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BIOLOGY CONTRIBUTION

CITRULLINE: A PHYSIOLOGIC MARKER ENABLING QUANTITATION AND MONITORING OF EPITHELIAL RADIATION-INDUCED SMALL BOWEL DAMAGE

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Purpose: Small bowel irradiation results in epithelial cell loss and consequently impairs function and metabolism. We investigated whether citrulline, a metabolic end product of small bowel enterocytes, can be used for quantifying radiation-induced epithelial cell loss.

Methods and Materials: NMRI mice were subjected to single-dose whole body irradiation (WBI). The time course of citrullinemia was assessed up to 11 days after WBI. A dose–response relationship was determined at 84 h after WBI. In addition, citrullinemia was correlated with morphologic parameters at this time point and used to calculate the dose-modifying factor (DMF) of glutamine and amifostine on acute small bowel radiation damage.

Results: After WBI, a time- and dose-dependent decrease in plasma citrulline level was observed with a significant dose–response relationship at 84 h. At this time point, citrullinemia significantly correlated with jejunal crypt regeneration ($p < 0.001$) and epithelial surface lining ($p = 0.001$). A DMF of 1.0 and 1.5 was computed at the effective dose 50 (ED50) level for glutamine and amifostine, respectively.

Conclusions: Citrullinemia can be used to quantify acute small bowel epithelial radiation damage after single-dose WBI. Radiation-induced changes in citrullinemia are most pronounced at 3½ to 4 days postirradiation. At this time point, citrullinemia correlates with morphologic endpoints for epithelial radiation damage.

Citrulline, Normal tissue damage, Small bowel, Assay, Glutamine.

INTRODUCTION

When abdominal or pelvic cancers are treated with radiotherapy, the small bowel is a major dose-limiting organ with regard to both acute and late treatment-related morbidity. The cellular mechanism of small bowel radiation injury has been studied extensively in the laboratory (1). The intestinal crypt cell and, more recently, the pericryptal endothelial cell (2) have been designated as target cells for epithelial radiation injury. We have focused our research on epithelial radiation sequelae as a target for intervention of both acute and late small bowel radiation injury.

Radiation damage to intestinal epithelium can be measured by morphologic and/or functional endpoints. The application of morphologic endpoints such as crypt cell regeneration (3), apoptosis (1, 2, 4), and mucosal surface measurements (5) in clinical practice is hampered by the need for tissue sampling. As a consequence, data are mainly derived from rodent studies. A wide diversity of functional disorders have been observed after ionizing radiation, such as changes in trans-epithelial transport processes (6, 7) or the absorption of various nutrients such as carbohydrates, amino acids, proteins, vitamins, and bile acid (8–13). Some of these functional changes have been correlated with the epithelial cell mass available for absorption (5, 12, 14, 15), suggesting a cellular basis in at least part of radiation-induced functional disorders. Subsequently, such functional tests were evaluated for their applicability as a clinical assay for intestinal damage related to radiation-induced epithelial cell loss (5, 14). However, as a routine in daily clinical practice these parameters are not suited for repeated measurements.

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Our aim is to develop a clinical assay for radiation-induced small bowel damage, i.e., an assay sensitive and quantifiable at clinically relevant doses. The assay must be applicable in daily clinical practice during the acute and late phases of radiation injury, enabling determination of temporal and/or permanent intestinal damage on a routine basis. Such an assay should then allow reliable assessments of the therapeutic window for new treatment strategies.

In this report, we describe the citrulline kinetics in NMRI mice in response to single-dose whole body irradiation (WBI). We hypothesized that the intestinal release of citrulline into the circulation decreases as a function of the radiation-induced reduction of epithelial cell mass. Thus, citrullinemia is tested as a quantitative marker for small bowel epithelial radiation injury. For this purpose, the time course for citrullinemia has been assessed at 1, 2, 4, 8, and 11 days after single WBI doses of 8–12 Gy, and a dose–response relationship for plasma citrulline level is determined at 84 h after single WBI doses between 0–14.9 Gy. Furthermore, citrullinemia is correlated with parameters for epithelial radiation damage and used for calculation of the dose-modifying factor for amifostine (WR-2721) (16, 17), a radioprotector for small bowel epithelium.

METHODS AND MATERIALS

Mice

Female NMRI mice 12–14 weeks old (23–33 g) were obtained from Harlan Netherlands B.V., Horst, The Netherlands. Mice were housed 3 per cage under standard 12-h light-dark cycle periods (7:30 am to 7:30 pm) with room temperature maintained at 25°C. The mice were fed standard mice chow and water *ad libitum*. All experiments were performed in accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals and approved by the Ethical Committee of Animal Research of the Catholic University of Leuven, Brussels.

