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# RADIOPROTECTIVE EFFECTS OF α–GALACTOSYLCERAMIDES

Kazuhiro Motoki, Eiichi Kobayashi, Masahiro Morita, Takeshi Uchida, Kohji Akimoto, Hideaki Fukushima, and Yasuhiko Koezuka*

*Pharmaceutical Research Laboratory, Kirin Brewery Co., Ltd.*
*3 Miyahara-cho, Takasaki-shi, Gunma 370-12, Japan*

**Abstract:** We examined the life-span-prolonging effects of five kinds of α-Galactosylceramides (α-GalCers) on mice irradiated with lethal dose (9 Gy) of X ray , and found that they show strong radioprotective activities even when they are administered to mice after the irradiation. These results suggest that α-GalCers are useful as radioprotective agents.

We previously reported that **AGL-517** having an α-GalCer structure showed strong antitumor activity against mice subcutaneously inoculated murine melanoma B16 or murine fibrosarcoma Meth A cells.[1,2] Since it was demonstrated that agelasphins having α-GalCer structures show potent immunostimulating activities as well as antitumor effects,[2-4] we examined the relationship between immunostimulatory and antitumor activities of several kinds of synthetic α-GalCers and found that they show antitumor effects via activating immune systems.[5] It is known that immunostimulating agents such as OK432 (*Streptococcus haemolyticus* preparation)[6,7] and AS101 (ammonium trichloro (dioxyethylene-O-O') tellurate)[8,9] possess radioprotective effects on lethally irradiated mice. These findings prompted us to examine whether α-GalCers such as **AGL-517** show radioprotective effects. To address the question, we performed the following experiments using five kinds of α-GalCers, **AGL-564** (β-GalCer) (Fig. 1), and OK432.

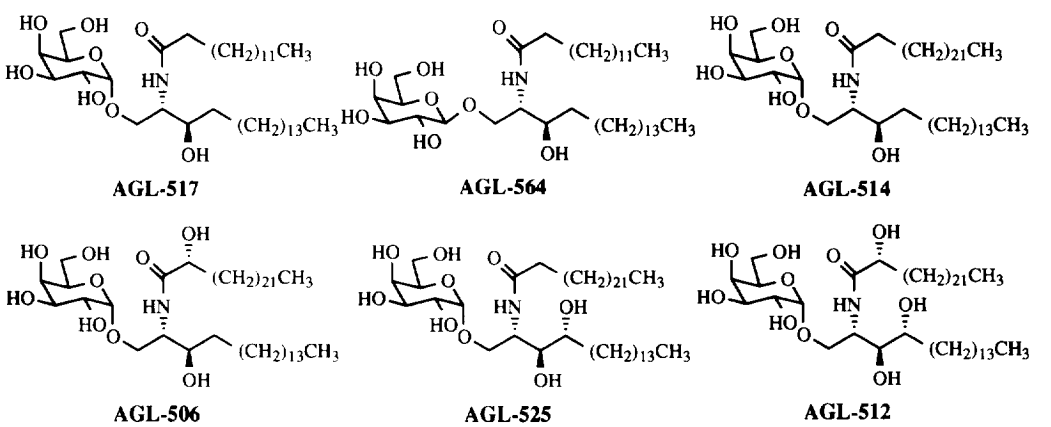


**Fig. 1.** Structures of **AGL-517**, **AGL-564**, **AGL-514**, **AGL-506**, **AGL-525**, and **AGL-512**

Since it is known that the pre-irradiation-treatment of OK432 which is commercially available or AS101 which is not commercially available shows stronger radioprotective effects than their post-irradiation-treatment, we, first, examined the life-span-prolonging effects of **AGL-517** and OK432 on mice irradiated with 9 Gy (900 rad) of X ray when they were administered prior to the irradiation. When **AGL-517** or OK432 were administered to mice two days prior to the irradiation, **AGL-517** markedly prolonged the life period of irradiated mice, though OK432 did not show any radioprotective effects. On the other hand, when they were administered one day before the irradiation, both compounds showed strong life-span-prolonging effects and the potency of **AGL-517** was stronger than that of OK432 (Fig. 2).

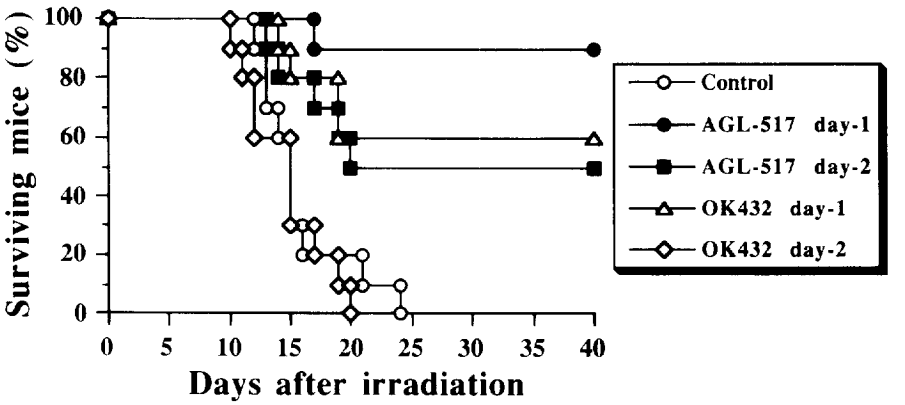


**Fig. 2.** Radioprotective effects of **AGL-517** and OK432 administered before the irradiation on mice irradiated with 9 Gy of X ray.

**AGL-517** (0.1 mg/kg) or OK432 (Chugai Pharmaceutical Co., Ltd., 1 KE/mouse) was intravenously administered into female $BDF_1$ mice (ten mice per group) one day or two days before the irradiation. Then, each mice was irradiated with 9 Gy of X ray on day 0. Control mice were treated only with the irradiation. Survival or death of each mice was observed daily.

