

THE IN SITU TUMOR RESPONSE TO RADIOSENSITIZATION OF A NOVEL SENSITIZER SODIUM GLYCIDIDAZOLE

ZHENG Xiulong, MENG Xiangshun, ZHAO Fang, LI Yu, GAO Jianguo

(*Institute of Radiation Medicine, 2nd Military Medical University, Shanghai 200433*)

Abstract The aim of this study was to see whether Sodium Glycididazole (CMNa) could enhance the in situ tumor efficacy of radiation, using 4 different tumor models bearing mice. The results showed that in comparison with the irradiated group alone, CMNa could decrease the velocity of tumor growth and increase the days of Tumor Growth Delay(TGD) evidently in all four tumor types. CMNa showed radiosensitizing activity for all tumors with SERs greater than 1.3. It demonstrates that the novel radiosensitizer can potentiate the in situ anti-tumor efficacy of radiation and has different radiosensitization effect among the tested tumors. Whether the new radiosensitizer CMNa has any effects on normal tissue such as radiosensitive bone marrow was tested. Survival of bone marrow stem cells(CFU-GM) due to radiation alone or in combination with the drug was assessed. Nevertheless it did not enhance the radiosensitizing effect on normal bone marrow and SER=1. The Therapeutic Gain Factor(TGF) defined as SER of tumor divided by SER of normal tissue bone marrow is greater than 1.3. Maybe CMNa will be an effective new type radiosensitive drug for clinical cancer therapy.

KEYWORDS Novel hypoxic radiosensitizer, Sodium Glycididazole, Four tumor models, CFU-GM

CLC R979.19

Radio-resistance of hypoxic cells in malignant tumors has been considered as one of the important limiting factors in the local control of human cancer by radiotherapy^[1]. At present, it is believed that radiosensitizers can selectively radiosensitize hypoxic cells and noticeably enhance the efficiency of radiation in controlling tumours. Because some of the radiosensitizing drugs now available have neurotoxicity, their clinical application is limited. The search for new types of radiosensitizers with higher efficiency and lower toxicity is therefore an important task for improving radiotherapy of solid tumours^[2].

CMNa [Sodium N, N-Bis (2-methyl-5-nitro-imidazol-1-ethoxy-carbonyl methyl)- glycinate trihydrate] was first synthesized in our laboratory^[3,4]. Preclinical studies with CMNa have showed not only enhanced DNA damage of tumor cells under hypoxic irradiation, but also inhibited DNA repair and Potentially Lethal Damage Repair (PLDR)^[5,6] yet. CMNa also showed a greatly enhanced hypoxic cell radiosensitizing efficiency in vitro^[7]. In the present study we evaluated the radiosensitizing efficiency of CMNa in vivo, which is currently undergoing clinical trials as a

Received: 19 July 1999, Accepted: 1 November 1999

radiation sensitizers under the authorization of the Ministry of Public Health of PRC. In addition the influence of CMNa on irradiated normal radiosensitizing tissue bone marrow was also studied.

1 Materials and methods

1.1 Murine tumors

C57BL/6, BALB/c, and Kunming male mice, each weighing 18~22gBW, were purchased from Shanghai Cihli-Bisa experimental animal Center of China. The animals were housed in institutional animal care facilities in our university. The Lewis lung cancer, B16 melanoma, EMT6 mammary carcinoma and S 180 sarcoma were obtained from donor mice maintained in Institute of Shanghai Pharmaceutical Industry, Cancer Institute of Chinese Academy Medical Sciences and Institute of Pharmacology Acta Sinica respectively. Lewis lung cancer, B16 melanoma, EMT6 mammary carcinoma and S180 sarcoma taken from corresponding donor mice were immediately sliced and implanted S. C. into the left hind limb of C57, BL/6, BALB/c and Kunming mice respectively. When the tumors became palpable, they were measured with vernier calipers 3 times a week in 2 orthogonal diameters. Tumor volume was calculated according to Steel's formula. When the tumor volume reached 200~400mm³, the mice were randomly divided into different treatment groups, ten mice in each group.

1.2 The effect of CMNa on normal tissue

The effect of CMNa on radiosensitive bone marrow hematopoiesis of the mice was measured with in vitro assay. Mice were injected for two weeks with CMNa (0.4 mmol/kg iv. twice a week) and then irradiated with 0~6Gy γ ray. Each dose group contained 6 mice. The mice were killed and the bone marrow taken for CFU-GM assay 2h after irradiation.

1.3 CMNa (Sodium Glycididazole)

CMNa [Sodium N, N-Bis (2-methyl-5-nitro- imidazole-1-ethoxy-carbonyl methyl) -glycinate trihydrate] is a pale yellowish powder with molecular weight of 573.45Da. It was dissolved in saline shortly before use. Drug dose was adjusted according to body weight for each mouse. The maximum volume injected into tail vein of mice was 0.4mL. A range of drug doses was used.

