Vitamin C as a Radioprotector Against Iodine-131 In Vivo

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The capacity of vitamin C (ascorbic acid) to mitigate radiation damage resulting from the tissue-incorporated radionuclide ^{131}I is examined. Spermatogenesis in mice is the experimental model and spermhead survival is the biological endpoint. When a small nontoxic amount of vitamin C was injected, followed by a similar injection of ^{131}I, the 37% spermhead survival dose (D_{37}) increased by a factor of 2.2 compared with the D_{37} in animals receiving only the radionuclide. Similar radioprotection was also observed when the animals were maintained on a diet enriched with 1% vitamin C (by weight). These results suggest that vitamin C may play an important role as a radioprotector against accidental or medical radiation exposures, especially when radionuclides are incorporated in the body and deliver the dose in a chronic fashion.

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The role of vitamin C as an antioxidant in the prevention and cure of diseases that result from free radicals has been of considerable interest and controversy lately (*1*). As an antioxidant, one would expect it to protect against radiation damage caused by free radicals that are produced when radiation interacts with living tissue. Damage by radiation-induced free radicals is predominant when the radiation is sparsely ionizing (e.g., beta, gamma and x-rays) (*2*). The literature on vitamin C as a radioprotector is limited. O'Conner et al. (*3*) observed a small radioprotective effect when cultured Chinese hamster ovary cells were acutely irradiated with 250 kVp x-rays in the presence of 0.3 mg/ml vitamin C. However, a recent review (*4*) indicates that the capacity of this compound to protect against radiation damage is inconclusive and therefore further experiments are called for. In this report, we examine the capacity of vitamin C to mitigate the effects of radiation in vivo using incorporated ^{131}I and external x-rays as sources of radiation.

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Iodine-131 is a well known radionuclide that is extensively used in medicine, particularly for the treatment of hyperthyroidism and thyroid cancer. The high yield of β^- particles ($\bar{E}$ = 191 keV) emitted by this radionuclide and its physical half-life of 8 days (*5*) has prompted its recent use in treating cancers with radiolabeled monoclonal antibodies (*6*). Unlike external beams of radiation, tissue-incorporated radionuclides generally deliver a radiation dose over an extended period of time which depends on their physical half-lives and biological half-times, the latter being highly dependent on the chemical nature of the radiocompound. The current literature on a variety of radioprotectors, including vitamin C, primarily deals with acute exposure to external radiation (*2*). No information is available concerning exposure to internal radionuclides. In view of the increasing use of radionuclides for medical diagnosis and therapy as well as concerns over occupational exposure and nuclear accidents, it is of interest to examine the potential of vitamin C to protect against internal radionuclide exposure.

METHODS

Experimental Model

The differentiated spermatogonial cells (types A_1-A_4, In and B) in mouse testes are the most radiosensitive cells in the animal. The process of spermatogenesis is well characterized (*7,8*) and is similar to that in humans, except for the time scale (about 5 wk for mouse compared to about 10 wk for humans) (*9*). Human spermatogonial cells are more sensitive to radiation than those of mice (*9*); therefore, this model is relevant to humans. It is well known that the precursors of the differentiated spermatogonia as well as the postgonial cells (spermatocytes, spermatids and spermatozoa) are relatively radioresistant (*8*). Hence, any initial radiation damage to the testis will result in a reduced testicular spermhead count after the time required for the spermatogonia to become spermatids of stages 12–16 (*8*). This time is 4–5 wk for mice. This highly sensitive in vivo model has been used extensively to study the biological effects of incorporated radionuclides (*10–12*).

Our experimental procedures and rationale have been described in detail previously (*10–12*). Male Swiss Webster mice, 8–9 wk old, weighing about 30 g, were used in these experiments. In order to establish the toxicity of the radionuclide, the mice were anesthetized under ether and, using a microsyringe, the right testes were injected with 3 μl of solution containing the radiochemical $H^{131}IPDM$ (N,N,N′-trimethyl-N′-(2-hydroxyl-3-

methyl-5-iodobenzyl)-1,3-propanediamine). This mode of administration requires very small amounts of radioactivity and allows us to calculate the testicular absorbed dose reliably without the complications of whole-body irradiation inherent in intravenous or intraperitoneal injections (*10*). To assess the radiotoxicity of ^{131}I in the presence of ascorbic acid, a nontoxic level of the vitamin (1.5 μg in 3 μl normal saline) was administered similarly 4 hr prior to the injection of the radiochemical. The intratesticular mode of administration was chosen over other modes to insure that all testes received the same initial amount of vitamin C. The amount of vitamin C injected had no effect on the spermhead count in the testis 29 days later. In a separate series of experiments, mice were placed on a vitamin C enriched (1% by weight) diet 5 days prior to the administration of the radionuclide. This diet was continued for 7 days after the administration of the ^{131}I compound, whereupon the animals were returned to their regular diet.

