Endothelial Cells and Radiation Gastrointestinal Syndrome

Paris et al. have quantified extensive apoptosis in endothelial cells of jejunal microvasculature (1). This raises questions about the pathobiology of tissue injury from irradiation, but their interpretation that these changes are the basis for postradiation demudation of the jejunal mucosa is not supported by relevant data.

If death of irradiated jejunal crypt cells were due principally to apoptosis of the capillary endothelial cells and consequent blockage of capillary blood flow, the mechanism would be hypoxic or ischemic death. In this scenario of severe jejunal hypoxia, the slope of the crypt cell survival curve (log cell survival fraction versus dose) would be steep for aerobic cells cell survival curve (log cell survival fraction versus dose) would be steep for aerobic cells hypoxic or ischemic death. In this scenario of capillary blood flow, the mechanism would be apoptosis of the capillary due principally to apoptosis of the capillary.

More than 2 days) that are inconsistent with intervals ranging from less than 1 hour to experimental data for crypt cell survival have been made in radiation-induced apoptosis in gut endothelial cells, and concomitantly in the endothelial-epithelial linkage remains un- known, although ischemia due to microvascular impairment likely plays a role in this process.

Hendry et al. also comment that there is little evidence that endothelial cells are more sensitive to apoptosis than clonogenic crypt epithelial cells. However, our data (2) showed that endothelial apoptosis plateaus at 4 hours after 15 grays (Gy), but that crypt epithelial cells displayed no discernible apoptosis, except at position 4 to 5, which is not associated with the GI syndrome (1). Further, recent dose-escalation experiments have shown that at 18 Gy, intensive epithelial apoptosis develops at positions 6 to 10 from the crypt base (3). This response is subject to different biochemical regulation than the en-
dothelial response, and is amenable to genetic and pharmacological manipulations different from those controlling endothelial apoptosis (3). These observations indicate that the epithelial clonogens of the crypt are indeed more resistant to lethal effects of radiation than the GI microvascular endothelium. The data also suggest a hierarchy in the sensitivity of critical tissue elements of the GI tract to different modes of radiation-induced cell death, activated in an orderly fashion as the dose increases (3).

Suit and Withers argue that the differential effects of radiation on the crypt and villus are inconsistent with our notion of microcirculatory impairment and ischemia in the pathogenesis of the GI syndrome. Concurrent damage to both crypts and villae would have rather been expected if blood flow were blocked. However, our results did not indicate an infarctlike, acute tissue necrosis. Instead, our data showed that even at doses that induce the GI syndrome, the severity of endothelial apoptosis was heterogeneous, with some regions exhibiting extensive damage and others showing mild or no apoptotic damage at all. In many instances, extensive endothelial apoptosis was observed at the upper half of the villus and none in the regions adjacent to the crypt, or vice versa. The paper’s figures clearly demonstrated these points (2). Hence, it appears that the microcirculatory damage leads to focal regions of ischemia of differing intensities, rather than to global tissue hypoxia.

Suit and Withers further suggest that if our model of microvascular dysfunction is correct, it would predict that curves of crypt regeneration versus dose would have progressively more shallow slopes as the number of radiation fractions increases. In fact, published data demonstrated a progressive flattening of jejunal crypt regeneration curves as the fraction number increased from 1 to 5, 10, and 20 fractions [figure 3 in (4)], but detailed statistical analysis of these curves was not provided. We have digitized the data from the original publication and statistically analyzed the slopes based on pairwise sets of points within a fraction. This analysis confirmed that the slopes were indeed different (5).

Suit and Withers also comment that data from a split radiation dose experiment published by Withers (6), in which two radiation doses were delivered at interfractional intervals of 1 to 48 hours, are inconsistent with our model. They argue that hypoxia produced by microvascular damage from the first radiation dose should have induced resistance to the second exposure, but this pattern was not observed. It should, however, be noted that in this particular experiment, the lead dose was only 660 cGy, a dose that, according to our data, produces minimal if any endothelial apoptosis and microvascular dysfunction (2). Hence, hypoxia-mediated radiation resistance to the second dose would not have been anticipated.

Finally, it should be emphasized that the hypothesis of a direct lethal effect of radiation on GI stem cells has never been proven experimentally. It is impossible to assess parameters of damage and repair, survival or death in a cell that has no markers and whose existence can only be inferred. The hypothesis on radiation-induced clonogenic stem cell death was derived by analogy from mathematical and biophysical models developed to fit survival data and explain patterns of mammalian cell responses to irradiation in tissue cultures. However, it should not be forgotten that the intestine is a complex organ, comprising many different interacting cell types and structures, with distinct response patterns to radiation. We believe that the overall response to radiation reflects a complex interplay of these cells, which cannot be interpreted at present by hypothetical models, but rather must be elucidated using molecular and genetic reagents in vivo.

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