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Pesticides, polychlorinated biphenyls and polycyclic aromatic hydrocarbons in cerebrospinal fluid of amyotrophic lateral sclerosis patients: a case-control study

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ABSTRACT

Neurotoxic chemicals including several pesticides have been suggested to play a role in the etiology of amyotrophic lateral sclerosis (ALS). We investigated the relation between organochlorine pesticides and their metabolites (OCPs), polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) in the etiology of sporadic ALS, determining for the first time their levels in cerebrospinal fluid as indicator of antecedent exposure.

We recruited 38 ALS patients and 38 controls referred to an Italian clinical center for ALS care, who underwent a lumbar puncture for diagnostic purposes between 1994–2013, and had 1 mL of cerebrospinal fluid available for the determination of OCPs, PCBs and PAHs.

Many chemicals were undetectable in both case and control CSF samples, and we found little evidence of any increased disease risk according to higher levels of exposure. Among males >60 years, we found a slight but statistically very unstable increased ALS risk with higher levels of the congener PCB 28 and the OCP metabolite p,p'-DDE.

Overall, these results do not suggest an involvement of the neurotoxic chemicals investigated in this study in disease etiology, although small numbers limited the precision of our results.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a multi-system, progressive and fatal neurodegenerative disorder characterized by degeneration of motoneurons (Boylan, 2015; Ingre et al., 2015; Mancuso and Navarro, 2015). Although great advances have been made in understanding the genetic causes of ALS among both familial as well as sporadic (non-familial) cases, environmental factors including chemicals may play a key role in disease etiology, possibly by interacting with genetic susceptibility (Al-Chalabi and Hardiman, 2013; Ingre et al., 2015; Bozzoni et al., 2016; Zufiria et al., 2016). Among the chemical contaminants potentially involved in ALS etiology, particular interest has been given to the role of pesticides and their metabolites in the residential and working environment (Bonvicini et al., 2010; Kamel et al., 2012; Malek et al., 2012, 2015; Vinceti et al., 2012a; Kang et al., 2014; Oskarsson et al., 2015; Beard et al., 2016; Su et al., 2016; Andrew et al., 2017), due to their persistence and accumulation in organisms and the environment and their potential etiologic involvement in a broad spectrum of neurodegenerative diseases (Dardiotis et al., 2013; Zaganas et al., 2013; Baltazar et al., 2014; Fernandez-Rodriguez et al., 2015).

Recognized neurotoxic pesticides include organochlorine pesticides

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(OCPs), a group of compounds used extensively from the 1940 to the 1970s, such as hexachlorobenzene (HCB) and dichlorodiphenyltrichloroethane (DDT) (Richardson et al., 2014). DDT was banned by the Stockholm Convention for agricultural uses worldwide, though its use is still permitted in small quantities in a few countries for disease control (Guimaraes et al., 2007; van den Berg, 2009), and some illegal use is also likely to still exist. It contains about 75% of the p,p′-isomer (p,p′-DDT), though the o,p′-isomer (o,p′-DDT) is also present in significant amounts (Venier and Hites, 2014). In mammals, after being absorbed by the oral and respiratory route, p,p′DDT is broken down into a few metabolites such as p,p′-dichlorodiphenyldichloroethylene (p,p′-DDE), a major degradation product and one of the most persistent due to its high lipophilicity. DDT may alter neuronal electrical excitability and increase susceptibility of nerve cells to stimulation (Guimaraes et al., 2007; Rinkevich et al., 2015).

Polychlorinated biphenyls (PCBs), a class of synthetic organic chemicals widely used in industry, have also been utilized in agriculture as adjuvants for pesticides mixtures from the 1920s until 2004, when they were banned for health concerns and neurotoxic properties (ATSDR, 2002; Korrick and Sagiv, 2008; Selvakumar et al., 2013). Due to their persistence and bioaccumulation, PCBs are still found in the environment and living organisms as mixtures of several individual chlorinated biphenyl congeners (Walkowiak et al., 2001; ATSDR, 2002).

Another class of neurotoxic chemicals includes polycyclic aromatic hydrocarbons (PAHs), ubiquitous compounds produced by the chemical industry, motor vehicles, cigarette smoking, and other incomplete combustion sources (Kim et al., 2013). PAHs have neurotoxic effects, particularly at high exposures (Tang et al., 2003), including harmful effects for the developing fetal brain and postnatal development (Perera et al., 2006; Sheng et al., 2010; Barrington-Trimis et al., 2013; Jurewicz et al., 2013; Peterson et al., 2015).

In this case-control study, we aimed to assess antecedent exposure to some neurotoxic pesticides and their metabolites, PCBs, and PAHs, among ALS patients and matched controls, by measuring the concentration of these chemicals in cerebrospinal fluid (CSF), which is rarely sampled in this disease.

