ORIGINAL ARTICLE

GERANYLGERANYLACETONE REDUCES RADIATION-INDUCED DEGENERATION OF SEMINIFEROUS EPITHELIUM IN MICE

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Abstract

Geranylgeranylacetone (GGA) has been shown to have cytoprotective effects against a variety of stressors via the induction of the heat shock response. In this study, male mice received GGA by gavage in doses of 250 mg/kg body weight (kg bw) and 1000 mg/kg bw daily for 5 days. The controls received the corresponding volume of vehicle. In the last day of treatment, all mice received a single dose of 4 gray (Gy) total body irradiation. Blood samples were collected before irradiation and at 2 and 8 days post-irradiation. The testes were removed after euthanasia, in the 8th day post-irradiation, for histological analysis. The results showed significant post-irradiation reduction of leukocyte and platelet counts, with no significant difference between treatment groups and controls. The seminiferous tubule proliferation status was significantly higher in mice treated with 1000 mg/kg bw GGA. In conclusion, GGA reduces radiation-induced degeneration of the seminiferous epithelium in mice.

Rezumat

Geranilgeranilacetona (GGA) prezintă efecte citoprotectoare faţă de diverse noxe prin inducerea răspunsului la şoc termic. În prezentul studiu, şoareci masculi au fost trataţi cu GGA prin gavaj, în doze de 250 mg/kg corp (kg c), respectiv 1000 mg/kg c pe zi timp de 5 zile. Lotul martor a primit volumul corespunzător de vehicul. În ultima zi a tratamentului, şoarecii au fost iradiaţi în monodoza de 4 *gray* (Gy). Au fost recoltate probe hematologice înainte de iradiere şi la 2, respectiv 8 zile postiradiere şi au fost numărate leucocitele şi trombocitele. Testiculele au fost prelevate la 8 zile postiradiere pentru analiză histologică. Rezultatele au arătat o reducere postiradiere semnificativă statistic a numărului de leucocite şi trombocite, fără diferenţe semnificative între loturile investigate şi martori. Şoarecii trataţi cu GGA 1000 mg/kg c au prezentat un status proliferativ al tubilor seminiferi semnificativ mai mare faţă de celelalte loturi. În concluzie, GGA reduce degenerarea postiradiere a epiteliului seminifer la şoareci.

Keywords: geranylgeranylacetone, heat shock proteins, radiation, seminiferous epithelium

Introduction

Geranylgeranylacetone (GGA) is an acyclic isoprenoid [15], with a very broad therapeutic index [7], used clinically in Japan as an antiulcer agent, in doses of 150 mg/day [23]. It has been shown that GGA has cytoprotective effects against various stressors, including ethanol [16], NSAIDs [2], acetic acid [5], ischemia [31], ischemia/reperfusion [17], photo-oxidative damage [25] and inhibition of the mitochondrial respiratory complex by nitropropionic acid [9]. GGA has been reported to be radio-protective *in vitro* [4, 6]. These effects have been explained by the induction of the heat shock response by GGA [26].

The heat shock response is a phylogenetically conserved adaptive reaction, found in all living organisms, consisting of the transcriptional activation of a specific set of proteins (heat shock proteins) by stressors that alter the tertiary structure of cytoplasmic proteins, exposing the hydrophobic residues of their constitutive aminoacids. These hydrophobic residues activate the substrate binding domain of heat shock protein 70 or 90 (Hsp70/90), releasing the heat shock factor 1 (HSF1), which initiates the heat shock response [12, 20]. GGA activates the same domain of Hsp70/90, thus inducing the heat shock response without proteotoxicity [18].

The heat shock proteins have cytoprotective roles by assisting the folding of nascent proteins and the refolding, the degradation or the sequestration of damaged proteins [12, 24]. They also block pro-apoptotic signalling by multiple mechanisms [11].

Regardless of the type of the initial stressor, the heat shock response offers transient cytoprotection against a subsequent stressor of greater intensity [13].

