Original Paper

Exposure to Atrazine Induces Lung Inflammation through Nrf2-HO1 and Beclin 1/LC3 Pathways

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Abstract
Background/Aims: Atrazine (ATR) is the second most widely used herbicide, after glyphosate, that is used to stop pre- and post-emergence broadleaf and grassy weeds. In 2007, it was included in the class of endocrine disruptors due to the impact its exposure had on human health. Occasional ATR exposure at work has been linked to an increased risk of respiratory problems, but the molecular mechanisms underlying this relationship has not yet been fully elucidated. ***Methods:*** Mice were exposed to an aerosol containing ATR. In particular ATR aerosol was prepared by dissolving 250 mg of ATR in a vehicle made with saline and 10% DMSO. Seven days after the aerosol exposure, the mice were sacrificed and lung tissue, bronchoalveolar lavage fluid (BALF), and blood samples were collected for histology and biochemical analysis. ***Results:*** ATR inhalation induces a generalized state of oxidative/nitrosative stress that leads to an increase in cytokines production and to a physiologically unstable antioxidant defense response evaluated by the alteration of Nrf-2 pathways. Moreover, it stimulates autophagy through Beclin 1/Lc3 expressions and increases lipid peroxidation and apoptosis. All these effects culminate in serious alterations in the tissue architecture of the lungs and to an increase in mucus production and mast cells degranulation. ***Conclusion:*** Our study shows, for the first time, the impact of ATR inhalation on lung tissue. This could represent the first step to also recognize this substance as a problematic air pollutant as well as a soil and water contaminant.

R. D'Amico, F. Monaco and R. Fusco contributed equally to this work.
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Introduction

There is increasing public interest in the effects of endocrine disrupting chemicals on public health, especially the effects of long-term low-dose exposures [1, 2]. The U.S. Environmental Protection Agency (EPA) has defined an endocrine disruptor (ED) as "an agent that interferes with the synthesis, secretion, transport, binding, or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development and/or behavior" [3]. Today, this definition is considered to be outdated; in fact, different studies conducted on animals as well as clinical observations and epidemiological studies have indicated that endocrine disruptors can affect reproductive systems, prostate, breast, liver, thyroid, metabolism, and lungs [4, 5]. EDs can be classified into the following three different groups: (i) food contact materials such as bisphenol A, (ii) chemicals in products such as phthalates or parabens, and (iii) pesticides such as atrazine (ATR) [6, 7]. Methods for predicting effects on populations and societies based on knowledge of effects on individuals must be established in order to understand the vulnerability and risk factors of people due to endocrine disruptors, as well as treatments approaches [2].

Every year, four million people die prematurely as a result of chronic respiratory diseases, 300 million people have asthma, and 210 million people have chronic obstructive pulmonary disease (COPD) [8-10]. As a result of this situation, stakeholders have advocated for a stronger battle against the main risk factors for respiratory diseases, such as air pollution [11, 12]. Occupational exposure to pesticides occurs during their production, transport, and storage; during user's preparation and application; as well as, during re-entry into treated fields, harvests, and equipment cleaning [13, 14]. The majority of pesticides used in agriculture enter the body through the skin, followed by the respiratory and oral paths. Inhalation of pesticides is most common during fumigation, mixture preparation, and/or application in enclosed spaces [15-18]. Occupational exposure of farmers, farm employees, and pesticide manufacturing industry workers may be the most important, and it has been extensively studied, but para-occupational and residential exposure are also worthy of consideration [15, 19-21].

The chemical name for ATR is 6-chloro-N-ethyl-N'-(1-methylethyl)-1,3,5-triazine-2,4-diamine, and it is the most commonly used broad-spectrum herbicide in agricultural crops such as corn, sorghum, and sugarcane [22]. Despite the fact that farm use of ATR is limited in the EU, it is still one of the most widely used pesticides in the world, with ATR being found in ground water in the United States and Europe on a regular basis [22, 23]. ATR has a half-life of 95–350 days and is resistant to degradation; in fact, following application, it can be present in the particulate and vapor phases of the air, and it can travel up to 186 miles from the application site [24]. Atrazine can be broken down in the air by reacting with hydroxyl radicals [25, 26]. ATR contamination has been linked to many different serious health issues such as dermatologic diseases, neurologic conditions, cancer, and respiratory problems [27-37]. In particular, the Agricultural Health Study (AHS), one of the most important studies of respiratory pathologies, found a correlation between wheeze and atrazine exposure (>20 days per year, OR 1.53, 95 percent CI 1.21–1.95) [38].

