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ANTI-OXIDANT VITAMINS REDUCE NORMAL TISSUE TOXICITY INDUCED BY RADIO-IMMUNOTHERAPY

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Our purpose was to determine whether the administration of anti-oxidant vitamins could reduce dose-limiting toxicity from radio-immunotherapy (RAIT) and thereby allow higher escalation of RAIT doses. Lipophilic vitamins A and E were administered i.p. and hydrophilic vitamin C was administered i.m. for 14 days (3 days pre-RAIT through 11 days post-RAIT) alone or with bone marrow transplantation (BMT) to either BALB/c mice for toxicity studies or to nude mice bearing s.c. GW-39 human colonic cancer xenografts for therapy studies. The maximal tolerated dose (MTD) of RAIT (^{131}I-MN-14 anti-CEA IgG) that results in no lethality was determined for mice that did not receive vitamins or BMT and those that did receive one or both interventions. Body weight, peripheral white blood cell (pWBC) and platelet (PLT) counts and tumor growth were also measured. Administration of vitamins (equivalent of 3.5 IU/day vitamin A, 0.107 IU/day vitamin E and 4.0 mg/day ascorbic acid) to mice along with BMT increased the MTD by 42% and reduced body weight loss associated with RAIT. Vitamins also reduced the magnitude of RAIT-induced myelosuppression. As early as day 7 after RAIT, vitamins increased WBC counts following both a 400 μCi and a 500 μCi dose. On day 14 after the 400 μCi dose of RAIT (day 7 post-BMT), the additive effect of BMT and vitamin could be detected. Tumor growth was not adversely affected by vitamin administration. *Int. J. Cancer* 86:276–280, 2000.

Radio-immunotherapy (RAIT) is a therapeutic approach that uses antibodies directed against tumor-associated antigens to carry cytotoxic radionuclides to antigen-expressing tumor tissue. Theoretically, high tumoricidal radiation doses can be achieved, while maintaining low normal tissue doses, resulting in a therapeutic response with minimal radiation toxicity to non-tumor tissue (Kaminski *et al.* 1996; Press *et al.* 1993). RAIT is limited by hematopoietic toxicity, *i.e.*, neutropenia and thrombocytopenia (Vriesendorp *et al.* 1996). The challenge of improving the clinical success of RAIT is to increase tumor accretion of radio-antibody and to decrease hematological toxicity (Blumenthal *et al.* 1995*b*). Complete remission rates and disease-free survival could potentially be improved if myelotoxicity were reduced and dose intensification achieved (Blumenthal *et al.* 1995*a*). Several approaches to accelerate recovery from or prevent hematopoietic toxicity by tumoricidal agents have been employed, including autologous bone marrow transplantation (BMT) (Blumenthal *et al.* 1994), peripheral blood stem-cell transplantation (SCT) (Kessinger *et al.* 1988) and administration of hematopoietic growth factors (Blumenthal *et al.* 1992).

Following reduction in RAIT-induced myelosuppression by cytokine intervention or BMT, secondary end-organ toxicity becomes problematic. Liver, kidney, lungs and gastrointestinal (GI) tract are potentially critical sites for secondary toxicity associated with RAIT (Langmuir and Sutherland, 1988). The normal tissues that are likely to be affected are in part determined by the antibody used, the form of immunoglobulin used [*e.g.*, for many antibodies, Fab' tends to concentrate in kidney and $F(ab')_2$ tends to concentrate in liver] and the radionuclide used (radiometals concentrate in liver and free radio-iodine distributes to the GI tract).

Radiation damage results in the generation of several types of reactive oxygen species (*e.g.*, hydroxyl radicals, superoxide anion radicals, hydrogen peroxide, singlet molecular oxygen). Vitamin E (tocopherols and tocotrienols), vitamin C (ascorbic acid) and vitamin A (retinol) are anti-oxidants that can be used to reduce DNA damage and diminish lipid peroxidation and increase tissue radioresistance (Frei, 1994; Sies and Stahl, 1995). With but few exceptions, toxicity, mutagenicity, teratogenicity and carcinogenicity are low after ingestion of large amounts of vitamin E, vitamin A and vitamin C (Diplock, 1995).