Irradiation

The effect of irradiation on plasma citrulline concentration was studied after a single dose of WBI. Three unanesthesized mice of 1 cage were placed in a prone position in a Perspex air-ventilated jig and exposed to 250-kV photons (Philips) at a dose rate of 0.86 Gy/min, using an anteroposterior and postero-anterior beam. During treatment the mice were loosely restrained with their legs stretched forwards and backward, respectively. A Perspex plate (4 mm thickness) on top and at the bottom of the jig ensured the homogeneity of the dose distribution (±3% dose variation).

Plasma citrulline level

Immediately after blood (0.2 mL) was sampled by a cardiac puncture, the mice were terminated for tissue sampling by cervical dislocation. Plasma was obtained by whole blood centrifugation at 8900 × *g* at 4°C for 5 min. For determination of amino acids, 100 μL of plasma was deproteinized by adding it to 8 mg of dry 5-sulfosalicylic acid, vortexed, frozen in liquid nitrogen, and stored at −80°C. Then the plasma citrulline level (μM) was measured by using high-performance liquid chromatography (18).

Morphology

In all experiments, mice were terminated by cervical dislocation. A 2-cm jejunal segment was excised and fixed in Bouin solution for a maximum of 24 h before the routine processing for histology. Three transverse paraffin sections per mouse were cut at a thickness of 4 μm and stained with hematoxylin and eosin.

Morphometric measurements

Three digitized images per mouse at 25× magnification from the jejunum were evaluated using the calibrated image analysis system (Leica Quantimet 570 C; Leica Q570 Qwin version V 02.01). After digital subtraction of intraluminal contents, the epithelial surface lining was demarcated and measured as a perimetrical length (Figs. 1A and 1B). Adjacent villar surfaces were digitally separated to enable measurement of the epithelial surface lining for individual villi. The epithelial lining of intraluminally located villar fragments was accordingly separately measured. The sum of the perimetrical lengths (mm) was then scored representing the total epithelial surface lining per transverse section of the jejunum. For statistical analysis, the mean averaged score of 3 images per mouse was used.

Morphologic assay

The microcolony assay, as introduced in 1970 by Withers and Elkind, was used (3). Strictly 84 h after irradiation, mice were terminated and 2 cm of jejunum was sampled, fixed, and prepared for sectioning. This time interval is enough to regenerate the crypts, and it is assumed that a crypt can regenerate from a single surviving stem cell. Crypts containing ≥10 cells were considered to be regenerating crypts. For each mouse, the number of regenerating crypts per circumference was scored (J.G.) in at least three transverse histologic sections. For statistical analysis, the mean averaged score of three transverse sections per mouse was used.

Experimental conditions

To assess the time course for citrulline after WBI, intervals were set at 1, 2, 4, 8, and 11 days between WBI and blood sampling. In this experiment, WBI doses between 8–12 Gy were applied using 6 mice per dose point at $t = 1$, 2, 4, and 8 days and 12 mice per dose point at $t = 11$ days after WBI, respectively.

A dose–response relationship for citrullinemia, jejunal crypt regeneration, and mucosal surface lining was assessed at strictly 84 h after WBI. This interval was chosen in accordance with the guidelines for the jejunal microcolony assay (3), used in the present experiment as one of the morphologic endpoints. WBI doses of 0–10.0 Gy (dose step 1 Gy) and 8.0, 9.8, 12.1, and 14.9 Gy were used. Three mice per dose point were used. No mice were lost due to the experimental procedure yielding data for all endpoints used.

