INT. J. RADIAT. BIOL., MAY, 2004, VOL. 80, NO. 5, 347–354

Taylor & Francis
healthsciences

Augmentation of wound healing by ascorbic acid treatment in mice exposed to γ-radiation

G. C. JAGETIA†, G. K. RAJANIKANT†, M. S. BALIGA†, K. V. N. M. RAO† and P. KUMAR‡

(Received 9 May 2003; accepted 18 February 2004)

Abstract.
Purpose: Because of the crucial practical importance of acute radiation exposure associated with combined injuries, the study was undertaken to investigate the effect of various doses of ascorbic acid on the survival and healing of wounds in mice exposed to whole-body γ-radiation.
Materials and methods: Animals were given double-distilled water or different doses of ascorbic acid by intraperitoneal injection before exposure to 0 or 10 Gy whole-body γ-radiation to evaluate the effect of ascorbic acid on radiation-induced mortality. The animals were monitored daily for the symptoms of radiation sickness and mortality. In a separate experiment, animals were administered with either double-distilled water or different doses of ascorbic acid before exposure to 0 or 6 Gy whole-body γ-radiation to investigate the effect of ascorbic acid on the irradiated wound. A full-thickness skin wound was created on the dorsum of the irradiated mice and the progression of wound contraction was monitored by capturing video images of the wound at various post-irradiation periods.
Result: Treatment of mice with various doses of ascorbic acid elevated survival of mice and a highest number of survivors (67 and 33% for 10 and 30 days post-irradiation) was observed for 250 mg kg^{-1} ($p < 0.002$ and < 0.02 for 10- and 30-day survival, respectively). Ascorbic acid treatment caused a dose-dependent elevation in the wound contraction and highest contraction was observed for 250 mg kg^{-1}. The wound contraction was significantly greater at 3 ($p < 0.005$), 6 (< 0.05) and 9 (< 0.05) days post-irradiation with 250 mg kg^{-1} ascorbic acid. The complete healing of the wound was effected by day 22.8 post-irradiation in the ascorbic acid-treated irradiation group.
Conclusion: Administration of ascorbic acid protected mice against radiation-induced sickness, mortality and improved healing of wounds after exposure to whole-body γ-radiation. Additional studies will be directed toward analysing the role of successive administration of ascorbic acid to protect non-target tissues during radiotherapy and in initiating and supporting the cascade of tissue repair processes in radiotherapy delayed wounds.

1. Introduction

The detonation of atomic (A)-bombs, nuclear reactor accidents, fallout from A-bomb tests and an accidental release of radioactive material from radiation therapy devices result in large-scale, uncontrolled exposure to radiation, which is accompanied by an undesirable deleterious side-effects in the form of mass casualties and mortality. The skin being the outer most tissue of the body bears the brunt of radiation in the form of erythema, desquamation, ulceration, telangiectasia, fibrosis and induction of skin carcinoma (Davis *et al.* 1989, Turesson and Thames 1989, Bernstein *et al.* 1993). The acute radiation exposure associated with combined injuries will act synergistically, resulting in much greater mortality than the radiation injury alone would have produced (Kumar and Jagetia 1994, Jarrett 1999). Interaction of ionizing radiation with wounded tissue will create a situation where normal responses to injury will be disrupted, leading to a protracted recovery period. Wound healing is a series of well-orchestrated cellular and molecular events, including inflammation, angiogenesis, fibroplasia, wound contraction, epithelialization and matrix remodelling (Clark 1985). When the response to injury is normal, wounds heal without any complications. However, exposure to ionizing radiation disrupts normal responses to injury and produces multiple negative effects on the wound-healing processes including diminished vascularity, impairment of the proliferative capacity of fibroblasts and decreased collagen synthesis (Rudolph *et al.* 1988, Doyle *et al.* 1996, Gu *et al.* 1998).

Although basic research has suggested many potential therapies for the treatment of irradiated wounds, little attention has been given to the effects of ordinary metabolites or metabolic intermediates on the radiation response of healing wounds. The use of dietary ingredients to protect against radiation burden and the repair of irradiated wounds is an attractive proposition because they have wide acceptability, better tolerance, do not have side-effects and can be safely manipulated for human use. Ascorbic acid is an essential ingredient of the daily human diet, which has been found to increase collagen synthesis (Gould and Woessner 1957) and promote wound healing in animals as well as in humans (Ringsdorf and Cheraskin 1982, Cabbabe and Korock 1986). Ascorbic acid treatment has also been reported to increase survival of irradiated cells *in vitro* and irradiated tissues *in vivo* (Redpath and Wilson 1973, Baverstock

*Author for correspondence; e-mail: gc.jagetia@kmc.manipal.edu
Departments of †Radiobiology and ‡Plastic Surgery, Kasturba Medical College, Manipal – 576 104, India

1979). It has been reported to have beneficial effect on the course of radiation-induced skin injuries (Decosse 1988). Because of the crucial practical importance of combined injuries, the present study was undertaken to investigate the effect of various doses of ascorbic acid (AA) on the survival and healing of wound in mice exposed whole-body γ-radiation.