1.4 γ ray irradiation

The tumors were irradiated with 4~32 Gy γ ray of ⁶⁰Co^[8]. The tumor-bearing mice were placed in a special plastic box. The tumor-bearing legs of the mice protruded in the radiation area and were irradiated with γ rays, at a dose rate 2Gy/min, and the rest of the mouse bodies were shielded from radiation by 10cm thick lead blocks. The source-to-surface distance was 100cm. The mice were administered by iv injection of drug with a dose of 0.1~1.8mmol/kg of CMNa respectively for 30 min^[9], and then arterial clamping tumor for 30 min prior to and during tumor local irradiation was carried out under hypoxic condition at room temperature.

1.5 Tumor growth delay

Tumor growth delay(TGD) was used to measure the radiosensitizing efficiency of the drug. After treatment, tumors were measured and growth delay was estimated by the time taken for each individual tumor to increase its volume by 5 fold. The mean delay for a dose group of 10 mice was then calculated.

2 Results

CMNa alone within 0.1~1.8 mmol/kg had little effect on tumor growth. However, when

given 30 min prior to the γ radiation with a dose range from 0 to 32Gy, these doses of the drug increased the anti-tumor effects of radiation in all 4 tumor models investigated. Fig.1 shows the dose-response curves for tumors irradiated with, or without CMNa given prior to irradiation. It is obvious that more delay is caused at all levels of radiation dose in all four tumor types, which had received the CMNa. Sensitizing Enhancement Ratio (SER) can be measured from these curves as a ratio of the radiation dose needed to provide tumor growth delay of ten days with respect to 5X original tumor size in the absence of CMNa to the isoeffect radiation dose given in the presence of CMNa. CMNa showed radiosensitizing activity in all tumor types with SERs mostly greater than 1.3 by growth time assay (Tab.1). The degree of radiosensitizations differed in different types of tumors.

In order to investigate whether CMNa in combination with radiation could lead to a therapeutic benefit, bone marrow toxicity due to radiation alone or in combination with the drug was assessed (Fig.2). Survival fractions of bone marrow stem cells (CFU-GM) in Kunming mice treated with a range of radiation doses alone was same as those with radiation plus a 1/6 LD50 CMNa given iv. 30min before radiation. The results demonstrated that this dose of CMNa did not increase the toxicity of γ irradiation.

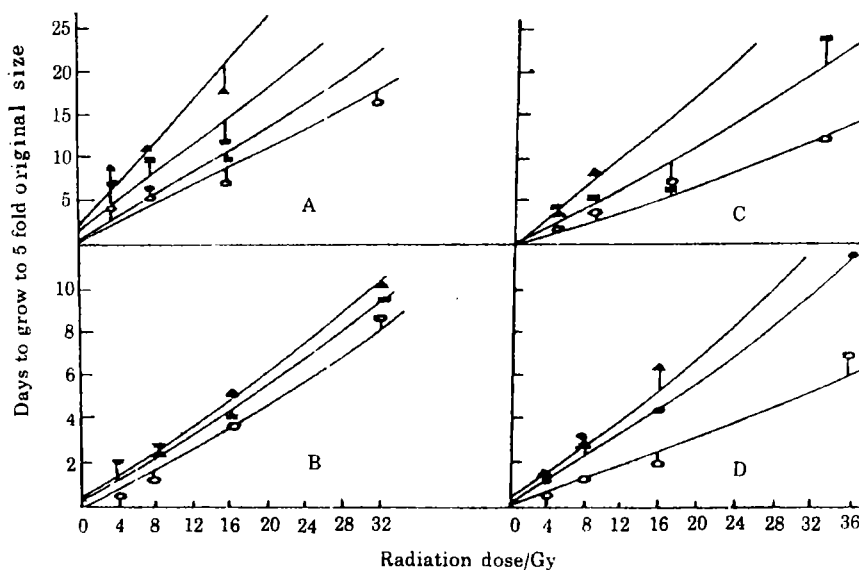
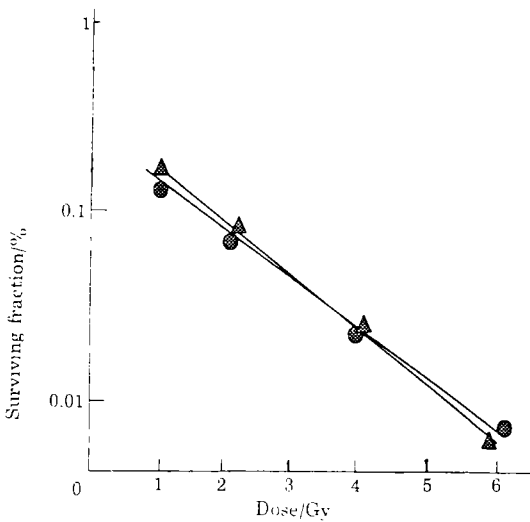


