Vincristine neuropathy : an electrophysiological and histological study

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Vincristine is an alkaloid derived from the periwinkle plant, *Vinca rosea*. It is a drug which is used in the treatment of acute leukaemia, lymphomas, and some other malignancies. Therapeutic doses of the drug nearly always cause peripheral neuropathy, the earliest clinical manifestations of which are paraesthesiae in the extremities and depression of the deep tendon reflexes; other toxic side-effects are alopecia, abdominal pain, constipation, and convulsions (Selawry and Hananian, 1963; Carbone, Bono, Frei, and Brindley, 1963; Karon and colleagues, 1966).

In order to study the characteristics and the natural course of the neuropathy caused by the drug, we performed serial motor and sensory nerve conduction studies on a group of patients treated with vincristine and studied the histological changes in the sural nerves. It is concluded that impairment of motor and sensory conduction results chiefly from axonal degeneration of nerve fibres, some of which sub-

sequently regenerate. A brief account of the work has been published previously (McLeod and Penny, 1968).

METHODS

Nine patients who had been treated with vincristine were studied. Vincristine was administered intravenously in courses which consisted of several weekly doses of 0.02 to 0.05 mg/kg of the drug; in no case were more than five successive doses of the drug given during a course of therapy. The clinical diagnoses and total dosage of vincristine given to each patient are shown in Table I.

ELECTROPHYSIOLOGICAL TECHNIQUES Motor conduction velocities were determined in the median, ulnar, and lateral popliteal nerves by means of standard techniques, using surface electrodes.

Sensory action potentials were recorded with surface electrodes from the median and ulnar nerves at the wrist on stimulating the index and little fingers respectively

TABLE I
SUMMARY OF DETAILS OF TREATMENT

	Patient	Sex and age (yr)	Diagnosis	Previous drug therapy	Vincristine		Side-effects of vincristine
					Course	Total dose (mg)	
1.	W.B.	M20	Acute myeloblastic leukaemia	6-Mercaptopurine, prednisone, methotrexate	1st 2nd	15.5 5.0	Paraesthesiae, abdominal pain, alopecia, loss of ankle jerks
2.	I.H.	F43	Acute myeloblastic leukaemia	6-Mercaptopurine, prednisone, methotrexate		7.0	Paraesthesiae, abdominal pain, alopecia, loss of ankle jerks
3.	E.D.	F43	Acute myeloblastic leukaemia	Prednisone, 6-mercaptopurine, methotrexate		7.5	Paraesthesiae, abdominal pain, loss of ankle jerks
4.	N.D.	M18	Acute lymphoblastic leukaemia	Prednisone, 6-mercaptopurine, methotrexate	1st 2nd	10.0 12.5	Paraesthesiae, loss of ankle jerks
5.	D.C.	M18	Acute lymphoblastic leukaemia	Prednisone, 6-mercaptopurine, methotrexate	1st 2nd	10.0 7.5	Paraesthesiae, abdominal pain, loss of ankle jerks
6.	W.W.	F79	Acute on chronic lymphatic leukaemia	Prednisone		9.0	Paraesthesiae, loss of ankle jerks
7.	A.S.	M29	Hodgkin's disease	Nitrogen mustard Chlorambucil, vinblastine, methyl hydrazine, prednisone		6.4	Paraesthesiae, abdominal pain, loss of ankle jerks
8.	J.H.	M74	Reticulum cell sarcoma	Chlorambucil		4.75	Loss of ankle jerks
9.	A.N.	M57	Follicular lymphoblastoma	Chlorambucil, prednisone		10.0	Paraesthesiae, abdominal pain, postural hypotension, loss of ankle jerks

through ring electrodes (Dawson, 1956). The mixed motor and sensory nerve action potential was recorded with surface electrodes from the ulnar nerve above the level of the elbow on stimulating at the wrist (Gilliatt and Sears, 1958), and the mixed nerve action potential was recorded from the lateral popliteal nerve by means of the technique described by Gilliatt, Goodman, and Willison (1961). The H reflex was recorded from the calf muscles after stimulation of the medial popliteal nerve in the popliteal fossa (Mayer and Mawdsley, 1965).

The electrical stimulus was a square wave of duration 0.2 msec derived from a Disa Ministim. The recording electrodes were connected to a Tektronix FM122 pre-amplifier and displayed on the upper beam of a Tektronix 502A oscilloscope; a time scale derived from a Digitimer (Devices Ltd.), was displayed on the lower beam. Photographic records were made on 35 mm film.

The skin temperature of the upper and lower limbs was measured at every examination with a thermistor and ranged from 32° to 35°C.

