

Fractionated Irradiation Combined with Carbogen Breathing and Nicotinamide of Two Human Glioblastomas Grafted in Nude Mice

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This study addressed the potential radiosensitizing effect of nicotinamide and/or carbogen on human glioblastoma xenografts in nude mice. U-87MG and LN-Z308 tumors were irradiated with either 20 fractions over 12 days or 5 fractions over 5 days in air-breathing mice, mice injected with nicotinamide, mice breathing carbogen, or mice receiving nicotinamide plus carbogen. The responses to treatment were assessed using local control and moist desquamation. In U-87MG tumors, the enhancement ratios (ERs) at the radiation dose required to produce local tumor control in 50% of the treated mice (TCD₅₀) with nicotinamide and/or carbogen ranged from 1.13 to 1.24 for irradiation in 20 fractions over 12 days. In LN-Z308 tumors, the ERs at the TCD₅₀ with nicotinamide and/or carbogen ranged from 1.22 to 1.40 for irradiation in 5 fractions over 5 days and from 1.11 to 1.30 in 20 fractions over 12 days, respectively. Skin injury was slightly enhanced, with ERs ranged from 1.06 to 1.15 when radiation was combined with carbogen and/or nicotinamide. Thus carbogen and nicotinamide can slightly improve the radiation response of human glioblastoma xenografts. © 2001 by Radiation Research Society

INTRODUCTION

Glioblastomas are among the most malignant brain tumors. Patients have an average survival time of around 10 to 12 months (1), and radiotherapy very rarely produces long-term local control (2). Potential causes of treatment failure are tumor cell repopulation, hypoxia and intrinsic cellular radioresistance. Accelerated radiotherapy, usually using several fractions a day to reduce overall treatment time, may be effective in overcoming rapid tumor cell proliferation. Breathing carbogen (95% O₂ plus 5% CO₂) before and during irradiation has been shown to promote reoxygenation of chronically hypoxic cells distant from

blood vessels (3–7). In addition, nicotinamide may overcome the unfavorable effects of acute hypoxia resulting from intermittent closure of blood vessels (8–10) and at high doses may also inhibit DNA repair (11).

In a few rodent tumor models, very effective radiosensitization with enhancement ratios (ERs) of 1.83 to 2.1 has been observed for single and fractionated irradiations by adding carbogen breathing plus systemic administration of nicotinamide (12–15). Accelerated radiotherapy combined with carbogen and nicotinamide (ARCON) is currently being evaluated in Phase I–II clinical trials with the aim of increasing the therapeutic effect of radiotherapy on rapidly proliferating tumors containing radiation-resistant hypoxic cells (16–20). Hypoxia has been hypothesized to be one of the most important factors in the radioresistance of human malignant brain tumors (21, 22), but this has not been proven. Furthermore, preclinical data on radiosensitization of human brain tumor models with carbogen and/or nicotinamide are limited (23). Therefore, this study was designed to investigate whether carbogen and/or nicotinamide can sensitize human glioblastomas xenografted into nude mice.

MATERIALS AND METHODS

Cell Lines

The human glioblastoma cell lines U-87MG and LN-Z308 were obtained from the Department of Neurosurgery at our hospital (24). These cell lines originated from glioblastoma multiforme, which is a rapidly growing and highly invasive human brain tumor. U-87MG cells are characterized by the presence of wild-type TP53, while LN-Z308 cells do not express endogenous TP53 protein (24). The cells were grown as a monolayer in Eagle's MEM with 10% FCS, 2 mM L-glutamine and 1% penicillin/streptomycin. A suspension of about 5×10^6 cells was inoculated subcutaneously in the dorsum of Swiss nude mice. Tumors were passaged at least three times in nude mice prior to experiments.

Tumor Model

All experiments with nude mice were performed according to Swiss animal welfare legislation and were approved by the official committee of surveillance of animal experiments. Seven- to 9-week-old female Swiss homozygous nu/nu mice were given a subcutaneous transplantation in the midline of the back at 2 cm from the tail of about 30 mm³ of freshly excised, minced U-87MG or LN-Z308 tumor. Two to 3 weeks after inoculation, the mice bearing tumors with a mean volume of ap-

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