Effect of Ifosfamide Treatment on Glutathione and Glutathione Conjugation Activity in Patients with Advanced Cancers

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ABSTRACT

Several studies have suggested that the glutathione/glutathione S-transferase (GSH/GST) system is involved in resistance of tumors toward ifosfamide and other cytostatic agents. Besides, ifosfamide metabolites (in vitro) as well as ifosfamide treatment (in vivo) have been shown to decrease cellular GSH availability. In the present study, the in vivo effects of three different ifosfamide treatment schedules on the GSH/GST system were studied in patients with advanced cancers (n = 24): continuous i.v. infusions of 1300 mg/m² daily for 10 days and 5000 mg/m²/day for 24 h, as well as a 4-h infusion of 3000 mg/m² daily for 3 days. The GSH/GST system was characterized by administering bromisoval, a probe drug to assess GSH conjugation activity in vivo, as well as by daily monitoring of GSH concentrations in blood cells and plasma. Bromisoval pharmacokinetics was assessed before and at the end of the ifosfamide treatment.

Blood cell GSH levels decreased significantly (P < 0.05) during the 3- and 10-day ifosfamide treatment schedules; the 24-h treatment had no effect. The ifosfamide treatment schedules had only minimal effects on bromisoval pharmacokinetics. Assuming that the kinetics of the probe drug provide an accurate reflection of enzyme activity, this suggests that GST activity remains unchanged. Because GSH conjugation of bromisoval enantiomers requires both GST activity and GSH availability, these results also indicate that, despite the 35% decrease in GSH in blood cells of two patient groups, the GSH availability of the cancer patients was not rate-limiting for GSH conjugation of bromisoval enantiomers. If GSH levels in blood cells reflect those in tumors/other tissues, the present results indicate that ifosfamide may be used clinically to decrease GSH levels. However, whether a 35% decrease is sufficient to increase tumor sensitivity toward (other) cytostatics remains uncertain.

INTRODUCTION

Ifosfamide is an alkylation oxazaphosphorine nitrogen mustard with activity against a wide range of tumors both in adults and children. It is a prodrug that requires biotransformation by cytochrome P450 isoenzymes to become cytotoxic. This metabolic process results in the formation of the active bifunctional alkylation metabolite, isophosphoramide mustard, and acrolein, a metabolite believed to contribute to the hemorrhagic cystitis associated with oxazaphosphorine treatment. Ifosfamide can also undergo side chain dealkylation, resulting in the formation of 2- and 3-dechloroethylifosfamide and equimolar amounts of the highly reactive chloroacetaldehyde (1-4). High plasma levels of chloroacetaldehyde have been suggested to be associated with ifosfamide-related neurotoxicity (5, 6). Resistance to cytostatic therapy is a common clinical problem in the treatment of cancer. A wide variety of mechanisms has been implicated in the aetiology of drug resistance, including detoxification by the GSH/GST4 system. The relationship between drug resistance and this system has been studied extensively in human cancer cell lines and tumor tissues (e.g., 7-13). However, evidence for involvement of the GSH/GST system in drug resistance in patients in vivo is limited. Such studies are generally restricted to the assessment of GSH content (tumors, blood cells) or GST profile/activity of tumors of patients (14).

So far, several in vitro studies have provided evidence for the involvement of GSH and GSTs in the detoxification of metabolites derived from ifosfamide and its structural analogue cyclophosphamide. The ifosfamide-derived 4-hydroxy intermediate and especially chloroacetaldehyde depleted intracellular GSH and inhibited the GST-catalyzed conjugation of 1-chloro-2,4-dinitrobenzene (15). Depletion of cellular GSH levels was also demonstrated for the 4-hydroxy metabolite of cyclophosphamide (16). Furthermore, GST conjugation of 4-hydroxycyclophosphamide and phosphoramide mustard was shown to be catalyzed by cytosolic GSTs, in particular GSTA1-1 (17, 18). Acrolein, a metabolite derived from both ifosfamide and cyclophosphamide, could be inactivated by human class α, μ, and in particular class π GST isoenzymes, either by conjugation with GSH or by covalent binding to the enzymes in the absence of GSH (19). In vivo, Lind et al. (15) demonstrated that an infusion...
of ifosfamide in a patient resulted in a 70% decrease of GSH in lymphocytes.

Based on the above-mentioned in vitro and in vivo data, it was hypothesized that changes in the GSH/GST system may affect tumor resistance toward ifosfamide. Furthermore, GSH conjugation of ifosfamide (metabolites) may also lead to consumption of GSH, thereby making ifosfamide a potentially GSH-lowering cytostatic agent, which may be used in combination with (another) cytostatic agent(s)—for which involvement of the GSH/GST system in resistance is a major contributing factor—with the aim to improve the efficacy of the cytostatic treatment.

In the present study, the in vivo effects of ifosfamide treatment on the GSH/GST system were studied in patients with advanced cancers. Three different ifosfamide treatment schedules were studied, i.e., a continuous i.v. infusion of 1300 mg/m² daily for 10 days or 5000 mg/m²/day for 24 h and a 4-h infusion of 3000 mg/m² daily for 3 days. The GSH/GST system of patients in vivo was characterized by administering 2-bromoisovalerylurea (bromisoval), a probe drug to assess GSH conjugation activity in vivo (20, 21), as well as by daily monitoring of GSH (plasma and blood cells) and GSSG (blood cells) concentrations.

PATIENTS AND METHODS

The study protocol was approved by the Ethics Review Board of the Leiden University Hospital (Leiden, the Netherlands). The study was an open trial. Twenty-four patients participated after each gave oral informed consent before study entry.

Patients

Patients of either sex, 75 years old or less, with histologically proven advanced cancer and for whom no standard therapy was available were eligible. Furthermore, patients had to have normal renal function, no major disturbance of liver function, adequate bone marrow function, no active infection, or central nervous system, cardiac, or pulmonary disease, as well as those with a second malignant disease, were ineligible.

All patients, except patient 301, had received prior treatment consisting of radiotherapy, surgery, and/or treatment with other cytostatic agents or hormones. None of the patients, except patient 102, had received ifosfamide therapy on a previous occasion (Table 1).

Ifosfamide Treatment Schedules

Three different ifosfamide infusion schedules were studied, i.e., continuous infusions of 1300 mg/m² daily for 10 days (12 patients with in general gastrointestinal primary tumors) and 5000 mg/m²/day for 24 h (6 patients with various primary tumors) as well as a 4-h infusion of 3000 mg/m² daily for 3 days (6 patients with in general head/neck primary tumors; Table 1). Ifosfamide infusions were administered along with the uroprotectant mesna. The infused solution always consisted of a mixture of mesna and ifosfamide. After the end of the ifosfamide treatment period, mesna infusion was continued at a lower dose for 1 day. For the prolonged ifosfamide (and mesna) infusions, external infusion pumps (CADD-I pump) were used, which were connected to a Port-A-Cath (Pharmacia Deltec Inc., Woerden, the Netherlands) i.v. device. The ifosfamide and mesna solutions were supplied by the Leiden University Hospital Pharmacy.