2. Material and methods

Animal care and handling were carried out according to the guidelines set by the World Health Organization (Geneva, Switzerland) and the Indian National Science Academy (New Delhi, India). Eight- to 10-week-old male Swiss albino mice weighing 30–36 g were selected from an inbred colony maintained under the controlled conditions of temperature ($23 \pm 2^{\circ}$C), humidity ($50 \pm 5\%$) and light (10 and 14 h of light and dark, respectively). The animals had free access to sterile food and water. The levels of AA were considered to be normal. Four animals were housed in a polypropylene cage containing sterile paddy husk (procured locally) as bedding throughout the experiment. The study was approved by the institutional animal ethical committee.

2.1. *Preparation of drug and mode of administration*

Ascorbic acid was procured as fine crystals from Sigma Chemical Co. (St Louis, MO, USA; catalogue no. A4544). The required amount of AA was dissolved in sterile Milli-Q (Millipore India Ltd, Bangalore, India) water, and the animals were given $0.01\,\mathrm{ml\,g^{-1}}$ body weight of double-distilled water (DDW) or AA intraperitoneally for all experiments.

2.2. *Experimental protocol*

2.2.1. *Experiment 1: Acute toxicity studies.* The acute toxicity of AA was determined according to Prieur *et al.* (1973) and Ghosh (1982). Briefly, the animals were allowed to fast by withdrawing food and water for 18 h. The fasted animals were divided into several groups based on the dose of AA. The animals from each group were administered with 1, 1.5, 1.75, 2, 2.25, 2.5, 2.75 or $3\,\mathrm{g\,kg^{-1}}$ body weight (bw) AA by intraperitoneal injection only once during the whole experimental period. The symptoms of toxicity like lethargy, reduced food and water intake, changes in body weight and mortality was monitored up to 14 days post-treatment. Ten animals were used in each group for each dose of AA.

2.2.2. *Experiment 2: Chronic toxicity studies.* The animals were injected intraperitoneally daily with DDW or $250\,\mathrm{mg\,kg^{-1}}$ bw AA, consecutively for 5, 8, 10 and 20 days and the symptoms of toxicity like lethargy, reduced food and water intake, changes in the body weight and mortality were monitored up to 14 days post-treatment. Ten animals were used in each group.

2.2.3. *Experiment 3: Survival studies.* A separate experiment was conducted to evaluate the effect of various doses of AA on radiation-induced mortality. The animals were injected intraperitoneally with 0, 31.25, 62.5, 125, 250, 500 or $1000\,\mathrm{mg\,kg^{-1}}$ bw AA 45 min before exposure to 0 or 10 Gy γ-radiation. The animals were monitored daily for the symptoms of radiation sickness and mortality. A minimum of 24 animals was used for each dose of AA in concurrent groups.

2.2.4. *Experiment 4: Wound contraction studies.* A separate experiment was carried out to investigate the effect of AA on irradiated wounds where the animals were administered 0, 62.5, 125, 250 or $500\,\mathrm{mg\,kg^{-1}}$ bw AA intraperitoneally before exposure to 0 or 6 Gy γ-radiation.

The animals were divided into following groups:

- DDW + irradiation: animals were injected with DDW before exposure.
- AA + irradiation: animals received different doses of AA before exposure.

2.2.4.1. *Irradiation.* Forty-five minutes after the injection of DDW or AA, each animal was placed into a specially designed well-ventilated acrylic restrainer and the whole body of the animals was exposed to 0, 6 or 10 Gy at $1.35\,\mathrm{Gy\,min^{-1}}$ from a ^{60}Co teletherapy source (Theratron, Atomic Energy Agency, Ontario, Canada). Eight animals were used in each group.

2.2.4.2. *Production of full-thickness skin wound.* The fur of the dorsum (below the rib cage) of each animal was removed with a cordless electric mouse clipper (Wahl Clipper Corp., Sterling IL, USA) before exposure to radiation and a full-thickness skin wound was produced on the dorsum (below the rib cage) of the animal as described by Kumar and Jagetia (1995) within 10 min of irradiation. Briefly, the animals were anaesthetized using diethyl ether and the skin of the entire body was cleaned and decontaminated by wiping the whole body

with sterillium solution (Bode Chemie, Hamburg, Germany). The cleared dorsal surface of the skin was marked with a sterile rectangular (15×25 mm) stainless steel stencil. A full-thickness wound was created by excising the skin flap in an aseptic environment using sterile scissors and forceps. Each wounded animal was housed in a separate sterile polypropylene cage.

2.2.4.3. *Wound contraction studies.* Wound contraction was monitored by capturing the video images of each full-thickness wound with a charge-coupled display camera connected to a computer. The first images of wounds from the different groups were acquired 1 day after wounding, and that day was considered as day 0. The subsequent images were captured on 3, 6, 9, 12 and 15 days after wounding. The wound area was calculated using Auto CAD R14 (Autodesk, Inc., San Rafael, CA, USA) software.

2.2.5. *Experiment 5: Mean wound healing time.* A separate experiment was performed to evaluate the effect of AA on mean healing time after exposure to 0 or 6 Gy whole-body γ-radiation. The grouping of animals and the production of a wound were as described in experiment 4. All animals in each group were monitored until the complete healing of wounds and the day at which each wound healed was recorded. The mean of all healed wounds was determined and expressed as the mean wound healing time (days). Eight animals were used in each group.

