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An *In Vitro* Assessment of the Effect of Cytotoxic Drugs Upon the Intestinal Absorption of Nutrients in Rats

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Abstract—*The effect of anti-cancer drug pretreatment on the rate of uptake of various ^{14}C-labelled nutrients has been investigated using intestinal everted sac preparations. The cytotoxic drugs decreased the efficiency of the barrier to passive penetration of the intestine by the nutrients. The rate of uptake of all nutrients except monopalmitic acid was reduced by a decrease in active absorption. There was no significant difference in tryptophan uptake between methotrexate or 5-fluorouracil pretreated animals. It was concluded that cytotoxic drugs reduce intestinal absorption by inhibiting carriers involved in "active-transport" mediated uptake.*

INTRODUCTION

RECENT investigations indicate that the administration of various commonly-used cytotoxic anti-cancer drugs to rats at a level comparable with the therapeutic dose in man, reduce the absorption of antipyrine *in vivo* [1]. The elimination of antipyrine from plasma is used for the estimation of hepatic metabolism [1, 2] and appeared to be of potential diagnostic value in indicating gastrointestinal malabsorption. Reports of anorexia associated with chemotherapy of certain malignant conditions have been reviewed recently [3]. In this experiment, therefore, the penetration of glucose, tryptophan, monopalmitic acid, vitamin A and vitamin C into everted intestinal sacs prepared from cytotoxic drug-pretreated and control animals was monitored. Non-aerated preparations were used to estimate "passive penetration" of these nutrients, and intestinal sacs were aerated to quantitate active absorption processes.

MATERIALS AND METHODS

Chemicals

L-[Methylene-^{14}C] tryptophan, D-[U-^{14}C] glucose, [1-^{14}C] monopalmitic acid and [carboxyl-^{14}C] vitamin A were purchased from the Radiochemical Centre, Amersham, Bucks., and L-[1-^{14}C] vitamin C from New England Nuclear, Boston, Mass., U.S.A. Ouabain and 5-fluorouracil were obtained from the Sigma Chemical Co., Poole, Dorset. Sodium methotrexate from Cyanamid, N.Y., U.S.A. Instagel® and Soluene-350® from Packard Instrument Co., Caversham, Bucks. All other chemicals were of the purest grade available and supplied by BDH Chemicals Ltd., Poole, Dorset.

Animals and dosing

Male Sprague–Dawley rats of body-weight 300–400 g were purchased from A. Tuck and Son, Rayleigh, Essex and maintained on PRM diet (Dixons, Ware, Herts.). Groups of 3 animals received an intramuscular injection of either 5-fluorouracil (12.5 mg/kg) or methotrexate (1 mg/kg) in saline 2 ml/kg once daily for 5 successive days. Control animals were given a similar dose of vehicle only.

Everted sac incubations

Twenty-four hours after cessation of treatment the animals were sacrificed by cervical dislocation and the whole of the small intestines surgically removed and everted sacs prepared from 4 cm segments essentially as described by Wiseman [4]. The sacs containing tyrode solution only were suspended in a test meal comprising: glucose (3.4 g), bovine serum albumen (1 g) and glycerol (1.8 g) in 100 ml in tyrode solution. The individual nutrient being studied was added as a ^{14}C-radioactive solution to give a final concentration of 0.1 μCi/ml. The sacs were incubated at 37°C and slowly rotated in the test

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meal through which a fine stream of 95% oxygen: 5% carbon dioxide was passed. The rate of absorption of various substrates varies according to the position of the intestine [5]. In this experiment, therefore, segments from treated and control animals to be compared were taken from the same position of the respective intestines. Three everted sacs, one of which had originated from each of the treated individuals, were removed from the test meal at the times indicated in Tables 1, 2 and 3. In the case of glucose only, additional everted sacs derived from treated and control animals were immersed in test meal containing the ATPase-inhibitor ouabain (0.1 mg/ml) [6]. After incubation all the sacs were washed by brief immersion in fresh tyrode solution and each sac was solubilised separately in "Soluene 350". The resultant solution was neutralised and decolourized before estimation of the radioactive content in Instagel.

Radiochemical analysis

Samples were counted for 10 min duration and 2 cycles in a Packard 4250 liquid scintillation spectrometer. Counting efficiency was determined by external standardisation.

