RADIATION PROTECTION BY AN ISOFLAVONE, GENISTEIN: A STUDY ON THE SURVIVABILITY OF MICE

by

Arvind Lal BHATIA, Ajay GAUR, and Avadhesh SHARMA

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The radioprotective effects of an acute administration of the isoflavone genistein (4, 5, 7-trihydroxyflavone), have been investigated in the present study. Male mice were administered with different doses (100, 200, 300, and 400 mg/kg body weight) of genistein 24 hours prior to 8 Gy gamma irradiation and the 200 mg/kg dose of genistein was determined to offer the maximum survivability and was used as an optimum dose for further experimentation. The 0.5 ml dose of genistein (200 mg/kg) was administered intraperitoneally to 2 different groups of mice, 15 minutes and 24 hours prior to gamma irradiation. In the mice treated with genistein with the optimum dose 24 hours before irradiation, a significant increase in 30 day survival has been recorded in contrast to the mice treated with genistein 15 minutes before the irradiation. The longer survivability (i. e. 20% for a period of more than 30 days) has been observed in the 24 hour group as compared to that of 15 minutes (i. e. 20% for 22 days). Although the radioprotective effect of genistein was evident in both groups, it was of greater magnitude in the group with a longer interval, indicating thereby an efficacy with longer retention with the possible minimum toxicity, unlike hitherto known other radioprotective agents.

Key words: survivability, genistein, radioprotection, soy foods, radiation

INTRODUCTION

The radioprotective agents need to be used to minimize or prevent the damage from solar radiation exposure experienced by astronauts, pilots, other flight personnel, and frequent fliers. The radioprotective agents can also be utilized for protection from accidental radiation exposure caused by nuclear power facilities, other radiation generating facilities including those for food irradiation, or by a detonation of an atomic bomb or other device that releases radiation. Also, they can be used to confer protection to the personnel involved in cleaning up the areas of such radiation accidents or disposal facilities. The radioprotective agents

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Authors' address: Radiation Biology Laboratory University of Rajasthan, Jaipur – 302004, Rajasthan, India L. B. S. College of Pharmacy, Jaipur, Rajasthan, India

E-mail address of corresponding author: arvindlalbhatia@yahoo.com (A. L. Bhatia) of the present discovery are also of use in reducing the toxic effects of inhaled or ingested radionuclides and in reducing toxicity caused by radiation produced by electronic devices of non-ionizing nature of radiation, such as cellular telephones and microwaves. Rapidly growing interventional radiological procedures such as dilatation of stenosed vessels or recanalization or vascular-angio-anastomoses would also benefit from the use of radioprotectors. Hence, there is a necessity to search for a radioprotector that could have multidimensional advantages. The present invention is aimed to the protection of normal cells and tissues in a mammal from therapeutic or diagnostic radiation exposure by administration of an isoflavone compound. This enables larger, more effective doses of radiation to be given to the patient.

Genistein, an isoflavone found in soy, has been reported to have weak estrogenic and antiestrogenic properties, to be an antioxidant, to inhibit topoisomerase II and angiogenesis, and to induce cell differentiation. Genistein (4, 5, 7-trihydroxyflavone), a naturally occurring isoflavone found in soybeans, has gained increasing attention because of its association with beneficial effects in treatment of cardiovascular diseases, high blood pressure, osteoporosis, breast cancer, and prostate cancer [1]. The ideal radioprotector would be nontoxic and would not degrade its performance. Naturally occurring dietary components also offer opportunities for development as effective chemopreventive and radioprotective agents because of their potential low toxicity [2, 3]. It is speculated that breast cancer protection in Asian women consuming a traditional soy containing diet is derived from early exposure to soybean products containing genistein [4]. Recently, Hyunki and collaborators [5] have observed the antibacterial activity of the soy isoflavone genistein, which is found to block the invasion of pathogenic bacteria in mammalian epithelial cells. The direct effect of genistein was studied in the survival and growth of the probiotic Lactobacillus reuteri and selected opportunistic bacteria in vitro as a prelude to in vivo use for managing postirradiation sepsis. Their results demonstrated the in vitro antimicrobial activity of genistein and suggested the use of genistein in combination with probiotics, which may augment the effectiveness of antimicrobial therapies, currently used in the management of infections, including those induced by ionizing irradiation.

Genistein is also classified as a phytoestrogen, a plant derived nonsteroidal compound that possesses estrogen like biological activity. Genistein and its glycosides are mainly found in legumes, such as *Glycine max* (soy beans) and *Cicer arietinum* (chickpeas). Soy beans and soy foods are the major dietary sources of these substances [6]. Genistein has been found to have a number of antioxidant activities. It is a scavenger of reactive oxygen species and inhibits lipid peroxidation. It also inhibits superoxide anion generation by the enzyme xanthine oxidase. In addition, in animal experiments, genistein has been found to increase the activities of the antioxidant enzymes superoxide dismutase, glutathione peroxidase, catalase, and glutathione reductase [7-10].

