SPANDIDOS PUBLICATIONS

Hsp27 may allow prediction of the response to single-agent vinorelbine chemotherapy in non-small cell lung cancer

HELEN K. BERRIEMAN, LYNN CAWKWELL, SARA L. O'KANE, LAURA SMITH and MICHAEL J. LIND

Postgraduate Medical Institute of the University of Hull in association with the Hull-York Medical School, University of Hull, Hull, UK

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Abstract. One of the most valuable objectives for oncologists is the ability to predict patient response to chemotherapy before drugs are administered in order to maximise the therapeutic benefit of treatment whilst limiting the toxicity. This is particularly relevant in non-small cell lung cancer as the initial treatment decision is important due to the inherent drug resistance of many tumours and short survival times of patients. We established a homogeneous series of pre-treatment archival biopsy samples from patients receiving first-line single-agent vinorelbine for non-small cell lung cancer. Cases were selected following strict inclusion criteria and patient response was assessed using the Response Evaluation Criteria in Solid Tumours guideline. The expression of 7 proteins was investigated and correlated with response data. Chi-square analysis revealed no association between expression of Bcl-2, Bcl-X_L, Bad, Bak, Bid or p53 proteins and response to vinorelbine therapy. There was a trend for Hsp27-positive tumours to show progression but this did not reach significance (p=0.068). The results suggest that Hsp27 expression may be useful as a predictor of response to single-agent vinorelbine chemotherapy in non-small cell lung cancer patients but a larger study is required to confirm this.

Introduction

One of the most valuable objectives for oncologists is the ability to predict patient response to chemotherapy before drugs are administered in order to maximise the therapeutic benefit of treatment whilst limiting the toxicity. Whilst chemotherapy for advanced non-small cell lung cancer has been shown to have benefits in terms of improved survival and quality of life (1), many patients fail to respond to the treatment but suffer the toxicity of chemotherapy. Therefore, a predictive test would be of enormous clinical benefit to patients where treatment decisions are difficult. The predictive clinical test would ideally be suitable for use on limited amounts of formalin-fixed, paraffin-embedded tissue, as this is the type of sample most likely to be available at the time of treatment decision. Immunohistochemistry is ideal in this respect, and has the advantage that it is a technique which is routinely used in histopathology departments. The technique can be used to detect any protein to which antibodies can be raised, revealing both its cellular and tissue localisation and allowing direct assessment of the tumour cells, thus avoiding conflicting results due to adjacent non-tumour tissue. It has been shown that IHC results from biopsy samples of NSCLC correlate well with those from resection samples from the same tumours, with >80% concordance between the two types of sample (2), demonstrating that biopsies are reliable samples for assessment of NSCLC tumour characteristics.

Although immunohistochemistry has been used on pre-treatment biopsy samples to assess the protein expression in relation to the chemotherapy response in NSCLC, the results are not conclusive. For example, p53 expression has been associated with both a better (3) and a worse (4) response to chemotherapy. Studies of this type typically have relatively small sample sizes and often include patients treated with a number of chemotherapy regimes which may involve combination therapies (4,5). In addition, biopsy samples from various sites, including distant metastases, have been included (6), and these may not accurately reflect the characteristics of the primary tumour. In order to more accurately investigate markers of response to chemotherapy, a homogeneous series of samples from patients treated with the same (preferably single-agent) chemotherapy regime would be required.

The aim of this study was to establish a homogeneous series of pre-treatment formalin-fixed, paraffin-embedded biopsy samples from patients receiving first-line single-agent vinorelbine chemotherapy for NSCLC, and to assess response to therapy in association with protein expression assessed by IHC. Single-agent vinorelbine therapy was chosen because it has been shown to improve the survival and quality of life in the elderly and in poor performance status NSCLC (7).

Proteins for investigation (Bcl-2, Bcl-X_L, Hsp27, p53, Bak, Bad and Bid) were selected on the basis of results obtained from the analysis of protein expression in a series of NSCLC resection samples (8). All of the proteins selected are involved in the control of apoptotic pathways. Bcl-2, Bcl-X_L and the heat shock protein, Hsp27, are capable of inhibiting apoptosis,

Correspondence to: Dr Lynn Cawkwell, R&D Building, Castle Hill Hospital, Hull, HU16 5JQ, UK
E-mail: l.cawkwell@hull.ac.uk

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whereas p53, Bak, Bad and Bid are all pro-apoptotic molecules. To our knowledge, Bcl-X_L, Bak, Bad and Bid have not previously been investigated in relation to response to chemotherapy in clinical NSCLC samples.

