Effect of Pesticide Exposure on HER-2/neu Overexpression Seen in Patients with Extensive Stage Small Cell Lung Carcinoma

Anil Potti,1 Apar Kishor Ganti, Kaley Sholes, Eric Langness, Vijay Koka, Linda Horvarth, and Michael Koch

Department of Medicine, University of North Dakota School of Medicine, Fargo, North Dakota 58102 [A. P., A. K. G., K. S., E. L., V. K.], and Department of Pathology [M. K.], Division of Oncology, Veterans' Affairs Medical Center [L. H.], Fargo, North Dakota 58102

ABSTRACT

Purpose: The aim of this study was to evaluate the relationship, if any, between pesticide exposure and overexpression of the HER-2/neu oncoprotein in extensive stage small cell lung cancer (ESSCLC).

Experimental Design: The records of all patients with a diagnosis of ESSCLC from January 1991 through April 2001 were reviewed in our retrospective study. Pesticide risk (herbicide and insecticide) was assessed by telephone interviews using a predetermined questionnaire with emphasis on type of exposure, use of protective measures, and duration of exposure. An exposure index was calculated (h/day × days/year × years), and patients with an index > 2400 h were considered as exposed. HER-2/neu overexpression was assessed by immunohistochemistry using the Hercep test developed by Dako. Statistical analysis was performed using SPSS-10.

Results: A total of 193 patients (84 females and 109 males), with a mean age of 68.5 years (range, 42–90 years) were included in the study. Of these, 57 (29.5%) revealed HER-2/neu overexpression by immunohistochemistry. After adjusting for age, smoking, Eastern Cooperative Oncology Group score, and treatment, HER-2/neu overexpression was associated with a statistically significant diminished survival (P < 0.001; Mann-Whitney U test). We contacted 53 of 57 patients with overexpression and 121 of 136 patients without HER-2/neu overexpression to ascertain a history of pesticide exposure. Forty-one of 57 (72.4%) patients with HER-2/neu overexpression and 47 of the 121 patients without overexpression (38.8%) were exposed to pesticides. We found that patients with history of pesticide exposure had a higher risk of having HER-2/neu overexpression (odds ratio, 5.38; P < 0.01, 95% confidence interval, 2.5–11.2).

Conclusions: HER-2/neu is overexpressed in ~30% patients with ESSCLC and is associated with decreased survival. Also, pesticide exposure seems to be related to HER-2/neu overexpression seen in our patient population. Future studies are needed to validate our findings and also to determine which pesticide(s)/pesticide components are actually responsible for HER-2/neu overexpression seen in ESSCLC.

INTRODUCTION

Despite recent advances, lung cancer remains the leading cause of death from cancer in the United States (1, 2). SCLC, which comprises of 20% of lung cancer, is characterized by a rapid growth rate and early metastases (3, 4). The high chemosensitivity of SCLC and recent advances in therapy have resulted in a 20–30% 2-year survival in limited stage SCLC (4), whereas ESSCLC leads to a median survival of only 10.1 months (5). Given this dismal survival rate of ESSCLC, other factors, including biomarker profiles and clinical features at initial presentation, need to be evaluated in attempt to define survival characteristics.

We had previously described that overexpression of HER-2/neu was seen in ~29% of patients with extended stage SCLC and was associated with increased mortality (6). Micke et al. (7) found a definite amplification of c-erbB-2 oncogene in 13% of patients with SCLC; however, this was predominantly in ESSCLC. They also felt that HER-2/neu overexpression was more important in advanced stages of the disease (7). However, Schneider et al. (8) found minimal or no overexpression of HER-2/neu in four of four cell lines derived from SCLC. Similarly, other studies have shown little or no overexpression of HER-2/neu SCLC (9). Bunn et al. (10) evaluated 11 SCLC cell lines and found no evidence of HER-2/neu overexpression. We had hypothesized in our previous report that the difference in the methods used to detect overexpression (IHC versus fluorescent in situ hybridization) and the population studied (exclusively ESSCLC in our study) could have been responsible for the difference found in overexpression (6). However, we were unable to definitively explain the possible mechanisms responsible for this high rate of HER-2/neu overexpression seen in our study. Because our study population is mainly rural (North Dakota and western Minnesota), we decided to investigate the possible causative factors for the rate of HER-2/neu overexpres-

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1To whom requests for reprints should be addressed, at University of North Dakota School of Medicine and Health Sciences, 1919 Elm Street North, Fargo, ND 58102. Phone: (701) 293-4131; Fax: (701) 293-4145; E-mail: apotti@medicine.nodak.edu. Present address: Duke University Medical Center, Box 3841, DUMC, Durham, NC 27710.