Keeping in mind the foregoing observations, the possibility of its efficacy as radioprotective agent cannot be ruled out. Hence, the present study deals with the effect of genistein in irradiated mice vis-à-vis its retention in the body.

MATERIALS AND METHODS

Male Swiss albino mice (6-8 week old) were selected from an inbred colony and maintained on standard mouse feed (Hindustan Lever Ltd., New Delhi) and water *ad labitum*. Mice were maintained at constant temperature (22 1 °C) and light (12L:12D). Genistein, obtained from L. C. Laboratories, New Boston St., USA, was used 100-400 mg/kg body weight 0.5 ml, intraperitoneally (IP). Genistein was administered 15 minutes and 24 hours before gamma irradiation. The animals were exposed to 8 Gy gamma radiation dose of Cobalt-60 at the rate of 1.02 Gy/min.

The experiment was performed in following phases.

Phase 1. Separate groups of mice were used to assess the acute toxicity of genistein in non-irradiated mice. Once an acceptable nontoxic dose of genistein was determined, radioprotection studies were performed. To determine the most effective and optimal dose of genistein, the male mice were administered with different doses (100, 200, 300, and 400 mg/kg body weight) of genistein 24 hours prior to 8 Gy of gamma irradiation and the dose at which maximum survivability occurred was selected as an optimum dose.

Phase 2. For testing the genistein efficacy against radiation, male mice were divided into following groups of 10 mice each:

Group 1: Control group (only irradiated),

Group 2: Dimethyl sulphoxide (DMSO) solvent treated group (24 hours prior to irradiation),

Group 3: Genistein treated group (15 minutes prior to irradiation), and

Group 4: Genistein treated group (24 hours prior to irradiation).

Mice were administered IP a single injection of genistein of a dose of 200 mg/kg body weight 15 minutes and 24 hours prior to a lethal dose of gamma radiation (8 Gy of Cobalt-60 at 1.02 Gy/min). The 0.5 ml dose of solvent (group 2) and genistein (in solvent) were administered IP to the groups 3 and 4 before gamma irradiation. The animals were observed for their survivability for more than 30 days under the standard laboratory conditions. For the statistical evaluation, the correlation coefficient has been used.

RESULTS AND DISCUSSION

The mice treated with genistein (200 mg/kg body weight) 24 hours before irradiation demonstrated a significant increase in 30 day survival, in contrast to the mice treated with genistein 15 minutes before irradiation. The $LD_{50/30}$ value for the mice treated with genistein (200 mg/kg) 24 hours before irradiation was 21 days while LD_{50/30} value for mice treated with genistein 15 minutes before irradiation was 14 days. Additionally, the acute toxicity of genistein evaluated in non-irradiated mice administered with a single IP injection of genistein of a dose of 200 mg/kg showed no adverse effects when compared with the mice in the control group. The results as shown in figs. 1 and 2 indicate that a single IP injection of the genistein (200 mg/kg body weight) administered 24 hours before irradiation offered better protection than the one administered 15 minutes before irradiation because in the 24 hour group genistein is properly absorbed and becomes able to offer better protection against radiation damage.

Optimum dose selection. The mice administered with different doses (100, 200, 300, and 400 mg/kg body weight) of genistein before 8 Gy gamma irradia-

tion showed the maximum survivability at 200 mg/kg, as shown in the graph (fig. 1). The dose of 400 mg/kg, on the other hand, showed the maximum mortality of mice followed by the doses of 300 and 100 mg/kg. It clearly establishes that 200 mg/kg is an optimum dose level which could have been taken into account for the experiments. Also, a single IP injection of genistein of the dose of 200 mg/kg does not show any adverse effect.

Survivability against radiation exposure. As shown in fig. 2, the mice treated with genistein (200 mg/kg body weight) 24 hours before irradiation demonstrated a significant increase in 30 day survival, in contrast to the mice treated with genistein 15 minutes before irradiation. The LD₅₀ value for the mice treated with genistein (200 mg/kg) 24 hours before irradiation was 21 days while LD₅₀ value for the mice treated with genistein 15 minutes before irradiation was 14 days. The results indicate that a single IP injection of genistein (200 mg/kg body weight) administered 24 hours before irradiation offers better protection than the one administered 15 minutes before irradiation; it might have been due to the proper/complete absorption occurring within 24 hours vis-à-vis the radiation damage occurrence. As shown in tab. 1,



Figure 1. The determination of optimum dose of genistein vis-à-vis control and solvent



Figure 2. The survivability curves of mice with genistein 15 minutes and 24 hours prior to 8 Gy gamma irradiation vis-à-vis control and solvent

the longer survivability (i. e. 20% for a period of more than 30 days) has been observed in the 24 hour group as compared to the one of 15 minutes (i. e. 20% for 22 days). Although the radioprotective effect of genistein was evident in both groups, it was, however, of greater magnitude in the group with a longer interval prior to irradiation indicating thereby an efficacy with longer retention with the possible minimum toxicity, unlike hitherto known other radioprotective agents. The trend lines in fig. 3 for the two genistein treated groups show an increased survival rate compared to the genistein non-treated groups. The statistics in tab. 2 shows how well a straight line describes the data. R² interpreted as the fraction of the variability explained by the day variable x, shows that in all cases it is quite close to 1, which indicates that the slope of regression line is highly significant.