Vitamin E is the most important lipophilic anti-oxidant. In comparison, vitamin A has lower reactivity toward radicals, is more lipophilic and is likely to be present at the interior of membranes or lipoproteins, which enables it to scavenge radicals within the lipophilic compartment more efficiently than vitamin E (Niki *et al.* 1995). *In vitro* studies on lipid peroxidation have demonstrated a synergistic interaction between vitamins A and E (Teshima *et al.* 1997), due at least in part to reciprocal protection against consumption of the other vitamin. Vitamin E alone is not effective at preventing radiation-induced GI toxicity in rats, whereas 2 water-soluble radioprotectors are effective (Schwartz, 1995).

Vitamin C is hydrophilic and is a most important free-radical scavenger in extracellular fluids, trapping radicals in the aqueous phase and protecting biomembranes from peroxidative damage. In addition, it appears to play a role in cellular redox processes mediated by glutathione (Harapanhalli *et al.* 1996). The choice of whether to use one vitamin or a combination of vitamins and at what dose schedule relative to radiation exposure is a subject of debate. In one murine study, both vitamins C and E demonstrated radioprotective effects (reduced frequency of micronuclei and reduced chromosomal aberrations post-radiation), but vitamin E afforded greater protection. The effect was greatest when administered 2 hr before radiation or immediately afterward but not 2 hr afterward (Sarma and Kesavan, 1993). Some reports, however, have shown that vitamin E alone was ineffective in rats and mice but vitamin C alone was radioprotective (Rostock *et al.* 1980; Umegaki *et al.* 1994). Possible causes of these differences may be radiation dose or route of vitamin administration. In addition to its anti-oxidant effects, vitamin C is involved in the regeneration of tocopherol from tocopheroxyl radicals in the membrane. Thus, vitamins C and E can have interactive effects (Stoyanovsky *et al.* 1995). β-Carotene and α-tocopherol also have synergistic inhibitory lipid-peroxidation effects. Studies in humans have shown that either β-carotene or vitamin C ingestion in combination with vitamin E can significantly enhance the concentration of circulating vitamin E above that seen when vitamin E is used alone; this may be a good reason to combine vitamin regimens. Similarly, when studying the chemopreventive effects of dietary supplements, which act in part through anti-oxidant functions of these supplements, the combination of carotenoid with vitamin E, vita-

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min C and glutathione may be more effective (Toma *et al.* 1995). Finally, radioprotective effects of a mixture of vitamin C, vitamin E and β-carotene against γ-ray-induced DNA damage in mouse bone marrow and bladder cells, as measured by the micronucleus assay, was reported (Konopacka *et al.* 1998). The vitamin mixtures worked well as both a pre-treatment and a post-treatment. In addition to the anti-oxidant effects, the vitamin mixtures appeared to improve the kinetics of repair of radiation-induced DNA damage.

Limited work has been performed on the efficacy of vitamins as radioprotectors against tissue-incorporated radionuclides. In one study, both dietary and injected vitamin C demonstrated radioprotective effects against ^{131}I and ^{125}I but not against α emissions from ^{210}Po (Narra *et al.* 1993). The efficacy of vitamin C was greater with lower-dose-rate than higher-dose-rate radiation, supporting its use with RAIT. The same team of researchers has also shown that β-carotene is an effective radioprotector against tissue-incorporated ^{125}I but not against ^{210}Po (Harapanhalli *et al.* 1994). This report addresses the application of anti-oxidant vitamins to reduce normal tissue toxicity resulting from low-dose-rate RAIT.

