Changes in absorptive and peptide hydrolase activities in rat small intestine after administration of 5-fluorouracil

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(Received 1 April 1977; accepted 12 October 1977)

Summary

1. Glucose absorption, water absorption and dipeptide hydrolase activities have been determined in isolated rat small intestine at 1, 3, 5 and 21 days after a single intraperitoneal injection of 5-fluorouracil.

2. Absorption rates and enzyme activities were elevated 1 day after treatment, but were reduced to 40% of control values at 3 and 5 days. Changes were seen regardless of whether absorption was expressed per unit length or per unit dry weight of intestine.

3. There were highly significant positive correlations between glucose or water absorption rates and peptidase activities, especially in proximal jejunum. The most significant correlation was observed between water absorption rate and jejunal L-Leu-Gly hydrolase activity.

4. Malabsorption may account for some of the gastrointestinal side effects associated with treatment with 5-fluorouracil. Enzyme measurements may be useful as an index of intestinal function.

Key words: absorption, 5-fluorouracil, intestine, peptide, peptidase, peptide hydrolase.

Introduction

The pyrimidine 5-fluorouracil is an antimetabolite used in the treatment of certain epithelial tumours.

However, undesirable gastrointestinal side effects including anorexia, nausea, weight loss and diarrhoea may be so severe as to restrict the dose that can be used (Calabresi & Parks, 1975). Although these toxic effects are presumed to relate to inhibition of nucleic acid synthesis by 5-fluorouracil their actual cause remains unknown. Intestinal epithelial cells which have a short half-life are potentially vulnerable to this class of cytotoxic agent. The malnutrition and diarrhoea might be explained by impaired intestinal absorption. Hence rational attempts to minimize these side effects clearly depend on knowledge of the functional abnormalities produced.

Histological damage and changes in absorptive and enzymic activities in the small intestines of animals and man after administration of 5-fluorouracil (Levin, 1968; Roche, Bognel, Bognel & Bernier, 1970; Bounous, Gentile & Hugon, 1971a; Bounous, Hugon & Gentile, 1971b; Hartwich, Domschke, Matzkies, Pesch & Prestele, 1974) are generally similar to those produced by ionizing radiation (Robinson, 1972). We have studied the time course of the effects of a single intraperitoneal dose of 5-fluorouracil on subsequent glucose and water absorption by isolated rat small intestine. Dipeptide hydrolase activities in cytoplasmic fractions of mucosal homogenates were also measured to determine whether they changed in a similar fashion to absorption, and thus whether enzyme measurements might provide a useful index of intestinal function.
Methods

Animals

Adult female rats, 180–220 g, of a local Wistar strain (Centre for Laboratory Animals, Easter Bush, Penicuik, Midlothian, Scotland, U.K.) were fed with continual free access to diet 86 (Oxoid Ltd, London) and water until they were killed. They were kept in conditions of controlled temperature and lighting for at least a week before use.

5-Fluorouracil treatment

Under ether anaesthesia rats were weighed and injected intraperitoneally with a sterile solution containing 384 μmol of 5-fluorouracil/ml (50 mg/ml) (Roche Products Ltd) as the Tris salt. The mean dose was 1-45 mmol (189 mg)/kg body weight. Control rats received no treatment.

Measurement of glucose and water absorption

Isolated whole segments of jejunum plus ileum (approximately 100 cm) were perfused in a single pass through the lumen (Fisher & Gardner, 1974). The perfusion medium was the modified Krebs–Henseleit bicarbonate solution containing glucose (28 mmol/l) and phenol red (141 nmol/ml; 50 µg/ml) used by Fisher & Gardner (1974), and their precautions to avoid temporary hypoxia were rigorously adopted. If any animal died during the setting-up procedure that experiment was abandoned (Gardner, 1978). Water absorption was determined directly from the weight of fluid secreted on to the serosal surface of the preparation, and glucose absorption (i.e. disappearance from the intestinal lumen) was determined from the concentrations of glucose in the inflowing perfusate and in the luminal effluent together with the weights of luminal effluent and serosal secretion. Four measurements of absorption rates were made on each intestine over consecutive 5 min periods commencing 5 min after the intestine had been placed into the organ chamber. Absorption rates were expressed in terms of both unit length of unstretched intestine and unit dry weight of whole intestine.

Analysis and assays

Glucose analysis. Glucose in the luminal perfusate and effluents was estimated on an Autoanalyzer with a glucose oxidase (EC 1.1.3.4)–peroxidase (EC 1.11.1.7) method with gum guaiacum as chromogen (Fisher & Gardner, 1974). Each sample was estimated in duplicate and in mirror-image sequence between standards to eliminate systematic effects of drift.

