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EFFECT OF VINBLASTINE SULPHATE ON SISTER CHROMATID EXCHANGES IN HUMAN CHROMOSOMES

RACHEL THOMAS-JACOB and O. S. REDDI
Institute of Genetics and Hospital for Genetic Diseases, Osmania University, Hyderabad 500 016, India.

IN recent years, the reciprocal exchanges between sister chromatids occurring in somatic cells have been the subject of intensive study, particularly in human cells. The incorporation of bromodeoxyuridine results in differential staining of the sister chromatids after two cell cycles[1,2]. In the present communication, we report our observations on sister chromatid exchanges [SCE] frequency with vinblastine sulphate, an anti-neoplastic agent used in the treatment of a wide variety of carcinomas.

Human lymphocyte cultures were set up with peripheral blood samples obtained from ten healthy donors following the method of Moorhead *et al*[3]. 5-Bromodeoxyuridine (BrdU) (0.1 ml) at a final concentration of 30 μg/ml was added after 24 hr and all cultures were maintained in the dark to avoid photolysis. Vinblastine sulphate (VLB) (Sigma) was added to the cultures under dim light, 24 hr before termination at concentrations of 1.25, 2.50, 3.75 and 5 μg/ml respectively. Air-dried preparations of the chromosomes were further processed for SCE studies after a minimum period of three days by the method of Perry and Wolff[2]. The number of SCEs/cell was counted in randomly selected, well scattered metaphase plates.

Table 1 shows the frequency of SCEs *in vitro* in PHA stimulated lymphocytes treated with VLB. A concentration-dependent increase in the frequency of SCEs was observed in VLB-treated cells.

VLB produced a dose- and time-dependent increase in various chromosomal aberrations, including chromatid breaks and SCEs in Don lung cells from Chinese hamsters[4]. One large dose of cyclophosphamide (1000 mg) and vinblastine (10 mg) administered at weekly intervals resulted in a dramatic increase in the number of SCEs/cell. The frequency of SCEs returned to the initial level about ten days after termination of treatment[5]. Kucerova and Polivkova[6] using the fluorescence plus Giemsa technique noted that at 10^{-7} mg/ml of VLB, the frequency of SCEs in the cultured lymphocytes did not increase.

There are indications that a close relationship may exist between DNA synthesis, DNA repair processes and SCE[7–11]. The frequency of SCEs may be increased by mutagenic agents, either because of impaired DNA repair function or because the extent of DNA damage (*e.g.* cross-links) exceeds the capacity of the repair enzymes to remove them from DNA during subsequent cell cycles. The increase in the number of SCEs in VLB-treated cells may be due to the inhibition of DNA replication by VLB.

Financial assistance received from CSIR, New Delhi, is gratefully acknowledged.

1 December 1983; Revised 13 January 1984

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Table 1 *Frequency of sister chromatid exchanges in PHA stimulated lymphocytes after treatment with vinblastine sulfate*

Cytostatic drug	Concentration (μg/ml)	No. of cells observed	No. of SCEs observed	Mean SCEs/cell (Mean ± S.E.)
Control (only BrdU)	30.0	500	5250	10.5 ± 0.3
Vinblastine Sulphate	1.25	50	1110	22.2 ± 0.3
	2.50	50	1170	23.4 ± 0.3
	3.75	50	1210	24.2 ± 0.2
	5.00	50	1250	25.0 ± 0.2

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PROSPECTIVE TREATMENT FOR *MYCOBACTERIUM ULCERANS* INFECTION (BURULI ULCER) IN RAT AND MICE THROUGH COMBINED THERAPY WITH RIFAMPICIN AND SEPTRAN

A. SRIVASTAVA, N. B. SINGH and S. K. GUPTA
Division of Microbiology, Central Drug Research Institute, Lucknow 226 001, India.

'BURULI ULCER'/Bairnsdale ulcer of man was described in 1948[1], but the effective treatment against this infection even today, is far from perfection. Some success has been obtained when the treatment is started early, otherwise the mainstay of treatment is surgery—excision or curettage with limited success[2]. The organism is reported to be resistant to many of the antituberculars[3]. We report here a combination therapy with rifampicin and septran which has arrested the pathogenicity of the organism in both rats and mice.

Random bred animals were used in the study. Duckrey rats weighing between 40–60 g were divided into 8 groups (table 1). The left hind footpad of each animal was inoculated with 1 mg (approx. 2.3×10^8 cfu) of *Mycobacterium ulcerans* in 0.03 ml of diluent (M/15 disodium hydrogen phosphate pH 8.7). Treatment was started after 54th day of infection and 6 injections per week were given. In all, 53 injections were given rifampicin dose was kept constant at 3 mg/kg but the dose of septran was kept varying at 10, 20 and 30 mg/kg alone and the same dosage in combination with rifampicin (table 1). The drugs were emulsified in 1 drop of tween 80 in saline and were given intraperitoneally. The criterion of effectiveness of the drug dose was reduction in foot pad inflammation after treatment since *M. ulcerans* produces severe inflammation of the foot pad. The thickness of both normal and the infected foot pads was measured weekly by means of Vernier Calipers and the difference in thickness between infected and normal foot pad actually represented the degree of inflammation.

In a second experiment the combination of septran and rifampicin was tested in mice. Park strain of mice bred randomly weighing 20 g ± 2 from CDRI animal house were taken for the study.

They were divided into ten groups (table 2). The dose of septran was kept constant at 30 mg/kg level and the dosages of rifampicin were varied at 2.5, 5, 10 and 15 mg/kg. The route of administration of the drugs was subcutaneous in this case. Mice were infected intravenously with 1 mg of *M. ulcerans* (approx. 2.3×10^8 cfu). Treatment with the drugs started from the day of infection and 6 injections per

Table 1 *Combined effect of rifampicin and septran against Mycobacterium ulcerans infection in rat foot pad model.*

Treatment	Dose mg/kg	Footpad inflammation in cm			Percent decrease in footpad inflammation
		9th week	11th week	14th week	
Untreated control	Nil	0.17 ± 0.01	0.13 ± 0.01	0.14 ± 0.02	18
Septran	10	0.15 ± 0.03	0.11 ± 0.05	0.09 ± 0.01	40
Septran	20	0.18 ± 0.06	0.15 ± 0.04	0.08 ± 0.01	56
Septran	30	0.18 ± 0.03	0.12 ± 0.03	0.08 ± 0.01	56
Rifampicin	3	0.17 ± 0.04	0.12 ± 0.02	0.06 ± 0.01	62
Septran + Rifampicin	10 + 3	0.31 ± 0.09	0.11 ± 0.04	0.14 ± 0.05	53
Septran + Rifampicin	20 + 3	0.32 ± 0.11	0.12 ± 0.03	0.06 ± 0.02	81
Septran + Rifampicin	30 + 3	0.31 ± 0.10	0.13 ± 0.03	0.06 ± 0.02	80