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Original Paper

Intrapatient Comparison of Single-agent Epirubicin with or without Lonidamine in Metastatic Breast Cancer

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The aim of this study was to determine if lonidamine (LND) supplementation to single-agent epirubicin (EPI) could reverse anthracycline resistance in patients with metastatic breast cancer. 45 patients with metastatic breast cancer were treated with EPI 120 mg/m² by intravenous (i.v.) bolus every 3 weeks. Patients who progressed were given the same chemotherapy regimen on day 4 in combination with oral LND, 150 mg on day 1, 300 mg on day 2 and 450 mg on days 3–5. Among the 40 evaluable patients, 6 complete responses (CR) and 14 partial responses (PR) were achieved with EPI treatment alone for an overall response rate of 50%. The median duration of response was 6.5 months. Among the 25 patients treated with EPI+LND, 5 PR (21% of 24 evaluable patients) were observed with a median duration of response of 7 months. The median survival in patients receiving both treatments was 20 months. The survival for all patients was 18 months. The survival of patients receiving LND was not significantly longer than for the other patients. Myelotoxicity was the most common side-effect followed by alopecia, nausea and vomiting, and stomatitis. LND-related toxic effects were mild-to-moderate epigastralgia and myalgia. Anthracycline-related toxicity was the same in the two treatment groups. This study indicates that LND may circumvent clinical resistance to EPI without altering the pattern or severity of the toxicity of this anthracycline. Continued investigation of the clinical modulation of EPI resistance by LND in breast cancer is warranted, hopefully in patients with known multidrug resistance status.

Key words: epirubicin, lonidamine, breast cancer, multidrug resistance, chemotherapy
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INTRODUCTION

ANTHRACYCLINES ARE widely considered to be the most effective drugs in the treatment of breast cancer. However, repeated courses of these agents are frequently associated with a decreased therapeutic response accompanied by the emergence of drug-resistant cell lines, ultimately limiting the treatment's usefulness. Acquired drug resistance to the anthracyclines is characterised by simultaneous resistance of cancer cells to other chemically unrelated compounds. This phenomenon has been termed "multidrug resistance" (MDR), and is often associated with overexpression of a membrane protein, the P-glycoprotein [1, 2]. A high incidence of P-glycoprotein overexpression has been reported in patients with breast cancer, especially in those previously exposed to chemotherapy [3, 4], and in some instances, a strong P-glycoprotein-positive staining was significantly correlated with poor response to cytotoxic treatment [5].

Reversal of MDR has been accomplished *in vitro* by a variety of agents, including verapamil, trifluoperazine, quinidine and cyclosporin A [6]. Although the mechanism of action of these agents has not been fully elucidated, they are believed to function by competitively inhibiting the drug binding to P-glycoprotein, which results in increased intracellular concentration of the cytotoxic agent. Since P-glycoprotein is an energy-dependent multidrug efflux pump, agents influencing the energy metabolism of the cell may be of value in overcoming drug resistance.

Lonidamine (LND), a dichlorinated derivative of indazole-3-carboxylic acid, has been reported to interfere selectively with the energy metabolism of neoplastic cells by inhibiting their aerobic lactate production probably via damage to mitochondrial membranes [7]. *In vitro* studies have demonstrated that LND, used in combination with either doxorubicin [8] or epirubicin (EPI) [9], is able to reverse MDR in a human breast cancer cell line resistant to doxorubicin.

It has been suggested that EPI at high doses in advanced

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breast cancer is probably as effective as any of the empirically derived combination regimens containing this drug, with lower toxicity [10]. Therefore, it was considered of interest to perform a clinical study of this disease to evaluate the reversing properties of LND in patients treated with high dose EPI and receiving the same cytotoxic treatment supplemented with LND, once drug refractoriness of the disease had been documented.

PATIENTS AND METHODS

45 patients with biopsy-proven metastatic breast cancer were entered into the study. Eligibility criteria included age <75 years, WHO performance status ≤2 and progressive disease with measurable lesions. Patients may have had prior chemotherapy without anthracyclines and/or hormonal therapy and/or radiotherapy. An initial white blood cell (WBC) count ≥4000/μl and platelets ≥100 000/μl, as well as adequate hepatic and renal function (bilirubin ≤1.5 mg/dl, creatinine ≤1.5 mg/dl) were required. Patients were excluded from the study if they had a history of congestive heart failure, active cardiac disease or a left ventricular ejection fraction (LVEF) at rest <45%, a history of other malignancies (except for carcinoma *in situ* of the cervix or basal cell carcinoma of the skin), central nervous system involvement or active infection. Informed consent was obtained from all patients. The study was approved by the institutional ethics committee.

