Radio-Protective Effects of Some bis-Thiosemicarbazone Compounds

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Abstract

Background: GTS, PTSM and ATSM are bis-thiosemicarbazone ligands used in the preparation of copper radiopharmaceuticals. Chemical structure of these materials indicates that they should have radio-protective effects.

Objective: To study the radio-protective effects of GTS, PTSM and ATSM.

Methods: This study has focused on radio-protective effects of these compounds at different doses (20, 40 and 80 µg) and with time intervals of 1 and 4 h before the whole-body gamma-irradiation. The survival curves were plotted for different groups after one month post-irradiation. The effective doses of these compounds were also calculated from the survival study. In the next step, biochemical markers of hepatic function, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined in a hepatotoxicity study.

Results: Administration of ATSM did not cause any serious side effects and hepatotoxicity in a mice model—AST and ALT enzyme levels in a group of animals that received 80 µg of ATSM showed no significant difference with that in the control group. However, AST and ALT enzymes rose significantly in those mice that received 40 µg of GTS compared to the control group even 7 days post-injection.

Conclusion: It seems that ATSM is a better candidate on which to carry out further research for protection against irradiation.

Keywords

Radio-protective agents; ATSM; PTSM; GTS; AST; ALT

Introduction

Radiation therapy is used for curative and palliative purposes [1]. It is estimated that almost 50%–70% of clinical oncology treatments are performed by either radiotherapy alone or a combination of radiotherapy and chemotherapy [2, 3]. Despite all precautions made for adjusting the dosage of gamma ray or electron beams on the target area and making planning treatment schedules for giving an appropriate dose, harmful effects of radiation are inevitable so that even in the most optimistic scenario, the damage to adjacent tissues is seen. Since early years of radiotherapy, researchers have studied utilization of effective agents for decreasing undesirable effects of radiation on living tissues. Such agents include a wide range of chemicals and natural remedies. Since the main cause of damage to cells is the exposure to irradiation that produces peroxide radicals [4], the most important radio-protective agents would be free radical scavengers. There are several compounds with such properties. For example, the following compounds are well known for their free radical scavenging properties.

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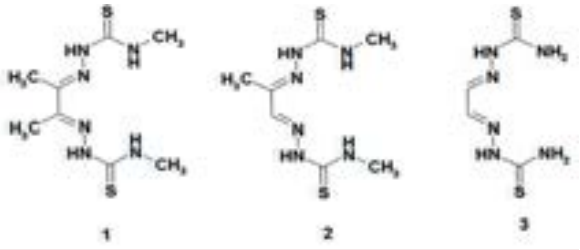

Figure 1: Molecular structure of 1) ATSM, 2) PTSM, and 3) GTS

1. **Vitamins:** vitamins C, A and K are natural source materials in the body with radio-protective effects [5].
2. **Sulfur-containing amino acids:** methionine and cysteine are the two with high radio-protective effects [6].
3. **Plant alkaloids:** these nitrogen-rich compounds have high radical scavenging properties [7].
4. **Ploy-phenyls:** these compounds are naturally abundant in tea and chocolate and have free radical scavenging properties [8].
5. **Sulfur-containing materials:** compounds with high amounts of sulfur such as milk and yoghurt proteins, garlic, onion, and cabbage have radio-protective effects [9].
6. **Some synthetic materials:** the main field of research on synthetic materials is focused on chemical containing electrophile elements such as phosphorus, sulfur, and nitrogen. The most important drug from this group for reducing side effects of radiation is amifostine [10].

According to theoretical studies, most of the above-mentioned agents have free radical scavenging properties due to S- and NH-groups in their structure [5].

Recently, through quality control and the synthesize of drugs and several radio-pharmaceuticals, researchers have studied and produced compounds such as bis-thiosemicarbazone, thiosemicarbazones, thiosemicarbazides, and a variety of hetero-cyclic compounds with thiol amine groups [11]. Considering chemical structures of these materials, they are expected to have degrees of radio-protective effects (Fig. 1).

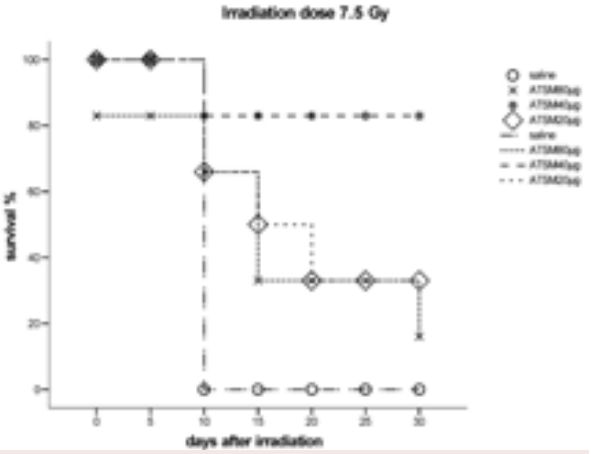

Figure 2: Survival curve for mice receiving different concentrations of ATSM, 1 h before exposure to radiation compared to the control group (n=6)

In this study the newly synthesized bis-thiosemicarbazone derivatives, ATSM, PTSM and GTS were examined for their radio-protective properties in an animal model.