The ionizing radiation causes generation of reactive oxygen species, whose deleterious effects on cellular macromolecules lead to cell death during metaphase or cell death preceded by multi-nucleation, phenomena formerly known as “mitotic catastrophe”, which share several molecular events with intrinsic apoptosis [10].

The radio-sensitivity of various tissues is usually dependent on their turn-over speed, the most sensitive being the hematopoietic system, lympho-poietic system [29] and germ cells [19].

Induction of the heat shock response by heat treatment has been shown to protect lymphocytes against ionizing radiation [13]. However, heat treatment has a wide impact on various molecular processes and activates the heat shock response through proteotoxicity [12], making targeted pharmacological induction of heat shock an attractive approach. In this study we assessed GGA protective effects against ionizing radiation.

Materials and Methods

Eighteen adult male Swiss mice (39 ± 4 g) were obtained from the Târgu-Mureş University of Medicine Biobase. The animals were divided in 3 groups of 6 individuals each (group 1 = controls, group 2 = 250 mg GGA/kg bw, group 3 = 1000 mg GGA/kg bw), kept in plastic cages of 43 x 33 x 18 cm, under constant temperature and humidity conditions, with food and water *ad libitum*. All procedures were performed in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and approved by the local ethics committee.

GGA was purchased from Tokyo Chemicals International (Zwijndrecht, Belgium) and was administrated by gastric gavage, with sunflower oil as vehicle. The treatment groups received GGA in doses of 250 mg/kg bw or 1000 mg/kg bw, respectively, while the control group received the corresponding volume of vehicle (5 mL/kg bw). Solutions were prepared and administrated daily, for 5 days.

In the 5th day of GGA treatment, the mice were placed in a 43 x 33 cm plastic box and received 4 Gy total body irradiation (γ-radiation, 1 Gy/min, 1.25 MeV) from a cobalt source (Theratron elite 100) in the Oncology Department of Sibiu County Emergency Hospital, Romania.

Blood samples (40 μL) were collected by tail tip amputation under Sevoflurane anaesthesia at the end of the GGA treatment (before irradiation - day 0) and 2 and 8 days after irradiation. Platelet (PLT) and leukocyte (WBC) counts were performed with a Sysmex KX-21N haematology analyser.

In the 8th day post-irradiation, the mice were euthanized under sevoflurane anaesthesia. After euthanasia, both testes from each animal were removed, weighed and fixed fresh in 10% formalin for the histological preparation. The results are expressed as testicular index, representing the weight of both testes (mg), divided by the weight of the whole animal (g) [22]. Parasagittal 4 μm-thick slides were stained with haematoxylin-eosin and examined in blind with a Nikon Eclipse E100 microscope. Seminiferous tubules in transversal section were counted in 5 microscope fields at a magnification of 100x. The clonogenic activity of the spermatogonia after irradiation was assessed by noting the tubules with 3 or more layers of cells as positive and the ones with less than 3 layers of cells as negative (Figure 1). The resulting percentage of positive tubules was termed seminiferous tubule proliferation status.

