was used in all sets of experiments. The day of tumour transplantation was taken as day 0. On the 6th day mice-bearing palpable tumour was treated i p. with 4 repeated injections of either 20, 40 or 60 mg/kg of ascorbic acid The injections were administered on alternate days. The survival period of tumour-bearing mice and the percentage of tumour-free survivors were observed in each group (10 mice in each group). For combination therapy cisplatin was dissolved freshly in balanced salt solution. Different groups of mice were treated with single i p. injection of subtherapeutical dose of cisplatin (3 mg/kg) along with repeated injections of ascorbic acid (20, 40 or 60 mg/kg). Cisplatin was injected on 6th day following tumour transplantation. On the same day a single injection of ascorbic acid (20, 40 or 60 mg/kg) was also given followed by three repeated injections of ascorbic acid on alternate days. The mean survival time of tumour-bearing mice and the percentage of tumour-free survivors were studied in each group of mice. In the experimental batch, the percentage of increased life span was calculated by the following formula[17]

Percentage of increased life span = $[(T - C)/C] \times 100$; $T$ = mean survival time of treated mice, $C$ = mean survival time of untreated control mice.

**Table 1.** Increased survival time and percentage of increased life span of tumour-bearing mice after treatment with ascorbic acid

| Treatment | Mean survival time in days* ± SD | Increased life span** (%) | 60/more than 60 day survivors*** | Tumour-free survivors |
|---|---|---|---|---|
| None | 13 33 ± 2 62 | – | – | – |
| 20 mg/kg ascorbic acid | 19.66 ± 4.19 | 48 | – | – |
| 40 mg/kg ascorbic acid | 18.66 ± 6.18 | 40 | – | – |
| 60 mg/kg ascorbic acid | 13 66 ± 5 90 | 3 | – | 2 |

*Including 60 day survivors

**$[(T–C)/C] \times 100$; $T$: mean survival days of treated mice
$C$: mean survival days of untreated control mice.

***Number of survivors out of 10 examined mice in a group at 60 days after treatment with ascorbic acid.

**Table 2.** Increased survival time and percentage of increased life span of tumour-bearing mice after treatment with cisplatin alone or in combination with ascorbic acid

| Treatment | Mean survival time in days* ± SD | Increased life span** (%) | 60/more than than 60 day survivors*** | Tumour-free survivors |
|---|---|---|---|---|
| None | 13.33 ± 2 62 | – | – | – |
| 3 mg/kg cisplatin | 35.40 ± 6.80 | 166 | – | – |
| 3 mg/kg cisplatin + 20 mg/kg ascorbic acid | 51.67 ± 6.02 | 287 | 2 | 4 |
| 3 mg/kg cisplatin + 40 mg/kg ascorbic acid | 50.00 ± 4.32 | 275 | | 4 |
| 3 mg/kg cisplatin + 60 mg/kg ascorbic acid | 66.50 ± 1.50 | 399 | 5 | 5 |

*Including 60 day survivors

**$[(T–C)/C] \times 100$; $T$: mean survival days of treated mice
$C$: mean survival days of untreated control mice.

***Number of survivors out of 10 examined mice in a group at 60 days after treatment with cisplatin alone or in combination with ascorbic acid.

Tumour-bearing mice when treated with ascorbic acid (20 or 40 mg/kg × 4) exhibited a slight increase in their mean survival time compared to the control (Table 1). When animals were treated with a high dose of ascorbic acid (60 mg/kg × 4), there was no significant increase in their survival time; however, 20–25% animals appeared as tumour-free survivors. Subtherapeutical dose of cisplatin was able to enhance the survival time of tumour-bearing animals, their life span increased to 166% compared to controls. None of the animals was found to be tumour-free. Therapeutical potential of low dose of cisplatin was increased when combined with different concentrations of ascorbic acid. Mice treated with cisplatin along with ascorbic acid (20 or 40 mg/kg × 4) exhibited an increase in their mean survival time from 13 days in control to 50 days in treated animals. Both the concentrations of ascorbic acid in combination with cisplatin were able to produce 40% tumour-free survivors (Table 2). However, when the dose of ascorbic acid was increased (60 mg/kg × 4) and combined with subtherapeutical dose of cisplatin, all the animals receiving such treatment survived beyond 60 days, 50% of the animals appeared as tumour-free survivors without any sign of tumour and its reappearance, the rest of them died at a later stage exhibiting about 400% increase in their life span.