2.3. *Analysis of data*

Statistical significance between treatments was determined using one-way analysis of variance for wound contraction studies. The Solo 4 Statistical Package (BMDP Statistical Software, Inc., Los Angeles, CA, USA and Ireland) was used for data analysis. All data are expressed as the mean $\pm$ standard error of the mean. A z-test was used for survival studies (Abramowitz and Stegun 1972) by using the following formula::

$$z = \frac{\hat{p}_1 - \hat{p}_2}{\sqrt{\hat{p}(1-\hat{p})(1/n_1 + 1/n_2)}},$$

where $\hat{p}$ = (number of successes)/total sample size.

n_1 = number of animals survived

n_2 = number of animals died

3. Results

3.1. *Experiment 1: Acute toxicity studies*

Administration of AA up to $1.75\,g\,kg^{-1}$ did not induce mortality in animals. However, animals showed temporary lethargy and a drop in body temperature. A further increase in drug dose to $2\,g\,kg^{-1}$ resulted in a 10% death rate for the animals. While a 50% mortality rate was observed at $2.25\,g\,kg^{-1}$ AA treatment, 80% of animals died after administration of $2.5\,g\,kg^{-1}$ and a 100% mortality rate was observed at a dose of $2.75\,g\,kg^{-1}$ AA or above.

3.2. *Experiment 2: Chronic toxicity studies*

The animals of all groups were monitored daily for the development of chronic toxicity symptoms. All animals were healthy and did not show any symptoms of drug-induced toxicity at $250\,mg\,kg^{-1}$ bw AA administered for 5, 8, 10 or 20 days.

3.3. *Experiment 3: Survival studies*

The whole-body exposure of animals to 10 Gy induced symptoms of radiation sickness characterized by a reduction in food and water intake, irritability, watering of the eyes, epilation, weight loss, emaciation, lethargy, diarrhoea and facial oedema. An approximate 26.7% survival rate was observed by day 10, which was further reduced by up to 6.7% by day 30 post-irradiation. The treatment of various doses of AA reduced the symptoms of radiation-induced sickness and increased survival in a dose-dependent manner. The highest number of survivors was observed at the end of 10 (66.7%) or 30 (33.3%) days post-irradiation for $250\,mg\,kg^{-1}$ AA ($p < 0.002$ and < 0.02-, for 10- and 30-day survivals, respectively). A further increase in AA dose caused a reduction in survival when compared with $250\,mg\,kg^{-1}$ AA (figure 1).

3.4. *Experiment 4: Wound contraction studies*

The progression of the healing of excision wounds could be evaluated by the periodic assessment of wound contraction. The area of each wound at a specific time was expressed as the percentage of its original size on day 0. The mean relative area for each group was plotted as a function of days after wounding (figure 2).

The results discussed here address sham-irradiation control groups. Treatment of mice with DDW or various doses of AA resulted in a steady contraction of excision wounds with time in both DDW + sham

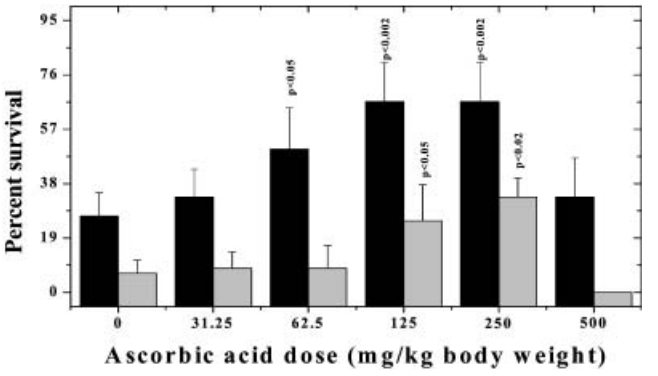

Figure 1. Alteration in the survival of mice by intraperitoneally administered AA 45 min before exposure to 10 Gy γ-radiation. Solid bars indicate 10-day survival and light grey bars indicate 30-day survival. p values indicate comparisons of DDW + irradiation group to AA + irradiation groups. Error bars indicate the standard error of the mean (95% confidence limit).

irradiation and AA + sham irradiation groups (0 Gy). The administration of 62.5, 125, 250 or 500 mg kg^{-1} bw AA enhanced the wound contraction significantly at 3, 6 and 9 days post-irradiation depending on the drug dose when compared with non-drug treated controls. The wound contraction increased progressively with time and AA treatment resulted in an early closure of wound when compared with the non-drug treatment group. Wound contraction increased with an increase in AA dose up to 250 mg kg^{-1} when compared with other drug doses. A further increase in drug dose did not alter this pattern significantly (figure 2, a–d). AA treatment showed a progressive reduction in scab formation with increasing dose and scab formation was almost absent for 250 mg kg^{-1} AA and thereafter. In contrast, the non-drug-treated animals (DDW + sham irradiation) showed a thick scab formation (data not shown).