MATERIAL AND METHODS

Dietary vitamins

Anti-oxidants were administered at 10-fold above nutritional requirements, while taking into account the amount of each vitamin found in the standard rodent diet. Recommended concentrations for vitamin A (retinol) are 2.4 IU/g diet and for vitamin E (RRR-α-tocopherol), 0.032 IU/g diet (Subcommittee on Laboratory Animal Nutrition, 1995). The laboratory autoclavable rodent diet 5010 from PMI Feeds (St. Louis, MO) contains 34.1 IU/g vitamin A (75% is lost upon autoclaving, leaving only 8.5 IU/g). Vitamin A is in the acetate form, and activity is determined analytically in retinol equivalents. The feed also contains 0.066 IU/g vitamin E (19% is lost upon autoclaving, leaving 0.0535 IU/g) in the di-α-tocopheryl acetate form and analytically is represented as tocopherol units. The feed has no vitamin C since it is not a required nutrient for mice, but it is important as a part of the anti-oxidant cascade. Thus, the daily administered vitamin doses were in the range of 21.3 IU/mouse vitamin A acetate (in mineral oil), which were converted to retinol, and 0.107 IU/mouse vitamin E acetate (in mineral oil). Based on previous work (Toma *et al.* 1995), 4 mg ascorbic acid were administered daily in saline. Vitamins A and E were administered i.p. and vitamin C i.m. Intra-gastric dosing was not used because of potential GI toxicity caused by RAIT that might limit intestinal absorption of the vitamins.

Radiolabeling and quality assurance

MN-14 anti-CEA monoclonal antibody (MAb) (Sharkey *et al.* 1993) was radio-iodinated by the chloramine-T method (McConahey and Dixon, 1966). Bound iodine was separated from free iodine by passage over a PD-10 column (Pharmacia, Piscataway, NJ) equilibrated with 0.04 M PBS (0.04 M phosphate, 0.15 M NaCl, 0.02% NaN_3), pH 7.4, containing 1% human serum albumin. Specific activity of the labeled product was 12 to 15 mCi/mg. Quality assurance of labeled MAb revealed no detectable aggregates and 2% to 4% free radio-iodine by size-exclusion HPLC using a Zorbax GF-250 (Dupont, Wilmington, DE) column; 75% to 95% of the radiolabeled MAb bound to CEA-Affigel. Radioantibody was administered by i.p. injection at a dose of 400 or 500 μCi in a minimum of 200 μl buffer.

Bone marrow transplantation

Bone marrow was harvested, using a sterile technique, from untreated donor mice. Total cells were counted with a hemocytometer. Cells were diluted, and 1×10^7 cells were injected i.v. into recipient mice 7 days post-radio-antibody administration in 100 μl buffer. The number of cells and the time for BMT were previously established (Blumenthal *et al.* 1995*a*).

Peripheral blood counts

Non-tumor-bearing BALB/c mice were studied for survival over a 4-week period, including measurement of body weight and blood counts. Mice were weighed weekly and changes in body weight recorded. The same mice were bled retro-orbitally at weekly intervals and 50 μl processed as done previously (Blumenthal *et al.* 1994, 1995*a*). RBCs were osmotically lysed and WBCs and platelets were washed in PBS (pH7.4 + 0.2% NaN_3) and fixed in PBS plus 1% formalin. Blood counts were made using a Becton Dickinson (Mountain View, CA) flow cytometer, with total and differential WBC counts determined. A 1:10 dilution was prepared, and the gains and threshold were adjusted to count platelets. The mean ± SD was recorded for groups of 5 to 10 mice in each treatment group. Results were evaluated by a single-factor analysis of variance (*F*-test).

Tumor measurements

Athymic nude mice were implanted s.c. with 200 μl of a 10% GW-39 human colonic tumor cell suspension and allowed to grow for approx. 2 weeks until average initial size was 0.5 cm^3. Tumors were measured on the day of treatment and then weekly, with a caliper in 3 perpendicular planes and the product of the 3 measurements expressed in cubic centimeters. The change in tumor size was calculated and the mean ± SD for each treatment group graphed. For s.c growth studies, data from an individual tumor line were aggregated for growth curve analysis. Within a tumor treatment group, the growth pattern was either linear or exponential. When the growth pattern was linear, linear regression was used for the statistical analysis. When the growth pattern was exponential, non-linear regression based on asymptotic approximation was used for the analysis. In comparing a linear growth pattern with an exponential one, area under the curve (AUC) was the end point of comparison rather than the slope, which is not constant over time in exponential growth. In comparing 2 linear growth patterns, the slope was the end point of comparison, provided the intercepts were not significantly different in the 2 groups.

