Potentiation of Radiation Effects on Two Murine Tumors by Lonidamine

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ABSTRACT

Lonidamine is a potent inhibitor of spermatogenesis and a hyperthermic sensitizer. The principal established locus of biochemical action of lonidamine is a selective inhibitory effect of the energy metabolism either in NAD-linked reactions in germ cell mitochondria, as well as the glycolytic metabolism of a variety of tumor cell lines by means of inhibition of mitochondrially bound hexokinase. We carried out in vivo tumor experiments to determine whether lonidamine when combined with radiation could potentiate the cytotoxic effects of radiation on two murine tumors. The combined effects of single acute lonidamine (100 mg/kg) and single dose X-irradiation were evaluated on the transplanted methylcholanthrene-induced fibrosarcoma in BALB/c mice and on the radiation-induced fibrosarcoma in C3H/He mice. The radiosensitizing effect by lonidamine was maximal when lonidamine was administered immediately prior to or after X-irradiation. The dose modifying factor of lonidamine is estimated to be 1.36 for methylcholanthrene-induced fibrosarcoma tumors and 1.25 for radiation-induced fibrosarcoma tumors. There was no disproportionately enhanced skin reaction following the combined treatments. The present results of the potentiating effects of radiation may be attributed, in part, to the findings of cell culture studies that lonidamine is a potent inhibitor of repair of potentially lethal damage.

INTRODUCTION

When it is successful, the clinical application of radiation therapy for cancer makes use of a differential sensitivity between neoplastic and normal tissues to ionizing radiation. In most instances, however, any intrinsic differential radiosensitivity between cancerous and adjacent non-cancerous tissues exists only within a narrow range. Over several decades, there have been extensive laboratory and clinical research efforts to find chemical agents that would selectively enhance the cytotoxic effects of radiation in the treatment of human cancers. Numerous clinical trials have been carried out attempting to improve the therapeutic effectiveness of radiation by combining it with conventional cytotoxic drugs such as Adriamycin, methotrexate, and cisplatin. The combined use of cytotoxic agents and radiation has not as yet shown any selective therapeutic effect on tumors; increased injury to normal tissues produced by the combined regimens has been a major limiting factor. More recently, hypoxic cell radiosensitizers such as the nitroimidazoles have been shown to be intensively studied as potential radiation sensitizers, since solid tumors are thought to contain a small fraction of cells that are radioresistant because of local hypoxia. Although these studies have given promising results in model systems, the clinical utility of the nitroimidazoles has not been demonstrable in randomized prospective assessment (1). Although the emphasis on the hypoxic mechanism of radioresistance has merit, it is appropriate also to consider drugs which may have a broader metabolic target (2), including the large fraction of the tumor cell population that is not hypoxic.

Lonidamine (1-[2,4-dichlorophenyl]-3H-indazole-3-carboxylic acid) is a potent inhibitor of spermatogenesis in various mammalian species and possesses embryotoxic and anti-tumor effects (2, 3). Since there was the striking morphological change of mitochondria in germ cells following lonidamine treatment, biochemical studies of drug utilization have focused upon changes in energy metabolism of a variety of tumor cell lines as well as normal cells (4–6). Floridi et al. (5, 6) demonstrated inhibitory effects of lonidamine upon respiration and aerobic and anaerobic lactate production, using Ehrlich ascites tumor cells. Under identical in vitro conditions, the drug produced negligible effects on hepatocytes. Further studies appeared to indicate that the major glycolytic inhibition was mediated through effects on the mitochondrial bound hexokinase (5, 6).

More recently, lonidamine has been investigated as a hyperthermic potentiating agent. The observations of Kim et al. (7) and Silvestrini et al. (8) demonstrated lonidamine to be a remarkably effective hyperthermic sensitizer both in vitro and in vivo. The hyperthermic sensitizing effect of lonidamine was greatly dependent upon the acidity of culture medium; since an acidic pH is known to exist in the interstitial space of solid tumors, >1 cm in diameter, the pH dependency of the drug's effects may provide therapeutic selectivity in tumors in vivo (9, 10).