 Table 1. Percentage survivability of mice after gamma irradiation

	Survivability [%]				
Groups	50	40	30	20	
Control group	4 days	5 days	6 days	7 days	
Solvent (vehicle) treated group	7 days	8 days	10 days	13 days	
Genistein treated group (15 minutes)	14 days	18 days	20 days	22 days	
Genistein treated group (24 hours)	21 days	26 days	30 days	>30 days	



Figure 3. The linear trends of the survivability curves of mice with genistein 15 minutes and 24 hours prior to 8 Gy gamma irradiation vis-à-vis control and vehicle

Hence, the present study demonstrates that genistein is an effective nontoxic radiation protective agent against radiation induced lethality in mice. Plans for further development of genistein to be used as an effective radioprotective drug can be initiated. A study by Landauer *et al.* [11] on the radioprotective and behavioural effects of an acute administration of the isoflavone genistein made on adult CD2F1 male mice showed a significant increase in 30 day survival for animals receiving

No.	Groups	Regression coefficient R ²	Zero order correlation coefficients R	Survivability y
1	Control	0.9717	-0.986	y = -10x + 94
2	Vehicle	0.8665	-0.989	y = -4.1671x + 82.468
3	Genistein (15 minutes)	0.9772	-0.970	y = -3.6615x + 98.277
4	Genistein (24 hours)	0.9317	-0.963	y = -2.1958x + 91.034

Table 2. Statistics showing regression coefficient \mathbb{R}^2 , zero order correlation coefficients \mathbb{R} and survivability y on the linear trend shown in fig. 3

genistein doses of 25 to 400 mg/kg (p < 0.001). In contrast, the 30 day survival rates of mice treated with genistein 1 hour before irradiation were not significantly different from the ones in the control group. Our results are also in conformity with their results.

Developments in the use of radioactive materials during the past decades have increased amazingly, which disturbs natural balance. At present, there has not been a single effective radioprotective drug that could show its efficacy with respect to a longer retention in the body in relation to time of exposure either as radioprotective or radiosensitive. Till now, the body load of the drug has been first created either before irradiation through chronic administration or the drug has been administered just shortly before or after administration. The findings of the present work will likely cover up such shortcomings that exist in drug testing studies for and against radiation.

The isoflavones in soy beans possess about 0.2% of the estrogenic activity of estradiol, the principle human estrogen. Soy isoflavones actually bind to estrogen receptor sites in the human body. Their weak estrogenic action is actually an antiestrogenic effect, in that the soy isoflavones prevent the binding of the body's own estrogen to the receptor site. This effect does not disrupt the normal reproductive and fertility functions of estrogen, but it may counteract some of the hormone cancer causing potential [12]. Oncogenes are potential cancer cells that produce enzymes that may cause cells to become cancerous. One of the main enzymes produced by oncogenes is protein-tyrosine kinase, an enzyme that stimulates the undifferentiated growth of cells. Genistein is a very potent protein-kinase inhibitor and blocks the signal from the receptors that control cell growth. Genistein blocks protein-tyrosine kinase and other enzymes that trigger tumour formation [13]. In studies of the mammary glands of immature rats, Lamartiniere [4] showed that genistein upregulates the expression of the epidermal growth factor receptor shortly after treatment, which may be responsible for the increased cell proliferation seen at that age. It was hypothesized that the early genistein action promotes cell differentiation that results in a less active epidermal growth factor signalling

pathway in adulthood that, in turn, suppresses the development of mammary cancer. Hence it can be believed that early events are essential for the benefits of cancer protection.

