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Doxorubicin plus Ionidamine: *in vivo* metabolic effects on the rat heart

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Lonidamine (LND) is a new drug that interferes with mitochondrial functions, thereby inhibiting cellular oxygen consumption and energy metabolism in both normal and neoplastic cells. These metabolic actions of LND seem to increase the cytotoxic effect of antitumor agents such as doxorubicin (Dx). Dx is a widely used antitumor agent, but the specific cardiac toxicity which develops at a critical cumulative dose is the major limiting factor in its long term use. So far, nothing is known about a possible synergic action of LND and Dx on the metabolism of cardiac cells. The purpose of this study was to verify in an experimental model *in vivo*, whether LND could increase the toxicity of Dx on rat heart. Groups each consisting of 10 female Wistar rats (weighing 100–150 g) were injected ip with a single dose of Dx (10 mg/kg), LDN (50 mg/kg), or Dx plus LND and dimethylsulfoxide (DMSO) (ratio LND:DMSO = 1/10). After 24 h, oxygen uptake (QO_2) and intracellular concentrations of ATP and GTP indices of cardiac metabolic impairment, were measured on heart slices in Warburg apparatus and by high-pressure liquid chromatography. Dx, significantly ($p < 0.01$), reduced QO_2 (34%) and intracellular concentration of ATP and GTP (32–57%). LND alone only partially reduced cardiac QO_2 (23%) and intracellular ATP–GTP concentration (16–31%). By contrast, the combination of the two agents did not enhance Dx-related metabolic cardiac toxicity.

Key words: Cardiac toxicity, doxorubicin, Ionidamine.

Introduction

Over recent years, the chemotherapy of solid tumors, particularly of the breast, gastrointestinal and urinary tracts, has not given completely satisfactory results: even the use of new anticancer drugs such as 4′-epidoxorubicin, mitoxantrone, carboplatin and ifosfamide has not significantly improved the objective response rate.[1,2] Promising initial results have, however, been obtained with the use of lonidamine (LND), a heterocyclic aromatic compound, in conjunction with standard combination chemotherapy.[3] LND is a new generation drug with a low toxicity which interferes with cellular energy metabolism and, unlike the antiproliferative agents, does not have nucleic acids as its target.[4]

LND acts on the mitochondria of tumor cells, inhibiting oxygen consumption and decreasing aerobic glycolysis by its effects on mitochondrially bound hexokinase.[5] Through this metabolic activity LND slows the repair processes of cancer cells, thus potentiating the cytotoxic action of ionizing radiation, hyperthermia and some antiproliferative agents.[6–13]

One of these agents is doxorubicin (Dx) the cytotoxicity of which is significantly potentiated by LND.[9] The underlying mechanisms of this marked synergism are a higher permeability of the outer mitochondrial membrane to Dx in Ehrlich ascites tumor cells and an increased intracellular retention of the anthracycline in MCF7 cells.[14,15]

This is a particularly interesting finding if we consider the fact that Dx is, without doubt, the drug with the broadest spectrum of antitumor activity.[16] Nevertheless, it is equally well known that dose-dependent, irreversible cardiotoxicity is a strong limiting factor in the prolonged use or high doses of Dx.[17]

Dx not only interacts with nucleic acids but also acts on cytoplasmic membranes and inhibits the mitochondrial respiratory chain. This results in a decreased oxygen consumption in cardiac muscle cells and a fall in the intracellular concentration of ATP and phosphorylcreatine.[18,19]

The aim of the present study was to determine in an experimental model, already used for evaluating the cardiac and organ toxicity of cytotoxic agents,[18,20,21] whether the cardiotoxicity of Dx is increased when it is combined with LND.

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Materials and methods

Animals

Female Wistar rats, weighing between 100 and 150 g were used. All were kept under the same environmental and nutritional conditions. The animals were divided into six groups of 10 rats each, including one group of untreated control animals who received NaCl 0.9% 250 μl ip. The other five groups were treated with Dx, DMSO, LND-DMSO, Dx plus LND-DMSO, and Dx plus DMSO, respectively. Each rat was treated with a total volume of 250 μl administered ip as a single dose: three days later the rats were decapitated, the hearts quickly removed and used for oxygen uptake measurements and for nucleotide analysis.