In view of the profound biochemical effects of lonidamine on energy metabolism of tumor cells and the drug's potent hyperthermic potentiating effect, experiments were performed to determine whether lonidamine would potentiate the cytotoxic effects of radiation on murine tumor systems. As reported in a preliminary communication (11), the drug does enhance radiation effects in transplanted tumors; this report offers a detailed characterization of the interaction.

MATERIALS AND METHODS

Two murine tumors were used in the present study. Meth-A was grown in 5–7 weeks isogenic BALB/c (H-2b) male mice, conditions under which the tumor doubling time is 1.5 days. The Meth-A tumor is moderately immunogenic, relatively radioresistant, and capable of metastasis to the lung. The second tumor was a RIF grown in male C3H/He mice. We obtained the line from Dr. C. W. Song, University of Minnesota. It is non-immunogenic, radioresistant, and metastasizes only at a relatively advanced stage of growth. BALB/c mice were inoculated with single cell suspensions of Meth-A cells obtained from donor mice, in which the tumor was maintained in the ascites form. For tumor transfer, cells were harvested in the ascites

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form, washed in phosphate-buffered saline, centrifuged, and resuspended in heparinized minimum essential medium for inoculation. Viability of the cells was determined by the ability of intact cells to exclude trypan blue dye and was regularly more than 99%. All suspensions were morphologically homogeneous with slight variation in cell size; they consisted exclusively of single cells. Cell numbers were counted with a Coulter counter and, following appropriate dilutions, $10^6$ cells were transferred intramuscularly to the distal thigh region.

The procedure of tumor transplantation of RIF cells was similar to the Meth-A, except that exponentially growing cells maintained as in vitro passages were carried out with Waymouth's medium supplemented with 15% fetal calf serum.

Irradiation was carried out on anesthetized mice (ketamine HCl, 125 mg/kg) post-transplantation. A G. E. Maxitron operated at 300 KVP and 20 mA with a 2-mm copper filtration (half value layer = 1.88 mm copper) at a dose rate of 80 cGy/min and a target skin distance of 16 cm was used.

Lonidamine was supplied as a powder at a purity of 99.5% by the F. Angelini Research Institute. It was suspended in carboxymethyl cellulose at a concentration of 100 mg/ml and administered into the peritoneal cavity of mice at selected time intervals according to the respective experimental protocol; control and radiation control mice received vehicle alone.

The tumor volume analyses were carried out by measuring maximal diameters of the tumor-bearing thighs. Tumor volumes were calculated and adjusted by a sliding scale for the normal leg volume component. All experimental tumor volume data points were plotted as the log of the average tumor volume per group versus days post-treatment. A solid line was determined by the least square method and best represented as a Gompertzian function for untreated control tumors. Dose dependent tumor growth delays were defined as the time required for post-treatment mean tumor volumes to regrow to the respective initial treatment volumes or 1.0 cm³ end points. Local tumor control was defined as a nonpalpable tumor at 120 days post-treatment.

Dose response curves of normal tissue response (e.g., skin damages) were obtained, using the criteria of Fowler and Denekamp (12), in which the gross clinical response was scored as erythema, desquamation, and ulceration. Using this technique, average skin reactions were plotted as a function of total dose.

Serum pharmacokinetics of lonidamine in treated mice were determined by sacrificing five mice each at $\frac{1}{2}$, 1, 2, 3, 4, 5, 6, and 18 h and obtaining blood by cardiac puncture. Lonidamine content was analyzed by a modification of the high-performance liquid chromatographic method of LeClaire et al. (13).

