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Original article

Phase II study. Concurrent chemotherapy and radiotherapy with nitroglycerin in locally advanced non-small cell lung cancer

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ABSTRACT

Background: Nitroglycerin, a nitric oxide donor agent, reduces the expression of hypoxia-inducible factor-1α (HIF-1α) and could be a normalizer of the tumor microenvironment. Both factors are associated with chemo-radio-resistance. The aim of this study was to determine the safety profile and efficacy of nitroglycerin administration with chemo-radiotherapy in patients with locally advanced non-small cell lung cancer (NSCLC).

Methods: This is a phase II trial of locally advanced NSCLC patients treated with cisplatin and vinorelbine plus concurrent nitroglycerin with radiotherapy. A 25-mg NTG patch was administered to the patients for 5 days (1 day before and 4 days after chemotherapy induction and consolidation) and all day during chemo-radiotherapy. VEGF plasmatic level was determined before and after two cycles of chemotherapy.

Results: Thirty-five patients were enrolled in this trial. Sixty-three percent of patients achieved an overall response after induction of chemotherapy, and 75% achieved an overall response after chemo-radiotherapy. The median progression-free survival was 13.5 months (95% CI, 8.8–18.2), and the median overall survival was 26.9 months (95% CI, 15.3–38.5). Reduction of VEGF level was associated with better OS. The toxicity profile related to nitroglycerin included headache (20%) and hypotension (2.9%).

Conclusions: The addition of nitroglycerin to induction chemotherapy and concurrent chemoradiotherapy in patients with locally advanced NSCLC has an acceptable toxicity profile and supports the possibility to add nitroglycerin to chemotherapy and radiotherapy. A randomized trial is warranted to confirm these findings.

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Lung cancer is the leading cause of death from cancer worldwide, including Mexico [1,2], with most cases diagnosed in advanced stages. The standard treatment for locally advanced non-small cell lung cancer (NSCLC) is concurrent chemoradiation (C-RT) with conventional fractionation [3]. This approach was supported, according to several studies (including a meta-analysis), because chemotherapy (CT) added to radiotherapy (RT) produces a 4% absolute 2-year survival benefit [4]. The prognosis of patients with locally advanced NSCLC is poor, with a median survival of 14–17 months and a 3-year survival of approximately 25% [5]. However, in a recent study that evaluated different doses of radiotherapy (RTOG 0617), the overall survival reached 28.7 months in the standard dose arm [6]. The search for new modalities of treatment that may improve the outcome in this group of patients is important [7,8]. Additionally, different drugs like cetuximab, have been found to enhance the effect of radiotherapy in an experimental model, however, these have failed to demonstrate benefit in results of clinical trial [9].

Organic nitrates, such as nitroglycerin (NTG), act through the liberation of nitric oxide (NO), producing vasodilation and increasing blood flow, and the biological effect of NO is dependent on its concentration at the site of action, which in turn decreases hypoxia [10]. Several studies have shown that NO reduces the expression of the hypoxia-inducible factor-1α (HIF-1α) [11], which is associated with both chemo and radio-resistance [12]. In addition, tumor tissue has a low oxygen tension [13,14], a condition that promotes the activation and stabilization of HIF-1α; this, in turn, activates transcription factors such as vascular endothelial growth factor (VEGF), which promotes angiogenesis, erythropoiesis, cellular growth, and metastasis [15]. Recent studies have demonstrated that anti-VEGF therapy can normalize the organization of tumor blood vessels by adjusting the structure and function of the vasculature network [16]. NO promotes HIF-1α proteasomal degradation by the activation of HIF prolyl hydroxylase and HIF asparaginyl

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hydroxylase. HIF-1α degradation followed by a decrease in VEGF improves the delivery of anticancer drugs through vascular normalization and alteration of the oncotic pressure gradient.

In addition, NTG application, which has been shown to increase vascular flow and drug delivery in hypoxic tissue, may favor the penetration of chemotherapy in tumor tissue and potentially improve their cytotoxic effect [17–19]. Moreover, it was previously reported that NO donors can increase the response to radiotherapy [20,21] (RT) and chemotherapy (CT) [12,22].

The aim of this study was to evaluate the efficacy and safety profile of NTG administered concurrently with C-RT to patients with locally advanced NSCLC.

