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Altered plasma creatine phosphokinase activity in vincristine-treated rats

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A side effect of vincristine, when used for treatment of a variety of human malignancies, is the development of muscle lesions which are secondary to nerve damage [1]. In rodents the myopathic features appear to be more severe [2], so that administration of vincristine to rats may be used to study the relationships between morphological and biochemical changes during the development of primary muscle lesions.

A previous study revealed biochemical changes in vincristine myopathy similar to those reported for several naturally occurring hereditary myopathies [3]. A prominent feature of some of these myopathies is a raised creatine phosphokinase activity (CPK; EC 2.7.3.27) in the blood. Consequently, we have measured plasma CPK activity (PCPK) in rats at various intervals following a single i.p. dose of vincristine at 0·6 mg/kg body wt.

Male Wistar rats (Carworth (Europe) Ltd.) weighing initially 275–310 g were injected with vincristine sulphate (Oncovin®, Eli Lilly) at a single i.p. dose of 0·6 mg/kg body wt. Control rats were given an equivalent volume of diluent (supplied by Eli Lilly to dilute vincristine) containing 0·9% NaCl and 0·9% benzyl chloride as a preservative. Although the vincristine treated animals were provided with Oxoid pellets and water *ad lib.* they lost weight during the course of the experiment, with mean losses as follows—Day 1: 12 g, Day 2: 20 g, Day 3: 25 g, Day 4: 33 g and Day 5: 32 g.

Blood was withdrawn at 1, 2, 3, 4 and 5 days after the vincristine injection from the heart under ether anaesthesia into a heparinized syringe and vials. The plasma was separated from the cells within 1 hr and stored at −20° for a maximum of 2 weeks prior to analysis. PCPK activity is stable under these conditions for at least 2 weeks [4].

Different rats were used for each time interval, and the complete experiment was repeated on 3 groups of control and 3 groups of treated animals except for the 5-day sample where 2 different groups of each were used. All injections and blood sampling were performed between 10 and 12 a.m.

PCPK was determined by the method of Worthy *et al.* [5] except that the recommended *p*-chloromercuribenzoate concentration was doubled, since we found that colour formation was erratic at the concentration recommended by these authors. The activity was calculated as international units/l. plasma.

Several workers have reported a non-linear activation of CPK activity with dilution [5, 6]. To minimize errors due to this dilution effect, all plasmas were diluted 1:5 with water prior to analysis. Although this would result in higher values for plasmas from the controls and an underestimation of the enzyme in plasmas with activity above the linear range of the method, we did this in an attempt to standardize comparisons between high and low values of PCPK activity.

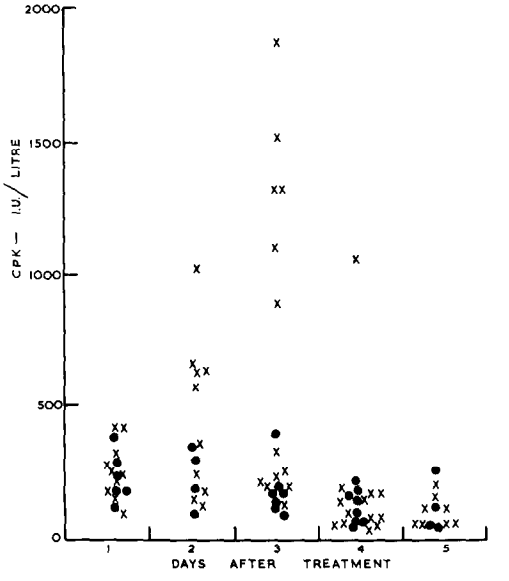

Fig. 1. Distribution of PCPK activities in control (●) and treated (×) rats following a single i.p. dose of vincristine at 0·6 mg/kg. body wt.

The Mann–Whitney *U*-test [7] was used for the statistical comparison of the results obtained 2 and 3 days following treatment. Because of the small number of control samples at the 2-day period, the control values from both these times were combined for this test.