Zhou and Mi [14] confirmed the radiation protection and stimulating haematopoietic recovery by oral administrations of genistein, 160 mg/kg body weight, once daily for seven consecutive days before whole body gamma ray irradiation of adult male BALB/c mice. The survival of irradiated mice protected by genistein was significantly increased and statistically higher than that of mice pretreated with DES. The effects of genistein on promoting recovery of bone marrow nucleated cells, leukocytes and lymphocytes were significantly higher than those of DES. EndoCFU numbers in mice pretreated with genistein was 3.47-fold higher than that in the irradiated control group. It could be deduced from their study that the radioprotective action against death is induced by a possible process of enhanced regeneration of the haematopoietic stem cells due to not only strengthened radioresistance and increased numbers of remained haematopoietic cells, but also enhanced postirradiation repair or promoted proliferation of the haematopoietic stem cells. Suzuki et al. [15] found that GPx activation might be one of the important characteristics of the effects of genistein on prostate cancer cells. They examined the effect of genistein on human prostate cancer (LNCaP and PC-3) cells. Proliferation of both cell lines was inhibited by genistein treatment in a dose dependent manner. The glutathione peroxidase (GPx)-1 gene expression level was the most upregulated. Quantitative real time polymerase chain reaction revealed significant elevation of transcript levels of GPx-1 in both LNCaP and PC-3 cells. Upregulation of gene expression levels accompanied elevation of GPx enzyme activities. It also indicates the better survival of animals against radiation induced free radicals in the present experiment.

However, Akimoto and collaborators [16] reported the effect of genistein on radiosensitivity, especially focusing on survival signal transduction pathways. Genistein greatly enhanced radiosensitivity of two human esophageal squamous cancer cell lines, TE-1 (p53, mutant) and TE-2 (p53, wild) by suppressing radiation induced activation of survival signals, p42/p44 extracellular signal regulated kinase and AKT/PKB. Their study suggested that survival signals, including p42/p44 ERK and AKT/PKB, might be involved in determining radiosensitivity, and genistein would be a potent therapeutic agent that had an enhancing effect on radiation.

Nevertheless, we are of the opinion that the treatment of malignant tumours through the use of radiation is often limited due to damage to non-tumour cells. Damage to the non-tumour cells can exceed the effectiveness of the radiation therapy. The dominant consideration in establishing radiation doses for cancer radiotherapy is the assessment of tolerance of the most radiosensitive normal tissue or organ in the treatment field. This assessment, together with the expected radiation dose required to eradicate a tumour, determines the feasibility of the treatment strategy, and whether a cure or palliation is to be attempted. Often, the maximum tolerable doses are insufficient to eradicate the tumour. Thus, the use of a radioprotective agent would greatly increase the tolerable dose, and therefore the prospects for eradication of tumours and treatment of cancer. Genistein as a radioprotective agent may thus be useful in eliminating or reducing the severity of deleterious cellular effects in normal cells caused by environmental or therapeutic exposure to radiation, cancer radiation therapy and diagnostic tests utilizing radiation where it might prove quite deleterious to tumour tissue (as indicated by Akimoto et al. [16] in cancer cell line). However, it warrants further study and careful evaluation.

However, the genistein may be a protective agent to minimize or prevent the damage from solar radiation exposure experienced by astronauts, pilots, other flight personnel, and frequent fliers. It can also be utilized in protection from accidental radiation exposure caused by nuclear power facilities, other radiation generating facilities including those for food irradiation, or by a detonation of an atomic bomb or other device that releases radiation. Also, they can be used to confer protection to the personnel involved in cleaning up the areas of such radiation accidents or disposal facilities.

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Арвинд Лал БХАТИЈА, Аџај ГАУР, Авадхеш ШАРМА

РАДИОПРОТЕКЦИЈА ГЕНИСТАЈНОМ ИЗУЧАВАЊЕ ПРЕЖИВЉАВАЊА МИШЕВА ТРЕТИРАНИХ ИЗОФЛАВОНОМ

У раду су разматрани радиопротективни ефекти једнократно дате дозе изофлавона генистајна (4", 5, 7-трихидроксифлавона). Мужјаци мишева подвргнути су различитим дозама генистајна (100, 200, 300 и 400 mg по килограму телесне масе) двадесетчетири часа пре излагања гама зрачења од 8 Gy и утврђено је да доза од 200 mg по килограму телесне масе обезбеђује максимално преживљавање, те је у даљем експериментисању била коришћена као оптимална. Петнаест минута и двадесетчетири часа пре гама озрачивања, доза генистајна од 0.5 ml (200 mg/kg) била је дата интраперитонеално двема различитим групама мишева. Код мишева третираних оптималном дозом генистајна двадесетчетири часа пре озрачивања забележен је значајан пораст преживљавања у току тридесет дана, насупрот мишевима подвргнутим генистајну петнаест минута пре озрачивања. Продужено преживљавање (20%-тно преживљавање у периоду дужем од тридесет дана) примећено је у групи "24 часа", у поређењу са групом "15 минута" (20%-тно преживљавање за двадесетдва дана). Мада су радиопротективни ефекти генистајна били евидентни у обе групе, домет је био већи у групи са дужим временским интервалом, указајући на ефикасност продуженог задржавања уз могућу минималну токсичност, различиту од других до сада познатих радиопротективних средстава.

Кључне речи: преживљавање, генистајн, радиопротекција, соја, зрачење