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Review

Mechanism of antineoplastic activity of lonidamine

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ABSTRACT

Lonidamine (LND) was initially introduced as an antispermatogenic agent. It was later found to have anticancer activity sensitizing tumors to chemo-, radio-, and photodynamic-therapy and hyperthermia. Although the mechanism of action remained unclear, LND treatment has been known to target metabolic pathways in cancer cells. It has been reported to alter the bioenergetics of tumor cells by inhibiting glycolysis and mitochondrial respiration, while indirect evidence suggested that it also inhibited L-lactic acid efflux from cells mediated by members of the proton-linked monocarboxylate transporter (MCT) family and also pyruvate uptake into the mitochondria by the mitochondrial pyruvate carrier (MPC). Recent studies have demonstrated that LND potently inhibits MPC activity in isolated rat liver mitochondria (K_i 2.5 μM) and cooperatively inhibits L-lactate transport by MCT1, MCT2 and MCT4 expressed in *Xenopus laevis* oocytes with $K_{0.5}$ and Hill coefficient values of 36–40 μM and 1.65–1.85, respectively. In rat heart mitochondria LND inhibited the MPC with similar potency and uncoupled oxidation of pyruvate was inhibited more effectively (IC_{50} ~ 7 μM) than other substrates including glutamate (IC_{50} ~ 20 μM). LND inhibits the succinate-ubiquinone reductase activity of respiratory Complex II without fully blocking succinate dehydrogenase activity. LND also induces cellular reactive oxygen species through Complex II and has been reported to promote cell death by suppression of the pentose phosphate pathway, which resulted in inhibition of NADPH and glutathione generation. We conclude that MPC inhibition is the most sensitive anti-tumour target for LND, with additional inhibitory effects on MCT-mediated L-lactic acid efflux, Complex II and glutamine/glutamate oxidation.

Contents

Abbreviations: LND, lonidamine; MIBG, meta-iodobenzyl guanidine; FCCP, carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone; GSH, glutathione; ROS, reactive oxygen species; SDH, succinate dehydrogenase; SQR, succinate-ubiquinone reductase; TCA, tricarboxylic acid; TTFA, 4,4,4-trifluoro-1-(2-thienyl)-1,3-butanedione; QH_2, ubiquinone dihydride; NAD, nicotinamide adenine dinucleotide; FAD, flavin adenine dinucleotide; MRS, magnetic resonance spectroscopy; MCT, monocarboxylate transporter; MPC, mitochondrial pyruvate carrier; ETC, electron transport chain; PPP, pentose phosphate pathway; pHi, intracellular pH; pHe, extracellular pH; i.p., intraperitoneal; i.v., intravenous; s.c., subcutaneous; NTP, nucleoside triphosphate; CHC, α-cyano-4-hydroxycinnamic acid.

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1. Introduction

Lonidamine (LND), first introduced in 1979 as an antispermatogenic agent [1], has limited antineoplastic activity as a single agent but has exceptional potential in modulating the activities of conventional chemotherapeutic agents such as N-mustard alkylating agents [2–7] and anthracyclines [8] as well as hyperthermia [9–11], radiation therapy [12,13] and photodynamic therapy [14]; it may also enhance response to targeted therapeutics such as vemurafenib. The most critical property of LND is its selective activity against a broad range of tumors with little to no effect on normal tissues provided that doses are below a threshold level of ~400 mg/m^2 (oral or i.v. doses) [15,16]. At such doses LND causes selective intracellular cytosolic acidification of tumors while diminishing tumor ATP levels.

Current evidence indicates that LND inhibits lactate export by the proton-linked monocarboxylate transporter(s) (MCT) and pyruvate uptake into mitochondria via the mitochondrial pyruvate carrier (MPC), whereas inhibition of respiration involves both diminished mitochondrial uptake of pyruvate via the MPC as well as inhibition of the mitochondrial electron-transport chain at Complex II and perhaps also Complex I, in both instances at the ubiquinone reduction step. There is also evidence that the drug may indirectly inhibit hexokinase [17–20] as well as possibly other glycolytic and pentose shunt enzymes as a result of cytosolic acidification. LND produces a substantial increase in total tumor lactic acid levels with most of the lactate being trapped in the cytosol as indicated by a pronounced decrease in intracellular pH (pHi); there is also a slight decrease in extracellular pH (pHe) reflecting a small extent of leakage of lactate through the MCT [6–8]. However, direct evidence for LND inhibition of the MPC, any of the four MCT isoforms known to transport lactic acid [21] as well as inhibition of mitochondrial electron-transport has until recently been lacking. In this review article, we present data addressing these issues.