Then, we compared radioprotective effects of **AGL-517** and OK432 on lethally irradiated mice when they were administered after the irradiation. As shown in Fig. 3, when **AGL-517** was administered within two hours after the irradiation, it markedly prolonged the life period of mice and its potency was as same as that of its one-day-before-treatment. On the other hand, OK432 did not show any radioprotective effects in this experimental condition in contrast to the report that the post-irradiation-treatment of OK432 markedly prolonged the life period of mice irradiated with 8.5 Gy of X ray.[7] These results suggested that α-GalCers such as **AGL-517** may be quite useful as radioprotective agents because potent radioprotective effects were observed with the treatments of **AGL-517** not only prior to but also after the irradiation.

Herein, various types of β-GalCers were isolated from marine organisms[10,11] or organ tissues[12,13], though their radioprotective effects have not been reported yet. The findings prompted us to examine whether β-GalCers possess radioprotective effects. To address the question, we compared life-span-prolonging activities between **AGL-517** and **AGL-564** which has the same ceramide portion as **AGL-517** but galactose is combined to the ceramide in the β-configuration.[1] As shown in Table 1, **AGL-517** markedly increased

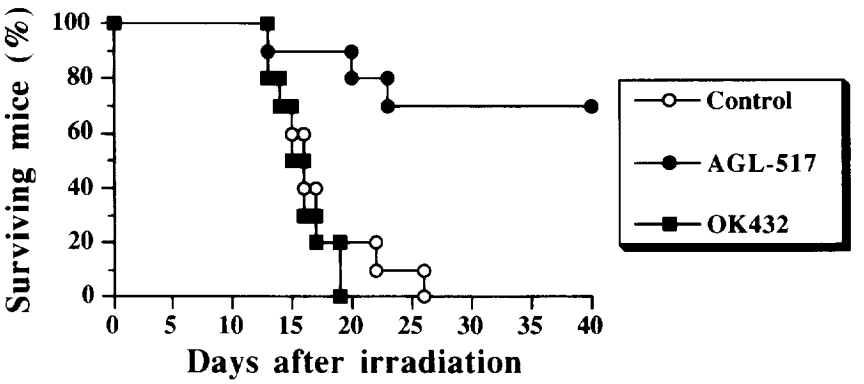


**Fig. 3.** Radioprotective effects of **AGL-517** and OK432 administered after the irradiation on mice irradiated with 9 Gy of X ray.

Female $BDF_1$ mice (ten mice per group) were irradiated with 9 Gy of X ray on day 0. **AGL-517** (0.1 mg/kg) or OK432 (1 KE/mouse) was intravenously administered into mice within 2 h after the irradiation on day 0, and also on day 4 and day 8. Control mice were treated only with the irradiation. Survival or death of each mice was observed daily

the ratio of surviving mice (90 %), though **AGL-564** did not show marked radioprotective effects. This result resembles our previous finding that **AGL-517** showed stronger immunostimulatory activities than **AGL-564**.[5] These results demonstrated that the manner of combination between galactose and ceramide plays an important role in radioprotective effects as well as immunostimulatory activities of GalCers.

**Table 1.** Radioprotective effects of **AGL-517**, **AGL-564**, **AGL-514**, **AGL-506**, **AGL-525**, and **AGL-512** adimnistered after the irradiation on mice irradiated with 9 Gy of X ray.

| | Surviving mice (%) | | | | | | |
|---|---|---|---|---|---|---|---|
| Sample | day 10 | day 15 | day 20 | day 25 | day 30 | day 35 | day 40 |
| Control | 100 | 60 | 20 | 0 | 0 | 0 | 0 |
| **AGL-517** | 100 | 100 | 90 | 90 | 90 | 90 | 90 |
| **AGL-564** | 100 | 70 | 20 | 20 | 20 | 20 | 20 |
| Control | 100 | 90 | 50 | 30 | 10 | 0 | 0 |
| **AGL-514** | 100 | 100 | 100 | 90 | 90 | 90 | 90 |
| Control | 100 | 50 | 10 | 0 | 0 | 0 | 0 |
| **AGL-506** | 100 | 100 | 100 | 90 | 90 | 90 | 90 |
| Control | 100 | 50 | 20 | 0 | 0 | 0 | 0 |
| **AGL-525** | 100 | 100 | 90 | 90 | 90 | 90 | 90 |
| **AGL-512** | 100 | 100 | 100 | 90 | 80 | 80 | 80 |

Female $BDF_1$ mice (ten mice per group) were irradiated with 9 Gy of X ray on day 0. Each sample (0.1 mg/kg) was intravenously administered into mice within 2 hr after the irradiation on day 0, and also on day 4 and day 8. Control mice were treated only with the irradiation. Survivial or death was observed daily.

Since it is important to confirm that α-GalCers show potent radioprotective effects, we examined the radioprotective activities of four kinds of α-GalCers (**AGL-506** (4-deoxy), **AGL-512**, **AGL-514** (2',4-dideoxy), and **AGL-525** (2'-deoxy), Fig. 1) which were synthesized in our laboratory and possess strong immunostimulatory activities.[14] As shown in Table 1, all compounds markedly prolonged the life period of irradiated mice and their potencies were similar. Since the compounds have the same fatty acid (C'-24) and long chain base (C-18) but different hydroxyl groups, this result demonstrated that the 2'- and 4-hydroxyl groups in the ceramide portion do not play an important role in their radioprotective activities as well as their immunostimulating effects.[14]

The above-mentioned results demonstrated that α-GalCers are useful as radioprotective agents. This is the first finding that α-GalCers possess capabilities that prolong the life period of lethally irradiated mice.

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