The pretreatment evaluation included a clinical history and physical examination, automated blood cell count, biochemical profile, chest X-ray, ECG (electrocardiogram), radionuclide angiography, liver ultrasound echography and a skeletal survey. When indicated, additional studies were made. Blood counts were obtained on day 1 and then at least every 7 days. Physical examination, blood chemistry tests and ECG reading were repeated at each course, whereas LVEF was determined every three courses and 4–8 weeks after the end of the treatment. The chest X-ray and liver ultrasound scanning were repeated at every alternate course if initially abnormal. Other scans and X-rays were performed every three courses.

All patients received EPI 120 mg/m^2 by intravenous (i.v.) bolus every 3 weeks. Patients who progressed were given the same chemotherapy regimen on day 4 in combination with oral LND, 150 mg on day 1, 300 mg on day 2 and 450 mg on days 3–5. Escalated doses of LND were used in an attempt to prevent or reduce drug-related toxicities, such as myalgia, epigastralgia and photophobia.

EPI administration was postponed if the WBC counts were <3000/μl and/or the platelet counts were <100 000/μl on day 22. Drug dose was reduced by 25% in instances of grade 4 haematological toxicity with infection and/or grade 4 stomatitis/mucositis, and by 50% if serum bilirubin was 1.5–3 mg/dl. Treatment was discontinued if the bilirubin was >3 mg/dl, or if there was a decrease in LVEF of >20% from basal values. Standard anti-emetics, generally metoclopramide, were used in all patients.

Cycles were repeated until disease progression, until severe toxicity precluded further therapy or until an EPI cumulative dose of 1000 mg/m^2 was reached. After reaching this cumulative dose, it was left to the responsible physician to continue treatment under careful cardiac monitoring.

Response and toxicity were evaluated according to WHO criteria [11]. Survival curves were calculated by the method of Kaplan and Meier.

RESULTS

Of 45 patients who entered this study, 40 were considered evaluable for efficacy (3 lost to follow-up after the first cycle; 2 lost due to refusal). Toxicity data were available for 42 patients. Patients' characteristics are summarised in Table 1.

In the EPI group, 6 patients achieved complete response (CR) and 14 partial response (PR) for an overall response rate of 50% (95% confidence interval (CI) 34–66%). In 14 patients, the disease remained stable. The response rate according to the dominant site of disease was 89% in 9 patients with soft tissue lesions, 40% in 20 patients with visceral disease, and 36% in 11 patients with dominant disease site in the bones. With respect to the number of disease sites, there was no significant difference between patients with one site of disease and those with two or three sites. The median duration of response was 6.5 months. The median time to response and median time to progression were, respectively, 2 and 7.5 months.

Of the 40 assessable patients treated with EPI, 25 received the combined treatment EPI + LND, and 24 were evaluable for response (1 patient developed atrial fibrillation after the first treatment cycle). The reasons for not giving the combination regimen were refusal of further anthracycline treatment, laboratory cardiotoxicity and reaching the cumulative EPI dose.

The response rate in the EPI + LND group was 21% (5 PR). Responses occurred only in patients with soft tissue lesions. Of these responses, four occurred in patients who achieved a response to EPI treatment, whereas one was observed in a patient who progressed after two cycles of EPI. The median duration of response was 7 months. 9 patients achieved stable disease lasting 4 months. The median survival in patients receiving both treatments was 20 months. The survival for all patients (n = 40) was 18 months.

Toxicity

Haematological and gastrointestinal toxicity was common (Table 2). The treatment had to be discontinued in 1 patient

Table 1. *Patients' characteristics*

	EPI	EPI + LND
Entered/evaluable	45/40	25/24
Median age, years (range)	51 (36–73)	56 (37–71)
Pre/postmenopausal	5/40	4/21
Median WHO performance status (range)	1 (0–2)	1 (0–2)
Patients with a disease-free interval of		
>1 year	18	13
1–5 years	20	8
> 5 years	7	4
Dominant disease site		
Soft tissue	10	9
Bone	11	4
Visceral	24	12
No. of disease sites		
1	19	11
2	21	11
3	5	3
Previous treatment		
Surgery	38	23
Radiotherapy	14	9
Hormonal therapy	21	10
CMF: adjuvant/advanced	16/11	10/4
None	4	0

EPI, epirubicin; LND, lonidamine; CMF, cyclophosphamide, methotrexate, 5-fluorouracil.

