Increased serum concentrations of growth factor receptors and Neu in workers previously exposed to asbestos

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Abstract

Objectives—Epidermal growth factor receptor (EGFR) and oncogene Neu belong to a family of growth factor receptors which may play a part in carcinogenesis. Although increased serum concentrations of Neu and EGFR have been shown in several patients with asbestosis who later developed cancer, serum concentrations have not been studied in workers exposed in the past to asbestos but without asbestos related diseases.

Methods—Serum concentrations of secreted growth factor receptors were studied in 300 workers exposed in the past to asbestos and the results were compared with those of 70 controls.

Results—In the controls 4.3% (3/70) had EGFR values >912 units/ml, compared with 39% (117/299) of the exposed group (p < 0.001). The difference in high values was even more pronounced for Neu with 4.3% of controls having Neu values >2580 fmol/ml compared with 72% (216/299) of the exposed workers (p < 0.001). Pleural plaques predicted lower serum concentrations of EGFR but not lower Neu concentrations, and this finding remained significant after adjustment for age, exposure time, smoking, and time from initial exposure.

Conclusions—Enhanced secretion of EGFR and Neu was found in a large cohort of retired asbestos workers with a wide range of exposure and latency periods. They did not have asbestosis or cancer and their EGFR values were higher in those without plaques. Further studies are needed to confirm our results, to determine the source of the secreted growth factor receptors, and to study their possible value as risk factors in the development of cancer.

(*Occup Environ Med* 1999;**56**:114–117)

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The epidermal growth factor receptor (EGFR/c-erbB) and the oncogene Neu (Neu/c-erbB-2) belong to same family of growth factor receptors, which were identified by their resemblance to the oncogene V-erb, encoded by the ovarian erythroblastosis virus.[1][2] The proteins produced by the genes for EGFR and Neu have been shown to have a close resemblance in their sequence of amino acids but do not bind to the same ligands. Both have transmembrane, extracellular, and intracellular regions, and are secreted into the serum. The intracellular region has tyrosine-kinase activity. Overexpression of these glycoproteins leads to their dimerisation, tyrosine-kinase activation, receptor autophosphorylation, and the subsequent activation of kinase substrates involved in the signal transduction mechanisms which eventually affect the nuclear transcription of genes regulating the cell cycle.[3][4] Recently,[5] asbestos fibres were found to induce autophosphorylation of the EGFR, an event triggering mitogen activated protein (MAP) kinases and extracellular signal regulated kinase (ERK) cascades, thus enhancing cell proliferation.

It is biologically plausible that EGFR and Neu play a part in carcinogenesis. Induction of their increased expression leads to tumours of NIH 3T3 cells.[6][7] Transgenic mice have a high cellular expression of the receptors and develop breast adenocarcinomas.[8] Furthermore, increased expression of EGFR has been found in several types of human malignancies: breast, prostate, lung, ovary, and larynx, and predicts a poor prognosis.[3] Neu is also highly expressed in human malignancies including breast, stomach, and ovarian cancers[4] and is associated with a poorer prognosis in women with breast cancer.[9] The extracellular regions of Neu and EGFR are probably shed or secreted from the tumour cells, and their high concentrations have been detected in serum samples derived from mice bearing malignancies[10] and from women with breast cancer.[11]

There is evidence that EGFR and Neu may play a part in the development of cancer in workers exposed in the past to asbestos. It has been shown that 50%–80% of the mesotheliomas expressed EGFR[12–14] and EGF itself has been shown to serve as a mitogen to mesothelioma cell lines.[15][16] Furthermore high serum concentrations of Neu and EGFR have been found in several patients with asbestosis who later developed cancer.[17–19] We are unaware, however, of studies which have measured the compounds in serum samples from workers exposed in the past to asbestos but without asbestosis or cancer.

In this study we have used immunological enzyme linked immunosorbent assay (ELISA) for EGFR and Neu, to study retired workers without diseases related to asbestos who were exposed to asbestos in the past. Our aim was to

Table 1 *Serum concentrations of Neu and EGFR in workers exposed to asbestos compared with controls*

	Exposed (n=300) median (25%–75%)	Controls (n=70) median (25%–75%)	p Value
Neu (units/ml)	3600 (2500–5200)	1533 (1163–1812)	<0.001
EGF receptor (fmol/ml)	800 (650–1225)	409 (274–599)	<0.001

Table 2 *Factors influencing high concentrations of EGFR in workers exposed in the past to asbestos*

Variables	High EGF-receptor (n=117)	Norm. EGF-receptor (n=182)	p Value
Age (y, mean (SD))	60.1 (10.9)	58.7 (9.5)	0.267
Exposure time (y, mean (SD))	10.0 (11.0)	9.2 (11.1)	0.577
Time from initial exposure (y, mean (SD))	30.8 (7.0)	30.6 (7.6)	0.774
Current smokers (n (%))	22 (18.8)	48 (26.4)	0.131
Plaques (n (%))	23 (19.7)	57 (31.3)	0.005