Characterization of GSH Conjugation

Characterization of the GSH/GST system was performed by administering the racemic drug bromisoval as well as by daily assessment of GSH (plasma and blood cells) and GSSG (blood cells) concentrations. In addition, the GSTM1 phenotype of all patients was determined.

Bromisoval. Bromisoval pharmacokinetics (plasma pharmacokinetics of the bromisoval enantiomers and urinary excretion of their corresponding mercapturates) was assessed on the day prior to the start of the ifosfamide infusion (day 0), the last day of the 3 (3000 mg/m² daily)-, and 10 (1300 mg/m² daily)-day ifosfamide treatment period, and the day after the 24-h (5000 mg/m²/day) ifosfamide infusion. On both bromisoval study days, patients received a capsule containing 600 mg (2.69 mmol) racemic bromisoval after an overnight fast. The bromisoval (Dutch Pharmacopoeia) capsules were supplied by the Leiden University Hospital Pharmacy. Patients refrained from all food and drinks (except plain mineral water ad libitum) until 3 h after bromisoval administration. To ensure sufficient urine production, the patients were asked to drink regularly. Patient 102 (24-h ifosfamide treatment) did not receive bromisoval because this patient refused to participate in the bromisoval study on the ifosfamide pretreatment day. Patient 304 (3-day ifosfamide treatment) did not receive bromisoval because this patient had a jejunal fistula.

Blood and Urine Sampling. On the ifosfamide pretreatment day (day 0), before the start of the first bromisoval study period, an i.v. cannula (Venflon, BOC Ohmeda AB, Sweden) was inserted into a suitable forearm vein. The cannula was kept patent by intermittent flushing with saline solution containing heparin. All blood samples were collected in 10-ml lithium heparin tubes.

On both bromisoval study days, bromisoval blood samples (10 ml/sample) were collected before and at 0.5, 1, 2, 3, 4, 5, 6, 8, 12, and 24 h after bromisoval administration. Plasma was obtained after centrifugation (6 min at 3000 × g) immediately after blood collection. The bromisoval plasma samples were stored at −20°C until analysis. Urine was collected quantitatively in fractions up to 24 h after bromisoval intake. The end of the collection periods was 3, 4, 5, 6, 7, 8, 9, 10, 15, 12, 15, and 24 h after administration of racemic bromisoval. Of each urine fraction, the volume was measured and an aliquot (10 ml) was stored at −20°C until analysis.

Blood samples (10 ml/sample) for the assessment of GSH (plasma and blood cells) and GSSG (blood cells) levels were collected daily after an overnight fast at approximately 09:00 a.m. from the day before until the last day of (day after for the 24-h ifosfamide infusion) the ifosfamide treatment period. However, in patients 1–6 (Table 1A; 10-day ifosfamide treatment), blood for the GSH assay was collected only on the pretreatment day...
### Table 1 Demographic data of patients with advanced cancers treated with ifosfamide