Cytoplasmic peptide hydrolase assays. Immediately after the rest of the small intestine had been set up for absorption measurement and before the animal was killed, segments (about 1 cm) of proximal jejunum adjacent to the ligament of Treitz and distal ileum adjacent to the ileo-caecal junction were rinsed with cold sodium chloride solution (154 mmol/l; saline) and placed in saline on ice. Within 2 h the mucosa was scraped from each segment with a microscope slide and was homogenized in 1.5 ml of ice-cold saline. The homogenate was centrifuged at 20 000 g at 4°C for 1 h and the supematant taken for peptide hydrolase (peptidase) assay. If this was not to be performed immediately individual portions were stored at −40°C for up to 2 days. Control experiments showed that supernatants lost up to 80% of peptidase activity in 30 min at 37°C, or 5% at room temperature, but were stable at −40°C for at least 3 weeks (R. R. Samson & A. Pryde, unpublished work). Therefore all manipulations were made as rapidly as possible under standardized conditions. Estimations of sucrase activity indicated that less than 3% of the peptidase activity measured in our cytoplasmic fractions could arise from brush-border contamination.

The peptidase assays followed the method of Fujita, Parsons & Wojnarowska (1972), in which free amino acids liberated during hydrolysis of dipeptide substrates are estimated spectrophotometrically by an amino acid oxidase/o-dianisidine method. The following substrates were purchased from the Sigma (London) Chemical Co. Ltd: L-leucyl-L-leucine, glycyl-L-methionine, L-leucyl-glycine, glycyl-L-phenylalanine, glycyl-L-leucine and L-valyl-L-leucine. Portions (0.1 ml) of an appropriate dilution (in the range 1:20–1:160) of mucosal supernatant were incubated for 30 min at 37°C with 0.4 ml of substrate (30 mmol/l) in Tris/HCl buffer (50 mmol/l) at pH 8.4 (Fujita et al., 1972). The released free amino acid assay was also as described by Fujita et al. (1972) except that the amino acid oxidase preparation was from Crotalus adamanteus venom (Sigma catalogue no. V6875). Calibration curves with appropriate free amino acids were prepared each day. Almost all assays were conducted in duplicate or triplicate.

Peptidase activities were expressed in terms of enzyme units per mg of protein, where 1 unit is defined as the amount catalysing the hydrolysis of
5-Fluorouracil and intestinal absorption

1 μmol of substrate/min under the conditions of temperature and pH given by Fujita et al. (1972).

Estimation of protein. The protein content of 0.1 ml of a 1:5 dilution of the mucosal supernatants was estimated by the method of Lowry, Rosebrough, Farr & Randall (1951) with crystalline bovine serum albumin (Sigma) used as standard.

Timing of experiments

Since there are diurnal rhythms in intestinal absorptive and enzymic activities (Fisher & Gardner, 1976; Furuya & Yugari, 1974; Saito, 1972) all absorption experiments were performed between 10.15 and 12.00 hours G.M.T. Likewise all injections of 5-fluorouracil were administered between 11.00 and 11.30 hours G.M.T.

Statistical tests

Mean absolute values of enzyme activities and of absorption rates were compared with the corresponding mean values obtained in control (untreated) animals by the unpaired $t$-test. Values are given as mean ± SEM.

Results

Glucose and water absorption rates and tissue dry weights

The mean rates of glucose and water absorption measured in control experiments on 13 untreated animals were $15.7 ± 0.67\ \mu\text{mol}\ h^{-1}\ \text{cm}^{-1}$ and $131.5 ± 5.75\ \mu\text{l}\ h^{-1}\ \text{cm}^{-1}$ respectively. The mean dry weight of whole intestine after perfusion was $13.5 ± 0.65\ \text{mg/cm}$. These values are closely similar to previous values obtained in this laboratory (Fisher & Gardner, 1974, 1976).