Table 3 *Factors influencing high concentrations of Neu*

Variables	High Neu (n=216)	Normal Neu (n=83)	p Value
Age (y, mean (SD))	58.1 (10.3)	60.7 (10.7)	0.210
Exposure time (y, mean (SD))	9.6 (11.2)	9.3 (11.2)	0.854
Time from initial exposure (y, mean (SD))	30.8 (7.4)	30.5 (7.0)	0.769
Current smokers (n (%))	57 (26.4)	13 (15.7)	0.0498*
Plaques (n (%))	58 (26.9)	22 (26.5)	0.952

*Not significant after correction for multiple comparisons.

find out whether high Neu or EGFR serum concentrations are present in such workers.

Patients and methods

PATIENTS

All living retired workers of a single factory that makes asbestos cement products were contacted by mail and offered a comprehensive physical examination including chest *x* radiography and blood tests. The factory had started regular manufacture of asbestos cement products in 1953, primarily large pipes for irrigation, plumbing, and sewage as well as flat and corrugated sheets for walls, ceilings, and canopies. The ratio of chrysotile to crocidolite fibres had been 10:1 until the past few years when crocidolite was eliminated from the production process. In the production and handling sites, concentrations of 4-40 fibres/ml were documented in the 1960s and 1970s; since 1977, concentrations have been consistently lower than 1 fibre/ml. Of 1119 workers tested, 80 (7.2%) had pleural plaques and of those with pleural plaques, six had interstitial abnormalities.

The cohort consisted of 300 former workers exposed in the past to asbestos for variable periods, chosen from a larger cohort of 1119 workers examined. From the larger cohort all those with plaques (80) were tested. The other 220 former workers were chosen at random. They had a median age of 60, range 34–86, had been exposed for 1–40 years, with a median of 4 years, and were examined 12–51 years (median of 32 years) after the first exposure. The routine examinations included chest radiography and recording of occupational and smoking history (70 (23.3%) were current smokers). Serum samples were collected and stored at -70°C. The asbestos workers were compared with a healthy control group who were relatives of patients seen in the outpatient clinics, and compared with the asbestos workers had similar social conditions and age range

EGFR AND NEU MEASUREMENTS

Serum samples were analysed for EGFR and Neu with the ELISA assays (Oncogene Science). The EGFR assay used a mouse monoclonal capture antibody and a rabbit detector antibody.[20] It did not cross react with the Neu oncoprotein. The detector antibody was then reacted with a goat antirabbit antibody linked to horseradish peroxidase which catalysed the conversion of the substrate, O-phenylenediamine to a coloured product; this was measured by spectophotometry at a wavelength of 490 nm. The results are given as fmol/ml. The Neu assay was also based on a sandwich technique, in which the capture primary antibody was bound to the microtitre plates and the secondary antibody, recognising another epitope of the receptors, was added after incubation with the serum samples. The coloured product in the Neu assay was measured at a wavelength of 490 nm, and the results are given as human Neu units/ml. Each assay was carried out twice, in duplicates.

STATISTICAL ANALYSIS

Continuous variates were compared with the student's *t* test, whereas non-parametric data were compared with the χ^2 test with Yates correction. All the variates were also entered into a logistic regression and mulivariate linear regression models.

Results

The concentrations of the two receptors (EGFR and Neu) in the serum are shown in table 1. We arbitrarily defined high values as those found in ≤5% of the controls. In the controls EGFR values >912 fmol/ml were found in 3/70 (4.3%) , and Neu values >2580 units/ml were also found in 4.3% (3/70) compared with 117/299 (39.1%) for EGFR and 72% (216/299) for Neu in the exposed workers (p<0.001 in both cases). The median, with 25-75 percentiles for the exposed workers and controls can be seen in table 1. With univariate analysis, those with high EGFR values were compared with those with normal values (table 2). Pleural plaques were found significantly less often in those with high EGFR values. This was not the case for high Neu values where pleural plaques were found equally often in those with high and normal Neu values, but smoking was more prevalent in those with high Neu values (table 3). After correction for multiple comparisons, however, this difference was not significant (p=0.0498 with a significant p value defined as 0.05 divided by 5 or 0.01)

A logistic regression model (table 4) showed that EGFR values were more often lower in those with pleural plaques after controlling for the other variates. There was a trend for increasing age and smoking to be associated with low EGFR values. Latency and exposure were not associated with high EGFR values. Adding high Neu to the model showed that a