Whole-body irradiation of animals to 6 Gy whole-body irradiation resulted in a thick scab formation in DDW + irradiation group. Treatment of animal with various doses of AA caused thin scab formation and early falling off of the scab. The scab formation was almost absent in animals treated with 250 mg kg^{-1} AA before 6 Gy irradiation (data not shown). Exposure of animals to 6 Gy whole-body irradiation resulted in a significant delay in wound contraction when compared with sham-irradiation control at all observed post-irradiation days. Treatment of mice with different doses of AA enhanced wound contraction in a dose-dependent manner up to 250 mg kg^{-1}, where a greatest wound contraction was observed (figure 2, a–d). Wound contraction was significantly greater at 3 ($p < 0.005$), 6 (< 0.05) and 9 (< 0.05) days post-irradiation in animals treated with 250 mg kg^{-1}

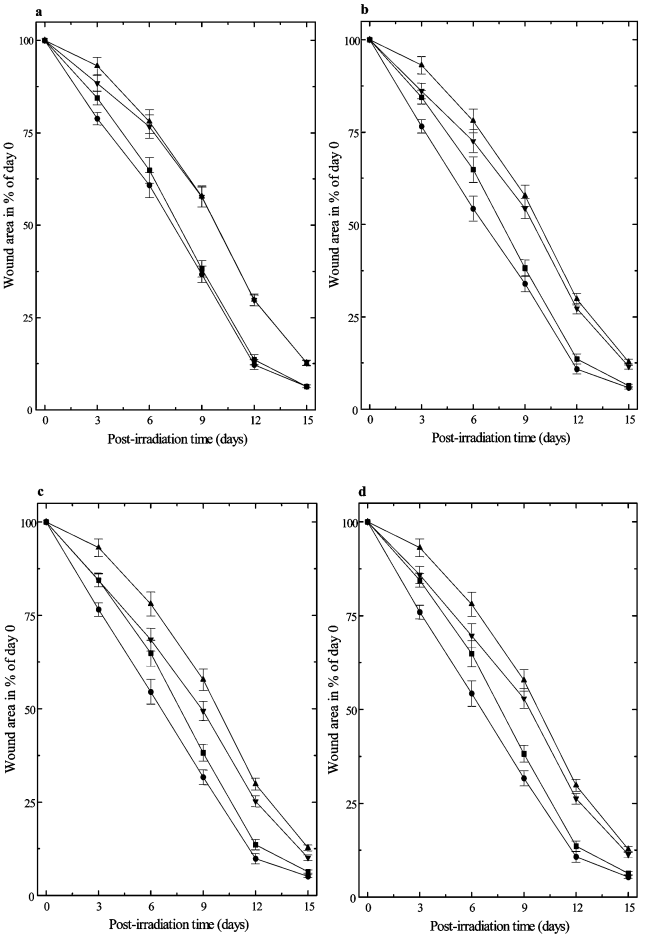

Figure 2. Effect of different doses of intraperitoneally administered AA on wound contraction in mice 45 min before exposure to 6 Gy whole-body γ-radiation: (a) 62.5, (b) 125, (c) 250 and (d) 500 mg kg^{-1} bw AA. Squares indicate DDW + sham irradiation, circles indicate AA + sham irradiation, up-triangles indicate DDW + irradiation and down-triangles indicate AA + irradiation. Error bars indicate the standard error of the mean.

AA before 6 Gy irradiation (figure 2, c). At other drug doses (62.5, 125 and 500 mg kg^{-1}), the significant contraction of wound was not observed.

3.5. *Experiment 5: Mean wound healing time*

The complete closure of wounds was observed on 17.3 ± 0.3 days post-irradiation in the DDW + sham irradiation group. Treatment of mice with 62.5 mg kg^{-1} AA (17.3 ± 0.3 days post-irradiation) did not alter the mean healing time significantly when compared with the DDW + sham irradiation group. The mean healing time of 16.5 ± 0.3 days post-irradiation was observed for 250 mg kg^{-1} AA ($p < 0.05$). A further increase in AA dose, i.e. 500 mg kg^{-1}, did not alter the mean healing time

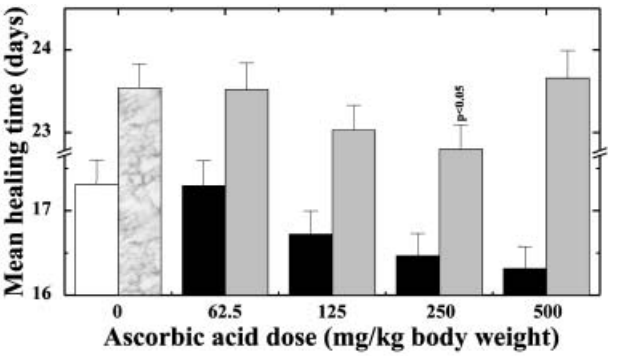

Figure 3. Effect of different doses of intraperitoneally administered AA on mean healing time in mice 45 min before exposure to 6 Gy whole-body γ-radiation. Plaid bar indicates DDW + sham irradiation, white marble bar indicates DDW + irradiation, solid bars indicate AA + sham irradiation and light grey bars indicate AA + irradiation. p values compare DDW + irradiation groups are compared with AA + irradiation groups. Error bars are the standard error of the mean.

(16.3 ± 0.3 days post-irradiation) when compared with $250\,mg\,kg^{-1}$ (figure 3).

The whole-body exposure of mice to 6 Gy γ-radiation significantly delayed the complete closure of wounds. As a result, the mean wound healing time was also more (23.5 ± 0.3 days post-irradiation) for DDW + irradiation group. The treatment of animals with $250\,mg\,kg^{-1}$ AA resulted in an early healing of wounds and a borderline effect on the mean healing time was noticed (22.8 ± 0.3 days post-irradiation) when compared with the DDW + irradiation group. At other drug doses (62.5, 125 and $500\,mg\,kg^{-1}$), even the borderline effect on mean healing time was also not observed (23.5 ± 0.3, 23.0 ± 0.3 and 23.7 ± 0.3 days post-irradiation for 62.5, 125 and $500\,mg\,kg^{-1}$ AA doses, respectively) (figure 3).