RESULTS

Sequence and Timing of Radiation and Lonidamine. Prior to proceeding to a determination of the dose modifying factor of lonidamine, we wanted to determine which specific treatment sequence and time of lonidamine administration with respect to X-irradiation would produce a maximal local control or tumor growth delay. The drug’s effects were analyzed both in terms of the percentage of local control (Meth-A tumor) and in terms of growth delay when regrowth occurred (RIF tumor). Fig. 1 shows the percentage of tumor control of radiation alone or in combination with a single dose of lonidamine applied before or after X-irradiation treatment. A single dose of lonidamine (100 mg/kg) administered 4 h prior to radiation did not enhance the radiation effect. However, when lonidamine was administered either 1 h before or 1, 2, and 3 h after irradiation, a highly significant increase in the percentage of tumor control relative to radiation alone was observed. Fig. 2 presents the data obtained with RIF tumors grown in C57-H/He. The RIF tumor showed better results at 1 h post-irradiation than 1 h pre-irradiation, but the optimal results were achieved with drug immediately before irradiation. This is slightly different from the results obtained with the Meth-A tumor. Since the tumor is inherently less responsive to irradiation, the drug’s effects were manifested more as enhanced growth delay than as increased local control. Moreover, the distribution between the immediate dosing and the 4-h delay is more clearly defined. As with Meth-A tumor, there was no significant growth delay observed with lonidamine alone (100 mg/kg).

Fig. 3 displays the pharmacokinetic data on serum levels of lonidamine in BALB/c mice that were given injections i.p. of lonidamine suspension (10, 50, and 100 mg/kg) in carboxymethyl cellulose. Peak sera concentrations occurred within $\frac{1}{2}$ h post drug administration. The drug concentration in sera exhibited a distribution between the immediate dosing and the radiation treatment. When the radiation dose that produced local tumor control dose of animals 50% was compared for Meth-A bearing mice receiving radiation plus lonidamine (100 mg/kg) or single dose radiation alone, we obtained a dose modifying factor of 1.36 (Fig. 4). Comparably treated RIF tumor bearing animals exhibited dose modifying factor...
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Fig. 3. Pharmacokinetic data of serum level of lonidamine in BALB/c mice.

Fig. 4. Probability of tumor recurrence as a function of single dose irradiation. Lonidamine (100 mg/kg) was administered into Meth-A tumor bearing mice immediately before single dose radiation. The dose modification factor is 1.36 (50% tumor control dose: 30/22 Gy = 1.36).

Fig. 5. Probability of tumor recurrence of RIF fibrosarcoma as a function of X-irradiation. The drug was given immediately prior to X-irradiation. Two different concentrations of lonidamine (50 and 100 mg/kg) were used.

Fig. 6. Mean skin reaction as a function of single dose irradiation with or without lonidamine administration. The drug was given either before (1 and 3 h) or after irradiation. Bars, SD.

Table 1 shows the tumor control rate of Meth-A tumor as a function of fractionated radiation treatments. It is apparent that the potentiating effect of the drug is also seen with fractionated irradiation.

Normal Tissue Effects. Fig. 6 shows the average skin reactions following radiation or radiation plus lonidamine. The contra-lateral thighs of tumor bearing mice were exposed to varying doses of radiation alone and in conjunction with the drug. There was no disproportionately enhanced skin reaction from the combined treatments. Rather, there was a tendency for the normal tissue reaction from the combination to be slightly less than those of radiation alone. More detailed studies with other organs and tissues are needed to firmly establish this observation.

DISCUSSION

The data herein presented demonstrate that the combination of radiation with lonidamine produces increased tumor growth delay and local control rates, apparently without a comparable increase in the reaction of the normal tissues. Lonidamine did not need to be present at the time of irradiation to produce at least partial increase in the radiation-induced antitumor effect (Figs. 1 and 2); in this regard, it differs from the electron affinic radiation enhancing agents, indicating that its effects are produced by a different mechanism. It has been suggested that lonidamine interferes with some aspects of the repair of radiation induced injury; the theoretical basis for that suggestion is the drug's established inhibitory effects on energy metabolism and the energy-dependent nature of the repair processes. Hahn et al. (14) have provided experimental support for the concept, reporting that the drug inhibits recovery from radiation-induced potential lethal damage.

The magnitude of lonidamine-induced potentiation of radiation-
induced tumor regression was dependent both on the tumor variety and on the timing and sequence of drug administration and irradiation treatment. In single dose studies the maximal effect was observed when the drug was administered immediately prior to or after irradiation. This is consistent with drug interference in a process that is initiated immediately following irradiation, but one that continues for at least several hours thereafter.