2The abbreviations used are: SCLC, small cell lung carcinoma; ESSCLC, extensive stage SCLC; IHC, immunohistochemistry; OR, odds ratio; DDT, 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane; o,p'-DDT, o,p'-dichlorodiphenyltrichloroethane.
sion seen in our patients with ESSCLC, with special relevance to pesticide exposure.

MATERIALS AND METHODS

After approval from the University of North Dakota Institutional Review Board and Human Subjects Committee, a two-phase retrospective study was performed. The records of all patients with a diagnosis of SCLC from January 1991 through April 2001 were reviewed. The data collected included the following variables regarding the patient’s cancer history: age; sex; socioeconomic status; family history of cancer (if positive, type of cancer); smoking, performance score (Eastern Cooperative Oncology Group); stage of cancer; treatment modality/modalities for SCLC; metastatic sites (other organs) involved; and recurrent disease: symptoms at first recurrence age of recurrence, months from initial diagnosis and stage of disease at recurrence, treatment received for the recurrence, cause of death, other comorbidities (nonmalignant illnesses), and associated malignancies (if any) in the same patient. In addition, we conducted a population-based epidemiological study in an effort to establish a relationship, if any, between pesticide exposure, ESSCLC, and HER-2/neu overexpression.

Pesticide risk assessment interviews were performed (by a single member of the team for consistency) via telephone on the basis of a predetermined questionnaire investigating occupations and hobbies with special emphasis on: (a) contacts with pesticides, including insecticides and herbicides, organic solvents (paints, varnishes, solvents, and glues) and petroleum products (diesels, petrol, oils, greases, dyes, inks, and colorings); (b) type of exposure (preparation and/or spraying of pesticide solutions and direct handling of solvents containing materials or petroleum products); (c) use of protective measures in the workplace (dissolving or spraying the pesticide with pressurized containers, using glues or varnishes with adequate ventilation, and so forth) in the presence of these effective protection measures the subject was considered as nonexposed; (d) duration of exposure. An exposure index was calculated for each interviewed subject according to the following formula: h/day × days/year × years (11). Patients with an exposure index > 2400 h were considered as exposed. The 2400 h cutoff value is chosen on the basis of previous reports, indicating that this figure represents heavy exposure to genotoxic agents (12, 13).

HER-2/neu oncoprotein overexpression was assessed by IHC. Adequate pathological samples were available on 193 subjects. IHC staining was carried out on formalin-fixed, paraffin-embedded material, using the Hercep test developed by Dako. Immunostaining was classified as follows: 0 = no staining; 1+ = faint, incomplete membranous pattern; 2+ = moderate, complete membranous pattern; and 3+ = strong membranous pattern (14). A trained pathologist (M. K.), who was blinded from the clinical and exposure history of the patient, interpreted the IHC results. An IHC score of 2+ or greater was considered as HER-2/neu overexpression (15, 16).

Statistical analysis was carried out using the χ² test to evaluate the association between pesticide exposure and HER-2/neu overexpression and the Mann-Whitney U test to assess the impact of HER-2/neu overexpression on survival. Overall survival was calculated from the date of diagnosis of lung carcinoma by the Kaplan-Meier product limit method (17). Logistic regression analysis was performed to estimate the magnitude of the effect of pesticide exposure on HER-2/neu status. A multivariate analysis was performed to analyze the prognostic impact of HER-2/neu. Statistical analysis was obtained using SPSS-10.

RESULTS

Between 1991 and 2001, 223 patients with ESSCLCs were identified of whom 209 patients had complete records of treatment. The most common chemotherapeutic regimen involved using either cisplatin/carboplatin and etoposide or carboplatin and paclitaxel. The most common second line agent used was docetaxel. A total of 193 patients (84 female and 109 males), with a mean age of 68.5 years (range, 42–90 years) had adequate pathological specimens available for definitive HER-2/neu testing by IHC.

