RESEARCH COMMUNICATION

Radioprotective Effects of Troxerutin against Gamma Irradiation in V79 Cells and Mice

Xu Ping[1*], Jia Junqing[1], Jia Junfeng[1], Jiang Enjin[2]

Abstract

The purpose of this study was to determine radioprotective effects of troxerutin. Cell experiments were carried out to test the cytotoxicity of troxerutin on V79 cells and to observe effects on apoptosis caused by 60Co γ rays. A model of 8 Gy ray-caused damage of mice was established to observe the effect that troxerutin has on the physical symptom of irradiated mice and to calculate the 30-day survival rate. It showed that troxerutin had no obvious cytotoxicity at the level of less than 20 μg/ml; but had a redioprotective effect in dose-dependence on viability of V79 cells at the range of 0.2-5 μg/mL irradiated by 5 Gy ray of 60Co γ ray. After the 8 Gy irradiation, the mice lost some weight, were dried up in fur and feather, low spirit, awkward in movement, shrinking in body and handicapped in sight, while mice with troxerutin were much better. So it was clear that troxerutin could increase the 30-day survival rates of irradiated mice dramatically. These results collectively indicate that troxerutin is an effective radioprotective agent.

Keywords: Troxerutin - cell survival - apoptosis rate - survival rate- radioprotection - V79 cells

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Introduction

Radiotherapy, which is a chief modality to treat cancer, faces a major drawback because it produces severe side effects generated as a result of damage to normal tissues (Maunch et al., 1995). The design of strategies capable of protecting normal host tissues from the lethal actions of radiation without compromising their anticancer activity is of considerable interest in radiation medicine (Umadevi, 1998; Nair et al., 2001). The development of effective, inexpensive, and nontoxic radioprotective agents is still an active area of research (Weiss, 1997; Dumont et al., 2010). At present, however, the list of agents endowed with such properties remains rather limited (Hosseinimehr, 2007; Weiss and Landauer, 2009). So it is necessary to find more agents that can be used as good radioprotectors during radiotherapy.

The flavanoid derivative troxerutin {2-[3,4-bis(2-hydroxyethoxy)phenyl]-3[(6-deoxy-α-L-manno-pyranosyl)-β(-D-glucopyranosyl)-oxy]-5-hydroxy-7-(2hydroxyethoxy)-4H-1-benzo-pyran-4-one} has been used therapeutically for treating chronic venous insufficiency (CVI) (Biosseau et al., 1986; Auteri et al., 1990; Vin et al., 1992; Wadworth and Faulds, 1992; Rehn et al., 1993; Vin et al., 1994; Boisseu et al., 1995; Incandela et al., 1996;), varicosity(Schuller-Petrovic et al., 1994), and capillary fragility (Krupinski et al., 1988; Glacet-Bernard et al., 1994; Gueguen-Duchesne et al., 1996). It has anti-erythrocytic, antithrombotic, fibrinolytic (Boisseu et al., 1995), odemaprotective (Vanscheidt et al., 2002), and rheological activity (Biosseau et al., 1986; Vin et al., 1992). Troxerutin scavenges oxygen-derived free radicals (Blasi et al., 1987; Blasig et al., 1988; Wenisch, 2001; Kessler et al., 2002). It has been reported that during radiotherapy of head and neck cancer, the administration of a mixture of troxerutin and coumarin offers protection to salivary glands and mucosa(Grotz et al.,1999). The structure of troxerutin is given in Figure1. The present study focuses on the effect of troxerutin on gamma-radiation-induced cell damage and survival rate in mice.

Materials and Methods

Animals

Male BALB/C mice (4–6 weeks old, weighing l8–22 g) were purchased from the Experimental Animal Center of Military Medical Sciences. Animals were housed at 10 per cage with ad libitum access to water and food pellets.

Drugs and reagents

Troxerutin (Institution of Pharmaceutical and Biological Products, China, Lot :100416 -201004); Cell Counting Kit-8 (CCK-8) was manufactured by Dojindo Laboratories (Kumamoto, Japan); "523" was obtained from Institute of Radiation Medicine(Beijing, China); Binding buffer was from an Annexin V-FITC apoptosis detection kit (Baosai Biotechnology Co., Ltd /China).

Cell culture

V79 Chinese hamster lung fibroblast cells were

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Table 1. Experimental Design

Drug delivery	Time (days)	Positive drug dose (agent "523")	Doses of irradiation (Gy)	Dose of troxerutin (mg/kg)
Before irradiation	624 h;	5 mg/kg	8	5 mg/kg 10 mg/kg 20 mg/kg

