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TIGHT JUNCTIONAL CHANGES UPON MICROWAVE AND X-RAY IRRADIATION*

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Tight junctions (*zonulae occludentes,* ZO) are cellularly regulated dynamic structures sensitive to environmental stress agents including ionizing radiation. Radiation induced pathological alterations of the small intestine (gastrointestinal radiation syndrome) are related to altered ZO-mediated paracellular transport. We carried out a quantitative morphological evaluation of the murine jejunal epithelial tight junctional structure in freeze fracture replicas as changed upon whole body X-ray irradiation and low energy microwave exposition. X-ray treatment (4 Gy, 1, 24 h) brought about a partial dearrangement of the ZO strand network which regenerated only partially by 24 h. This observation is in line with data on paracellular permeability increases and ZO-bound calcium drop caused by X-ray irradiation. On the other hand, microwave treatment (16 Hz-modulated 2.45 GHz wave, 1 mW/cm^2 power density, 1 h exposition, samples at 1 and 3 h after exposition) did not cause dearrangement but, rather an increase in the integration of thight junctional structure, which is in agreement with an increase in cytochemically detectable ZO-bound calcium.

Keywords: Tight junction – zonula occludens – freeze-fracture – X-ray – microwave

INTRODUCTION

Since 1956 when Quastler described [28] the gastrointestinal radiation syndrome as the mode of acute radiation death, exceptional attention has been payed to the role of intestinal injury in this deadly phenomenon that may follow either accidental or therapeutic ionizing irradiation. Damage to the gastrointestinal system is of primary concern for local irradiation of abdominal or pelvic tumors as well as for whole-body irradiation before bone marrow transplantation [1, 7, 12, 23]. Depending on its dose, irradiation causes a partial denudation and changes of the intestinal luminal surface which results in a fluid and electrolyte imbalance, bacteremia and consequent endotoxemia [7, 8, 38]. It is generally accepted now that ionizing radiation death is a direct consequence of the gastrointestinal epithelial damage [12, 15, 25, 29, 38] that has been thought to depend solely or mainly on the number of the surviving crypt

*Dedicated to Professor János Kovács on the occasion of his 70th birthday.

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cells [25–27]. Recent research, however, revealed that irradiation induce a more complex series of alterations in the gastrointestinal structure and function [31], including immediate and delayed changes of enterocytic organelles [30]. Due to the pathophysiological importance of intestinal epithelial integrity with special regard to the regulation of the paracellular transports, structural and functional investigations into the radiation-induced alterations of enterocytic tight junctions deserve morpho-functional studies. In his early freeze fracture study, Porvaznik [24] observed a significant enlargement of the mean tight junctional depht (zonule width) 3 days after X-irradiation (3 and 5 Gy). In this paper we present our morphometrical data on the changes of thight junctional complexity upon X-ray (4 Gy) and modulated microwave irradiation.

MATERIALS AND METHODS

Tissue samples were taken from whole body X-irradiated (4 Gy, samples 1 and 24 hours after irradiation) or microwave exposed (16 Hz-modulated 2.45 GHz wave, 1 mW/cm^2 power density, 1 hour exposition, 1 and 3 hours after exposition) animals.

Pieces of the jejunum were fixed by immersion in phosphate-buffered 2.5% glutaraldehyde solution for 2 hours at 4 °C. After rinsing in the vehicle buffer, the samples were sequentially infiltrated with 10, 20 and 30 per cent glycerol diluted in the buffer, for 10, 20 and 30 minutes, respectively, then transfered to specimen holders, frozen in liquid Freon 22, and stored in liquid nitrogen. Fracturing and replication was performed in a Balzers freeze-etching apparatus. The fracturing temperature was –110 °C. For the preincubated and incubated samples a total of twenty replicas was examined. In most cases the replicated tissue appeared uniformly well-preserved,

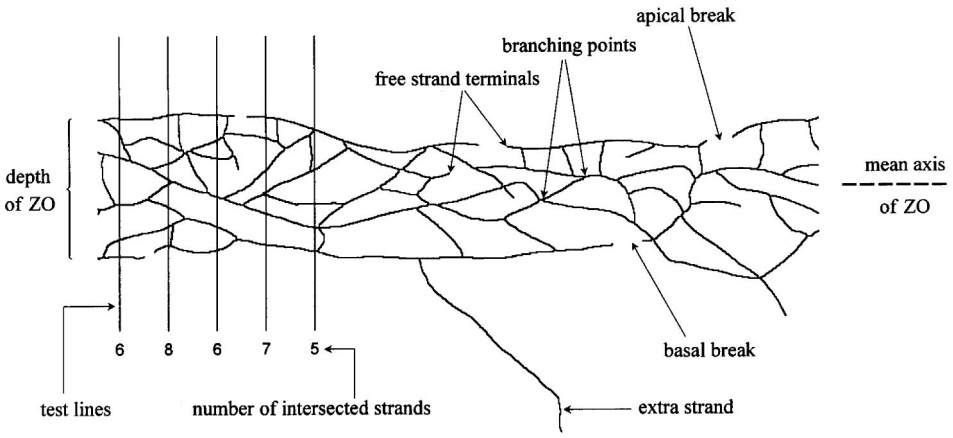

Fig. 1. Schematic drawing demonstration of the quantitative analyzed morphological parameters of the tight junction (ZO)

since signs of unspecific damage (for example, clustering of intramembrane particles in the plasma membrane; ER vesiculation) were not evident. For the studies reported in this paper only the replicas of uniformly well-preserved tissue were considered. Thight junction P fracture faces were superimposed by transparent paper and their strand network superdrawn to obtain samples for electronic image analysis at a magnification of ×100 000. The evaluated morphological parameters of tight junctional network are defined and summarized in Fig. 1 and Table 1.