4. Discussion

Toxicity of AA differs from species to species and depends greatly on the mode of administration (Du Bruyn *et al.* 1977, Hanck 1982). Acute toxicity studies demonstrated that administration of AA up to $1.75\,g\,kg^{-1}$ intraperitoneally did not cause any mortality in animals. However, the administration of $2.25\,g\,kg^{-1}$ AA reduced the survival rate of mice up to 50%. AA treatment caused a significant drop in the body temperature (data not shown) at higher doses ($>2\,g\,kg^{-1}$) and the animals were unable to recover from this shock. This could be the cause of early deaths (day 1) of animals treated with higher dose of AA.

The experience with radioprotectors world wide is that animal death as the endpoint after whole-body irradiation is most confirmatory. A 30-day survival period clearly indicates the capacity of the drug being tested to modulate the recovery and regeneration of the gastrointestinal epithelium and the haemopoietic progenitor cells in the bone marrow, the two most radiosensitive organs essential for sustenance of life. AA treatment prevented gastrointestinal deaths in a dose-dependent manner up to $250\,mg\,kg^{-1}$ as evidenced by a higher number of survivors in this group when compared with the concurrent control. A similar effect was observed for bone marrow death, where AA protected mice against radiation-induced mortality. AA has been reported to protect against radiation-induced damage (Redpath and Wilson 1973). Some other compounds like WR-2721, 2-mercaptopropionylglycine (MPG) and copper glycinate (Yuhas and Storer 1969, Nagata *et al.* 1972, Jagetia *et al.* 1993) have also been reported to offer protection against radiation-induced damage. In the present study, optimum protection was observed at $250\,mg\,kg^{-1}$ AA and a further increase in drug dose resulted in the decline in animal survival. The earlier studies on radioprotection have shown that an agent in test (for radioprotective action) acts only at a particular dose range and above which it might not afford protection and sometimes can even be toxic (Thomson 1962, Jagetia *et al.* 2002, 2003a). The reason might be that after a particular concentration, a radioprotector instead of being an antioxidant might act like pro-oxidant, thus increasing the toxic effects of radiation insult and ultimately causing death. A similar effect of higher dose of AA ($500\,mg\,kg^{-1}$) in the present study cannot be ruled out. The antioxidant property of AA has been reported to be concentration dependent (Wayner *et al.* 1986, Bijur *et al.* 1997).

Wound contraction can be defined as the centripetal movement of the edges of a full-thickness wound to facilitate closure of the defect (Peacock 1984). The progression of wound healing can be judged by the periodic assessment of the contraction of excision wounds. AA treatment caused a dose-dependent elevation in wound closure. Numerous earlier investigations have reported the beneficial effect of AA on wound healing (Ringsdorf and Cheraskin 1982, Cabbabe and Korock 1986). The observed delay in wound contraction after exposure to whole-body radiation is in good agreement with earlier reports (Grillo and Potsaid 1961, Stromberg *et al.* 1968, Kumar and Jagetia 1995). Treatment of mice with different doses of AA before whole-body irradiation resulted in a dose-related enhancement in wound healing, as evident by an early closure of wounds in the AA + irradiation group and an optimum effect was observed at $250\,mg\,kg^{-1}$. These

observations are corroborated by survival studies where a similar effect has been observed. AA treatment has been reported to increase the survival rate earlier in mice (Redpath and Wilson 1973). The studies about the amelioration of wound healing by AA treatment after whole-body irradiation are unavailable. However, AA pretreatment has been reported to enhance wound healing after fractionated hemibody irradiation (Jagetia *et al.* 2003b). Studies on the use of ordinary metabolites in the enhancement of radiation-impaired wound healing are scanty. Vitamin A supplementation has been reported to improve acute radiation-induced delay in wound healing (Levenson *et al.* 1984). Similarly, phenytoin sodium has been reported to accelerate healing of irradiated wounds (Song and Cheng 1997). Whole-body irradiation might cause bone marrow depression and produce significant effects on the clinical course of skin wounds. AA treatment might have arrested the radiation-induced bone marrow depression in the present study and might have helped in the accelerated healing of wounds. This effect is evident by increased survival in the AA-treated irradiation group when compared with the DDW + irradiation group.