HISTOLOGICAL TECHNIQUES Using a technique similar to that described by Dyck and Lofgren (1966), a fascicular biopsy of the sural nerve was performed on one patient (case 4). A portion of sural nerve was obtained at necropsy from two other patients (cases 1 and 9), and from one control subject. The nerves were divided into two portions each about 1 cm in length, and the segments splinted on to cards. One segment was fixed immediately in Flemming's solution for 24 to 36 hours, dehydrated in alcohol, embedded in paraffin wax, and cut transversely in serial sections of 5 μ thickness. The sections were stained with Kultschitsky's haematoxylin and counterstained with van Gieson's stain (Gutmann and Sanders, 1943). They were then dehydrated, cleared, and mounted. Photomicrographs were made of selected fascicles and printed on photographic paper at an enlargement of $\times 1,000$. The total number of myelinated fibres in each fascicle was counted and the external diameter of each myelinated fibre was measured with a rule. The area of each fascicle was measured with a planimeter and the fibre density was calculated as the number of fibres/sq. mm of intraperineurial area (Swallow, 1966). Histograms of fibre size were constructed according to the methods of Swallow (1966) and Fullerton and O'Sullivan (1968). Fibres of external diameter of 2 μ or less were grouped together, and larger fibres were subdivided into 1 μ groups.

The second segment of each nerve was fixed in 10% formol-saline. It was subsequently stained for 24 hours in 1% osmic acid, macerated in glycerol, and teased apart under a dissecting microscope in order to isolate single nerve fibres which were mounted on slides (Thomas, 1955). Measurements were made of internodal length and diameter of the individual fibres (Vizoso and Young, 1948), and the results plotted in the manner described by Fullerton, Gilliatt, Lascelles, and Morgan-Hughes (1965).

RESULTS

CLINICAL MANIFESTATIONS OF VINCRISTINE NEUROTOXICITY Of the nine patients examined, eight

reported sensations of tingling and numbness in the fingers and toes after one to three doses of the drug; six patients experienced abdominal pain and constipation; one patient developed severe postural hypotension. In all patients, the ankle jerks were absent after completion of the course of vincristine (Table I), but no other abnormal neurological signs were noted.

ELECTROPHYSIOLOGICAL STUDIES In all but one patient (case 9) the progress of the peripheral neuropathy caused by vincristine was followed by performing electrophysiological studies before the commencement of the drug and at intervals during and after the course of treatment. The results are summarized in Fig. 1 and in Table II. Although vincristine caused changes in motor conduction and in the amplitudes and latencies of sensory action potentials, the changes were not significant ($P > 0.05$, t test). However, in individual cases it was observed that during a course of treatment progressive slowing of motor conduction and reduction in the amplitude of the sensory action potentials occurred and continued after cessation of therapy (Figs. 2, 3). In the case of one patient who was studied for a period of 16 months (case 4, N.D.), it was observed that some recovery in sensory conduction had taken place eight months after completion of a course of vincristine; a second course of therapy temporarily reversed the improvement (Fig. 3).

A significant reduction occurred after treatment with vincristine in the amplitudes of the mixed nerve action potentials recorded from the ulnar and lateral popliteal nerves (Table II).

Ankle jerks could not be elicited in any of the nine patients immediately after completion of a course of treatment with vincristine, but in all except one patient (case 1) an H reflex was recorded at a time when the deep tendon reflexes were absent. The amplitude of the maximum H response in the eight patients ranged from 0.40 mV to 10.3 mV and the latency ranged from 26 msec to 35 msec.

HISTOLOGICAL STUDIES Fibre densities of the sural nerves of three patients treated with vincristine and of one control subject were calculated. The control subject was a female aged 48 with no clinical evidence of neurological disease who died of myocardial infarction. The fibre densities of the sural nerves of the three patients were 3.63 thousand fibres/sq. mm (case 1), 4.13 thousand fibres/sq. mm (case 4), and 2.97 thousand fibres/sq. mm (case 9). The fibre density of the sural nerve from the control subject was 6.12 thousand fibres/sq. mm. These figures may be compared with the mean fibre density and standard deviation of 6.13 ± 1.11 and 5.78 ± 0.90

FIG. 1. Motor conduction velocity, amplitude of sensory action potentials, and amplitude of mixed nerve action potentials in patients before (A) and after (B) treatment with vincristine. Horizontal bars represent mean figure for each group.

thousand fibres/sq. mm given by O'Sullivan and Swallow (1968) for the human sural nerve in the 17 to 39 and 40 to 59 age groups respectively. A

FIG. 2. W.B. (case 1). Effect of vincristine therapy on motor conduction velocity in median, ulnar and lateral popliteal nerves (A) and on amplitude of lateral popliteal nerve action potential (B). Solid black bars represent duration of vincristine therapy; total dosage of vincristine is indicated above each bar.

portion of the transverse section of the control nerve and one of the pathological nerves is shown in Fig. 4.

