Radioprotective Effect of Amifostine (WR 2721) and Vitamin E on Whole-Body-Irradiated Rat Liver

Amifostin (WR 2721) ve E Vitamininin Tüm Vücut Işınlaması Uygulanmış Sıçanların Karaciğeri Üzerindeki Radyoprotektif Etkisi

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ABSTRACT Objective: The aim of this study was to assess whether amifostine with/or without vitamin E could protect the normal liver from the effects of ionizing radiation. **Material and Methods:** Six groups were included in the study, each consisting ten, healthy, male, Wistar rats. The first group (control group) did not receive any radiation, amifostine (WR 2721), or vitamin E (∝ tocopherol acetate). The second group received an intraperitoneal (i.p.) infusion of 200 mg/kg amifostine (WR 2721) and subcutaneous (s.c.) infusion of 100 IU/kg vitamin E (∝ tocopherol acetate). The third group received only 8 Gy total body irradiation (TBI). The fourth group received irradiation and an intraperitoneal (i.p.) infusion of 200 mg/kg amifostine, administered half an hour prior to the irradiation. The fifth group received irradiation and subcutaneous (s.c.) infusion of 100 IU/kg vitamin E (∝ tocopherol acetate), administered one hour prior to the irradiation. The last (sixth) group received irradiation and amifostine and vitamin E infusion under the same conditions. We measured thiol and malondialdehyde (MDA) levels in plasma and MDA levels in liver tissue. **Results:** Plasma MDA levels were not different in the control and the irradiated groups. Plasma thiol level was lowest in the third [irradiation alone (R)] group, and it was significantly different from the first (C) group ($p< 0.001$). Plasma thiol level of the fifth (irradiation plus vitamin E [R + V - E]) group was lower than the level in the first (C) group and the difference was significant ($p= 0.019$). The comparison of the plasma thiol level of the third (R) group with the fourth (R + A) and the sixth (R + A + V - E) groups revealed much higher levels in the fourth (R + A) and the sixth (R + A + V - E) groups than in the third (R) group; this difference was significant ($p< 0.001$). The highest liver MDA level was in the third (R) group and the difference compared with the first (C) group was significant ($p< 0.001$). In the third (R) group, the level of liver MDA was significantly higher than in the fourth (R + A) and sixth (R + A + V - E) ($p= 0.001$ and $p= 0.003$ respectively) groups. **Conclusion:** Amifostine and vitamin E are effective in protecting the liver against the damage induced by irradiation.

Key Words: Radiation protection; amifostine; tocopherols

ÖZET Amaç: Bu çalışmada, amifostinin (WR-2721) E vitamini (∝ tokoferol asetat) ile birlikte ya da tek başına iyonize radyasyona maruz kalan normal karaciğer dokusunu radyasyonun olumsuz etkilerinden koruyup korumadığı araştırıldı. **Gereç ve Yöntemler:** Her birinde 10 sağlıklı Wistar albino sıçanı bulunan 6 grup bu çalışmaya dahil edildi. Birinci gruba radyasyon, amifostin ve E vitamini verilmedi; bu grup kontrol grubunu oluşturdu. İkinci gruba, 200 mg/kg amifostin intraperitoneal (i.p) olarak ve 100 IU/kg E vitamini subkütan (s.k) yoldan uygulandı. Üçüncü gruba sadece 8 Gy tüm vücut ışınlaması yapıldı. Dördüncü gruba, 8 Gy tüm vücut ışınlamasından yarım saat önce, 200 mg/kg amifostin i.p uygulandı. Beşinci gruba irradyasyondan bir saat önce 100 IU/kg E vitamini s.k. verildi. Altıncı gruba da aynı şartlar altında hem radyasyon hem amifostin hem de E vitamini uygulandı. Plazmada, tiyol ve malondialdehid (MDA); karaciğer dokusunda da MDA seviyelerine bakıldı. **Bulgular:** Hem kontrol grubunda hem de radyasyon uygulanan grupta, plazma MDA seviyeleri arasında fark yoktu. Plazma tiyol seviyeleri üçüncü [sadece radyasyon (R)] grupta en düşüktü ve kontrol grubuna (K, 1. grup) kıyasla aralarındaki fark anlamlı idi ($p< 0.001$). Beşinci gruptaki (R + V - E) plazma tiyol seviyesi birinci (K) grupta olduğundan daha düşüktü ve aralarındaki fark anlamlıydı ($p= 0.019$). Plazma tiyol düzeyleri, dördüncü (R + A) ve altıncı (R + A + V - E) grupta, üçüncü (R) gruba kıyasla anlamlı ölçüde daha yüksekti ($p< 0.001$). En yüksek karaciğer MDA seviyesi üçüncü (R) grupta idi ve birinci (K) grupla kıyaslandığında aralarındaki fark anlamlı idi ($p< 0.001$). Üçüncü (R) grupta karaciğer MDA seviyesi, dördüncü (R + A) ve altıncı (R + A + V - E) grupta olduğundan anlamlı olarak daha yüksekti (sırasıyla $p= 0.001$ ve $p= 0.003$). **Sonuç:** Amifostin, E vitamini ile birlikte ya da tek başına radyasyona bağlı karaciğer hasarına karşı koruyucu bir maddedir.

