Biochimica et Biophysica Acta 902 (1987) 93–100
Elsevier

BBA 73635

Decreased transport of D-glucose and L-alanine across brush-border membrane vesicles from small intestine of rats treated with mitomycin C

Masayuki Mizuno, Hiromi Yoshino, Mitsuru Hashida and Hitoshi Sezaki

Department of Basic Pharmaceutics, Faculty of Pharmaceutical Sciences, Kyoto University, Kyoto 606 (Japan)

(Received 24 February 1987)

Key words: Glucose transport; Alanine transport; Cotransporter, Na^+/D-glucose; Brush-border membrane; Mitomycin C; Sodium gradient; (Rat small intestine)

To elucidate the mechanisms underlying the dysfunctions of intestinal absorption induced by antitumor drugs, the effect of pretreatment with mitomycin C on sodium gradient-dependent D-glucose and L-alanine transports was studied in rat brush-border membrane vesicles. 24, 48, 96, or 120 h following a single intravenous injection of mitomycin C, brush-border membrane vesicles were prepared from rat small-intestines. The uptake of D-glucose and L-alanine was shown to be Na^+ gradient-dependent even in the case of vesicles obtained from mitomycin C-treated rats, but uptake rates measured at 15 s and magnitude of overshooting effect in uptake of both solutes were decreased in vesicles maximally from 48 h mitomycin C-treated rats. The rate of D-glucose uptake calculated at 15 s recovered to the control level in vesicles prepared at 96 h and 120 h after mitomycin C-treatment, indicating that the effect of mitomycin C on Na^+ gradient-dependent D-glucose transport would be fully reversible. Tracer exchange experiments under Na^+ and D-glucose equilibrated conditions indicated that the Na^+/D-glucose transporters were similarly operative in the vesicles from control and 48 h mitomycin C-treated rats. Rates of $^{22}Na^+$ uptake measured at 15 s in vesicles from 48 h mitomycin C-treated rats, however, were increased. The increased permeability to Na^+ might bring about a more rapid dissipation of the Na^+ gradient in these vesicles and this would secondarily cause the decrease in Na^+-dependent D-glucose uptake in vesicles from mitomycin C-treated rats.

Introduction

Mitomycin C, an alkylating agent which inhibits DNA synthesis of malignant cells [1], also injures the bone marrow and the gastrointestinal epithelium [2]. Therefore, the clinical utility of this agent is often limited by these undesirable side-effects [3]. The influence of antitumor drugs on the intestinal handling of other drugs and nutrients has been widely investigated. However, less attention has been paid to their effects on transport systems for nutrients at a brush-border membrane level.

Brush-border membrane vesicles have been employed to analyze the mechanisms underlying the alterations of the transepithelial transport in certain disease state [4–8] or in developmental course [9] without the interference of the cellular metabolism or of the basolateral membrane [10]. In our previous studies [11,12], mitomycin C-induced disorders in drug and nutrient absorption

Abbreviations: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid; Tris, 2-amino-2-hydroxymethylpropane-1,3-diol.

Correspondence: H. Sezaki, Department of Basic Pharmaceutics, Faculty of Pharmaceutical Sciences, Kyoto University, Kyoto 606, Japan.

from rat gastrointestinal tract were investigated. We demonstrated that rates of Na^+ gradient-dependent D-glucose uptake into small-intestinal brush-border membrane vesicles calculated at 15 s were significantly decreased in rats having mitomycin C-pretreatment at 48 h before the preparations of vesicles [11]. Furthermore, it was revealed that in vivo administration of mitomycin C, but not in vitro incubation of vesicles with mitomycin C, induces the change in Na^+ gradient-dependent D-glucose transport [11]. In the present study, the uptakes of D-glucose and L-alanine by brush-border membrane vesicles were examined in mitomycin C-treated rats to further characterize the nature of the obvious transport defect. The results suggested that transport alterations could be attributed to the secondary changes accompanied with the increased permeability of brush-border membranes to Na^+ by mitomycin C treatment rather than to the direct effect of mitomycin C on Na^+/solutes cotransporters.

