Cyclophosphamide priming reduces intestinal damage in man following high dose melphalan chemotherapy

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Summary: A small pre-treatment 'priming' dose of cyclophosphamide will reduce gut damage due to high dose i.v. melphalan in mice and sheep but efforts to demonstrate this effect in man have been hampered by difficulty in the measurement of gut damage. We have evaluated the ⁵¹ chromium EDTA absorption test, a new method for measuring intestinal permeability, as a means of assessing damage due to high dose melphalan. The test was reliable, with a narrow normal range, easy to use and well tolerated. It detected an increase in intestinal permeability after high dose melphalan with a maximum occurring between 9 and 15 days after treatment and subsequently returning to normal. It was shown in 19 patients that a pre-treatment dose of cyclophosphamide was capable of significantly reducing the abnormalities in intestinal permeability which resulted from high dose melphalan.

Methods and patients

Informed consent was obtained. Patients fasted from midnight and emptied their bladders before the test. A sample of this urine was used to measure background activity. At 9 am ⁵¹ chromium EDTA (specific activity 37 MBq 10 ml⁻¹, Amersham, Bucks; half life 27.7 days) prepared to activity of 4 MBq was drunk by the patient in a tasteless, odourless drink followed by ~100 ml of water. An aliquot of the ⁵¹ chromium EDTA solution was removed to serve as a standard. Patients then fasted for a further 2 h, after which they could eat and drink freely. Urine was collected for 24 h. The total volume of urine was noted and aliquots of 4 ml each were counted on a Kontron gamma counter for 10 min. The percentage of ⁵¹ chromium EDTA excreted over 24 h was then calculated from the formula:

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\text{cpm urine} \times \frac{\text{weight of standard}}{\text{cpm standard}} \times \frac{\text{weight of dose}}{\text{volume measured standard}} \times \frac{\text{volume of diluted sample}}{100}. \]

Creatinine was measured in the same urine sample and a simultaneous blood sample. Creatinine clearance was calculated to estimate renal function.

Thirty-three cancer patients (18 M; 15 F) of mean age 32 yrs (range 10–59 yrs) who had received no cytotoxic treatment for at least 1 month formed the control population. Three of these gave a history of heavy alcohol intake immediately before testing and they were subsequently excluded from the control group (Draper et al., 1983). In three patients, repeated tests were possible before any treatment was given and the results compared.

We applied the test sequentially at 5–8-day intervals in two groups of patients:

1. Nineteen patients (Table I) treated with a single injection of melphalan, i.v. with autologous bone marrow grafting. Fifteen patients were randomly allocated either to receive melphalan 200–200 mg m⁻² or to receive a priming dose of cyclophosphamide 300–400 mg m⁻² i.v. followed by melphalan 200–220 mg m⁻² seven days later. Four patients received 180 mg m⁻² alone.

2. Two patients treated with cyclophosphamide 7 g m⁻² i.v. without bone marrow grafting.
Results

Acceptability

The test was generally well tolerated. The EDTA solution is odourless and colourless and none of the untreated patients were unable to take it. After intensive cytotoxic treatment, two patients were completely unable to tolerate the test because of nausea and mouth soreness. Two patients who are already nauseated by their cytotoxic treatment given 5 days earlier, managed to swallow the $^{51}$Cr EDTA but remained nauseated and vomited a few hours after the test began on this occasion and these results are excluded. They completed the test on all other occasions and those results are included in the analysis. In one patient the test failed on day 15 because of nausea. She was very ill with infection, jaundice and fluid imbalance and subsequently died. The completeness of the urine collections in her case was uncertain and she is excluded from analysis.

Normal range

In 30 untreated patients, the mean 24 h $^{51}$Cr EDTA excretion in urine was 1.7% of administered dose with standard deviation 0.58% (Figure 1). Three other untreated patients gave a history of heavy alcohol intake and their excretion values were 4.1, 5.6 and 5.9%. These three are excluded from our normal range which extends up to 2.9%. In the three untreated patients in whom the test was done twice the results agreed closely.

Duration of excretion of $^{51}$Cr EDTA

In eight patients, urine was collected for 3 days and the daily excretion of $^{51}$Cr EDTA estimated (Figure 2). The excretion fell rapidly and had reached low levels during the third day. However, a significant proportion of the dose was excreted between 24 and 48 h for some patients and so an initial 24 h period of collection may underestimate the total absorption of EDTA in these patients.

Serial collections after cytotoxic treatment

The test was performed sequentially after treatment at intervals of ~5 days.