Anahtar Kelimeler: Radyoproteksiyon; amifostin; vitamin-E

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Amifostine (WR-2721) (Ethanethiol; 2-3 aminopropyl-amino-dihydrogen phosphate ester), which is an aminothiol free radical scavenger, is a selective cytoprotective agent for normal tissue from the toxicities associated with chemotherapy and irradiation.[1] Indeed, WR-1065, the major active metabolite of amifostine, which is selectively produced by normal cells through dephosphorylation by membrane-bound alkaline phosphatase at a preferentially neutral pH,[2] provides cytoprotection by at least three different mechanisms. First, it can bind directly to, and thus detoxify, the active alkylating species[3] and platinum agents.[4] Second, it acts as a potent scavenger of drug-or radiation-induced oxygen free radicals. Third, when administered after exposure to radiation and/or several chemicals, it can markedly reduce injury-induced apoptosis.[5,6] It is debatable whether the protection includes the tumor cells. Although some studies in animal models have shown minimal protection of the tumor,[7,8] most of the preclinical data suggest that destruction of the tumor cells is not compromised.[9,10]

Radioprotective activity of amifostine has been demonstrated in most normal tissues except the central nervous system.[11] One study in rats has shown that systemic administration of amifostine protects hepatocytes from reproductive cell death with a dose modification factor of 2,[12] and that the liver is protected from fibrosis with a dose modification factor that is greater than 2.[13]

Lipid peroxidation (LP) has been suggested as one of the main causes of ionizing radiation damage, thus toxicity of irradiation to hepatocytes may result in part from radical-mediated tissue damage. The biological relevance of markers for LP in rats, eg, malondialdehyde (MDA), which is an aldehydic by-product of LP, can be used as a marker that shows liver damage induced by irradiation.[11,13,14]

Vitamin E is a free radical scavenger that acts as first line of defense against peroxidation of polyunsaturated fatty acids. In the tissues, it reacts very rapidly with molecular oxygen and free radicals and protects polyunsaturated fatty acids (especially those in membranes) from LP.[15]

In a number of experimental studies, it has been demonstrated that, vitamin E can increase the growth inhibitory effect of various tumor treatment modalities such as radiation, chemotherapeutic agents, and hyperthermia.[16] There are, however, conflicting reports in the literature on radioprotective effects of vitamin E on normal tissue.[17]

In this study we aimed to show whether amifostine with or without vitamin E could act as a radioprotector agent in rat liver when administered alone or in combination, prior to whole body irradiation. We used plasma thiol, plasma MDA, and liver tissue MDA levels, which were known to be influenced by ionizing radiation, as damage markers.

MATERIAL AND METHODS

EXPERIMENTAL ANIMALS

Fifty male Wistar rats, which were purchased from the Animal House of Faculty of Medicine, Erciyes University, weighing 270-430 g, were included in the study. The experimental protocol used was approved by the Department of Animal Care and Use Committee of the Turkish Ministry of Agriculture and adhered to the European Community Guiding Principles for the Care and Use of Animals. All animals were conditioned at room temperature at natural photo-period (14 h/10 h: light/dark) for one week before the initiation of the experiment. A commercial, balanced diet and tap water, ad libitum, were provided. After one week of acclimatization, all rats were food restricted (without water restriction) for 12 h before the experiment. This was done to put all animals into a similar metabolic state.

EXPERIMENTAL PROCEDURE

The animals were divided into six groups and each group consisted of 10 animals:

First group: First group was the control group (C). C did not receive any radiation, amifostine (WR 2721), or vitamin E (μ tocopherol acetate). C was given the vehicle only.

