Bonga, J. M. and Durzan, D. J.), Martinus Nijhoff Publishers, Dordrecht, 1987, vol. 3, pp. 300–315.
4. Lakshmi Sita, G., Chattopadhyay, S. and Tejavathi, D. H., *Plant Cell Rep.*, 1986, **5**, 266.
5. Vibha Dhawan and Bhojwani, S. S., *Plant Cell Rep.*, 1985, **4**, 315.
6. Natarja, K. and Sudhadevi, A. M., *Indian J. Plant Physiol.*, 1984, **27**, 255.
7. Khattar, S. and Mohan Ram, H. Y., *Indian J. Exp. Biol.*, 1983, **21**, 251.
8. Gharya, P. K. and Maheshwari, S. C., *Naturwissenschaften*, 1982, **68**, 379.
9. Tomar, U. K. and Gupta, S. C., *Plant Cell Rep.*, 1988, **7**, 70.
10. Skolman, R. G., in *Biotechnology in Agriculture and Forestry 1. Tress I* (ed. Bajaj, Y. P. S.), Springer-Verlag, Berlin, 1985, pp. 375–384.
11. *Tropical Legumes: Resources for the Future Crop*, National Academy of Sciences, Washington DC, 1979, pp. 221–231.
12. Gamborg, O. L., Miller, R. A. and Ojima, K., *Exp. Cell Res.*, 1968, **50**, 151.
13. Murashige, T. and Skoog, F., *Physiol. Plant*, 1962, **15**, 473.
14. Sarita Patri, Bhatnagar, S. P. and Bhojwani, S. S., *Phytomorphology*, 1988, **38**, 41.

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Vincristine can cause giant cell formation in rat testis

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While it is known that several cancer chemotherapeutic regimens containing the *Vinca* alkaloid vincristine bring about abnormalities in male reproduction, like azoospermia, gynaecomastia, etc., the precise mode of toxicity of vincristine is not known. We administered vincristine sulphate to sexually mature Wistar strain male albino rats, and show that vincristine causes absence of sperm in seminiferous tubules, depletion of germinal epithelial elements, and formation of hypertypic giant cells. We discuss the origin of the giant cells in the light of the antimitotic activity of vincristine, and suggest that vincristine affects spermatogonial mitosis, consequent upon which there is endomitosis, resulting in giant cells.

COMBINATION chemotherapy is one of the most advocated therapeutics for cancer. MOPP (mustine, oncovin, procarbazine and prednisone)[1], COPP (cyclophosphamide, oncovin, procarbazine and prednisone)[2], MOMP (mustine, oncovin, methotrexate and prednisone)[3], CVP (cyclophosphamide, vincristine and prednisone)[4], MVPP (mustine, vinblastine, procarbazine and prednisone)[5], CMM (cyclophosphamide, methotrexate and mercaptopurine)[6] and MCCB (mustine, cyclophosphamide, chlorambucil and busulphan)[7], are examples of combination cancer chemotherapeutic regimens. Most of these drugs are by nature cytotoxic; they are either alkylating agents, antimetabolites, antibiotics or mitotic spindle poisons[8]. There have also been reports implicating these regimens in several side-effects like nausea and vomiting, leukopenia, alopecia, stomatitis, peripheral neuropathy, cardiopathy, hepatocellular damage and pulmonary fibrosis[9]. Male gonadal dysfunction including azoospermia, oligospermia, gynaecomastia and germinal aplasia, probably culminating in male sterility[3,10–14], has also been reported.

Vincristine is advocated as one of the drugs in combination chemotherapy[1–4]. It is established as a mitotic-spindle poison and is believed to prevent cancer growth by arresting mitotic-spindle formation through prevention of tubulin polymerization and disruption of microtubules. Use of such spindle poisons in cancer chemotherapy is also likely to affect other dividing cells, including those connected with spermatogenesis[15]. While alkylating agents, antimetabolites and antibiotics—chlorambucil[16], cyclophosphamide[17], prednisone, methotrexate, 5-fluorouracil, mitomycin C, actinomycin D, procarbazine[18] and cisplatin[19]—, when administered in cancer chemotherapy, have been shown to lead to testicular atrophy, azoospermia, germinal aplasia and sterility, the specific gonadal toxicity of spindle poisons like vincristine has not yet been studied. On the other hand, administration of total alkaloids of *Vinca rosea* (*Catharanthus roseus*) (West Indian periwinkle, Apocynaceae) to adult male rats and mice has been shown to bring about arrest of spermatogenesis, regression of Leydig cells, and derangements in sperm[20–24]. Vincristine being one of the binary indole-indolin alkaloids isolated from this plant, it is highly probable that this drug, when used in cancer treatment, might cause testicular derangements. In this paper we report that vincristine causes giant-cell formation in seminiferous tubules of rat.