RESULTS

The maximum tolerated dose (MTD) for ^{131}I-MN-14 in non-tumor-bearing mice was 350 μCi. We evaluated 400, 450 and 500 μCi doses in the presence of either vitamins, BMT or both interventions together. Table I summarizes results on survival after dose escalation. Both vitamins alone and BMT alone increased the percent of mice surviving from dose intensification, though BMT resulted in a greater percentage of mice surviving than vitamins alone. Survival was further improved when both BMT and vitamins were used together compared with either intervention used alone. A 500 μCl dose of radio-antibody results in 100% lethality within 18 days. The use of vitamins or BMT resulted in a 20% or 70% survival, respectively. Use of both interventions resulted in 100% survival and a 42% increase in total dose tolerated or a 13% increase above that observed with BMT alone. When the dose was

TABLE I – SURVIVAL OF NON-TUMOR-BEARING NUDE MICE GIVEN A SINGLE DOSE OF RAIT (^{131}I-MN-14 ANTI-CEA IGG)

	No additions	+ Vitamins[1]	+ BMT[2]	+ Vitamins + BMT
350 μCi[c3]	100%	—	—	—
400 μCi	20%	70%	100%	100%
450 μCi	—	50%	100%	—
500 μCi	0%	20%	70%	100%
550 μCi	—	0%	20%	60%

[1]Daily dose of 21.3 IU/mouse vitamin A acetate (in mineral oil), 0.107 IU/mouse vitamin E acetate (in mineral oil) i.p.; 4 mg/mouse ascorbic acid administered in saline i.m. on days −3 through +11 relative to RAIT.–[2]Donor bone marrow cells (10^7) infused i.v. on day 7.–[3]MTD in non-tumor-bearing nude mice (75 μCi above the MTD previously reported in tumor-bearing mice).

further escalated to 550 μCi of radio-antibody with BMT, only 20% survived, while the combination of vitamins and BMT resulted in 60% survival.

One area in which vitamins appear to have a clear radioprotective effect is in intestinal damage, as measured by percent body weight loss. Table II shows weight loss data on days 7 and 14 after either a 400 or a 500 μCi dose of RAIT. Untreated mice gained 10.2 ± 1.9% of their initial body weight within 7 days and 18.1 ± 1.4% within 14 days. Treatment with 500 μCi of RAIT resulted in a 10.0 ± 2.0% loss within 7 days and a 20.7 ± 4.1% loss within 14 days. BMT alone did not influence weight loss. Vitamins alone reduce the observed weight loss to 1.8 ± 1.3% on day 7 ($p < 0.001$ compared with RAIT alone) and to 1.4 ± 2.8% on day 14 ($p < 0.001$). The combination of BMT with vitamins did not afford any additional protection against weight loss.

Administration of vitamins as described above also reduced the magnitude of RAIT-induced myelosuppression. Figure 1 illustrates the effect that vitamins, BMT and the combination of both had on pWBC counts following a 400 μCi and a 500 μCi dose of ^{131}I-IgG. As early as day 7 post-RAIT, vitamins increased WBC counts from 1,464 ± 418/mm^3 to 3,023 ± 987/mm^3 ($p < 0.02$) following a 400 μCi dose and from 1,235 ± 705/mm^3 to 2,673 ± 638/mm^3 ($p < 0.01$) after a 500 μCi dose. On day 14 post-RAIT (400 μCi dose, day 7 post-BMT), the additive effect of BMT and vitamins could be detected. WBC counts in mice given RAIT alone were 154 ± 43, while those given RAIT + BMT were 588 ± 203 ($p < 0.01$). Mice given vitamins had 1,259 ± 148 WBCs/mm^3 and those given both BMT and vitamins had 1,734 ± 588 cells/mm^3 ($p < 0.001$ compared with those given only BMT). Similar additive effects were noted at 21 days post-RAIT. Figure 2 demonstrates that vitamins also protect the platelet population and that vitamins and BMT have additive effects. A 400 μCi dose of RAIT reduced platelets from 504 × 10^3/mm^3 to 262 × 10^3/mm^3 by day 14. BMT or vitamin intervention resulted in a platelet count of 468 × 10^3 and 467 × 10^3/mm^3, respectively. The combination of BMT and vitamins resulted in a platelet count of 605 × 10^3/mm^3, which is significantly higher than that found in the untreated group. The additive effect of BMT and vitamins could also be observed on day 21 with the 400 μCi dose group and on days 14 and 21 with the 500 μCi dose group.