The symptoms at presentation included weight loss in 61 patients (31.6%), cough in 53 (27.5%), dyspnea in 33 (17.1%), mass on chest radiograph in 18 (9.3%), and chest pain was present in 15 patients (7.7%). Fourteen patients (7.2%) were asymptomatic, whereas 29 patients (15.0%) had other symptoms, including weakness, lymphadenopathy, hoarseness of voice, and paraneoplastic syndromes. In our study, weight loss and cough (59.0%) were the two most common presenting complaints that showed a trend toward a decreased survival (P = 0.03) in patients with ESSCLC. Of the 193 specimens, 57 (29.5%) revealed HER-2/neu overexpression by IHC, whereas the remaining 136 (70.5%) did not overexpress HER-2/neu (Table 1). After adjusting for age, smoking, Eastern Cooperative Oncology Group score and treatment, HER-2/neu overexpression was also associated with a statistically significant diminished survival (P < 0.001; Mann-Whitney U test; Ref. 6).

Of the 57 patients with HER-2/neu overexpression, we were able to ascertain pesticide exposure in 53 patients, whereas of the 136 without HER-2/neu overexpression, 121 were contacted. Forty-one of the 53 (77.4%) patients with HER-2/neu overexpression had a history of significant pesticide exposure, whereas the 121 patients without HER-2/neu overexpression, only 47 (38.8%) were exposed to pesticides. The pesticide exposure occurred anytime between 2 and 21 years before the

| Table 1 Demographic data and symptoms at initial presentation in relation to HER-2/neu overexpression (n = 193) |
|---------------------------------|-----------------|-----------------|
| Mean age at diagnosis (years)   | 65              | 66              |
| Mean age at death (years)       | 67              | 68              |
| Sex                             |                 |                 |
| Male (n = 109)                  | 71              | 38              |
| Female (n = 84)                 | 65              | 19              |
| Performance score               |                 |                 |
| Scores 0–2 (n = 112)            | 82              | 30              |
| Scores 3–4 (n = 81)             | 54              | 27              |
| Common symptoms at presentation |                 |                 |
| Cough                           | 36 (21.7%)      | 17 (29.3%)      |
| Dyspnea                         | 26 (10.2%)      | 7 (12.2%)       |
| Weight loss                     | 47 (28.3%)      | 14 (24.5%)      |
diagnosis of the lung cancer. Patients with an exposure index > 2400 h were considered as exposed, although the length of exposure varied from 2400 to 7100 h. We found that patients with history of pesticide exposure had a higher risk of having HER-2/neu overexpression (OR, 5.38; P < 0.01, 95% confidence interval, 2.5–11.2; Fig. 1). After adjusting for age, gender, and smoking history, the OR for developing a HER-2/neu positive tumor after pesticide exposure was still significant (OR, 15.7; 95% CI, 5.3–46.6; P < 0.01).

**DISCUSSION**

The HER-2/neu (also known as c-erbB-2) oncogene is the second member of the epidermal growth factor receptor family. It has been found to be overexpressed in many different types of human malignancies, notably, breast, lung, ovarian, gastric, pancreatic, colorectal, and cancers of the female genital tract (18–25). Overexpression of HER-2/neu in breast cancer has been associated with poor overall survival and has been shown to enhance malignancy and the metastatic phenotypes. HER-2/neu overexpression also seems to induce chemoresistance in certain experimental conditions (18). These findings suggest the HER-2/neu may serve as an excellent target for developing anticancer agents specific for HER-2/neu overexpressing cancer cells, and the benefit of the monoclonal antibody against HER-2/neu (trastuzumab) in the treatment of advanced female breast carcinoma has been demonstrated (26).

Although animal studies demonstrate that many pesticides are carcinogenic, (e.g., organochlorines, creosote, and sulfalate), whereas others (notably, the organochlorines DDT, chlordane, and lindane) are tumor promoters, human data, however, are limited by the few studies that evaluate individual pesticides (27). In a case control conducted to evaluate the hypothesis that past exposure to the pesticide lead arsenate led to an excess mortality from respiratory cancer, Wicklund et al. (28) found that neither presence, intensity, or duration of lead arsenate exposure differed between case and control subjects. McDuffie et al. (29) reported an absence of correlation of lung cancer risk with occupational exposure to any specific pesticide or pesticides grouped by chemical composition. In contrast, Blair et al. (30) studied the mortality experience of a cohort of 3827 white men licensed to apply pesticides in Florida to investigate health effects associated with chronic exposure to pesticides. They found an increasing risk of lung cancer with number of years licensed, suggesting that some pesticides may be carcinogenic in humans (30). Similarly, De Stefani et al. (31) found that workers in the construction industry exposed to DDT may have an excess risk of lung cancer. Becher et al. (32) also showed an increased overall cancer mortality and mortality of respiratory cancer after long-term exposure to phenoxy herbicides and dioxins. Pesatori et al. (33) showed that the risk of lung cancer was greater among those who worked as pest control operators than nonpest control workers.