We examined stained sections of testes from rats that had been given intraperitoneal injections of vincristine sulphate (see Figure 1). The typical histoarchitecture of seminiferous tubules of normal rats show cells arranged in spermatogenic sequence (Figure 1,*a*). In rats treated with 10 or 20 μg (per animal) of vincristine, the seminiferous tubules were thoroughly disorganized, highly regressed, and contained far fewer layers of cells (Figure 2,*b*). Meiotic elements were never seen in the cells. Most of the tubules contained hypertypic giant cells of different sizes along the border of the widened lumen as well as lying free in the lumen (Figure 1,*c*). There were a few tubules in which the size of the germinal epithelial cells increased towards the luminal border (Figure 1,*d*), in contrast to the decrease in size of

cells in tubules of normal testis. There was no trace of necrosis of the germinal epithelium or cytolysis of normal or giant cells in the lumen.

Formation of giant cells has never been reported in normal testis. Multinucleated and uninucleated hypertypic giant cells have been reported in the testis of rodents, monkey and man exposed to irradiation[25-27], rats subjected to hyperthermia[28-29], rats and mice treated with pesticides[30-32] and mice treated with cimetidine[33]. As regards the formation of multinucleated giant cells in the testis, three explanations have been offered: (i) coalescence of spermatids, either in Golgi phase or cap phase[29]; (ii) multiple nuclear division in the absence of cell cleavage[27,34]; and (iii) marcophages swallowing young spermatids[25]. Origin of hypertypic giant cells (large cells with a single large nucleus) in testis has been attributed to failure of pachytene spermatocytes to differentiate further and their subsequent hypertrophy[29]. The giant cells noticed in the present study are clearly hypertypic and not multinucleated. The histological picture suggests that meiotic division was not initiated after treatment with vincristine. This is inferred from the increase in the size of the cells from the tunica towards the lumen and the absence of meiotic figures. The particular appearance of the nucleus of the giant cells is indicative of endomitosis. It is pertinent to point out in this context the proposed possible anticancer action of vincristine. The drug is supposed to substoichiometrically bind to tubulin at the high-affinity binding site and thereby prevent tubulin polymerization and mitotic-spindle formation; contrarily it renders the tubulin monomers form into tubulin crystals and acts on the microtubule-associated protein, converting microtubules into stable spiral structures, without affecting chromosome duplication[15,35]. Spermatogenic cells engage in two phases of division, mitosis during the proliferative phase and meiosis during the chromosome reduction phase. Our observations suggest that vincristine affects spindle formation during spermatogenic mitosis, leading to repeated duplication of chromosomes without the nucleus or cell actually dividing.

Thus it is apparent that vincristine, as much as preventing mitosis in cancerous cells, can exert a similar influence on other dividing cells[15], including those of the seminiferous epithelium. The finding also offers a possible explanation for the infertility reported in cancer patients[14] who are treated with chemotherapeutic regimens containing vincristine. In fact it would be surprising to find drugs that affect cell division not affecting spermatogenesis.