RESULTS

The effect of pretreatment with cytotoxic drugs upon the rate of uptake of tryptophan, vitamin A, vitamin C, monopalmitic acid and glucose in everted segments of rat intestine is given in Tables 1, 2 and 3. For most nutrients in aerated preparations, the absorption rate was greater in the control than the pretreated animals, but with non-aerated samples the rate of penetration is greater in the treated animals than in the controls. Thus, the amount of tryptophan absorbed into aerated intestinal sacs derived from the 5-fluorouracil pretreated animals was only 50% of the control animals, whereas in the non-aerated preparations, 4 times the amount of tryptophan penetrated the sacs derived from pretreated animals as the controls. Using tryptophan as nutrient, the results obtained were not significantly different whether the animals were pretreated with either 5-fluorouracil or methotrexate.

Monopalmitic acid was the only nutrient which penetrated the everted sacs at the same rate regardless of whether or not they were aerated. Thus, in the pretreated animals the uptake of monopalmitic acid was reduced to 46–58% of the level in the controls.

Glucose was the most rapidly absorbed of the substrates studied (Table 3) and its uptake was particularly sensitive to aeration being increased 2–3 fold in the oxygenated preparations. Pretreatment with 5-fluorouracil decreased active absorption (aerated samples) to 31% of the control and increased passive penetration (non-aerated samples) to 164% of the control level. When ouabain was included in the test meal the results obtained were similar to those obtained with non-aerated preparations, suggesting that the 5-fluorouracil mediated decrease in absorption could be the result of the inhibition of specific carriers.

DISCUSSION

During the past 20 yr chemotherapy has achieved major importance in the control of cancer [7, 8]. Cytotoxic drugs have been divided into groups according to their mode of action [9, 10]. The drugs used in this experiment, 5-fluorouracil and methotrexate, are antimetabolites which attack cells in the S period of their reproductive cycle by interfering with the biosynthesis of the purine or pyrimidine bases. The host toxicity of such drugs is frequently associated with the inhibition of the fastest dividing cells such as those of the bone marrow and gut epithelium. Although bone marrow toxicity [11] and neurotoxicity [12, 13] has been the subject of detailed investigation, gastrointestinal damage was accepted as an unavoidable consequence associated with oral administration [10] or direct contact with the cytotoxic drugs [14]. In a previous experiment [1] it was demonstrated that drug absorption was reduced by a similar amount by various different cytotoxic drugs, of differing modes of action, administered by different routes. Since in this experiment the cytotoxic drugs were administered intramuscularly, the observed decrease in nutrient uptake is probably associated with a decrease in gastrointestinal cell metabolism rather than the ulcerative effect associated with enteral administration.

In vitro techniques have been used to study the active absorption of a wide variety of substances, but it is not generally accepted that everted sac techniques can measure passive diffusion mechanisms [5]. However, under some anoxic conditions *in vitro* some transport phenomena have been reported [15]. Presumably in this experiment the accumulation of radioactivity monitored is not mediated through an energy-requiring carrier, and reflects the cytotoxic drug-initiated de-

Table 1. The effect of pre-treatment with anti-cancer drugs on the absorption of nutrients through aerated intestinal sacs

Nutrient	Dis/min per sac at time (min) 5	10	15	20	25	30	60
Tryptophan	4767 ± 352	4358 ± 330	6384 ± 448	8280 ± 1048	10,130 ± 477	10,786 ± 1263	12,952 ± 406
	4213 ± 432	3594 ± 443	3982 ± 502	5453 ± 566	4687 ± 769	5895 ± 524	6919 ± 1201
b	3991 ± 232	4151 ± 318	4990 ± 352	5441 ± 406	5611 ± 602	5923 ± 1062	6142 ± 839
Vitamin A	4747 ± 751	5712 ± 431	7332 ± 882	10,024 ± 842	9038 ± 920	10,970 ± 1487	18,069 ± 1830
	3329 ± 302	3548 ± 352	5764 ± 327	6516 ± 791	17,095 ± 792	30,948 ± 1967	30,022 ± 1860
Vitamin C	1130 ± 156	1356 ± 174	1730 ± 112	1840 ± 161	2455 ± 254	2939 ± 377	4109 ± 412
	1301 ± 154	1507 ± 233	2053 ± 143	2029 ± 244	2338 ± 370	2634 ± 213	3385 ± 317
Monopalmitic acid	4143 ± 515	5695 ± 332	6904 ± 722	7398 ± 971	10,883 ± 957	10,675 ± 1561	18,226 ± 1525
	2624 ± 209	3445 ± 403	3693 ± 494	5063 ± 446	5059 ± 490	5258 ± 686	10,891 ± 992

Results quoted are the mean and S.D. of the sac and contents taken from 3 different animals, for each nutrient the upper figures are the control values, the lower ones represent the treated animals.
b Animals pretreated with methotrexate (1 mg/kg), all others pretreated with 5-fluorouracil (12.5 mg/kg).

