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European Journal of Cancer 41 (2005) 1645–1648

European Journal of Cancer

www.ejconline.com

Cyclooxygenase-2 expression predicts survival in malignant pleural mesothelioma

S.L. O'Kane [a], L. Cawkwell [a,*], A. Campbell [b], M.J. Lind [a]

[a] *Postgraduate Medical Institute of the University of Hull in association with the Hull-York Medical School, University of Hull, UK*
[b] *Histopathology, Hull Royal Infirmary, Hull, UK*

Received 21 November 2004; received in revised form 18 April 2005; accepted 22 April 2005
Available online 16 June 2005

Abstract

The expression of cyclooxygenase 2 (COX-2) protein is increased in many tumours and may be associated with a more aggressive phenotype. We aimed to assess COX-2 expression in a large series of archival mesothelioma specimens. Archival tissue was obtained from 86 malignant pleural mesothelioma samples (histological subtype: 42 epithelial, 28 biphasic and 16 sarcomatoid). Overexpression of COX-2 was detected by immunohistochemical analysis. Positive staining was located in the cytoplasm of malignant tumour cells. Overall 51/86 (59%) tumour sections demonstrated COX-2 overexpression. The frequency varied with histological subtype with 31/42 (73%) of epithelial sections, 14/28 (50%) of biphasic sections and 6/16 (37%) of sarcomatoid sections recorded as positive. Kaplan Meier survival analysis indicated that overexpression of COX-2 was significantly related to improved prognosis ($P < 0.001$) and was an independent prognostic factor in multivariant analysis. Overexpression of COX-2 protein may confer a survival advantage in mesothelioma patients.

Keywords: Cyclooxygenase; Immunohistochemistry; Mesothelioma

1. Introduction

Mesothelioma is a relatively rare, aggressive disease affecting the mesothelium (lining surrounding the main internal organs), which is caused primarily by industrial exposure to asbestos fibres. It typically presents as a unilateral pleural mass with a moderate-to-large pleural effusion. Despite the rarity of this disease, it is increasing in incidence [1,2]. Asbestos is responsible for approximately 80% of all cases of malignant pleural mesothelioma (MPM) [3]. Although MPM has a latency period of 15–40 years [4], it has very poor prognosis with a survival rate of only 10% at 3 years and the average survival time is generally accepted as 4–12 months [6]. In the UK there were 1755 recorded deaths from mesothelioma in 2002 (www.cancerresearchuk.org).

The molecular basis of the disease is unknown and treatment tends to focus on traditional methods including supportive care, surgery, radiotherapy and chemotherapy. It is unclear if any currently established form of treatment has any significant impact on the progression of mesothelioma, although modern chemotherapeutic agents such as pemetrexed are showing promising results [6]. Many clinical prognostic factors have been suggested for MPM including performance status, age and gender, but much of the data produced is conflicting [7]. The most significant clinical prognostic factor to date is histological profile, as non-epithelial types have shorter survival times [5,8–10].

Non-steroidal anti-inflammatory drugs (NSAIDs), *e.g.*, aspirin, is known to decrease the incidence of

* Corresponding author. Present address: R&D Building, Castle Hill Hospital, Hull, HU16 5JQ, UK. Tel.: +44 1482 875875x3617; fax: +44 1482 622398.
E-mail address: L.Cawkwell@hull.ac.uk (L. Cawkwell).

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doi:10.1016/j.ejca.2005.04.019

colorectal [11,12], oesophageal [13] and lung [14,15] tumours. These drugs are thought to exert their action by inhibiting cyclooxygenases, which are enzymes are involved in the production of prostaglandins from arachidonic acid [16]. At least two isoforms of cyclooxygenase (COX) exist. COX-1 is constitutively expressed in many normal tissues, whereas COX-2 is only seen at the site of inflammation. COX-2 is an initial response and is inducible by many factors including hypoxia and growth factors. COX-2 expression has been previously reported to be involved in the inhibition of apoptosis with decreased COX-2 expression increasing the levels of apoptosis [17]. COX-2 may also play a role in angiogenesis, as selective COX-2 inhibitors have been shown to be antiangiogenic [18,19]. The abnormal overexpression of COX-2 has been reported in many tumour types [20,21] including mesothelioma [22]. A significant association with prognosis has also been reported in mesothelioma patients [23].

In this study, COX-2 overexpression was analysed by immunohistochemistry in a large retrospective series of MPM and correlated with survival and histological subtype to clarify its role as an independent prognostic factor.

2. Materials and methods

2.1. Samples

A list was obtained from the Histopathology records (Hull Royal Infirmary) of all patients diagnosed with MPM within the Hull and East Yorkshire NHS Trust from 1995 to 2000. Formalin-fixed, paraffin-embedded blocks were obtained where possible from pathology archives, and a series of samples from 86 patients was established, containing 42 epitheliod cases (48%), 28 biphasic cases (32%) and 16 sarcomatoid cases (18%). Clinicopathological data was collected for all patients (Table 1). Survival times were calculated from date of diagnosis to date of death. Local Research Ethics Committee approval was obtained for the research.