1. Devita, V. T. Jr., Lewis, B. J., Rozenweig, M. and Muggia, F. M., *Cancer*, 1978, **42**, 979.
2. Morgenfeld, M. C., Pavlosky, A., Suarez, A. *et al.*, *Cancer*, 1975, **36**, 1241.
3. Sherins, R. J., Olweny, C. L. M. and Ziegler, J. L., *New Eng. J. Med.*, 1978, **299**, 12.
4. Bagley, C. M. Jr., Devita, V. T. Jr., Bearad, C. W. *et al.*, *Ann. Intern. Med.*, 1972, **72**, 227.
5. Whitehead, E., Shalet, S. M., Blackedge, G., et *al.*, *Cancer*, 1982, **49**, 418.
6. Hinkes, E. and Plotkin, D., *J. Am. Med. Assoc.*, 1973, **222**, 1490.
7. Roeser, H. D., Stocks, A. E. and Smith, A. J., *Aust. N. Z. J. Med.*, 1978, **8**, 250.
8. Curie, G. and Curie, A., *Cancer: the Biology of Malignant Disease*, Edward Arnold, 1982.
9. Perry, M. C., *Semin. Oncol.*, 1982, **9**, 1.
10. Sherins, R. J. and Devita, V. T., *Ann. Intern. Med.*, 1973, **79**, 216.
11. Asbjornsen, G., Mopne, K., Klepp, O. and Aakvaag, A., *Scand. J. Haematol.*, 1976, **16**, 66.
12. Olweny, M., Katongle-Mbidde, Kilre, *et al.*, *Cancer*, 1978, **42**, 787.
13. Chapman, R. M., Sutcliffe, S. B., Ress, L., *et al.*, *Lancet*, 1979, **1**, 285.
14. Charak, B. S., Gupta, R., Mandrekar, P., *et al.*, *Med. Ped. Oncol.*, 1990, **4**, 263.
15. Dustin, P., *Microtubules*, Springer-Verlag, New York, 1984.
16. Miller, D. G., *J. Am. Med. Assoc.*, 1971, **217**, 1662.
17. Kumar, R., Biggart, J. D., McEnvoy, J. and McGedown, M. G., *Lancet*, 1972, **1**, 1212.
18. Meistrich, M. L., Marcia, F., da Cunha, M. F., *et al.*, *Cancer Res.*, 1982, **42**, 122.
19. Oshio, S., Tomomasa, H., Amemiya, H., *et al.*, *Arch. Androl.*, 1990, **24**, 113.
20. Joshi, M. S. and Ambaye, R. Y., *Indian J. Exp. Biol.*, 1968, **6**, 256.
21. Lambert, B. and Ericksson, S., *Mutat. Res.*, 1979, **68**, 275.
22. Chinoy, N. J. and Geetha Ranga, M., *Comp. Physiol. Ecol.*, 1983, **8**, 41.
23. Murugavel, T., Ruknudin, A., Thangavelu, S. and Akbarsha, M. A., *Curr. Sci.*, 1989, **58**, 1102.
24. Murugavel, T. and Akbarsha, M. A., *Indian J. Exp. Biol.*, 1991, **29**, 810.
25. Amoroso, E. C., in *Radiation Effects in Physiochemistry and Biology* (eds. Evert, M. and Howard, A.), North-Holland, Amsterdam, 1963.
26. Lacy, D., in *Effects of Ionizing Radiation on Reproductive System* (eds. Carlson, W. P. and Gassner, F. X.), Pergamon, London, 1964.
27. Rao, A. R. and Srivatsava, P. N., *Experientia*, 1967, **23**, 381.
28. Colins, P. and Lacy, D., *Proc. R. Soc. Biol.*, 1969, **172**, 17.
29. Kanwar, K. C., Bawa, S. R. and Singal, P. K., *Fertil. Steril.*, 1971, **22**, 11.
30. Dikshith, T. S. S. and Datta, K. K., *Acta Pharmacol. Toxicol.*, 1971, 20.
31. Dikshith, T. S. S. and Datta, K. K., *Exp. Pathol.*, 1972, **7**, 309.
32. Nigam, S. K., Larrad, B. C., Karnik, A. B., *et al.*, *Bull. Environ. Contam. Toxicol.*, 1979, **23**, 431.
33. Biswas, S., Sareen, M. L. and Gill, A., *Curr. Sci.*, 1990, **59**, 432.
34. Montgomery, P. O. B., Karney, D., Reynalds, R. C. and McClendon, D., *Am. J. Pathol.*, 1964, **44**, 727.
35. Donoso, A. J., Haskins, K. M. and Himes, R. H., *Cancer Res.*, 1979, **39**, 1604.
36. Humason, G. L., *Animal Tissue Techniques*, W. H. Freeman & Co, Sanfrancisco, 1979.

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