Chemicals

Doxorubic hydrochloride, was supplied by Farmitalia-Carlo Erba, Milan, Italy. The drug was dissolved in distilled water just before use. LND, supplied by the Francesco Angelini Research Institute, Rome, Italy, was dissolved in dimethylsulfoxide (DMSO) in the proportion of 1/10. The dose of Dx (10 mg/kg) and of LND (50 mg/kg) administered ip to each rat, corresponded to the dose usually used in human anticancer therapy.

Oxygen uptake

Oxygen uptake was measured manometrically in a Warburg apparatus. Heart slices from control and treated rats, weighing about 30 mg (wet weight) were incubated in flasks containing 3 ml of Krebs–Ringer phosphate solution, pH 7.4, in air for 60 min.[22]

Nucleotide assay

Following Bergmeyer's method,[23] we used deproteinized extracts to establish the ATP and GTP concentrations in the hearts of control and treated rats. Assays were undertaken using high-pressure liquid chromatography (HPLC; Waters Associates, Milford, MA, USA) equipped with a 590 solvent delivery system, a U6 K universal liquid chromatograph injector, a Z-module radial compression separation system, a 481 variable wavelength detector set at 254 nm, and a model 730 data module.

A 100 × 8 nm internal diameter Radial-Pak cartridge prepacked with quaternary-amine (SAX) bonded to 10 μl silica (Waters Associates) was used for the chromatographic analysis of all samples. During the mobile phase, 0.25 M Na_2HPO_4 + 0.5 M NaCl, pH 4.9, were filtered through a hydrophilic Durapore 0.45 μm filter unit (Millipore, Bedford, MA, USA).

Water was prepared for use as a chromatography eluent by passage through a Milli-Q water purification unit (Millipore) and pumped through the column at a flow rate of 6 ml/min. All separations took place at room temperature. The volume of samples injected varied from 100 to 400 μl. Over the range explored, namely, 5–200 nmol injected, the relationship between the nucleotide's peak area and amount of ATP was linear.

Statistical analysis

Results were expressed as means ± standard error of the mean; p values were calculated from Student's t-test, and values >0.05 were not considered significant.

Results

The oxygen consumption of heart slices of treated and control rats is reported in Table 1. The greatest inhibition of QO_2 occurred after 60 min incubation in rat hearts treated with Dx (−34%), the difference being statistically significant compared to the controls ($p < 0.01$). The same level of QO_2 inhibition was obtained in rats treated with Dx plus

Table 1. Endogenous respiration of heart slices of treated rats

Treatments	QO_2 at incubation time	
	30 min	60 min
None (controls)	2.66 ± 0.10	4.57 ± 0.13
Doxorubicin	1.73 ± 0.08	3.02 ± 0.17
Dimethylsulfoxide	2.61 ± 0.11	4.40 ± 0.35
Lonidamine dimethylsulfoxide	2.20 ± 0.09	3.50 ± 0.19
Doxorubicin plus dimethylsulfoxide	1.79 ± 0.11	3.20 ± 0.21
Doxorubicin plus lonidamine–dimethylsulfoxide	1.82 ± 0.09	3.17 ± 0.17

Rates of oxygen uptake (μl/mg wet weight) measured by the manometric Warburg apparatus at 38°C for 60 min. Entries refer to mean ±SE of 9–12 determinations.

Table 2. Intracellular concentrations of adenosine triphosphate and guanosine triphosphate in heart slices of treated rats

Treatments	ATP	±%[a]	GTP	±%[b]
Controls	2775 ± 88		309 ± 55	
Doxorubicin	1885 ± 36	−32	132 ± 25	−57
Doxorubicin + DMSO	1927 ± 61	−30	141 ± 29	−59
Doxorubicin + LND-DMSO	2006 ± 59	−28	156 ± 27	−49
DMSO	2341 ± 59	−16	217 ± 39	−30
LND-DMSO	2321 ± 51	−16	214 ± 34	−31

ATP and GTP concentrations (μmol/g wet weight) measured by HPLC. Entries refer to mean ± SE of 9–12 determinations.
[a] Change (%) in ATP level compared to controls.
[b] Change (%) in GTP level compared to controls.