2.2. Immunohistochemistry

The method of staining has been described previously [24]. In brief, formalin-fixed, paraffin-embedded 4 μm thick sections were cut onto SuperFrost® Plus slides (Menzel-Glaser, Germany) and dried overnight at 37 °C. Slides were dewaxed in warm Histoclear II (National Diagnostics, Hull, UK) for 10 min, followed by two changes of Histoclear II (at room temperature) for 10 s each and rehydrated by passing through three changes of 100% ethanol for 10 s each and rinsed in running tap water for 1 min. Endogenous peroxidase activity of red blood cells was blocked using 2% hydrogen peroxide in methanol. Antigen retrieval was achieved by boiling the slides in 1500 ml distilled water containing 15 ml Antigen Unmasking Solution (Vector Laboratories Inc., CA, USA) in a pressure cooker for 3 min at 15 psi. Slides were transferred to Tris buffered saline (TBS) (pH 7.6). Non-specific staining was blocked by incubation with 100 μl of 1× casein diluted in TBS added to each slide. Endogenous avidin and biotin were blocked using the Avidin/Biotin Blocking Kit (Vector Laboratory Inc.). One hundred microliter of primary antibody (COX-2, Clone 33; BD Biosciences, CA, USA) was diluted in 0.2× casein in TBS to a final dilution of 1:50 and applied to the test sections. A negative control with 100 μl of 0.2× casein was included. The slides were incubated at room temperature for 2 h. Antibody detection was carried out using the avidin–biotin complex (ABC) method using the Duet Kit (DakoCytomation Ltd., High Wycombe, UK). The slides were incubated with the chromagen–3,3′-diaminobenzidine tetrahydrochloride (DAB) and 0.01% hydrogen peroxide as enzyme substrate. The slides were incubated for up to 30 min to allow the development of the brown immunostain. Sections were counterstained with Harris Haematoxylin, dehydrated in 100% ethanol and mounted with Histomount (National Diagnostics, Hull, UK). Immunohistochemical analysis was carried out without previous knowledge of survival details of the patients. Sections were blindly assessed by two independent researchers, and positive overexpression was recorded if cytoplasmic staining was seen in greater than 10% of malignant tumour cells.

Table 1
Clinicopathological details for 86 MPM patients

Histological subtype	Male	Female	Age range (mean age)	Mean survival time (days)
Epithelial	38	4	45–86 (70)	405
Biphasic	27	1	44–87 (61)	220
Sarcomatoid	15	1	50–94 (71)	142

2.3. Statistical analysis

SPSS software version 11.0 (SPSS, Chicago, USA) was used for statistical analysis. Univariate survival analysis was calculated for both COX-2 expression and histological subtype using Kaplan Meier survival curves. Multivariable analysis was calculated using Cox regression analysis.

3. Results

COX-2 staining was found in the cytoplasm of tumour cells. Staining of the reactive spindle cells in the sections acted as an internal positive control. The sections stained with varying intensity. Overall 51/86 (59%) of the sections demonstrated COX-2 overexpression. The frequency of

overexpression varied with histological subtype where 31/42 (73%) of epithelial sections, 14/28 (50%) of biphasic sections and 6/16 (37%) of sarcomatoid sections recorded as positive.

Univariate analysis indicated that overall survival was strongly influenced by histological subtype ($P < 0.001$; Fig. 1) and expression of COX-2 ($P < 0.001$; Fig. 2).

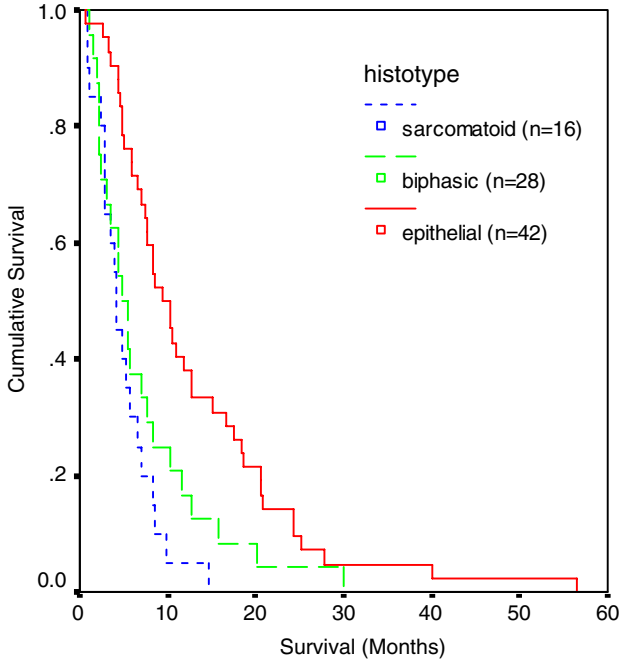

Fig. 1. Kaplan Meier survival plot for histological subtype ($P < 0.001$).

