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0014-4754/90/070704-05$1.50 + 0.20/0

Increase in the survival time of mice exposed to ionizing radiation by a new class of free radical scavengers

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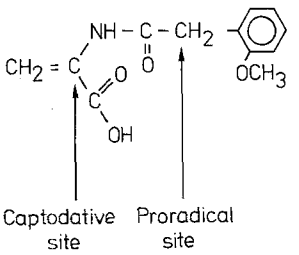
Received 17 July 1989; accepted 23 January 1990

Summary. N-acyl dehydroalanines react with and scavenge mainly superoxide radical ($O_2^{\cdot-}$) and hydroxyl radical ($HO^{\cdot}$). The ortho-methoxyphenylacetyl dehydroalanine derivative, indexed as AD-20, protects mice against damage resulting from total body X-irradiation, as measured by the increase in their survival time. AD-20 increases the LD_{50} at 30 days from 6.1 to 7.3 Gy in animals exposed to a wide range of X-rays (6 to 10 Gy). The dose reduction factor (D R F) of AD-20 is 1.20. We postulate that such radioprotective effect may result from its free radical scavenging activity.
Key words. Oxygen-derived free radical scavenger; N-acyl dehydroalanines; ionizing radiation toxicity; radioprotective effect.

Ionizing radiation, through production of oxygen radical species, can result in DNA damage, especially by forming thymidine hydroperoxide [1]. Moreover, ionizing radiation may overwhelm the balance between the enzymatic protective defense and the production of endogenous oxidizing species such as superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($HO^{\cdot}$) and singlet oxygen ($^1O_2$), leading to a metabolic amplification of the initial physico-chemical damage.

Recently a new way to modulate the reactivity of free radicals has been proposed [2]. Olefins which are substituted at the geminal carbon atom by both an electron-donating and an electron-withdrawing group (captodative substitution), have been shown to inactivate free radicals by forming stabilized free radical adducts, which do not polymerize but disappear mostly by dimerization or by reacting with another free radical [3]. Among these molecules, we have reported that the N-acyl dehydroalanines (indexed as AD compounds) react with and scavenge $O_2^{\cdot-}$ and $HO^{\cdot}$ [4,5]. They inhibit both in vitro and in vivo processes mediated by free radicals [6–8]. AD compounds inhibit lipid peroxidation initiated when rat liver microsomal suspensions are exposed to gamma rays [9], showing that they may give protection against the deleterious effects of ionizing radiation.

In the present work we examined the capacity of AD 20 (see structure of (ortho-methoxyphenylacetyl)-dehydroalanine) to inhibit in vivo radiation damage. The parameters observed in order to analyse the radioprotective effect of AD-20 were the percentage of survivors and the survival time of animals exposed to a total body X-ray irradiation.



Structure of AD-20.

Materials and methods

Animals. Female NMRI mice, weighing approximately 25 g, were obtained from Animalerie Centrale – UCL. They were housed in groups of 10 in plastic cages ($40 \times 25 \times 15\ cm^3$) and fed with standard food (AO4, UAR, France) and tap water ad libitum.

Irradiation procedures. Mice were exposed in groups of 10 to 250 kV X-rays (Phillips, RT 200/250) while restrained in perforated lucite cages placed on a rotatory disk at 60 cm from the source. The disk rotated in a horizontal plane during exposure. The filtration was 1.0 mm of copper, and the dose rate was 0.6 Gy/min. Animals were observed during a period of 30 days, and mortality was recorded daily. Mice were not left to die spontaneously; when they had become paralysed and

were near to death, they were sacrificed by cervical dislocation and recorded as dead the next day.

Experimental protocol. AD-20 was synthesized by Prof. Viehe at Louvain-la-Neuve, and it was administered i.p. to animals as a suspension in water by using 2% gum arabic as vehicle. It was injected at the indicated dosage 15 min before irradiation. Each experimental group consisted of 10 female mice, and experiments were performed at least 3 times. Animal survival data were pooled from separate experiments. The parameters observed were the number of survivors at the end of the experiment (day 30), and the mean survival time (MST), as described by Geran et al.[10]. The dose reduction factor DRF) of AD-20 was calculated by using the ratio of the values for LD_{50} at 30 days for control and AD-pretreated groups as decribed by Petkau and Pleskach[11].