Although a drug dose-effect relationship was not examined in the more radiosensitive tumor, in the RIF fibrosarcoma a dosage of 100 mg/kg was clearly superior to 50 mg/kg (Fig. 5). The pharmacokinetic data demonstrate that both the respective peak concentrations and the height and duration of the steady state levels are dose dependent. The enhanced effect might relate to either the higher initial peak concentration or to maintenance of a drug level on a more prolonged basis. The impact of chronic drug exposure was examined by giving lonidamine on a daily ×5 basis with both single and fractionated dose irradiation. The potentiating effect of multiple fractions of radiation by the drug appears to be maintained (Table 1). This effect also differentiates lonidamine from the hypoxic radiosensitizers wherein the drug-induced enhancement ratio by electron affinic agents decreases with reoxygenation during fractionated irradiation (15). The fact that radiation enhancement effectiveness of lonidamine was maintained with fractionated irradiation is consistent with the dose modifying factor curves in Figs. 4 and 5. The drug’s effects are readily detectable in the lower dosage range of 20 Gy for the Meth-A tumor and 36 to 40 Gy for the RIF fibrosarcoma.

Recent cell culture studies of human tumor cells suggested that a high level capacity to repair PLD might be responsible for the radioresistance of human tumors (16); this would be an appropriate enzymatic target for radiation-enhancing drug therapy. Several well described inhibitors of PLD repair have been tested in in vitro tumor systems as candidate radiosensitizers. Purine nucleoside analogues (e.g., deoxyadenosine, 9-ß-D-arabinofuranosyladenine) were found to enhance tumor control rates (17); the magnitude of the potentiation is similar to that we have reported here following a single dose of lonidamine. Hahn et al. (14) reported inhibition of PLD repair in Chinese hamster cells at drug levels of 10 and 25 µg/ml. These drug concentrations are well within the range we have observed in the serum of drug levels of 10 and 25 µg/m. These drug concentrations are (14) reported inhibition of PLD repair in Chinese hamster cells at drug levels of 10 and 25 µg/ml. These drug concentrations are dose modifying factor curves in Figs. 4 and 5. The drug’s effects are readily detectable in the lower dosage range of 20 Gy for the Meth-A tumor and 36 to 40 Gy for the RIF fibrosarcoma.

Lonidamine has been in clinical trial in cancer patients for some years (18, 19). These studies suggest that lonidamine causes minimal inhibitory effects on the rapidly proliferating systems, including hematopoietic and gastrointestinal systems. The principal observed side effects have been limited to the skeletal muscle discomfort. The plasma concentration achieved with 430 mg/m²/day (given in 3 divided doses) is at the level of about 15 µg/ml (19, 20). This plasma concentration is sufficient to inhibit the PLD repair observed in cell culture experiments. Since the PLD repair process is prominent with low doses of radiation, it is expected that the radiation enhancement of lonidamine would increase with fractionated irradiation. Cell culture data obtained with studies with multi-cellular tumor spheroids show that lonidamine enhancement of growth inhibition of these in vitro tumors increases with fractionation schedules. This finding may be very significant in the clinical setting, where most radiation treatment schedules involve multiple fractions given over several weeks.

Although the radiation enhancing effect of lonidamine is firmly established, both following single doses and fractionated irradiation with chronic drug administration, many practical and biochemical considerations remain to be resolved. When the drug is given chronically how long should it be administered for optimal effect? What dose-effect relationships apply in chronic usage? What is the range of tumors that can be affected? Throughout all the above discussion it has been assumed that the drug is altering tumor cell sensitivity to irradiation; it is also conceivable that irradiation could affect cellular metabolism in a manner that would sensitize cells to the effects of the drug. Although the drug is not broadly cytotoxic, testicular cells are extremely sensitive to its effect. The question is readily explicable in cell culture systems; results of studies in tumor cell spheroids which suggest the existence of a radiation-induced alteration in sensitivity to lonidamine will be presented in a subsequent report.

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