Materials and Methods

Materials. Mitomycin C was kindly supplied by Kyowa Hakko Kogyo Co. (Tokyo, Japan). D-[1-^{3}H]Glucose (6.44 Ci/mmol) and L-[2,3-^{3}H]alanine (56.0 Ci/mmol) were purchased from Amersham International (Amersham, U.K.). $^{22}Na^+$ (as ^{22}NaCl) (513.31 mCi/mg) was purchased from New England Nuclear (Boston, MA). [U-^{14}C]Sulfanilic acid (3.90 mCi/mmol) was obtained as a gift from Daiichi Pure Chemicals Co. (Tokyo, Japan). 1,6-Diphenyl-1,3,5-hexatriene and ouabain were supplied from Nakarai Chemicals (Kyoto, Japan). Gramicidin D (from *Bacillus brevis*) and bovine serum albumin were obtained from Sigma Chemical Co. (St. Louis, MO). All other chemicals used for the experiments were of reagent grade and were used without further purification.

Animals. Age (6–7 weeks old) and weight (180–220 g) matched groups of male Wistar rats were used. They were fed a normal breeding diet (Oriental Yeast Kogyo Co., Tokyo, Japan) and given tap water, ad libitum. All animals were maintained on 12 h light-dark cycles. They were not used earlier than 3 to 7 days after arrival from the supplier to acclimatize to laboratory conditions. Then, they were weighed and given a bolus intravenous injection of mitomycin C (3 mg · kg^{-1}) in sterile 0.9% (w/v) NaCl solution (saline) into the left femoral vein under light ether anesthesia. Control animals received a comparable volume of saline. Details concerning the dosing of animals were reported in our previous paper [11].

Preparation of brush-border membrane vesicles. 24, 48, 96 or 120 h following the adminsitration of mitomycin C or saline, brush-border membrane vesicles were prepared from the entire length of the samll intestine according to the calcium precipitation method of Kessler et al. [13]. A detailed procedure for the preparation was reported in our previous paper [11], and all preparative procedures were carried out on ice or in a cold room at 4° C. For transport studies, typically, the purified brush-border membrane vesicles were preincubated in 300 mM mannitol, 10 mM Hepes/Tris (pH 7.5) (buffer A) to give a protein concentration of 6–12 mg/ml. The purity of the membrane preparations obtained from control and mitomycin C-pretreated rats was evaluated by the specific activity of the marker enzymes. In separate experiments, brush-border membrane lipids were extracted according to the method of Folch et al. [14], and total phospholipid contents were calcualted from the amount of inorganic phosphate determined with the method of Bartlett [15].

Determination of enzyme activities. The activities of enzymes known to be characteristics of the brush-border membrane (alkaline phosphatase (EC 3.1.3.1), aminopeptidase (EC 3.4.11.2)) and the basolateral membrane (($Na^+ + K^+$)-ATPase (EC 3.6.1.3)) were measured. Alkaline phosphatase was assayed with *p*-nitrophenyl phosphate as a substrate according to the method of Murer et al. [16]. Aminopeptidase activities were measured with leucine-*p*-nitroanilide as a substrate according to the method of Kramers and Robinson [17]. ($Na^+ + K^+$)-ATPase was assayed according to the method of Wilson and Treanor [18] and liberated inorganic phosphate was determined by the method of Fiske and SubbaRow [19].

Transport assay. Uptake of ^{3}H-labeled D-glucose and L-alanine, ^{14}C-labeled sulfanilic acid and $^{22}Na^+$ by the freshly prepared brush-border membrane vesicles was determined by a rapid filtration method [20]. The incubation medium was com-

posed of 300 mM mannitol, 10 mM Hepes/Tris, 200 mM NaSCN and one of the substrates. When the uptake of $^{22}Na^+$ in membrane vesicles was measured, NaSCN was replaced isosmotically by mannitol. Transport studies were initiated by the addition of 20 μl of the medium to 20 μl of the vesicle suspension at 25 °C. At the stated times, 2 ml of an ice-cold stop solution (250 mM NaCl, 1 mM Tris/HCl (pH 7.5)) was added to the mixture. Then, the resulting mixture was immediately filtered through pre-wetted 0.45 μm filters (Fuji Photo Film, Tokyo, Japan) and washed twice with 5 ml of ice-cold stop solution. Background value was determined by the addition of substrate medium (20 μl) to 2 ml ice-cold stop solution containing 20 μl of membrane vesicles followed by filtration. This value was subtracted from the uptake data.