Fig. 1 shows the mean rates of glucose and water absorption and intestinal dry weights measured 1, 3, 5 and 21 days after a single intraperitoneal injection of 5-fluorouracil (1.45 mmol/kg; 189 mg/kg). Rates were determined per unit length of intestine and then expressed as a percentage of the corresponding mean control values measured in untreated animals. There was a significant ($P < 0.001$) increase in the absorption rates of both glucose and water 1 day after administration of 5-fluorouracil. At 3 and 5 days absorption rates had fallen to some 40% of their control values ($P < 0.001$), but had recovered by day 21, when water absorption was slightly, but significantly, greater than in the control intestines ($P < 0.01$). The tissue dry weight had fallen to some 45% of their control values ($P < 0.001$), but had recovered by day 21, when tissue dry weight was slightly, but significantly, greater than in the control intestines ($P < 0.01$).
dry weights were significantly reduced at 1, 3 and 5 days \( (P < 0.01) \) but were not significantly different from control values at 21 days \( (P > 0.15) \).

When the glucose and water absorption rates are expressed in terms of unit dry weight of intestine a biphasic pattern, which is qualitatively similar to that in Fig. 1, is again seen. Water absorption at 1 day was elevated to 202% of the control values and at 5 days reduced to 48%, significantly different from control values \( (P < 0.001) \). Similar changes occur for glucose absorption \( (P < 0.001) \). At 21 days after administration of 5-fluorouracil both water and glucose absorption rates per mg dry weight were above their control values, the difference being significant for water absorption \( (P < 0.01) \).

**Dipeptide hydrolase activities**

Table 1 summarizes the mean enzyme activities of each of the dipeptidases measured in proximal jejunum and distal ileum in the control (untreated) rats. Fig. 2 and Fig. 3 show the variation in the mean peptide hydrolase activities (per mg of protein) in proximal jejunum and distal ileum respectively at 1, 3, 5 and, in some cases, 21 days after administration of 5-fluorouracil. Variability was so large that no values at 1 day were significantly different from control values (i.e. \( P > 0.1 \)). However, significant depression below control

<table>
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<tr>
<th>Peptide substrate</th>
<th>Activity (units/mg of protein)</th>
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<tbody>
<tr>
<td></td>
<td>Jejunum</td>
</tr>
<tr>
<td>L-Leu-L-Leu</td>
<td>11.6 ± 1.3</td>
</tr>
<tr>
<td>Gly-L-Met</td>
<td>38.5 ± 2.9</td>
</tr>
<tr>
<td>L-Leu-Gly</td>
<td>8.94 ± 0.70</td>
</tr>
<tr>
<td>Gly-L-Phe</td>
<td>25.7 ± 1.41</td>
</tr>
<tr>
<td>Gly-L-Leu</td>
<td>51.1 ± 7.95</td>
</tr>
<tr>
<td>L-Val-L-Leu</td>
<td>20.0 ± 1.95</td>
</tr>
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![Fig. 2. Mean dipeptide hydrolase activities in proximal jejunum in control rats and at 1, 3, 5 and 21 days after a single injection of 5-fluorouracil. Substrates: ●—●, L-Leu-Gly; △—△, L-Val-L-Leu; ■—■, Gly-L-Met; ○—○ Gly-L-Phe; △—△, L-Leu-L-Leu; ■—■, Gly-L-Leu. Mean values ± SEM are plotted with numbers adjacent showing the number of animals for each point.](image-url)
5-Fluorouracil and intestinal absorption

Fig. 3. Mean dipeptide hydrolase activities in distal ileum in control rats and 1, 3, 5 and 21 days after a single injection of 5-fluorouracil. Substrates: · · · , L-Leu-Gly; Δ Δ Δ, L-Val-L-Leu; ■ ■ ■, Gly-L-Met; ○ ○ ○, Gly-L-Phe; Δ Δ Δ, L-Leu-L-Leu; ■ ■ ■, Gly-L-Leu. Mean values ± SEM are plotted with numbers adjacent showing the number of animals for each point.

Values was observed at 3 days for jejunal peptide hydrolase activity to Gly-Met (P < 0.001), Leu-Gly (P < 0.05) and Val-Leu (P < 0.01), and for ileal hydrolase activity to Gly-Met (P < 0.05) and Val-Leu (P < 0.02). At 21 days the jejunal Gly-Met (P < 0.01), Leu-Gly (P < 0.05) and Val-Leu (P < 0.01) hydrolase activities were significantly greater than the corresponding control values.

Histological examination

Tissue sections from stomach, proximal jejunum, terminal ileum and colon were stained with haematoxylin and eosin after routine formaldehyde fixation and paraffin embedding. At day 1 there was degeneration and necrosis of the epithelial cells at the bases of the crypts of the jejunum, ileum and colon and at the bases of the gastric glands without disturbance of the gross mucosal morphology. This was the only time at which obvious abnormality was seen in the sections from the stomach. At days 3, 5 and 21 the small intestinal mucosa showed villous atrophy, which was mild at days 3 and 21 and moderate on day 5. This villous atrophy, which was never total, was associated with a mild to moderate mixed inflammatory cell infiltrate of the mucosal lamina propria. The colonic mucosa at days 3 and 5 showed features suggesting regeneration but at day 21 appearances were normal.