Table 4 Logistic regression of factors predicting high concentrations of EGFR

Variates	Coefficient (SEM)	Odds ratio (95% CI)	p Value
Constant	0.419 (0.812)		0.21
Exposure (10 y)	0.114 (0.138)	1.12 (0.86 to 1.47)	0.41
Time from intial exposure (10 y)	0.187 (0.234)	1.21 (0.76 to 1.91)	0.43
Current smoking (yes/no)	−0.505 (0.308)	0.60 (0.33 to 1.10)	0.10
Age (10 y)	−0.310 (0.162)	0.73 (0.53 to 1.01)	0.056
Pleural Plaques (yes/no)	−0.722 (0.280)	0.49 (0.28 to 0.84)	0.010

Table 5 Logistic regression of factors predicting high concentrations of Neu receptor

Variates	Coefficient (SEM)	Odds ratio (95% CI)	p Value
Constant	1.41 (0.91)		0.120
Exposure (10 y)	0.06 (0.149)	1.07 (0.8 to 1.43)	0.660
Time from initial exposure (10 y)	0.257 (0.248)	1.29 (0.80 to 2.10)	0.300
Current smoking (yes/no)	0.628 (0.352)	1.86 (0.94 to 3.74)	0.074
Age (10 y)	−0.239 (0.165)	0.79 (0.57 to 1.09)	0.143
Pleural Plaques (yes/no)	0.036 (0.300)	0.99 (0.56 to 1.74)	0.904

high Neu value predicted a high EGFR value (odds ratio (OR)=4.61, 95% confidence interval (95% CI) 2.58 to 8.22). This did not change the other ORs (not shown). High Neu values (table 5) had a tendency to be associated with smoking, but not with the other variates (exposure, latency, age, pleural plaques). Adding high EGFR values to the model showed that EGFR predicted a high Neu value (OR=1.99, 95 CI 1.13 to 3.49). In this model smoking also became a significant predictor (OR 2.03, 95% CI 1.01 to 4.09).

Finally multiple regression was used to predict both EGFR and Neu values after logarithmic transformation. Both were normalised with Wilk-Shapiro values of 0.9727 for the log of Neu values and 0.910 for the log of EGFR values. The results did not improve on those of the logistic regression models (results not shown).

Discussion

Asbestos fibres are human carcinogens with undefined mechanisms of action. Recently, asbestos has been found to cause autophosphorylation of the EGFR, triggering signal transduction pathways which may lead to uncontrolled cell proliferation.[5] Overexpression of the soluble extracellular domains of EGFR have been found in the serum of patients with several types of malignancies, including those associated with asbestos exposure—such as mesothelioma.[10 11 17–19]

We have concentrated on a population which consisted of exposed retired workers, most of whom were symptom free with a wide range of exposure and latency periods. The major finding of this study was the proportion of subjects with increased concentrations of EGFR and Neu in the serum samples of retired asbestos workers compared with controls. A second unexpected finding was the negative correlation between high serum EGFR and the existence of plaques. Although soluble EGFR could serve as a marker of human malignancies its role in the pathogenesis of diseases induced by asbestos is not known. A possibility could be that as with other soluble receptors (soluble interleukin-2 receptor, soluble tumour necrosis factor α receptor), serum EGFR could compete with the cellular receptor on binding with its substrate, EGF, and protect the cells from uncontrolled proliferation. In this case, healthy asbestos workers with enhanced production and activation of EGFR/Neu, may have secreted the extracellular portion of EGFR as a protective mechanism, which could be defective in those with plaques.

The high concentration of EGFR and Neu in retired workers with such a wide range of exposure and latency periods, with no significant associations between receptor values and these variables may suggest that amphibole fibres, which are not biodegradable and remain in the lungs, trigger EGFR and Neu production after even a short exposure.

We found a correlation between the serum concentrations of EGFR and Neu. This is not surprising as the two proteins belong to the closely related group of growth factor receptors.[1–4] Some cross reactivity between the antibodies in the two assays could result in such correlation although this is unlikely to explain this finding entirely. Furthermore plaques were associated with lower EGFR but there was no correlation of Neu with plaques, suggesting a separate role for each of them, as has already been shown in patients with asbestosis.[19]

Our findings should be interpreted with caution. There was considerable selection bias due to exclusion of those who had died and to inclusion of only those workers who volunteered for the examination. This could decrease differences between the study group and the control group if growth receptors are markers of disease, but have the opposite effect if increased serum receptor concentrations are part of a protective mechanism. Although the large differences between the controls and the study group suggest that our results are not due to chance or to methodological problems, it is likely that the positive predictive value of increased serum receptor concentrations for future development of cancer will be low because of the high prevalence of such findings in a relatively healthy cohort. The relevance of the high receptor concentrations can be determined only in a follow up study.

In summary, we found an enhanced secretion of EGFR and Neu in a large cohort of retired asbestos workers with a wide range of exposure and latency periods. They did not have asbestosis or cancer and their EGFR values were higher in those without plaques. Further studies are needed to confirm our results, to determine the source of the secreted growth factor receptors, and to study their possible value as risk factors in the development of cancer.

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