Histograms of the fibre diameters of the sural nerves of the three patients and of the control subject were constructed. It can be seen from Fig. 5 that there is a loss of both large and small diameter fibres. The most severe damage to large diameter fibres occurred in case 1, in whom there was progressive slowing of motor conduction (Fig. 2) and in whom an H reflex could not be recorded.

Single fibres teased from the sural nerve of one patient (case 4, N.D.) five weeks after completion of a second course of vincristine were seen to be undergoing active Wallerian degeneration (Fig. 6a and b). A few fibres were undergoing remyelination, in some of which there was marked variability of internodal length (Fig. 7a).

Single fibres were also teased from the nerve of another patient (case 9, A.N.) eight months after a course of vincristine had been given. Numerous

FIG. 3. N.D. (case 4). Effect of vincristine therapy on amplitudes of sensory action potentials of median and ulnar nerves. Solid black bars represent duration of vincristine therapy; total dosage of vincristine is indicated above each bar.

fibres were seen to be undergoing remyelination, and in a few fibres there were the pathological changes associated with segmental demyelination (Fig. 6c, d). In some regenerating fibres there was a pronounced variability of internodal length; in other fibres there were abnormally short internodes such as are seen to occur in fibres regenerating after Wallerian degeneration (Vizoso and Young, 1948) (Fig. 7b). Fibres teased from the nerve of case 1 were not undergoing active degeneration, but a number of them showed the pathological characteristics of remyelination.

DISCUSSION

Clinical manifestations of peripheral neuropathy are a very common side-effect of vincristine therapy (Selawry and Hananian, 1963; Carbone *et al.*, 1963; Warot, Goudemand, and Habay, 1965; Karon *et al.*, 1966). Since the toxicity of vincristine is dose related (Carbone *et al.*, 1963) an attempt was made in the present study to prevent the occurrence of severe neuropathy by employing an intravenous dosage of vincristine of 0.05 mg/kg weekly and by limiting the course of therapy to four or five injections

TABLE II
RESULTS OF NERVE CONDUCTION STUDIES ON EIGHT PATIENTS
BEFORE AND AFTER TREATMENT WITH VINCRIStINE

	Motor conduction velocity			Sensory action potential				Nerve action potential			
	Median nerve	Ulnar nerve	Lateral popliteal nerve	Median nerve		Ulnar nerve		Ulnar nerve		Lateral popliteal nerve	
	(m/sec)	(m/sec)	(m/sec)	Latency (msec)	Amplitude (μV)	Latency (msec)	Amplitude (μV)	Latency (msec)	Amplitude (μV)	Latency (msec)	Amplitude (μV)
Before treatment (Mean ± S.D.)	58.6 ± 4.6	57.3 ± 5.2	46.1 ± 7.8	3.2 ± 0.3	14.3 ± 4.7	2.5 ± 0.4	10.4 ± 2.6	5.6 ± 0.8	31.8 ± 10.4	6.6 ± 0.6	4.3 ± 3.9
After treatment (Mean ± S.D.)	56.6 ± 4.7	53.8 ± 4.9	43.3 ± 3.5	3.4 ± 0.4	9.9 ± 2.5	2.7 ± 0.4	7.3 ± 3.8	6.0 ± 0.8	19.0 ± 5.3	6.8 (1)	0.4 ± 1.0
Significance of difference (Student <i>t</i> test)*	<i>P</i> > 0.05	<i>P</i> > 0.05	<i>P</i> > 0.05	<i>P</i> > 0.05	<i>P</i> > 0.05	<i>P</i> > 0.05	<i>P</i> > 0.05	<i>P</i> > 0.05	<i>P</i> < 0.001		<i>P</i> < 0.001

**P* > 0.05 is not significant.

P < 0.001 is highly significant.

FIG. 4. Portions of transverse sections of sural nerves from: (a) control and from (b) N.D. (case 4). Flemming—Kultschitsky—van Gieson. Scale 50 μ .

FIG. 5. Distribution of diameters of myelinated fibres in sural nerves of control subject, case 4, case 9, and case 1.

of the drug. Although all patients experienced paraesthesiae and developed impairment of deep tendon reflexes, the only patient who was seriously handicapped by the neurotoxic effects of the drug was one who developed postural hypotension.

In the majority of patients, only minor degrees of slowing of motor conduction resulted from the administration of a single course of the drug. These findings are consistent with the observations of other workers (Hildebrand and Coërs, 1965; Tobin and Sandler, 1966). The amplitudes of the median and ulnar nerve sensory action potentials were not significantly reduced after a course of vincristine. The most pronounced electrophysiological changes occurred in the amplitude of the mixed motor and sensory nerve action potentials of the ulnar and lateral popliteal nerves. These findings suggest that, although motor conduction velocities remained within the normal range, a significant number of motor fibres were nevertheless affected by the drug. The results of repeated conduction studies over prolonged periods of time on motor and sensory nerves of patients with vincristine neuropathy do not appear to have been previously reported. The results of the present investigation indicate that the neuropathy progresses in severity after cessation of a course of vincristine, but that after several months some recovery occurs; such findings are consistent with clinical observations (Karon *et al.*, 1966).

