Br. J. Cancer (1978) **37**, Suppl. III, 237

THE EFFECT OF THE RADIOSENSITIZER MISONIDAZOLE ON MOTOR NERVE CONDUCTION VELOCITY IN THE MOUSE

D. G. HIRST, B. VOJNOVIC, I. J. STRATFORD* AND E. L. TRAVIS

From the Gray Laboratory of the Cancer Research Campaign, Mount Vernon Hospital, Northwood, Middlesex, HA6 2RN

Summary.—The clinical use of misonidazole as a hypoxic cell radiosensitizer is at present limited by its neurotoxicity at high doses (Urtasun *et al.*, 1977; Dische *et al.*, 1977). An *in vivo* neurological end point, *viz.* measurement of nerve conduction velocity, has been developed to examine sensitizer action. Conduction velocity in mice was measured as a function of time after a single dose of misonidazole and as a function of drug dose. Doses greater than 0·33 mg/g produced significant transient reductions in velocity. The time course of the reduction in velocity closely followed the uptake/excretion profile of misonidazole from blood serum.

THE *in vitro* sensitizing efficiency of many nitroaromatic compounds has been correlated with reduction potential (Adams *et al.*, 1976*a*). Also, chronic aerobic cytotoxicity shows a similar correlation (Adams *et al.*, 1976*b*). *In vivo*, reduction potential will certainly play the dominant role in determining radiosensitizing efficiency. However, other parameters, such as lipophilicity, which determines the rate of uptake and excretion of a compound might profoundly influence the chronic effects on the host. Therefore, in the development and evaluation of potential sensitizers for clinical use further *in vitro* and *in vivo* end-points are required to determine the efficiency of any particular compound.

Clinical trials being conducted on the radiosensitizer misonidazole report an enhancement of tumour radiation response which corresponds well with predictions from *in vitro* data (Dische *et al.*, 1977). However, the dosage of drug which can be administered and, therefore, the enhancement achieved is limited by the neurological effects of the drug in man. This limitation has stimulated the development of an *in vivo* neurological assay for investigating sensitizer action in an experimental animal. Changes in nerve conduction velocity (NCV) were investigated as an index of the neurotoxic action of misonidazole.

MATERIALS AND METHODS

The sciatic nerve and soleus muscle of 6-week-old male CBA mice were exposed under penthrane anaesthesia and NCV in the sciatic nerve was determined using the soleus muscle as a detector of nerve impulses. The nerve was stimulated sequentially at two different locations and the muscle responses as well as the distance between the stimulation points (~10 mm) were recorded. This distance divided by the differential stimulus-response time delay gave a value for the conduction velocity of the nerve. This technique eliminated delays in the muscle and in the neuromuscular junction. Precise location of the stimulation points was obtained by using drawn glass micropipettes, filled with potassium chloride solution and with tip diameters of the order of 2 μm. The resistance of the electrodes was 1–3 MΩ; the capacitance at the electrode tip was approximately 10 pF. The electrode thus acted like a low pass filter with a time constant of approximately 20 μs.

* Present Address: Physics Division, Institute of Cancer Research, Royal Marsden Hospital, Sutton, Surrey.