Materials and methods

Selection of cases. Pre-treatment samples from patients receiving single-agent vinorelbine chemotherapy for NSCLC in the Hull & East Yorkshire NHS Trust were selected using pharmacy and oncology records. Patients received vinorelbine 30 mg/m^2 on days 1, 8, 15 and 21 for a maximum of 4 cycles. The vinorelbine dose was reduced to 25 mg/m^2 if the patient experienced haematological or non-haematological common toxicity grade III or IV toxicity. Local ethical approval was granted for the study. In order to establish a homogeneous series of samples, cases had to meet the following inclusion criteria: primary lung tumour; histologically proven NSCLC; patient treated with first-line single-agent vinorelbine chemotherapy; pre-treatment formalin-fixed, paraffin-embedded biopsy sample from lung/bronchus or pleura available. Clinical data were obtained from the patient's hospital notes by an experienced oncologist (M.J.L.) and response was assessed using the standard Response Evaluation Criteria in Solid Tumours (RECIST) guideline (9).

Immunohistochemistry. Immunohistochemical assays were carried out essentially as described previously (8,10). Briefly, 4-μm sections of tissue were cut onto SuperFrost Plus slides and dried overnight at 37˚C. Sections were dewaxed in Histoclear (National Diagnostics, Hull, UK) and rehydrated in alcohol before blocking endogenous peroxidase by incubating in 400 ml methanol containing 8 ml of hydrogen peroxide (30% v/v). Antigen retrieval was carried out by boiling slides in a pressure cooker for 3 min at 15 psi in 1500 ml distilled water with 15 ml Antigen Unmasking Solution (Vector Laboratories Ltd., Burlingame, CA). Non-specific protein was blocked by incubation with 1X casein (Vector) diluted in TBS for 10 min and endogenous biotin and avidin were blocked by successive 15 min incubations with avidin and biotin, respectively (Avidin Biotin Blocking Kit; Vector). The primary antibody was diluted in 0.2X casein in TBS and applied for 2 h at room temperature. Antibody suppliers and dilutions are given in Table I. The primary antibody was omitted from the negative control. Antibody detection was carried out using the Duet Kit (Dako cytomation Ltd., Ely, UK) according to the manufacturer's instructions. Finally, sections were counterstained with haematoxylin, dehydrated in alcohol and mounted with Histomount (National Diagnostics).

Scoring of sections and statistical analysis. Samples were scored as positive or negative by two independent investigators and discrepancies in the results were resolved by consensus following re-assessment of the slide by both investigators. For p53, Bcl-2, Bcl-X_L and Hsp27, a section was considered positive if >10% of the tumour cells stained (11,12), as over-expression of these proteins inhibits apoptosis and is therefore detrimental. For Bak, Bad and Bid, where loss of expression is detrimental, sections were scored as negative if expression was lost in >50% of the tumour cells (13). Associations between protein expression and chemotherapy response were assessed by Fisher's exact test using SPSS (v.11).

Table I. Details of antibodies used.

Antibody	Clone	Supplier	Catalogue no.	Dilution
Bcl-2	100	Serotec	MCA1550	1:50
Bcl-X_L	7D9	Neomarkers	MS-1334	1:75
Bak		Neomarkers	RB-1520	1:50
Bad	48	BD Biosciences	610391	1:50
Bid	7	BD Biosciences	611528	1:100
Hsp27	G3.1	Neomarkers	MS-101	1:100
p53	D01	BD Biosciences	554293	1:100

Table II. Summary of clinical details of the 24 cases included in the study.

Sex (%)	
Male	16 (67)
Female	8 (33)
Histology (%)	
AC	7 (29)
SCC	11 (46)
N/D	6 (25)
Response (%)	
PR	3 (13)
SD	8 (33)
PD	13 (54)
PR/SD	11 (46)
Stage (%)	
II	3 (13)
IIIA	4 (17)
IIIB	7 (29)
IV	8 (33)
Recurrent	2 (8)
Age (years)	
Range	49-76
Mean	64.5
Median	64

N/D, not determined.

Results

Selection of cases. A total of 24 cases met the full criteria for inclusion and the clinical details are summarised in Table II. Patients were divided into two groups according to the clinical benefit gained from treatment: those with progressive disease (PD, n=13) and those with either stable disease or a partial response (SD/PR, n=11) as shown in Table III. No patients attained a complete response (CR) to treatment.

Distribution of cases between the PD and PR/SD

	PD (n=13)	PR/SD (n=11)
Sex (%)		
Male	11 (85)	5 (46)
Female	2 (15)	6 (54)
Histology (%)		
AC	2 (15)	5 (46)
SCC	8 (62)	3 (27)
N/D	3 (23)	3 (27)
Stage (%)		
II	2 (15)	1 (9)
IIIA	3 (23)	1 (9)
IIIB	4 (31)	3 (27)
IV	4 (31)	4 (36)
Recurrent	0 (0)	2 (18)
Age (years)		
Range	49-76	58-72
Median	64	64

N/D, not determined.

Table IV. Analysis of protein expression in relation to response to chemotherapy (Fisher's exact test).