These appearances are consistent with an acute injury to the proliferating epithelial cells of the mucosa manifest by subsequent villous atrophy in the small intestine and followed by a degree of compensatory hyperplastic regeneration. The histological findings were similar to those reported in detail by Bounous et al. (1971b).

Correlations between absorption rates and peptide hydrolase activities

The correlation between the rates of glucose and water absorption is highly significant (r = 0.955 with 48 degrees of freedom; P < 0.0001), and the data for control intestines and at 1, 3, 5 and 21 days after 5-fluorouracil all conform to the same relationship, i.e. with similar slopes and intercepts.

Table 2 summarizes the correlation coefficients between the rates of glucose or water absorption [whether expressed (a) per unit length or (b) per unit dry weight of intestine] and the various dipeptidase activities measured in jejunum and ileum. A number of highly significant positive correlations (P < 0.001) are evident.
TABLE 2. Correlation coefficients (a) between glucose absorption rates in isolated small intestine and various dipeptide hydrolase activities measured in either jejunal or ileal sections, and (b) between water absorption rates in isolated small intestine and various dipeptide hydrolase activities measured in either jejunal or ileal sections.

Enzyme activities were expressed in units/mg of protein, glucose absorption rates in either $\mu$mol h$^{-1}$ cm$^{-1}$ length (A) or $\mu$mol h$^{-1}$ mg$^{-1}$ dry weight (B) and water absorption rates in either $\mu$l h$^{-1}$ cm$^{-1}$ length (C) or $\mu$l h$^{-1}$ mg$^{-1}$ dry weight (D). Number of observations are given in parentheses. ***P < 0.001; **P < 0.01; *P < 0.02; NS, P > 0.02 (not significant).

<table>
<thead>
<tr>
<th>Peptide substrate</th>
<th>Correlation coefficients</th>
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<tr>
<td></td>
<td>(a)</td>
</tr>
<tr>
<td></td>
<td>Glucose absorption</td>
</tr>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Jejunum</td>
<td></td>
</tr>
<tr>
<td>Gly-Met (31)</td>
<td>0.354 NS</td>
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<tr>
<td>Leu-Gly (31)</td>
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<tr>
<td>Val-Leu (31)</td>
<td>0.584***</td>
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<tr>
<td>Leu-Leu (16)</td>
<td>0.697**</td>
</tr>
<tr>
<td>Gly-Phe (16)</td>
<td>0.766***</td>
</tr>
<tr>
<td>Gly-Leu (16)</td>
<td>0.720**</td>
</tr>
<tr>
<td>Ileum</td>
<td></td>
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<tr>
<td>Gly-Met (31)</td>
<td>0.426*</td>
</tr>
<tr>
<td>Leu-Gly (31)</td>
<td>0.227 NS</td>
</tr>
<tr>
<td>Val-Leu (31)</td>
<td>0.263***</td>
</tr>
<tr>
<td>Leu-Leu (16)</td>
<td>0.352 NS</td>
</tr>
<tr>
<td>Gly-Phe (16)</td>
<td>0.630**</td>
</tr>
<tr>
<td>Gly-Leu (16)</td>
<td>0.584*</td>
</tr>
</tbody>
</table>

Discussion

Our results confirm that administration of 5-fluorouracil can profoundly depress absorptive and enzymic activities of rat small intestine. Biphasic effects on glucose and water absorption were observed with increased rates 1 day after the drug and decreased rates on the third and fifth days. However, these changes were reversible and absorption rates at 21 days were above control values. The changes were seen regardless of whether the rates were expressed per unit length or per unit dry weight of intestine, and thus contrast with the findings by Levin (1968) that 5-fluorouracil did not affect absorption per unit dry weight of intestine. The tissue dry weights determined in our study included the intestinal muscle, and it might be that if mucosal dry weights had been used the absorption rates per unit weight at days 3 and 5 might have been unchanged. Therefore we cannot certainly determine if the number of absorbing cells or the absorptive capacity of individual cells had been reduced. Roche et al. (1970) observed a correlation between absorption rate and the number of cells per villus. Their data and those of Levin (1968) suggest that the number of absorbing cells had been reduced.

