The Pharmacokinetics of Prednimustine and Chlorambucil in the Rat

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Summary. In the rat prednimustine, the prednisolone ester of chlorambucil, is much less toxic than equimolar doses of chlorambucil, when administered subcutaneously (SC). This is due to differences in alkylating agent pharmacokinetics. Prednimustine injected SC produced low plasma concentrations (< 5 μM) of the alkylating metabolites chlorambucil and phenyl acetic mustard, which were maintained for 48 h. No unhydrolysed prednimustine could be detected. Chlorambucil, in contrast, was rapidly absorbed, peak levels (40 μM) occurring within 2 h, after which chlorambucil and phenyl acetic mustard plasma levels decreased with half-lives of 2.4 h and 2.9 h respectively.

The toxicity of chlorambucil could be similarly reduced by administering either the methyl ester of chlorambucil or by giving chlorambucil in a multi-treatment low-dose schedule. Neither of these treatments inhibited the Yoshida alkylating agent-resistant tumour, however, whereas prednimustine or a combination of chlorambucil and prednisolone produced significant tumour growth inhibition. Prednisolone did not alter chlorambucil pharmacokinetics. Thus the reduced toxicity of prednimustine is due to chlorambucil esterification and the subsequent alteration in pharmacokinetics, whilst inhibition of alkylating agent-resistant tumours results from the combination of chlorambucil and prednisolone.

Introduction

Chlorambucil [4(4-bis(2-chloroethyl)amino phenyl)butyric acid] is a bifunctional alkylating agent used widely in the treatment of human cancers [10, 17]. Prednimustine [pregna-1,4-diene-11β,17α,21-triol-3,20-dione,21 (4(4-bis(2-chloroethyl)aminophenyl)-butyric acid)], the prednisolone ester of chlorambucil, was synthesised by A. B. Leo (Helsingborg, Sweden) [9], in an attempt to improve the antitumour selectivity of chlorambucil by facilitating uptake into tumours possessing high concentrations of glucocorticoid receptors. In practice, prednimustine has proved effective in the management of a number of leukaemias and lymphomas [1].

We have previously shown [5] that prednimustine is much less toxic than chlorambucil when given SC to rats. Although the concomitant administration of prednisolone does significantly reduce chlorambucil-induced mortality, the combination of chlorambucil and prednisolone is consistently more toxic than the ester prednimustine [5]. This latter observation has been confirmed in an independent study [3]. In an attempt to explain the differential toxicities of chlorambucil and prednimustine further, their pharmacokinetics and tissue distributions have been investigated following SC administration to the rat.

Metabolism and distribution of chlorambucil in the rat have been investigated extensively. Chlorambucil is metabolised by β-oxidation of the butyric side chain [4, 11, 13], the products of β-oxidation, phenyl acetic mustard [2(4-bis(2-chloroethyl)aminophenyl)acetic acid] and an intermediate, 3,4-dehydrochlorambucil, constituting the major metabolites in rat blood 1 h after administration [11]. Radiochemical studies have shown that chlorambucil is excreted mainly by the kidneys, with 40%–60% of the administered dose being present in the urine at 24 h [7, 12, 13]. These studies also demonstrate that active uptake of chlorambucil into specific tissues does not occur, so that plasma concentrations generally exceed tissue levels [7, 13].

The metabolism of prednimustine in the rat has not previously been reported. However, in vitro
studies from this laboratory have shown that the ester linkage is rapidly hydrolysed by esterases present in a number of tissues, including rat plasma [19]. The hydrolysis of prednimustine releases equimolar quantities of chlorambucil and prednisolone, whereupon the chlorambucil moiety is available for \( \beta \)-oxidation.

In the present study, the comparative pharmacokinetics and tissue distributions of chlorambucil and prednimustine have been investigated. This work has been facilitated by a recently developed high-performance liquid chromatography (HPLC) technique, which allows the simultaneous estimation of prednimustine, chlorambucil and phenyl acetic mustard in biological fluids [15]. These pharmacokinetic studies have allowed more accurate definition of the factors responsible for the toxicological and antitumour properties of prednimustine.

Materials and Methods

**Chemicals.** Prednimustine, both radioactive and non-radioactive, was a gift from A. B. Leo (Helsingborg, Sweden). \(^{14}\)C/\(^{3}\)H-prednimustine was universally \(^{14}\)C-labelled in the chloroethyl side chains and \(^{3}\)H-labelled in the 6 and 7 positions of the prednisolone molecule. Chlorambucil was a gift from the Wellcome Foundation (Beckenham, Kent, England). Phenyl acetic mustard was synthesised by Prof. W. C. J. Ross [2], and the methyl ester of chlorambucil by Dr M. Jarman, both at the Institute of Cancer Research (London, England). \(^{3}\)H-Chlorambucil, \(^{3}\)H-labelled in the ring ortho to the butyric side chain, was also prepared by Dr M. Jarman [8]. Prednisolone (11\(\beta\),17\(\alpha\),21-trihydroxy-1,4-pregnadiene-3,20-dione) was obtained from the Sigma Chemical Company Ltd (Poole, Dorset, England). All other chemicals were supplied by BDH Chemicals Ltd (Poole, Dorset, England), Fisons Scientific Ltd (Loughborough, England); or Hopkin and Williams, Romford, Essex, England) and were of analytical grade.