Wound healing involves a cascade of well-orchestrated biochemical and cellular events leading to the growth and regeneration of wounded tissue in a specific manner. Ionizing radiation produces multiple negative effects on wound healing including diminished vascularity, impairment of the proliferative capacity of fibroblasts and haematopoietic cells, and decreased collagen synthesis (Rudolph *et al.* 1988, Kumar and Jagetia 1995, Doyle *et al.* 1996, Gu 1998). There are several possible explanations for alterations in wound healing after irradiation. One such possibility is a delay in the fixation of the wound edge to the underlying tissue, which might be due to a lack of fibroblast proliferation and a decrease in fibroblast function in the granulation bed. The contraction of open excised wounds as a function of contractile fibroblasts known as myofibroblasts (Gabbiani *et al.* 1972). Local irradiation is thought to impair wound healing in skin through its cytotoxic effect on fibroblasts (Gorodetsky *et al.* 1988). Fibroblast function might also depend on a competent bone marrow, since some fibroblasts of the normal subcutaneous connective tissue participating in wound healing were shown to take their origin from the bone marrow (Vasil'eva *et al.* 1978, Lange *et al.* 1979). Radiation has adverse effects on fibroblasts and endothelial cells through bone marrow depression, since radiation has been reported to diminish haematopoiesis in a dose- and time-dependent manner (Zelman *et al.* 1969, Vegesna *et al.* 1993). A similar effect cannot be ruled out in the present study where the whole body of the animal was irradiated during exposure. AA might have provided strength to the regenerating wound by increasing cellular proliferation, collagen deposition and vascular supply, causing early closure of the wound in the AA + irradiation group, indicating its non-specific action. The earlier studies reported the beneficial effect of AA on wound healing through changes in cell regeneration and collagen synthesis (Gould and Woessner 1957, Ringsdorf and Cheraskin 1982, Cabbabe and Korock 1986). The inhibition of the delay in contraction of irradiated wounds by AA might be due to its antioxidant, free-radical scavenging and radioprotective activities (Redpath and Wilson 1973, Beverstock 1979, Frie *et al.* 1989, Jagetia *et al.* 2003c). It might also have stimulated the fibroblasts causing early healing of irradiated wounds. The antioxidant property of AA against ionizing radiation is concentration dependent and at higher doses it might act as a pro-oxidant. This is evident from survival, wound contraction and mean healing time studies where a further increase in AA dose ($500\,mg\,kg^{-1}$) to above $250\,mg\,kg^{-1}$ decreased the animal survival, contraction and mean healing time of irradiated wound. The reason might be that after an optimum AA dose, it might act like a pro-oxidant instead of an antioxidant, augmenting the toxic effects of ionizing radiation. A borderline effect on mean healing time in the AA + irradiation group can be explained on the basis of additional trauma induced by a skin wound, which uses AA more rapidly than non-irradiated animals. At the cellular level, AA has been reported to mitigate the deleterious effects of reactive oxygen species, which deplete endogenous antioxidants (Fuchs *et al.* 1989), elevate intracellular lipid peroxidation (Meffert *et al.* 1976), and induce specific signal transduction pathways that can modulate the inflammatory, immune suppressive or apoptotic processes in the skin (Buttke and Sandstrom 1994).

Acute toxicity studies demonstrated that administration of AA up to $1.75\,g\,kg^{-1}$ did not cause any mortality in animals. However, the administration of $2.25\,g\,kg^{-1}$ AA reduced the survival rate of mice to 50%, which if converted to an acceptable daily intake for humans, corresponds to approximately 169 mg day^{-1} if a safety factor of 1000 is applied on a conservative scale (Conine *et al.* 1992). The present dose of $250\,mg\,kg^{-1}$ is approximately 10% of the LD_{50} dose. Therefore, the daily human dose in this range will be less than $169\,mg\,day^{-1}$, which is in the acceptable daily intake range. It has been demonstrated that AA ($1\,g\,kg^{-1}$) given intraperitoneally with vitamin K3 ($10\,mg\,kg^{-1}$) increased the therapeutic effect of radiation on solid tumours without causing

any signs of toxicity due to the vitamins in mice (Taper *et al.* 1996). Further, the AA is used faster during oxidative stress (Hubel *et al.* 1997), necessitating exogenous supplementation to make the loss and to maintain a normal plasma level. Since irradiation induces oxidative stress, AA administration is required to overcome this stress and provide optimum protection against radiation-induced damage in the present study. However, it is not possible to draw an analogy between mice and human owing to differences in the species.

Mass casualties resulting from acute radiation exposure and/or combined injuries produce serious clinical problems not encountered by most military and civilian physicians. In such circumstances, administration of AA could be an important therapeutic strategy to ameliorate radiation-induced sickness and to improve healing of chronically irradiated wounds. Additional studies will be useful towards analysing the role of successive administration of AA to protect non-target tissues during radiotherapy and in initiating and supporting the cascade of tissue-repair processes in radiotherapy-delayed wounds.

Acknowledgements

The authors thank Dr M. S. Vidyasagar, Professor and Head, and Dr J. Velumurugan, Department of Radiotherapy and Oncology, Kasturba Medical College, Manipal, India, for providing the necessary irradiation facilities and for help in dosimetry. The authors also thank Professor B. Srinivas, Head, and Mrs Geetha J. Nayak, Department of Mechanical Engineering, Manipal Institute of Technology, Manipal, India, for expert help in calculating the wound area. The financial assistance provided by the Defense Research and Development Organization (DRDO), Ministry of Defense, Government of India, New Delhi, India, to carry out the above study is gratefully acknowledged.

References

Abramowitz, M. and Stegun, I. A., 1972, *Handbook of Mathematical Functions* (New York: Dover).

Baverstock, K. F., 1979, Radioprotection by vitamin C. *British Journal of Radiology*, **52,** 592–593.

Bernstein, E. F., Sullival, F. J., Mitchell, J. B., Salomon, G. D. and Glatstein, E., 1993, Biology of chronic radiation effect on tissues and wound healing. *Clinics in Plastic Surgery*, **20,** 435–453.

