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Short Communication

Amifostine ameliorates recognition memory defect in acute radiation syndrome caused by relatively low-dose of gamma radiation

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This study examined whether amifostine (WR-2721) could attenuate memory impairment and suppress hippocampal neurogenesis in adult mice with the relatively low-dose exposure of acute radiation syndrome (ARS). These were assessed using object recognition memory test, the terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling assay, and immunohistochemical markers of neurogenesis [Ki-67 and doublecortin (DCX)]. Amifostine treatment (214 mg/kg, i.p.) prior to irradiation significantly attenuated the recognition memory defect in ARS, and markedly blocked the apoptotic death and decrease of Ki-67- and DCX-positive cells in ARS. Therefore, amifostine may attenuate recognition memory defect in a relatively low-dose exposure of ARS in adult mice, possibly by inhibiting a detrimental effect of irradiation on hippocampal neurogenesis.

Keywords: acute radiation syndrome, amifostine, hippocampus, memory impairment, neurogenesis

Clinical interest in the consequences of acute full-body radiation exposure as a result of a nuclear accident or significant partial-body irradiation by radiotherapy is increasing. People who experience a single exposure to a broad dose range of ionizing irradiation are described as having acute radiation syndrome (ARS) [1]. The primary consequence of ARS is a transient or permanent depletion of proliferating stem cells and/or immature progenitor cells in specific tissues [5].

Irradiation exposure of the adult brain results in variable degrees of cognitive impairment, even though less histological injury is apparent [5]. The cognitive deficits are associated with inhibition of adult hippocampal neurogenesis in experimental animals [5]. The rate of neurogenesis may be altered by several factors including age, genetic influence, chemicals, and radiation [4-6].

Amifostine (WR-2721) is a thiophosphate prodrug that is converted into the radioprotective thiol, N-2-mercaptoethyl-1,3-diamino propane (WR-1065) by the action of membrane-bound alkaline phosphatase [9]. Amifostine may have free radical scavenging properties [7].

The aim of this study was to analyze whether amifostine could attenuate recognition memory impairment and inhibition of hippocampal neurogenesis in adult mice with a relatively low-dose exposure ARS.

Forty-one male, 8-week-old ICR mice (Orient Bio, Korea) were used in this experiment. All experimental procedures were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals at Chonnam National University.

To observe the effect of amifostine (214 mg/kg, i.p. 30 min before irradiation) on the recognition memory defect in ARS, the experimental groups of mice (n = 7 in each group) were sham irradiated (0 Gy; control) or were whole-body irradiated with 2 Gy ^{60}Co gamma-rays using a Gamma-cell Elan 3000 (Nordion International, Canada) at a dose-rate of 3.1 Gy/min. An object recognition memory test representing a hippocampus-dependent learning paradigm was performed as previously described [5].

To discern the effect of amifostine on apoptotic cell death and the inhibition of neurogenesis in the adult hippocampus with ARS, mice (n = 4 mice/group) were sham irradiated (control) or whole-body irradiated with 0.5 or 2 Gy. The mice were sacrificed 12 h after irradiation, and the brain were then dissected from each mouse.

Five micron-thick coronal sections of paraffin wax-embedded brain were deparaffinized and allowed to react with immunohistochemical markers for neurogenesis

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using a monoclonal rabbit anti-Ki-67 antibody (DRM004; Acris Antibodies GmbH, Germany) and polyclonal rabbit anti-DCX antibody (Cell Signaling Technology, USA), as previously described [5].

The number of cells showing specific characteristics of proliferating cells (immunopositive for Ki-67) and immature progenitor cells (immunopositive for DCX) in the hippocampi was scored by an observer blinded to the identity of the sample using a histomorphometric approach [5]. The level of DNA fragmentation was detected by *in situ* nick end-labeling (terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling; TUNEL) using an ApopTag *in situ* apoptosis detection kit (Millipore, USA) according to the manufacturer's instructions.

The data is reported as mean ± SE and analyzed using one-way analysis of variance followed by a Student-Newman-Keuls post hoc test for multiple comparisons. In all cases, a p value < 0.05 was considered significant.