However, until today, few reports have explored the molecular mechanisms underlying ATR induction of lung problems. Some of the most accredited hypothesis that could explain the mechanism of toxicity induced by ATR is the production of reactive oxygen species (ROS) [22, 39-43]. In particular, some of the study results that prompted us to investigate the ways by which ATR exerts its lung toxicity were the discovery by Zhao and colleagues that ATR could induce a response activated by nuclear factor erythroid 2-related factors 2 (Nrf2); the increase in the malondialdehyde levels as well as in the activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) found by Batti et al.; and finally the results by Song et al. that demonstrated that ATR enhanced the apoptotic and autophagic processes [22, 39, 44]. These studies (and many others) have in common the fact that ATR was administered by oral gavage. However, considering that some ATR is released into the atmosphere as a result of its preparation, manufacturing, and disposal, and that it also enters

the environment through the loss of applied herbicide until it enters the soil surface, as well as the particle distribution of ATR-containing dust, and considering that ATR volatilization after application to fields has been estimated to be up to 14% of the applied volume, it is critical to investigate the effects in the lungs of occasional air exposure. Understanding the molecular basis of ATR-induced oxidative stress, apoptosis, and autophagic processes is critical for the development of therapeutic approaches to limit compromised lung function. With this aims in our mind, we investigated for the first time the impact of ATR aerosol inhalation.

Materials and Methods

Animals

CD1 male mice (8 weeks old, 18–24 g) were acquired from Envigo (Milan, Italy) and located in a controlled environment. The study was approved by the Review Board of the University of Messina for the care of animals. In addition, the experiments on mice complied with U.S. (Animal Welfare Insurance No. A5594-01, Department of Health and Human Services, Washington, DC, USA), European (OJ of ECL 358/1 12/18/1986), and Italian (DM 116192) regulations.

Experimental Design and Groups

The ATR aerosol was prepared by dissolving 250 mg of ATR in a vehicle made with saline and 10% DMSO. After complete solubilization, a Lovelace nebulizer (In-Tox Products, Albuquerque, NM, USA) was used to create an atmosphere in an exposure chamber (Research and Consulting Co., AG, Basel, Switzerland) [45, 46]. In detail, each mouse was carefully inserted into an animal tube with the nose pointing to the aerosol outlet. The animal tubes were specifically designed to contain one mouse per tube. Using the plunger in the tube, the mouse was gently immobilized in the correct position. This phase was very important to allow the animal to breathe properly. After being immobilized, a known volume of vehicle or ATR (pro kilo) was placed in the nebulization until it was completely nebulized.

The mice were randomly divided into the following two groups:

(I) Sham group, i.e., animals that were exposed to the vehicle (saline with 10% of DMSO).

(II) ATR group, i.e., animals that were exposed to 250 mg of ATR for 1 hour for 1 day.

After exposure, the mice were housed in individual cages (six per cage) and maintained under a 12:12 hours light/dark cycle at 21 ± 1 °C and 50 ± 5% humidity. Standard laboratory litter, diet, and water were available ad libitum. Additionally, the mice were weighted and observed for any clinical symptoms, and the information was recorded by the animal care staff. Seven days after the aerosol exposure, the mice were sacrificed and lung tissue, bronchoalveolar lavage fluid (BALF), and blood samples were collected for histology and biochemical analysis, as previously described [40, 42, 43, 47-49]. The ATR dosage was chosen based on other previous studies, but for the first time, ATR was not administered by oral gavage but instead by aerosol, because there is still limited knowledge of the effects of ATR on the lungs [50-52].

Western Blot Analysis of Cytosolic and Nuclear Extracts

Extracts of the cytosol and nucleus were prepared, as previously mentioned [53-57]. The following primary antibodies were used: anti-NRF-2 (1:500, Santa Cruz Biotechnology, Heidelberg, Germany, #sc-365949), anti-heme oxygenase 1 (HO-1; 1:500, Santa Cruz Biotechnology, Heidelberg, Germany, #sc-136960), anti-Bax (1:500, Santa Cruz Biotechnology, #sc7480), anti-Bcl-2 (1:500, Santa Cruz Biotechnology, #sc7382), anti-Beclin 1 (1:500, Santa Cruz Biotechnology, #sc48341), and anti-MAPLC3 (1:500, Santa Cruz Biotechnology, #sc271625) in 1× PBS, 5% w/v non-fat dried milk, and 0.1% Tween 20, at 4 °C overnight. For the cytosolic fraction, Western blots were also probed with antibody against β-actin protein to ensure that they were filled with equivalent amounts of proteins (1:500, Santa Cruz Biotechnology). The same methods were used for nuclear fraction with lamin A/C (1:500, Sigma-Aldrich Corp., Milan, Italy). Signals were examined with an enhanced chemiluminescence (ECL) detection system reagent, according to the manufacturer's instructions (Thermo, Monza, Italy). The relative expression of the protein bands was quantified by densitometry with BIORAD ChemiDocTM XRS⁺ software and standardized to the β-actin and lamin A/C levels.