Fluorescence polarization studies. The purified brush-border membrane fraction was suspended in phosphate-buffered saline containing 154 mM NaCl, 4 mM KCl, 0.5 mM $CaCl_2$ and 5 mM sodium phosphate (pH 7.4) [21]. The fluorescent hydrocarbon 1,6-diphenyl-1,3,5-hexatriene was used as a probe for monitoring fluidity in brush-border membrane lipid layers. The procedure of fluorescence labeling of vesicles was the same as reported by Schachter and Shinitzky [22]. In brief, brush-border membranes equivalent to 100–200 μg protein were incubated in 2 ml of phosphate-buffered saline containing 1 μM 1,6-diphenyl-1,3,5-hexatriene suspension for 4 h at 37 °C. Thereafter, the fluorescence polarization and fluorescence intensity were determined at 25 °C with an excitation wavelength of 360 nm and an emission wavelength of 430 nm (Shimadzu RF-540 spectrofluorometer equipped with a polarizer unit P/N 204, Kyoto, Japan).

Analytical method. In D-glucose, L-alanine and sulfanilic acid uptake experiments, radioactivities remained on the filters were measured by a liquid-scintillation counter (Aloka LSC-900, Tokyo, Japan). For the measurement of $^{22}Na^+$ uptake, NaI well type scintillation counter (Packard Auto Gamma 500) was used. Protein was assayed by the method of Lowry et al. [23] using bovine serum albumin as a standard.

Statistical analysis. The results were analyzed statistically with the Student's *t*-test. Differences with P values of less than 0.05 were considered to be significant.

Results

Table I summarizes the specific activities of marker enzymes in brush-border membranes obtained from control and mitomycin C-pretreated rats.

Final preparations of brush-border membranes were purified approximately 10-fold as compared to corresponding crude mucosal homogenates. Relatively high specific activities of a basolateral membrane marker enzyme, $(Na^+ + K^+)$-ATPase, indicate slight contamination by contraluminal membrane fragments. The relative purities of the membranes judging by the enrichment extent of alkaline phosphatase were not significantly different in all cases. To evaluate the lipid/protein composition of the brush-border membrane preparations, phospholipids were determined as a marker of the membrane lipid. As shown in Table I, each value for vesicles obtained from mitomycin C-pretreated rats was not significantly different from one for control rats and these values were compatible with those previously reported by Forstner et al. [24]. The yield of brush border membrane protein from mitomycin C-treated rats was similar to that from controls, although the yield from 48 h mitomycin C-treated rats tended to increase.

Table II shows the time-courses of D-glucose and L-alanine uptakes by small-intestinal brush-border membrane vesicles isolated from the control and mitomycin C-treated rats in the presence of a 100 mM NaSCN gradient (outside to inside). Equilibrium uptakes of both solutes at an equilibrated stage (30 min) were not different between the control and mitomycin C-pretreated vesicles, suggesting that intravesicular volume was not changed by mitomycin C-treatment. In all cases, D-glucose and L-alanine uptakes by brush-border membrane vesicles transiently reached a value greater than each equilibrium value observed after 30 min (overshoot phenomenon). The rates of D-glucose uptake measured at 15 s were significantly reduced in vesicles obtained from animals which received in vivo mitomycin C injection 24 h or 48 h before the preparation. Thereafter, the

TABLE I

ENZYME ACTIVITY AND PURIFICATION OF BRUSH-BORDER MEMBRANE VESICLE PREPARATIONS FROM CONTROL AND MITOMYCIN C-PRETREATED ANIMALS

S.A., specific activity (μmol/min per mg protein; μmol/h per mg protein for ($Na^+ + K^+$)-ATPase). The enrichment factor refers to S.A. in vesicle preparation comapred with S.A. in the whole homogenate. Phospholipids/protein ratio means contents of phospholipids mg per 100 mg protein in each vesicle preparation. Protein recovery represents the percentage of protein recovered in the vesicle preparations compared with initial homogenate. Blank space; not done. Each value represents the mean ± S.E. for three or four experiments.