The radiochemical $H^{131}IPDM$ was prepared following the procedures described by Lui et al. (*13*). This beta-emitting radiochemical was selected because of its low-LET nature and its favorable biological clearance pattern (*12,14*). All assays of ^{131}I activity were carried out using a NaI(Tl) well detector. The vitamin C (purity 99%) used for intratesticular injections was from Aldrich Chemical Co. (Milwaukee, WI). Mouse food enriched with vitamin C was obtained from Dyets Inc. (Bethlehem, PA).

External irradiation of the testes was carried out with acute 120 kVp x-rays generated with an overhead fluoroscopy unit (GE Televi × 2, Schenectady, NY) (*15*). The whole body was protected from the radiation using custom designed lead shields (*15*). The vitamin C groups were treated in the same manner as described above.

Testicular Clearance of $H^{131}IPDM$

Mice were killed in groups of four under ether at different times after initial injection of small amounts of $H^{131}IPDM$. The testicular activity was determined using the characteristic 365 keV photopeak of ^{131}I. The presence of vitamin C in the testes had no effect on the biological clearance of the radiochemical (Fig. 1). The biological half-life of $H^{131}IPDM$ was found to be independent of the amount of radiochemical injected.

Survival of Spermatogonial Cells

The spermhead survival assay was used as an indicator of spermatogonial cell survival (*8,10–12*). The optimal time for this assay is when the testicular spermhead population reaches a minimum after the initial radiation insult. This optimal time was determined for each experimental condition and found to be 29 days for both external x-rays and the radiopharmaceutical. About 40 mice were injected with 31.7 kBq of $H^{131}IPDM$, and killed at different postinjection times. The injected testes were removed, placed in 1 ml of deionized water, homogenized for 15 sec and sonicated for 30 sec. The spermheads, which are resistant to sonication, were counted under a microscope using a hemocytometer. A minimum of 200 were scored. To verify that the presence of vitamin C had no effect on the optimal day for spermhead survival assay, the entire experiment was repeated with vitamin C treated animals. The postinjection time required to achieve the minimum spermhead count was not affected by the presence of vitamin C.

In order to establish the dose-response relationship for $H^{131}IPDM$, mice (groups of four) were injected with different concentrations of the radiochemical using standard 3-μl volumes. The animals were killed 29 days later and the injected testes were removed and processed for spermhead counting (*10–12*). The radiotoxicity studies were repeated with animals preinjected with vitamin C as well as with those animals maintained on a vitamin C enriched diet. Untouched mice, those injected with normal saline and animals injected with a nontoxic level (1.5 μg in 3 μl) of vitamin C served as controls. There were no chemotoxic effects observed when the mice were injected with nonradioactive HIPDM at a concentration equal to the highest level of injected $H^{131}IPDM$. Similarly, no chemotoxic effects were observed for vitamin C.

The average absorbed dose to the testes was calculated following the Medical Internal Radiation Dose (MIRD) procedures (*16*) using the ^{131}I radiation data given elsewhere (*5*). The biological half-time of the radiochemical was obtained from the clearance data (*12*). Absorbed fractions for all radiations were obtained using the computer code of Howell et al. (*17*). Most of the absorbed dose to the testes comes from the energetic beta particles, with ~1% coming from the penetrating gamma radiations. By using these data, spermhead survival (S) was determined as a function of the average absorbed dose (D) to the testes.