Histopathological Evaluation with Hematoxylin/Eosin, Toluidine Blue, and Periodic Acid Schiff

The lung tissues were dehydrated, embedded in paraffin, stained with hematoxylin and eosin (H&E) and toluidine blue, and analyzed using a light microscopy (Leica DM6, Milan, Italy) associated with an Imaging system (LasX Navigator, Milan, Italy); lung tissues were blindly scored and mast cells counted by two investigators. The degree of inflammation was evaluated according to a score from 1 to 3, as previously described [58, 59]. The mast cell counts were assessed, as previously described by Fusco et al. [60]. Lung tissue was also analyzed with PAS to assess goblet cell hyperplasia according to a score from 0 to 4, as previously described by Wang et al. [58, 59].

Evaluation of Tissue Lipid Peroxidation

Malonaldehyde (MDA) levels was assessed, as previously described for lung tissue, at the end of the experiments. Briefly, after homogenization, MDA absorbances was measured at 650 nm, using a spectrophotometer and expressed in mill-units per 100 milligram weights (mU/100 mg) of wet tissue [61-67].

Assessment of Plasma Parameters

ELISA kits were used to test the activity of superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), reactive oxygen species (ROS), reactive nitrogen species (RNS), and hydrogen peroxide (H_2O_2) in plasma, as previously described [68]. The manufacturer's manuals provided specific instructions for the test procedure. Every test were made in triplicate.

Assessment of Cytokines Production

ELISA kits were used to test the inflammatory cytokine production, in particular IL-1β, IL-6, IL-18, IL-10, and IL-4, in BALF after ATR induction, as previously described [69]. The manufacturer's manuals provided specific instructions for the test procedure. Every test were made in triplicate.

Materials

Unless otherwise stated, all compounds were purchased from Sigma-Aldrich (city, country).

Statistical Evaluation

In this study, the data are expressed as the average ± SEM and represent at least 3 experiments carried out in different days. For *in vivo* studies, N represents the number of animals used. The number of animals used for *in vivo* studies was carried out by G * Power 3.1 software (Die Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany). Data were analyzed by an experienced histopathologist, and all the studies were performed without knowledge of the treatments. The results were analyzed by t-tests followed by two-tailed calculations. Group data are normalized to mean values for mice. Homoscedasticity not been assessed. A *p*-value less than 0.05 was considered to be significant.

Results

ATR Induces a Generalized State of Oxidative/Nitrosative Stress

In the plasma, through the ELISA analysis, we found that a generalized state of oxidative and nitrosative stress was established which would probably spread through the blood as well as into other parts of the body. In particular, we found that ATR induced a significant increase in ROS (Fig. 1A), H_2O_2 (Fig. 1B), and RNS (Fig. 1C) as compared with animals not exposed to ATR.

ATR Exposure Enhanced Cytokines Production

Through the use of ELISA kits, on the one hand, we found that ATR exposure increased the level of proinflammatory cytokines in BALF, in particular, IL-1β (Fig. 2A), IL-6 (Fig. 2B), and IL-18 (Fig. 2C) and, on the other hand, we found a significant decrease in anti-inflammatory cytokines IL-10 (Fig. 2D) and IL-4 (Fig. 2E) as compared with the sham groups.

ATR Exposure Compromised the Physiological Antioxidant Response

In order to further investigate how ATR induced the antioxidant response of cells, by Western blot analysis, we investigated the expression of Nrf2 and HO-1 in lung tissue and, consequently, by ELISA kits, we also investigated the stimulation of antioxidant enzymes in plasma. We found that ATR stimulated the physiological response of Nrf2 expression (Fig. 3A, see densitometric analysis Fig. 3A') as compared with the sham group. Similar results were found by the analysis of the expression of HO-1 (Fig. 3B, see densitometric analysis Fig. 3B'), one of the most important enzymes regulated by Nrf2. Additionally, ATR stimulates the body's physiological antioxidant defense trying to fight the establishment of a generalized condition of oxidative stress. In particular, it increases the activity of CAT (Fig. 3C) as well as of SOD (Fig. 3D) and GPx (Fig. 3E).