Bijur, G. N., Ariza, M. E., Hitchcock, C. L. and Williams, M. V., 1997, Antimutagenic and promutagenic activity of ascorbic acid during oxidative stress. *Environmental and Molecular Mutagenesis*, **30,** 339–345.

Buttke, T. M. and Sandstrom, P. A., 1994, Oxidative stress as a mediator of apoptosis. *Immunology Today*, **15,** 7–10.

Cabbabe, E. B. and Korock, S. W., 1986, Wound healing in vitamin C deficient and non-deficient guinea pig: a pilot study. *Annals of Plastic Surgery*, **17,** 330–334.

Clark, R. A. F., 1985, Cutaneous tissue repair: basic biologic considerations. *Journal of the American Academy of Dermatology*, **13,** 701–725.

Conine, D. L., Naumann, B. D. and Hecker, L. H., 1992, Setting health-based residue limits for contaminants in pharmaceuticals and medical devices. *Quality Assurance: Good Practice, Regulation and Law*, **1,** 171–180.

Davis, M. M., Hanke, C. W., Zollinger, T. W., Montebello, J. F., Hornback, N. B. and Norins, A. L., 1989, Skin cancer in patients with chronic radiation dermatitis. *Journal of the American Academy of Dermatology*, **20,** 608–616.

Decosse, J. J., 1988, Antioxidants. *Progress in Clinical and Biological Research*, **279,** 131–134.

Doyle, J. W., Li, Y. Q., Salloum, A., Fitzgerald, T. J. and Walton, R. L., 1996, The effects of radiation on neovascularization in a rat model. *Plastic and Reconstructive Surgery*, **98,** 129–134.

Du Bruyn, D. B., De Klerk, W. A. and Liebenberg, N. W., 1977, High dietary ascorbic acid levels and oxalate crystallization in soft tissues of baboons. *South African Medical Journal*, **52,** 861–862.

Frie, B., England, L. and Ames, B. N., 1989, Ascorbic acid is an outstanding antioxidant in human blood plasma. *Proceedings of the National Academy of Sciences, USA*, **86,** 6377–6381.

Fuchs, J., Huflejt, M., Rothfuss, L. M., Wilson, D. S., Carcamo, D. S. and Packer, L., 1989, Impairment of enzymatic and non-enzymatic antioxidants in skin by UVB irradiation. *Journal of Investigative Dermatology*, **93,** 769–773.

Gabbiani, G., Hirschel, B. J., Ryan, G. B., Statkov, P. R. and Majno, G., 1972, Granulation tissue as a contractile organism: a study of structure and function. *Journal of Experimental Medicine*, **135,** 719–734.

Ghosh, M. N., 1984, Toxicity studies. In M. N. Ghosh (ed.), *Fundamentals of Experimental Pharmacology* (Kolkatta, India: Scientific Book Agency), pp. 153–158.

Gorodetsky, R., McBride, W. H. and Withers, H. R., 1988, Assay of radiation effects in mouse skin as expressed in wound healing. *Radiation Research*, **116,** 135–144.

Gould, B. S. and Woessner, F. J., 1957, Biosynthesis of collagen. The influence of ascorbic acid on the proline, hydroxyproline, glycine and collagen content of regenerating guinea pig skin. *Journal of Biological Chemistry*, **226,** 289–300.

Grillo, H. C. and Potsaid, M. S., 1961, Studies on wound healing – IV, retardation of contraction by local X-irradiation and observations relating to the origin of fibroblast in repair. *Annals of Surgery*, **154,** 741–750.

Gu, Q., Wang, D., Cui, C., Gao, Y., Xia, G. and Cui, X., 1998, Effects of radiation on wound healing. *Journal of Environmental Toxicology Oncology*, **17,** 117–123.

Hanck, A., 1982, Tolerance and effects of high doses of ascorbic acid. Dosis facit venenum. *International Journal for Nutrition and Vitamin Research*, **23 (Suppl.),** 221–238.

Hubel, C. A., Kagan, V. E., Kisin, E. R., McLaughlin, M. K. and Roberts, J. M., 1997, Increased ascorbate radical formation and ascorbate depletion in plasma from women with preeclampsia: implications for oxidative stress. *Free Radical Biology and Medicine*, **23,** 597–609.

Jagetia, G. C., Baliga, M. S., Aruna, R., Rajanikant, G. K. and Jain, V., 2003a, Effect of abana (a herbal preparation) on

the radiation-induced mortality in mice. *Journal of Ethnopharmacology*, **86,** 159–165.

JAGETIA, G. C., BALIGA, M. S., MALAGI, K. J. and KAMAT, M. S., 2002, The evaluation of radioprotective effect of Triphala (an ayurvedic rejuvenating drug) in the mice exposed to γ-radiation. *Phytomedicine*, **9,** 99–108.

JAGETIA, G. C., GANAPATHI, N. G. and UNNIKRISHNAN, M. K., 1993, Copperglycinate protects mouse exposed to various doses of gamma radiation. *Strahlentherapie und Oncologie*, **5,** 323–328.

JAGETIA, G. C., RAJANIKANT, G. K. and RAO, S. K., 2003b, Evaluation of the effect of ascorbic acid treatment in the artificially wounded mouse exposed to different doses of fractionated gamma radiation. *Radiation Research*, **159,** 371–380.

