A phase 1 and pharmacokinetic study of didox: A ribonucleotide reductase inhibitor

D. Veale1, 2, J. Carmichael2, B.M.J. Cantwell2, H.L. Elford3, R. Blackie4, D.J. Kerr4, S. B. Kaye4 & A.L. Harris2

1Department of Respiratory Medicine, Regional Cardiothoracic Centre, Freeman Hospital, Newcastle-upon-Tyne NE7 7DN; 2University Department of Clinical Oncology, Newcastle General Hospital, Westgate Road, Newcastle-upon-Tyne NE4 6BE, UK; 3Molecules for Health, Inc., 3313 Gloucester Road, Richmond, Virginia 23227, USA; 4Department of Medical Oncology, Gartnavel General Hospital, Glasgow G12 0YN, UK.

Summary A phase 1 study of a new ribonucleotide reductase inhibitor, didox, was performed by administration of escalating doses of the drug by slow i.v. injection. Thirty-four patients with unresponsive metastatic carcinoma received the drug. There were 13 escalations of dosage, from a starting dose of 192 mg m⁻² to 10 gm m⁻². Dose limiting toxicity was encountered at 7.5 gm m⁻² where disturbances of hepatic and renal function were observed, in addition to severe gastrointestinal toxicity. Pharmacokinetic studies showed that a peak level of didox was achieved within 5 minutes of injection. At 1,728 gm m⁻² the data best fitted a 1\/-2 distribution with a \(\text{t}_{1/2} \) of 5.2 min, with a \(\text{t}_{1/2} \) of 41.3 min. Less than 10% of the drug was excreted unchanged in the urine and the majority of this excretion was within 6h. Didox can therefore be safely given by slow i.v. injection at a dose of 6 gm⁻².

The biosynthesis of deoxyribonucleotides from ribonucleotides is the first reaction in the biosynthetic pathway that is specifically committed to DNA synthesis (Thelander & Reichard, 1979). Thus, this reaction represents a prime target for the development of cytotoxic drugs because it is one of the rate controlling reactions in the biochemical pathway that is specifically committed to DNA synthesis (Elford et al., 1981).

In mammalian cells the pool size of deoxyribonucleotides is not adequate to support DNA synthesis for more than a brief period (Skoog et al., 1973). The enzyme ribonucleotide reductase catalyses the reductive conversion of ribonucleotides to deoxyribonucleotides with the level of this enzyme closely correlated with the replicative rate of the cell (Turner et al., 1968), with enzyme activity very low in non-proliferating cells and exhibiting an increase on conversion of the cell to a rapidly proliferating state (Elford et al., 1970).

The only specific inhibitor of ribonucleotide reductase presently available for clinical use is hydroxyurea (Thurman, 1964). However, the effectiveness of hydroxyurea is limited because it is a weak inhibitor of ribonucleotide reductase in vitro (Elford, 1968), and inhibitory levels are difficult to maintain in vivo. Elford et al. have tested many analogues of hydroxyurea for cytotoxic action, including many substituted hydroxamic acids (Elford et al., 1979). One of the more active of these compounds is didox (N,3-4-trihydroxybenzamide), which has anti-neoplastic activity in L1210 leukaemia bearing female mice (Van’Riet et al., 1979) and has been shown to be active in the NCI tumour panel and was tested against L1210 and P388 leukaemias, B16 melanoma, Lewis Lung, Colo38, CD3, mammary tumour and several human tumour xenographs (Elford & Van’Riet, 1985). Didox has been shown to exhibit greater inhibition of ribonucleotide reductase compared to hydroxyurea (Elford et al., 1979), with toxicity studies in mice showing an LD₁₀ of 243 mg/kg when given as a single dose (CRC Sponsored Toxicity Report, 1984). Based on these experimental studies a phase 0 study of didox in humans was performed.

Patients and methods

Patients with histologically confirmed metastatic carcinoma which was progressive despite conventional first line therapy, for any existed, were studied. They were of good performance status (ECOG Grade 0-2 [WHO, 1979]) and given informed consent. They had received no potentially myelosuppressive therapy within 3 weeks of treatment and liver and renal function were within normal limits prior to treatment.