Fig. 1. Exposure to ATR induces oxidative/nitrosative stress conditions. (A) ROS; (B) H2O2; (C) RNS. By ELISA kits, in the plasma, we found that ATR exposure induced a significant generalized state of oxidative/nitrosative stress. Values are means ± SEM of 6 mice for all groups. Each experiment was repeated 3 times on 3 different days. Group data are normalized to mean values for mice. Each sample was loaded in triplicate. See manuscript for further details. *** $p < 0.001$ vs. sham and ** $p < 0.01$ vs. sham.
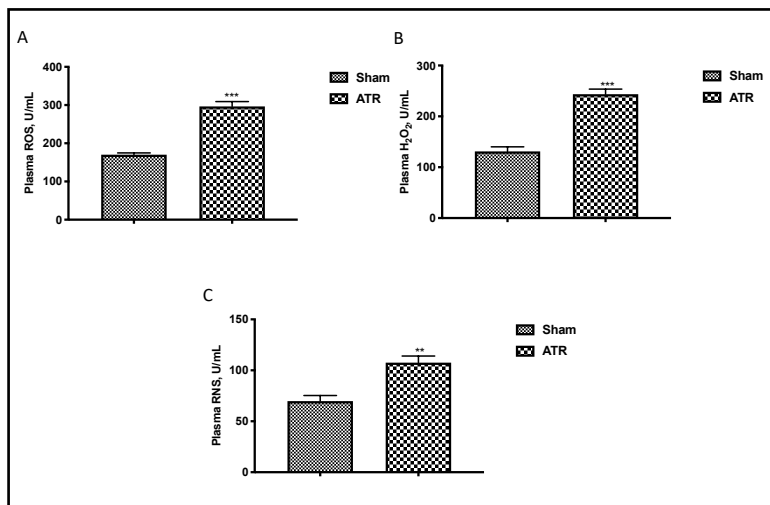

Fig. 2. Exposure to ATR affects cytokine production. (A) IL-1β; (B) IL-6; (C) IL-18; (D) IL-10; (E) IL-4. ATR administration was characterized by an increase in several proinflammatory cytokines and a decrease in anti-inflammatory cytokines into the BALF as compared with the sham group. Values are means ± SEM of 6 mice for all group. See manuscript for further details. *** $p < 0.001$ vs. sham.

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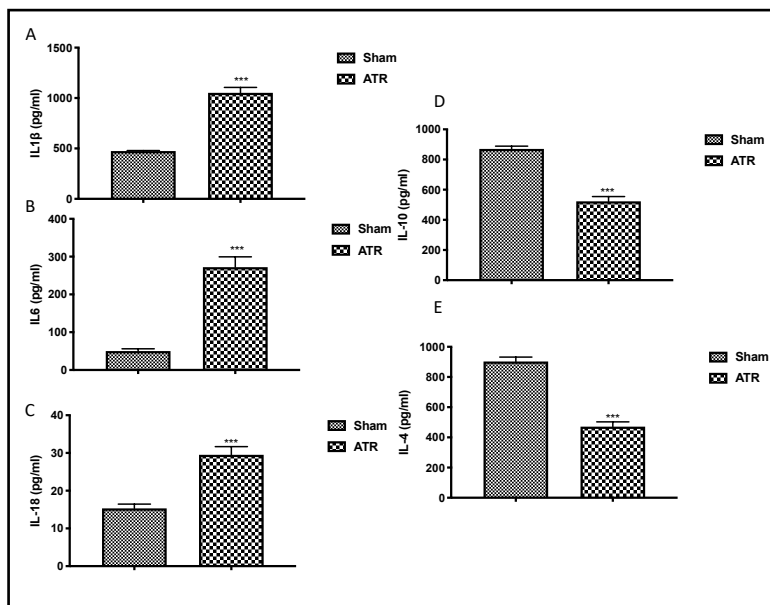D'Amico et al.: Atrazine Inhalation Induces Lung Damage

