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The effect of dietary amino acids on the gastrointestinal absorption of melphalan and chlorambucil*

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Summary. Previous studies have demonstrated that the biovailability of melphalan and chlorambucil may be reduced under non-fasting conditions, and that the gastrointestinal and cellular absorption of melphalan is an active process, while that of chlorambucil is passive. In view of these findings, the effect of dietary amino acids on the gastrointestinal absorption of these two drugs was investigated using the in situ rat intestine model. The segment lengths used in the study were (mean ±SD) 47.1 ±3.8 cm. Experimentation was carried out in a randomised fashion and involved monitoring the absorption of drug from control intestinal segments and from segments perfused with L-glycine (1 and 10 m*M*) and L-leucine (1 and 10 m*M*). For chlorambucil, absorption was carried out from segments perfused with the 10 m*M* concentration of amino acids only. Aliquots of gut-perfusing solution were removed at 5-min intervals over 30 min and assayed for drug content using a high-performance liquid chromatography (HPLC) method which was selective for each agent.

Values recorded for the absorption of melphalan were (mean ± SD percentage absorption per centimetre segment length over a 30-min period) 1.11%±0.07% cm^{-1} (control); 1.18%±0.20% cm^{-1} (1 m*M* L-glycine); 0.99%±0.27% cm^{-1} (1 m*M* L-leucine); 0.80%±0.25% cm^{-1} (10 m*M* L-glycine); and 0.60%±0.23% cm^{-1} (10 m*M* L-leucine). Chlorambucil absorption from control animals was 1.77%±0.11% cm^{-1} gut length, as against 1.77%±0.08% cm^{-1} in 10 m*M* L-glycine and 1.69%±0.16% cm^{-1} in 10 m*M*-L-leucine-perfused segments. The only statistically significant observation was a reduction in melphalan absorption from perfusate containing 10 m*M* leucine ($P<0.005$). The experimental data suggest that competitive inhibition by amino acids may be one of the mechanisms involved in the observed reduction in melphalan bioavailability under non-fasting conditions, but that it has no effect on chlorambucil absorption.

Introduction

It is generally accepted that bifunctional alkylating agents such as melphalan and chlorambucil exert their cytotoxic action by interacting with DNA to form interstrand, intrastrand or DNA-protein cross links [10, 16]. It therefore seems that intracellular, and hence systemic levels of drug are important factors in effecting cell death.

Cellular uptake of melphalan has been shown to occur by way of an energy-dependent system [13, 14]. A detailed analysis of the interaction between melphalan transport and amino acids was first demonstrated by Vistica et al. [20–22]. It was suggested that L-leucine and L-glutamine, in sharing a common transport system with the drug, competed for carrier sites, reduced drug influx, and protected the cell from cytotoxicity. Conversely, Hill [15] and Begleiter and Goldenberg [6] have noted that chlorambucil undergoes cellular absorption by passive diffusion and therefore is not subject to competitive inhibition in its uptake.

Because chlorambucil and low-dose melphalan are usually administered orally [12, 17], the question of factors that affect their oral biovailability is an important one. The pharmacokinetic studies of Tattersall et al. [19], Alberts et al. [5], and Woodhouse et al. [24] highlighted the erratic nature of melphalan bioavailability, which, it was suggested, might have been the cause of poor tumour response. Recent studies on oral absorption have shown that the rate and efficiency of absorption are reduced when melphalan [8] and chlorambucil [4] are taken with food.

The aim of the present study was to investigate the influence of the two amino acids, L-glycine and L-leucine, on the gastrointestinal absorption of both cytotoxic drugs. Our previous study [1] on the gastrointestinal absorption of melphalan and chlorambucil showed the involvement of an energy-dependent mechanism for melphalan and a passive absorption process for chlorambucil. The present work extends the use of the in situ rat intestinal model to consider the affect of amino acids on the bioavailability of melaphalan and chlorambucil.

Materials and methods

In situ intestinal model. The method adopted for the study was an in situ rat intestinal model based on that described by Doluisio et al. [11] and Swintosky and Pogonowska-Wala [18]. A similar protocol to the earlier studies on melphalan and chlorambucil absorption [1] was adopted. Albino Wistar rats weighing 228–290 g were fasted for 16–24 h before experimentation, except that water was allowed ad libitum. The animals were anaesthetised by i.p. injection of pentobarbitone sodium (60 mg/kg), and a midline incision was made so that two L-shaped glass cannulae could be inserted through small slits at the duodenal and ileal

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ends of the small intestine. The cannulae were secured with linen sutures and were connected to 20-ml syringes via three-way stopcocks. This arrangement allowed fluid to be withdrawn from and replaced into the intestinal segments as required. Exposed abdominal areas of animals were covered with cotton wool moistened with normal saline and were maintained at 37 °C by an infrared lamp.

