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Lonidamine: *In Vitro/In Vivo* Correlations

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LONIDAMINE, 1[(2,4-dichlorophenyl)methyl]-1H-indazole-3-carboxylic acid, was first prepared and studied in the mid-1970s as an anti-spermatogenic agent [1]. It was soon recognised that mitochondria and, consequently, cellular energy metabolism were targets for the observed anti-spermatogenic activity. The potential application of lonidamine to malignant disease was quickly realised, and preclinical development of lonidamine in cancer began [2]. Lonidamine is interesting as an anti-cancer agent for two reasons: (1) it has a unique mechanism of action and (2) it has a unique spectrum of normal tissue toxicities.

Numerous careful and elegant studies on the mechanism of lonidamine cellular effects focused on the mitochondria and oncellular energetics. These studies identified mitochondrial hexokinase as an enzymatic target for this drug [3–6]. Later studies, however, found that lonidamine alters properties of the inner surface of the plasma membrane of cells as well as damaging both the inner and outer mitochondrial membranes, resulting in

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the inhibition of cellular respiration and glycolysis [7, 8]. As data accrued, it became evident that lonidamine was not equally effective in all cell types, and that the mitochondrial effects of lonidamine can be reversible if the drug is removed after a short period of exposure [9]. As a single agent, lonidamine was an active anti-cancer agent in some of the traditional transplantable tumours, but was inactive in others [2].

Given its effect on cellular energy metabolism, and non-overlapping normal tissue toxicity profile, lonidamine appeared to have the potential to be an important component in combination chemotherapy and combined modality regimens. It was hypothesised that inhibition of cellular energy production by lonidamine would prevent energy-dependent repair of the damage to cellular DNA produced by genotoxic treatments. Cell culture studies carried out in laboratories around the world demonstrated positive effects, i.e. increased cell killing with combinations of lonidamine with radiation, hyperthermia, antitumour alkylating agents and anthracyclines [10–12]. Most of these *in vitro* experiments involved relatively acute (24 h or less) exposure of the cells to relatively high concentrations (500–500 μM) of lonidamine prior to, during and after a short (1 h or less) exposure to the cytotoxic therapy. Scheduling studies with lonidamine with chemotherapeutic agents showed that, in general, lonidamine treatment for a short period (24 h or less), beginning immediately after the cytotoxic therapy, achieved maximal cell killing. These findings are completely consistent with the proposed hypothesis that inhibition of cellular energy-dependent repair processes by lonidamine results in fixation of DNA damage, strand breaks or crosslinks, by the genotoxic treatments. Kim and colleagues [13, 14] carried out *in vivo* tumour experiments to determine whether lonidamine, when combined with radiation, could potentiate the cytotoxic effects of radiation on two murine solid tumour models. The combined effects of single acute lonidamine (100 mg/kg) and single-dose X-irradiation were evaluated. The radiosensitising effect by lonidamine was maximal when it was administered immediately prior to or immediately after X-irradiation. The potentiating effects of lonidamine on radiation therapy may be attributed, in part, to the findings of cell culture studies that lonidamine is a potent inhibitor of repair of potential lethal damage.

Resistance to many anti-cancer agents involves alterations at the level of the plasma membrane including: (1) the multidrug-resistance efflux pump (GP170) in doxorubicin, vincristine, vinblastine, etoposide, etc. resistant cell lines; (2) altered amino acid and choline transporters in melphalan and nitrogen mustard resistant cell lines, (3) an altered membrane transporter in antifolate (methotrexate) resistant cell lines and (4) reduced drug uptake by an, as yet, not fully defined mechanism in cisplatin-resistant cell lines. Many of these transport/efflux processes are energy requiring. Several reports have shown that co-exposure and/or immediate sequential exposure of drug-resistant cells to lonidamine at concentrations at 100–500 μm (24 h or less) can increase the sensitivity of the cells to the drug. In most cases, the sensitivity of the parental (wild type) cells to the cytotoxic drug was also increased by co-exposure to lonidamine, but in most cases, complete reversal of resistance to the cytotoxic drug by co-exposure to lonidamine in the resistant cell line was not achieved [15–20].

