feeding with a vitamin E–enriched diet also yielded significant protection of the intestinal mucosa against acute radiation damage.

In the context of lumenal effects, interpretation of the results of oral pretreatment is ambiguous. Vitamin E is absorbed from the gut and circulates systemically. It concentrates in lipids and lipid bilayer membranes. Studies with radiolabeled alpha-tocopherol have indicated that absorption is incomplete.[23] It is unknown whether the unabsorbed vitamin remains physiologically active throughout the full length of the gut lumen. If so, vitamin E would reach the enterocytes directly from the lumen as well as through the systemic circulation.

Acute radiation enteritis is common and causes severe but generally self-limiting symptoms. Late complications in the form of stricture, ulceration, or malabsorption are relatively infrequent but cause severe morbidity and significant mortality. These delayed injuries are progressive and are merely palliated by operative interventions. The relationship between acute injury and late complications is uncertain. Studies are underway to address the question of whether amelioration of acute damage eventuates in diminished late effects.

Most of the experimental studies regarding radioprotectors and the gastrointestinal tract have involved systemic administration of drugs. These agents generally cause substantial toxicity and have the inherent potential to protect target tumor cells.

In clinical trials that have involved marginally effective doses of systemic WR-2721, the most thoroughly studied radioprotectant, multiple side effects, including hypotension, somnolence, nausea, and vomiting, were observed. These were severe enough to make the drug impractical for clinical use.[24]

The experimental procedure of injecting high concentrations of vitamin E directly into the lumen cannot be duplicated clinically. Use of oral vitamin E as a radioprotectant could solve some of the problems encountered previously in the development of a practical pharmacologic regimen. High doses of this agent have essentially no adverse side effects, acute or chronic. Whether vitamin E can reduce damage to target malignancies will require extensive study.

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Discussion

DR. WILEY W. SOUBA (Boston, Massachusetts): I rise to congratulate Dr. Delaney on a fine study. This is an area that he

has been studying for quite some time, and I appreciate the opportunity to review the manuscript prior to the meeting.

As nicely appointed out, the authors have demonstrated the radioprotective effect of a relatively safe and a relatively inexpensive naturally occurring nutrient—vitamin E—on the small bowel mucosa.

Ionizing radiation generates toxic oxygen free radicals; that is, an oxygen molecule containing an unpaired electron in its outer orbital. It is this unpaired electron that makes the superoxide unstable, conferring to it the capacity to induce damage when it reacts with other molecules in the cell to find a mate for this unpaired electron.

Vitamin E is a naturally occurring nonenzymatic antioxidant that helps keep reactive oxygen derived molecular species at a minimum. Vitamin E is crucially important in protecting against lipid peroxidation by scavenging superoxide radicals. I have three questions.

First, the model employed here uses a single dose of 1100 rads, a relatively high dose of radiation, particularly as a one-time dose. In patients, multiple lower doses are used, usually about 200 rads per day over a 25- or 30-day period. This lower dose in part allows the cells to undergo some repair prior to the next treatment. Have you examined the use of vitamin E in a model that more approximates this clinical setting?

My second question you alluded to and centers around the concept that other forms of nutrition can be used to modulate this radioprotective effect. Both cysteine and glutamine can enhance the production of glutathione, which is an antioxidant tripeptide, which has properties, and in addition the mineral selenium, is a cofactor that functions to assist the enzymatic capacity of several antioxidant enzymes. Other potentially useful nutrients include vitamin C, carotenes, and taurine. Would you comment about the potential use of combination therapy? Do these agents act synergistically to protect the mucosa?

Finally, can these antioxidants confer any beneficial effect if given after the radiation has been delivered? Or is prophylaxis the best way of affording protection?

DR. FRANK G. MOODY (Houston, Texas): I have a methodologic question, I guess, that relates to the guts of your experiment. I understand that you did four segments that you tied off. Were they tied off throughout the whole 5 days in which you observed the animals? Could there be other variables in terms of, for example, accumulation of additional fluid within the lumen that might affect this crypt phenomenon?