Fig. 3. Exposure to ATR induces limits to anti-oxidant defense. Western blots and quantification of lung tissue from NRF2 (A and A') and HO-1 (B and B'), respectively. ELISA kits of CAT (C), SOD (D), and GPx (E) levels after ATR administration. Values are means ± SEM of 6 mice for all groups. Each experiment was repeated 3 times on 3 different days. Group data are normalized to mean values for mice. Each sample was loaded in triplicate. See manuscript for further details. *** $p<0.001$ vs. sham and ** $p<0.01$ vs. sham.
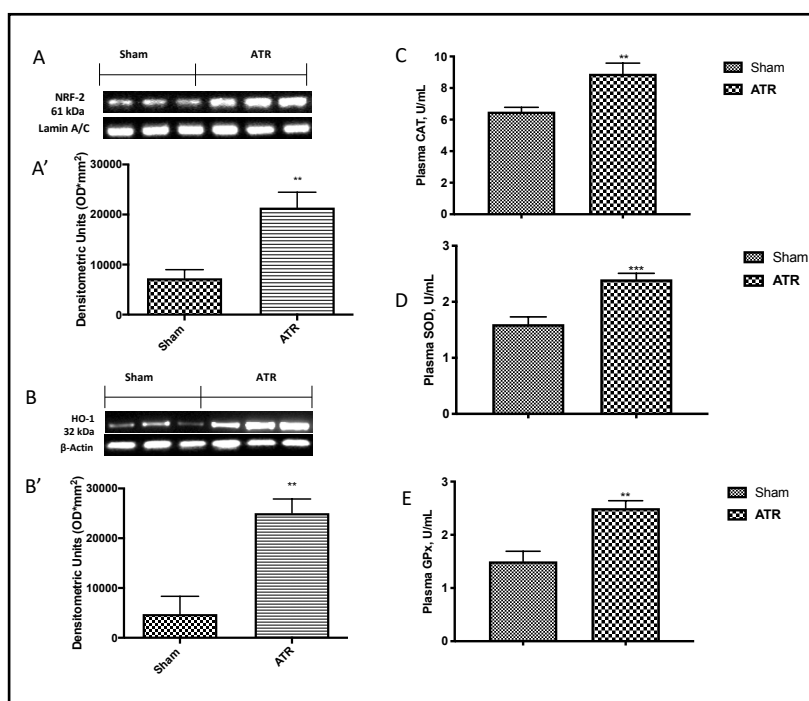
ATR Stimulates Autophagy through Beclin 1/LC3 Expressions

Microtubule-associated protein light chain 3 (LC3) and Beclin 1 are specific marker proteins of autophagy; therefore, we investigated them by Western blot analysis. In our study, we found that ATR stimulates the initiation of the autophagic process, as demonstrated by an increase in Beclin 1 (Fig. 4A and densitometric analysis Fig. 4A') and LC3 (Fig. 4B and densitometric analysis Fig. 4B') expressions.

ATR Induces Lipid Peroxidation and Apoptosis

Therefore, we investigated the effect of ATR in lung tissue by MDA levels and by Western blot for Bax and Bcl-2, As expected, on the one hand, we found a significant increase in lipid peroxidation as well as in pro-apoptotic protein Bax (Fig. 5B and densitometric analysis Fig. 5B'), on the other hand, we found a decrease in anti-apoptotic Bcl-2 (Fig. 5C and densitometric analysis Fig. 5C')