Materials and methods

Patient selection

This is a prospective, non-randomized, phase II trial of patients with locally advanced NSCLC. Patients with confirmed NSCLC in stages IIIA and IIIB according to the American Joint Committee on Cancer (AJCC) 2007 criteria were eligible for participation in the study. Before 2008, patients were staged using computed tomography, bone scanning and MRI. Thereafter, patients were staged using PET–CT and MRI. Inclusion criteria comprised an Eastern Cooperative Oncology Group (ECOG) performance status of 0–2, no prior cytotoxic chemotherapy (CT) or radiotherapy (RT) for NSCLC, aged 18 years or older, adequate laboratory measurements, measurable disease according to Response Evaluation Criteria in Solid Tumors (RECIST), and a life expectancy >12 weeks. The study protocol was approved by the Institutional Scientific and Bioethics Committees (INCAN/CC/162/08) and is registered at www.clinicaltrials.gov (number NCT00886405).

Induction chemotherapy, concurrent chemoradiotherapy and consolidation chemotherapy

The treatment plan consisted of two cycles of induction CT; in the absence of progression, patients were treated with concurrent C-RT and two cycles of consolidation CT (a total of 6 cycles of chemotherapy). Every CT cycle consisted of 25 mg/m^2 vinorelbine on days 1 and 8 and 70 mg/m^2 of cisplatin on day 1 every 21 days.

All patients received conformal 3D treatment using a linear accelerator with 6 and 15 MV, and the treatment plans were performed on the ECLIPSE™ (Varian™) treatment planning system. The GTV considered all gross disease (primary tumor and positive mediastinal nodes). A 6-mm margin and an 8-mm margin were used for squamous cell carcinoma and adenocarcinoma, respectively, to create the CTV, and a 1-cm margin was added to create the PTV. The GTV was created from the postchemotherapy volume in most cases because using the pre-chemotherapy volume would result in very large treatment volumes. Because we used 3D treatment plans, all of the patients were treated using 4–6 fields, always maintaining the cord dose below 46 Gy. The dose to the primary tumor was 60 Gy on average. Lower doses were 58 Gy, higher doses were 63 Gy, and the dose to the positive mediastinal nodes was 54 Gy. The dose constraint goals were as follows: total lung V20 below 37% and V5 below 60%. The mean contralateral lung dose was maintained below 8 Gy. This was accomplished in all patients. Patients with T4N2 lesions were deemed to have stage IIIB disease. In these cases, only the ipsilateral nodes were treated. In all cases, we used between 4 and 6 fields to maintain the cord dose below 46 Gy, but the dose to the mediastinal lymph nodes was increased to 54 Gy in most cases. All patients were treated with the same tecnic during all the recruitment phase.

After completing the treatment scheme, all of the patients were reevaluated by a multidisciplinary team; those with an objective response, adequate lung function, and residual tumor were candidates for surgical treatment.

Nitroglycerin administration

NTG transdermal patches (25 mg daily) are widely and safely used in the treatment of coronary arterial disease and heart failure. Therefore, we used 25 mg NTG transdermal patch daily. NTG was used for 5 days (1 day before and 4 days after CT) in every CT cycle (induction and consolidation) and daily during concurrent C-RT. NSAIDs were used in the presence of NTG headache. Patients with severe headache (grade 3) NTG were suspended.

Toxicity, response evaluation and follow up

Patients were evaluated every three weeks during induction and consolidation chemotherapy and every week during concurrent C-RT. All toxic events were recorded according to the Common Toxicity Criteria for Adverse Events (CTCAE) ver. 3. Six weeks after completion of a treatment, computed tomography or PET/CT was performed. Response evaluation was performed according to the RECIST criteria. After the treatment plan, all the patients were followed clinically and with laboratory testing every 6 weeks. Computed tomography was performed every twelve weeks during the first 3 years of follow up. Patients who presented clinical deterioration underwent computed tomography.

VEGF plasma measurement levels

Blood samples were taken before and after 2 cycles of chemotherapy. These were performed using the enzyme-linked immunosorbent assay (ELISA) method with the INVITROGEN® KHG0111 Kit. All the samples were incubated with the anti-VEGF antibody previously attached to the plates. VEGF present in the plasma of the patient and control samples, and controls were conjugated with the antibody, followed by the addition of the biotin-streptavidin horseradish peroxidase (HRP) complex. Samples were measured at 450-nm absorbance level.