Second group: This group received an intraperitoneal (i.p.) infusion of 200 mg/kg amifostine

(WR 2721) and a subcutaneous (s.c.) infusion of 100 IU/kg) vitamin E (μ tocopherol acetate) (A + V - E).

Third Group: This group only sunderwent total body irradiation (TBI) with 8 Gy (R), and was also given the vehicle.

Fourth group: This group received irradiation, with i.p. infusion of 200 mg/kg amifostine (WR 2721), administered half an hour prior to irradiation (R + A).

Fifth group: This group also received irradiation with s.c. infusion of 100 IU/kg vitamin E (μ tocopherol acetate), administered one hour prior to irradiation (R + V - E).

Sixth group: This group was both irradiated and given amifostine and vitamin E infusions under the same conditions that were described above (R + A + V - E).

RADIATION TECHNIQUE

Mild hypnosis of the animals for immobilization was achieved by intramuscular administration of Ketamine (50 mg/kg B.W.), 5 minutes prior to the irradiation, ensuring spontaneous respiration throughout the procedure. Then the animals were paired and placed in supine position on a Plexiglas board, so that two animals would be irradiated at a time. Rats were exposed to a single dose of 8 Gy TBI of gamma radiation from a ^{60}Co source (Theratron 780-C), at a dose rate of 0.52 Gy/min, administered at 1.5 cm depth below the skin, the source-skin distance being 80 cm.

Following their exposure to ionizing radiation, the animals were placed individually into metabolic cages. After an interval of 36 hours, all rats were sacrificed by general anesthesia (50 mg/kg, i.p. ketamin). Blood samples were collected from each rat and were cooled in ice water. Samples were centrifuged at 3000 *g* for 10 min at 4°C to isolate serum and were stored as aliquots at -10°C until testing. Livers were excised immediately and were homogenized in ice-cold 100 mM phosphate buffer (pH 7.4) using a Potter-Elvehjem homogenizer fitted with a Teflon plunger. Homogenates were centrifuged at 11 000 x g for 20 min and resulting supernatants were stored at -80°C.

Analytical Methods

Chemicals: Thiobarbituric acid, 5,5-dithio bis (2-nitrobenzoic acid), malondialdehyde, n-butanol, pyridine and sodium dodecyl sulfate were all obtained from Sigma (St. Louis, MO, USA).

Assessment of antioxidant status

The plasma free thiol groups may be important components of the extracellular antioxidant defense system.

Plasma thiol measurement: Thiol levels were measured in plasma samples obtained from the rats using the method which was developed by Koster et al.[18] The method is based on the property of free sulfydryl compounds (SH) that enables them to react with 5,5-dithio-bis-2-nitrobenzoic acid (DTNB), developing a stained complex (TNB), which can be measured spectrophotometrically, with an absorbance peak at 412 nm, which is directly proportional to its concentration.

Plasma MDA measurement: According to the method developed by Wong at al,[19] MDA, which is an LP product, reacts with thiobarbituric acid, developing a pink colored stain complex, which can be measured spectrophotometrically with an absorbance peak at 532 nm, which is directly proportional to its concentration.

Liver MDA measurement: The levels of MDA in liver tissue were assessed according to the method of Ohkawa et al.[20] The assay procedure for MDA level in rat liver included the addition of 0.2 ml of 8.1% sodium dodecyl sulphate (SDS) and 1.5 ml of 20% acetic acid solution to samples less than 0.2 ml of 10% (w/v) tissue homogenate. pH was adjusted to 3.5 with NaOH and 1.5 mL of 0.8% aqueous solution of thiobarbituric acid (TBA). The final volume was brought to 4.0 mL with distilled water and then the sample was heated in an oil bath at 95°C for 60 min using a glass ball as a condenser. After cooling with tap water, 1.0 mL of distilled water and 5.0 mL of the mixture of *n*-butanol and pyridine (15:1 v/v) were added and the mixture was shaken vigorously. After centrifugation at 4000

rpm for 10 min, the organic layer was taken and its absorbance at 532 nm was measured. MDA levels were expressed in nanomoles MDA per milliliter in plasma (nmol MDA/mL) and per milligram of protein in tissue homogenates (nmol MDA/mg protein).