	Control	Time after mitomycin C pretreatment (h)			
		24	48	96	120
Alkaline phosphatase					
S.A.	5.48 ± 0.97	5.46 ± 0.54	7.95 ± 0.74	4.86 ± 0.86	5.23 ± 0.57
Enrichment factor	9.18 ± 0.52	7.80 ± 1.42	9.92 ± 0.96	8.53 ± 0.50	8.65 ± 0.57
Aminopeptidase					
S.A.	0.69 ± 0.02		0.54 ± 0.01		
Enrichment factor	9.35 ± 0.40		8.08 ± 0.51		
($Na^+ + K^+$)-ATPase					
S.A.	7.14 ± 1.86	7.75 ± 1.24	4.73 ± 0.20	6.64 ± 1.52	7.60 ± 0.51
Enrichment factor	1.73 ± 0.45	1.95 ± 0.12	1.12 ± 0.11	1.76 ± 0.38	1.58 ± 0.03
Phospholipids/protein ratio	10.7 ± 1.9	6.9 ± 0.6	8.7 ± 1.6	8.7 ± 2.8	10.5 ± 1.3
Protein recovery	1.27 ± 0.15	1.63 ± 0.17	2.18 ± 0.16	1.15 ± 0.07	1.62 ± 0.20

TABLE II

TIME-COURSE OF D-GLUCOSE AND L-ALANINE UPTAKE BY BRUSH-BORDER MEMBRANE VESICLES ISOLATED FROM THE CONTROL OR MITOMYCIN C-TREATED RATS

Mitomycin C (3 mg·kg^{-1}) was given intravenously 24, 48, 96, 120 h before the preparation of vesicles. The vesicles were incubated at 25 °C with media containing 300 mM mannitol, 10 mM Hepes/Tris and 200 mM NaSCN (pH 7.5). The final concentration of D-glucose and L-alanine was 0.1 mM. Uptake was measured at 15 s, 30 s and 30 min. Results are expressed as the mean ± S.E. of four experiments. Mit C, mitomycin C.

Solute	Group	Uptake (pmol/mg protein)		
		15 s	30 s	30 min
D-Glucose	Control	152.8 ± 16.1	135.6 ± 17.8	18.4 ± 2.3
	Mit C			
	24 h	113.7 ± 1.8 [b]	80.0 ± 1.0 [b]	17.7 ± 3.7
	48 h	49.5 ± 4.5 [a]	45.2 ± 5.3 [a]	12.8 ± 2.9
	96 h	133.3 ± 21.1	101.2 ± 6.7	16.6 ± 1.3
	120 h	141.8 ± 14.7	106.6 ± 10.3	19.6 ± 2.3
L-Alanine	Control	38.5 ± 1.7	35.3 ± 3.1	16.8 ± 1.1
	Mit C			
	48 h	23.0 ± 1.9 [a]	26.1 ± 1.8 [b]	14.8 ± 0.3

[a] $P < 0.01$.

[b] $P < 0.05$ compared with the uptake into vesicles of the control rats at each time point (Student's t-test).

uptake rate measured at 15 s returned to the control level in 96 h and 120 h mitomycin C-pretreated vesicles. At 48 h after mitomycin C-treatment, the rate of L-alanine uptake in the presence of a NaSCN gradient was also significantly decreased compared with the control. Consequently, overshoot magnitude (ratio of uptakes at 15 s/ones at 30 min) was much lower in mitomycin C-treated vesicles than in controls. These observations suggested that Na^+ gradient-dependent D-glucose uptake was most strongly depressed at 48 h after dosing with mitomycin C. Therefore, the following experiments were carried out employing only membrane vesicles prepared from animals killed 48 h after the dosing.