Materials and Methods

The experimental protocol was in accordance with the guidelines for care and use of laboratory animals as adopted by the Ethics Committee of the School of Medicine, Tehran University of Medical Sciences, Tehran, Iran. Syrian male mice with mean weight of 25 g were obtained from Karaj Razi Institute.

Materials and equipment were prepared from radiobiology laboratory of agricultural, medical and industrial research center, Karaj nuclear medicine research and dosimetry and monitoring groups. Thin layer chromatography was carried out using Watman paper 1×1 cm and ammonium stat solvent 10% + methanol (1:1) as the mobile phase to control chemical purity of the mixture. All chemical mixtures were supplied by Aldrich, USA. Moreover, chromatography of marked materials was conducted on thin layer of aluminum-based silica gel (TLC Ready Foils Schleicher and Schuell F 1500/LS 254, 20×20 cm model).

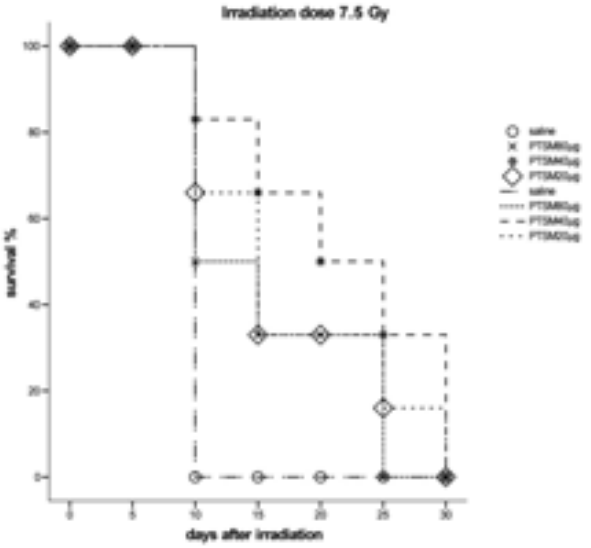

Figure 3: Survival curve for mice receiving different concentrations of PTSM, 1 h before exposure to radiation compared to the control group (n=6)

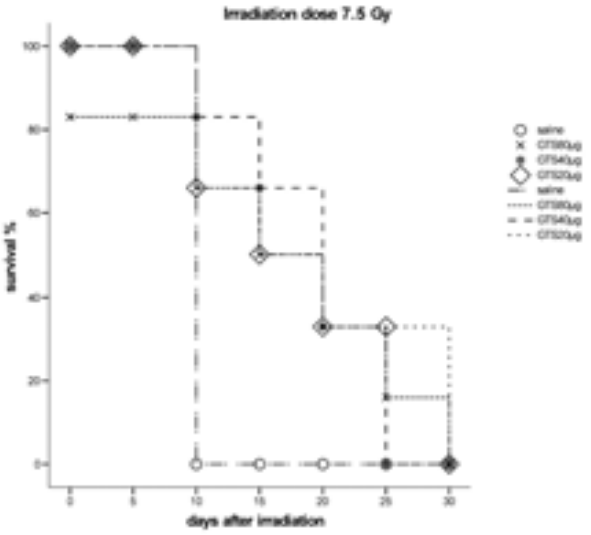

Figure 4: Survival curve for mice receiving different concentrations of GTS, 1 h before exposure to radiation compared to the control group (n=6)

Synthesizing of bis-Thiosemicarbazone Compounds

ATSM, PTSM and GTS compounds were synthesized through the recently reported method [11]. Briefly, pyruvaldehyde solvent was added gradually to N-4 methyl bis-thiosemicarbazone solution in a mixer. Then, ethanol was added and the reaction was cooled, the mixture was then cooled and finally the sedimentation was washed by diethyl ether and 5 mL water afterward. The sediment was then crystallized in hot ethanol; the obtained products were confirmed by spectrometry of HNMR, IR, MASS and mass spectroscopy and melting determination.

Formulation of the Mixture for Administration

Purified powder of crystals was ground to produce finer powder. Considering low solubility of the powder in water, dimethyl sulfoxide (DMSO) was used as solving facilitator. At first, a 1 mg/mL solution was made of each substance in normal saline; then DMSO 5% was added and the mixture was placed in ultrasound bath for 15 min. The obtained clear yellow solution was sterilized immediately by being passed through anti-microbial filter. The solutions were then diluted in normal saline to produce concentrations of 20, 40 and 80 µg in 50 µL volume sucked into sterilized insulin syringes ready for injection. The control mice were only injected with the same volume of normal saline and DMSO (as vehicle).