2. Contemporary background

In 1981, Floridi et al. [22] reported that LND inhibited respiration as well as aerobic and anaerobic glycolysis in Ehrlich ascites tumor cells but had no effect on normal rat sertoli cells. They attributed the selective inhibition of glycolysis in tumor cells to LND binding to and inhibiting mitochondrial bound hexokinase that the Pedersen lab had shown [23] existed mainly in tumor cells. In 1982, Floridi et al. [24] demonstrated that LND affected respiration of Ehrlich ascites cells only in the uncoupled state but not in the coupled state. Scatchard analysis indicated two classes of binding sites in uncoupled mitochondria, a high affinity site with dissociation constant (K_d) of 3.2 μM and a weaker but more highly populated site with K_d of 45 μM. Binding of LND to normal liver mitochondria was qualitatively similar but with lower affinity than binding to tumor mitochondria. Further studies of LND inhibition of the mitochondrial electron transport chain of Ehrlich ascites cells were reported by Floridi and Lehninger in 1983 [25]. They concluded that LND was bound to mitochondria in state 4 but did not inhibit respiration in this state except at concentrations above 200 μM. Half-maximal inhibition of respiration of FCCP (carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone)-stimulated tumor mitochondria occurred at 32 μM LND with almost complete inhibition at LND concentrations greater than 200 μM. They concluded that LND shifted mitochondrial NAD(P) into a more oxidized steady state. Subsequent addition of an uncoupler or ADP, which would normally induce respiration, increased LND binding and inhibition of reduction of NAD(P) by mitochondrial dehydrogenases. The precise mechanism of interaction with the mitochondrial electron transport chain was not clearly defined although the authors noted that LND appears to affect the state 4 to state 3 transitions. It is noteworthy that the authors recognized that LND inhibits the reduction of NAD(P) by various NAD-linked substrates (pyruvate + malate, α-ketoglutarate and glutamate) and that it inhibits succinate dehydrogenase at some point prior to the reduction to ubiquinone.

In 1995 Ben-Horin et al. [26] reported ^{31}P and ^{13}C NMR (nuclear magnetic resonance) spectroscopic studies of isolated perfused MCF7 breast cancer cells immobilized by encasement in calcium alginate beads. By incorporating Pi (inorganic phosphate) into the perfusate, the authors were able to detect two Pi resonances, which they used to simultaneously monitor both the pHi and pHe, respectively. LND produced a decrease in pHi with no effect on pHe. Furthermore, ^{13}C NMR demonstrated that this decrease in pHi was accompanied by accumulation of lactic acid in the intracellular compartment and depletion of extracellular lactate. The evidence clearly pointed to inhibition of lactate export (i.e., via MCTs) as a key mechanism responsible for LND-induced tumor acidification. Rather than inhibiting glycolysis, the accumulation of intracellular lactate indicated that LND was stimulating glycolysis, although kinetic inhibition of certain steps along the glycolytic pathway might still be occurring since it is well known that enzymes such as phosphofructokinase are subject to allosteric H^+ inhibition. These investigators also noted that LND decreased NTP (nucleoside triphosphate) levels in the tumor, which also decreased phospholipid metabolites as a consequence of diminution of choline and ethanolamine kinase activities. The decrease in the bioenergetic status of the tumors was attributed to the effect of LND on mitochondrial metabolism that had been reported by Floridi et al. [25].

Mardor et al. [27] combined NMR studies of MCF7 cells with diffusion-weighted imaging of perfused cells, a method that eliminates signals from metabolites in the perfusate. This ability to distinguish between intracellular and extracellular compartments further supported the conclusions of Ben-Horin et al. [26].

These NMR studies of breast cancer cells were confirmed and extended by studies of 9 L glioma cells and 9 L glioma xenografts by Ben-Yoseph et al. [28]. Using ^{31}P NMR, these investigators demonstrated that LND produced intracellular acidification and de-energization both in culture and *in vivo* and showed that the *in*