To examine whether decreases in the uptake rate calculated at 15 s and in the overshoot magnitude were directly derived from the inhibition of activity of the Na^+/solute cotransporter, tracer exchange experiments were performed under the condition in which both Na^+ and D-glucose were equilibrated across the membrane vesicles. Gramicidin D (6 μg/mg protein) was also added to the membrane vesicles for clamping membrane potential developed by Na^+-coupled D-glucose flux at zero.

As shown in Table III, no significant difference was observed between D-glucose uptake values in the control and mitomycin C-treated vesicles. These results suggest that the activity of Na^+/D-glucose transporters was uninfluenced by mitomycin C-pretreatment and that the decrease in Na^+ gradient-dependent D-glucose might be due to a more rapid dissipation of Na^+ gradient in 48 h mitomycin C-treated vesicles.

Table IV shows $^{22}Na^+$ uptake by the control and mitomycin C-treated vesicles as an index of Na^+ permeability in the absence of solute that could be cotransported with Na^+.

As is evident from the table, $^{22}Na^+$ uptake at 15 s and 30 s was significantly greater in membrane vesicles isolated from 48 h mitomycin C-treated rats than those in the control vesicles. Equilibrium values of $^{22}Na^+$ uptake at 30 min were not different significantly between both groups.

In our previous report [11], we demonstrated increased passive permeability of brush-border membranes to D-glucose in 48 h mitomycin C-treated vesicles. To determine whether mitomycin C-pretreatment uniformly increases brush-border membrane permeability to any other solute, therefore, the uptake of sulfanilic acid (4-aminobenzenesulfonic acid) was measured at pH 7.5 in the control and 48 h mitomycin C-treated vesicles.

As shown in Table V, no significant difference between the two groups was noted at any time point, suggesting that mitomycin C-treated brush-border membranes efficiently served as a diffusion barrier to this strong sulfonic acid.

To assess the degree of the fluidity of brush-border membrane lipid layer, fluorescence polarization studies were performed in the control and mitomycin C-treated vesicles with the aid of the fluorescent hydrocarbon probe 1,6-diphenyl-1,3,5-hexatriene. Fluorescence polarization of 1,6-diphenyl-1,3,5-hexatriene was expressed as the fluorescence anisotropy, r, and as the anisotropy parameter, $((r_0/r) - 1)^{-1}$. The value of r_0, the maximal limiting anisotropy, was reported to be 0.362 for 1,6-diphenyl-1,3,5-hexatriene [22]. Values of r and $((r_0/r) - 1)^{-1}$ for the control and 48 h mitomycin C-treated brush borders were 0.243 $\pm$ 0.01, 0.246 $\pm$ 0.01 (mean $\pm$ S.E., $n = 5$ per group) and 1.90 $\pm$ 0.19, 2.14 $\pm$ 0.13 (mean $\pm$ S.E., $n = 5$ per group), respectively. These two parame-

TABLE III

EFFECT OF MITOMYCIN C PRETREATMENT ON TRACER EXCHANGE OF D-GLUCOSE UPTAKE

Intestinal brush-border membrane vesicles were prepared from rats given physiological saline (control) or 3 $mg \cdot kg^{-1}$ mitomycin C (Mitomycin C-treated) intravenously 48 h before the experiment. These vesicles were preincubated for 60 min at 25°C with 300 mM mannitol, 100 mM NaSCN, 0.1 mM D-glucose, 10 mM Hepes/Tris buffer (pH 7.5), and 6 μg per mg protein gramicidin D. The uptake was started by the addition of 20 μl of preincubated vesicles to 20 μl of incubation media containing 300 mM mannitol, 100 mM NaSCN, 0.1 mM D-glucose, 10 mM Hepes/Tris buffer (pH 7.5), and 50 μCi/ml radiolabeled D-glucose. Data represent the mean $\pm$ S.E. for four determinations.

Time (min)	D-Glucose uptake (pmol/mg protein)	
	Control	Mitomycin C-treated
0.25	4.98 $\pm$ 1.09	7.51 $\pm$ 0.85 [a]
0.50	12.01 $\pm$ 2.70	10.42 $\pm$ 1.25 [a]
1	15.15 $\pm$ 3.70	15.05 $\pm$ 1.63 [a]
30	17.79 $\pm$ 0.86	18.68 $\pm$ 3.22 [a]

[a] Not significantly different compared with each control (Student's t-test).