The segments in each case were first flushed with 20 ml normal saline at 37 °C to clear any debris. Buffered perfusion solutions (12 ml) containing the drug under investigation were then added to the segments and drug absorption was monitored. Experimentation was carried out in a randomised fashion and involved the study of melphalan and chlorambucil absorption from control animals and from test segments perfused with buffer containing drug plus amino acids.

Sample collection and storage. Samples of drug solutions (0.5 ml) were removed from the intestinal segment at 5-min intervals up to 30 min, and the volume of perfusate was maintained by replacement with 0.5 ml of original drug solution. The latter solution was incubated at 37 °C from the beginning of each experiment to ensure that the rate of spontaneous hydrolysis of the cytotoxic agents was the same as that in the luminal perfusate. During collection all samples were stored on ice and then frozen at −70 °C prior to their analysis on the same day by high-performance liquid chromatography (HPLC). This ensured that drug was not lost by in vitro hydrolysis during storage. The HPLC methodology used was selective for the parent alkylating agents and their products of hydrolysis. Details of these procedures are given elsewhere [2, 3].

Perfusing media. The perfusing vehicle for both drugs was an iso-osmotic (263 mosmol/kg) phosphate buffer (sodium phosphate monobasic/sodium phosphate dibasic) at pH 5.20. Because both alkylating agents are sparingly soluble in aqueous solution and are subject to rapid spontaneous hydrolysis, stock solutions of 0.1 mg/ml were prepared in methanol. The intestinal perfusing solution was a mixture of methanolic drug solution (2.5% v/v) and iso-osmotic phosphate buffer. The perfusion concentration for each alkylating agent was 2.5 μg/ml. The total dose administered to each animal was 30 μg, which approximates to the equivalent adult human daily dose (mg/kg) for intermittent low-dose therapy.

Test melphalan solutions were prepared in a similar manner to control perfusates, in addition containing L-leucine (1 and 10 m*M*) or glycine (1 and 10 m*M*). In the case of chlorambucil only the 10 m*M* amino acid consentration was examined. The amounts of amino acid used in each case correlated well (on a milligram-to-kilogram basis) with their composition values in the standard meal used in clinical bioavailability studies [4, 8].

Data treatment. Loss of intact drug from the intestinal segment is brought about by processes of absorption and spontaneous hydrolysis. To account for the latter effect, in vitro hydrolysis rate constants were determined for each alkylating agent in the perfusate at 37 °C, and subtracted from observed in situ rate constant for drug disappearance (absorption and hydrolysis). This enabled the process of drug absorption to be quantified separately, and the data presently reported relate solely to the absorption of parent compounds.

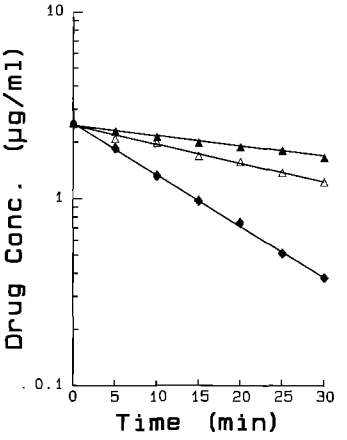

Fig. 1. Log concentration vs time profiles (mean values shown) for the absorption of melphalan (△-control; ▲10 mM L-leucine) and chlorabucil (◆-control) from intestinal segments. Data have been corrected for hydrolysis and represent absorption only. Other amino acid-drug combinations have been omitted for clarity

Control and test data for both drugs were compared statistically using analysis of variance. When statistical differences were found within a data set, the Neuman-Keuls multiple range test was used to ascertain significance values between differing data pairs.

Results

In each case the drug absorbed was calculated as a percentage of the initial dose. These values were obtained by regression analysis of log drug concentration-versus-time curves (Fig. 1). Upon completion of each experiment the intestinal segment was dissected out and measured. To account for the variations in segment length, the percentage of drug absorbed is also calculated as the percentage of drug absorbed per centimetre of gut length. A summary of absorption data for each drug is given in Tables 1 and 2.

The percentage of melphalan absorbed from intestinal segments perfused with 10 m*M* leucine was significantly lower than the drug absorbed from controls ($P<0.005$), 1 m*M* glycine ($P<0.005$) and 1 m*M* leucine ($P<0.025$). However, no significant difference in absorption was noted between segments perfused with leucine 10 m*M* and those perfused with glycine 10 m*M* ($P>0.2$). Perfusing solutions containing 10 m*M* glycine reduced melphalan absorption, although the effect was smaller than that observed for 10 m*M* leucine, and just failed to reach statistical significance ($P>0.05$). The absorption of chlorambucil was unaffected by the amino acid component in test segments ($P>0.05$). The coefficient of variation (CV; given in parentheses in Tables 1 and 2 for the percentage of drug absorbed and percentage of drug absorbed per centimetre of gut length) in control animals perfused with melphalan is very low and is close in value to the variability observed for chlorambucil. However, the CV for melphalan absorption increased when amino acids were present in perfusate and was highest in value with high concentrations of amino acid. These data agree with the variable nature of melphalan absorption as noted in previous clinical studies [5, 7, 19, 24]. Variation in chlorambucil absorption remained constant even in the presence of amino acids.