Since the histological techniques which were used for fixation and staining of sural nerves are similar to those employed by O'Sullivan and Swallow (1968),

the fibre density and fibre diameter distribution of the sural nerves in the present study may be compared with their published data for control nerves. The fibre density of the control nerve was close to their mean value, but the fibre density of the pathological nerves was reduced. Histograms of fibre diameter distribution in the nerves obtained from patients treated with vincristine demonstrated a reduction in the density of fibres, of both large and small diameter. The damage caused by vincristine to fibres of all diameters may be contrasted with the selective loss of large fibres which has been reported to occur in thalidomide neuropathy (Fullerton and O'Sullivan, 1968).

The most pronounced pathological change in the fibres teased from sural nerves of the three patients treated with vincristine was that of axonal or Wallerian degeneration. Similar observations have been made by other workers in experimental animals (Uy, Moen, Johns, and Owens, 1967; Gottschalk, Dyck, and Kiely, 1968) and in man (Moress, D'Agostino, and Jarcho, 1967; Gottschalk *et al.*, 1968). A number of fibres in the nerves were seen to be regenerating after damage caused by the drug. Although the majority of abnormal fibres showed the pathological changes of axonal degeneration, a few fibres in all the nerves were seen to have undergone segmental demyelination; the marked variability of internodal length in some regenerating fibres also indicated that segmental demyelination had been the primary pathological process (Fullerton *et al.*, 1965). Although peripheral neuropathy may

FIG. 6.
Teased single fibres stained with 1% osmic acid N.D. (case 4).
a, b. Fibres at different stages of axonal degeneration show segmentation and beading, and formation of ovoids and clumps of myelin. A.N. (case 9)
c. Fibre undergoing remyelination after segmental demyelination and d. Re-myelinating fibre. Arrows indicate sites of nodes of Ranvier.

FIG. 7. Distribution of internodal length in single fibres teased from sural nerves of (a) case 4 and (b) case 9. Lengths of internodes in individual fibres are plotted against diameter of widest internode, and joined by a vertical line.

be present in association with leukaemia and lymphomas (Williams, Diamond, Craver, and Parsons, 1959), the development of neuropathy in the patients studied in the present investigation was clearly related in time to the administration of vincristine.

Gilliat (1966) has emphasized that the pathological changes of segmental demyelination are often associated with pronounced degrees of slowing of conduction in peripheral nerves, whereas axonal degeneration may result in only minor abnormalities

of nerve conduction. The fact that gross slowing of conduction was not observed in any of our patients who had developed peripheral neuropathy following vincristine therapy is consistent with the pathological finding of axonal degeneration.

In eight of the nine patients studied, the H reflex was present at a time when the ankle jerk was absent. Tobin and Sandler (1966) reported similar findings, and deduced that during the early stages of vincristine neurotoxicity either the large diameter afferent fibres in the region of the muscle spindle or the small diameter fibres of the fusimotor system were selectively damaged. However, the histological studies on the sural nerves in the present work do not support their conclusions since both large and small diameter fibres are affected by vincristine, and the damage is not confined to the muscle spindle region. It is possible that the preservation of the H reflex at a time when the ankle jerk cannot be elicited may be explained by the occurrence of differing degrees of dispersion of the volleys in the two reflex arcs. It seems likely that a minor degree of damage to large fibres results in a greater degree of dispersion of the relatively asynchronous volley after a mechanical tap than that of the synchronous volley which follows the application of an electrical stimulus.

Experimental studies on rats indicate that vincristine acts primarily on the nerve cell body and that peripheral nerve degeneration is a secondary phenomenon (Uy *et al.*, 1967). The mode of action of the Vinca alkaloids is not certain, but they appear to inhibit cell division by interfering with the synthesis of nucleic acids (Richards, Jones, and Beer, 1966; Cline, 1968). The finding of segmental demyelination in a few nerve fibres in the present study suggests that vincristine interferes to some extent with the metabolism of the Schwann cell as well as that of the nerve cell.

SUMMARY

Electrophysiological studies have been performed on patients treated with the Vinca alkaloid, vincristine. The drug caused slowing of motor conduction and impairment of sensory conduction in peripheral nerves. In a number of patients the neuropathy progressed after cessation of therapy but in some cases recovery occurred. Histological studies on sural nerves indicated that fibres of large and small diameter were damaged by vincristine. The predominant pathological change in the fibres was that of axonal degeneration, although segmental demyelination also occurred. Regeneration of fibres occurred after treatment with vincristine was discontinued.

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