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Wastewater-based epidemiological evaluation of the effect of air pollution on short-acting beta-agonist consumption for acute asthma treatment

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ABSTRACT

Asthma, one of the most common chronic diseases in the world and a leading cause of hospitalization among children, has been associated with outdoor air pollution.

We applied the wastewater-based epidemiology (WBE) approach to study the association between the use of salbutamol, a short-acting beta-agonist used to treat acute bronchospasm, and air pollution in the population of Milan, Italy.

Composite 24-h samples of untreated wastewater were collected daily and analyzed for human metabolic residues of salbutamol by liquid chromatography tandem mass spectrometry. Corresponding daily outdoor concentrations of particular matter up to 10 µm (PM_{10}) and 2.5 µm ($PM_{2.5}$) in aerodynamic diameter, nitrogen dioxide, ozone, sulfur dioxide, and benzene were collected from the public air monitoring network. Associations at different lag times (0–10 days) were assessed by a log-linear Poisson regression model.

We found significant direct associations between defined daily doses (DDD) of salbutamol and mean daily concentrations of PM_{10} and $PM_{2.5}$ up to nine days of lag time. The highest rate ratio, and 95% confidence interval (CI), of DDD of salbutamol was 1.06 (95% CI: 1.02–1.10) and 1.07 (95% CI: 1.02–1.12) at seven days of lag time and for an increase of 10 $\mu g/m^3$ of PM_{10} and $PM_{2.5}$, respectively. Reducing the mean daily PM_{10} concentration in Milan from 50 to 30 $\mu g/m^3$ means that 852 (95% CI: 483–1504) daily doses of salbutamol per day would not be used. These results confirm the association between asthma and outdoor PM_{10} and $PM_{2.5}$ and prove the potential of the WBE approach to quantitatively estimate the relation between environmental exposures and diseases.

1. Introduction

Asthma is one of the most common chronic diseases in the world and one of the leading causes of hospitalization among children, its prevalence has increased during the second half of the 20th century and is expected to rise further in the next 15–20 years (Eder et al., 2006; Masoli et al., 2004). It is a complex immunologic and inflammatory disease comprising different overlapping phenotypes with defined clinical and physiological characteristics (Wenzel, 2006). Current pharmacological management includes the treatment of acute episodes and the control of chronic symptoms. The acute episodes are mainly treated using short-acting beta-agonists (SABAs), which cause reactive relief of bronchospasm, whereas, for the control of the chronic symptoms, long-acting beta-agonists (LABAs) are used regularly on a daily basis. Nowadays the combinations of inhaled corticosteroids and

Abbreviations: ATC, anatomic therapeutic chemical; ARPA, Regional Agency for Environmental Protection; CI, confidence interval; DDD, defined daily dose; IS, internal standard; LABAs, long-acting beta-agonists; $PM_{2.5}$, particulate matter with aerodynamic diameter ≤ 2.5 µm; PM_{10}, particulate matter with aerodynamic diameter ≤ 10 µm; RR, rate ratio; SABAs, short-acting beta-agonists; WBE, wastewater-based epidemiology; WHO, World Health Organization

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LABAs have become established as the most efficacious treatment (Chung, 2015). However, asthma is not cured by any therapeutic regimen and not all patients respond well to current treatments (Adcock et al., 2008). Thus, primary prevention strategies to avoid the determinants of asthma are fundamental to reduce the morbidity and mortality of this disease (Beasley et al., 2015).

In the last decade scientific evidence has emerged about an association between air pollution and exacerbations of existing asthma and new-onset asthma attacks in children and adults (Kunzli et al., 2009; McConnell et al., 2010; Weinmayr et al., 2010). The mechanisms by which the pollutants induce these effects are not completely understood, although four main mechanisms have been proposed: 1) oxidative stress and damage, 2) airway remodelling, 3) inflammatory pathway and immunological responses, and 4) enhanced respiratory sensitization to aeroallergens (Guarnieri and Balmes, 2014).

Most studies on the association of air pollution with asthma assessed asthma as self-reported, emergency department visits and hospitalizations, and fewer have examined surrogate markers for asthma morbidity, such as medicine sales or prescriptions. SABA prescriptions have been seen to be closely associated with emergency department visits, and hospital admissions for asthma (Naureckas et al., 2005), and ecological studies based on respiratory drug sales may have higher statistical power than studies on mortality or hospital admissions (Pitard et al., 2004). However, the association between anti-asthmatic sales or prescriptions and air pollution may underestimate the effect of air pollution, since patients suffering from asthma are generally using self medications by using a SABA drug always available in case of incipient asthma attacks (Urbano, 2008).