Table 1. Summary (means ± SD) of absorption data for melphalan in the absence and presence of the amino acids glycine and leucine

Group	Number of animals	Intestinal segment length (cm)	Residual amount of drug (μg)	Percentage of dose absorbed	Percentage of dose absorbed per cm segment length
Control	5	47.4±3.2	14.2±1.2	52.5± 4.0 (7.6)	1.11±0.07 (6.3)
Glycine 1 m*M*	5	46.6±3.6	13.5±2.7	54.9± 9.0 (16.4)	1.18±0.20 (17.0)
Leucine 1 m*M*	5	47.4±4.9	16.1±3.5	46.5±11.5 (24.7)	0.99±0.27 (27.3)
Glycine 10 m*M*	5	47.6±3.0	18.6±3.7	38.0±12.5 (32.9)	0.80±0.25 (31.3)
Leucine 10 m*M*	5	48.6±4.7	21.4±2.6	28.6± 8.8 (30.8)	0.60±0.23 (38.3)

Figures in parentheses give the coefficient of variation in each case. Absorption data given for a 30-min period

Table 2. Summary (means ± SD) of adsorption data for chlorambucil in the absence and presence of the amino acids glycine and leucine

Group	Number of animals	Intestinal segment length (cm)	Residual amount of drug (μg)	Percentage of dose absorbed	Percentage of dose absorbed per cm segment length
Control	5	46.6±2.9	5.2±1.7	82.5±5.7 (6.9)	1.77±0.11 (6.2)
Glycine 10 m*M*	5	45.4±3.1	6.0±1.3	80.2±4.2 (5.2)	1.77±0.08 (4.5)
Leucine 10 m*M*	5	47.2±4.7	6.1±2.1	79.5±6.9 (8.7)	1.69±0.16 (9.5)

Figures in parentheses represent the coefficient of variation in each case. Absorption data given for a 30-min period

The intestinal segment lengths did not differ significantly between control and test segments for either melphalan or chlorambucil ($P > 0.05$). The amino acid component in the perfusate did not affect drug hydrolysis. The in vitro hydrolysis rate constants for melphalan at 37 °C in control, 1 m*M* leucine, 10 m*M* leucine, 1 m*M* glycine and 10 m*M* glycine perfusate solutions were −0.01246, −0.01240, −0.01287, −0.01275 and −0.01254 min^{-1}, while those for chlorambucil in control, 10 m*M* leucine and 10 m*M* glycine perfusate solutions were −0.03810, −0.03975 and −0.03696 min^{-1}, respectively. The slopes of these log concentrations/time profiles were linear (minimum correlation coefficient = −0.947). These data indicate that hydrolysis of each drug occurred by first-order kinetics and was not affected by the amino acid content of the perfusate.

Discussion

The present results indicate that the gastrointestinal absorption of melphalan follows first order kinetics and may be significantly reduced in the presence of dietary amino acids such as L-leucine (Fig. 1). The principle of competitive inhibition therefore may be one of the mechanisms involved in the reduction in bioavailability noted by Bosanquet and Gilby [8] when melphalan was taken with food. In view of the amino acid moiety contained within the melphalan molecule and the active nature of gastrointestinal [1] and cellular uptake of drug [12, 13], it is reasonable to suggest that competition for absorption sites takes place within the gastrointestinal tract.

Competitive inhibition of drug uptake by food constituents has previously been demonstrated for the amino acid transport processes; for example, it has been shown that L-dopa (which is absorbed and transported by the same mechanisms as transport neutral amino acids) absorption may be reduced by dietary amino acids, leading to a poor therapeutic response [23].

Our previous report [1] showed that chlorambucil underwent gastrointestinal absorption via a passive process, and in that respect agreed with the cellular uptake studies of Hill [15] and Begleiter and Goldenberg [6]. The present data show that chlorambucil absorption from the gastrointestinal segment is unaffected by the amino acid content. It is clear, therefore, that the mechanism by which food causes a reduction in the bioavailability of chlorambucil may differ from that affecting the bioavailability of melphalan.

The comprehensive study by Chatterji et al. [9] on the stability of chlorambucil has shown the drug to undergo rapid breakdown with a half-life in the order of 16–25 min. These authors noted that at a luminal pH of 3.0, 10% of the dose would degrade in 4 min. Food is known to decrease the gastric emptying rate and therefore slow drug absorption [23], and indeed a recent clinical study of chlorambucil absorption [4] showed that the time taken (mean ± SD) to attain peak plasma levels increased from 44±10 min under fasting conditions to 102±25 min in non-fasting patients. In view of these findings it is likely that hydrolysis, in addition to the other possible effects of food, e.g., drug binding to or adsorption by food components, may be responsible for the poor bioavailability of chlorambucil under non-fasting conditions.

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