Therapeutic and protective effect of oxymetholone against mortality induced by γ irradiation in mice

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Abstract

Introduction: Oxymetholone can stimulate bone marrow cells and increase the blood cells in the peripheral blood vessels. Previously we showed that administration of oxymetholone as a single dose increased survival rate in mice against a lethal dose of γ irradiation. In this study, we determined the percentage survival rate in irradiated mice treated with oxymetholone at divided low dose and prior and after irradiation.

Material and methods: Oxymetholone at different doses (40, 80, 160 and 320 mg/kg) was administered to mice by gavage with starting time at 24 h before or 1 h after exposure to a lethal dose of γ irradiation for four consecutive days. Mortality was recorded daily for 30 days. The percentage survival rates were compared with two-sample test for proportions.

Results: At 30 days after treatment, the percentage of animal survival in each group was between 15 and 25%, compared to 0% in control-irradiated mice. Administration of oxymetholone at a single dose of 640 mg/kg increased survival rate to 40%. Oxymetholone treatment increased survival rate in mice when administered after irradiation at divided low doses.

Conclusions: Oxymetholone had potential effects on increasing survival rate when administered after γ irradiation in mice at divided low doses. With the therapeutic effects of oxymetholone, it can be used for reducing mortality induced by γ irradiation after exposure to irradiation.

Key words: radioprotective, therapeutics, oxymetholone, survival, radiation.

Introduction

Exposure to elevated ionizing radiation to the whole body causes injury to the haematopoietic system that will be reflected in the peripheral blood cells and platelets [1]. These reduced circulating blood cells can cause septicaemia, haemorrhage, anaemia and death [1-3]. Administration of some compounds before irradiation can protect and reduce side effects induced by ionizing radiation to critical organs including the haematopoietic system. Thiol synthetic compounds which act by a free radical scavenging effect reduced the side effects induced by the reactive substrate in mammalian organs [4, 5]. These agents have limited application due to their toxicity at optimum dose for example in hypotension, vomiting and nausea [6]. These compounds have a protective effect and these are less effective if administered after irradiation. Immunomodulation and proliferation of haematopoietic stem cells is one of the most common strategies for increasing survival rate in mammalian subjects. Agents with this mechanism stimulate the haematopoietic pluripotent cells for production and proliferation and then increase the peripheral blood cells. Cytokines and 5-androstenediol stimulate the haematopoietic system and

reduce side effects and mortality induced by irradiation [1, 7].

Oxymetholone (OXM) is a 17 α-alkylated anabolic-androgenic steroid derived from testosterone. Oxymetholone exhibits higher anabolic activity and lower androgenic activity compared to other testosterone derivatives. This drug can stimulate bone marrow cells and increases blood cells in peripheral blood vessels [8]. Currently, oxymetholone has been approved by the US Food and Drug Administration (FDA) for the treatment of anaemia caused by low red cell production. In addition, acquired or congenital aplastic anaemia, myelofibrosis and hypoplastic anaemias due to the administration of myelotoxic anticancer drugs often respond to this medication [8]. Recently we showed that a single oral administration of OXM at 24 h prior to γ irradiation increased survival rate in mice. We have observed a dose-dependent effect with OXM in protective effect [9]. Oxymetholone increased mainly red blood cells and platelets in peripheral blood vessels [9]. In a previous study, OXM was administered as a single dose prior to exposure to γ irradiation in mice. The administrative effect of OXM after irradiation is unclear. It is difficult to be watchful and monitor the time that irradiation happened, because prediction of accidents is usually impossible. Administration of the drug after irradiation helps this agent to be used for therapeutic effects.

The aim of this study is the administration of OXM at divided low doses prior to and after irradiation to determine optimal oral dose of OXM for survival enhancement in mice exposed to whole-body γ irradiation.