In the object recognition memory test, the sham controls, vehicle-treated mice, and amifostine-treated mice displayed an equal preference to the two objects during training (1 day after irradiation, Fig. 1A). During testing (1 day after training), vehicle-treated mice showed memory deficits in the object recognition memory test ($p < 0.05$ vs. sham

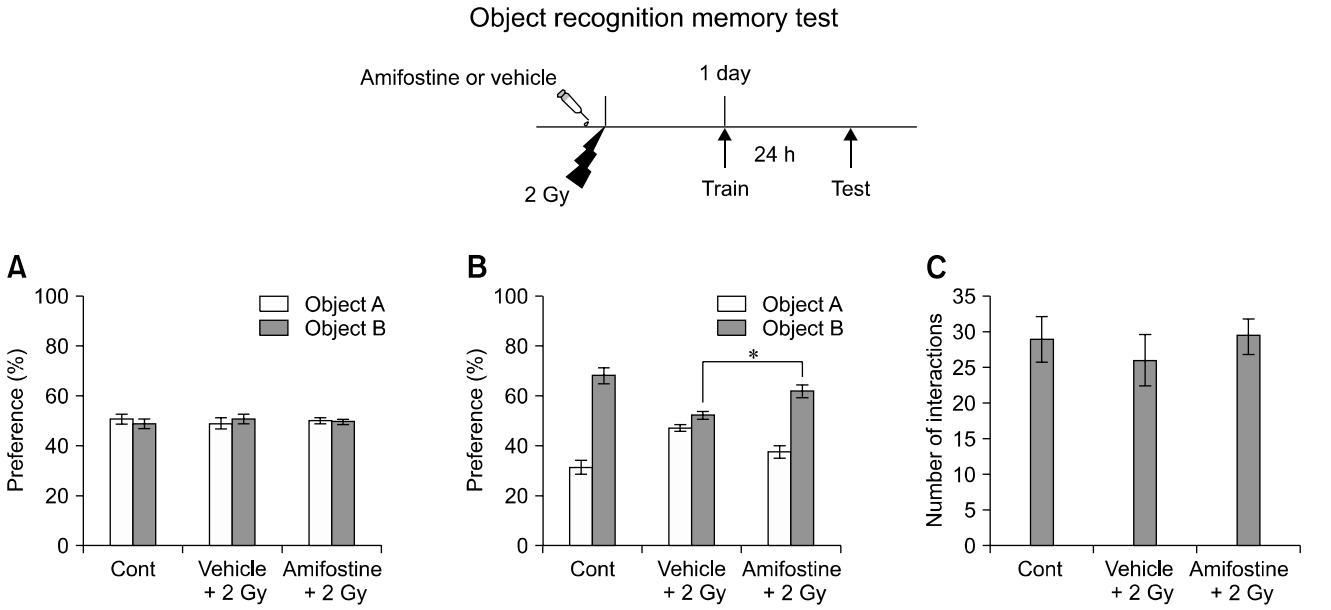

Fig. 1. Pretreatment of amifosine significantly attenuates object recognition memory defects in mice with acute radiation syndrome (ARS). The sham controls (sham irradiation of 0 Gy), vehicle controls (vehicle + 2 Gy), and amifostine-treated mice (amifostine + 2 Gy) were examined (n = 7 for each group). *$p < 0.05$.