Treatment was started at a dose of 192 mg m⁻², 10% of the LD₁₀ in mice for an intravenous dose. The dose was escalated according to a modified Fibonacci scheme (Goldsmith et al., 1975), with didox given as a single bolus intravenously until the dose rose to 4 gm m⁻². In view of solubility difficulties at higher doses, the drug was thereafter infused intravenously over 30 min in 500 ml of 0.5N dextrose saline solution. Patients were treated every 3 weeks, with escalation permitted 3 weeks after the previous dosing level, with a minimum of 3 doses at each increment.

Blood samples were taken from an indwelling cannula at varying times after injection of didox. Samples were stored on ice and centrifuged at 3,000 rpm for 5 min at 4°C within 15 min. Plasma was separated and stored at −20°C, with aliquots of urine collected for 4×6 hourly periods after injection of didox, and stored at −2°C until estimation of didox levels were performed.

Analytic methods

Didox levels were estimated in plasma and urine by HPLC using a Beckman/Altek 100A pump and stainless steel column (25 cm × 0.46 cm) packed with µ Bondapack C18, 10µ particle size. Detection was achieved with an electro-chemical detector, using a glassy carbon electrode with applied potential of 800 mV. Chromatograms were recorded at 1mV, and automatic injection, integration and step-gradient were controlled by a Beckman/Altex programmer Model 420. After addition of 2 µg internal standard, plasma samples were extracted twice with 10 ml ethyl acetate vortexing for 20 min. The organic phases were combined, evaporated to dryness and reconstituted in 20% methanol prior to injection. Separation of didox and internal standard was achieved using a step-gradient elution system consisting of the following buffers, at a flow rate of 1.5 ml min⁻¹:

Buffers

1. 0.1M sodium phosphate, 1mM EDTA pH 3.5 for 2 min.
2. As above +5% acetonitrile for 2 min.
3. As above +10% acetonitrile for 7 min.

The column was equilibrated with buffer 1 for 5 min prior to
adding the next sample. Retention times of 6 min and 10.4 min were observed for didox and internal standard respectively. This method was found to have a detection limit of 100 ng ml\(^{-1}\), with an extraction efficiency of greater than 80% and was linear up to 100 \(\mu g\) ml\(^{-1}\). Urine samples were injected directly onto the column and eluted isocratically with 0.1% sodium phosphate, 1 mM EDTA, pH 3.5. Following elution of didox and internal standard, the column was washed with phosphate buffer containing 15% acetonitrile and equilibrated for 7 min with Starting buffer prior to injection of the next sample.

The plasma concentration-time profiles (1,728 mg m\(^{-2}\); \(n=6\)) were fitted to a 2 compartment open model by the method of least squares using an ‘in house’ programme based on the Marquardt algorithm. The drug clearance and steady state volume of distribution were calculated from the microscopic rate constants; \(k_{10}, k_{12}\) and \(k_{21}\).

**Results**

Patient details are shown in Table I. Thirty-four patients were entered into the trial with a mean age of 56 years (range 37-74). There were 23 male and 11 female patients. All the patients had evidence of metastatic disease but were of good performance status, all being self caring (ECOG scale 0-2). The majority of patients had carcinoma of the bronchus or carcinoma of unknown origin, with 13 patients having received previous chemotherapy.

Eleven patients had only one injection of didox, treatment being stopped because of progressive disease in the majority of cases. Fourteen patients had 2 courses, 7 had 3 courses and 2 had 4 courses of didox. There were 13 increments of dosage and the highest dosage achieved was 10 g m\(^{-2}\). No clinical responses were observed at any dosage level.

Minor toxicity was initially noted at 2,304 mg m\(^{-2}\) (Table II), with severe nausea and vomiting almost universal above 7 g m\(^{-2}\). Abnormalities of liver function were dose limited at doses at 7.5 g m\(^{-2}\) and above. The only abnormality of liver function seen at lower doses was elevated alkaline phosphatase, but at higher doses increases in transaminases and bilirubin were seen, with no evidence of haemolysis.

Difficulty and delay in passing urine were the major renal toxicities, although transient elevations in urea were observed in 2 patients receiving 7,500 mg m\(^{-2}\) and one patient at 9,500 mg m\(^{-2}\). One patient who died 4 days after receiving didox was noted to have abnormal renal and liver function tests. Autopsy revealed the presence of diffuse tumour infiltration of the liver, but the kidneys were normal histologically. All other hepatic and renal toxicities were reversible. Severe diarrhoea (grade 4) was noted in 2 patients. This commenced prior to the completion of the didox infusion and stopped within 6 h. One of these patients, who received 7.5 g m\(^{-2}\) became severely hypotensive immediately following the didox infusion. The patient was anuric for 8 h following this and developed transient abnormalities in renal function tests, although these settled within 24 h. An increase in white cell count was observed in 4 patients following didox, with total counts in excess of \(2 \times 10^9\) 1\(^{-1}\) seen, but these increases were not related to the dose of didox. None of these patients were receiving corticosteroids and none had clinical symptoms suggestive of infection.