Statistical analysis

The null hypothesis for this study assumed a 2-year actuarial overall survival (OS) rate of 30% (based on the 2-year survival rates for stage IIIA/IIIB patients in SWOG 8805) [23]. The study had a single-stage trial design and was powered at 80% (two-tailed test, alpha = 0.05) to detect a 2-year OS rate of 55%. This required 30 patients, assuming an accrual over 2 years with a 1-year follow-up. The progression-free survival (PFS) and OS were analyzed using the Kaplan–Meier method, whereas comparisons among subgroups were analyzed using the log-rank or Breslow tests, if the two survival curves crossed. The significance value was set at a p value less than or equal to 0.05. The SPSS ver. 17 software package (SPSS, Inc., Chicago, IL, USA) was employed for data analysis.

Results

From November 2006 to May 2011, 35 patients were enrolled in this trial. The median follow up was 18.9 months (SD ± 15.5 months). The mean age was 59.9 years (SD ± 10.9 years), 51.6% of patients were males, and 68.6% of patients were smokers. The ECOG performance status was 0 in 22.9%, 1 in 65.7%, and 2 in 11.4% of patients. Histopathology was adenocarcinoma in 68.6%, squamous cell carcinoma in 17.1%, and undifferentiated carcinoma in 14.3% of patients. The stage distribution was IIIA in 57.1% and IIIB in 42.9% of patients. Seven (20%) patients were staged using

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Table 1
Patient characteristics.

Characteristic	No. (%)
Age (y)	59.9 ± 10.9 (range, 40–81)
Sex (%)	
Masculine	51.6
Feminine	48.6
Smoking history (%)	
Yes	68.6
No	31.4
Histology (%)	
Adenocarcinoma	68.6
Squamous cell	17.1
Other	14.3
Stage	
IIIA	20 (57.1)
T3N1M0	1
T1N2M0	1
T2N2M0	4
T3N2M0	14
IIIB	15 (42.9)
T4N0M0	2
T4N2M0	5
T3N3M0	3
T4N3M0	5
ECOG score (%)	
0	22.9
1	65.7
2	11.4

ECOG = Eastern Cooperative Oncology Group.

computed tomography, bone scanning and MRI, and 28 patients (80%) were staged using PET–CT and MRI (Table 1).

No patients had progression during induction chemotherapy, and all completed the treatment schedule. The planned dose of radiotherapy in this study was 60 Gy in 30 fractions to the primary tumor and 54 Gy to the mediastinal positive nodes. Thirty-one patients received the planned dose. Two patients received 63 Gy in 35 fractions to produce a lower dose per fraction and decrease toxicity (1.8 Gy instead of 2 Gy per fraction). Two patients did not complete RT and received only 56 Gy due to toxicity. The mean ± SD of the GTVs was 148.7 ± 99.9 [range 36–409], and the V20 was 25.6 ± 9.5 [7–44]. After completing the treatment schema, 4 patients underwent surgical treatment.

The toxicity profile related to NTG was headache in 20% (grades I and II) and hypotension in 2.9% (grade I) of patients. NTG discontinuation was not necessary in any patient, and thrombosis and bleeding were not reported. Table 2 shows the toxicity during induction CT, C-RT and consolidation CT. Global toxicity grades III and IV in any phase of treatment were neutropenia in 60%, esophagitis in 17.6%, nausea/vomiting in 8.7% and pneumonitis in 2.9% of patients. Dose reduction of chemotherapy was necessary during induction CT, C-RT and consolidation CT in 11.6%, 11.6% and 0%, respectively, of patients.

Sixty-three percent of the patients achieved a partial response, and none attained a complete response after CT. However, 51.7% and 23% of patients did so after C-RT, respectively (supplement 1). With a response rate to 2 years of 51.3%.

The median PFS was 13.5 months (95% CI, 8.8–18.2 months), and the 2 and 3-years PFS was 38.7% (95% CI, 22.5–55.9) (Fig. 1). The median PFS for stages IIIA and IIIB was 21.9 months (95% CI, 2.5–47.9 months) and 10.8 months (95% CI, 8.5–13), respectively ($p = 0.087$). The median OS was 26.9 months (95% CI, 15.3–38.5), and the 2-year OS was 51.3% (95% CI, 33.3–69.3) (Fig. 2). Regarding stage IIIA disease, the median OS was 29.7 months (95% CI, 19.2–40.3) and 16.1 months in stage IIIB (95% CI, 0–34) ($p = 0.04$) (supplement 2). The median OS according to the treatment response was 29.7 months (95% CI, 19.8–39.6) for patients with an overall response and 12.6 months (95% CI, 4.3–20.9) for patients with stable disease after induction CT ($p = 0.047$) (supplement 3).