2. Methods

2.1. Subjects

Newly-diagnosed cases defined as having clinically definite or probable ALS (Brooks et al., 2000) at the ALS Center of the Modena University Neurological Department from 1994 to 2013, residing in the Emilia-Romagna region of Northern Italy and undergoing a lumbar puncture during diagnostic procedures, were eligible for this study. Patients with 'possible' ALS form were not included, to avoid potential misclassification of the disease. This group comprised 72 patients with sporadic ALS, some of whom were enrolled in previous studies (Mandrioli et al., 2006; Vinceti et al., 2013). Among these, we enrolled the 38 patients (21 males and 17 females with a mean age of 61.9 and 61.3 years, respectively) having at least 1 mL CSF available when the present pilot study was designed. These 38 patients were similar in clinical and demographic characteristics from those who could not be enrolled due to the lack of enough CSF. Analysis of their clinical data showed that there were 11 cases with bulbar onset (29%), 14 with onset at the upper limb (37%) and 13 at the lower limb (34%), while the phenotype was bulbar in 11 patients, classic in 14 cases, flail in 5 and upper motor neuron-dominant in 8. Twenty-nine of the ALS patients were deceased at time of submission of this manuscript.

The control series comprised consecutive patients who resided in the Emilia-Romagna region and were admitted to the Modena University Neurological Department between 1998 and 2013, underwent lumbar puncture because of suspected but later disconfirmed neurological disease, and had a sample of at least 1 mL of CSF still available. The mean age of these subjects was 61 years. Among these eligible individuals, we randomly sampled one control from the set of controls who matched each of the 38 ALS cases on age (within 10 years, though most were within 5 years) and sex. Signs or symptoms that led to neurological examination and lumbar puncture were: headache or neck/back pain (n =19), paresthesias (n =8), isolated neuropathies (n =5), transient confusion (n =3), visual impairment (n =3). All controls were subsequently discharged from hospital without a diagnosis of a major disease; final diagnosis was primary headache in 13 individuals, benign intracranial hypertension (n =3), idiopathic isolated neuropathies (n =5), somatoform disorder (n =5) and a symptomatic diagnosis with negative instrumental tests in the remaining 12 individuals. Informed consent for diagnostic lumbar puncture was obtained from all patients, and utilization of the CSF specimens for the present study was approved by the Modena Ethics Committee.

2.2. Analytical procedures

The lumbar puncture was performed according to established procedures to avoid sample contamination. The caring neurologist and assisting nurse located the L3-L4 interspace together with the interspaces above and below, wearing non sterile gloves, and mask, cap and coat. Skin swabs and antiseptic solution were used to clean the skin in a circular fashion, and an adhesive sterile drape to create a sterile and clean field on the patient. After a local anesthetic, a 20-gauge needle was used to withdrawn CSF, orienting the bevel parallel to the longitudinal dural fibers to increase the chances that the needle would separate the fibers rather than cut them. Withdrawing the stylet, 6–8 mL of CSF (depending on diagnostic/clinical requirements) were collected in sterile polypropylene tubes. After CSF collection, the stylet was replaced and needle removed and eliminated. The specimens were divided into 1 mL aliquots which were stored at −80 °C for future research. Samples were kept continuously frozen in polypropylene tubes until transport by air courier to the Laboratory of Forensic Science & Toxicology, University of Crete, Heraklion, Greece for toxicological analysis. Overall, we analyzed 26 chemicals. We measured the level of 6 DDT metabolites (o,p′-DDT, o,p′-DDE, o,p′-DDD, p,p′-DDT, p,p′-DDE and p,p′-DDD), HCB, 7 PCB congeners (PCB 28, PCB 52, PCB 101, PCB 118, PCB 138, PCB 153 and PCB 180), and 12 PAHs (acenaphtylene, benzo(a)anthracene, benzo(k)fluoranthene, benzo(a) fluoranthene, benzo(a)pyrene, benzo(g,h,i)perylene, dibenz(a,h)anthracene, indeno(1,2,3-CD)pyrene, benzo(k)fluoranthene, benzo(a) fluoranthene, benzo(a) pyrene and chrysene) using gas chromatography mass spectrometry.

Stock solutions of each OCP-DDT and PCB analyte individually were prepared in hexane at a concentration level of 10 ppm. The mix stock solution of PAHs was in methylene chloride at 50 ppm. Further dilutions were performed to prepare mix working solutions at concentration levels 1.0, 0.1 and 0.01 ppm. All solutions were stored at −20 °C, in the dark. Ultrapure water (obtained by a Direct-Q 3UV water purification system) free of the checked compounds was used for the preparation of spiked solutions at concentrations 0, 0.5, 1, 2.5 and 5 ng/mL. These spiked solutions were used for the preparation of spiked calibration curves which were constructed using the ratio of the area of each analyte to the area of internal standard. The instrument has a linear response for all analytes both for standards and spiked solutions at the concentration range from 0 to 2.5 ng and 0–5 ng/mL, respectively.

In 0.5 mL of the sample, 20 ng of 1,2,3,4-tetrachloronaphthalene (IS), 0.2 gr of NaCl and 1.5 mL of ultrapure water were added and placed in 8 mL solid phase microextraction vials. The vials were sealed with Polytetrafluoroethylene/silicon septum caps and placed in the gas chromatography mass spectrometry tray. Online extraction was done with an extraction fiber (65 μm polydimethylsiloxane/divinylbenzene metal alloy fiber from Supelco (Bellefonte PA, USA)) at 90 °C for 30 min with an agitation speed at 250 rpm. After that, the fiber was