Peak levels of didox in the plasma were attained within 5 min of injection. At the first dose of 192 mg m\(^{-2}\) peak levels of 8.8 g m\(^{-2}\) were recorded, and at the highest dose at which samples were taken (7.5 g m\(^{-2}\) peak levels of 166 g ml\(^{-1}\) were achieved. More detailed information was obtained at 1,728 mg m\(^{-2}\) (\(n=6\)) as shown in Figure I. The data was appropriately fitted by a 2 compartment open model. Drug clearance and steady state volume of distribution were calculated from the microscopic rate constants; \(k_{101}, k_{12}\) and \(k_{21}\) as shown in Table III. There was minimal variation in plasma didox concentrations between patients as is shown in Figure I, and these levels were dose related as illustrated in Figure 2. Less than 10% of the administered dose of didox was excreted unchanged in the urine, with the majority of this excretion within the first 6 h (data not shown).

**Discussion**

A phase I study of didox, a novel inhibitor of ribonucleotide reductase, was performed. Toxicity was minor below 7 g m\(^{-2}\), but severe dose limiting organ toxicity was initially encountered at 10 g m\(^{-2}\), but on reduction of the didox dosage similar toxicities were observed at 7.5 g m\(^{-2}\). The major dose limiting toxicity was hepatic, although whether this toxicity related to the peak level of drug or area under the curve exposure (AUC) remains unclear. Renal toxicity was observed in some patients although its aetiology remains unclear. It would seem unlikely to be related to solubility of the drug, as at doses greater than 4 g m\(^{-2}\) didox was dissolved in a large volume of fluid, and all administrations were via a bacteriological filter. In these patients the renal toxicity would appear to be predominantly pre-renal, although a renal component could not be completely excluded.

The levels of didox achieved in the plasma were dose related and relatively constant between patients. At a dose of 1,728 mg m\(^{-2}\), didox has rapid \(a\) and \(b\) half lives of 5.2 and
Table III  Pharmacokinetic parameters derived from a 2 compartment model fitted to plasma concentration data at 1.728 mg m$^{-2}$ (n=6). The results are expressed as mean ± 1 s.d.

|                 | Steady state volume of distribution (l m$^{-2}$) | t$_{1/2}$ (min) | t$_{1/2}$ (min) | Area under the curve (0→∞) (l m$^{-2}$ min$^{-1}$) |
|-----------------|-------------------------------------------------|-----------------|----------------|---------------------------------------------------|
| Total body Clearance (l min$^{-1}$ m$^{-2}$) | l$_{1/2}$ | l$_{1/2}$ |                      |
| 0.71 ± 0.19    | 27.9 ± 6.2 | 5.2 ± 2.8 | 41.3 ± 18.6 | 2.674 ± 1.022 |

Figure 1  Plasma didox levels as measured in patients receiving 1.728 mg m$^{-2}$. Each symbol represents a separate patient.

Figure 2  Plasma didox levels as measured in a single patient following administration of didox at 3 different doses:

- □ -1.344 mg m$^{-2}$
- ● 1.728 mg m$^{-2}$
- ■ 2.304 mg m$^{-2}$

41.3 min respectively, with the data best fitted by a 2 compartment open model. Peak plasma levels at this dose are approximately 300 μM, and these levels correspond favourably with the levels used in experimental systems.

an enzyme study 30 μM didox was shown to cause 50% inhibition of the target enzyme, and in vitro concentrations of 100 µM or less have been shown to be growth inhibitory in a number of cell lines. These experiments have been performed using continuous drug exposure, however, and the didox levels achievable clinically using prolonged infusions will be of interest. The 3 major metabolites formed in rats are 3,4-dihydroxybenzamide, 3-hydroxy-4-methoxybenzohydroxamic acid and 3-methoxy-4-hydroxybenzohydroxamic acid (Van’t Riet, personal communication). The metabolism in man is unknown, although less than 10% of the dose is excreted unchanged in the urine.