Elevated absorption rates 1 day after 5-fluorouracil were not reported by Levin (1968) or by Roche et al. (1970). Transient increases in phenylalanine absorption have also been noted after administration of methotrexate (Robinson, Antonioli & Vannotti, 1966b) and neomycin (Robinson, Antonioli & Fasel, 1966a), and also in intestinal esterase and cathepsin activities after whole-body irradiation (Spiro & Pearse, 1964). This phenomenon may be related to food intake. Although we did not measure daily food intake and body weight Bounous et al. (1971b) noted a drastic decrease in the food consumption and growth in the 3 days after an injection of 5-fluorouracil in rats. Starvation can increase both intestinal absorption rates (Kershaw, Neame & Wiseman, 1960) and cytoplasmic peptidase activities (Kim, McCarthy, Lane & Fong, 1973). However, in their detailed study of various semi-starvation and refeeding regimes Kershaw et al. (1960) never observed depression of absorption rates. Our results at day 3 are thus unlikely to be related to food intake.

The depression of absorption rates between 1 and 3 days after injection of 5-fluorouracil is consistent with the known kinetics of intestinal epithelial turnover, as the mean life of duodenal and
ileal epithelial cells in the rat is about 1-5 days (Leblond & Stevens, 1948). Roche et al. (1970) also observed maximal depression of glucose transport about 3 days after 5-fluorouracil, and similar effects were reported for the intestinal damage induced by methotrexate and neomycin and by irradiation (Robinson et al., 1966a, b; Spiro & Pearse, 1964).

At 21 days the water absorption rate and the jejunal activities of Gly-Met, Leu-Gly and Val-Leu hydrolases were all significantly greater than the corresponding control values (P < 0·01; P < 0·01; P < 0·05; P < 0·01 respectively). A raised glucose absorption rate and raised activities of ileal peptidases also occurred at 21 days, although these were not statistically significant, and Roche et al. (1970) also noted an insignificant rise in glucose absorption 7 days after administration of 5-fluorouracil, the maximum time studied.

Dipeptidase activities (Fig. 2 and Fig. 3) showed a generally similar response to the absorptive activity, with elevations, though not statistically significant, at day 1 and depression at day 3 (with the exception of ileal enzymes, particularly Leu-Leu peptidase). Indeed there was a highly significant positive correlation between the activities of most of the peptide hydrolases studied, especially those in proximal jejunum, and the rates of glucose and water absorption observed in the isolated whole small intestine (Table 2). The most significant correlation observed (r = 0·626; n = 31) was between jejunal Leu-Gly hydrolase activity and water absorption rate which implies that measurement of this enzyme (e.g. in biopsies) might afford a reliable index of intestinal functional integrity. Decrease in intestinal dipeptidase (Gly-Val and Gly-Leu) activities 1 and 3 days after 375 mg of 5-fluorouracil/kg and in sucrase activity at 3 days was reported by Bounous (1971b). They also noted a threefold increase in sucrase activity at 1 day, although Hartwich et al. (1974) reported depressed activities of sucrase, maltase and lactase at both 1 and 3 days after injection of 5-fluorouracil at 0·767 and 1·53 mmol/kg (100 and 200 mg/kg).

It should be stressed that the peptide hydrolase activities in the present study were associated with considerable variability, so that a single measurement of enzyme activity in a single animal will not give an unequivocal estimate of absorptive activity. Furthermore measurement of peptidase activities in tissue specimens is fraught with technical difficulties, e.g. the loss or leakage of enzymes from tissue in vitro even at reduced temperatures (Silk & Kim, 1976; Lindberg, Norén & Sjöström, 1975; Josefsson & Sjöström, 1966) and the loss of enzyme activity at room temperature (see the Methods section). These pitfalls require rigorous standardization of technique but should be relatively unimportant where comparisons are made with internal control systems as in the present work, although they have probably contributed to the high variability in the results.

The substantial impairment of intestinal absorption seen after administration of 5-fluorouracil is likely to account for at least some of the undesirable side effects of this drug, and so attempts to alleviate these effects should be directed towards maintenance of normal intestinal function.

Acknowledgments

We are indebted to the Medical Research Council and Eaton Laboratories for financial support to M.L.G.G. and R.C.H. respectively, and to Dr Lennox-Smith, Roche Products Ltd for a gift of 5-fluorouracil. We are also grateful to Dr H. M. Gilmour for histological studies, and to Miss Elizabeth Middleton and Mrs Anne Pryde for skilled technical assistance.

References