Statistical Analysis

The data were tested with Kolmogorov Simirnov test to measure its suitability to normal distribution. Due to its suitability for normal distribution, the data were presented as means ± standard deviation (SD). All statistical evaluations were carried out with the SPSS 10.0 (Statistical Packages for Social Sciences; SPSS Inc, Chicago, Illinois, USA). Statistical comparison of the data from the five groups was made by analyses of variance (ANOVA) and post-ANOVA (Scheffe's procedure) tests. A p value of less than 0.05 was considered significant.

RESULTS

All results obtained from the study groups were shown in Table 1. In the second group (amifostine plus vitamin-E without irradiation [A + VE]), the plasma thiol level was not significantly different from the level in the first group [control group (C)]. Plasma thiol level was lowest in the third [irradiation alone (R)] group, and it was significantly different from the level in the first (C) group ($p< 0.001$). Plasma thiol level of the fifth group (irradiation plus vitamin E group [R + V - E]) was significantly lower than that of the first (C) group ($p= 0.019$). The difference between the first (C) and the fifth (R + V - E) and the sixth [irradiation plus amifostine plus vitamin-E (R + A + V - E)] groups was not significant ($p= 0.234$ and $p= 0.774$ respectively). The plasma thiol level was significantly higher in the fourth (R + A) and sixth (R + A + V - E) groups than in the third (R) group ($p< 0.001$). In the fifth (R + V - E) group, plasma thiol level was higher than in the third (R) group, but this difference was not significant ($p> 0.079$). Plasma thiol level of the fifth (R + V - E) and the sixth (R + A + V - E) groups was significantly lower than the level in the fourth (R + A) group ($p< 0.001$).

In all groups, plasma MDA levels were not statistically different from each other.

The highest liver MDA level was in the third (R) group, and when compared with the first (C) group, the difference was significant ($p< 0.001$). However, the differences between liver MDA levels were not statistically significant between the first (C) and the fourth (R + A) and the sixth (R + A + V - E) groups ($p= 0.618$ and $p= 0.430$ respectively). In the third (R) group, the level of liver MDA was significantly higher than in the fourth (R + A) and the sixth (R + A + V - E) groups ($p= 0.001$ and $p= 0.003$ respectively). The differences were not statistically significant between the sixth (R + A +

TABLE 1: Liver tissue malondialdehyde (MDA), plasma MDA and plasma thiol values of study groups*

Groups	n	Plasma thiol	Plasma MDA	Liver MDA
1. Control (C)	10	483.4 ± 58.6	1.56 ± 0.4	3642.7 ± 590.2
2. A + VE - without irradiation (A + VE)	10	433.5 ± 51.4 [a]	1.44 ± 0.3	3598 ± 566.7 [a]
3. Irradiation (R)	10	268.0 ± 46.7[a]	2.22 ± 0.3	5748 ± 1087.5[a]
4. Irradiation + amifostine (R + A)	10	561.7 ± 111.6[b]	1.8 ± 0.4	4192 ± 783.5[b]
5. Irradiation + vitamin E (R + VE)	10	365.6 ± 41.4[a,c]	1.8 ± 0.8	4931.3 ± 691[a]
6. Irradiation + amifostine + Vitamin E (R + A + VE)	10	439.9 ± 81.5[b,c]	1.7 ± 0.4	4307.6 ± 457[b]
F (ANOVA)		32.53	0.24	336.58
p		< 0.001 or 0.000	> 0.05 or 0.915	< 0.001 or 0.000

*Values are: **mean ± SD (Standart deviation)**

Statistical comparisons:

[a]: Comparisons with control group,

[b]: Comparisons with irradiation group,

[c]: Comparisons with irradiation + amifostine group

TABLE 2: Liver tissue MDA, and plasma thiol values of study groups* (significantly different from control group and each other)

Groups	n	Plasma thiol	Liver MDA
1. Control (C)	10	483.4 ± 58.6	3642.7 ± 590.2
3. Irradiation (R)	10	268.0 ± 46.7[a]	5748 ± 1087.5[a]
4. Irradiation + amifostine (R + A)	10	561.7 ± 111.6[b]	4192 ± 783.5[b]
5. Irradiation + vitamin E (R + VE)	10	365.6 ± 41.4[a,c]	4931.3 ± 691[a]
6. Irradiation + amifostine + Vitamin E (R + A + VE)	10	439.9 ± 81.5[b,c]	4307.6 ± 457[b]
F (ANOVA)		32.53	336.58
p		< 0.001 or 0.000	< 0.001 or 0.000

*Values are: **mean ± SD (Standart Deviation).**
Statistical comparisons:
[a]: Comparisons with control group,
[b]: Comparisons with irradiation group,
[c]: Comparisons with irradiation +Amifostine group

V - E) and the fourth (R + A) or the fifth (R + V - E) groups for the liver MDA levels (p= 0.998 and p= 0.322 respectively). In the second (A + V - E) group, the liver MDA level was not significantly different from the first (C) group (p= 0.415). Table 2 shows significant differences between all groups.