A risk of using radioprotective anti-oxidant vitamins to reduce toxicity is that therapeutic efficacy to the tumor will be adversely affected. We used the GW-39 tumor-bearing nude mouse model to evaluate tumor growth in mice given 400 μCi ^{131}I-MN-14 + BMT without vitamins or a matched dose of RAIT + BMT with the same dose schedule of vitamins as was used for survival and toxicity measurements in mice not bearing tumors. Figure 3 summarizes the results of this study. Untreated tumors increased 3.66 ± 0.67-fold in size over a 3-week period. RAIT held tumor growth to a 1.2 to 1.5-fold increase above the initial size from day 14 to day 49 post-treatment. Similarly, tumor growth was held constant between 0.9- and 1.3-fold of starting size in mice given RAIT + vitamins over the same time frame. Using the GW-39 tumor model, there was no significant difference in pattern of growth between the 2 treatment groups, and both were significantly different from the untreated growth profile.

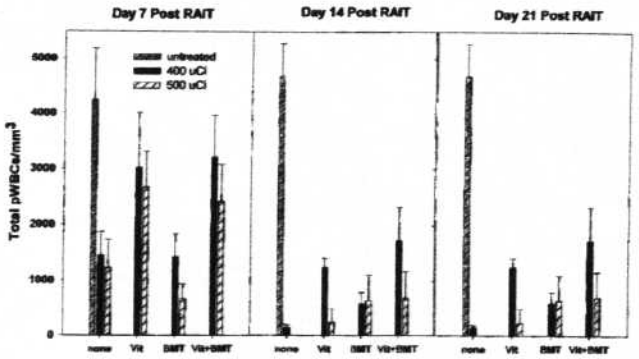

FIGURE 1 – Peripheral white blood cell (pWBC) counts on days 7, 14 and 21 post-RAIT (either a 400 or a 500 μCi dose of ^{131}I-MN-14 IgG). Mice were either left untreated or given 10^7 bone marrow cells from a donor mouse on day 7 post-RAIT or given a 14-day schedule of anti-oxidant vitamins or given both vitamins and BMT as described above. Average of 10 mice in each treatment group (2 studies pooled of 5 mice/group).

DISCUSSION

We have demonstrated that incorporation of anti-oxidant vitamins into radio-antibody therapy results in less normal tissue toxicity and is additive with BMT at reducing RAIT-induced hematopoietic suppression. In these studies, we have found that vitamin administration together with BMT permitted a 150 μCi dose above the MTD for mice without intervention (43% increase) and a 50 μCi increase above that observed with BMT alone (11.1% increase). The actual advantage afforded by each vitamin individually remains to be determined. Since the mouse diet already contains 350% of the recommended daily dose of vitamin A, it is not clear whether a further increase to 10× the dose found in the food provides any further radioprotection. Additional work is needed to further optimize the use of anti-oxidant vitamins: *e.g., (i)* selection of vitamins and *(ii)* dose schedule of vitamins, when using a single dose of a low-energy β emitter (*e.g.,* ^{131}I) or a high-energy β emitter (*e.g.,* ^{90}Y) or fractionated doses of radio-antibody. We hypothesize that the combination of all 3 vitamins will be superior to any one vitamin alone or any combination of 2 vitamins because free radicals from both the lipophilic and hydrophilic compartments can be scavenged and the combination allows vitamin E to be regenerated for re-use.

Further, since large differences in volume of distribution exist between mice and humans, a 50 μCi dose will likely translate into much higher doses in patients. This situation has been shown to pertain to BMT/SCT in mice and humans (*i.e.,* BMT permits an approx. 30% increase in radio-antibody dose in mice (Blumenthal *et al.* 1995*a*) and SCT permits a 300% to 400% higher radio-antibody dose in humans (Press *et al.* 1996). Thus, an 11% increase in radio-antibody dose in mice might translate to a significantly higher dose in humans.