Effects of ATR Administration on Lung Tissue

Finally, we directly investigated alterations in lung tissue. We found that, after ATR administration, lung tissue (Fig. 6B and high magnification Fig. 6B', see score Fig. 6C) appeared to be significantly inflamed as compared with the sham group (Fig. 6A and high magnification Fig. 6A', see score Fig. 6C). In order to better understand the effect of ATR-induced airway inflammation, we also performed PAS staining. On the one hand, there were no pathological changes observed in the lung tissue of the control group (Fig. 2A, see score Fig. 2B). On the other hand, after ATR exposure, we found significant goblet cell proliferation in the bronchial epithelium with increased production and secretion of mucus (Fig. 7A', see score Fig. 7B). Additionally, we decided to investigate whether mast cells were also recalled to the lungs following exposure to ATR. As previously highlighted by Mizoda and Ueda [70], we found that ATR induced significant mast cell recall following exposure (Fig. 7C', see score Fig. 7D)., which headed towards the site of inflammation and also underwent significant degranulation, as evidenced by the staining. No degranulated mast cells were found in the control animals (Fig. 7C, see score Fig. 7D).
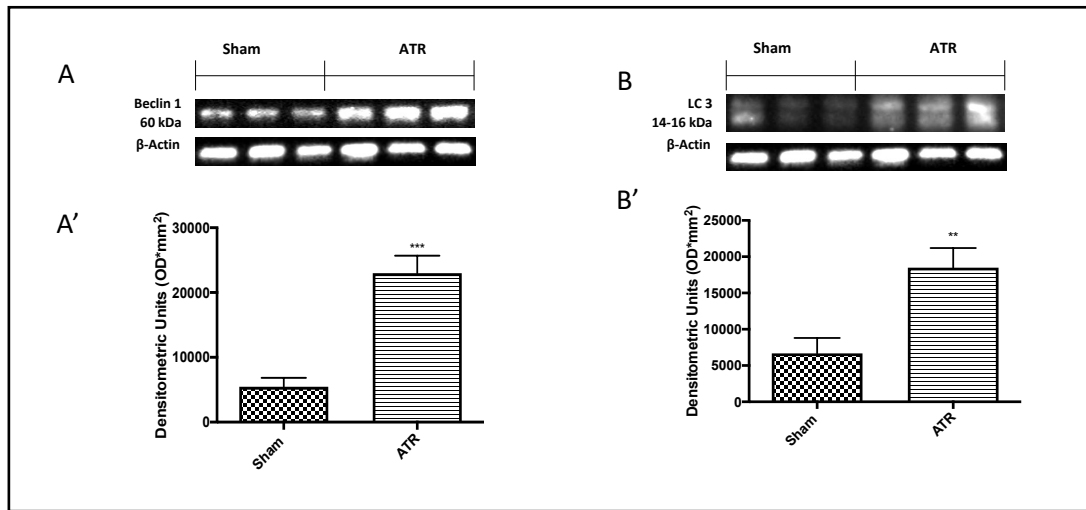
Fig. 4. Exposure to ATR increases Beclin 1 and LC3 expressions. Western blots and quantification of lung tissue from Beclin 1 (A and A') and LC3 (B and B'), respectively, after ATR administration. Values are means ± SEM of 6 mice for all groups. Each experiment was repeated 3 times on 3 different days. Each sample was loaded in triplicate. Group data are normalized to mean values for mice. The photo shown is representative of the results obtained. See manuscript for further details. *** p<0.001 vs. sham and ** p<0.01 vs. sham.

Fig. 5. Exposure to ATR induces lipid peroxidation and apoptosis. MDA (A) and Western blots, respectively, quantification of lung tissue for Bax (B and B') and Bcl-2 (C and C') after ATR administration. Values are means ± SEM of 6 mice for all groups. Each experiment was repeated 3 times on 3 different days. Each sample was loaded in triplicate. Group data are normalized to mean values for mice. The photo shown is representative of the results obtained. See manuscript for further details. ** p<0.01 vs. sham.

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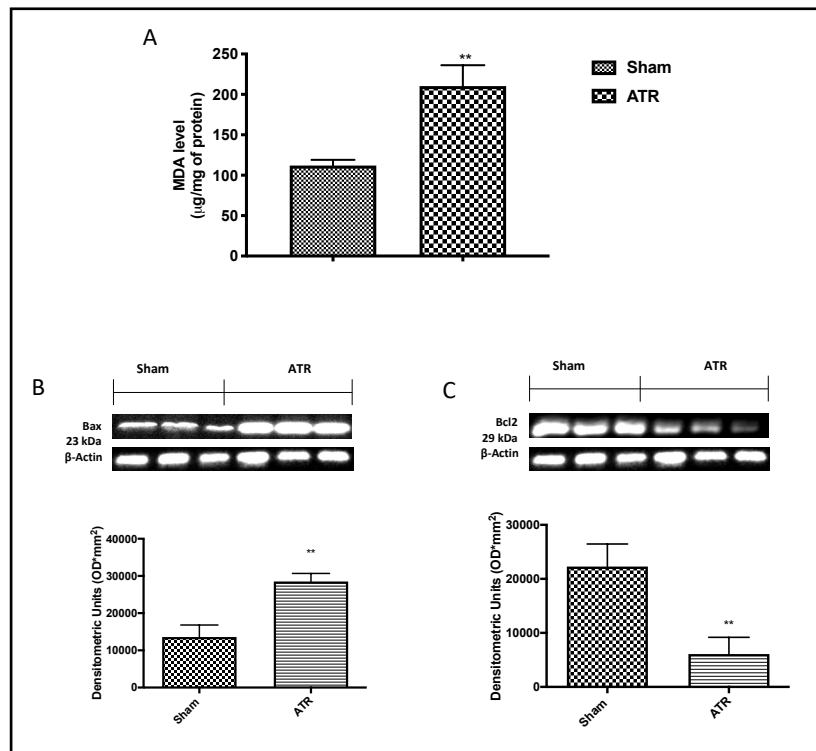D'Amico et al.: Atrazine Inhalation Induces Lung Damage