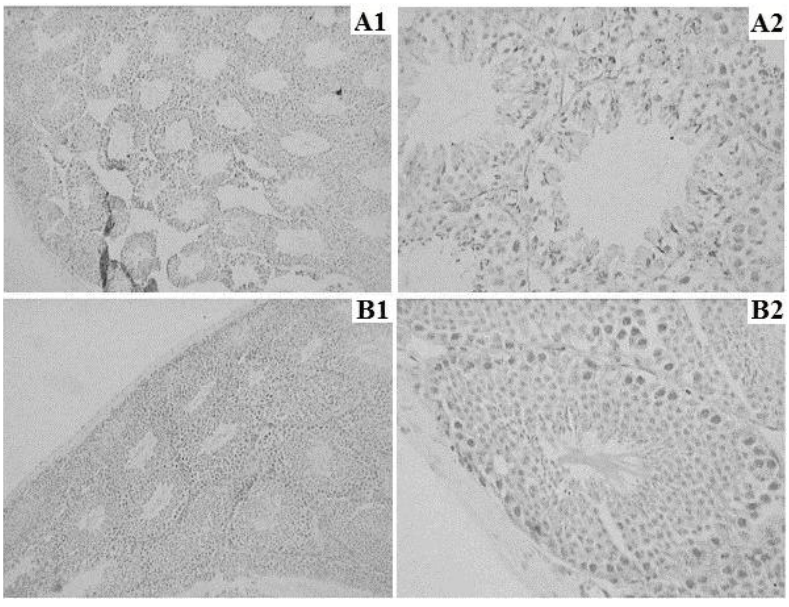

Figure 1.
Histologic aspect of seminiferous tubules

A1, A2: Control with seminiferous tubules with negative proliferation status at 100X (A1) and 400X (A2).
B1, B2: Lot treated with 1000 mg/kg bw GGA, with seminiferous tubules with positive proliferation status at 100X (B1) and 400X (B2).

Required group size was determined by Mead's resource equation. Results are expressed as arithmetic mean ± SD. A p - value under 0.05 determined by two-tailed Student t test was considered significant.

Results and Discussion

Evolution of leukocyte (WBC) and platelet (PLT) counts post-irradiation

The leukocyte (WBC) and platelet (PLT) counts are frequently used to estimate the biological impact of radiation exposure and the efficacy of radiation countermeasures, due to the high radio-sensitivity of the immune and hematopoietic systems [29]. The induction of the heat shock response by thermal treatment has been shown to have radio-protective effects on lymphocytes [13].

The WBC and PLT counts registered significant post exposure reductions, after 2 (Figure 2) and 8 days (Figure 3), respectively.

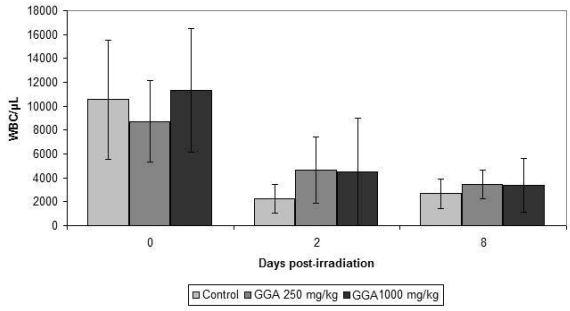

Figure 2.
White blood cell count post-irradiation

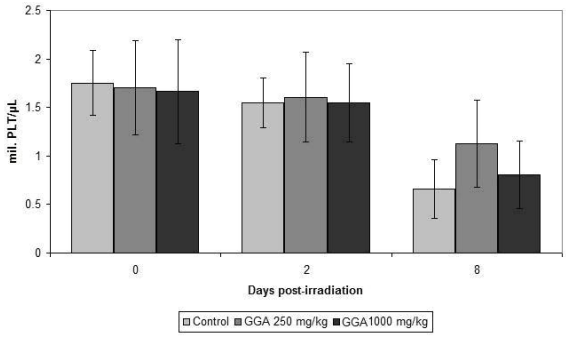

Figure 3.
The evolution of platelet counts post-irradiation

Two days after irradiation, controls showed a decrease from 10566 ± 4982 to 2240 ± 1219 WBC/µL ($p = 0.015$), while the 250 mg/kg bw GGA group decreased from 8720 ± 3410 to 4633 ± 2792 WBC/µL ($p = 0.038$) and the 1000 mg/kg bw GGA group decreased from 11333 ± 5168 to 4533 ± 4234 WBC/µL. Eight days after irradiation, PLT counts decreased from 1.753 ± 0.334 to 0.657 ± 0.304 10^6 PLT/µL in controls ($p = 0.0004$), from 1.703 ± 0.485 to 1.125 ± 0.45 10^6 PLT/µL in the 250 mg/kg bw GGA group and from 1.663 ± 0.536 to 0.806 ± 0.346 10^6 PLT/µL in the 1000 mg/kg bw GGA group ($p = 0.014$). These results are consistent with the effects of sub-lethal irradiation as described by previous studies [3, 30, 32].