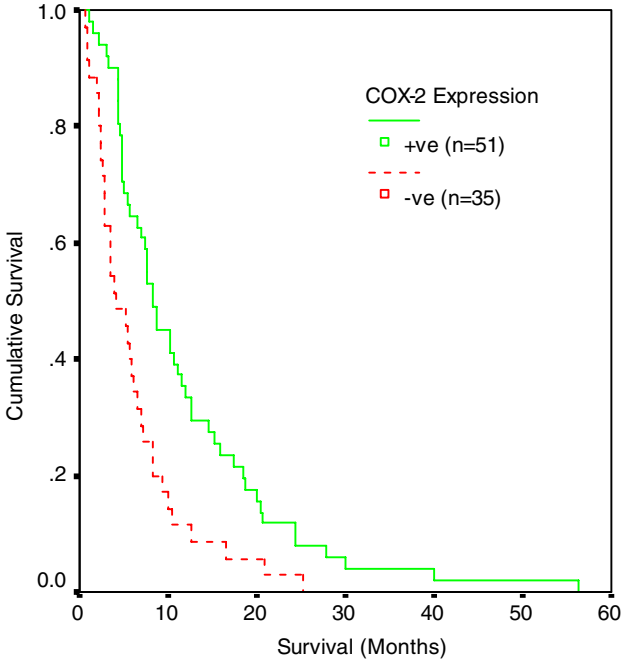

Fig. 2. Kaplan Meier survival plot for COX-2 expression ($P < 0.001$).

Multivariant Cox regression analysis showed that COX-2 overexpression was independent of histological subtype (COX-2 expression: regression coefficient 0.705, $P = 0.002$; Histological subtype: regression coefficient 0.535, $P < 0.001$). In this series of MPM tumours, patient age and sex were not significant prognostic factors.

4. Discussion

This study was designed to analyse the expression of the COX-2 protein in archival MPM sections. Fixation of the samples was achieved by routine processing in a histopathology laboratory, and intensity of staining was not analysed due to the possibility of inter block variation. The scoring scheme utilised in this study recorded sections as positive or negative according to the percentage of positive cells (over expression was recorded if $\geqslant$10% of tumour cells were positively stained). Overall 51/86 (59%) of the sections demonstrated COX-2 overexpression, however there appeared to be a difference in the frequency between histological subtypes. We identified overexpression in 73% of epithelial tumours but in only 37% of sarcomatoid tumours. This finding requires confirmation in a larger series of MPM cases.

Using immunohistochemistry, Marrogi and colleagues [25] had previously reported strong COX-2 expression in 30/30 flash-frozen, paraffin embedded cases (23 epithelial, 4 biphasic and 3 sarcomatoid). The study used the same monoclonal antibody as used in our research therefore we can only suggest that the difference in observed expression may be due to sample preparation or methodological differences.

Baldi and colleagues [23] assessed COX-2 expression in 29 formalin-fixed, paraffin-embedded mesothelioma samples consisting of 16 epithelial, 7 biphasic and 6 sarcomatoid tumours. Using a polyclonocal antibody, they reported high COX-2 expression in 66% of sections, which is in agreement with the level of expression observed in our study of a similar series of archival MPM. From this relatively small series of samples, they reported that COX-2 overexpression was an indicator of poor prognosis even though histological subtype did not prove to be a significant prognostic factor in their series.

We have also analysed our immunohistochemical results in relation to clinicopathological features (age, sex, histological subtype and survival) to identify factors which may be important in assessing prognosis in MPM patients. Histological subtype was found to be a significant prognostic factor, which is in agreement with previous reports [5,8–10]. We confirmed in this series that epithelial tumours are associated with a better prognosis than sarcomatoid tumours.

Our study, with a relatively large sample size, indicates that overexpression of COX-2 in MPM is associated with improved prognosis ($P < 0.001$). However,

this finding is counter-intuitive in that inhibition of COX-2 in human tumour cell lines generally results in increased apoptosis and reduced angiogenesis and matrix invasion [26–28]. Overexpression of COX-2 in MPM would be expected to enhance the malignant phenotype by reducing apoptosis, enhance angiogenesis and matrix invasion and thereby, reduce survival. However, in the final analysis whether a cell undergoes apoptosis will depend on the exact balance of pro- and anti-apoptotic factors.

Further work will be required to assess the role of COX-2 in MPM and to confirm its relationship with patient prognosis. Larger series of archival MPM samples will need to be analysed in order to confirm our data since we have identified subtype differences in COX-2 overexpression and multivariant analysis is required to ascertain if any factors are independent of histological subtype which, as we have confirmed, is an established prognostic factor in MPM.

The downstream effects of overexpression of COX-2 are complex, including the up- and down-regulation of many such factors (*e.g.*, bcl-2 and survivin). Selective COX-2 inhibitors have been shown to inhibit the growth of mesothelioma cell lines and induce apoptosis in these cells [29]. However, there may be other mechanisms by which COX-2 inhibitors exert an effect on apoptosis, other than by inhibiting COX-2. *In vitro* analyses will need to performed in MPM cell lines to establish the COX-2 pathway and the mechanisms which lead to inhibition.

Conflict of interest statement

None declared.

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