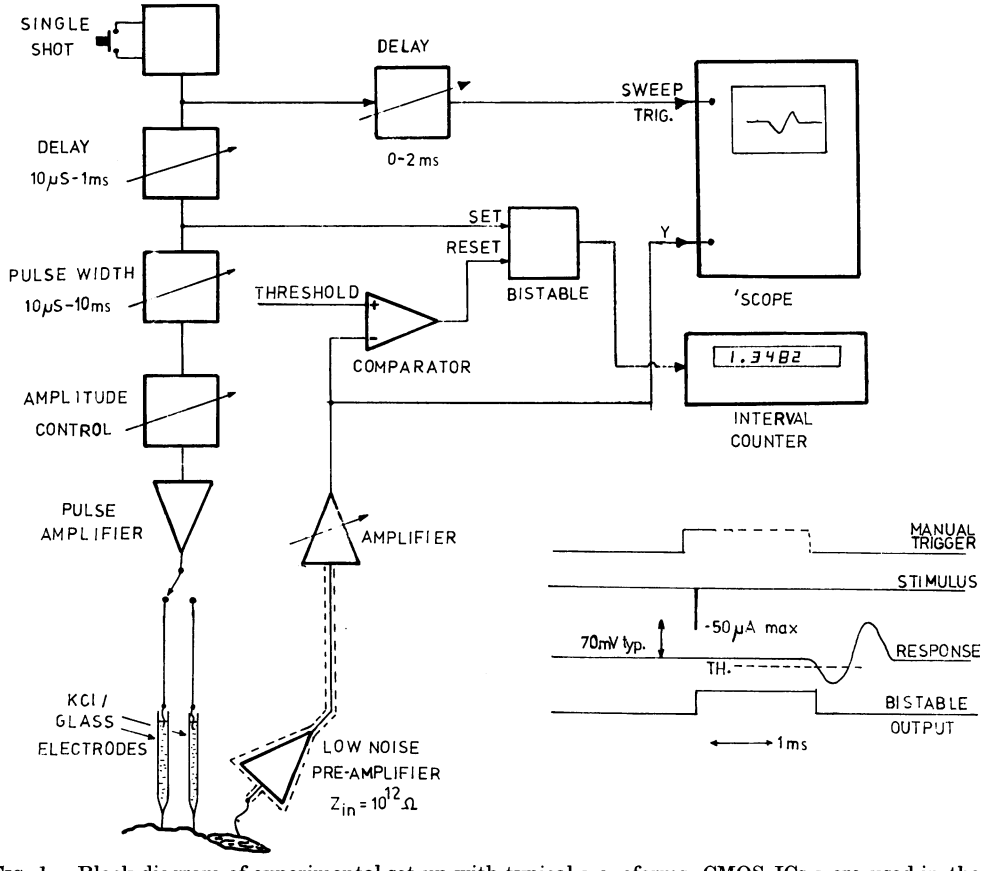

FIG. 1.—Block diagram of experimental set-up with typical waveforms. CMOS ICs were used in the timing sections. The signal amplifier has a variable gain (10–1000). The comparator switches when a threshold level is exceeded and resets a bistable circuit to obtain the stimulus response delay.

Pulse widths of 20–30 μs were used so that the maximum charge deposited on to the nerve fibre was 1·5 nC. High-amplitude short pulses were used to reduce any second order errors which might dominate when measuring differential time delays of 200 μs. The recording of the muscle response was achieved by detecting the local muscle action potential with a platinum electrode. Changes in this potential were fed to a high-input impedance ($>10^{12}\Omega$) F.E.T. low-noise pre-amplifier situated close to the recording electrode. Both stimulation and recording were performed using an unbalanced circuit configuration with the surrounding tissue and body fluid earthed. The remainder of the equipment consisted of battery-operated electronics for the generation of the stimulus and the processing of the response signal (Fig. 1). The response was recorded on a storage oscilloscope or photographically and a typical response is shown in Fig. 2.

NCV is temperature-dependent, and all values reported in the present work were corrected to a standard temperature of 25°C. The temperature coefficient of the conduction process was determined over the range 23·5–27·5°, which required correction factors between 1·07 and 0·89 to be applied.

RESULTS

Each experimental animal was subject to about ten successive determinations of NCV and the mean NCV from 12 untreated mice was $29{\cdot}2 \pm 1{\cdot}0$ m. s^{-1}. Following i.p. administration of 1 mg/g misonidazole, NCV fell to $21{\cdot}6 \pm 1{\cdot}1$ m. s^{-1} 1 h