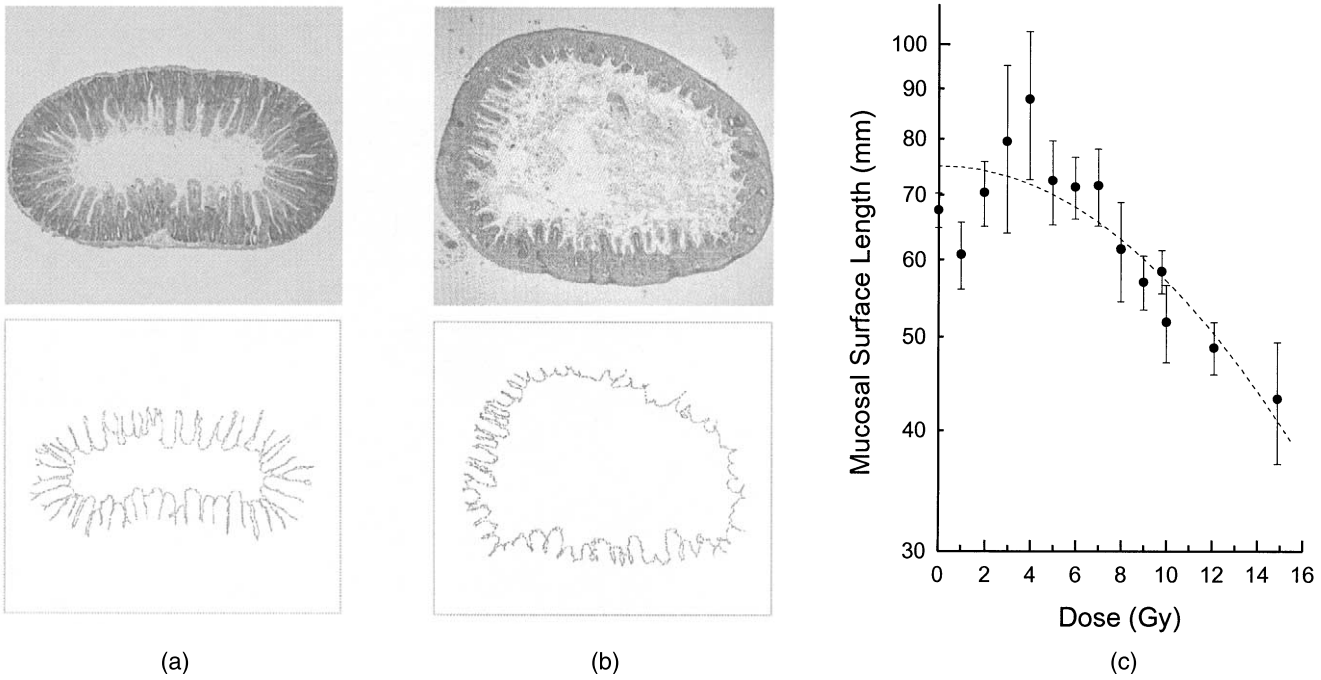

Fig. 1. Mucosal surface lining measurements. (a) Light microscopic image at 25× magnification. The image was digitized using a calibrated image analysis system (Leica Quantimet 570C; Leica Q570 Qwin version V 02.01). (b) The length (mm) of the digitally demarcated mucosal surface lining was used for statistical analysis. The images shown were obtained at 84 h after WBI with doses of 9.8 Gy (left) and 14.9 Gy (right) revealing an epithelial surface lining of 54.3 mm and 38.6 mm and corresponding citrulline levels of 15.8 μM and 9.3 μM, respectively. (c) Dose–response curve for the mucosal surface lining (mm) 84 h after WBI doses of 0–14.9 Gy. Data are presented as mean ± SEM. One-way ANOVA: $p = 0.002$.

Plasma citrulline level was used as a secondary endpoint to assess the effect of enteral glutamine (19) on intestinal radiation damage in mice after single-dose WBI. The amino acid was supplied as a 3%-glutamine enriched drinking water solution, starting 4 days before radiation until termination at t = 84 h after WBI. Amifostine (WR-2721) was used as a positive control (16, 17). Amifostine (Ethyol, USB Pharma, Nijmegen) was stored at 4°C. Immediately before the experiment, 500 mg was dissolved in 10 mL 150 mM NaCl solution (pH 6.0). Mice were randomly assigned to radiation only (WBI dose 8.0–15.0 Gy), enteral glutamine feeding (WBI dose 8.7–15.7 Gy), or 10 mg amifostine intraperitoneally 30 min before radiation (WBI dose 11.1–25.2 Gy), respectively. The DMF of parenteral amifostine on jejunal epithelial radiation damage was determined using jejunal crypt regeneration and plasma citrulline level assessed at 84 h after WBI as endpoints. Six mice were used per dose point.