Material and methods

Animals

Eight-week old male NMRI mice (Razi Institute of Iran) weighing 28 ±3 g were used. A standardized pellet diet was given and tap water was *ad libitum*. The animals were housed for one week in a quarantine facility. All of the mice were kept under controlled lighting conditions (light : dark, 12 : 12 h) and temperature (22 ±1°C) in the university animal house.

Oxymetholone

Oxymetholone powder was prepared from Alhavi pharmaceutical company (Iran). This drug was suspended in distilled water containing 0.5% methyl cellulose (MC). This suspended drug was delivered by gavage tube. Mice were fasted for 4 h, then given one gavage of 0.3 ml oxymetholone or vehicle (0.5% methyl cellulose in water).

Irradiation

Whole-body irradiation was performed with a cobalt-60 γ-radiation source (Theratron 780, Canada). Mice were placed in a well-ventilated Perspex box and irradiated in groups of ten mice simultaneously. The source-to-skin distance was 80 cm with a dose rate of 1.35 Gy/min at room temperature (23 ±2°C). After irradiation, mice were placed, five mice to each group, in separated animal cages and transferred to the animal house.

Radioprotective effect of oxymetholone

Mice were divided into three type categories. The mice were administered orally with 0, 40, 80, 160, 320 mg/kg of OXM that started, firstly, at 24 h before and secondly at 1 h after whole-body exposure to 8.2 Gy of γ irradiation, and OXM administration was continued for four consecutive days. Control groups of mice were treated by vehicle and 320 mg/kg of OXM without irradiation for four consecutive days. Thirdly, mice were treated orally with 160, 320, 640 mg/kg of a single dose of OXM at 24 h before whole-body exposure to 8.2 Gy of γ irradiation. A total of 280 mice were used for this survival rate study. Twenty animals were used for each group. Mortality of the animals was monitored daily for 30 days after irradiation. This research was approved by Novin Institute Research committee.

Statistical analysis

The percentage survival of various doses was compared using two-sample test for proportions with Fisher's exact test [10].

Results

Exposure to 8.2 Gy γ irradiation induced mortality. Twenty animals died within 30 days in the non-irradiated control group. Mice treated with OXM had lower mortality induced by radiation. The percentage survival rate in each group was between 15 and 25% at various doses of OXM at 24 h before and 1 h after 8.2 γ irradiation for four consecutive days (Table I). Survival rate was increased in all doses of OXM-treated groups compared to the control irradiated group ($p < 0.05$). We did not observe any dose-dependent effect with OXM treatment on survival rate. Administration of OXM after irradiation was not significant for survival rate in mice compared to before treatment. The lowest and highest doses of OXM were 40 and 320 mg/kg that were administered before and after exposure to a lethal dose of γ irradiation for several consecutive days. The survival rates were not significant in mice treated with OXM at dose 40 mg/kg with other doses up to 320 mg/kg for 4 consecutive days. This result showed that better protection was observed at dose 40 mg/kg than other doses for four consecutive days.

Pre-treatment of mice with OXM at a single dose reduced mortality induced by γ irradiation (Table I).

Table I. Radioprotective effects of oxymetholone against lethality induced by γ irradiation at dose 8.2 Gy in mice

Treatment	Window[1]	Duration administration[2]	Survival of mice at 30 days [%]
Irradiation	–	–	0
40 mg/kg OXM + IR	24 h prior	4 consecutive days	25
80 mg/kg OXM + IR	24 h prior	4 consecutive days	20
160 mg/kg OXM + IR	24 h prior	4 consecutive days	25
320 mg/kg OXM + IR	24 h prior	4 consecutive days	25
40 mg/kg OXM + IR	1 h after	4 consecutive days	15
80 mg/kg OXM + IR	1 h after	4 consecutive days	20
160 mg/kg OXM + IR	1 h after	4 consecutive days	15
320 mg/kg OXM + IR	1 h after	4 consecutive days	20
160 mg/kg OXM + IR	24 h prior	Single	20
320 mg/kg OXM + IR	24 h prior	Single	25
640 mg/kg OXM + IR	24 h prior	Single	40
640 mg/kg OXM	–	Single	100
320 mg/kg OXM	–	4 consecutive days	100

[1]Preirradiation period (in hours) in which the agent was applied, [2]Frequency of time that agent was administered

Maximum survival rate was observed with 640 mg/kg of OXM singly 24 h prior to irradiation (40%). 640 mg/kg enhanced survival rate statistically significantly compared to other doses and time schedules.