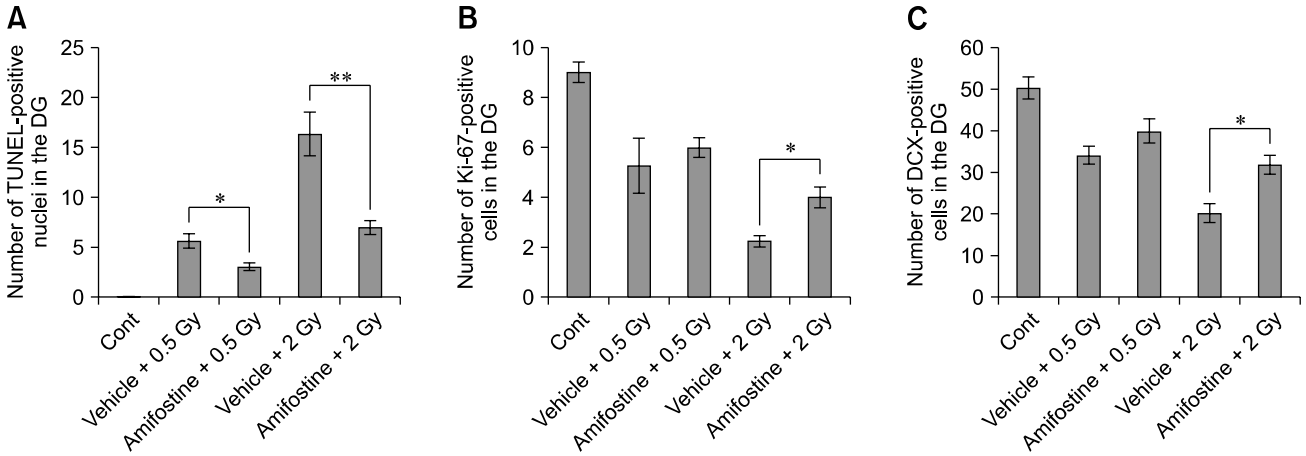

Fig. 2. Histological analyses for the sham controls (12 h after sham irradiation of 0 Gy), vehicle-treated, and amifostine-treated mouse hippocampe 12 h after gamma irradiation of 0.5 and 2 Gy. *$p < 0.05$, **$p < 0.01$.

controls), whereas amifostine-treated mice did not (Fig. 1B; $p < 0.05$, amifostin-treated group vs. vehicle- treated group). There was no significant difference in the total number of interactions during training between the three groups (Fig. 1C), suggesting comparable levels of attention, motivation, and visual perception.

TUNEL-positive apoptotic cells were measured 12 h after irradiation (Fig. 2A). In vehicle-treated mice, the number of TUNEL-positive cells in the hippocampus significantly increased in mice irradiated with gamma ray doses of 0.5 Gy and 2 Gy. However, pretreatment with amifostine markedly blocked the number of apoptotic nuclei in mice irradiated with 0.5 Gy ($p < 0.05$ vs. vehicle-treated mice) and 2 Gy ($p < 0.01$ vs. vehicle-treated mice).

Immunhistochemically, Ki-67- (Fig. 2B) and DCX-positive cells (Fig. 2C) were constitutively expressed in the DGs of adult hippocampi in control mice. Ki-67-positive cells significantly declined in the hippocampus 12 h after irradiation with gamma ray doses of 0.5 Gy and 2 Gy. DCX-positive cells significantly declined in the hippocampus 12 h after irradiation with 0.5 Gy and 2 Gy. However, amifostine pretreatment markedly increased the number of Ki-67-positive cells ($p < 0.05$ vs. vehicle-treated mice) and DCX-positive cells ($p < 0.01$ vs. vehicle-treated mice) only in mice irradiated with 2 Gy (Figs. 2B and C).

There is consensus in the literature the inhibition of neurogenesis in the DG of the adult hippocampus is associated with cognitive impairment, with progenitor neural cells being particularly vulnerable to irradiation [8,10]. Therefore, it is important to clarify which radioprotective agent is effective in rescuing neural stem/ progenitor cells in the adult hippocampus from ARS.

Radiation injury in specific organs is initiated by DNA damage and oxidative stress involving reactive oxygen species (ROS) [10]. Therefore, substances capable of decreasing the production of ROS or scavenging excess ROS have gained interest in recent years [10]. Amifostine specifically protects normal tissues from damage caused by radiation and chemotherapy [11]. *In vitro* and *in vivo* studies of developing cerebellar granular cells showed that amifostine suppressed radiation-induced cell death [2,3]. In this study, amifostine can significantly block the radiation-induced decrease of hippocampal neurogenesis in adult mice, suggesting amifostine protects progenitor neural cells in the adult hippocampus from ionizing radiation *via* a cytoprotective mechanism that includes free radical scavenging.

Therefore, we conclude that amifostine attenuates radiation-induced recognition memory dysfunction in adult mice, possibly by inhibiting the detrimental effect of irradiation on hippocampal neurogenesis.

Acknowledgments

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