| Patient | Sex | Age (yr) | Weight (kg) | Height (cm) | Primary tumor | Metastases | Prior treatment | Performance status (WHO) | Ifosfamide total dose (g) | Mesna total dose (g) | GSTM1
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<tbody>
<tr>
<td>A. Continuous ifosfamide infusion of 1300 mg/m² daily for 10 days</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>F</td>
<td>48</td>
<td>91</td>
<td>159</td>
<td>Rectum</td>
<td>Abdomen/other</td>
<td>S, R</td>
<td>1</td>
<td>25.0</td>
<td>27.5</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>45</td>
<td>87</td>
<td>171</td>
<td>Rectum</td>
<td>Abdomen</td>
<td>S, R (5-FU)</td>
<td>1</td>
<td>27.0</td>
<td>29.7</td>
<td>-</td>
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<tr>
<td>3</td>
<td>F</td>
<td>68</td>
<td>63</td>
<td>155</td>
<td>Colon</td>
<td>Liver, muscle</td>
<td>S</td>
<td>1</td>
<td>20.0</td>
<td>20.2</td>
<td>+</td>
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<tr>
<td>4</td>
<td>F</td>
<td>57</td>
<td>72</td>
<td>161</td>
<td>Colon</td>
<td>Liver</td>
<td>S, C (5-FU and levamisole)</td>
<td>0</td>
<td>22.7</td>
<td>25.0</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>45</td>
<td>64</td>
<td>172</td>
<td>Colon/rectum</td>
<td>Liver</td>
<td>S</td>
<td>0</td>
<td>22.8</td>
<td>25.5</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>63</td>
<td>78</td>
<td>171</td>
<td>Stomach (small cell)</td>
<td>Abdomen/liver</td>
<td>C (etoposide)</td>
<td>1</td>
<td>18.5</td>
<td>20.4</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>55</td>
<td>55</td>
<td>178</td>
<td>Unknown</td>
<td>Lymph node (neck)</td>
<td>R</td>
<td>1</td>
<td>22.0</td>
<td>24.2</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>56</td>
<td>65</td>
<td>180</td>
<td>Thyroid</td>
<td>Bone, neck</td>
<td>S, R</td>
<td>1</td>
<td>24.0</td>
<td>26.4</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>57</td>
<td>76</td>
<td>167</td>
<td>Colon</td>
<td>Abdomen (peritoneal)</td>
<td>S, R</td>
<td>1</td>
<td>24.0</td>
<td>25.2</td>
<td>-</td>
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<tr>
<td>10³</td>
<td>M</td>
<td>58</td>
<td>109</td>
<td>194</td>
<td>Rectum</td>
<td>Liver, lung</td>
<td>S, R</td>
<td>1</td>
<td>18.0</td>
<td>19.5</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>M</td>
<td>41</td>
<td>77</td>
<td>178</td>
<td>Colon (appendix)</td>
<td>Skin</td>
<td>S, R (5-FU and leucovorin)</td>
<td>0</td>
<td>25.0</td>
<td>27.5</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>F</td>
<td>57</td>
<td>65</td>
<td>181</td>
<td>Colon/rectum</td>
<td>Lung</td>
<td>S, R</td>
<td>1</td>
<td>21.0</td>
<td>23.1</td>
<td>+</td>
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<tr>
<td>Mean ± SD</td>
<td>53 ± 8</td>
<td>72 ± 11</td>
<td>168 ± 9</td>
<td></td>
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<tr>
<td>Median</td>
<td></td>
<td>55</td>
<td>72</td>
<td>171</td>
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<tr>
<td>B. Continuous ifosfamide infusion of 5000 mg/m²/day for 24 h</td>
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<tr>
<td>101</td>
<td>F</td>
<td>54</td>
<td>77</td>
<td>165</td>
<td>Endometrium</td>
<td>Lung</td>
<td>S, R, C (doxorubicin, cisplatin)</td>
<td>2</td>
<td>9.2</td>
<td>6.9</td>
<td>-</td>
</tr>
<tr>
<td>102³</td>
<td>F</td>
<td>53</td>
<td>64</td>
<td>160</td>
<td>Sarcoma</td>
<td>Lung</td>
<td>S, C (doxorubicin, ifosfamide)</td>
<td>1</td>
<td>8.4</td>
<td>6.0</td>
<td>+</td>
</tr>
<tr>
<td>103</td>
<td>F</td>
<td>56</td>
<td>47</td>
<td>165</td>
<td>Colon</td>
<td>Abdomen/liver</td>
<td>S</td>
<td>1</td>
<td>7.2</td>
<td>5.9</td>
<td>-</td>
</tr>
<tr>
<td>104</td>
<td>M</td>
<td>74</td>
<td>69</td>
<td>172</td>
<td>Head/neck</td>
<td>Abdomen/liver</td>
<td>S</td>
<td>1-2</td>
<td>9.0</td>
<td>6.0</td>
<td>+</td>
</tr>
<tr>
<td>105</td>
<td>M</td>
<td>41</td>
<td>73</td>
<td>184</td>
<td>Pancreas</td>
<td>Abdomen/liver</td>
<td>S</td>
<td>2</td>
<td>9.6</td>
<td>7.4</td>
<td>-</td>
</tr>
<tr>
<td>106</td>
<td>F</td>
<td>67</td>
<td>68</td>
<td>155</td>
<td>Thyroid</td>
<td>Lung, skin</td>
<td>S, R, C (epirubicin, doxorubicin)</td>
<td>1</td>
<td>8.2</td>
<td>6.2</td>
<td>+</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>58 ± 12</td>
<td>66 ± 10</td>
<td>167 ± 10</td>
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<td>Median</td>
<td></td>
<td>55</td>
<td>69</td>
<td>165</td>
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<tr>
<td>C. 4-h ifosfamide infusion of 3000 mg/m² daily for 3 days</td>
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<td>301</td>
<td>F</td>
<td>51</td>
<td>74</td>
<td>177</td>
<td>Unknown</td>
<td>Liver</td>
<td>0</td>
<td>1</td>
<td>17.3</td>
<td>10.4</td>
<td>+</td>
</tr>
<tr>
<td>303</td>
<td>F</td>
<td>54</td>
<td>65</td>
<td>178</td>
<td>Head/neck</td>
<td>Skin (stoma)</td>
<td>S, R</td>
<td>2</td>
<td>16.2</td>
<td>16.2</td>
<td>+</td>
</tr>
<tr>
<td>304³</td>
<td>F</td>
<td>47</td>
<td>52</td>
<td>163</td>
<td>Head/neck</td>
<td>Skin</td>
<td>S, R</td>
<td>2</td>
<td>13.8</td>
<td>8.8</td>
<td>-</td>
</tr>
<tr>
<td>305</td>
<td>M</td>
<td>51</td>
<td>51</td>
<td>171</td>
<td>Head/neck</td>
<td>Skin</td>
<td>S, R</td>
<td>2</td>
<td>14.7</td>
<td>8.8</td>
<td>+</td>
</tr>
<tr>
<td>306</td>
<td>F</td>
<td>60</td>
<td>58</td>
<td>170</td>
<td>Breast</td>
<td>Abdomen, bone</td>
<td>C³</td>
<td>1</td>
<td>15.0</td>
<td>9.0</td>
<td>+</td>
</tr>
<tr>
<td>308</td>
<td>M</td>
<td>60</td>
<td>50</td>
<td>174</td>
<td>Head/neck</td>
<td>Abdomen</td>
<td>S</td>
<td>1</td>
<td>14.4</td>
<td>5.8</td>
<td>+</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>54 ± 5</td>
<td>58 ± 10</td>
<td>172 ± 5</td>
<td></td>
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<tr>
<td>Median</td>
<td></td>
<td>53</td>
<td>55</td>
<td>173</td>
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³ GSTM1 phenotype: +, GSTM1 nondeficient; -, GSTM1 deficient.
³ Female: M, male: S; surgery: R; radiation; C, chemotherapy; 5-FU, 5-fluouracil; C, cyclophosphamide.
³ The ifosfamide infusion was stopped on day 7 of the ifosfamide treatment because of neurotoxicity. The average total ifosfamide and mesna dose were calculated without patient 10.
³ Patient 102 refused to participate in the bromosoval study on the ifosfamide pretreatment day.
³ Patient 304 did not receive bromosoval because of the presence of a jejunal fistula.
³ FEC (5-FU, epirubicin, cyclophosphamide) and hormonal therapy (tamoxifen, aminoglutethimide, hydrocortisone).
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both the first and second course of the ifosfamide therapy. (The
treatment on a subsequent course, OSH blood cell levels of
different ifosfamide treatment schedules on GSH levels. To get
after the results of the first six patients were available; daily
The decision to extend the OSH sampling scheme was made
in both the first and second course of the ifosfamide therapy. (The
second course started approximately 5 weeks after the end of
In addition, in both patients 306 (first and second
course of ifosfamide treatment) and 308, blood for the assess-
ments of GSH blood cell levels was collected more frequently,
(i.e.), prior to and at 2, 4, 6, and 8 h after the start of each
ifosfamide infusion. The latter scheme would, in addition to
the daily GSH sampling, also give an impression on the effects
of ifosfamide treatment within the separate treatment days. After
collection, the blood samples were immediately pretreated and
stored at −80°C until analysis (see below). A separate blood
sample was always collected along with the daily GSH sample
for the assessment of Hb and hematocrit values, to enable the
calculation of GSH and GSSG blood cell concentrations (see
below). Blood (approximately 500 μl) for the assessment of the
GSTM1 phenotype was collected only on the ifosfamide pre-
treatment day (day 0) (collected along with the first GSH sample).

Analytical Methods

(R)- and (S)-Bromisoval Enantiomers (Plasma) and Their Corresponding Mercapturates (Urine). (R)- and (S)-
bromisoval enantiomers in plasma were separated by normal
phase HPLC on a chiral column (Chiralcel OD column; Daicel
Industries, Tokyo, Japan) and detected spectrophotometrically
at 210 nm (22).

The diastereomeric bromisoval mercapturates were as-
sayed by direct injection (after dilution) on a reversed phase
HPLC using a bromine generation cell and electrochemical
detection (23). Because human urine, compared with rat urine,
contains compounds which interfere with the chromatography
of the mercapturates, the eluent was slightly changed. It con-
sisted of buffer (0.1 m sodium nitrate, 0.01 m potassium bromide,
0.01 m citric acid, and 11 μm sodium decane-1-sulfonic acid)
mixed with methanol and acetonitrile (180:9:9).

GSH (Plasma and Blood Cells) and GSSG (Blood
Cells). Immediately after collection of the blood sample, 100
μl blood were added to 1.9 ml 1 mM EDTA, after which 2 ml 0.8
M perchloric acid were added to the blood/EDTA mixture. After
centrifugation (6 min, 3000 × g) of the remaining blood sample,
plasma was also diluted with 0.8 M perchloric acid (1:1). All
pretreated samples were frozen in liquid nitrogen as soon as
possible (within 30 min after sample collection) and stored at
−80°C until analysis (within 1 month after collection of the
sample).

The GSH (plasma and blood) and GSSG (blood) concen-
trations were assayed on a reversed phase HPLC using a bro-
mine generation system and electrochemical detection (23). The
eluents consisted of buffer (0.1 mM EDTA disodium salt, 0.1 m
sodium nitrate, 0.01 m potassium bromide, and 2.5 mM sodium
octane-1-sulfonic acid) mixed with 11.8 mM acetic acid and meth-
anol (175:1:4). The pH of the eluent was adjusted to 2.4.

