Pteridines
Vol. 7, 1996, pp. 148 – 150

5,6,7,8-tetrahydroneopterin (NH₄) Acts like an Endogenous Superoxide Dismutase

Suguru Sawada^{1,3,§}, Takeshi Morimoto¹, Tsuyoshi Kito¹, Osamu Taguchi², Mikio Yasumura³,
and Hajime Hirose³

Department of Gastroenterological Surgery¹ and Laboratory of Experimental Pathology²,
Aichi Cancer Center 1-1 Kanokoden, Chikusa-ku, Nagoya, 464, Japan First
Department of Surgery, Gifu University School of Medicine³ 40 Tsukasa-machi, Gifu, 500, Japan

(Received September 10, 1996)

Summary

The protective effect of 5,6,7,8-tetrahydroneopterin (NH₄) against radiation injury in mice was studied. (C57BL/6×A/J)F₁ mice received whole body irradiation with a single fatal dose of 8.0 Gy X-ray. NH₄ was injected intraperitoneally into irradiated mice at various protocols. NH₄ was significantly effective to prolong the survival of the irradiated mice. The most effective protocol of NH₄ administration was 3 times injections (10 minutes before and after irradiation and additional one 12 hours after that.) with a dose of 30 mg/kg body weight for each. Some mice that received such treatment survived more than 30 days after irradiation. In conclusion, these results indicate that NH₄ has an inhibitory potential on radiation injury as an endogenous superoxide dismutase like substance.

Key words : 5,6,7,8-tetrahydroneopterin(NH₄), Superoxide dismutase, Radiation injury

Introduction

The surgical intervention combined with intraoperative radiation therapy for advanced carcinomas such as pancreatic carcinoma have been carried out in several hospitals (1-3). Since several severe side effects such as myelopathy and necrosis of normal tissue are often observed in patients who are receiving radiation therapy, development of protective methods against post-radiation injury of normal tissues needs immediate study.

It is believed that the major chemical lesion induced by irradiation is production of superoxide radicals. Many studies have consistently shown that exogenously administered enzymatic scaveng-

ers of species of reduced molecular oxygen, such as superoxide dismutase, can protect organisms against the effects of ionizing radiation both *in vitro* and *in vivo* (4-7). Recently, it was reported that pteridines such as neopterin and 5, 6, 7, 8-tetrahydroneopterin (NH₄) play important roles as endogenous superoxide dismutase like substances, scavenging for the superoxide anion radical (9). However, protective effect of post-irradiated injury by neopterin is not examined so far. In this report, we investigated this point using whole-body irradiated mice.

Materials and Methods

Eight weeks old (C57BL/6×A/J)F₁ male mice were used and housed in a specific pathogen-free fa-

§ Author to whom correspondence should be addressed.

cility at the Aichi Cancer Center.

All mice used in this study received whole-body irradiation of a single dose of 8.0 Gy by using an X-ray unit (MBR1520R, HITACHI, Tokyo Japan).

NH₄ was from Torii Pharmaceutical Co., Ltd. Tokyo Japan, and was dissolved in a constant volume of 7.5 mg/ml of phosphate-buffered saline.

In the first experiment, NH₄ was intraperitoneally injected into mice 10 minutes before and after irradiation at a dose of 3 mg/kg ($n=3$), 30 mg/kg ($n=6$), and 150 mg/kg ($n=5$) body weight.

In the second experiment, NH₄ at a dose of 30 mg/kg was intraperitoneally injected into mice from one to four times as follows, 10 minutes before irradiation ($n=5$), 10 minutes before and after irradiation ($n=9$), 10 minutes before and after irradiation and 12 hours after irradiation ($n=12$).

In the third experiment, NH₄ at a dose of 30 mg/kg was intraperitoneally injected into mice 10 minutes before and after irradiation then one more injection was performed at 1 ($n=7$), 12 ($n=12$) or 24 ($n=4$) hours after irradiation.

Generalized Wilcoxon test was used to evaluate the survival days between NH₄ injected mice and non-injected control mice ($n=18$). Differences were considered to be significant when the probability (p) value was $p < 0.05$.

Results

In the first experiment, the mean survival days of each group of mice that received injection of different dose of NH₄ at 10 minutes before and after irradiation were as follows; NH₄ non-injected control, 11.1 days; NH₄ 3 mg/kg body weight, 10 days; NH₄ 30 mg/kg body weight, 13.7 days ($p < 0.005$); NH₄ 150 mg/kg body weight, 2.5 days (significantly shorter life than NH₄ non-treated control mice. $p < 0.04$).

In the second experiment, the mean survival days of each group of mice that received injection of NH₄ at different times with a dose of 30 mg/kg body weight were as follows; NH₄ single injection at 10 minutes before irradiation, 9.6 days; two times injection at 10 minutes before and after irradiation, 13.7 days ($p < 0.005$); three times injection at 10 minutes before and after radiation and 12 hours after irradiation, 16.3 days ($p < 0.001$). Four out of 12 mice of the last group lived more than 30 days.

In the third experiment, the mean survival days of each group of mice that received 3 times injection of NH₄ at 10 minutes before and after irradiation and additional one at different time

were as follows; additional one at 1 hour after irradiation, 6.3 days (significantly shorter life than NH₄ non-injected control mice. $p < 0.004$); additional one at 12 hours after irradiation, 16.3 days ($p < 0.001$); additional one at 24 hours after irradiation, 13.5 days ($p < 0.05$).

Discussion

(C57BL/6 $\times$ A/J)F1 male mice that received whole body irradiation with a single dose of 8.0 Gy X-ray died 11.1 days on average after exposure. The results of this study clearly indicate that administration of a proper dose of NH₄ on proper time to fatally irradiated mice had an inhibitory potential on radiation injury resulting in prolonged survival time or escaped acute death. The results of this experiment suggest that NH₄ acts as an endogenous antioxidant.

In this first experiment, we injected NH₄ twice to mice 10 minutes before and after irradiation. The most effective dose of NH₄ to prolong irradiated mouse life was 30 mg/kg body weight. Administration of 10% of this dose had no protective effect. While, administration of quintuple dose was fatal dose for not only irradiated but also non-irradiated (C57BL/6 $\times$ A/J)F1 male mice (data not shown).

In the second experiment, we examined effective NH₄ administration times for protection. Single injection of NH₄ at 10 minutes before irradiation had no effect towards protection. As shown in the first experiment, NH₄ twice injections at 10 minutes before and after irradiation was significantly effective. Furthermore, triple administration of NH₄ at 10 minutes before and after irradiation and 12 hours after that was most effective.

In the last experiment, we had altered the last injection of second experiment to different time. A negative effect was induced when the last injection was performed at 1 hour after irradiation. This protocol may induce fatal blood concentration of NH₄. Positive effect was observed when the last injection was performed at 24 hours after irradiation, however, this protocol was less effective compared to that of the last injection performed at 12 hours after irradiation.

These results altogether indicated that the most effective protocol for administration of NH₄ to protect post-radiation injury was as follows. 3 times injection of NH₄ at 10 minutes before and after irradiation and 12 hours after that with a dose of 30 mg/kg body weight each. This pro-

tocol may induce best blood concentration of NH₄ for protection of post-radiation injury. NH₄ would be useful as a therapeutic agent against post-radiation injury for patients in the near future.

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