Survival Study

Before injection, mice were kept in a 12:12 h light:dark cycle for three days in sterilized environment and on a standard diet. Mice where anesthetized by ether and received. intraperitoneal (IP) injection of studied substances. The mice received different doses (20, 40, and 80 µg) of ATSM, PTSM and GTS at either 1 or 4 h before the whole-body gamma-irradiation. In the survival study, we used 6 mice in each group (a total of 108 mice). The whole-body exposure of studied mice was performed by a ^{60}Co gamma source (7.5 Gy) with mice kept in specially designed cages for 40 min 80 cm away from the radiation source with a radiation field of 30×30 cm. The animals were then taken to their cages after exposure to be fol-

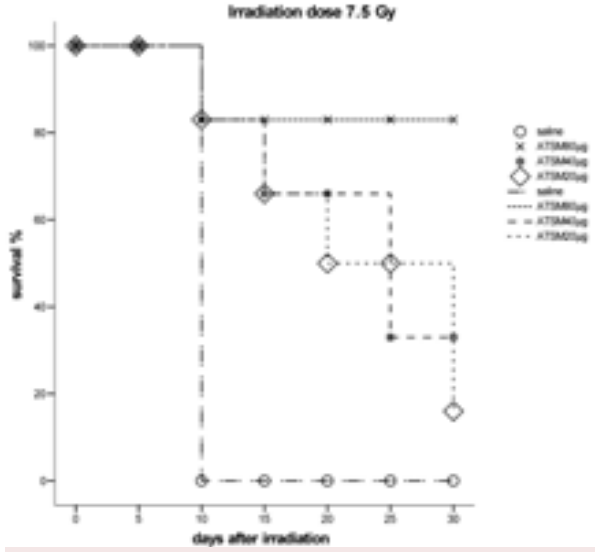

Figure 5: Survival curve for mice receiving different concentrations of ATSM, 4 h before exposure to radiation compared to the control group (n=6)

lowed for a month with access to proper diet and descent lighting.

Hepatotoxicity Tests

To investigate the hepatotoxicity of the studied bis-thiosemicarbazone compounds, the effective doses of radio-protective compounds found from survival study was administered to healthy mice. The studied mice (the study and control groups, n=5) were sacrificed one week after the injection and the whole blood of the samples was collected through aorta. Blood samples were put in heparinized test tubes and immediately transferred to biochemistry reference laboratory. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzymes were measured by colorimetric tests.

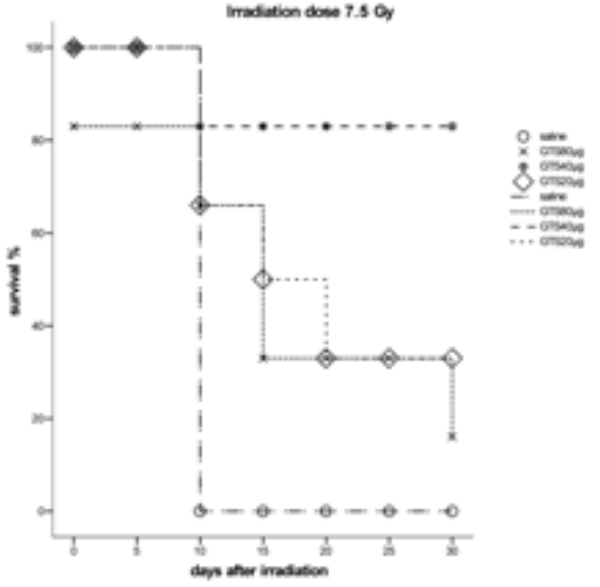

Figure 6: Survival curve for mice receiving different concentrations of GTS, 4 h before exposure to radiation compared to the control group (n=6)

Statistical Analysis

Results are presented as mean±SEM (standard error of the mean) of at least eight animals per group. One-way analysis of variance (ANOVA) was performed to compare means among three or more groups. Tukey's multiple comparison test was used as *post hoc* test. A p value <0.05 was considered statistically significant.

Results

Eighty-three percent of mice that received 40 µg ATSM 1 h before irradiation survived after a month (Fig. 2). For other concentrations of ATSM and different concentrations of other compounds administrated 1 h before irradiation, no significant difference in survival rate was observed (Figs 2, 3 and 4). Eighty-three percent of mice that received 80 µg ATSM or 40 µg GTS 4 h before irradiation survived after a month (Figs 5 and 6). Also, we did not observe any significant survival rate difference for PTSM administration (Figs 3 and 7).