After centrifugation of the pretreated plasma GSH samples,
the supernatant was directly injected on the HPLC. For the
assessment of blood GSH concentrations, pretreated blood sam-
pies were 25-fold diluted with mobile phase and centrifugated:
the supernatant was injected on the HPLC. The additional
dilution with mobile phase was not necessary for the GSSG analy-
ysis. All blood (GSH and GSSG) and plasma (GSH) analyses
were performed in duplicate. Quantification was performed
using external standards (GSH and GSSG solutions in water
diluted with perchloric acid for plasma, mobile phase for GSH
blood, and EDTA/perchloric acid (1:1) for GSSG blood analy-
sis) which were freshly prepared prior to the analysis of a
sample (in duplicate). GSH and GSSG levels are expressed as
μmol/g Hb. Because RBCs are the most predominant blood
cells, the GSH and GSSG blood cell levels presented in this
article will mainly reflect those in RBCs. The expression of
GSH/GSSG levels in μmol/g Hb was considered to be necessary
because: (a) the largest amount of GSH in blood is present in
blood cells (μm versus μm range for cells and plasma, respec-
tively) and (b) all three ifosfamide treatment schedules caused a
statistically significant decrease in Hb and hematocrit values
(see below).

GSTM1 Polymorphism. The GSTM1 phenotype was
determined with an ELISA (MUKIT; Medlabs, Dublin, Ireland)
which required 200 μl whole heparinized blood.

Bromisoval Pharmacokinetic Analysis

The pharmacokinetic analysis was performed with Siphar
(Release 3.3; Simed, Créteil, France) software (the interactive
program for exponential models and urinary data analysis).
Pharmacokinetic parameters were calculated separately accord-
ing to standard procedures (24) for both bromisoval enantiomers
in plasma and both bromisoval mercapturates in urine. All
calculations were performed under the assumption of complete
absorption of the parent compound. The Ci of the mercapturates
of (R)- and (S)-bromisoval was calculated as the ratio of the
cumulative amount of mercapturate excreted in urine and the
AUC of the corresponding bromisoval enantiomer in plasma.
The (S):(R) ratios in plasma were calculated by dividing the
AUC values of both bromisoval enantiomers (either the AUC
extrapolated to infinity or the AUC values up to 3 h after
administration). The (R):(S) ratios in urine were calculated by
dividing the cumulative amount of the (R)- and (S)-bromisoval
mercapturates in urine (cumulative amount excreted in 24 h).

Statistical Analysis

The statistical analysis was performed with SPSS/PCT
(Release 5.0; SPSS Inc., Chicago, IL) statistical software. Pa-
rameters are reported as mean ± SD. For each ifosfamide
treatment schedule, the bromisoval pharmacokinetics of the
ifosfamide pretreatment day were compared with those of the
last ifosfamide treatment day (day after for the 24-h ifosfamide
treatment schedule) by paired t tests. The significance level was
set at α = 0.05.
Fig. 1. GSH levels (mean ± SD) in plasma (upper panels) and blood cells (lower panels) of the patients receiving a continuous ifosfamide infusion of 1300 mg/m² daily for 10 days (n = 11). GSH levels of patient 10 are shown separately: the ifosfamide infusion in this patient was stopped on day 7 (i.e., after 6 days of treatment) because of neurotoxicity. Left panels, average GSH concentrations of all patients on the pretreatment day (day 0), day 7, and the last ifosfamide treatment day (day 10). Daily monitoring of GSH levels during the ifosfamide treatment was started from patient 7 onward (right panels). *, P < 0.05. Left panels, □, mean (n = 11); □, patient 10. Right panels, ●, mean (n = 5); ▲, patient 10.

The effect of each ifosfamide treatment schedule on GSH and GSSG concentrations in blood cells and plasma were analyzed by two-way ANOVA using subjects as a random and days as a fixed factor. The significance level was set at α = 0.05. To maintain the probability of type I error at 0.05, a subsequent analysis, comparison of each day to the pretreatment day (day 0), was only performed if the analysis of variance showed significant differences between days.

RESULTS

Clinical Effects

All ifosfamide treatment schedules were reasonably well tolerated by all patients. Remarkably, all three ifosfamide treatment schedules caused a significant decrease in Hb values (P < 0.001 for the 10- and 24-h treatment and P < 0.05 for the 3-day ifosfamide treatment): the decreases in Hb were significant from day 2 (1300 mg/m² for 10 days), day 1 (5000 mg/m² × 24 h), and day 3 (3000 mg/m² for 3 days) onward. The average decreases in Hb levels (expressed as percentage of the pretreatment level) were about 22% for the 10-day, 17% for the 24-h, and 8% for the 3-day ifosfamide treatment schedule.

In patient 10 (1300 mg/m² ifosfamide daily for 10 days), the ifosfamide infusion was stopped on day 7 of the ifosfamide infusion period (i.e., after 6 days of ifosfamide treatment) because of neurotoxicity, which was probably related to the ifosfamide treatment. Neurotoxicity symptoms included confusion, disorientation, inability to concentrate, visual hallucinations, and tremors in the hands and legs. Furthermore, patient 301 (3000 mg/m² ifosfamide daily for 3 days) was suspected of neurotoxicity. However, the neurotoxicity symptoms of this patient, consisting of disorientation and confusion, became manifest after the end of the ifosfamide treatment period.

The concomitant medication of the patients during the ifosfamide treatment period consisted in general of antiemetic, analgesic (e.g., acetaminophen), and anxiolytic (e.g., benzodiazepines) drugs. The antiemetic drugs used were dexamethasone plus granisetron (Kytril) in the 24-h and 3-day ifosfamide treatment groups and metoclopramide (Primperan) or domperidom (Motilium) in the 10-day ifosfamide treatment group.
A 600-mg p.o. dose of racemic bromisoval caused slight sedation in some patients. None of the patients experienced any bromisoval-related adverse events.

**Characterization of the GSH/GST System**

**Glutathione (Blood Cells and Plasma) and the GSTM1 Phenotype**

Both the 10- and the 3-day ifosfamide treatment schedules caused a statistically significant decrease in blood cell GSH levels ($P = 0.03$ and $P < 0.001$, respectively), whereas the 24-h ifosfamide treatment schedule did not have a statistically significant effect on blood cell GSH levels (lower panels of Figs. 1–3 and Table 2). Compared with the levels measured on the pretreatment day, blood cell GSH levels were significantly lowered ($P < 0.05$) from day 2 of the 3-day and day 5 of the 10-day ifosfamide infusion schedule onward. The average decrease in blood cell GSH levels for both treatment schedules was $\approx 35\%$. In patients 306 and 308, receiving the 3-day ifosfamide treatment and in whom blood cell GSH levels were measured more frequently on each ifosfamide study day (see “Patients and Methods”), blood cell GSH levels gradually decreased on each ifosfamide treatment day: the highest levels were measured on day 1 and the lowest levels on day 3, i.e., the last day of the ifosfamide treatment. In addition, during the second course of ifosfamide treatment in patient 306, the height of the blood cell GSH levels and the profile of its decrease on each ifosfamide treatment day were more or less identical to those measured during the first course of ifosfamide treatment.