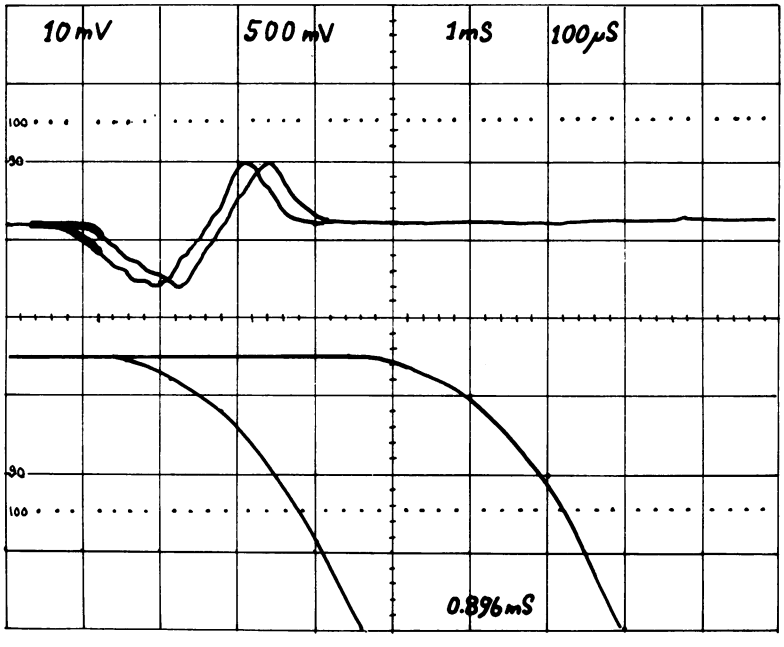

FIG. 2.—Typical response recording using Tektronix 7844 dual beam scope with 7B85 and 7B81 time bases. The top two traces are the muscle responses after sequential stimulation; the bottom traces show the onset of the responses (bright portion of top trace). The bottom read-out shows the stimulus-response delay.

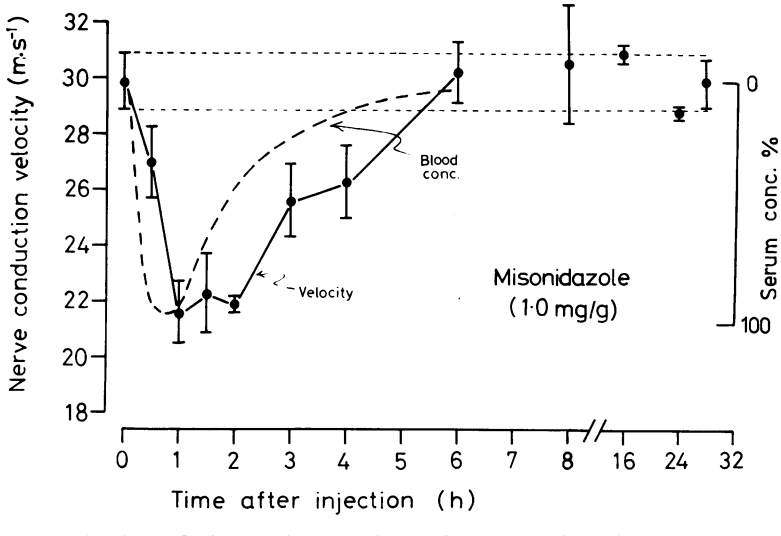

FIG. 3.—Nerve conduction velocity at different times after an i.p. injection of 1·0 mg/g misonidazole (solid line) and serum level of misonidazole after 1 mg/g i.p. injection (dashed line). Error bars represent ± 1 s.e. mean.

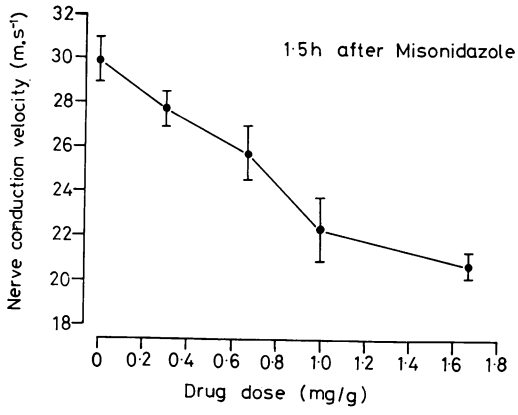

FIG. 4.—Nerve conduction velocity plotted as a function of the dose of misonidazole administered (mg/g) and tested 1·5 h after injection of drug.

after injection (Fig. 3). Reduction of NCV to this level persisted for 1 h and then recovered over the next 4 h reaching the normal value 6 h after injection. The time course of this effect showed a similar time profile to that of the serum concentration of the drug, which is shown in Fig. 3 for comparison (Flockhart and Sheldon, unpublished data).