The WBC counts showed visible differences between control and both treatment groups in the second day post-irradiation. Also, PLT counts of the 250 mg/kg bw treatment group were visibly higher compared to the control or the 1000 mg/kg bw treatment group in the 8th day post irradiation. However, none of the differences between groups reached statistical significance.

Testicular weight and seminiferous tubule proliferation status

The seminiferous epithelium is highly radiosensitive, studies showing significant apoptosis of spermatogonia following irradiation with low doses as 1 Gy [19]. Although heat shock proteins (HSP) usually fulfil cyto-protective roles, their function in spermatogenesis remains to be clarified [14, 28] and evidence of cyto-protection by HSP induction in the seminiferous epithelium is scarce.

The testicular weight index is sometimes used to estimate seminiferous epithelium status in irradiation experiments [22]. The results were 5.75 ± 0.91 mg/g for controls, 5.01 ± 0.53 mg/g for the 250 mg/kg bw GGA group and 5.52 ± 0.85 mg/g for the 1000 mg/kg bw GGA group. The present study found no significant weight differences between treatment groups and controls (Figure 4).

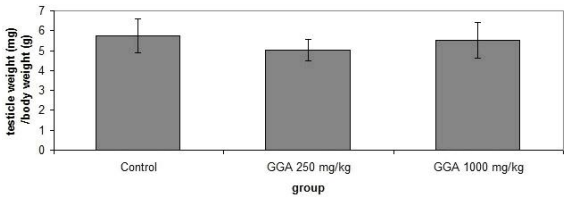

Figure 4.
Testicular index

Histologically, the proliferation status of the seminiferous tubules was significantly higher in the 1000 mg/kg bw treatment group (52.8 ± 11.5% positive), compared with the 250 mg/kg bw treatment group (32.5 ± 14.1% positive) ($p = 0.022$) and controls (23.9 ± 10.4% positive) ($p = 0.0011$) (Figure 5).

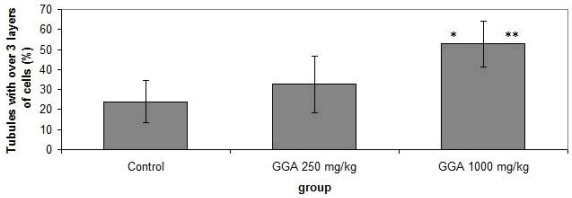

Figure 5.
Seminiferous tubule proliferation status
*p < 0.05 *versus* control; **p < 0.05 *versus* 250 mg/kg bw GGA treatment group

This finding is consistent with a large body of evidence supporting the cytoprotection of various tissues by both GGA [2, 5, 6, 9, 15, 16, 17, 25, 31] and heat shock proteins [8, 20], although there is some debate regarding the effects of heat shock induction on spermatogenesis [21, 28]. Other mechanisms of cytoprotection may be involved, some studies suggesting that GGA inhibits protein 53 (p53) [4], inhibits mitochondrial membrane permeabilization [1] and maintains membrane fluidity [27] independent of HSP.

Conclusions

The results suggest a radio-protective effect of GGA (1000 mg/kg bw) on the murine seminiferous epithelium, shown by the higher proliferation status of the seminiferous tubules. The WBC and PLT counts showed no radio-protective effect of GGA (250 mg/kg bw or 1000 mg/kg bw) in mice.
Whether GGA protects the seminiferous epithelium through HSP induction or another mechanism, whether the heat shock response is beneficial or deleterious to spermatogenesis and what are the hereditary effects of heat shock response modulation during irradiation remain to be clarified in future studies.

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