The "wastewater-based epidemiology" (WBE) approach (Daughton, 2001) involves chemical analysis of human metabolic residues in the raw wastewaters produced by a population, in order to study the collective use of a wide range of substances. This approach has been successfully applied to drugs of abuse (Castiglioni et al., 2006; van Nuijs et al., 2011; Zuccato et al., 2008), alcohol consumption (Reid et al., 2011; Rodriguez-Alvarez et al., 2015) and tobacco (Castiglioni et al., 2015), and is potentially applicable to other classes of chemicals, including therapeutic drugs, once the drug environmental sources, urinary excretion, metabolism and stability have been carefully assessed (Castiglioni et al., 2013).

In this study, we applied the WBE approach to study the association between the use of SABAs and air pollution. Salbutamol, one of the most popular SABAs, was the chemical measured in wastewaters. We report the results of a pilot phase of data collection for three months.

2. Methods

2.1. Sampling and data collection

The study area was Milan, the second largest city in Italy, where, on a large number of days during the year, air quality limits are exceeded with some very strong pollution episodes (Masiol et al., 2014).

Composite 24-hour samples of untreated wastewater were collected daily, from September 30 to December 22, 2013, from the inlet of the wastewater treatment plant at Nosedo, the largest in Milan, with a treatment capacity of about 1,100,000 inhabitant equivalents. Wastewater samples were collected every 15–30 min for 24 h with an automatic computer-controlled sampling device and were stored frozen at −20 °C until analysis.

In the same period, daily concentrations of particulate matter up to 10 μm (PM_{10}) and 2.5 μm ($PM_{2.5}$) in aerodynamic diameter, nitrogen dioxide, ozone, sulfur dioxide, and benzene were collected from the monitoring network of the Lombardy Regional Environmental Protection Agency (ARPA, 2016); in addition, meteorological variables (temperature and relative humidity) were recorded from ARPA. Data from three monitoring stations in three different areas in Milan were used and averaged to cover the metropolitan area served by the wastewater treatment plant.

2.2. Analytical method

Wastewater samples were analyzed for salbutamol within few days from their arrival to the laboratory using solid phase extraction and liquid chromatography tandem mass spectrometry. The method was adapted from that of previous studies (Castiglioni et al., 2005; Zuccato et al., 2000). In brief, wastewater samples (100 mL) were filtered, acidified to pH 2.0 with 37% HCl, spiked with labelled internal standard (IS) and solid-phase extracted using mixed reverse-phase cation exchange cartridges (Oasis-MCX, Waters Corp., Milford, MA). Cartridges were then vacuum-dried for 10 min and eluted with 2 mL of methanol and 2 mL of a 2% ammonia solution in methanol. The eluates were pooled, dried under a nitrogen stream and re-dissolved in ultrapure water for instrumental analysis.

A high-performance liquid chromatograph 1200 Series (Agilent Technologies, Santa Clara, CA, USA) coupled to a 6410 QqQ mass spectrometer equipped with an electrospray ionization ion source (Agilent Technologies, Santa Clara, CA, USA) was used. The analysis was run in positive ionization mode using the selected reaction monitoring mode. Quantification was done by isotope dilution using the most abundant precursor/product ion transition (quantifier ion) and comparing the area with its corresponding IS. The conditions used for analysis and quantification and the validation parameters of the method are detailed elsewhere (Re Depaolini et al., 2016). A stability tests showed that salbutamol was stable during sampling and for up to eight weeks during storage at −20 °C (Re Depaolini et al., 2016).

2.3. Data analysis

We transformed salbutamol wastewater concentrations to the number of daily doses consumed, by using the defined daily dose (DDD) of the WHO (WHO, 2016) and the metabolic excretion rate for salbutamol (Dollery, 1991) by the following formula:

$$\text{No DDD} = \frac{\text{C} \times \text{F}}{E \times DDD}$$

Where: No DDD = number of DDD excreted daily; C = concentration of salbutamol in wastewaters (mg/l); F = flow rate of wastewaters entering into the treatment plant (L/day); E = metabolic extraction rate of salbutamol (0.3); DDD = 0.8 mg.

We also calculated the average number of salbutamol DDD prescribed daily for the Milan population, using the pharmacy prescription database of the Lombardy Region. Prescription data for 2012 were used because data were not available for the subsequent years. The prescription database contains the medication name and anatomic therapeutic chemical (ATC) classification code, quantity and dispensation date. Data were analyzed for the Milan Local Health Authority, in order to identify prescriptions of salbutamol by its ATC code (R03AC02) and determine the numbers of boxes dispensed for each salbutamol pharmaceutical specialty.

We provided descriptive statistics for all the variables of interest (number of doses of salbutamol, air pollutants, and meteorological variables), calculated the correlation between them (Pearson's correlation coefficient), and plotted salbutamol versus each air pollutant by a linear regression model.

To assess the association between salbutamol and air