There have been fewer reports concerning lonidamine in combination with other anti-cancer treatments in *in vivo* model systems [14, 21–24]. Most of the published preclinical solid tumour studies with lonidamine in combination with radiation, hyperthermia or chemotherapeutic agents have involved relatively acute, high doses (50–100 mg/kg per day) of lonidamine administered essentially concurrently with the cytotoxic therapy. When lonidamine was combined with multiple-dose chemotherapeutic regimens, it was administered once to twice daily on the same schedule as the cytotoxic agent [21–24]. The results of these studies indicate that lonidamine has the potential to increase the efficacy of anti-neoplastic alkylating agents, without a reduction in the dosage of the alkylating agents, and that a greater potentiation of the effect of the alkylating agents may occur in the tumour compared with the bone marrow.

In the current volume, Villa and colleagues (pp. 1534–1540) have shown that, in two human colon carcinoma cell lines, exposure to a relatively high concentration of lonidamine (150–225 μM) for 24 h immediately after a 1-h treatment with mitomycin or BCNU could lead to increased killing of the tumour cells.

Lonidamine has been in clinical trials in cancer patients for more than 5 years [25–34]. By necessity, most of these trials have been in patients with advanced disease. The trials have included many of the major solid tumours including non-small cell lung cancer, head and neck cancer and colorectal cancer. The studies have included combinations of lonidamine with radiation therapy, single chemotherapeutic agents and combination chemotherapy regimens. In many of these clinical trials, lonidamine was administered chronically for 3 months or until progression of disease. The strategy of administering lonidamine chronically was probably derived from the early history of this drug as an anti-spermatogenic agent, and because it is possible to maintain patients on lonidamine chronically without untoward toxicity. The drawbacks of scheduling lonidamine administration in this manner in cancer therapy may be 2-fold. First, acute, high intratumoral concentrations of lonidamine may not be achieved. Second, chronic exposure of tumours to a drug often leads to resistance to the drug. Although cell lines resistant to lonidamine have not been reported, biochemical pathways to resistance, such as increased levels of hexokinase, altered hexokinase, or altered pathways of energy production, can be envisaged to occur in tumour cells leading to, at least, tolerance toward lonidamine. Many of these clinical trials have shown marginally positive results for patients maintained chronically on the drug. The randomised trial of MACC (methotrexate/adriamycin/cyclophosphamide/CCNU) chemotherapy with and without lonidamine in advanced non-small cell lung cancer reported by Buccheri and associates (pp. 1424–1431) in this volume may represent the definitive study of this type.

In contrast, Franchi and colleagues (pp. 1420–1423) have attempted to mimic a treatment schedule as described herein by Villa and colleagues (pp. 1534–1540), and similar to many preclinical *in vitro* and *in vivo* studies, giving lonidamine acutely at high dose (900 mg once) during the 24 h after BCNU and mitomycin C. The rationale here, like the laboratory rationale, is to inhibit, during a short critical time period, the energy-dependent repair processes related to the DNA damage produced by BCNU and mitomycin C. The study of Gadducci and colleagues (pp. 1432–1435) is also an interesting departure from the traditional chronic administration schedule for lonidamine. Gadducci and colleagues (pp. 1432–1435) were treating refractory or recurrent ovarian cancer with epidoxorubicin. In these patients, drug efflux via the multidrug resistance pump GP170 may be anticipated, therefore, lonidamine was administered for 2 days prior to, during and, to inhibit repair processes, for 2 days after administration of the anthracycline. The studies of

Franchi and colleagues (pp. 1420–1423) and Gadducci and colleagues (pp. 1432–1435), albeit relatively small in patient numbers, provide promising clinical results.

The translation of laboratory findings to the clinic is a process frought with pitfalls. The best advice may be to keep in the forefront of planning the desired biological effect. With lonidamine, as we understand its action as a modulator in combination therapy, the acute, maximal deprivation of cellular energy at the critical moment when repair of DNA damage is required for cellular survival, would argue for short high-dose regimens rather than lower dose prolonged treatments. Lonidamine, as evidenced by the four papers presented in this volume (pp. 1420–1423, 1424–1431, 1432–1435, 1534–1540), is a unique and interesting agent with the potential to be an important addition to cancer therapy.

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