Table 2. Haematological and gastrointestinal toxicity (% of patients)

Toxicity	EPI	EPI + LND
Leucopenia		
G1	12	8
G2	43	52
G3	38	32
G4	7	8
Thrombocytopenia		
G1	5	16
G2	5	4
Anaemia		
G1	52	52
G2	17	8
Nausea and vomiting		
G1	36	20
G2	21	60
G3	26	12
Stomatitis		
G1	9.5	16
G2	31	16
G3	7	

EPI, epirubicin; LND, lonidamine.

who developed a bilirubin increase to 6.2 mg/dl after three courses of therapy. Moderate nausea and vomiting were recorded in the majority of patients. Severe mucositis was experienced by only 3 patients. Although leucopenia was the most common haematological side-effect, WBC counts $<1000/\mu l$ were recorded only in a few instances and required a dose reduction in 1 patient who developed a febrile episode of undetermined origin. Alopecia was universal.

Laboratory cardiac toxicity was evaluated in 30 patients who had at least two radionuclide angiographies. Absolute decreases in LVEF of >20% were recorded only in 3 patients, 2 in the EPI group and 1 in the EPI + LND group. Treatment was discontinued in these patients, and their LVEF improved to normal in the following months. There was one instance of ventricular dysrhythmia (ventricular quadrigeminy) in the EPI group, and one of atrial fibrillation in the EPI + LND group. No patient developed congestive heart failure. The median dose of EPI administered was 840 mg/m^2, with 10 patients receiving $\geq$960 mg/m^2. Overall, there was no significant difference in anthracycline-related toxicities between the two treatment groups. Other toxicities were due to the LND treatment: mild to moderate epigastralgia in 7 patients and myalgias in 24 patients.

DISCUSSION

Despite the great advances made in the chemotherapy of metastatic breast cancer, many patients fail to respond to cytotoxic agents or relapse after a good initial response. These treatment failures are generally associated with the development of drug resistance, and attempts to overcome this phenomenon have included either the administration of higher doses of anticancer drugs or the use of agents capable of reversing MDR.

In this study, both these approaches were evaluated, and the intrapatient comparison was selected since it seems to offer some advantages over randomised trials. The number of patients required is smaller and clinical resistance to particular cytotoxic agents can be unequivocally determined, allowing a more appropriate evaluation of the effects of the reversing agent.

The 50% response rate observed in this trial with EPI as single agent is in the range of that obtained with conventional doses of 75–90 mg/m^2 [12–14], and does not significantly differ from the response rates obtained in studies using a median EPI dose of 120 mg/m^2 [14–17]. This is not surprising since the maximum tolerated dose of EPI determined in earlier trials [18] was clearly underestimated, being significantly lower than the recommended dose for phase II trials (135–150 mg/m^2) determined in more recent studies [19, 20]. However, 21% of the patients receiving EPI + LND achieved a further response, therefore benefiting from a longer response duration. Unfortunately, in this study, survival of patients receiving LND was not significantly longer than that of patients given other treatments after failing EPI alone.

Only in a few studies has reversal of MDR in breast cancer patients been evaluated [21–23]. No positive results have been observed, but the design of these studies makes it difficult to assess the role, if any, of the reversing agent. In contrast, the results of the present trial clearly demonstrate that LND can overcome drug resistance in some anthracycline-resistant patients, since the only variable in the two segments of the study was LND supplementation. It is difficult, however, to establish the exact mechanism of action of this agent. LND can inhibit cell repair of sublethal damage induced by some anticancer drugs [24] or may circumvent drug resistance by increasing cellular drug accumulation, either by reduced drug efflux or by increased drug uptake [8, 25]. This may be related to a direct interaction with the P-glycoprotein or an indirect impairment of biological functions of this drug transporter through the interaction with the lipid bilayer structure and the permeability of the cell membranes [26]. Unfortunately, the MDR status of our patients was not assessed and the clinical significance of P-glycoprotein in breast cancer remains to be determined.

It may well be expected that the results of this trial can be improved. Since EPI and LND have different toxicities which were generally moderate in our patients, increased doses of both drugs can be safely given. In addition, alternative schedules of LND administration may be used [27].

Continued investigation on the clinical modulation of anthracycline resistance by LND, as well as by other agents, appears to be warranted. However, every effort should be made to analyse the MDR expression in breast cancer tissues, as well as the functional significance of P-glycoprotein. In this disease, drug resistance may be unrelated to MDR type and MDR expression does not necessarily result in refractoriness to the agent used. Only with this information will we be able to determine whether overcoming MDR will improve the effectiveness of chemotherapy in breast cancer patients.

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