Statistical analysis

SPSS for Windows software (release 11.0) was used for statistical analysis. For investigation of the dose–response relationship of citrullinemia, raw data of plasma citrulline levels per dose point were used. For examination of a relationship between plasma citrulline levels and morphologic endpoints, mean values per dose point were used. For the number of regenerated crypts as a function of dose, an exponential relation can usually be assumed and a straight line (in log/linear coordinates) can be usually fitted through the experimental points corresponding to less than 70–80 regenerated crypts. This results from the fact that at these (high) dose levels the number of regenerated crypts corresponds to the number of surviving stem cells, so that the dose–effect relation for crypt regeneration fits the (exponential) distal part of the stem cell survival curve. To assess a linear or non-linear relationship between parameters, bivariate correlations procedure was used for calculation of Pearson's correlation coefficient or Spearman's rho, respectively. One-way analysis of variance (ANOVA) with additional Tukey's post hoc testing was used to analyze a correlation of plasma citrulline level and jejunal epithelial surface lining with WBI dose at 84 h after irradiation. A time and dose effect of plasma citrulline level was tested by two-way factorial ANOVA. The significance level was set at $\alpha = 0.05$.

RESULTS

Mucosal surface lining

The mean (± SEM) mucosal surface lining in 6 control mice (WBI dose 0 Gy) was 67.5 ± 2.5 mm (range: 58.9–75.3 mm). Mucosal surface lining decreased as a function of time after irradiation (two-way ANOVA; $p < 0.001$). After 8 Gy, WBI nadir values were measured at Day 2 and Day 11

(58.0 ± 2.9 mm and 53.3 ± 2.8 mm; p = NS), whereas for the 9 Gy dose level the nadir value was measured at Day 8 (58.0 ± 6.7 mm; one-way ANOVA: $p = 0.2$). In contrast, nadir values for the 10, 11, and 12 Gy dose levels were measured at 4 days (52.4 ± 4.7 mm, 38.3 ± 2.7 mm, and 45.3 ± 4.3 mm; one-way ANOVA: $p = 0.034$, $p < 0.001$, and $p = 0.001$, respectively). Similarly, a significant dose–response relationship was observed for mucosal surface lining at the 4-day time point only (Pearson: $r = -0.77$) whereas no correlation was seen at the 1, 2, 8, and 11-day time points, respectively (Pearson r: −0.18, 0.19, −0.11, and −0.15).

A dose–response curve for mucosal surface lining was then assessed at 84 h after WBI using doses between 1–14.9 Gy (Fig. 1c). The mucosal surface lining ranged between 38.5 and 109.3 mm and declined as a function of irradiation dose (one-way ANOVA; $p = 0.002$). At this time point, a strong correlation between mucosal surface lining and jejunal crypt regeneration was also observed (Pearson r: 0.99).

Citrullinemia

The mean (± SEM) plasma citrulline level in control mice (WBI dose 0 Gy; $n = 6$) was 82.5 ± 2.5 μM (range: 50.1–106.5 μM). The time course of plasma citrulline level as a function of WBI dose was assessed at 1, 2, 4, 8, and 11 days after WBI doses between 8 and 12 Gy. Up to 4 days after WBI no mouse died, whereas radiation-induced mortality increased to 16% and 57% (8 Gy), 33% and 66% (9 Gy), 66% and 83% (10 Gy), 50% and 43% (11 Gy), and 50% and 75% (12 Gy) at Days 8 and 11 after WBI, respectively.

Plasma citrulline level decreased as a function of dose and time after WBI (two-way ANOVA; $p = 0.002$ and $p = 0.001$, respectively). Whereas the time effect was significant for all dose levels used, a significant dose-effect relationship was present only at Day 4 after WBI (Pearson: $r = -0.88$; Fig. 2a).

As shown in Fig. 2b, starting 1 day after WBI the plasma citrulline level progressively decreased irrespective of WBI dose ($p = 0.23$). After WBI doses between 8–10 Gy, the nadir plasma citrulline level (mean ± SEM) was observed at 2 days after WBI (36.7 ± 6.8 μM [8 Gy]; 34.3 ± 1.7 μM [9 Gy]; 30.0 ± 6.5 μM [10 Gy]); one-way ANOVA: $p = 0.006$, $p < 0.001$, and $p = 0.047$, respectively). In contrast, citrullinemia nadir values after WBI doses of 11 and 12 Gy were measured at 4 days after WBI (13.8 ± 2.7 μM [11 Gy] and 9.6 ± 2.2 μM [12 Gy]; one-way ANOVA: $p < 0.001$ and $p < 0.001$, respectively). Although a slight recovery of citrullinemia was noticed thereafter, plasma citrulline levels remained significantly lower at the 11-day time point compared with control mice (44.4 ± 8.7 μM [11 Gy] and 28.7 ± 3.8 μM [12 Gy]; one-way ANOVA: $p = 0.006$ and $p = 0.004$, respectively; Fig. 2b).