Antibody	PD	SD/PR	p-value
Bcl-2			
-	8	8	
+	5	3	0.679
Bcl-X_L			
-	2	3	
+	11	8	0.630
Bad			
-	2	1	
+	11	10	1.000
Bak			
-	2	1	
+	11	8	1.000
Bid			
-	4	2	
+	9	7	1.000
Hsp27			
-	0	3	
+	13	7	0.068
p53			
-	7	5	
+	6	5	1.000

Immunohistochemistry. Due to the limited amounts of tissue, it was not possible to assess the expression of every protein in each case. One case was not assessed for Bak and Bid expression and a second case was not assessed for expression of Bak, Bid, Hsp27 and p53. Sample sizes were, therefore, 24 for Bcl-2, Bcl-X_L and Bad, 23 for Hsp27 and p53, and 22 for Bak and Bid.

Factors predictive of response to chemotherapy. Chi-square analysis revealed no association between protein expression and response to chemotherapy for Bcl-2, Bcl-X_L, Bad, Bak, Bid or p53 (Table IV). There was a trend for patients with Hsp27-positive tumours to demonstrate disease progression but this did not reach significance (p=0.068). No significant association was revealed between disease stage, sex or histological type and response to treatment, although the sample size is too small to be able to completely rule out these factors.

Discussion

The most limiting factors in this study were selecting suitable cases and acquiring suitable samples for analysis. Despite the large number of lung cancer patients in the Hull and East Yorkshire area, there were relatively few cases which met the strict inclusion criteria for this study, which were necessary in order to ensure a homogeneous series of samples. A larger sample size may be obtainable by inclusion of cases from other centres and prospective as well as retrospective selection of cases.

As biopsy samples may contain only small amounts of tissue, comprising normal as well as tumour tissue, it has been suggested that sampling error may be a problem when using IHC to analyse biopsy specimens (5). However, it has previously been shown in SCLC that the percentage of cells showing positive staining for several biological markers was similar between trans-bronchial biopsy (TBB) specimens and open lung biopsy specimens (14). It has also been demonstrated that there is >80% concordance between IHC results obtained from paired NSCLC biopsies and corresponding resection samples (2) and, therefore, that biopsies are a reliable means of assessing tumour characteristics.

For the purposes of this study, patients were divided into two groups according to the benefit gained from treatment: those with stable disease or a partial response (benefit) and those with progressive disease (no benefit). This system is different to that used in most studies and clinical trials, where stable disease is grouped with progressive disease and only partial or complete responses are classified as responses. However, the modification to this system is justified in this case, as preventing disease progression and extending survival is arguably an important benefit to patients with a very poor prognosis and limited life expectancy. In addition, as most patients receiving single-agent vinorelbine have advanced disease and poor performance status, and as chemotherapy in lung cancer is only intended to be palliative in the majority of cases, very few patients will achieve a complete response (no complete responses were found in the patients included in this study) and a partial response or stable disease is the desired outcome.

The results presented here reveal no association between immunohistochemical detection of Bcl-2, Bcl-X_L, Bad, Bak, Bid or p53 and the outcome of treatment. Krug *et al* (6) also found no link between Bcl-2 or Bax expression and response to treatment with combined vinorelbine and docetaxel. Harada *et al* (3) found no association between Bcl-2 expression and response to a variety of platinum-based chemotherapy regimes. The detection of p53 using IHC has been reported to be associated with both increased response to platinum-based chemotherapy (3) and lack of response/resistance to platinum- or vinorelbine-based chemotherapy in NSCLC (4,15,16). To our knowledge, there have been no previous reports of studies comparing Bcl-X_L, Bad, Bak or Bid expression and response to chemotherapy in primary NSCLC samples.

This is also the first investigation of Hsp27 expression in clinical NSCLC samples in relation to chemotherapy response. The results indicate a trend for patients with Hsp27-positive tumours to show disease progression, although this did not reach statistical significance (p=0.068). Interestingly, if the 2 cases with recurrent tumours following previous resection were excluded from the analysis, the relationship between Hsp27 expression and response became significant (p=0.042). It is possible that this is due to biological differences between initial and recurrent tumours; however, the sample size is too small to draw any conclusions. Hsp27 is capable of inhibiting apoptosis by binding to cytochrome *c* and preventing its interaction with Apaf-1 and pro-caspase-9 (17). The result is therefore consistent with the role of Hsp27, as apoptosis would be inhibited in those tumours with Hsp27 over-expression, and chemotherapy would not have the desired effect of inducing cell death. This is in agreement with the results of a study of oesophageal SCCs treated with neo-adjuvant therapy (5-FU, cisplatin and radiotherapy), which revealed an increased therapeutic effect to be associated with negative Hsp27 status (18). In order to fully assess the role of Hsp27 in response to single-agent vinorelbine treatment in NSCLC, a larger study group would be required.

In conclusion, the results of this study have suggested that Hsp27 expression may be useful as a predictor of response to single-agent vinorelbine chemotherapy in NSCLC patients. A larger study group would be required in order to confirm this, which may necessitate cases and samples from other centres being included to sufficiently increase the sample size while maintaining the strict inclusion criteria necessary to ensure a homogeneous series. It would probably also be necessary to select patients prospectively as well as retrospectively.

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