Pharmacokinetic and metabolic studies of high-dose busulphan in adults*

M. Hassan 1, G. Öberg 2, H. Ehrsson 1, M. Ehrnebo 3, I. Wallin 1, B. Smedmyr 2, T. Tötterman 2, S. Eksborg 1, and B. Simonsson 2

1 Karolinska Pharmacy, Stockholm and
2 Department of Medicine, University Hospital and
3 Galenus AB, Uppsala, Sweden

Summary. The pharmacokinetics of high-dose busulphan was studied in adult patients with acute myeloblastic leukaemia after oral doses of 1 mg·kg⁻¹ every 6 h for 4 days. The mean steady-state plasma concentration was 1080 ng/ml⁻¹ during the treatment. Individual steady-state concentrations after the last dose on average were 32% lower than those predicted from total AUC measurements following the first dose. Mean elimination half-life in plasma was 2.3 h after the last dose and 3.4 h after the first dose which suggests that busulfan may increase its own metabolic rate on repeated treatment.

The cerebrospinal fluid/plasma concentration ratio of busulphan was 1.3. Busulphan showed insignificant protein binding in plasma (7.4%). About 2% of the dose was excreted unchanged in the urine.

For the first time sulpholane, 3-hydroxysulpholane and tetrahydrothiophene 1-oxide were identified as urinary metabolites of busulphan in man.

Key words: busulphan, leukaemia; high-dose pharmacokinetics, metabolism, bone marrow transplantation

Bone marrow transplantation (BMT) in the last decade has become one of the most efficient forms of therapy for acute leukaemia [1-2] and congenital bone marrow disorders [3]. Most BMT patients are treated with total body irradiation (TBI) and cyclophosphamide as the myeloablative regimen [4]. It allows full engraftment but the toxicity of TBI is high and the treatment can cause damage to the developing brain [5-6].

Busulphan, an anti-leukaemic and alkylating agent [7], has been suggested as an alternative to TBI [8-10]. The drug is used mainly in the treatment of chronic myelocytic leukaemia (CML) and polycythemia vera and is usually given in low daily doses (2-6 mg). The use of busulphan in high doses prior to BMT has become more common in the past few years. Despite its widespread and long clinical use, the pharmacokinetics of busulphan has only been studied in adult patients treated with low doses for CML [11], and in children conditioned with high doses prior to BMT [12]. Only scanty information is available about the metabolic fate of busulphan in man [13].

The present study deals with the pharmacokinetics of high-dose busulphan in adult patients treated with busulphan 1 mg·kg⁻¹ every 6 h for 4 days p.o. prior to autologous bone marrow transplantation (ABMT). Busulphan was assayed by gas chromatography with electron capture detection [14]. The distribution of busulphan to cerebrospinal fluid (CSF) and saliva was studied, and preliminary results were also obtained about the urinary metabolites of busulphan.

Patients and methods

Patients

Five female patients with acute myeloblastic leukaemia (AML; Table 1), of 38 years (range 32-47 years) were studied. As a preparation for ABMT high dose busulphan 1 mg·kg⁻¹ every 6 h p.o. was administered for 4 days (Days -8 to -5), followed by cyclophosphamide 60 mg·kg⁻¹·day⁻¹, i.v. for 2 days (Days -4 to -3). The marrow was infused on Day 0. Busulphan (25 mg tablets) was a gift from Wellcome Foundation Ltd. UK.
Table 1. Patient details and dose

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (year)</th>
<th>Weight (kg)</th>
<th>Busulphan dose (mg) every 6 h</th>
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<tbody>
<tr>
<td>1</td>
<td>47</td>
<td>81</td>
<td>70</td>
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<tr>
<td>2</td>
<td>32</td>
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<td>5</td>
<td>32</td>
<td>63</td>
<td>62</td>
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</table>

**Busulphan determination**

Busulphan in plasma, saliva, urine and CSF was measured by gas chromatography with electron capture detection (GC-ECD), which is a highly specific method [14]. At 10, 100 and 500 ng·ml⁻¹ concentrations the coefficients of variation (CV) were ±4, ±2 and ±3%, respectively. Busulphan was converted to 1,4-diiodobutane by direct reaction with sodium iodide in plasma in the presence of n-heptane as an organic solvent [14].

**Isolation and structural determination of urinary metabolites of busulphan**

Pooled urine (24 h, 3-4 l) was evaporated to dryness under reduced pressure at room temperature. The residue was washed twice with acetone 20 ml and the solvent was evaporated to about 5 ml, filtered and subjected to gas chromatography - mass spectrometry (GC-MS). The analysis was performed using the same equipment and conditions described previously [15]. The mass spectra were recorded in the electron impact mode (70 eV) and were processed using a Teknivent Vector Data System (St. Louis, USA). Urine was hydrolyzed by the addition of NaOH and extracted with CH₂Cl₂ [15] previously described.

**Protein binding**

Reversible binding to plasma proteins was studied using patient plasma and equilibrium dialysis as previously described [16]. The blood samples from the patients were collected at various times during treatment as described above. The plasma was separated and the dialysis was carried out for 5 h at 25°C. Concentrations were determined in plasma before dialysis and in the buffer and plasma compartments after dialysis using GC-ECD [14]. The sum of the busulphan concentrations in buffer and plasma was in good agreement with the initial plasma concentration (350–2750 ng·ml⁻¹).

**Pharmacokinetic calculations**

The plasma concentration data were treated according to a linear one-compartment open model. However, all the equations used were applicable to any linear pharmacokinetic model [17]. The apparent terminal slopes following the first and last doses were calculated by semilogarithmic regression analysis. The area under the plasma concentration - time curve (AUC) was estimated using the trapezoidal rule. At the first dosage interval, the total area from 0 to infinity, AUC included the remaining area after the last sampling point calculated by integration. Average plasma concentration during a dosage interval (C) were calculated as AUC divided by the duration of the dosage interval. The predicted average steady-state concentration on repeated dosing, based on pharmacokinetics values following the first dose, was calculated according to the standard equation [17]:

\[ C = \frac{AUC}{t} = \frac{f \cdot D}{CL_s \cdot t} = t = 1.44 \frac{f \cdot D \cdot t}{t_1/2} \]  

where t is the dosage interval, f the bioavailability fraction, D the dose, CLs the apparent systemic clearance, t₁/₂ the elimination half-life and V₁ the total distribution volume.

The renal clearance (CLR) was determined as:

\[ CLR = \frac{Ae}{AUC} \]  

where Ae is the cumulative amount excreted in the urine.