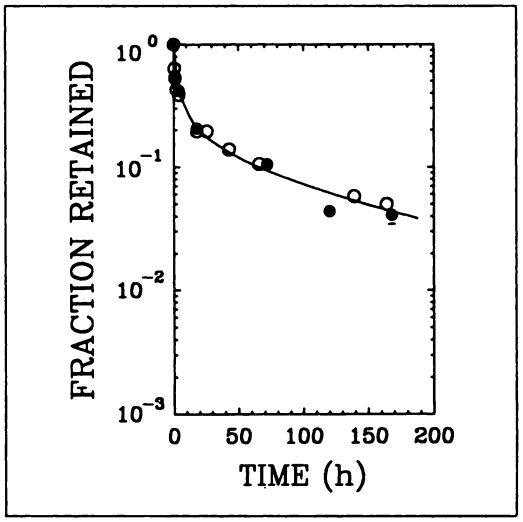

FIGURE 1. Testicular clearance of $H^{131}IPDM$ following injection of the radiochemical into mouse testis. The open circles represent the data for administration of $H^{131}IPDM$ alone, while the closed circles are for $H^{131}IPDM$ in the presence of intratesticularly injected vitamin C. The presence of vitamin C had no effect on the clearance pattern. A least-squares fit of the data to a three-component exponential expression yields:

$$f(t)=0.11\ e^{-0.693t/104}+0.35\ e^{-0.693t/23.3}+0.54\ e^{-0.693t/0.38},$$

where f is the fraction of radioactivity remaining in the testis at time *t* (hr) postinjection. The error bars fall within the representative symbols.

RESULTS

The testicular clearance of $H^{131}IPDM$ with and without vitamin C preinjection is illustrated in Figure 1. The data clearly show that injection of vitamin C does not affect the biological clearance of the radiochemical. About 50%

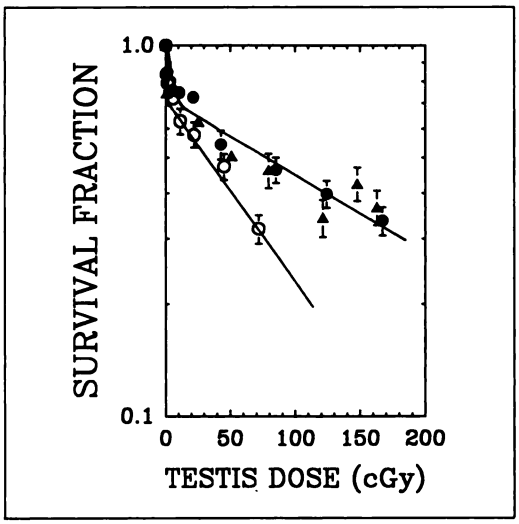

FIGURE 2. Spermhead survival in mouse testis as a function of average absorbed dose D to the organ from intratesticularly injected $H^{131}IPDM$ (*14*). Open circles represent the survival following intratesticular injection of $H^{131}IPDM$ alone. The closed circles represent survival in the presence of intratesticularly injected vitamin C and the closed triangles represent animals on a vitamin C enriched diet. Error bars represent the standard error of the mean for two to three independent experiments. A least-squares fit to the survival data yields:

$$S(H_{131}IPDM)=0.22\ e^{-D/0.67}+0.78\ e^{-D/81.6}$$

$$S(H^{131}IPDM+\text{vitamin C i.t. injected})=0.23\ e^{-D/1.06}+0.77\ e^{-D/183}$$

$$S(H^{131}IPDM+\text{vitamin C diet})=0.35\ e^{-D/0.48}++0.65\ e^{-D/260}.$$

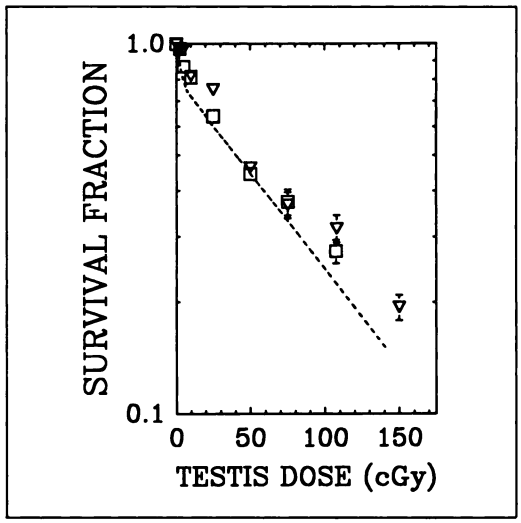

FIGURE 3. Spermhead survival as a function of absorbed dose D to the testes from acute external 120 kVp x-rays. The dotted line represents the dose response curve for x-rays, which was published previously (*15*). Open inverted triangles represent the data for vitamin C administered intratesticularly 4 hr prior to irradiation. The open squares represent animals fed a vitamin C enriched diet 5 days prior to irradiation and continued on the same diet for an additional 7 days. Data points represent the average of three independent experiments. The error bars represent standard error of the mean.