(i) High dose melphalan (180–220 mg m$^{-2}$) with autologous marrow grafting. The test became abnormal after one week and returned to normal after two weeks (Figure 3). The data suggest that the time of maximum increase in gut permeability was at about day 9. All of these patients experienced diarrhoea after their melphalan treatment, beginning after one and continuing for about one week as described by Cornbleet et al. (1983).

Gut damage increased with increased melphalan dose and the peak abnormality was significantly greater after melphalan 200 mg m$^{-2}$ than after melphalan 180 mg m$^{-2}$ ($P=0.05$ Wilcoxon Rank Sum Test). Among patients who received 200–220 mg m$^{-2}$ of melphalan, the first 8 received 200 mg m$^{-2}$ and the next 7 received 220 mg m$^{-2}$. They were randomly allocated within each dose level to receive a cyclophosphamide prime or not. The maximum measured excretion of EDTA was significantly lower in the patients who received a cyclophosphamide prime (Figure 1, $P<0.01$ test and Fishers exact test).

| No. patient | Treatment | Age | Sex | Diagnosis |
|------------|-----------|-----|-----|-----------|
| 1–4        | Melphalan 180 mg m$^{-2}$ | 44–60 | F | Breast cancer |
| 5          | Cyclophosphamide priming and melphalan 200 mg m$^{-2}$ | 52 | F | Melanoma |
| 6          | Cyclophosphamide priming and melphalan 200 mg m$^{-2}$ | 42 | F | Breast cancer |
| 7          | Cyclophosphamide priming and melphalan 200 mg m$^{-2}$ | 26 | M | Hodgkin's disease |
| 8          | Cyclophosphamide priming and melphalan 200 mg m$^{-2}$ | 50 | F | Breast cancer |
| 9          | Melphalan 200 mg m$^{-2}$ | 44 | F | Hodgkin's disease |
| 10         | Melphalan 200 mg m$^{-2}$ | 37 | M | Hodgkin's disease |
| 11         | Melphalan 200 mg m$^{-2}$ | 50 | F | Nasopharyngeal cancer |
| 12         | Melphalan 200 mg m$^{-2}$ | 48 | F | Breast cancer |
| 13         | Melphalan 220 mg m$^{-2}$ and cyclophosphamide | 32 | F | Hodgkin's disease |
| 14         | Melphalan 220 mg m$^{-2}$ and cyclophosphamide | 19 | M | Hodgkin's disease |
| 15         | Melphalan 220 mg m$^{-2}$ and cyclophosphamide | 21 | M | Wilms tumour |
| 16         | Melphalan 220 mg m$^{-2}$ and cyclophosphamide | 21 | M | Rhabdomyosarcoma |
| 17         | Melphalan 220 mg m$^{-2}$ | 42 | M | Angiofollicular hyperplasia |
| 18         | Melphalan 220 mg m$^{-2}$ | 42 | M | Hodgkin's disease |
| 19         | Melphalan 220 mg m$^{-2}$ | 21 | F | Soft tissue sarcoma |
CYCLOPHOSPHAMIDE AND MELPHALAN-INDUCED GI TRACT DAMAGE

(ii) **High dose cyclophosphamide** Two patients received cyclophosphamide 7 g m⁻² i.v. without bone marrow grafting. No abnormalities were observed in their intestinal permeability.

**Renal function** Since renal clearance of ⁵¹Cr EDTA might be expected to influence the speed of excretion of the absorbed oral dose, creatinine clearance was measured on each 24 h urine. Change in urine excretion of ⁵¹Cr EDTA in the first 24 h were not explained by changes in creatinine clearance.

**Discussion**

The ⁵¹Cr EDTA absorption test was easy to perform and generally well tolerated by our patients even after intensive cytotoxic therapies. ¹³¹I edetic acid is cheap, stable and widely used in Nuclear Medicine. The test was reliable and had a narrow normal range although interfering factors such as heavy alcohol intake must be identified (Bjarnason et al., 1984). Collection of urine for longer than 24 h might increase its sensitivity but is time consuming for patients and was not necessary for the purposes of this study.

Abnormalities due to melphalan were detected at doses above 180 mg m⁻² and seemed to be dose-dependent. The timing of these abnormalities was similar to the timing of histological gut abnormalities after cytotoxic treatment in serial biopsy studies in man (Lubitz & Ekert, 1979; Cornbleet et al., 1983). No intestinal abnormalities were shown after cyclophosphamide treatment which is in keeping with previous evidence for this drug (Shaw et al., 1979).

Although the numbers of patients are not great, we have demonstrated a significant reduction in gut damage after melphalan by cyclophosphamide priming. The effect is quite small and probably not greater than a dose change of 20–40 mg. Its mechanism is not known (Millar & McElwain, 1985) but further investigation of mechanism and of the optimal timing of the priming dose may allow further reduction in gut damage.

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