DISCUSSION

Ionizing radiation can be used to damage target cells or tissues. However, the irradiation also damages non-target cells or tissues. Since irradiation of the body results in oxidative stress due to the formation of oxygen radicals, damage due to irradiation could be controlled by antioxidants and antioxidative enzymes, resulting in a successful irradiation.[21] Due to the late damage on the normal tissue caused by radiation, the potential efficacy of it for the treatment of malignant tumors is limited. Radiation therapy sequels are still sometimes unavoidable and can cause great handicaps in a significant number of patients.[22,23]

In the past few years, amifostine (WR-2721) was introduced into cancer clinical trials to study its protective effects against normal tissue damage caused by irradiation and various chemotherapeutic agents.[24-26] The phosphorylated compounds serve as prodrugs for the active free aminothiols, e.g., WR-1065 (2-(3-aminopropylamino) ethanethiol) from WR-2721, and their corresponding disulfides formed in vivo. Phosphorothioates and other aminothiols, which are usually administered shortly before irradiation, have been hypothesized to act by one mechanism or by a combination of mechanisms: scavenging of radiation-induced free radicals before their reaction with biomolecules; induced hypoxia; formation of mixed disulfides; scavenging of metals; repair of DNA through hydrogen donation to carbon-centered radicals; and genome stabilization.[27] As a result of these activities, they can prevent or ameliorate cisplatin-induced nephrotoxicity[24] chemotherapy-related thrombocytopenia,[25] radiation-induced tissue damage,[26] etc.

A number of studies have been conducted which look into the radioprotection of liver by reducing LP and most of them were able to show the efficacy of amifostine. Symon et al, used amifostine in a rat liver tumor model to protect hepatocytes from radiation treatment selectively[9] and they reported that both systemic and portal venous administration of amifostine effectively protected hepatocytes from ionizing radiation, without compromising tumor cell kill, in a clinically relevant animal model. In our study, we found a 30% decrease in MDA levels with amifostine, which was caused by irradiation-induced LP. Addition of vitamin E had no effect on the results. Despite the fact that we applied very high total body irradiation, amifostine effectively protected the liver against the irradiation-induced LP. Mertsch et al,

studied amifostine in bovine aortic endothelial cell line and reported that amifostine prevented radical-induced membrane LP in endothelial cells injured by hypoxia/reoxygenation and pointed to the use of amifostine in the protection of the endothelium against oxidative stress, which is a new approach.[28]

After performing clinically relevant animal model studies, many authors made phase I and phase II clinical studies with amifostine in an attempt to protect normal tissue from ionizing radiation damage. Coia et al, designed a phase I study to establish the maximum tolerated dose (MTD) of amifostine, when given twice weekly with TBI and to define the toxicities of this combination and schedule.[29] They showed that 910 mg/m^2 was tolerated on a twice weekly schedule with TBI and the more effective or less toxic use of TBI in the treatment of non-Hodgkin's lymphoma, which may be potentially achieved with amifostine, would represent an important therapeutic option for the clinician. In another randomized clinical study, Bourhis et al showed that the concomitant use of amifostine was able to reduce the severity and duration of mucositis induced by a much accelerated irradiation regimen. However, the tolerance of this twice-daily amifostine schedule was relatively poor.[30]

Karbownik et al, performed a study to examine the potential protective effect of melatonin against whole body irradiation (8 Gy).[31] They took liver tissue samples 12 hours after irradiation but could not find any change in MDA levels between the control and melatonin groups. In our study, we could not see any difference in plasma MDA levels between the control and irradiation groups; however, liver MDA levels were markedly higher in the irradiation group than in the control group. This may result from the time interval after which we took plasma and liver samples, which was 36 hours after the irradiation. This interval was adequate for the detection of the liver MDA levels but not for plasma MDA. Thus, we agreed that we needed more than 36 hours if we wanted to detect plasma MDA levels. Former studies stated that the MDA increase depended on the irradiation dose and the time interval after which the samples were taken, but the optimal time and the optimal radiation dose were not specified.[14,26,27]