We have been doing radiation studies with much lower doses (8 Gy) in the rat, focusing on the immunophysiology, and you mentioned that what is important is what goes on behind the mucosa. Have you made any observations on the cellular infiltrate that must necessarily occur in this model and what effect that might have in terms of crypt development or rat development and also the production of oxygen derived free radicals?

DR. JOHN S. SPRATT (Louisville, Kentucky): I studied this problem at the Mallinckrodt Institute of Radiology in 1957 and 1958. The most sensitive tissue in the intestine is the endothelium of the capillaries and lymphatics with a reduction in the capacity to form granulation tissue. The long-term adverse effects of radiation on the gut can be seen microscopically with the sclerosis that occurs in the vessels. As a matter of fact, it should be looked for by frozen section if there is any question about whether or not gut has been radiated beyond its capacity to heal adequately.

We also found that if ulceration occurred, as it generally did at the doses we were using, it was fascinating how the bowel healed itself. It healed itself by longitudinal contracture after the omentum wrapped around the bowel and really brought about a spontaneous reanastomosis of proximal and distal, nonirradiated bowel.

But the principal point I wanted to make or the question I wanted to pose is whether or not they have looked at the protective effect on the endothelium of the microvasculature of the gut. Because if that is not protected, the radiation injury will still progress over time.

DR. WARD O. GRIFFEN, JR. (Lexington, Kentucky): Jack, just a practical question. It seems to me you could test this clinically if you could get your radiation therapy people to go along with it by giving vitamin E enemas to people getting pelvic irradiation to see if they could reduce the incidence of proctitis. That is something you could do tomorrow if you wanted to.

DR. JOHN P. DELANEY (Closing Discussion): The issue of single-dose radiation *versus* multiple doses is a very difficult one experimentally. In the radiologic literature, about the most you ever find is five or so doses of radiation because of the logistic difficulty of repeatedly radiating the same animal. Obviously, you cannot exteriorize the bowel over and over. There is a model now in which a scrotal hernia is created. A portion of the small bowel is brought down into the scrotal hernia and then is radiated repeatedly. We have not used this preparation yet, but may do so.

Dr. Souba, as some of you might know, has demonstrated the benefits of glutamine. And he made a unique observation that glutamine given after the radiation injury actually provided some protection. I do believe that is one of the very few experiments in which post-treatment rather than pretreatment resulted in radioprotection.

Using our model, we have verified his observation. One of the combinations we plan to use is glutamine plus vitamin E. Both of them are perfectly nontoxic, and both provide benefits. One could speculate that glutamine actually hastens recovery of the mucosa, whereas vitamin E diminishes injury. We have not used selenium. We have tried vitamin C in this particular model, and it provided no benefits. It seems that one of its main functions is to regenerate vitamin E—that is, it serves to reduce oxidized vitamin E.

Dr. Moody asked about the ties. The ties are taken off after irradiation then markers are put adjacent to the bowel to be able to identify the segments later on when we harvest the intestine.

He also asked about the possibility of fluid accumulation. We tested luminal osmolarity as a variable and found no enhancement of or protection against radiation injury.

Cellular infiltration certainly plays a part in the injury. One of the merits of this model is that all four segments get exactly

the same treatment in terms of radiation. They are at the same temperature, they are getting the same blood flow. It is obviously a fairly unphysiologic circumstance, but we are comparing apples to apples.

Dr. Spratt pointed out that vascular injury is important. It certainly is observed in late injuries to the small intestine. There are two schools of thought in the radiobiology literature. One holds that the vascular injury is primary and causative. The other holds that the vascular injury is a consequence of the sclerosis—that is, collagen deposition in the submucosa and in the muscle. But vascular damage certainly is a very important factor to study. We have a student starting in the laboratory soon who is going to devote himself specifically to attempting to quantitate vascular injury in a model of late injury.

Dr. Griffen pointed out the possibility of testing the easily observed part of the intestine, the rectum, by using enemas at the time of the radiation. We have a proposal in to our Human Experimentation Committee to use enemas of various agents, including glutamine and vitamin E. We might diminish injury to the rectal mucosa, which would be measurable. Diminishing injury to the small bowel mucosa will be very, very difficult to evaluate in the human. The rectum should be fairly easy.