The present study has shown that vitamin C is effective in treatment of Dalton's lymphoma in mice if combined with low dose of cisplatin. In the present system complete regression of tumour was observed in

20–25% of experimental animals when treated with *high dose of ascorbic acid (60 mg/kg × 4)*. However, low doses (20 or 40 mg/kg × 4) failed to regress tumour, showing no significant increase in the survival time of tumour-bearing mice. Similar stimulation of ascites tumour cell multiplication at low doses and inhibition at high doses has been reported earlier[18, 19] using ascorbic acid or dehydroascorbic acid. Recently, no therapeutical benefit in mice bearing C1300 neuroblastoma has been reported after a single i.p. administration of low dose (20 mg/kg × 9) of vitamin E, although the concentration of vitamin E was elevated in tissues and blood[20]. In the present system antitumour activity of cisplatin was enhanced in mice receiving ascorbic acid at different concentrations. Animals receiving subtherapeutical dose of cisplatin survived with tumour growth. However, it has been reported earlier that therapeutical dose of cisplatin when administered in tumour-bearing mice was able to produce 57% tumour-free survivors after 3–4 days of treatment, the rest of them died after a few days due to reappearance of tumour[16]. A similar type of tumour regression has also been reported when mice-bearing syngeneic fibrosarcoma was treated with single i.p. injection of cisplatin at therapeutical dose (9 mg/kg)[21]. It has been reported earlier that cisplatin when given in a dose of 8.5 or 10 mg/kg induces a rapid increase in the urinary protein and glucose associated with an increase in serum glucose and urea[22]. Systemic side effects of cisplatin on kidney injury have been frequently reported[22–25]. Besides, significant reduction in tumour burden, high dose of cisplatin result in significant host's toxicity[12, 13] with a carcinogenic risk at a later stage[26, 27] To avoid this risk and other side effects in the present system, subtherapeutical dose of cisplatin with repeated injections of ascorbic acid was used to evolve a nontoxic chemotherapy of this tumour. The most effective dose of ascorbic acid in combination with cisplatin was found to be 60 mg/kg which has given almost 100% tumour-free survivors up to 65 days. About 50% are still surviving without any sign of tumour. *Even low doses of ascorbic acid (20 or 40 mg/kg × 4)* were found to be effective when combined with subtherapeutical dose of cisplatin. This shows that ascorbic acid somehow increases the therapeutical potential of low dose of cisplatin, resulting in complete regression of tumour in most of the animals. Subtherapeutical dose of cisplatin alone resulted in an increase in the mean survival time of tumour-bearing animals without any tumour-free survivor. It is believed that the stimulation of helper T cells by vitamin E might enhance the antitumour activity of cisplatin[20]. Vitamin E (20 IU/kg × 7 days) has also been reported to enhance both lymphoproliferative reaction and the antitumour effect of adriamycin[28, 29]. This enhancement is probably due to increase in the intratumour vitamin E content[20] which acts at the sites on DNA or RNA of the tumour cells[30] like cisplatin[31]. It has already been reported that vitamin C decreases the rate of DNA synthesis[32]. From these studies it is clear that in the present system ascorbic acid enhances the tumour growth inhibition induced by cisplatin by modulating the permeability of tumour cell membrane, thus elevating the intratumour content of ascorbic acid. These cell surface changes may increase the uptake of cisplatin into the tumour cells facilitating the stronger action of cisplatin as an antitumour agent.


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ACKNOWLEDGEMENT S S thanks UGC, New Delhi for financial assistance

Received 17 November 1992, accepted 8 June 1993

# A rare condition of budding in bipinnaria larva (Asteroidea)

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**A rare situation of budding in bipinnaria larva was encountered in the plankton samples collected from coastal waters of Visakhapatnam in 1987. Elsewhere, paratomy is a regular feature of adult flatworms and annelids. The phenomenon seems to be rather frequent and widespread. However, the chances of encountering the larvae actually may have been minimal. It may also be that very few have had enough opportunities to discern them cloning. The present paper reports asexual reproduction through budding in echinoderm larvae.**

AMONG invertebrates the phenomenon of asexual reproduction is well established in several phyla. Turbellarians and some annelids like earthworms or aeolosomatids have remarkable power of regeneration and cloning. In some parasitic flatworms there is normal larval propagation by asexual reproduction, whereas in other groups like coelenterates larval propagation takes place by transverse fission, as in scyphistoma stage of *Aurelia*, producing ephyrae larvae. In echinoderms, asteroids reproduce by asexual method. A sea star may break into two by what is known as fissiparity, each piece regenerating into a complete individual. In our study on planktotrophic larvae in the coastal waters of Visakhapatnam we came across several specimens of bipinnaria larvae presenting evidence of budding.