Analysis of data. Statistical analysis of the results was performed with the analysis of variance (Anova) two ways with interaction dose/treatment. For statistical comparisons at a given dose, data were analysed by Student's t-test. The level of significance was set at $p < 0.05$. The computing of each LD_{50} was performed by probit analysis[11] using the Proc. Probit Log 10 computer program from SAS Institute Inc. (NC 27 511, USA, 1986).

Results and discussion

Survival data for mice after receiving a dose of 6.5 Gy X-rays is shown in table 1. When animals were pretreated with a single i.p. injection of AD-20 at different doses, a statistically significant radioprotective effect was observed at doses higher than 400 mg/kg ($p < 0.01$). Experimental evidence shows that the toxicity of ionizing radiation is mainly mediated by free radical species, especially the oxygen-derived free radicals[12-14]. Previous reports have shown that AD compounds inhibited two free radical-mediated processes, the degradation of deoxyribose in water[4] and lipid peroxidation in rat liver microsomal suspensions[9] by ionizing radiation (gamma rays). Moreover, AD compounds have been shown to protect in vitro M3-1 cells (Chinese hamster) irradiated under hypoxic conditions (unpublished results), suggesting that such protection is obtained by a mechanism which excludes the induction of a local hypoxia. Thus, the decreased radiation-induced mortality in AD-20 pretreated groups reflects a radioprotective effect which could be related, at least to some extent, to the inactivation of oxygen radicals resulting from the free radical scavenging activity of AD-20.

Table 1. Effect of increasing doses of AD-20 on survival of mice after receiving 6.5 Gy of X-rays

Treatment	Dose (mg/kg)	Survival (%)	MST (days)
Control	---	37	19.7 ± 0.2
AD-20	50	46	21.9 ± 0.8
AD-20	100	46	21.3 ± 0.9
AD-20	200	40	21.2 ± 0.3
AD-20	400	80	26.5 ± 0.7*
AD-20	500	90	27.5 ± 1.1*

Animal survival was followed for 30 days, and mortality was recorded daily. AD-20 was administered i.p. 15 min before X-irradiation. The control group received gum arabic i.p. Each experimental group consisted of 10 female mice, and the experiment was performed 3 times.
MST, mean survival time. Values are means ± SD from 3 separate experiments.
* Statistically significant difference from control values ($p < 0.01$).

Table 2. Effect of AD-20 on survival of mice after receiving increasing doses of X-ray

Dose (Gy)	MST (days) Control	AD-20 (50 mg/kg)
6.0	24.9 ± 1.7	29.5 ± 0.3
6.5	18.4 ± 1.7	27.4 ± 1.2
7.0	13.6 ± 1.7	25.1 ± 1.8
7.5	11.1 ± 0.6	19.3 ± 2.3
8.0	11.6 ± 0.6	14.6 ± 2.2
10.0[a]	7.5 ± 0.9	11.4 ± 1.5

NMRI mice were given an i.p. injection, 15 min before irradiation, of either gum arabic or 500 mg/kg b.wt of AD-20. Fifty female mice were used for doses of X-rays varying from 6 to 8 Gy, whereas 30 animals were used for the dose of 10 Gy. MST mean survival time. Values are means ± SD from 5 separate experiments. [a]Values are means ± SD from 3 separate experiments. ANOVA test: 2-factors interactions (dose, treatment), sig. level = 0.0482.

In the presence of molecular oxygen, water radiolysis generates mainly O_2^- and $HO^{\cdot}$[15]. Therefore, we hypothesized that the administration of AD-20 to mice before they were exposed to X-rays may inactivate these free radicals, and in this way it protects mice from damage due to ionizing radiation. The data presented in tables 1 and 2 support such a hypothesis; AD-20 increased the survival time of mice after receiving 6.5 Gy of X-rays, this effect being dependent of the dose of AD-20 administered. A radioprotective effect of AD-20 administered at one dose (500 mg/kg) was also observed when increasing the X-ray doses (from 6 to 10 Gy). In control animals the LD_{50} at 30 days was 6.1 Gy (5.94–6.33 Gy, 95% confidence limits). In AD-20-treated mice it was 7.3 Gy (6.99–7.64 Gy, 95% confidence limits), ($p < 0.01$). The treatment with AD-20 increased the LD_{50} with a dose reduction factor (DRF) of 1.2. When compared to AD-20, other structurally related dehydroalanines have been shown to increase significantly the survival time of animals exposed to 7.0 Gy of X-rays (data not shown).