$$S(\text{X-rays}+\text{vitamin C i.t. injected})=0.08\ e^{-D/16.0}+0.92\ e^{-D/88.5}$$

$$S(\text{X-rays}+\text{vitamin C diet})=0.12\ e^{-D/6.9}+0.88\ e^{-D/83.3}.$$

of the radioactivity cleared from the testes with a very short biological half-life (0.5 hr), while the remainder cleared more slowly. The surviving fraction of spermheads as a function of the average absorbed dose to the testes both in the presence and absence of vitamin C is shown in Figure 2. A least-squares fit of the data yielded a mean lethal dose of 62 ± 6 cGy at 37% survival for $H^{131}IPDM$ alone (*14*). In contrast, a D_{37} value of 134 ± 14 cGy was obtained when the $H^{131}IPDM$ injection was preceded with an intratesticular injection of vitamin C. Similarly, a diet of vitamin C enriched food resulted in a D_{37} of 146 ± 20 cGy. The corresponding dose modifying factors (DMF), being the ratio of absorbed doses with and without vitamin C at a given spermhead survival, are 2.2 ± 0.3 and 2.4 ± 0.4 at D_{37}, respectively. A DMF value of greater than unity indicates radioprotection. The observed DMFs are in agreement within the experimental uncertainties.

Figure 3 shows the survival curves for external irradiation of the testes with acute 120 kVp x-rays in the absence and presence of vitamin C. A least-squares fit of the data yielded a D_{37} of 67 ± 3 cGy for x-rays alone. Similar least-squares fits gave a D_{37} value of 72 ± 5 cGy when the mice were fed a vitamin C enriched diet, whereas 81 ± 8 cGy was obtained when the vitamin C was injected intratesticularly. The corresponding dose modifying factors at 37% survival are 1.1 ± 0.1 and 1.2 ± 0.1, respectively. Table 1 summarizes all of the observed D_{37} values and the resulting dose modifying factors.

The two-component survival curves are characteristic of this model for external x-rays as well as internal radionuclides (*11*). This is likely due to the differential radiosensitivity of the spermatogonial cell subpopulations (*18*). Similar two-component survival curves also have been observed by others (*19–21*).

TABLE 1
Dose Modifying Factors for Vitamin C

	D_{37} (cGy)*	DMF
$H^{131}IPDM$	62 ± 6	—
$H^{131}IPDM$ + vitamin C i.t. injection	134 ± 14	2.2 ± 0.3
$H^{131}IPDM$ + vitamin C enriched diet	146 ± 20	2.4 ± 0.4
120 kVp x-rays (15)	67 ± 3	—
120 kVp x-rays + vitamin C i.t. injection	81 ± 8	1.2 ± 0.1
120 kVp x-rays + vitamin C enriched diet	72 ± 5	1.1 ± 0.1

*Testicular absorbed dose required to achieve 37% survival

DISCUSSION

The radioprotection against H[131]IPDM provided by vitamin C in our in vivo system (DMF ~ 2.2) is significantly greater than the protection against acute external 120 kVp x-rays (DMF ~ 1.2). The reason for this difference is likely that the radiation dose to the testes from ^{131}I was delivered over a period of a few days, whereas the dose from external 120 kVp x-rays was delivered in a matter of minutes. This hypothesis is supported by the in vitro data of O'Connor et al. (*3*) where a DMF of only 1.4 was obtained for acute 250 kVp x-rays (1.9 Gy/min). Since free radical production is related to the dose rate and total dose, higher concentrations of vitamin C may be required when radiation is delivered acutely. Such high concentrations may not be feasible without being cytotoxic. These considerations, as well as the similar DMF value (~2) observed in our ongoing experiments with ^{125}I and vitamin C (*22*), render additional support for the hypothesis that this antioxidant is likely to offer enhanced protection against chronic irradiation of tissues.

The work presented in this report may not end the controversy surrounding vitamin C as a preventive-curative agent for many diseases (*1*). However, it does provide clear evidence for its capacity as a radioprotector against chronic irradiation which may be of practical value. Naturally, several questions come to mind:

1. What is the optimum time to administer vitamin C?
2. What is the ideal level of vitamin C in the testes?
3. Will vitamin C protect against radionuclides with short half-lives (e.g., ^{99m}Tc)?
4. Are other natural antioxidants such as vitamins A and E capable of providing similar protection?

These and other questions will be the subject of future investigations.

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