Fig. 6. Effects of ATR exposure on lung tissue. Hematoxylin/eosin on lung tissue. (A and A') sham group; (B and B') ATR group. After ATR exposure, inflammatory cells, edema, and lung tissue architecture alterations were evident in the ATR group. Values are means ± SEM of 6 mice for all group. Group data are normalized to mean values for mice. The photo shown is representative of the results obtained. See manuscript for further details. *** p<0.001 vs. sham.
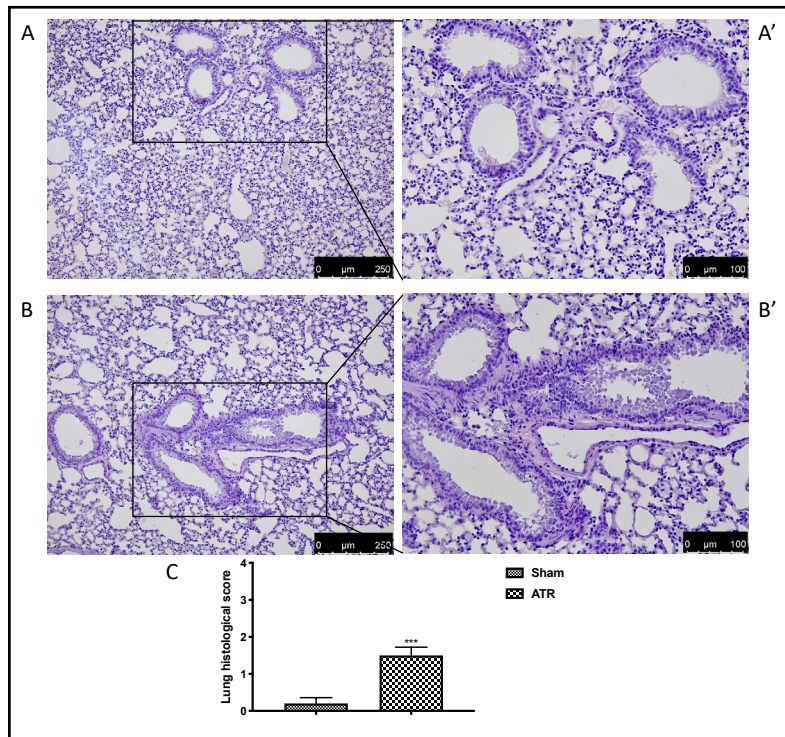

Fig. 7. Effects of ATR on goblet and mast cells. PAS staining on lung tissue. (A) sham; (A') ATR; (B) PAS score. Toluidine blue staining on lung tissue. (C) sham; (C') ATR; (D) mast cells count. PAS staining highlighted, in the ATR group, a marked goblet cell hyperplasia with increased mucus production and secretion. Additionally, by toluidine blue, we observed that ATR exposure was able to increase mast cells degranulation as compared with the sham group. Values are means ± SEM of 6 mice for all group. Group data are normalized to mean values for mice. The photo shown is representative of the results obtained. See manuscript for further details. *** p<0.001 vs. sham.
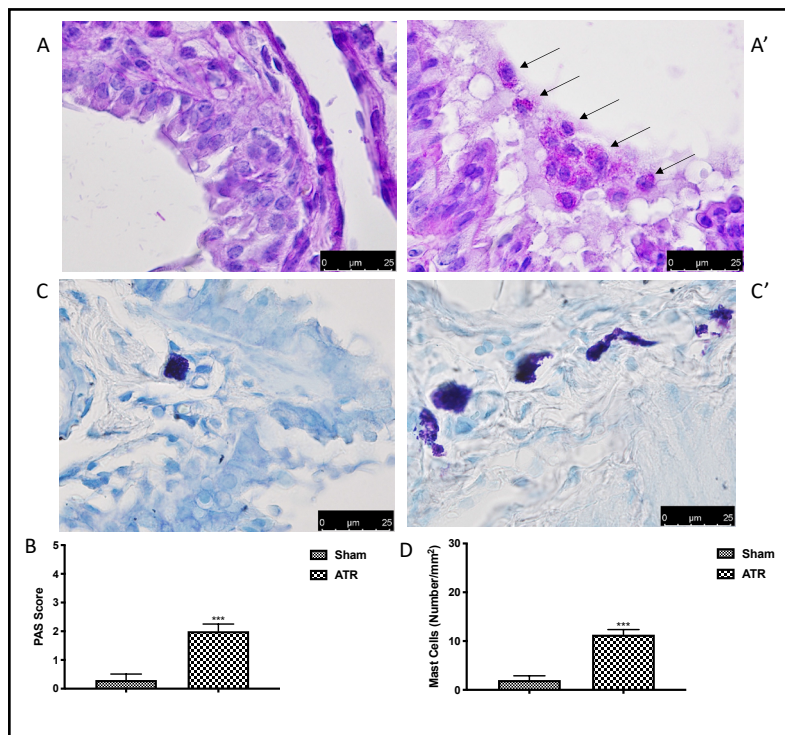
Discussion