Total body exposure to ionizing radiation, as well as treatment with anticancer drugs, are followed by a decrease of peripheral white blood cells[16]. The radioprotection produced by AD-20 (table 2) is obtained with doses known to affect hematopoiesis (6–8 Gy). Furthermore, it has been shown that AD-20 produces a fast recovery in mouse peripheral leukocytes after depletion induced by acute doses of doxorubicin[7]. These results suggest that the protective effect of AD-20 may be explained to some extent by the protection of bone marrow cells.

The available data suggest that AD-20 inactivates the oxygen-derived free radicals generated during water radiolysis (and probably the secondary carbon-centered free

radicals), and in this way it stops the free radical cascade which leads to radiation damage.

Acknowledgments. The authors thank I. Blave for her excellent technical assistance.

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0014-4754/90/070708-03$1.50 + 0.20/0

Possible involvement of indolamines in the glycogenic effect of the convulsant methionine sulfoximine in rat brain

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Received 16 October 1989; accepted 23 January 1990

Summary. The aim of the present investigation was to look for the mechanisms causing disturbances in carbohydrate metabolism during the action of the epileptogenic agent methionine sulfoximine. The levels of glucose, glycogen, and indolamines were measured in seven different regions of rat brain. Methionine sulfoximine induced a decrease in serotonin level which was roughly dose-dependent. There were no obvious changes in tryptophan and 5-hydroxyindoleacetic levels in any area. Methionine sulfoximine induced the known increase in glucose and glycogen levels. The direct precursor of serotonin, 5-hydroxytryptophan, and benserazide (a decarboxylase inhibitor) were then injected into rats in association with methionine sulfoximine. In this case, methionine sulfoximine failed to induce seizures. Moreover, the serotonin level was unchanged and the carbohydrate content did not significantly increase. There was only a rise in 5-hydroxyindoleacetic acid level. This work shows a striking parallelism between serotonin decrease and glycogen increase.
Key words. Methionine sulfoximine; epileptogenesis; serotonin; glycogen; glucose.

Methionine sulfoximine (MSO) is a potent epileptogenic agent in a variety of laboratory animals. Its particular interest is the existence of a long period of latency before the seizure onset. MSO has been described as a glycogenic agent in the central nervous system of rodents during the preconvulsive, convulsive, and post-convulsive periods [1 – 3]. In our laboratory, we looked for the mechanism responsible of glycogen accumulation in brain induced by this convulsant. We found that MSO increased the activity of the glycogenic enzyme fructose-1,6-diphosphatase [4]. The quantity, the de novo biosynthesis, and the immunostaining of this enzyme notably increased under the influence of MSO [5, 6]. Since glycogen particles and fructose-1,6-diphosphatase are exclusively localized in the same cells, we concluded that the gluconeogenesis induced by the enzyme may account for glycogen accumulation [7]. A decrease in glycogen level induced by indolamine has been reported by Quach et al. [8], Pennington and Pentreath [9], and Magistretti [10]. The selective stimulation of serotonergic pathways increases glucose utilization in rat brain [11]. Thus, it seemed interesting to investigate whether the indolamine neurotransmitter is involved in the mechanism of action of MSO on carbohydrate metabolism. Sellinger and Dietz [12] and Blizard and Balkoski [13] have already studied the effect of MSO on the indolamine system, with a particular interest in seizure onset. We pursued and extended this work essentially looking at the glycogenic effect; we used increasing doses of MSO in order to correlate changes in indolamine level and glycogen level. We reinforced serotonin synthesis by using its precursor, 5-hydroxytryptophan, in association with benserazide, a decarboxylase