DMSO (−30%) and Dx plus LND-DMSO (−31%). The degree of QO_2 reduction in rat hearts treated with LND-DMSO was less marked (−23%) but it was still significantly different from the control values ($p < 0.01$). DMSO alone, the solvent of LND, caused no significant changes in cardiac QO_2 (−4%).

Although Dx combined with LND-DMSO caused a marked decrease in QO_2 (−31%) with respect to the controls, it was not significantly different ($p > 0.05$) from the decrease in QO_2 obtained with Dx alone (−34%).

Table 2 shows the cardiac intracellular concentrations of ATP and GTP in rats undergoing different types of treatment. As in the case of endogenous cellular respiration, Dx caused a marked fall in the level of the two nucleotides (ATP −32%, GTP −57%) with respect to the untreated controls ($p < 0.01$). Similar results were obtained in rat hearts treated with Dx plus DMSO (ATP −30%, GTP −59%) and Dx plus LND-DMSO (ATP −28%, GTP −49%).

Treatment with DMSO alone and LND-DMSO caused the intracellular nucleotide concentrations to fall to a lesser extent (ATP −16% in both cases, GTP −30% and −31% respectively). The drop in the intracellular ATP and GTP levels was no greater with Dx plus LND-DMSO than with Dx alone (ATP −28% vs −32%; GTP −49% vs −57%) ($p > 0.05$).

Finally, it should be stressed that each type of treatment caused the cardiac GTP to decrease twice as much as the ATP.

Discussion

Over recent years we have seen a growing interest in the use of new drugs in cancer chemotherapy. One such drug is LND which potentiates the cytotoxic action of antiproliferative agents,[9–13] although it has only a narrow spectrum of antitumor activity in experimental systems used for screening drugs affecting cell division. A particular problem is encountered, however, in combining it with Dx without increasing serious side effects or cardiotoxicity. The main mechanisms involved in the cardiotoxic effects of the anthracycline antibiotics are cell membrane damage,[25] inhibition of endogenous cellular respiration[20] and a slowing of the mitochondrial oxidative pathway[26] with a consequent decrease in the intracellular levels of ATP.[18] No direct cardiotoxic effects have been reported for LND, which acts by disrupting the energy metabolism of tumor cells. It depresses cellular respiration and also decreases aerobic glycolysis by inhibiting mitochondrially bound hexokinase. We therefore thought it would be useful to evaluate the cardiac effects of Dx-LND combined, in view of the metabolic activities of the two drugs.

The results of this study first of all confirm published data on the effects of Dx on the energy metabolism of cardiac muscle cells. We found that it caused a marked decrease in endogenous cellular respiration (QO_2 −32%) and intracellular ATP levels (−35%) associated with a fall in the GTP levels almost twice that level (−57%).

This observation cannot be considered to be only casual. We may in fact suppose that in emergency situations occurring for instance in cardiac cells treated with Dx, the resultant ATP deficiency can be partly compensated by phosphocreatine, by glycolysis which in turn is activated by the decrease in oxygen consumption, and by the transformation of cardiac GTP into ATP.[27] At this point, however, Dx-induced mitochondrial damage would prevent optimal levels of GTP from being restored through

the Krebs cycle and would explain why particularly low levels of GTP are present during treatment.

As far as LND is concerned, we found, as may have been expected, a decrease in cardiac cellular respiration (−23%) and nucleotide content (ATP −16%, GTP −31%) with respect to the controls while DMSO, the solvent of LND, does not appear to interfere significantly with cardiac metabolism, either alone or combined with Dx.

The most noteworthy finding in our opinion was that the Dx–LND combination did not enhance cardiotoxicity on the basis of the indices used in our experimental model which has already in the past proved to be a reliable indicator of expected clinical results.[28] Both the QO_2 and the intracellular ATP and GTP levels fall to the same extent as with Dx alone and there was no significant difference between the effects of the two types of treatment ($p > 0.05$). The results of this study together with the data reported in the literature on the potentiation of the cytotoxic activity of Dx by LND offer the possibility of using anthracycline derivatives under optimal conditions.

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