While the serum levels of AST and ALT in the study and control groups that received ATSM were not significantly different, they increased significantly ($p<0.05$) in mice that received GTS (Figs 8 and 9).

Discussion

Ionizing radiation interacts with biological systems to produce free radicals or reactive oxygen species (ROS), which attack various cellular components including DNA, proteins and membrane lipids, leading to serious cel-

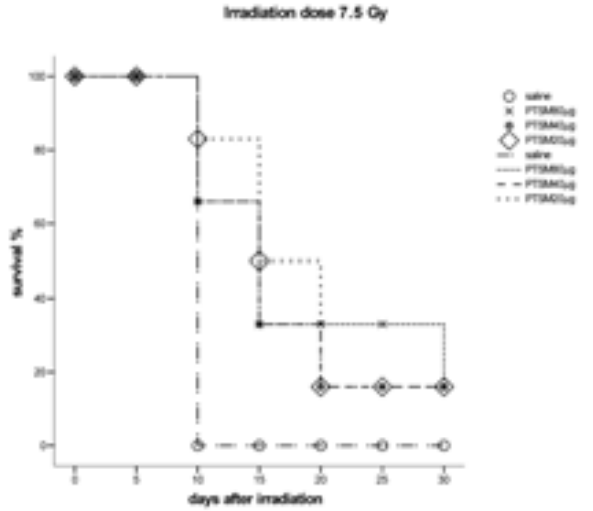

Figure 7: Survival curve for mice receiving different concentrations of PTSM, 4 h before exposure to radiation compared to the control group (n=6)

lular damage [12]. During the last 60 years, researches have extensively studied on radio-protective compounds for protecting healthy tissues during radiotherapy [13, 14]. Therefore, finding new radio-protectors, especially those with low toxicity is of paramount importance [15-17]. To the best of our knowledge, this study is the first of its kind to investigate the radio-protective properties of ATSM, PTSM and GTS.

A dose of 80 µg of ATSM resulted in the highest protective effect observed, however, GTS could do the same at a dose of 40 µg. Since GTS is more hydrophilic than ATSM, it has a higher solubility and distribution in bio-liquids and thus can provide protection at lower doses.

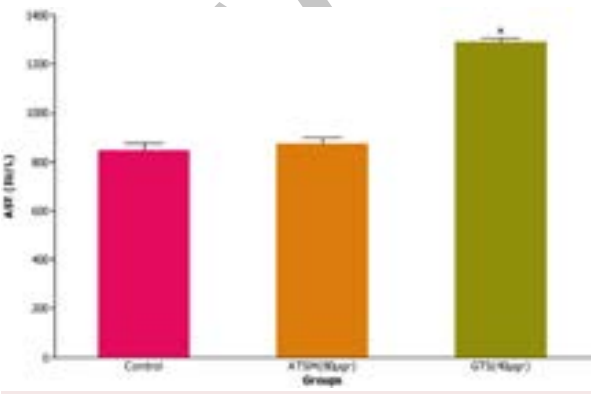

Figure 8: Comparison of mean level of AST in the control group and the mice that received 80 µg of ATSM or 40 µg of GTS, 1 wk after injection. Error bars represent the standard error of the mean; (n=5 animals per group) *p<0.05 (compared to the control group)

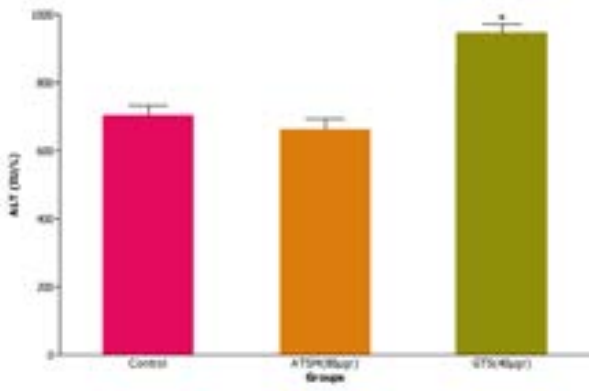

Fig 9: Comparison of mean level of ALT in the control group and the mice that received 80 µg of ATSM or 40 µg of GTS, 1 wk after injection. Error bars represent the standard error of the mean; (n=5 animals per group) *p<0.05 (compared to the control group)

As illustrated in Figures 8 and 9, there was no enzyme rise (hepatotoxicity) after administration of ATSM. However, GTS was clearly hepatotoxic.

In conclusion, we found that GTS and ATSM have considerable radio-protective properties compared to PTSM. With its significant radio-protection and little hepatotoxicity, it seems that ATSM at a dose of 80 µg is a promising candidate for future works in the field of radio-protection.

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