The first report on asexual reproduction in echinoderm larvae is that of Bosch *et al.*[1]. They reported the presence of highly modified posterolateral arms in the bipinnaria larvae (of the sea star *Ludia* sp.) of the Gulf stream and the western Sargasso sea in June–July 1987. We have found the same (Figure 1) in several specimens of bipinnaria larva encountered in our samples. The asteroid identity of the bipinnaria in our plankton collections (made in 1987) is, however, not clear at present. Typically, each larva has two

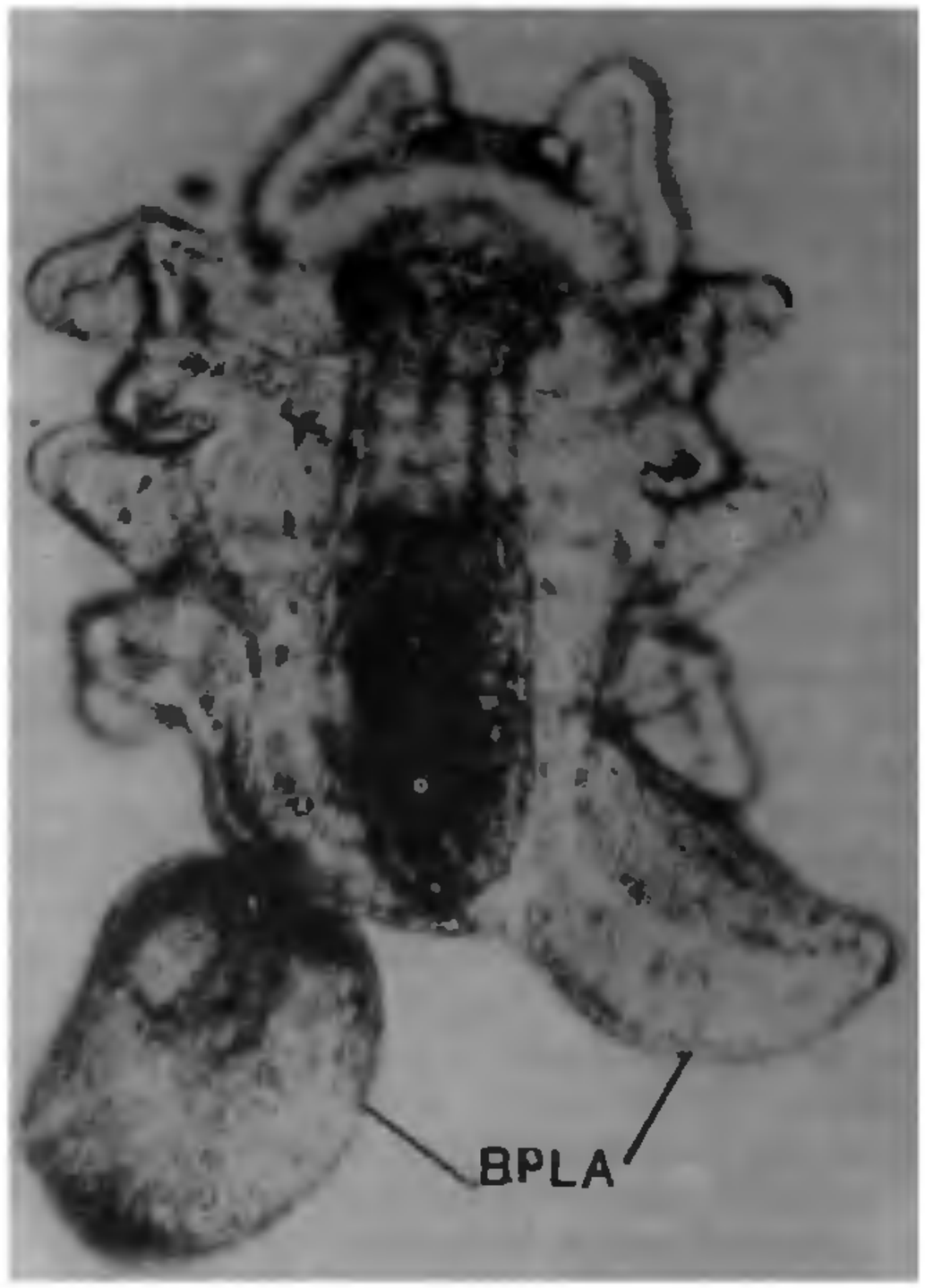


Figure 1. Bipinnaria larva showing budding of postero-lateral arms (BPLA)

fission zones, one on each postlateral arm. There are no ciliated bands, but rudiments of as-yet undifferentiated gut could be seen in each. Bosch *et al.* have discussed at length the details pertaining to the origin, formation and release of these fission products. It has been stated that at a stage when the stomodael invagination breaks through the undifferentiated digestive tract, the epithelium joining the primary and secondary larvae separates and the secondary larva is released.

There is no doubt that an exactly similar situation occurs in the bipinnaria encountered in our samples. This is the second report from the world and the first from India on paratomy, or larval propagation by fission, in echinoderm larvae. Both reports of budding in the bipinnaria are from warm waters (The Gulf stream and The Bay of Bengal). An evolutionary significance from the point of view of hazards in the completion of life cycle and survival of race may be implicated.

In the parasitic flukes, for instance, asexual reproduction in the larval stages (sporocysts and rediae) which are intramolluscan stages has definitely survival value for the species, and this could be directly related to hazards in the completion of life cycle. Mortensen[2] had already implied proliferation by budding in echinoderm larvae.