TABLE IV

EFFECT OF MITOMYCIN C PRETREATMENT ON THE TIME-COURSE OF $^{22}Na^+$ UPTAKE

Intestinal brush-border membrane vesicles of control (48 h after intravenous administration of physiological saline) and mitomycin C-treated (48 h after the administration of 3 $mg \cdot kg^{-1}$ mitomycin C) rats were preincubated for 10 min at 25°C with 300 mM mannitol and 10 mM Hepes/Tris buffer (pH 7.5). The reaction was started by the addition of 20 μl of preincubated vesicles to 20 μl of incubation media containing 700 mM mannitol, 10 mM Hepes/Tris buffer (pH 7.5) and $^{22}NaCl$ (50 μCi/ml, final concentration; 48.7 μM). Data represent the mean $\pm$ S.E. for four determinations.

Time (min)	$^{22}Na^+$ uptake (nmol/mg protein)	
	Control	Mitomycin C-treated
0.25	0.220 $\pm$ 0.014	0.317 $\pm$ 0.029 [b]
0.50	0.411 $\pm$ 0.024	0.619 $\pm$ 0.068 [b]
1	0.797 $\pm$ 0.036	0.822 $\pm$ 0.115 [a]
30	3.777 $\pm$ 0.428	3.456 $\pm$ 0.821 [a]

[a] Not significantly different compared with each control.
[b] $P < 0.05$ compared with each control (Student's t-test).

TABLE V

EFFECT OF MITOMYCIN C PRETREATMENT ON THE TIME-COURSE OF [U-^{14}C]SULFANILIC ACID UPTAKE

Intestinal brush-border membrane vesicles prepared from control (48 h after intravneous administration of physiological saline) and mitomycin C-treated (48 h after intravenous administration of 3 mg·kg^{-1} mitomycin C) rats were preincubated for 10 min at 25°C with 300 mM mannitol and 10 mM Hepes/Tris buffer (pH 7.5). The reaction was started by the addition of 20 μl of preincubated vesicles to 20 μl of incubation media containing 300 mM mannitol, 200 mM NaSCN, 10 mM Hepes/Tris buffer (pH 7.5) and radiolabeled sulfanilic acid (20 μCi/ml, final concentration; 2.57 mM). Data represent the mean ± S.E. for four determinations.

Time (min)	[U-^{14}C]Sulfanilic acid uptake (pmol/mg protein)	
	Control	Mitomycin C-treated
0.25	187 ± 19	158 ± 9 [a]
0.50	252 ± 14	258 ± 28 [a]
1	391 ± 30	304 ± 32 [a]
30	667 ± 47	650 ± 89 [a]

[a] Not significantly different compared with each control (Student's *t*-test).

ters were not significantly different between control and 48 h mitomycin C-treated vesicles.

Discussion

It is reasonable to consider that the clarification of precise mechanisms underlying the toxicities is the first requirement for deciding on the course to circumvent toxicity problems of anticancer agents. In order to obtain information on the toxic effects at the level of small-intestinal brush-border membrane where the active transport systems for many solutes are localized, we have examined uptake characteristics of D-glucose and L-alanine into the brush-border membrane vesicles. These two solutes were used because their transport across intestinal brush-border membrane vesicles had been well characterized [13,20,25].

In the course of our studies we used techniques previously available for normal membranes. So we checked the validity of experimental procedures at the start of our studies. The purity of brush-border membrnaes was evaluated by the use of marker enzymes (Table I). The specific activities of brush-border membrane marker enzymes were enriched approximately 10-fold over initial mucosal homogenates in control and mitomycin C-pretreated preparations. The specific activity of a basolateral membrane enzyme marker, ($Na^+ + K^+$)-ATPase, was only slightly increased compared with mucosal homogenate in all cases. As seen in Table I, the relative purities of the vesicles (enrichment factors of marker enzymes) from the control and mitomycin C-pretreated rats were almost identical, indicating that changes in solute transport were not due to changes in the purity of vesicles. Furthermore, we showed that there was no significant change in the relative amounts of phospholipids and proteins of membrane vesicles between the two groups. These results supported validity to express solute uptake data by normalizing with the amount of protein. In addition, we had revealed that D-glucose uptake represented the penetration into osmotically active spece rather than the binding onto surfaces of membrane vesicles obtained from both control and 48 h mitomycin C-pretreated rats [11]. Therefore, brush-border membrane vesicles are concluded to be suitable as a tool for studying transport processes in the rats pretreated with mitomycin C.