The three ifosfamide treatment schedules did not have a statistically significant effect on plasma GSH and blood cell GSSG levels. A tendency for plasma GSH levels to decrease during the ifosfamide treatment period seemed to be present in the 3-day ifosfamide treatment group (upper panels of Figs. 1–3, and Table 2).

Of all patients studied, 46% were deficient in GSTM1 isoenzymes (50% of the patients receiving the 10-day and 24-h treatment and 33% of the patients receiving the 3-day ifosfamide treatment; Table 1).

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**Fig. 2** GSH levels (mean ± SD) in plasma (upper panel) and blood cells (lower panel) of the patients receiving a continuous ifosfamide infusion of 5000 mg/m$^2$/day for 24 h ($n = 6$). Day 1 is the ifosfamide treatment day.

**Fig. 3** GSH levels (mean ± SD) in plasma (upper panel) and blood cells (lower panel) of the patients receiving a 4-h ifosfamide infusion of 3000 mg/m$^2$ daily for 3 days ($n = 6$). GSH levels were measured daily from the pretreatment day (day 0) up to the last ifosfamide treatment day (day 3). *, $P < 0.05$ compared to pretreatment.
tween 30 and 70%. GSH levels than a relatively short 24-h treatment with a higher

The plasma pharmacokinetics of both bromisoval enantiomers were highly variable on both bromisoval study days: the coefficient of variation of all pharmacokinetic parameters generally ranged between 30 and 70%.

**DISCUSSION**

**GSH Concentrations in Blood Cells.** In the present study, the effects of ifosfamide treatment on the GSH/GST system in cancer patients were studied. Statistically significant decreases of about 35% were observed for blood cell GSH levels in the patients receiving the 3- and 10-day ifosfamide treatments. In the patients receiving the 24-h ifosfamide treatment, blood cell GSH levels were hardly affected. Therefore, ifosfamide treatment of longer duration with a lower daily dose is associated with a more pronounced decrease in blood cell GSH levels than a relatively short 24-h treatment with a higher

**Urinary Excretion.** The urinary excretion of bromisoval mercapturates on the pretreatment day was not statistically significantly different from that on the last study day (day 10) in the patients receiving the 10-day ifosfamide treatment (Table 4A). For the 24-h and 3-day ifosfamide treatments, the cumulative amount excreted of the mercapturate derived from the (S)-bromisoval was significantly lower on the last day compared to the first bromisoval study day (Table 4. B and C). In addition, the (R):(S) ratio of the patients receiving the 24-hour ifosfamide treatment was significantly higher on the last bromisoval study day (Table 4B).

For all ifosfamide treatment schedules, similar tendencies could be observed in most of the individual urinary excretion profiles of the bromisoval mercapturates: the cumulative amount excreted of both bromisoval mercapturates as well as the total amount of mercapturates excreted in 24 h tended to be lower on the last day compared to the first bromisoval study day. Furthermore, the (R):(S) ratios tended to be increased on the last bromisoval study day. It should be mentioned that, like for the plasma pharmacokinetic parameters, all urinary excretion parameters of the mercapturates derived from both bromisoval enantiomers were highly variable on both bromisoval study days (coefficient of variation values in general <40%, except the values for the clearance of formation), although this variation seemed to be less than that observed in the plasma pharmacokinetic parameters.

**Bromisoval Pharmacokinetics and GSH Levels in the Neurotoxicity Patient**

In patient 10, receiving the 10-day ifosfamide treatment schedule, the ifosfamide infusion was stopped after 6 days (i.e., on day 7) of treatment because of neurotoxicity. Therefore, the bromisoval pharmacokinetics could only be assessed on the pretreatment day (day 0). Blood cell/plasma GSH and GSSG levels were measured from day 0 until day 9, i.e., up to 3 days after the end of the ifosfamide treatment.

On day 0, the plasma elimination pharmacokinetics of bromisoval enantiomers and the urinary excretion of their corresponding mercapturates were not different from those of the other patients. On the other hand, both plasma and blood cell GSH levels decreased: plasma levels from 16 μmol/L (day 0) to 3 μmol/L (day 9) and blood cell levels from 12 μmol/g Hb (day 0) to 3.5 μmol/g Hb (day 9; Fig. 1). The blood cell GSSG levels of this patient were not affected by the ifosfamide treatment, and they were also not different from those observed in the other patients (Table 2). Patient 10 was not deficient in GSTM1 isoenzymes (Table 1A).

**Table 2 GSH levels [mean ± SD (range)] in plasma and blood cells of patients receiving ifosfamide therapy**

<table>
<thead>
<tr>
<th>Ifosfamide treatment (mg/m² × time)</th>
<th>Patients (n)</th>
<th>Day</th>
<th>GSHp (μmol/L)</th>
<th>GSHb (μmol/g Hb)</th>
<th>GSSGb (μmol/g Hb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1300 × 10 days</td>
<td>11</td>
<td>0</td>
<td>6.8 ± 3.2</td>
<td>8.8 ± 3.0</td>
<td>0.19 ± 0.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(2.9-10.9)</td>
<td>(3.1-12.5)</td>
<td>(0.04-0.37)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>9.3 ± 3.1</td>
<td>6.4 ± 1.7</td>
<td>0.15 ± 0.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(2.4-20.1)</td>
<td>(3.6-8.8)</td>
<td>(0.05-0.32)</td>
</tr>
<tr>
<td>5000 × 24 h</td>
<td>6</td>
<td>0</td>
<td>5.4 ± 4.4</td>
<td>8.2 ± 2.6</td>
<td>0.18 ± 0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.4-11.8)</td>
<td>(5.7-11.7)</td>
<td>(0.13-0.27)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>6.2 ± 9.9</td>
<td>8.9 ± 2.7</td>
<td>0.18 ± 0.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.5-26.2)</td>
<td>(6.1-13.9)</td>
<td>(0.10-0.38)</td>
</tr>
<tr>
<td>3000 × 3 days</td>
<td>6</td>
<td>0</td>
<td>5.9 ± 3.2</td>
<td>11.9 ± 2.6</td>
<td>0.19 ± 0.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(2.9-10.0)</td>
<td>(9.8-16.9)</td>
<td>(0.09-0.44)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>3.1 ± 2.2</td>
<td>7.8 ± 3.1</td>
<td>0.15 ± 0.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.8-5.6)</td>
<td>(5.8-13.6)</td>
<td>(0.04-0.32)</td>
</tr>
</tbody>
</table>

- GSHp, GSH in plasma; GSHb and GSSGb, GSH and GSSG in blood cells, respectively.
- Without patient 10, dropout because of neurotoxicity. The ifosfamide infusion in this patient was stopped on day 7, i.e., after 6 days of treatment.
- Both the 10- and 3-day ifosfamide treatment schedules caused a statistically significant decrease in GSH blood cell levels (P = 0.03 and P < 0.001, respectively). Compared with the levels on the pretreatment day, the decrease was significant from day 2 of the 3-day and day 5 of the 10-day ifosfamide infusion onward.

**Bromisoval Pharmacokinetics**

In all patients, irrespective of the ifosfamide treatment schedule and bromisoval study day, the pharmacokinetics of bromisoval enantiomers was more or less similar to those observed in healthy male volunteers (21): the plasma elimination and urinary excretion of (R)-bromisoval and its mercapturate, respectively, were always higher than those of (S)-bromisoval and its mercapturate.