Fig. 4 shows NCV plotted as a function of drug dose. NCV fell progressively as the dose of misonidazole was increased from 0·33 mg/g; at the highest dose NCV was reduced to $20{\cdot}7 \pm 0{\cdot}5$ m.s^{-1}.

DISCUSSION

Clinically the neurotoxicity of misonidazole first manifests itself as peripheral neuropathy. Misonidazole is not alone among potential hypoxic cell radiosensitizers to exhibit this effect. The nitrofurans, nitrofurantoin and nitrofurazone, and the 5-nitroimidazole, metronidazole which have been used as chemotherapeutic agents, have produced peripheral neuropathy after chronic high dose administration (Le Quesne, 1975; Coxon and Pallis, 1976).

Peripheral nerve damage has been observed in rats after administration of Nitrofurantoin (Klinghardt, 1967) and particularly relevant to the present work is the finding that NCV was reduced in man after Nitrofuran treatment (Honet, 1967; Toole *et al.*, 1968). We have shown that administration of misonidazole can reduce NCV in mice. This occurs after single doses greater than 0·33 mg/g, but this is a higher dose than could be used in man; therefore, experiments are under way to determine whether reduction in NCV can be observed after chronic low doses to mice, a regime which would be more relevant to the clinical situation.

Nerve conduction velocity can be reduced by exposing larger areas of fibre at the firing nodes or the formation of more nodes through removal of parts of the myelin sheath. However, this is unlikely to account for the observed reduction in NCV since the effect is reversible. It is more likely that misonidazole invades the myelin sheath, so causing a change in the conductance and/or capacitance of the myelin which would alter its effectiveness as an electrical screen. This hypothesis might be tested by comparing the effects of drugs of differing lipophilicity, a parameter yet to be fully evaluated *in vivo* for determining the efficacy of any particular drug as a clinical hypoxic cell radiosensitizer.

We thank Roche Products Ltd., for providing the misonidazole.

REFERENCES

ADAMS, G. E., FLOCKHART, I. R., SMITHEN, C. E., STRATFORD, I. J., WARDMAN, P. & WATTS, M. E. (1976*a*) Electron-affinic Sensitization VII. A Correlation between Structures, One-electron Reduction Potentials and Efficiencies of Nitroimidazoles as Hypoxic Cell Radiosensitizers. *Rad. Res.*, **67**, 9.

ADAMS, G. E., CLARKE, E. D., JACOBS, R. S., STRATFORD, I. J., WALLACE, R. G., WARDMAN, P. & WATTS, M. E. (1976*b*) Mammalian Cell Toxicity of Nitro Compounds: Dependence upon Reduction Potential. *Biochem. biophys. Res. Comm.*, **72**, 824.

COXON, A. & PALLIS, C. A, (1976) Metronidazole Neuropathy. *J. Neurol. Neurosurg. Psychiat.* **39**, 403.

DISCHE, S., SAUNDERS, M. I., LEE, M. E., ADAMS, G. E. & FLOCKHART, I. R. (1977) Clinical Testing of the Radiosensitizer Ro-07-0582: Experience with Multiple Doses. *Br. J. Cancer*, **35**, 567.

HONET, J. C. (1967) Electrodiagnostic Study of a Patient with Peripheral Neuropathy after Nitrofurantoin Therapy. *Arch. Phys. Med. Rehabil.*, **48**, 209.

KLINGHARDT, G. W. (1967) Schädigungar des Nervensystems durch Nitrofurare bei der Ratte. *Acta Neuropathol., Berl.*, **9**, 18.

LE QUESNE, P. M. (1975) Neuropathy Due to Drugs. In *Peripheral Neuropathy*. Eds. P. J. Dyke, P. K. Thomas and E. H. Lambert. Philadelphia: Saunders. 1263–1280.

TOOLE, T. F., GERGEN, J. A., HAYES, D. M. & FELTS, J. H. (1968) Neural Effects of Nitrofurantoin. *Arch. Neurol.*, **18**, 680.

URTASUN, R. C., BAND, P., CHAPMAN, J. D., RABIN, H. R., WILSON, A. F. & FREYER, C. G. (1977) Clinical Phase 1 Study of the Hypoxic Cell Radiosensitizer Ro 07-0582 a 2-nitromidazole Derivative. *Radiology*, **122**, 801.