To further explore the dose–response relationship for citrullinemia, plasma levels were measured 84 h after single WBI doses between 0 and 14.9 Gy. As shown in Fig. 2c, plasma citrulline levels decreased as a function of WBI dose with an approximately linear response for the dose range of 3–12.1 Gy (Pearson: $r = -0.98$). No change in plasma citrulline level could be discriminated up to single WBI doses of 3 Gy, whereas no further decrease in plasma citrulline level was noticed for the 14.9 Gy dose point (4.7 ± 1.3 μM) as compared with the 12.1 Gy dose point (6.2 ± 0.3 μM).

Correlation of citrullinemia with mucosal surface lining and jejunal crypt regeneration

The correlation between plasma citrulline level and mucosal surface lining was examined for the 1, 2, 4, 8, and 11-day time points. A strong correlation was observed for the 4-day and 11-day time points (Pearson r: 0.96 and 0.75, respectively), whereas for the 1, 2, and 8-day time points the correlation coefficients were 0.41, −0.41, and 0.32, respectively. These results were confirmed for the data obtained at 84 h after WBI doses between 0–14.9 Gy. As shown in Fig. 2d, a linear correlation was observed between epithelial surface lining and plasma citrulline level (Pearson: $r = 0.77$; $p < 0.001$).

Citrullinemia was then tested as an assay for acute small bowel epithelial radiation injury in an experiment using enteral glutamine suppletion as a radioprotector. For this purpose, plasma citrulline level was compared with jejunal crypt survival as an endpoint for small bowel epithelial radiation injury at 84 h after single WBI. Amifostine was used as a positive control. Enteral glutamine had no effect on epithelial radiation damage (Figs. 3A and 3B). The DMF for glutamine (1.0) and amifostine (1.5) determined at the Effective Dose 50 (ED50) level were similar for the microcolony assay and citrulline assay (Figs. 3A and 3B). As shown in Fig. 3c, a significant correlation was observed between both endpoints for epithelial radiation damage (Spearman's rho 0.94).

DISCUSSION

A decrease of intestinal absorptive function after irradiation has been correlated to morphologic changes, i.e., a decrease in the number of functionally active cells, constituting the absorptive mucosal surface (5, 12, 14, 15). In an attempt to develop an assay for detecting radiation-induced intestinal damage related to epithelial cell loss, functional assays have been used such as changes in active glucose transport (5) and ^{99m}Tc-pertechnetaat (14). We chose plasma citrulline concentration in our study for its methodologic simplicity (20–23). Citrulline accounts for almost 30% of metabolized glutamine nitrogen in rat small intestine (24). The small intestinal enterocyte contains specific enzymes involved in citrulline production but lacks the enzymes necessary for its conversion to arginine (25–27). This unique enzymatic profile (21) and the fact that citrulline is not metabolized by the liver (28) are the reasons that the small bowel is the principal source of circulating citrulline. Consequently the plasma citrulline level is highly dependent upon the intestinal cell mass (25).

