Effectiveness of AMSA alone or in combination with radiation on murine fibrosarcoma pulmonary nodules

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The cytotoxic effects in vivo of 4'- (9-acridinylamino) methanesulfon- m-anisidine (AMSA), radiation or both modalities in combination on murine fibrosarcoma (FSa) cells grown as pulmonary tumors were determined. Fourteen days following the i.v. injection of viable FSa cells, recipient mice developed between 100 and 150 visible pulmonary nodules. At that time, tumor-bearing animals were exposed to either single or combined modality treatments, as well as single and fractionated dose regimens. Animals were sacrificed 1 hour after the last treatment. Tumor nodules were excised, made into a single cell suspension and separated on the basis of cell size by centrifugal elutriation. Flow microfluorometry (FMF) was used to determine the cell-cycle parameters and the relative synchrony of the separated populations, as well as the percentage contamination by normal diploid cells in each of the tumor cell populations. Known numbers of viable cells from each elutriator fraction were injected into recipient mice to determine their colony-forming efficiency (CFE). Surviving fractions were determined by comparing the CFEs of treated FSa cells from each of the separated elutriator fractions with those of appropriate untreated controls. Following a single i.v. dose of AMSA (30 mg/kg), populations of cells enriched in S phase were the most sensitive. When a single dose of AMSA was combined with a single dose of radiation (100 rad), there was a marked schedule dependence with the more effective sequence, especially if a 12 hour interval was chosen between doses, being AMSA followed by irradiation. No schedule dependence was observed if both modalities were combined and administered under a fractionated protocol of four fractions of AMSA (5 mg/kg per fraction) and four fractions of radiation (300 rad per fraction). Under these conditions the greatest reduction in CFE was in cell subpopulations most enriched in S and G2 + M phase cells.

Introduction

There is currently considerable interest in the development and application of combined modality approaches for use in the treatment of neoplastic disease. Through the judicious use of chemotherapy in combination with radiation it is hoped that an improved therapeutic ratio will be achieved. This might occur through the selective cytotoxic activity of each agent on different classes of neoplastic target cells thus giving rise to an additivity of response or through the sensitization of a class of tumor cells allowing for an increased efficiency in cell killing by the second agent. Unfortunately, in some instances an antagonism and/or a protection can be exhibited.

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through the combination of multiple agents. These concepts are elegantly described elsewhere [19].

Because of these factors, it is important that controlled studies be performed to test the efficacy of combined modality protocols. In particular, parameters such as the sequencing and timing of therapeutic agents need to be characterized. An in vivo procedure has been developed which allows for the rapid testing and evaluation of the phase-specific therapeutic effectiveness of selected cytotoxic agents on artificial pulmonary metastases [11–13]. Fourteen days following i.v. injection of tumor cells, test animals contain visible pulmonary tumor nodules. At this time, tumor bearing animals can be treated with a single modality or combination of modalities to test for cytotoxic effectiveness. At selected times during or after treatment, tumor cells can be harvested from lungs excised from test animals and then synchronized by centrifugal elutriation to ascertain phase-specificity, if any, of the agents used. In this manner questions relating to cytotoxic effectiveness, phase specificity, scheduling and timing of administration for optimum effect can be addressed.

In a previous communication, the effectiveness of vincristine in combination with fractionated doses of ionizing radiation was described [12]. The combination of both modalities exhibited a marked schedule dependence, with the most effective result occurring when vincristine was administered after irradiation. In particular, following treatment super-additive cell killing was evidenced in cell populations enriched in late S and G₂ as compared to additive or subadditive cytotoxicity in early and mid S populations.

Because of these findings, it was of interest to expand these studies to other chemotherapeutic agents, in particular the antineoplastic agent AMSA (4'–9 (acridinylamino)methanesulfon-m-anisidide). AMSA, an acridine derivative synthesized in 1974, was shown to have an antitumor activity against murine L1210 leukemia, B16 melanoma, and C₃H/HeJ spontaneous mammary tumors [3]. While activity has also been observed against cultured human colon carcinoma cells [4] and leukemia [1], it has not proven to be very effective against primary liver cancer [5] or head and neck cancer [6]. From these and other studies, it appears that its most effective utilization will be in its combination with other cytotoxic agents [17]. In this communication a variety of experimental treatment protocols are described in which AMSA is combined with radiation therapy with emphasis directed at maximizing cell killing in a murine artificial metastases system.

Materials and methods

The tumor and cell separation systems have been described in detail elsewhere [12]. Briefly, a methylcholanthrene induced fibrosarcoma (FSa) was used in this study. Sixth generation isotransplants were made into viable single cell suspensions and injected into (defined flora) female, C₃Hf/Kam mice so as to give rise in 14 days to 100–150 visible pulmonary nodules.

AMSA was obtained from the Division of Cancer Treatment, National Cancer Institute. The drug was administered i.v. to pulmonary tumor bearing animals either as a single dose of 30 mg/kg or in four fractions of 15 mg/kg per fraction, each fraction separated by a 6 hour interval. Irradiation was performed using a ¹³⁷Cs source having a dose rate of 240 rad/min [12]. Radiation was administered either in a single fraction of 1000 rad or in four fractions of 300 rad per fraction, with each fraction separated by a 6 hour interval. The experimental protocols used include: a single injection of AMSA; a single injection of AMSA either preceded or followed in