**Animal Studies.** Female Wistar rats (150–200 g) were used throughout the investigation. Toxicity studies were performed on non-tumour-bearing rats as previously described [5]. Metabolic studies were performed on rats bearing the sensitive strain of the Walker 256 carcinosarcoma grown as ascites. Antitumour studies were carried out in rats bearing the alkylation agent-resistant strain of the Yoshida sarcoma, as previously described [5].

For metabolic studies, tumour-bearing rats received either 10 mg \(^{3}\)H-chlorambucil/kg (25.4 mCi/m mole) or 20 mg \(^{14}\)C/prednimustine/kg (\(^{14}\)C: 3.01 mCi/m mole; \(^{3}\)H: 9.12 mCi/m mole). In a separate experiment to determine the effect of subsequent prednisolone administration on chlorambucil pharmacokinetics, animals received \(^{3}\)H-chlorambucil 40 mg/kg (4.11 mCi/m mole) followed by prednisolone (40 mg/kg) 4 h later. All drugs were dissolved in dimethyl sulphoxide (DMSO) and administered SC. Animals were anaesthetised with diethyl ether at various times after drug administration and exsanguinated by direct cardiac puncture. Blood was placed in heparinised tubes (10 ml) and plasma prepared by centrifuging at 600 g for 10 min at 4°C. Plasma was removed and stored at \(-20^\circ\)C prior to analysis. Bone marrow (aspirated from femurs) and tumour cells were counted on a Model ZF Coulter Counter (Coulter Electronics Ltd., Harpenden, Herts, England), and washed once in reticulocyte standard buffer (0.01 M Tris-HCl pH 7.4, 0.01 M NaCl, 0.0015 M MgCl\(_2\)) prior to storage at \(-20^\circ\)C.

**Sample Analysis.** Plasma levels of chlorambucil, phenyl acetic mustard and prednimustine were analysed by HPLC as previously described [15]. Samples (1 ml) of tissue homogenates (50% w/w) were extracted and analysed in a similar manner following homogenisation in ice-cold reticulocyte standard buffer in a Teflon/glass homogeniser. Compounds present in the effluent of the HPLC were quantitated by scintillation counting.

**Scintillation Counting.** Samples (0.1 ml) of plasma or tissue homogenates were counted following solubilisation in NCS solubiliser [14]. Fractions (1 ml) of HPLC effluent were collected directly into scintillation vials, 10 ml PCS scintillant (Hopkin and Williams, Romford, Essex, England) was added, and radioactivity was counted on a Model SL 30 Intertechnique Liquid Scintillation Spectrometer (Kontron Intertechnique, St. Albans, Herts., England). Counting efficiencies were determined by using \(^{3}\)H- and \(^{14}\)C-hexadecane (The Radiochemical Centre, Amersham, Bucks, England). In the calculation of molar quantities of prednimustine-derived compounds the \(^{13}\)C radioactivity was employed.

**Pharmacokinetic Analysis.** Where possible, plasma levels of chlorambucil and phenyl acetic mustard were fitted to a mathematical function, a non-linear least-squares analysis being used [16]. Following chlorambucil administration, levels of parent drug and phenyl acetic mustard were fitted to the exponential function:

\[
C = Ae^{-\beta t}
\]

where \(C\) is the drug plasma concentration, \(t\) is the time after dosing, \(A\) is a concentration constant and \(\beta\) the first-order disposition rate constant. The plasma terminal phase half-life, \(t_{1/2}\beta\), was calculated in the form:

\[
t_{1/2}\beta = \frac{0.693}{\beta}.
\]

After chlorambucil administration data were analysed following the initial absorption/distribution phase, i.e., from 2 h onwards. Following prednimustine administration chlorambucil plasma levels were fitted to the expression:

\[
C = A \left(\frac{a}{a - \beta}\right) (e^{-\beta t} - e^{-at})
\]

as described by Wagner [18], where in addition to the previously defined terms, \(a\) is the first-order absorption rate constant. Phenyl acetic mustard levels following prednimustine administration were plotted manually, the mean value being used for each time point.

The areas under the plasma concentration vs time curves were determined by the trapezoidal rule [18]. The extrapolated volume of distribution (\(V_D\)) was calculated as:

\[
V_D = \frac{Dose (\text{umoles/kg})}{A}
\]

where, as previously, defined, \(A\) is the time zero concentration constant.