Table 2. The effect of pre-treatment with anti-cancer drugs on the penetration of nutrients through non-aerated intestinal sacs

Nutrient	Dis/min per sac at time (min) 5	10	15	20	25	30	60
Tryptophan	673 ± 81	922 ± 218	1143 ± 173	1165 ± 213	1389 ± 222	1507 ± 370	2308 ± 692
	2818 ± 250	3121 ± 440	3876 ± 594	4766 ± 305	6032 ± 474	7266 ± 1588	9711 ± 1172
b	2860 ± 233	3635 ± 329	4480 ± 421	5085 ± 505	5683 ± 666	8696 ± 1363	12,170 ± 738
Vitamin A	4136 ± 306	4592 ± 377	6586 ± 460	5045 ± 275	5815 ± 519	11,670 ± 1457	13,305 ± 1136
	4592 ± 265	10,632 ± 335	18,404 ± 1461	15,379 ± 259	21,899 ± 252	25,525 ± 1193	37,689 ± 1933
Vitamin C	1122 ± 114	1688 ± 284	1842 ± 288	1911 ± 271	2199 ± 141	2294 ± 444	3208 ± 81
	964 ± 234	1492 ± 327	1795 ± 331	2252 ± 319	2539 ± 249	2829 ± 380	3948 ± 379
Monopalmitic acid	4413 ± 300	5248 ± 518	6932 ± 602	8971 ± 811	10,975 ± 720	13,987 ± 630	17,868 ± 1910
	3756 ± 418	4332 ± 531	4309 ± 406	4884 ± 413	7290 ± 402	8249 ± 934	9435 ± 1989

Results quoted are the mean and S.D. of the sac and contents taken from 3 different animals, for each nutrient the upper figures are the control values, the lower ones represent the treated animals.
b Animals pretreated with methotrexate (1 mg/kg), all others pretreated with 5-fluorouracil (12.5 mg/kg).

Table 3. The effect of pre-treatment with anti-cancer drugs on the absorption of glucose through everted intestinal sacs

Preparation	Dis/min per sac at time (min) 1	2	3	4	5
Aerated	4483 ± 339	7362 ± 654	9156 ± 1322	13,778 ± 1505	15,335 ± 1354
	2214 ± 299	3242 ± 559	3158 ± 479	3790 ± 528	5020 ± 546
Non-aerated	1522 ± 231	1891 ± 199	3469 ± 501	3738 ± 377	6091 ± 133
	2604 ± 242	3032 ± 369	5248 ± 470	7077 ± 1404	8690 ± 250
Ouabain	2604 ± 145	4272 ± 451	6161 ± 814	8122 ± 733	9161 ± 922
	3101 ± 308	5361 ± 424	7954 ± 863	9281 ± 502	10,113 ± 841

Results quoted are the mean and S.D. of the sac and contents taken from 3 different animals, the upper figures are the control values, the lower ones represent the treated animals.

crease in the integrity of the intestinal epithelium.

Recent evidence [16] suggests that administration of certain cytotoxic drugs, including 5-fluorouracil and methotrexate, results in no detectable histological damage to the intestine. Presumably if the replication of the cells lining the gastrointestinal epithelium (which are constantly being shed) is retarded, then this would result in a decrease in the effectiveness of the barrier to penetration as observed in these non-aerated *in vitro* preparations. With the exception of monopalmitic acid, all the nutrients in this experiment can be actively absorbed [17] and the decrease in their uptake, therefore, appears to be mediated by a decrease in the activity of the active transport mechanism. This suggests that 5-fluorouracil (and methotrexate) may poison metabolic carriers, as ouabain inhibited the carrier responsible for glucose uptake.

From this *in vitro* model it is concluded that absorption is not only decreased overall but that the uptake of some of these "digestive end-products" is retarded by differing amounts. This emphasizes the importance of current research for corrective nutritional support therapy for patients undergoing cytotoxic drug treatment [3], and also attempts to protect the gastrointestinal tract by administration of other drugs or metabolites [18] before commencement of cytotoxic drug therapy.

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