In a predominantly agricultural state such as North Dakota where farming is the main occupation, exposure to various pesticides and herbicides is an everyday occurrence. Tessier and Matsumara (34) have shown that in the human prostate cancer cell lines LNCaP and PC-3, erbB-2 kinase was activated by pesticides of different chemical classes: the organochlorine insecticides β-hexa-chlorocyclohexane; o,p’-DDT; heptachlor epoxide; the pyrethroid insecticide trans-permethrin; and the fungicide chlorothalonil. Similarly, in another study, Enan and Matsumara (35) examined the effect of o,p’-DDT, the most estrogenic congener of the DDT family of chemicals and β-hexa-chlorocyclohexane on protein phosphorylation activities in MCF-7, a line derived from human breast cancer cells. They found that o,p’-DDT activated c-neu (HER-2/neu protein) at extremely low concentrations (35). Despite the evidence on the role of pesticides in the pathogenesis of lung cancer, the exact molecular role of pesticides on tumorigenesis in humans is unclear at this time. Hence, we conducted this study to evaluate the relationship, if any, between pesticide exposure and overexpression of the HER-2/neu oncoprotein in patients with ESSCLC.

Although there are no studies in lung cancer, either in
patients or on cell lines demonstrating the biological effects of pesticides or their components, our results indicate that pesticide exposure may indeed be related to HER-2/neu overexpression in our select population with ESSCLC. We found that patients with HER-2/neu overexpression were significantly more likely to have been exposed to pesticides (41 of 53; 77.4%) than those without HER-2/neu overexpression (47 of 121; 38.8%; OR, 5.38; P < 0.01). Our findings seem to agree with the field cancerization theory of Gazdar (36), which states that all or much of the aerodigestive tract epithelium has been mutagenized, perhaps as the result of exposure to carcinogens leading to multiple molecular changes in individual cells ultimately resulting in carcinogenesis. Although there is a relative dearth of clinical studies, experimental studies using cancer cell lines revealed that HER-2/neu was activated by various insecticides and fungicides of different chemical classes (34, 35). However, whether this activation translates into clinical malignancy has not been studied thus far. Our data suggest that the findings of these experimental models may, in fact, be true in the clinical carcinogenesis of SCLC as well.

We realize that as with most questionnaire-based studies, a drawback of our study-design is the distinct possibility of a recall bias among the case population. In addition, in cases where the patient was deceased, the data were obtained from the next of kin, who may or may not have had detailed knowledge regarding pesticide exposure of the subject in question. Interviewing technique plays a central role in improving accuracy of the recall and is under the control of the investigator (37). We tried to minimize the effect of interviewing technique on the results by having one single investigator conduct all of the interviews. Also, we used prompted questions, rather than open-ended questions during the collection of exposure data because that has been shown to decrease recall bias (38). Finally, another potential drawback to our study was the fact that although we gathered enough data regarding the extent of pesticide (insecticide/herbicide) exposure while implementing our questionnaire, we failed to investigate the extent of exposure to specific pesticides/their components. However, it is possible that the effect of individual pesticides/pesticide components on HER-2/neu overexpression may be better evaluated initially using SCLC cell lines (34).

In conclusion, in our large study, HER-2/neu overexpression was seen in slightly less than one-third of patients with ESSCLC and was associated with decreased survival. Our preliminary data also suggests that pesticide exposure seems to be related to HER-2/neu overexpression and may explain the increased incidence of overexpression detected in our study population. Future epidemiological studies are needed to validate our findings and also to investigate which pesticide(s)/pesticide components are actually related to HER-2/neu overexpression and eventually to diminished survival so that proper preventative measures may be adopted.

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