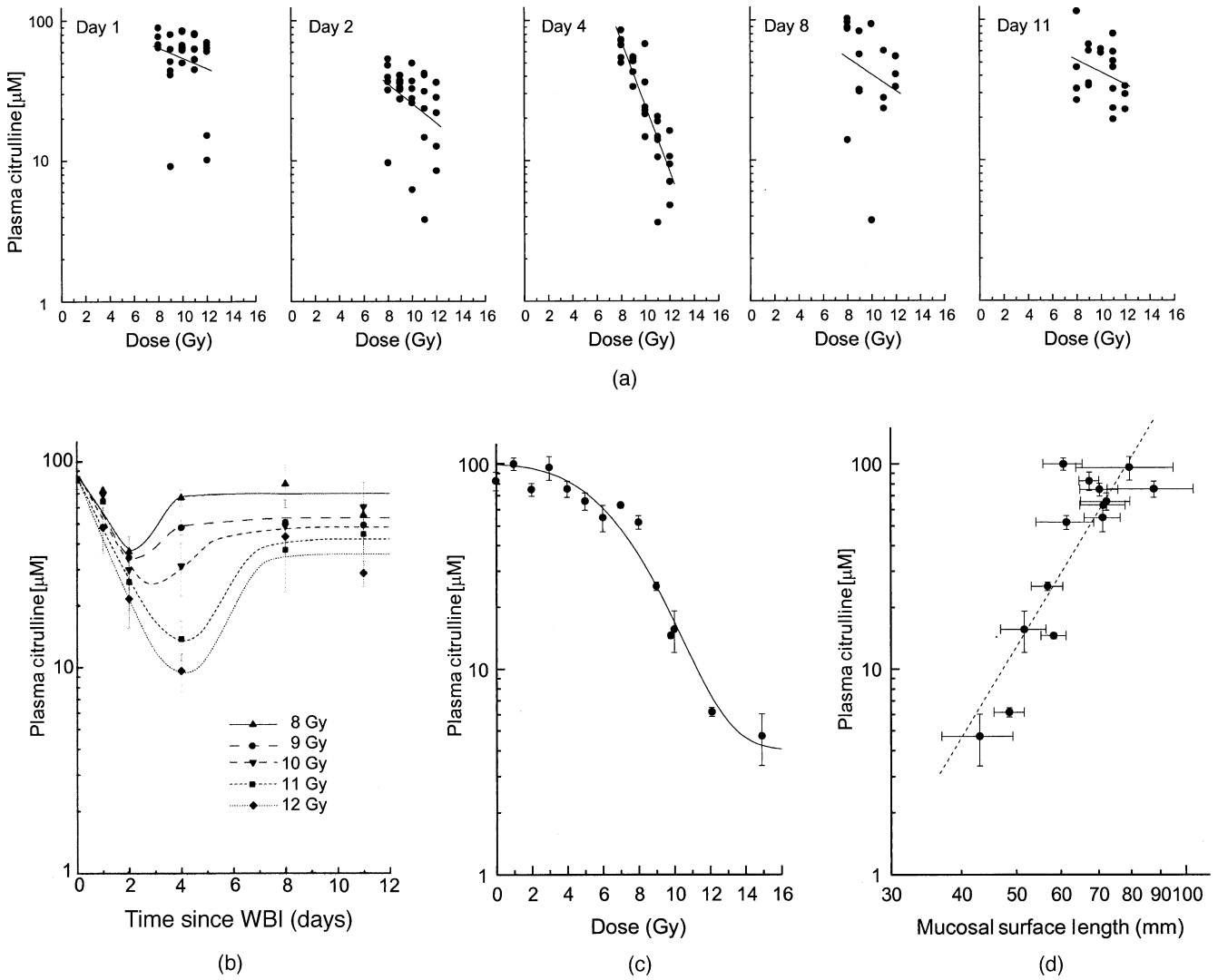

Fig. 2. Citrullinemia kinetics. (a) Scatter plots showing the dose–response relationships for plasma citrulline level at 1, 2, 4, 8, and 11 days after WBI with doses of 8–12 Gy. Pearson: $r = -0.23$, $r = -0.42$, $r = -0.88$, $r = -0.43$, and $r = -0.32$, respectively. The corresponding p values are 0.23, 0.02, <0.001, 0.09, and 0.18, respectively. (b) Mean (± SEM) plasma citrulline level (μM) as a function of time after single WBI doses of 8–12 Gy. Plasma citrulline level at Day 11 after WBI doses of 11 and 12 Gy are significantly lower as compared with control values ($p = 0.006$ and $p = 0.004$, respectively). (c) Plasma citrulline level at 84 h after whole body irradiation as a function of irradiation dose (Gy). Data points represent mean values ± SEM. One-way ANOVA; $p < 0.001$. (d) Correlation between plasma citrulline level and mucosal surface lining at 84 h after single WBI doses of 0–14.9 Gy. Data shown represent mean values ± SEM. Pearson: $r = -0.77$; $p < 0.001$.

The dependence of plasma citrulline level on epithelial cell mass observed in surgical experiments in rodents (20, 21) and human studies (22, 23) has been confirmed by us in mice after single-dose WBI. We found a linear correlation (Pearson: $r = 0.77$) between plasma citrulline levels and a representative parameter for epithelial cell mass, i.e., the epithelial surface lining. Remarkably, a strong correlation between the epithelial cell mass and jejunal crypt regeneration at 84 h after WBI was observed (Pearson: $r = 0.99$). The maximum decrease in epithelial surface lining in our time experiment was noticed on Day 4 after WBI. Although a further decrease between Day 4 and Day 8 cannot be ruled out, these observations are in agreement with previous studies using mucosal cell mass as an endpoint (5, 14).