The maximum tolerated dose of didox given by bolus injection in this study was 6 mg m$^{-2}$, although the optimal scheduling of the drug remains unclear. Hydroxyurea, the only ribonucleotide reductase inhibitor in clinical use, has previously been shown to be effective by prolonged 42 hour infusions with reduced toxicity (Veale et al., 1987). It is intended to further evaluate didox in phase I studies using alternative schedules such as 36 h infusions or daily times 5 injections.

Dadox may be of value in combination with other cytotoxic drugs, particularly with anti-metabolites, as it is known to deplete the pools of all deoxyribonucleotides by over 50% (Elford et al., 1981), whereas hydroxyurea only depleted pools of dGTP and dATP (Thelander & Reichard 1979). In addition didox has been shown to act synergistically with doxorubicin and cyclophosphamide in murine tumours (Elford et al., 1984).

No responses were seen in 34 patients, but only 3 patients received more than 1 course at doses of 6 mg m$^{-2}$ or greater, and it should be emphasised that the tumour types treated in this study are generally considered unresponsive to chemotherapy. Therefore, despite the apparent lack of activity, didox is worthy of further study in view of its potency for the inhibition of the target enzyme (Elford et al., 1970).

We thank the nursing staff on the oncology wards at Newcastle General Hospital for their support in this study. Didox was supplied through the CRC Phase I Committee, and we are grateful to Dr B. Van’t Riet for synthesis of the compound.

References

Cancer Sponsored Toxicity Report, 1984 not cited in refs (see p. 3).

ELFORD, H.L. (1968). Effect of hydroxyurea on ribonucleotide reductase. Biochem Biophys. Res. Commun., 33, 129.

ELFORD, H.L., FREESEE, M., PASSAMANI, E. & MORRIS, H.P. (1970). Ribonucleotide reductase and cell proliferation. J. Biol. Chem., 245, 5228.

ELFORD, H.L., WAMPLER, G.L. & VANT RIEI B. (1979). New ribonucleotide reductase inhibitors with antineoplastic activity. Cancer Res., 39, 844.

ELFORD, H.L., VANT RIEI B., WAMPLER, G.L., LIN, A.L. & ELFORD, R.M. (1981). Regulation of ribonucleotide reductase in mammalian cells by chemotherapeutic agents. Adv. in Enzyme Regul., 19, 151.

ELFORD, H.L., WAMPLER, G.L., VANT RIEI B. & BURCHENAL, J.H. (1984). Synergistic oncolytic drug combination involving polyhydroxypheyl compounds, cytoxan, other alkylating agents and Adriamycin. Proc. Amer. Assoc. Cancer Res., 25, 320 (Abstract).

ELFORD, H.L. & VANT RIEI B. (1985) Inhibition of nucleoside diphosphate reductase by hydrobenzohydroxamic acid derivatives. Pharmacol. Ther., 29, 239.

GOLDSMITH, M.A., SLAVIK, M. & CARTER, S.K. (1975). Quantitative prediction of drug toxicity in humans from toxicology in small and large animals. Canc. Res., 35, 1354.

SKOOG, L.K., ORDENSKJOLD, B.A. & BIURSELL, K.G. (1973). Deoxyribonucleoside triphosphate pools and DNA synthesis in synchronised hamster cells. Eur. J. Biochem., 33, 428.

THELANDER, L. & REICHARD, P. (1979). Reduction of ribonucleotides. Ann. Rev. Biochem., 48, 133.

THURMAN, W.G. (Ed) (1964). Symposium on hydroxyurea. Cancer Chemother. Rep., 40, 1.

TURNER, M.K., ABRAMS, R. & LIEBERMAN, I. (1968). Levels of ribonucleotide reductase during the division cycle of the cell. J. Biol. Chem., 243, 3725.

VANT RIEI B., WAMPLER, G.L. & ELFORD, H.L. (1979). Synthesis of hydroxy- and amino-substituted benzohydroxamic acids: Inhibition of ribonucleotide reductase and anti-tumour activity. J. Med. Chem., 22, 589.

VEALE, D., CANTWELL, B.M., KERR, N., UFPOLD, A. & HARRIS, A.L. (1987). Phase I and II study of high dose hydroxyurea in lung cancer. Canc. Chemother. Pharm. (in press).

WORLD HEALTH ORGANISATION (1979). Handbook for Reporting Results of Cancer Treatment. World Health Organisation, Geneva (WHO Offset publication 48).