**Plasma Pharmacokinetics.** In the 24-hour and 3-day ifosfamide treatment groups, the bromisoval plasma pharmacokinetics on day 0, i.e., the ifosfamide pretreatment day, were not statistically significant different from those on day 2 (after the 24-h ifosfamide treatment) and day 3 (last day of the 3-day ifosfamide treatment), respectively (Table 3, B and C). Significant (P < 0.05) differences between the plasma pharmacokinetics on the pretreatment day and last day of the ifosfamide treatment only occurred for the 10-day ifosfamide treatment: the Cmax of both (R)- and (S)-bromisoval and the AUC(∞) of (S)-bromisoval were significantly decreased, whereas the p.o. CI/F of the (S)-enantiomer was significantly increased on day 10 compared to day 0 (Table 3A).

Similar tendencies could be observed in the individual bromisoval plasma pharmacokinetic profiles of both enantiomers for, in particular, the 3- and 10-day ifosfamide treatment schedules: Cmax and AUC(∞) values as well as (S):(R)-ratios [AUC(∞) and AUC(∞)_p.o.] tended to be decreased, whereas p.o. CI/F values tended to be increased on the last day compared to the first bromisoval study day. However, the plasma pharmacokinetic parameters of both bromisoval enantiomers were highly variable on both bromisoval study days: the coefficient of variation of all pharmacokinetic parameters generally ranged between 30 and 70%.

**DISCUSSION**

**GSH Concentrations in Blood Cells.** In the present study, the effects of ifosfamide treatment on the GSH/GST system in cancer patients were studied. Statistically significant decreases of about 35% were observed for blood cell GSH levels in the patients receiving the 3- and 10-day ifosfamide treatments. In the patients receiving the 24-h ifosfamide treatment, blood cell GSH levels were hardly affected. Therefore, ifosfamide treatment of longer duration with a lower daily dose is associated with a more pronounced decrease in blood cell GSH levels than a relatively short 24-h treatment with a higher
Ifosfamide and Glutathione in Cancer Patients

Table 3: Pharmacokinetic parameters (mean ± SD) of the bromisoval enantiomers in plasma of patients receiving ifosfamide therapy

<table>
<thead>
<tr>
<th>Day</th>
<th>$C_{max}$ (μmol/liter)</th>
<th>$t_{max}$ (min)</th>
<th>AUC(0→t) (mmol/liter·min)</th>
<th>C/F</th>
<th>$t_{1/2}$ (min)</th>
<th>MRT (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Continuous ifosfamide infusion of 1300 mg/m² daily for 10 days*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(R)-bromisoval</td>
<td>0</td>
<td>1.4 ± 0.7</td>
<td>91 ± 99</td>
<td>0.16 ± 0.10</td>
<td>11.5 ± 6.5</td>
<td>60 ± 35</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.8 ± 0.5</td>
<td>142 ± 75</td>
<td>0.12 ± 0.12</td>
<td>20.6 ± 14.9</td>
<td>45 ± 31</td>
</tr>
<tr>
<td>(S)-bromisoval</td>
<td>0</td>
<td>11.4 ± 5.2</td>
<td>118 ± 91</td>
<td>2.28 ± 2.78</td>
<td>0.66 ± 0.26</td>
<td>121 ± 44</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>6.2 ± 4.1</td>
<td>154 ± 91</td>
<td>1.05 ± 0.41</td>
<td>1.53 ± 0.74</td>
<td>112 ± 81</td>
</tr>
<tr>
<td>(S):(R) ratio</td>
<td>AUC(0→t)</td>
<td>AUC(0→1h)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>17.6 ± 5.7</td>
<td>126 ± 6.2</td>
<td>17.6 ± 5.7</td>
<td>126 ± 6.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>15.1 ± 9.7</td>
<td>91 ± 7.4</td>
<td>15.1 ± 9.7</td>
<td>91 ± 7.4</td>
<td></td>
</tr>
<tr>
<td>B. Continuous ifosfamide infusion of 5000 mg/m²/day for 24 hf</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(R)-bromisoval</td>
<td>0</td>
<td>3.0 ± 4.0</td>
<td>96 ± 78</td>
<td>0.22 ± 0.23</td>
<td>12.5 ± 9.5</td>
<td>41 ± 6</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2.2 ± 1.0</td>
<td>90 ± 60</td>
<td>0.20 ± 0.04</td>
<td>6.9 ± 1.5</td>
<td>53 ± 28</td>
</tr>
<tr>
<td>(S)-bromisoval</td>
<td>0</td>
<td>11.7 ± 6.6</td>
<td>114 ± 68</td>
<td>2.57 ± 1.23</td>
<td>0.66 ± 0.37</td>
<td>145 ± 53</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>9.6 ± 4.7</td>
<td>114 ± 80</td>
<td>2.14 ± 0.98</td>
<td>0.86 ± 0.68</td>
<td>130 ± 18</td>
</tr>
<tr>
<td>(S):(R) ratio</td>
<td>AUC(0→t)</td>
<td>AUC(0→1h)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>17.0 ± 6.2</td>
<td>8.6 ± 3.9</td>
<td>17.0 ± 6.2</td>
<td>8.6 ± 3.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>10.6 ± 4.5</td>
<td>6.4 ± 2.6</td>
<td>10.6 ± 4.5</td>
<td>6.4 ± 2.6</td>
<td></td>
</tr>
<tr>
<td>C. 4-h ifosfamide infusion of 3000 mg/m² daily for 3 daysf</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(R)-bromisoval</td>
<td>0</td>
<td>1.6 ± 0.8</td>
<td>95 ± 62</td>
<td>0.27 ± 0.10</td>
<td>5.5 ± 1.6</td>
<td>85 ± 37</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1.3 ± 0.7</td>
<td>150 ± 99</td>
<td>0.21 ± 0.09</td>
<td>7.4 ± 3.5</td>
<td>76 ± 56</td>
</tr>
<tr>
<td>(S)-bromisoval</td>
<td>0</td>
<td>8.1 ± 4.7</td>
<td>124 ± 43</td>
<td>1.96 ± 1.25</td>
<td>0.94 ± 0.52</td>
<td>99 ± 20</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>5.8 ± 2.3</td>
<td>162 ± 66</td>
<td>1.07 ± 0.40</td>
<td>1.37 ± 0.38</td>
<td>85 ± 22</td>
</tr>
<tr>
<td>(S):(R) ratio</td>
<td>AUC(0→t)</td>
<td>AUC(0→1h)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>8.2 ± 5.6</td>
<td>5.9 ± 3.2</td>
<td>8.2 ± 5.6</td>
<td>5.9 ± 3.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>5.5 ± 2.5</td>
<td>5.1 ± 3.5</td>
<td>5.5 ± 2.5</td>
<td>5.1 ± 3.5</td>
<td></td>
</tr>
</tbody>
</table>

* Data of patient 2 (day 0, (R)- and (S)-bromisoval) and patient 9 (day 0, (R)-bromisoval, except $C_{max}$ and $t_{max}$) are missing (due to analytical problems and very low concentrations of (R)-bromisoval, respectively). Data of patient 10 [(R)- and (S)-bromisoval] were not included in the data analysis because the ifosfamide infusion was stopped on day 7 (neurotoxicity).

f $t_{max}$: time to reach $C_{max}$; MRT: mean residence time.