Fifty years ago, deficiency of vitamin E (i.e., a-tocopherol) was reported to be associated with abnormal repair of connective tissue, which resulted in the production of scar-like tissue in humans. Today, the antioxidant role of vitamin E in biological systems is well known. a-tocopherol is located primarily in the cellular membranes and is the most important antioxidant that protects membrane phospholipids from oxidative damage. Experimental and clinical evidence indicate that the development of fibrosis in the lung, kidney, and liver is generally associated with the overexpression of TGFβ-1, increased transcription of procollagen Type I, and LP of biological membranes, as shown by MDA production.[32] Vitamin E plays an important role in the protection against oxidative damage induced by carcinogenesis and exposure to ionizing radiation and chemotherapeutic agents.[27,33] There are many studies conducted both with animals and cancer patients receiving radical irradiation where vitamin E supplementation has been studied. Gitanjali et al, administered vitamin E supplementation to patients with cervical carcinoma, receiving radical radiotherapy (RRT).[15] They randomized fifty patients with biopsy-proven carcinoma of the cervix into two groups. Group I received vitamin E supplementation (100 mg orally daily) in addition to RRT. Following vitamin E supplementation in group I, serum MDA levels were reduced as compared to group II indicating that vitamin E supplementation was effective in reducing the LP. This also showed that the serum vitamin E level correlated with its in vivo effect on LP. The most effective radioprotectors, such as the phosphorothioates, are not protective when administered in the postirradiation period. Vitamin E belongs to another class of protectors (free radical scavenger or antioxidants) that are also active when administered during the postirradiation period.[27] These compounds probably modulate later reactions, for example, interactions of radiation-induced radicals of biomolecules with reactive oxygen species evolved during normal cellular

processes.[34] While we were getting a significant radioprotective effect with amifostine, we could not achieve a good response against radiation induced LP with vitamin E. This may be a result of various factors, such as route of administration, type of vitamin preparation, strain of rat, dose and administration time of vitamin E and/or total dose and dose rate of irradiation.

It should be emphasized that in our experiment, irradiation was given at a dose rate of 52 cGy/min. Since it has been reported that there is a greater radiation-induced LP at lower dose rates compared to higher dose rates,[17,27,35] it is possible that greater protection would be more readily observed at lower dose rates by protectors, such as vitamin E, that act mainly as a membrane antioxidant.

Additional studies are required to establish whether vitamin E would still be effective when mice/rats are exposed to higher dose rates of ionizing radiation. Another issue is that radioprotection by vitamin E may also involve immunological effects,[17,36] and it is not clear whether the radioprotective effects of vitamin E depend on its humoral features or antioxidant features. In fact, we could not get a good response against radiation induced LP with vitamin E and this led us to the thought that the radioprotective effects of vitamin E mainly depended on its humoral activity rather than its antioxidant effect. Another reason for our failure in achieving a good response with vitamin E could be the dose of the drug. Mainly, 100 IU/kg may not be an adequate dose for achieving radioprotection with vitamin E; thus, we suggest that further studies are needed with vitamin E regarding its dose of administration.

In conclusion, both amifostine and vitamin E are very important radioprotector agents against radiation-induced damage. They can protect the normal tissue from the harmful effects of ionizing radiation either alone or in combination.

To our knowledge, this is the first study showing a significant in vivo antioxidant effect of amifostine combined with vitamin E. The current findings of our study suggest that exogenously administered amifostine is highly effective in reducing the toxic effects of ionizing radiation. However, we could not achieve the same result with vitamin E administration to rats before TBI. As discussed earlier, this may be related with the dose of irradiation and/or vitamin E or with the timing of plasma sampling. Therefore, the remaining issues that need to be clarified are the timing, the dose of vitamin E and irradiation, and administration route of drugs (i.p., i.v., oral, venous, etc.).

With respect to liver, especially clinical use of amifostine may protect the liver from the damage of radio-chemotherapy if we reach optimal dose and schedule. This is the reason for the need for further preclinical or clinical studies to establish the optimal dose and schedule for amifostine and vitamin E.

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