The time course of plasma citrulline level after single-dose WBI (Figs. 2A and 2B) observed by us was comparable to that reported for other functional tests related to a reduced number of functional epithelial cells (5, 14). The rapid decline of plasma citrulline level seen at the first 2 days after treatment is independent of the WBI doses used. However, recovery was more rapid for the lowest dose (i.e., 8 Gy) and it was incomplete during the observation period for the highest dose levels used (i.e., 11 and 12 Gy; Fig. 2b). This time and dose pattern is in agreement with the radiation

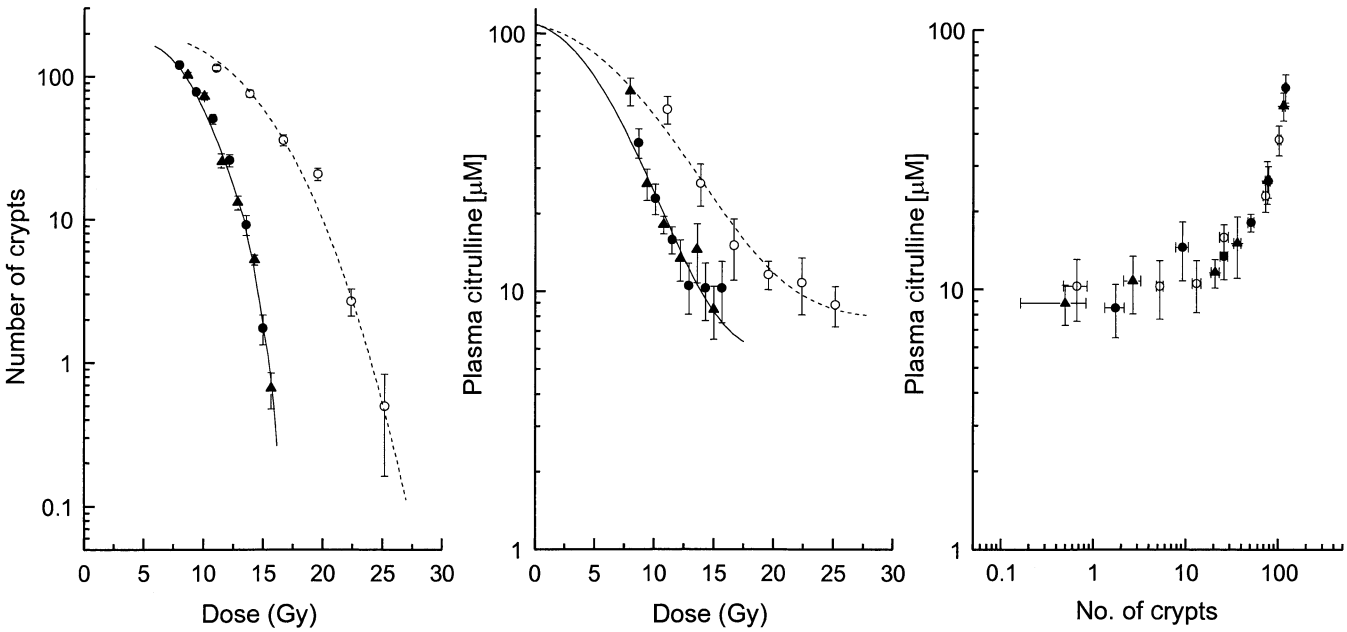

Fig. 3. Correlation between citrulline assay and microcolony assay. (a) Dose–response curve using the jejunal microcolony assay as an endpoint for acute small bowel radiation damage 84 h after WBI. Per dose point, 6 mice were used. Data points represent the mean number of crypts per circumference per dose point. ● = WBI only; ▲ = WBI plus 3%-glutamine enriched drinking water; ○ = 10 mg amifostine intraperitoneally 30 min before WBI. The dose modification factor computed at the level of 50 crypts per circumference for glutamine and amifostine was 1.0 and 1.5, respectively. (b) Dose–response curve using plasma citrulline level as an endpoint for acute small bowel radiation damage 84 h after whole body irradiation. Per dose point, 6 mice were used. The data points represent the mean plasma citrulline level (μM) per dose point. ● = WBI only; ▲ = WBI plus 3%-glutamine enriched drinking water; ○ = 10 mg amifostine intraperitoneally 30 min before WBI. The dose modifying factor for glutamine and amifostine determined at the ED50 level was 1.0 and 1.5, respectively. (c) Scatter plot showing a correlation between plasma citrulline level (μM) and crypt regeneration measured at 84 h after WBI doses of 8.0, 9.4, 10.8, 12.2, 13.6, and 15.0 Gy (WBI only), 8.7, 10.1, 11.5, 12.9, 14.3, and 15.7 Gy (WBI plus 3%-glutamine enriched drinking water), and 11.1, 13.9, 16.7, 19.6, 22.4, 25.2, and 28.0 Gy (WBI plus amifostine intraperitoneally 30 min before irradiation). Spearman's rho 0.83; $p < 0.001$.