Na^+ gradient-dependent D-glucose and L-alanine uptakes were decreased in brush-border membrane vesicles isolated from 48 h mitomycin C-pretreated rats compared with those from control rats. Decreased transport of D-glucose or amino acids following pretreatments with antitumor drugs other than mitomycin C has been confirmed in intact cell preparations in vivo and in vitro [26–29]. Although these findings are apparently compatible with reduced uptakes by brush-border membrane vesicles, it has not been elucidated whether abnormalities in intact cell preparations are due solely to a defect of Na^+ gradient-dependent solute transport in brush-border membranes. On the other hand, changes in D-glucose transport through renal or intestinal brush-border membranes have been reported in failures such as spontaneous idiopathic Fanconi syndrome [4,6], chronic renal failure [5], acute renal failure [7], transmissible gastroenteritis [8], and in the developmental course [9]. Gishan et al.

[9] have demonstrated that the initial uptake of D-glucose by intestinal brush-border membrane vesicles in the presence of Na^+ gradient was significantly greater in adolescent rats compared with suckling rats. They discussed that the diminution of D-glucose transport in suckling rats was most likely due to the increased permeability to Na^+ which resulted in a faster dissipation of the Na^+ gradient. In this case, the activity of D-glucose/Na^+ cotransporters was considered to be similar in suckling and adolescent rats. These findings are in good agreement with the present results (Table III, Table IV). Decreased Na^+ gradient-dependent L-alanine uptake was perhaps mediated, at least in part, by rapid dissipation of the driving force, while the nature of Na^+/L-alanine cotransporters in mitomycin C-treated rats remains to be determined.

Rates of D-glucose uptake by brush-border membrane vesicles measured at 15 s were diminished with a nadir occurring at 48 h and returned to normal 96 h after mitomycin C-injection (Table II). These changes induced by mitomycin C are characterized to be reversible and would primarily reflect the rapid proliferation rate of intestinal mucosa. In rodents, median transit time of columnar cells from the base of the crypts to the top of the villi was reported to be 2 to 3 days [30]. Thus, cells damaged at crypt level might be replaced with normal mature cells at the distal extrusion zone within a few days.

Changes in membrane fludiity may be associated with the increased permeability of brush-border membranes to Na^+, because the alterations in membrane fluidity have been shown to influence cation permeability in plasma membranes [31,32]. However, in the present experiments using a fluorophore 1,6-diphenyl-1,3,5-hexatriene, the fluidity of inner lipid layers was not changed by mitomycin C-pretreatment. Alteration in Na^+ permeability, therefore, is unlikely to be due to fluidity changes in these membranes, but studies using probes which label other regions of the membranes are required. In contrast with the case of Na^+, no significant change in uptakes of sulfanilic acid between control and mitomycin C-treated vesicles (Table V) would suggest that increased permeability of membranes can not be generalized for any solutes, but has some selectivity according to the physicochemical and/or the biological properties of solutes.

In conclusion, we have demonstrated the decrease in Na^+ gradient-dependent transport of D-glucose and L-alanine in small-intestinal brush-border membranes isolated from mitomycin C-pretreated rats. Increased permeability of mitomycin C-pretreated membranes to Na^+ would result in a rapid dissipation of Na^+ gradient, and this is more likely to be responsible for dysfunctions in D-glucose and L-alanine transport rather than the changes in the activity of Na^+/solutes cotransporters.

Acknowledgements

The authors especially thank Professor Toshikiro Kimura, Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, Okayama University, for his valuable suggestions to this work.