Previous studies have shown that cytokines (IL-1, GM-CSF) can also be used for dose intensification of RAIT (Blumenthal *et al.* 1997, 1999). The efficacy of this method of intervention is limited

TABLE II – PERCENT CHANGE IN BODY WEIGHT POST-RAIT ± VITAMINS (COMPARED WITH DAY 0)

	A Untreated	B RAIT	C RAIT + BMT	D RAIT + vitamins	E RAIT + BMT + vitamins
400 mCi (day 7)	+10.2 ± 1.9%	−5.5 ± 2.4%	−7.4 ± 0.7%	+0.6 ± 0.3% (p<0.001)[1]	+0.8 ± 2.4% (p<0.001)
500 mCi (day 7)	—	−10.0 ± 2.0%	−10.2 ± 1.3%	−1.8 ± 1.3% (p< 0.001)	−1.3 ± 4.7% (p< 0.01)
400 mCi (day 14)	−18.1 ± 1.4%	−0.4 ± 1.6%	−2.8 ± 1.5%	+12.5 ± 8.2% (p< 0.01)	+9.1 ± 1.9% (p<0.001)
500 mCi (day 14)	—	−20.7 ± 4.1%	−19.9 ± 8.2%	−1.4 ± 2.8% (p< 0.001)	+2.7 ± 0.9% (p<0.001)

[1] p values represent comparisons made between columns B and D and columns C and E.

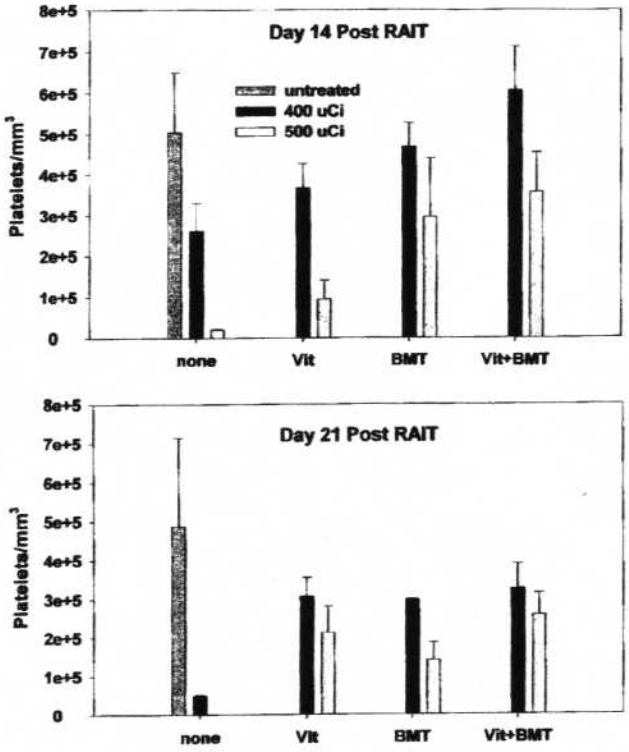

FIGURE 2 – Platelets measured on day 14 (upper panel) and day 21 (lower panel) in mice given either a 400 or a 500 μCi dose of radio-antibody. Mean of 10 mice in each treatment group (2 studies pooled of 5 mice/group).

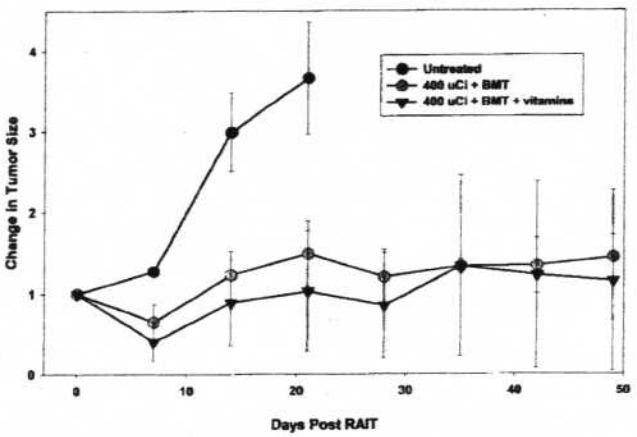

FIGURE 3 – GW-39 s.c. tumor growth in nude mice treated with 300 μCi ^{131}I-MN-14 IgG ± vitamins. Tumor size was measured weekly, and change in size was recorded for each mouse. Average of each group (n = 10) is graphed over a 7-week period.

to alleviating hematopoietic damage but does not affect GI toxicity or any other normal tissue injury. The vitamin approach may afford radioprotection of multiple forms of non-tumor toxicity. Vitamin administration is also preferable with respect to cost and side effects.