P < 0.05, significantly different compared to day 0, i.e., the ifosfamide pretreatment day.

f n = 5, patients 102 and 304 did not receive bromisoval.

Ifosfamide dose. Because blood cell GSH levels measured daily for 11 days in two healthy adults appeared to be stable (mean ± SD for the whole 11-day study period: 7.6 ± 0.6 and 5.8 ± 1.1 μmol/g Hb, respectively),5 the decrease observed in the patients receiving the 3- or 10-day ifosfamide treatment is likely to be caused by ifosfamide and/or its metabolites.

The data obtained in patients 306 and 308 (3-day ifosfamide treatment) support that the decrease in blood cell GSH levels is related to ifosfamide treatment: blood cell GSH levels of these two patients decreased gradually on each ifosfamide treatment day, and the height of and decrease in blood cell GSH levels were very similar during the first and second ifosfamide treatment course (patient 306 only). The latter also indicates that blood cell GSH levels were recovered from a decrease of about 40% in the period (i.e., about 5 weeks) between both ifosfamide treatment courses.

The absence of effects caused by the 24-h ifosfamide treatment on blood cell GSH levels seems to be in contrast to results obtained by Lind et al. (15). They reported a decrease of GSH to about 30% of its original value in lymphocytes isolated from one patient undergoing an 8-h ifosfamide infusion of 5 g/m² along with an equal dose of the uroprotector mesna. Apart from the fact that this observation was made in only one patient, assay differences may have contributed to this observed discrepancy: Lind et al. (15) used the monochlorobimane method to measure cellular GSH levels. However, GSH quantification by this assay is dependent on the GST isoenzyme composition of cells/tissues (25) and may therefore not accurately estimate the "real" GSH levels of lymphocytes.

Plasma GSH and blood cell GSSG levels were not significantly affected by any of the three ifosfamide infusion schedules. This may be partially related to the large variation observed in these levels: the coefficients of variation on each study day (irrespective of the treatment) were about 60% for both plasma GSH and blood cell GSSG levels compared to 30% for blood cell GSH levels. Because GSH concentrations in plasma are much lower than those in blood cells (µM versus mM range, respectively), a small extent of hemolysis may have a pronounced effect on the plasma GSH levels. Recently, it was even demonstrated that hemolysis during sample collection is inevitable and contributed on average 25% to "plasma" GSH (26).

Information about tissue or organ GSH availability in patients in vivo can in general only be obtained indirectly by monitoring of GSH levels in blood (cells)/plasma. However, it still remains to be established whether GSH levels in blood cells are related to those in tumors or other tissues. So far, a correlation has only been described between GSH levels in plasma and liver of rats and liver disease patients (27, 28). Furthermore, several factors may contribute to the intra- and interpatient variability in blood GSH levels: these include the nutritional

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status of the patients [in particular intake of ascorbate (29–31)], age (32), the presence of (additional) disease states associated with changes in GSH availability (e.g., disturbances in liver and thyroid function; Refs. 28 and 33–35), intra- and interpatient differences in concomitant medication (e.g., paracetamol), physical activity (36), and interpatient differences in expression of \( \gamma \)-glutamylcysteine synthetase (the rate-limiting enzyme in GSH biosynthesis; Ref. 37).

### GSH Conjugation Activity as Assessed by Bromisoval Pharmacokinetics

<table>
<thead>
<tr>
<th>Day</th>
<th>( t_{1/2} ) (min)</th>
<th>( \Sigma \text{Ae} ) (( \mu \text{mol} ))</th>
<th>( \Sigma \text{Ae} ) (% dose)</th>
<th>( \text{Cl}_\text{e}^b ) (liter/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Continuous ifosfamide infusion of 1300 mg/m(^2) daily for 10 days (^a)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mercapturate derived from (R)-bromisoval</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>99 ± 26</td>
<td>762 ± 162</td>
<td>28.3 ± 6.0</td>
<td>6.56 ± 3.88</td>
</tr>
<tr>
<td>10</td>
<td>124 ± 57</td>
<td>700 ± 192</td>
<td>26.0 ± 7.1</td>
<td>9.35 ± 4.44</td>
</tr>
<tr>
<td>Mercapturate derived from (S)-bromisoval</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>122 ± 37</td>
<td>176 ± 42</td>
<td>6.56 ± 1.57</td>
<td>0.09 ± 0.04</td>
</tr>
<tr>
<td>10</td>
<td>168 ± 88</td>
<td>163 ± 125</td>
<td>6.07 ± 4.66</td>
<td>0.16 ± 0.10</td>
</tr>
<tr>
<td>( \Sigma \text{Ae} ) total (% dose)</td>
<td></td>
<td></td>
<td>(R):(S) ratio</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>34.9 ± 7.3</td>
<td></td>
<td>4.42 ± 0.80</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>32.1 ± 10.3</td>
<td></td>
<td>5.18 ± 1.61</td>
<td></td>
</tr>
<tr>
<td>B. Continuous ifosfamide infusion of 5000 mg/m(^2)/day for 24 h (^b)</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Mercapturate derived from (R)-bromisoval</td>
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<td></td>
<td></td>
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<tr>
<td>0</td>
<td>138 ± 30</td>
<td>686 ± 239</td>
<td>25.5 ± 8.9</td>
<td>6.31 ± 4.10</td>
</tr>
<tr>
<td>2</td>
<td>109 ± 13</td>
<td>632 ± 145</td>
<td>23.5 ± 5.4</td>
<td>3.15 ± 0.58</td>
</tr>
<tr>
<td>Mercapturate derived from (S)-bromisoval</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>163 ± 15</td>
<td>201 ± 45</td>
<td>7.46 ± 1.66</td>
<td>0.10 ± 0.06</td>
</tr>
<tr>
<td>2</td>
<td>148 ± 30</td>
<td>132 ± 18 (^d)</td>
<td>4.89 ± 0.67 (^d)</td>
<td>0.08 ± 0.05</td>
</tr>
<tr>
<td>( \Sigma \text{Ae} ) total (% dose)</td>
<td></td>
<td></td>
<td>(R):(S) ratio</td>
<td></td>
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<tr>
<td>0</td>
<td>33.0 ± 10.3</td>
<td></td>
<td>3.37 ± 0.79</td>
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<tr>
<td>2</td>
<td>28.4 ± 5.0</td>
<td></td>
<td>4.95 ± 1.55 (^d)</td>
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<tr>
<td>C. 4-h ifosfamide infusion of 3000 mg/m(^2) daily for 3 days (^c)</td>
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<td>Mercapturate derived from (R)-bromisoval</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>111 ± 20</td>
<td>700 ± 30</td>
<td>26.0 ± 1.1</td>
<td>2.86 ± 0.91</td>
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<tr>
<td>3</td>
<td>127 ± 42</td>
<td>675 ± 130</td>
<td>25.1 ± 4.8</td>
<td>3.61 ± 1.58</td>
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<tr>
<td>Mercapturate derived from (S)-bromisoval</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>166 ± 21</td>
<td>185 ± 37</td>
<td>6.88 ± 1.37</td>
<td>0.12 ± 0.05</td>
</tr>
<tr>
<td>3</td>
<td>170 ± 71</td>
<td>112 ± 47 (^d)</td>
<td>4.17 ± 1.74 (^d)</td>
<td>0.12 ± 0.07</td>
</tr>
<tr>
<td>( \Sigma \text{Ae} ) total (% dose)</td>
<td></td>
<td></td>
<td>(R):(S) ratio</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>32.9 ± 2.5</td>
<td></td>
<td>3.89 ± 0.66</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>29.3 ± 5.7</td>
<td></td>
<td>7.08 ± 3.42</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Data of patient 10 not included (dropout on day 7 because of neurotoxicity).