RESULTS AND DISCUSSION

Our morphometric data are demonstrated in Table 1. X-ray irradiation caused a dearrangement of ZO network as indicated by a significant enlargement in the mean depth of tight junction zonules, increase of overall brake index (BI), elevation of number of breaks in apical and basal strands (N_a and N_b, respectively) (Table 1). According to our experience the so-called complexity index (CI: branching points per total strand length) introduced to quantitate tight junctional structure in correlation with permeability of epithelia [37] is not an indicator sensitive enough to detect tight junctional structural alteration in the present system with wide zonules of dense network of strands. CI was originally successfully used in the analysis of narrow zonules such as those found in endothelium [10, 16]. Since structural dearrangement involves not only a decreased abundance of branching points but, more characteristically, also an increased frequency of strand brakes, our brake indices are more informative in this respect. The geometrical dearrangements of tight junction network were rapid (detectable already 1 hour after irradiation), and we found only a partial regeneration of the tight junctional structure after 24 hours.

The breakdown of tight junction-regulated barrier was visualized in electron microscopical sections by means of lanthanum or ruthenium red tracers [12, 14, 32] because when the paracellular barrier is broken, these electron dense tracers can penetrate from the lumen to the intercellular space of epithelia. Parallelly, the loss of cytochemically detectable tigh junction-bound calcium occurs [32]. Our freeze-fracture study now has revealed the structural background of these permeability changes and confirmed the conclusion of earlier studies, i.e. that the tight juction is a dynamicly changing structure, rather than a simple inactive permeability barrier [2–3, 9, 12, 21, 29, 35–36]. An important output of this view is the search for intestinal permeability modifying agents to be used as drug release modifyers [4–6, 11, 13]. A quite recent finding [22] that tight junctions contain specific microdomains enriched in cholesterol-, ZO-1 and highly phosphorylated occludin put forward the idea, that dynamic changes in their structure may occur as changes in the relationship of their micromodules. As to the alteration of tight junctions it is of interest, that X-irradiation (3, 5 Gy) induced a cytochemically detectable time dependent disruption of the continuous occludin staining between differentiated HT-29 and MDCK cells (Somosy et al., unpublished data). X-irradiation induced cellular alterations may be the consequence of changes in the homeostasis of extracellular and consequently of

Table 1
Changes in tight junction morphometrical parameters after irradiation

	Control	X-ray 1 h	X-ray 24 h	Microwave 1 h	Microwave 3 h
D [nm]	303.3±61.0	256.4±67.1	307.9±68.1	253.5±53.2	273.4±63.0
N_i	5.836±1.137	5.178±1.136	5.377±1.186	5.647±1.116	5.566±1.226
L_t [nm/μm]	7798±911	6690±970	7639±1108	6942±856	7742±1194
l [nm]	82.18±13.20	68.29±9.52	78.09±10.91	76.42±7.59	72.62±9.74
N_e [1/μm]	1.094±0.921	1.488±1.195	2.507±1.504	0.983±0.667	1.416±1.249
L_e [nm/μm]	127.7±135.2	149.6±158.2	300.4±235.4	192.8±162.0	178.6±161.9
CI [1/μm]	7.646±1.147	7.916±1.208	7.529±1.440	8.071±0.825	8.820±1.018
BI [1/μm]	1.434±0.782	6.279±2.577	3.993±1.690	0.788±0.270	1.029±0.451
N_b [1/μm]	1.130±0.866	3.807±2.250	2.424±1.530	0.619±0.582	0.927±0.935
N_a [1/μm]	0.430±0.665	3.359±2.065	3.474±1.930	0.335±0.473	0.334±0.530

Parameters of tight junctional strand network: (D) – Depth of ZO; (N_i) – Number of intersected strands; (L_t) – Total strand length/Axis; (l) – Mean strand length; (N_e) – Number of extra strands/Axis; (L_e) – Total extra strand length/Axis; (CI) – Complexity Index: Number of branching points/Total strand length; (BI) – Break Index: Number of free strand terminals/Total strand length; (N_b) – Number of basal breaks/Axis; (N_a) – Number of apical breaks/Axis. Data are given as indicated ±SD.

intracellular signalling (for references see 31). Tight junctional changes in which the actin cytoskeleton plays an important role [17–21, 33], are most probable under the control of intracellular signals [22].

In contrast to the effect of X-irradiation, jejunal epithelial cells reacted to microwave exposure with an increase of structural integrity of tight junction as indicated by the considerable decrease of break indexes (Table 1). This observation is in line with a microwave-induced increase of tight junction-bound calcium concentration [34]. Thus, an opposite effect of ionizing and non-ionizing radiations on the tight junction was found in our study.

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