After two cycles of CT and nitroglycerin, the plasma VEGF levels decreased (median: 132 ± 79 vs. 53 ± 78 pg/ml; $p < 0.001$). The median change value was 96.8 ± 116 ng/dl (supplement 4) The median OS for patients who achieved a 50% VEGF level reduction

Table 2
Toxicity.

Toxicity	Chemotherapy induction (%)	Radiotherapy/chemotherapy concurrent (%)	Chemotherapy consolidation (%)
Anemia			
All grade	17.1	14.7	21.9
Grade 3/4	0	0	0
Neutropenia			
All grade	20	25.8	22.9
Grade 3/4	38	34.4	19.9
Nausea/vomiting			
All grade	51.8	47.1	22.8
Grade 3/4	8.7	8.7	0
Headache			
All grade	20	17	8.6
Grade 3/4	0	0	0
Diarrhea			
All grade	20	14.7	2.9
Grade 3/4	0	0	0
Pneumonitis			
All grade	0	2.9	26.1
Grade 3/4	0	0	2.9
Esophagitis			
All grade	0	58.9	23
Grade 3/4	0	17.6	2.9
Hypotension			
All grade	2.9	0	0
Grade 3/4	0	0	0

All toxic events were recorded according to the Common Toxicity Criteria for Adverse Events (CTCAE) ver. 3.

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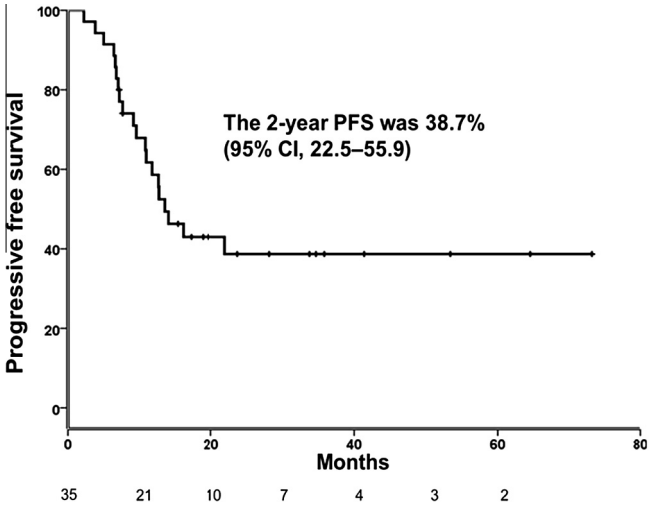

Fig. 1. Kaplan–Meier curve for progression free survival.

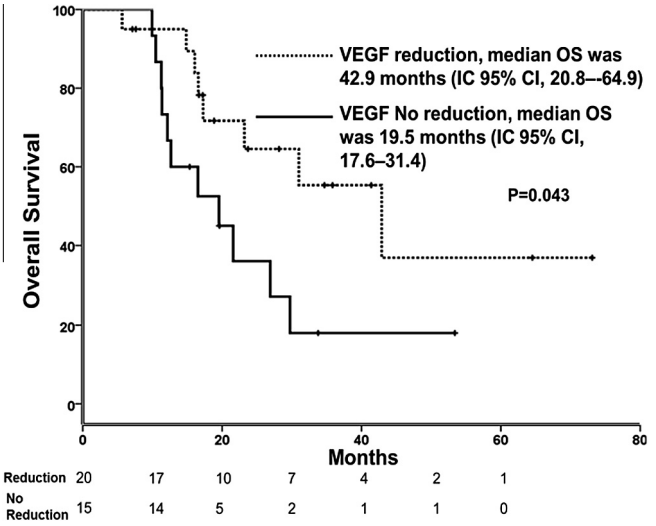

Fig. 3. Kaplan–Meier curves for overall survival (OS). According to VEGF levels.

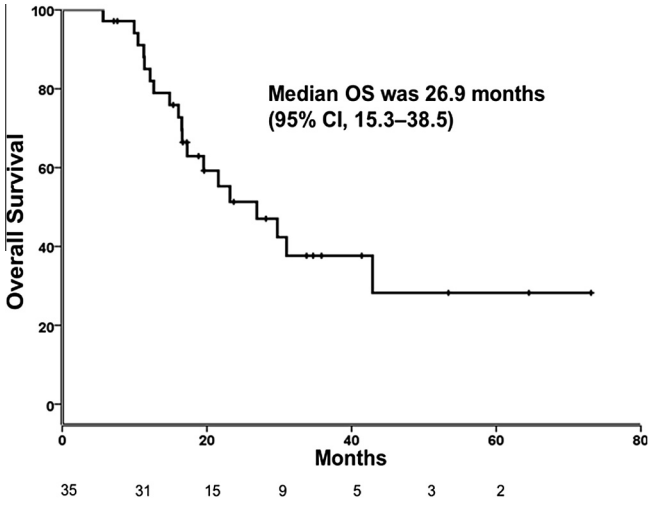

Fig. 2. Kaplan–Meier curves for overall survival (OS). Of the entire cohort.