effect on the hierarchically structured intestinal epithelium (1). Using the epithelial surface lining as a parameter did not yield significant changes except for the 4-day time point for the highest dose levels (i.e., 11 and 12 Gy). In contrast, for citrullinemia significant changes were observed for all dose levels used at the 4-day time point. Furthermore, plasma citrulline levels remained significantly decreased at the 11-day time point. For the dose range used in our experiments, mean values for mucosal surface lining ranged between 56% and 130% of control values, whereas for citrullinemia mean values ranged between 6% and 121% of control values. Thus citrullinemia seems to be more sensitive for detecting and monitoring small bowel radiation-induced epithelial cell loss than the representative morphologic endpoint used in these experiments.

We have demonstrated a significant dose–response relationship for citrullinemia at 84 h and 4 days (Fig. 2) after single-dose WBI. After WBI doses of 1–3 Gy, no effect on citrullinemia could be demonstrated. However, this parameter was inversely proportional to WBI doses of 3–12 Gy. The spread in baseline plasma citrulline level between mice may cover an effect at lower doses per fraction, which however might be demonstrated if repeated measurements in the same mouse are performed. The observation that plasma citrulline levels did not decrease any further beyond doses of 12 Gy is in agreement with the observation that the mucosal surface lining was intact, i.e., denudation was absent at the time point and dose levels used by us.

Encouraged by these results, the citrulline assay was compared with the microcolony assay as a test to prove its efficacy in discriminating different levels of small bowel epithelial radiation damage. Glutamine is the principal fuel for small bowel enterocytes (29). Enteral supplementation of this amino acid has been demonstrated to improve intestinal morphometrics (19) and survival (30) in rats submitted to single-dose whole abdominal irradiation. We have used glutamine accordingly to examine its effect on acute epithelial small bowel radiation damage in mice. In these experiments, amifostine (WR-2721), a compound of the class of sulfhydryl radioprotectors, was also used as a known positive control. In mice a linear dose–response effect for amifostine exists up to about 400 mg/kg body weight (16, 17). At this dose, administered 30 min before radiation by intraperitoneal injection, a DMF of 1.6 is consistently found for mouse jejunum using the microcolony assay to assess crypt cell regeneration (16, 17) with an accuracy on the DMF value better than ± 5% for the biologic procedure (31). We

observed a DMF of 1.5 using both jejunal crypt survival and citrullinemia as an endpoint, in complete agreement with data reported in the literature (16, 17). On the contrary, no effect was observed by us for glutamine on epithelial radiation damage (manuscript in preparation). For experimental conditions, as defined for the use of the microcolony assay (3), we found a strong correlation (Spearman's rho 0.94) between jejunal crypt regeneration and citrullinemia as parameters for epithelial radiation damage, independent of the intervention used (Fig. 3). Interestingly, enteral supplementation of glutamine did not have any impact on citrullinemia or crypt survival.

Although the DMF computed for amifostine for both endpoints was in complete agreement with literature data (16, 17), the citrulline assay seems less sensitive than the microcolony assay at the higher dose range such as typically used for the microcolony assay, as is demonstrated by a greater slope for the curve of the microcolony assay compared with the citrulline assay (Figs. 3B and 3C) and the dose–response relationship data (Fig. 2c). The opposite situation is true for the lower dose range where the threshold dose for the citrulline assay is significantly lower as compared with the microcolony assay, i.e., about 3 Gy vs. 8 Gy, respectively. Furthermore, in contrast to the microcolony assay, the citrulline assay permits repeated measurements within the same animal. Therefore, the citrulline assay and the microcolony assay are supplementary, both with regard to the dose range and with regard to their applicability.

In conclusion, our data demonstrate that after single-dose WBI plasma citrulline level kinetics are dose-dependent and in accordance with radiation injury to the clonogenic compartment of small intestinal epithelium. Citrullinemia is a simple and sensitive marker for monitoring small bowel epithelial radiation damage after single WBI doses between 8–12 Gy. Furthermore, this parameter enables quantification of epithelial cell loss after doses per fraction between 3–12 Gy. The kinetics observed for this parameter challenge its use as an assay for monitoring epithelial radiation-induced intestinal damage in clinical practice.

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