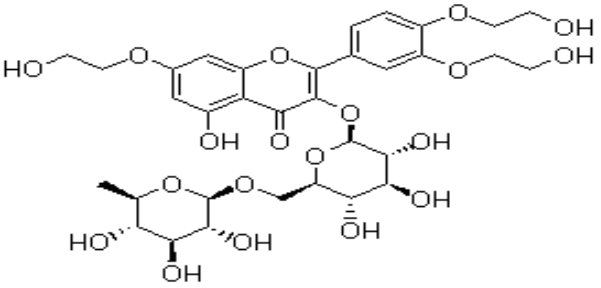

Figure 1. Structure of Troxerutin

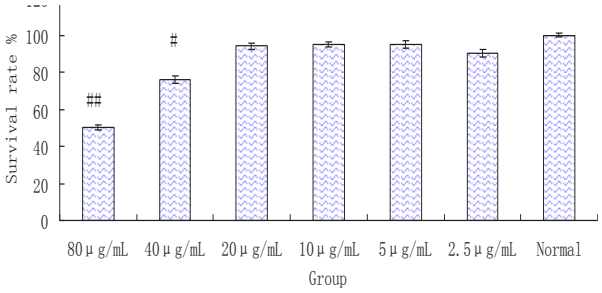

Figure 2. Cytotoxicity of Troxerutin on V79 Cells (##, $P < 0.01$; #, $P < 0.05$ compared with normal group)

cultured adherently in DMEM medium containing 10% fetal bovine serum, 100 U/mL penicillin and 100 U/mL streptomycin at 37°C in a humidified atmosphere of 5% CO_2.

Irradiation

Cells were irradiated at room temperature with 60Co γ-rays at a dose rate of 227.7 cGy/min for a total dose of 5 Gy. The animals were restrained in holders and exposed to 8 Gy total-body 60Co γ radiation at a dose rate of 228.27 cGy/min to determine their 30-day survival rate.

Detection of cell toxicity

100 μL/well of V79 cells (1×10^5 cells/mL) in logarithmic growth phase were seeded in 96-well plates containing 100 μL/well of drug at a final concentration of 2.5, 5, 10, 20, 40, and 80 μg/mL (referred to as the drug groups). The drug groups were compared with control unirradiated cells and with irradiated, untreated cells. CCK-8 solution 10 μL was added to each well at 48 h after drug intervention. The absorbance of each well at 490 nm was determined after a further 4 h, using a multifunctional microplate reader (Xu et al., 2003).

Radioprotective effects of different concentrations of troxerutin in V79 cells

V79 cells (1×10^5 cells/mL) in logarithmic growth phase were seeded in 96-well plates containing 100 μL/well of drug at final drug concentrations of 0.4, 0.8, 1.6, 3.2, 6.4, and 12.8 μg/mL(referred to as the drug groups). These were compared with control unirradiated cells and with irradiated, untreated cells. After 24 h, cells were irradiated with 60Co at a dose of 5 Gy. CCK-8 solution 10 μL was added to each well at 24 h after irradiation. The absorbance of each well was determined after a further 4 h, using a multifunctional microplate reader at 490 nm.

Detection of apoptosis by Annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) double-staining method

V79 cells (1×10^6 cells) in logarithmic growth phase were seeded in 6-well culture plates at a drug concentration of 6.4 μg/mL, according to the results of the experimental screening. After 24 h, cells were irradiated with 60Co at a dose of 5 Gy. Cells from the normal (unirradiated), irradiated (untreated), and drug groups were collected at 24 h after irradiation and centrifuged at 1,000 rpm for 10 min. The supernatant was removed, and cells were resuspended in 200 μL binding buffer after washing twice with pre-cooled phosphate-buffered saline. Annexin V-FITC 10 μL was added to the cells. The cell suspension was gently mixed and incubated in the dark for 15 min at room temperature. A total of 300 μL binding buffer and 5 μL PI were added, and the samples were analyzed by flow cytometry.

Determination of 30-day survival rate

Mice adapted to the environment were divided into normal (unirradiated), irradiated (untreated), positive control (treated with the known radioprotective agent "523"), and drug treatment groups. All drugs were administered orally. Mice in the normal and irradiation groups were given distilled water, and mice in the treatment groups received the appropriate drugs, as indicated in Table 1. All mice were observed for 30 days after irradiation.

Statistical analysis

Data were expressed as mean ± standard deviation (SD). The significance of differences between mean values for groups were analyzed using SPSS-16 (IBM in USA). The means of the treated groups were compared with those of the radiation-alone and unirradiated groups. A value of $P < 0.05$ was considered to be statistically significant.

Results

Detection of cell toxicity

The cell toxicities of troxerutin in V79 cells were tested. The survival rates at 48 h after drug intervention are shown in Figure 2. Troxerutin had no significant cytotoxicity in 2.5-20 μg/mL.