References

1 Lown, J.W. (1979) in Mitomycin C: Current Status and New Developments (Carter, S.K. and Crooke, S.T., eds.), pp. 5–26, Academic Press, New York
2 Philips, F.S., Schwartz, H.S. and Sternberg, S.S. (1960) Cancer Res. 20, 1345–1361
3 Bradner, W.T. (1979) in Mitomycin C: Current Status and New Developments (Carter, S.K. and Crooke, S.T., eds.), pp. 33–40, Academic Press, New York
4 Medow, M.S., Reynolds, R., Bovee, K.C. and Segal, S. (1981) Proc. Natl. Acad. Sci. USA 78, 7769–7772
5 Hruska, K.A., Klahr, S. and Hammerman, M.R. (1982) Am. J. Physiol. 242, F17–F22
6 Yanase, M., Orita, Y., Okada, N., Nakanishi, T., Horio, M., Ando, A. and Abe, H. (1983) Biochim. Biophys. Acta 773, 95–101
7 Hori, R., Takano, M., Okano, T. and Inui, K. (1985) J. Pharmacol. Exp. Ther. 233, 776–781
8 Keljo, D.J., MacLeod, R.J., Perdue, M.H., Butler, D.G. and Hamilton, J.R. (1985) Am. J. Physiol. 249, G751–G760
9 Ghishan, F.K. and Wilson, F.A. (1985) Am. J. Physiol. 248, G87–G92
10 Murer, H. and Kinne, R. (1980) J. Membrane Biol. 55, 81–95
11 Mizuno, M., Hamaura, T., Hashida, M. and Sezaki, H. (1986) Biochem. Pharmacol. 35, 1153–1158
12 Mizuno, M., Kawabata, S., Hashida, M. and Sezaki, H. (1986) J. Pharm. Pharmacol. 38, 663–667
13 Kessler, M., Acuto, O., Storelli, C., Murer, H., Müller, M. and Semenza, G. (1978) Biochim. Biophys. Acta 506, 135–154

14 Folch, J., Lees, M. and Sloane-Stanley, G.H. (1957) J. Biol. Chem. 226, 497–509
15 Bartlett, G.R. (1959) J. Biol. Chem. 234, 466–468
16 Murer, H., Ammann, E., Biber, J. and Hopfer, U. (1976) Biochim. Biophys. Acta 433, 509–519
17 Kramers, M.T.C. and Robinson, G.B. (1979) Eur. J. Biochem. 99, 345–351
18 Wilson, F.A. and Treanor, L.L. (1981) Gastroenterology, 54–60
19 Fiske, C.H. and SubbaRow, Y. (1925) J. Biol. Chem. 66, 375–400
20 Hopfer, U., Nelson, K., Perrotto, J. and Isselbacher, K.J. (1973) J. Biol. Chem. 248, 25–32
21 Brasitus, T.A. and Schachter, D. (1980) Biochemistry 19, 2763–2769
22 Schachter, D. and Shinitzky, M. (1977) J. Clin. Invest. 59, 536–548
23 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1955) J. Biol. Chem. 193, 265–275
24 Forstner, G.G., Tanaka, K. and Isselbacher, K.J. (1968) Biochem. J. 109, 51–59
25 Sigrist-Nelson, K., Murer, H. and Hopfer, U. (1975) J. Biol. Chem. 250, 5674–5680
26 Roche, A.C., Bognel, J.C., Bognel, C. and Bernier, J.J. (1970) Digestion 3, 195–212
27 Jolly, L.E. and Fletcher, H. (1977) Toxicol. Appl. Pharmacol. 62, 73–80
28 Chén, T.S. (1982) Toxicol. Appl. Pharmacol. 62, 73–80
29 Pinkerton, C.R. and Milla, P.J. (1984) Br. J. Cancer, 49, 97–101
30 Lipikin, M. (1981) in Physiology of the Gastrointestinal Tract (Johnson, L.R., ed.), pp. 145–168, Raven Press, New York
31 Papahadjopoulos, D., Cowden, M. and Kimelberg, M. (1973) Biochim. Biophys. Acta 330, 8–26
32 Srivastava, S., Phadka, R.S., Govil, G. and Rao, C.N.R. (1983) Biochim. Biophys. Acta 734, 353–362