Other non-nutritional approaches to ameliorate host side effects from radiation toxicity [*e.g.*, vascular modifying agents, cytokines, basic amino acids, glutamine (Klimberg *et al.* 1990; Orazi *et al.* 1996)] have been reported or are under consideration. For example, we have shown that basic amino acids, such as lysine and arginine, can block renal tubular peptide re-absorption by neutralizing the negative charges of the cell membrane on the proximal tubule, thus reducing renal toxicity associated with radiometal-labeled antibody fragments (Behr *et al.* 1995, 1997).

One of the most sensitive tissues to radiation toxicity aside from bone marrow is the intestine (Rubio and Jalnas, 1996). The striking reduction in RAIT-induced body weight loss by anti-oxidant vitamin intervention lends strong support to the need for further investigation.

One potential pitfall is that it is not known whether there is differential protection between normal tissues and neoplasms. If the vitamins were delivered equally to normal tissues and tumors, then an undesirable radioprotective effect against tumors would also be anticipated, thus reducing the therapeutic efficacy of the radio-antibody. Prior to any therapy study, we hypothesized that this would not be a real concern since vitamin distribution would be a function of blood flow to individual tissues and to tumor. Our experience has been that blood flow (μl/g/min) of xenografted tumors is much lower than that of normal tissues in mice (data not shown): liver = 735 ± 183, spleen = 475 ± 178, kidney = 2,073 ± 640, lung = 1,221 ± 356 and tumor = 40 to 200 (depending on the type and size of the tumor.

Based on the therapy study using GW-39 xenografts, it appears that vitamins do not protect against the tumor-directed therapeutic effects of radiation from RAIT. However, this conclusion is based on 1 tumor type, so additional tumor models would need to be evaluated to assure the reliability of the data. Work by others provides considerable insight to address this question and strongly suggests that vitamins may actually be a positive modulator, enhancing the cytotoxic effect in tumors. Certain vitamins, such as α-tocopheryl succinate (α-TS), the most active form of vitamin E, can induce apoptosis of cancer cells (Prasad and Kumar, 1996) or indirect apoptosis *via* differentiation to a normal phenotype (Hazuka *et al.* 1990; Yu *et al.* 1997). Vitamin C and β-carotene (Schwartz, 1995) can also have pro-apoptotic effects. Mechanistically, the high vitamin dose effect involves protein kinase C activity, oncogene expression, synthesis of TGF-β and activation of apoptosis-associated genes. Lower doses of individual vitamins can have anti-apoptotic effects both *in vitro* and in an animal model (Park, 1988), but the mechanism is not well understood. When mixtures of vitamins are used (*e.g.*, vitamin C, retinoic acid, α-TS), a growth-inhibitory effect occurs at doses where each vitamin alone is not sufficient to affect the growth of cells (Prasad and Kumar, 1996), and vitamin mixtures have never stimulated the growth of cancer cells. Studies in humans and other species show no evidence of an apoptosis-inducing effect in normal tissues.

When vitamins are combined with other agents (X-rays or chemotherapy) that induce apoptosis, an enhanced therapeutic effect is observed both *in vitro* and *in vivo*, while they protect normal tissues against apoptosis (Prasad and Kumar, 1996; Rostock *et al.* 1980). The difference in the selective effect of vitamins in tumor *vs.* normal tissue may be a function of the amount accumulated. It is likely that vitamins have an anti-oxidant protective effect in both tissue types. However, cancer cells accumulate much greater amounts, independently of tumor blood flow, which initiate intracellular events leading to apoptosis, growth inhibition and/or cancer cell differentiation (Teicher *et al.* 1994).

In summary, we believe that the radioprotective ability of combined lipophilic and hydrophilic anti-oxidant vitamins is a promising approach to explore for reduction of dose-limiting side effects from RAIT and may permit dose intensification without compromising the therapeutic benefit.

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