Radioprotective effects of different concentrations of troxerutin in V79 cells

The effects of different concentrations of troxerutin on the survival of V79 cells were examined. The survival

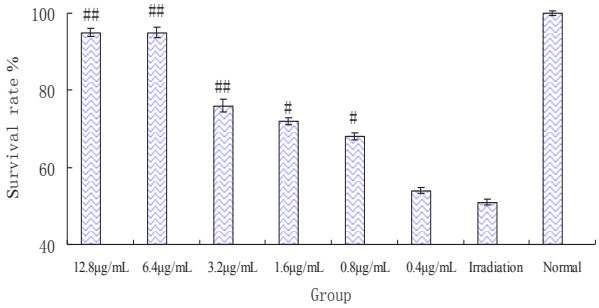

Figure 3. Survival Rates of V79 Cells Treated with Troxerutin of Different Concentrations (#, P< 0.05, ##, P < 0.01 compared with irradiation alone

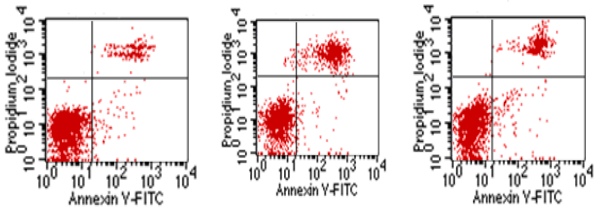

Figure 4. Apoptosis Rates in V79 Cells Treated with Normal Irradiation and Troxerutin (troxerutin, P < 0.01, compared with irradiation group)

rates at 24 h after irradiation are shown in Figure 3. The survival rates of the irradiated cells gradually increased with increasing concentrations of troxerutin from 0.4-6.4 μg/mL. The survival rate was highest at a concentration of 6.4 μg/mL, and this was therefore recommended as the final concentration.

Detection of apoptosis by Annexin V-FITC/PI double-staining method

The effects of troxerutin on apoptosis was investigated. The apoptosis rates at 24 h after irradiation are shown in Figure 4. Irradiation increased the apoptosis rate to 37%, while pretreatment with troxerutin before 5 Gy irradiation significantly reduced the rate of apoptosis to 12%, compared with the irradiation group.

Observation of 30-day survival rate in mice

The 30-day survival rates of mice are shown in Figure 5. Pretreatment with troxerutin of 5 mg/kg, 10 mg/kg, 20 mg/kg improved the survival rates by 20%, 40% and 10% respectively compared with untreated irradiated animals. These results indicate that pretreatment with troxerutin is needed to realize its radioprotective activity at the dose of 10 mg/kg .

Discussion

With the modernization of science and technology, nuclear power was widely applied to manufacturing, research, medical treatment and people's daily life. According to a report, about 70% tumour suffers were taken radiotherapy, but radiotherapy damaged normal tissues of the organism while killing tumour cells. So radiotherapy workers would suffer phototoxis in some extent. Though providing us convenience, radiotherapy also brought more and more harmful influence.

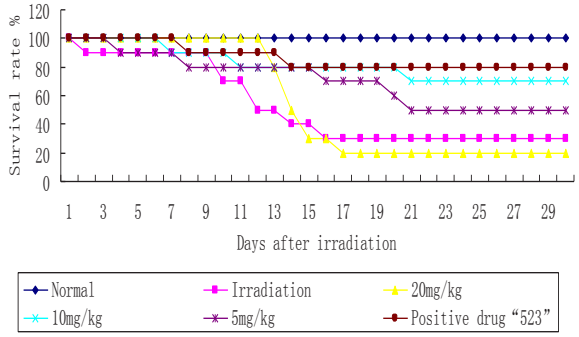

Figure 5. 30-day Survival Rates of Mice Pretreated with Troxerutin. Pretreatment with troxerutin of 5 mg/kg, 10 mg/kg, 20 mg/kg improved the survival rates by 20%, 40%, and 10% respectively, compared with untreated irradiated animals. These results indicate that pretreatment with troxerutin is needed to realize its radioprotective activity at the dose of 10 mg/kg

Therefore, it is important to develop anti-radiation medicine. The research on ionizable radioprotectant started at the early 20th century. Until now, many effective chemical medicines and biological pharmaceutics have been found, including WR-2721 (amifostine), superoxide dismutase (SOD), medal chelate Cu, Fe, Zn), potassium iodide, vitamin (Vc, Ve) and cellular factor medicine (G-CSF, IL-6, EPO, KGF etc).

Troxerutin is the most active component of hydroxide rutin. It had been found that troxerutin could cure venous disease (Rehn et al., 1996). Later it was also found that troxerutin had effects of anti-platelet aggregation and anti-thrombosis (Hladovec, 1986), so it was effective to the sequela of apoplexia and was still widely used clinically. In recent years, troxerutin was applied into the treatment of microangiopathy(Cesarone et al., 2003; Cosarone et al., 2003) and spread in clinical researches further by overseas doctors. Researches of the protective action of troxerutin on damaged DNA were carried out by some scholars with comet winding-up technology (Dharmendra et al., 2004). The aim of this research is to make a further study of the redioprotective effects of troxerutin by experiments in cells and mice.

Exposure to ionizing radiation significantly decreased cell survival and increased apoptosis rates in irradiated cells in dose-dependent manners. Troxerutin had no obvious cytotoxicity at the level of less than 20 μg/ml; but had a redioprotective effect in dose-dependence on viability of V79 cells irradiated by 5 Gy ray of 60Co γ ray at the range of 0.2-5 μg/mL. Similarly, exposure of mice to radiation at 8 Gy also had adverse effects, and pretreatment with troxerutin increased the survival rate to 70%, compared with 30% in the untreated irradiation group. And further studies are needed to investigate the potent radioprotective effects of troxerutin.

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