Fig.1 The radiosensitizing effect of CMNa on the tumor growth delay of Lewis lung cancer (A) EMT6 mammary carcinoma (B) B16 Melanoma (C) and S180 Sacoma (D) respectively

0.5h iv. injected prior to irradiation, $n=10$; (o) Radiation alone; (●, ■, ▲) represents 0.1, 0.3, 0.9mmol/kg dose of CMNa iv. Injected respectively prior to γ irradiation

Tab.1 Radiosensitizing effects of 0.1~1.8mmol/kg CMNa in four models of tumor bearing mice

	S180	Lewis	B16	EMT6
When $V_t : V_0=5$ TGD/days	1.7~11.9	4.4~17.4	5.7~24.9	1.0~10.1
When TGD=10days SER value	1.5~1.8	1.3~2.6	1.6~2.4	1.2~1.3



3 Disssussion

In the present study, we demonstrated that the novel hypoxic cell radiosensitizer CMNa could increase 10~25 days of TGD in tumor bearing mice (with four tumor models). when drug was given iv at 1/75-1/4 LD50 (2.7 g/kg) CMNa prior to 4~32 Gy irradiation (Fig.1). When tumors grew to 5 fold of theirF original volumes, and TGD=10 days. from the isoeffect we could calculate the SER values (Tab.1), which were almost all greater than 1.3. It showed that the non-toxic dose of CMNa had radiosensitization effect among the tested tumors (Tab.1).

Usually, in clinical tumor radiotherapy fractionated small radiation doses are used. Whether CMNa would have radiosensitizing effects under these conditions is not known.

In our other experiments,the radiosensitization by CMNa was observed using Lewis lung cancer-bearing mice with fractionated dose (8 Gy per day, twice per week) of radiation and compared with SR~2508 under identical experimental conditions. The results showed that there was a more significantly decreased tumor growth velocity(V_t/V_0) with 0.1mmol/kg CMNa than that with radiation alone. The radiosensitization of CMNa was also more effective than that of SR~2508^[10,11].

Whether the new radiosensitizer CMNa has any effects on normal tissues was tested. There was no obvious difference between the radiation alone group and the group given radiation combined with CMNa by a CFU-GM assay. The SER value was about 1. Previously, we have demonstrated that the murine solid tumor had a higher concentration of CMNa due to a higher concentration ability and slower metabolism in tumor^[12].

These findings demonstrate that the novel radiosensitizer CMNa can potentiate the in situ anti-tumor efficacy of γ radiation. At the same dosage, the sensitizing effect of CMNa was higher than that of potent control agent (SR-2508). Nevertheless it did not enhance the sensitizing effect on normal radiosensitive tissues bone marrow. Thus, in the pre-clinical model investigated, CMNa did give a therapeutic benefit, for its radiosensitizing effect (SER was greater than 1.3) was greater than that of normal tissue bone marrow {SER=1}. The Therapeutic Gain Factor (TGF) defined as SER of tumor divided by SER of normal tissue bone marrow is greater than 1.3. It is hopeful that CMNa will be an effective new type radiosensitive drug for clinical cancer

therapy.

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新增敏剂—甘氨双唑钠对体内肿瘤的放射增敏作用

郑秀龙 孟祥顺 赵 芳 李 雨 高建国

(第二军医大学放射医学研究室 上海 200433)

摘要 采用 4 种不同肿瘤模型的荷瘤小鼠, 观察了 CMNa 对受照小鼠肿瘤的作用。结果表明: 与单纯照射组相比, CMNa 能明显地减慢肿瘤的生长速度; 增加肿瘤生长的延迟天数, 4 种肿瘤模型均得到相同的结果。绝大多数 SER 值在 1.3 以上。实验结果表明, CMNa 对体内肿瘤亦有明显的放射增敏作用。然而在相似条件下, 以 CFU-GM 为指标, 在研究 CMNa 对受照正常小鼠对 γ 射线敏感的造血组织有否影响时, 却未观察到 CMNa 对正常组织的放射增敏效应, 与单纯照射组相比, CFU-GM 无变化, SER=1, 其治疗增益指数 (TGF) 均在 1.3 以上。提示: CMNa 将有希望成为临床肿瘤治疗有效的新型放疗增敏药。

关键词 新型乏氧放射增敏剂, 甘氨双唑钠, 4 种肿瘤模型, CFU-GM

中图分类号 R979.19