was 42.9 months (95% CI, 20.8–64.9) compared with 19.5 months (95% CI, 17.6–31.4) for patients who did not attain this reduction in VEGF level ($p = 0.043$) (Fig. 3).

Discussion

Radiation therapy is one of the most important treatments for solid tumors; however, the degree of hypoxia is inversely correlated with radiosensitivity [24]. The cause may be that ionizing radiation produces its damage through free-oxygen radicals. Therefore, in a lower oxygen tension environment, this mechanism could be impaired [25]. Radiotherapy induces VEGF tumor secretion, which in turn leads to some degree of radio-resistance, suggesting that tumor tissue protects itself using such a pathway. The remaining mechanism by which resistance could be mediated is decreasing the blood flow to the tumor tissue, leading to a decrease in antineoplastic drug concentration, particularly in the center of the mass [26]. Mitchell et al. showed that increasing the blood flow in tumors with NTG would improve tumor tissue pO_2 and enhance radiotherapeutic sensitivity [27]. Furthermore, NO could increase the radiosensitivity of the cell through DNA damage by reacting with free radicals formed by ionizing radiation [28].

NO donor administration reduces tumor hypoxia-related resistance by decreasing HIF-1α levels [29] and exerts several effects on tumor cells, including oxygen pressure enhancement, blood flow increase, p53 activation, apoptosis, and synergizes with ionizing irradiation to enhance tumor radioresponsiveness in an experimental model [30]. We found a promising response rate (75%) to C-RT plus NTG in patients with locally advanced NSCLC that was associated with longer PFS and OS with a response rate to two years 51.3%, near the proposal survival according to the number of sample calculation; however, the small size of our study does not allow confirmation of this finding. Similarly, Yasuda et al. reported encouraging results using NTG in patients with metastatic NSCLC. These authors found that patients treated with NTG plus vinorelbine and cisplatin had an overall response (OR) of 72% vs. a 42% response in patients treated without NTG ($p \leqslant 0.001$) [12]. Additionally, a retrospective study suggested that application of nitroglycerin plus docetaxel and carboplatin in patients with operable lung adenocarcinoma increases the response with decreased expression of HIF-1α and VEGF [31]. To our knowledge, this is the first clinical study to combine NTG and C-RT for locally advanced NSCLC.

NTG administration concomitant with C-RT might exert a beneficial effect on blood vessels. As recently shown in preclinical studies, anti-VEGF therapy can transiently "normalize" abnormal tumor vessels, leading to reduced vascular permeability, edema, and hypoxia, as well as improved delivery and efficacy of various therapies [32]. Yasuda et al. found that VEGF levels were lower in patients treated with NTG. Interestingly, we found that patients with a greater decrease in VEGF plasma levels had a better OS than those who did not, suggesting that the potential benefit of NTG might have an effect in anti-VEGF therapy [8]. In addition, the application of NTG was shown to increase blood flow in the tumor and enhance delivery of macromolecular antitumor drugs in an experimental model [14]. Thus, NTG could elevate the concentration of vinorelbine and cisplatin intratumoral and synergize the effect of concurrent chemotherapy and radiotherapy in our patients.

Regarding toxicity, Yasuda et al. reported up to 30% of grade 3 and 4 neutropenia [12]. In our study, we found a higher frequency of grade 3 and 4 neutropenia (60%). In addition, NTG could potentiate systemic toxicity due to chemotherapy. However, the frequencies of esophagitis and pneumonitis are consistent with those in other studies.

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Our study suggests that the addition of NTG to induction CT and concurrent C-RT could have a positive effect on the tumor vasculature by inhibiting the VEGF pathway and reducing hypoxia in patients with locally advanced NSCLC. This treatment has an acceptable toxicity profile and supports the possibility to add nitroglycerin to chemotherapy and radiotherapy. A randomized trial is warranted to confirm these findings.

Conflicts of interest

None of the authors of this paper has a financial or pèrsonal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.radonc.2014.01.021.

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