JAGETIA, G. C., RAJANIKANT, G. K., RAO, S. K. and BALIGA, S. M., 2003c, Alteration in the glutathione, glutathione peroxidase, superoxide dismutase and lipid peroxidation by ascorbic acid in the skin of mice exposed to fractionated gamma radiation. *Clinica et Chimica Acta*, **332,** 111–121.

JARRETT, D. G., 1999, *Medical Management of Radiological Casualties Handbook* (Bethesda: Armed Forces Radiobiology Research Institute).

KUMAR, P. and JAGETIA, G. C., 1994, A review of triage and management of burns victims following a nuclear disaster. *Burns*, **20,** 397–402.

KUMAR, P. and JAGETIA, G. C., 1995, Modulation of wound healing in Swiss albino mice by different doses of gamma radiation. *Burns*, **21,** 163–165.

LANGE, M. A., VASIL'EVA, T. V. and MICHURINA, T. V., 1979, Origin of fibroblasts and macrophages of skin wound granulation tissue. *Arkhiv Anatomii, Gistologii i Embriologii*, **77,** 22–28.

LEVENSON, S. M., GRUBER, C. A., RETTURA, G., GRUBER, D. K., DEMETRIOU, A. A. and SEIFTER, A., 1984, Supplemental vitamin A prevents the acute radiation-induced defect in wound healing. *Annals of Surgery*, **200,** 494–512.

MEFFERT, H., DIEZEL, W. and SONNICHSEN, N., 1976, Stable lipid peroxidation products in human skin: detection, ultraviolet induced increase, and pathogenic importance. *Experientia*, **32,** 1397–1398.

NAGATA, H., SUGAHARA, T. and TANAKA, T., 1972, Radiation protection by 2-mercaptopropionylglycine in mice. *Journal of Radiation Research*, **13,** 163–166.

PEACOCK, E. E., 1984, Contraction. In E. E. Peacock (ed.), *Wound Repair*, 3rd edn (Philadelphia: W. B. Saunders), pp. 39–55.

PRIEUR, D. J., YOUNG, D. M., DAVIS, R. D., COONEY, D. A., HOMAN, E. R., DIXON, R. L. and GUARINO, A. M., 1973, Procedures for preclinical toxicologic evaluation of cancer chemotherapeutic agents: protocols of the laboratory of toxicology. *Cancer Chemotherapy Reports*, **4,** 1–28.

REDPATH, J. L. and WILSON, R. L., 1973, Reducing compounds in radioprotection and radiosensitization: model experiment using ascorbic acid. *International Journal of Radiation Biology and Related Studies in Physics, Chemistry and Medicine*, **23,** 51–65.

RINGSDORF, W. M. JR and CHERASKIN, E., 1982, Vitamin C and human wound healing. *Oral Surgery, Oral Medicine, Oral Pathology*, **53,** 231–236.

RUDOLPH, R., VANDE BERG, J., SCHNEIDER, J. A., FISCHER, J. C. and POOLMAN, W. L., 1988, Slowed growth of cultured fibroblasts from human radiation wounds. *Plastic and Reconstructive Surgery*, **82,** 669–675.

SONG, S. and CHENG, T., 1997, The effect of systemic and local irradiation on wound macrophage and repair promoting action of phenytion sodium. *Zhonghma Yi Xue Za Zhi*, **77,** 54–57.

STROMBERG, L. W., WOODWORD, K. T., MAHIN, D. T. and DONATI, R. M., 1968, Combined surgical and radiation injury: the effect of timing of wounding and whole body gamma irradiation on 30 day mortality and rate of wound contraction in the rodent. *Annals of Surgery*, **167,** 18–22.

TAPER, H. S., KEYEUX, A. and ROBERFROID, M., 1996, Potentiation of radiotherapy by nontoxic pretreatment with combined vitamins C and K3 in mice bearing solid transplantable tumor. *Anticancer Research*, **16,** 499–504.

THOMSON, J. F., 1962, *Radiation Protection in Mammals* (New York USA: Reinhold).

TURESSON, I. and THAMES, H. D., 1989, Repair capacity and kinetics of human skin during fractionated radiotherapy: erythema, desquamation and telangiectasia after 3 and 5 years follow-up. *Radiotherapy and Oncology: Journal of the European Society for Therapeutic Radiology and Oncology*, **15,** 169–188.

VASIL'EVA, T. V., MICHURINA, T. V. and KHRUSHCHOV, N. G., 1978, Immunofluorescent study of the origin of connective tissue cells in xenogenic radiation chimeras under normal conditions and in aseptic inflammation. *Ontogenez*, **9,** 288–290.

VEGESNA, V., WITHERS, H. R., HOLLY, F. E. and MCBRIDE, W. H., 1993, The effect of local and systemic irradiation on impairment of wound healing in mice. *Radiation Research*, **135,** 431–433.

WAYNER, D. D. M., BURTON, G. W. and INGOLD, K. U., 1986, The antioxidant efficiency of vitamin C is concentration dependent. *Biochemica et Biophysica Acta*, **884,** 119–123.

YUHAS, J. M. and STORER, J. B., 1969, Chemoprotection against three modes of radiation death in the mouse. *International Journal of Radiation Biology*, **15,** 233–237.

ZELMAN, D., SONG, I. C., PORKOUS, D. D. and BROMBERG, B. E., 1969, The effect of total body irradiation on wound healing and hematopoietic system. *Bulletin of the New York Academy of Medicine*, **45,** 293–300.