Discussion

In this study we showed that administration of OXM by gavage at low dose at divided dose for four consecutive days reduced mortality induced by γ irradiation. Time-dependency of radioprotective effects was not observed with OXM. Oxymetholone significantly increased survival rate when it was administered 1 h after irradiation. This result showed that OXM has therapeutic effects as well as protective effects. Previously we observed protection with oxymetholone when it was administered in a single oral dose at 24 h before exposure to γ irradiation at dose 8 Gy in mice. The survival rate, 30 days after irradiation, in the group treated with 640 mg/kg of oxymetholone, was 75%, vs. 15% in the control group [9]. In this study good protection was observed with administration of OXM at a single dose of 640 mg/kg. These data are in agreement with our old results. In the last study, we observed a higher survival rate compared to this study with OXM, while last study, animal irradiated at dose 8 Gy [9]. In this study we showed that OXM at divided low dose significantly reduced mortality. We have not observed a higher survival rate in animals treated with four consecutive days' treatment. In this study we established that application of OXM at divided low doses or a single dose has the same effect. For example, administration of OXM at a single dose of 320 mg/kg 24 h prior to γ irradiation has a 20% survival rate, which is the same with 80 m/kg for 4 consecutive days before or after irradiation. Therapeutic effect of OXM is the most fruitful result of this study, since OXM reduced mortality when administered after γ irradiation. Amifostine as a good radioprotective agent is not effective if administered after irradiation [11]. Therapeutic effects help to use a drug after radiation accident and reduce mortality induced by a lethal dose of irradiation. Now, cytokines are biological compounds that are used for treatment of side effects induced by γ rays; these agents are administered after irradiation [11, 12]. Unfortunately, some cytokines have disadvantages that limit their use in clinical practice. Also, some of them have adverse side effects, such as proinflammatory activity or immunogenicity. These agents had less of an effect when administered systemically [1].

Oral administration of oxymetholone ameliorated the radiation-induced decrease in circulating platelets and erythrocytes, but had less of an effect on the number of white blood cells (WBC). This drug has advantages, such as: it can be administered orally; it has an extended window of effect and is less toxic [9]. Oral application of OXM before γ irradiation increased RBC up to normal level at 20 days after irradiation. Platelet numbers were elevated after γ irradiation in OXM-treated animals [9]. Although many compounds were tested and proved as radioprotective agents, most of these

compounds are not effective when taken orally, such as amifostine. Oxymetholone is considered as an effective anabolic steroid in eugonadal patients with AIDS-associated wasting. This drug at a 150 mg/kg/day regime is effective in terms of weight gain, body cell mass and lean body mass and is associated with lower liver toxicity [14]. Oxymetholone has been studied extensively for toxicology and carcinogenesis by the National Toxicology Program (NTP) in 1999 [15]. The administration of OXM by gavage has not shown any mortality in a 16-day study in mice at a dose of 5000 mg/kg and in a 14-week study at 2500 mg/kg. All treated mice survived until the end of the study and did not show any mortality [15].

In conclusion, the present study demonstrates that oxymetholone treatment increased survival rate in irradiated mice when administered after irradiation at divided low doses. It has less effect at divided low doses in comparison with a high single dose. The higher survival rate was not obtained in different doses and time schedule administration; this may be due to a threshold effect of oxymetholone for protection against mortality induced by γ irradiation. With the therapeutic effects of oxymetholone, it can be used after exposure to irradiation for reducing mortality induced by γ irradiation.

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