\(^b\) Data of patients 2 (day 0, (R)- and (S)-bromisoval) and 9 (day 0, (R)-bromisoval) were not included because of missing AUC data.

\(^c\) \( n = 5 \); patients 102 and 304 did not receive bromisoval.

\(^d\) \( p < 0.05 \), significantly different compared to day 0, i.e., the ifosfamide pretreatment day.

In all three treatment groups, i.e., irrespective of the ifosfamide infusion schedule, the plasma elimination pharmacokinetics of (R)- and (S)-bromisoval and urinary excretion of their corresponding mercapturates were not significantly affected by the ifosfamide treatment, although certain trends could be observed. The bromisoval pharmacokinetics of cancer patients on the ifosfamide pretreatment day were even comparable to those assessed in healthy male volunteers (21), which indicates that GSH conjugation of bromisoval enantiomers in these cancer patients \( \text{in vivo} \) was also not affected by their disease state. Because bromisoval pharmacokinetics are not influenced by the presence or absence of GSTM1 isoenzymes (21), differences in GSTM1 phenotype will not have influenced the present results. Assuming that the kinetics of the probe drug indeed provide an accurate reflection of GSH enzyme activity, the bromisoval data therefore suggest that GST activity remains unchanged and that...
the GSH availability of the cancer patients was not rate-limiting in GSH conjugation of bromisoval enantiomers.

Comparison of the individual pharmacokinetic profiles of the bromisoval enantiomers and their mercapturates on both study days revealed similar tendencies for all patients, in particular those receiving the 3- and 10-day ifosfamide infusion schedules: the \( C_{\text{max}} \) and AUC values were lower and, correspondingly, the oral CI/F was higher on the last day compared to the first bromisoval study day. Assuming complete absorption of bromisoval enantiomers on both bromisoval study days, these changes in bromisoval pharmacokinetics may be caused by (slightly) increased activity of specific GST isoenzymes. If ifosfamide and/or its metabolites cause increases in the activity of specific GST isoenzymes (and/or changes in GST profiles) after long-term exposure, this may lead to development of drug resistance toward specific cytostatic agents (including ifosfamide) for which the GSH/GST system is involved in bioactivation.

For all three ifosfamide treatment schedules, the cumulative amount of mercapturates excreted tended to be lower on the last day compared to the first bromisoval study day, which does not seem to be in agreement with the apparently higher p.o. CI/F of both bromisoval enantiomers. However, because the second bromisoval study day coincided with the last day of the ifosfamide treatment for the 3- and 10-day treatment schedules, an influence of ifosfamide and its metabolites on the bromisoval pharmacokinetics on this study day cannot be excluded. In the patients receiving the 24-h ifosfamide treatment, an interaction between ifosfamide (metabolites) and bromisoval on the day after the ifosfamide treatment can also not be ruled out [plasma \( t_{1/2} \) of ifosfamide after i.v. administration of 5 g/m²/day for 24 h: \( \approx 4.5 \) hours (39)].

Changes in blood cell GSH levels did not seem to affect GSH conjugation of bromisoval enantiomers on the second bromisoval study day. This may be related to the presence of a low \( K_{\text{m}} \) of GST isoenzymes for GSH: in rats, 70–80% of hepatic GSH must be depleted before the GSH availability becomes rate limiting for in vivo GSH conjugation (40). A decrease in blood cell GSH levels of approximately 70% was observed in the patient (patient 10) exhibiting severe neurotoxicity. However, the clinical condition of this patient did not allow bromisoval administration on the last day of the ifosfamide treatment, and, consequently, the effects of this possibly rate-limiting decrease in GSH blood cell levels on the in vivo GSH conjugation of bromisoval enantiomers could not be studied. Nevertheless, this large decrease in GSH blood cell (and plasma) levels may have contributed to the neurotoxicity experienced by this patient. Renal dysfunction, as detected by elevated serum creatinine levels (>200 μM/liter; range, 70–133 μM/liter), may also have been a contributing factor (6).

Concomitant Medication and Autoinduction of Ifosfamide Metabolism. Ifosfamide requires biotransformation by cytochrome P450 isoenzymes to exert its alkylating action. Recently, studies using human liver microsomes revealed the involvement of CYP3A4 as the major cytochrome P450 responsible for both ifosfamide 4-hydroxylation (bioactivation) and \( N \)-dechloroethylation (a detoxification pathway; Refs. 41 and 42). In this respect, it is important to mention that the CYP3A inducer dexamethasone was administered along with granisetron (Kytril) as antiemetic therapy in almost all patients receiving the 24-h and 3-day ifosfamide treatment schedules. Whether the administration of dexamethasone had any effect on the therapeutic efficacy of ifosfamide is not clear. Nevertheless, the administration of dexamethasone may have contributed, by increasing the presence of ifosfamide metabolites, to the observed decrease in GSH blood cell levels in the patients receiving the 3-day ifosfamide treatment. Furthermore, the possible occurrence of autoinduction of ifosfamide metabolism in the course of ifosfamide therapy (4, 43) may (partially) explain why only the longer ifosfamide treatment schedules, i.e., the 3- and 10-day treatment regimens, were associated with a significant decrease in blood cell GSH levels.

Conclusions. Characterization of the GSH/GST system in cancer patients in vivo by administering the probe drug bromisoval showed that the influence of all three ifosfamide treatment schedules on the pharmacokinetics of both bromisoval enantiomers was only limited. On the other hand, the two longer treatment schedules, i.e., the 3- and 10-day ifosfamide treatments, were associated with a significant decrease in GSH blood cell levels of approximately 35%. Since lowering of GSH levels may sensitize (resistant) tumors to cytostatic agents, these data suggest that ifosfamide may potentially be used for this purpose during cytostatic therapy. However, whether blood cell GSH levels reflect those in tissues and whether a decrease of 35% is sufficient for sensitization of (resistant) tumors need to be studied in greater detail. Furthermore, it should be mentioned that the complexity of ifosfamide metabolism [dependence on CYP3A isoenzymes for bioactivation, autoinduction of the hepatic oxygenase system, and stereoselectivity in ifosfamide metabolism (4, 44)] may make this GSH-modulating approach of combined administration of ifosfamide and (another) cytostatic agent(s) difficult. In addition, because resistance of tumors to cytostatic therapy [including ifosfamide (45)] is in general multifactorial, modulation of GSH levels will only be effective for those tumors and for those cytostatic treatments for which involvement of the GSH/GST system in resistance is a major contributing factor (14).

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