To investigate possible changes in PCPK activity associated with weight loss, 20 control rats were fed a restricted diet so that their body wt at the time of blood sampling was similar to the treated animals, while 9 were fed *ad lib.* No significant difference in PCPK activity was observed between the two groups, the values obtained being 185 ± 100 I.U/l. and 137 ± 72 I.U/l. respectively ($P > 0·2$, Student's *t*-test).

Results are shown in Fig. 1. PCPK activities levels were significantly higher than control values at 2 days ($0·025 > P > 0·01$) and 3 days ($0·01 > P > 0·001$). At both

these intervals activity was increased in only 50 per dent of the animals. Several factors could contribute to this, e.g. (a) transient release of CPK from damaged muscle which is rapidly cleared from the plasma, (b) variable time responses in different animals, (c) defective absorption of drug from the peritoneal cavity.

The temporal development of PCPK changes in this study are similar to those of the muscle pathology as muscle necrosis is first observed 2 days after treatment [8]. Although, histologically only a few fibres showed changes, the release of CPK is sufficient to measurably increase the level in the plasma. The mechanism of vincristine myopathy is at present unknown, but because of the short time interval involved, the results suggest a myogenic rather than a neurogenic lesion. Findings supporting this view are the unchanged levels of PCPK up to two weeks in rats after peroneal or sciatic nerve section [9], where muscle atrophy is a continuous process, and also following a subcutaneous injection of the neuromuscular blocking agents *d*-tubocurarine and succinylcholine [10].

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Diabetogenic activity of deoxy-2-[[(ethylnitrosoamino) carbonyl]amino]-D-glucopyranose

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The diabetogenic agent streptozotocin, is composed of the cytotoxic moiety 1-methyl-1-nitrosourea attached to the carbon-2 position of glucose. In addition to producing a permanent diabetic state in animals, mediated through the specific destruction of the pancreatic beta cell [1], this compound has demonstrable clinical activity against human islet cell carcinomas [2]. The diabetogenic activity of streptozotocin has been correlated with an inhibition of nicotinamide adenine dinucleotide (NAD) synthesis in the pancreatic islets of Langerhans [3, 4] with subsequent beta cell necrosis. While all compounds having an $R—\overset{\overset{NO}{|}}{N}—(CH_2)_{1-2}H$ end group have been demonstrated to depress hepatic NAD concentrations, only streptozotocin has been shown to be diabetogenic [5]. To explore further these structure–activity relationships and the importance of the glucose carrier for diabetogenicity, the pharmacologic properties of deoxy-2-[[(ethylnitrosoamino)carbonyl]amino]-D-glucopyranose (DENU; Upjohn U-30,964, NSC-174793) [6], a glucose-containing nitrosourea, identical in structure to streptozotocin except for the presence of an ethyl end group, were studied.

Male Swiss mice weighing 17–26 g and maintained on Purina laboratory chow pellets and water *ad lib.* were used for all studies. DENU was dissolved in 0·005 M citrate buffer, pH 4·5, immediately prior to use; each dose was administered intravenously at a volume of 0·1 ml/10 g of body weight. Animals were fasted for 18 hr prior to drug administration. Control animals received equal volumes of the citrate buffer diluent.

Five days after drug administration, mice were sacrificed and plasma glucose [7] and immunoreactive insulin concentrations [8] were determined on blood obtained by car-

Table 1. Mean plasma glucose and immunoreactive insulin concentration 5 days after treatment with intravenous DENU administered at doses of 500–2500 mg/kg in Swiss mice

Dose (mg/kg)	Plasma glucose* (mg/100 ml)	P†	Plasma insulin* (μU/ml)	P†
Control	128		86	
500	132	>0·1		
1000	181	<0·05		
1500	174	<0·05		
2500	276	<0·01	21	<0·01

* Mean value for five mice.
† Compared to control.