ATR is a commonly used herbicide for controlling broadleaf weeds. It is a man-made compound that does not exist naturally and is widely used on corn crops in the United States and Europe. The total projected agricultural use in the United States is 76.4 million pounds per year, with corn accounting for 86 percent of that total [71]. The United States Environmental Protection Agency (EPA) has designated ATR as a restricted use pesticide (RUP), meaning that only licensed herbicide users can purchase or use it due to its persistence in water and various adverse health effects on humans. The maximum contamination level (MCL) for atrazine is set at 3 ppb (0.003 g/L) by EPA regulations [71]. Unlike the United States, Europe has stricter regulations on the use of ATR. A pesticide directive issued by the European Union (EU), in 1991, restricted the use of chemicals that were accused of causing harm to human health, groundwater, or the atmosphere. In addition, during acceptable usage, the scientific committee examining atrazine found, in 2003, that the herbicide had the potential to contaminate groundwater levels above the permitted 0.1 g/L. As a result of this discovery, a regulatory ban on ATR was enacted in 2005, affecting all EU member states. As a result, Europe is embarking on a continent-wide agricultural experiment without the use of ATR [72]. Significant quantities of ATR that are not absorbed by plants do end up in the environment. ATR is only weakly adsorbed by soil particles after application, and thus mainly leaves the field in runoff water. Rainfall washes large quantities of ATR out of the soil and into nearby areas, such as streams, reservoirs, and other waterways. Moreover, after it is added to the soil, small quantities of ATR may reach the air [73]. Humans are mainly exposed to ATR by the intake of tainted drinking water. However, inhalation exposure may occur during application on rare occasions. ATR's negative effects are still being studied [74]. In humans, increased risk of intrauterine growth retardation, decreased semen content, and spontaneous abortions were found in many peer-reviewed studies, as were demasculinization and hermaphrodism in frogs [75-79]. In particular, it has been demonstrated that ATR can induce oxidative stress conditions following an imbalanced condition of physiological antioxidant [39, 42, 47, 70, 80, 81].

Oxidative stress is characterized as an imbalance between oxidant and antioxidant species in a system where oxidant species predominate [82]. It has only recently been studied as a potential mediator of ED-related outcomes [83-85]. Many oxidative stress measurements can be collected using minimally invasive methods, for example, in blood, making these methods reasonably simple to use in both animal and human studies [86]. Our first results demonstrate the onset of a generalized state of increased oxidative and nitrosative stress. The respiratory system regulate and express inflammatory pathways in such a way that the respiratory system's primary functions are preserved, while still protecting it from invasion by foreign infective agents or antigens. A previous study has shown that oxidative stress can act as an initiator of cytokine release and cell damage [87]. Cytokines, which are expressed by a variety of cells ranging from immune cells like macrophages and lymphocytes to endothelial cells and fibroblasts, during inflammation, act as communicators between immune cells for regulating cell growth, maturation, and responsiveness [88, 89]. In particular, several cytokines are released from monocytes and macrophages, and recruit neutrophils into lung tissues, which is vital for host defense and contributes to the development of lung injury [90, 91]. On the other hands, IL-4 and IL-10, which are potent anti-inflammatory cytokines, could suppress the activity of many inflammatory molecules [91, 92]. Our study demonstrated, for the first time, that inhalation of ATR, on the one hand, significantly enhanced the release of proinflammatory cytokines and, on the other hand, reduced the production of anti-inflammatory cytokines. In order to preserve redox homeostasis in the cell, ROS and RNS are counterbalanced by complex antioxidant systems. SOD, CAT, and GPx are the most important antioxidant protagonists involved in this response initiated by the cells. Nrf2, which is stimulated with HO-1 in the case of an imbalance in physiological/pathological ROS production, is one of these regulatory networks [93-95]. Nrf2 is a transcription factor that is present in epithelium and alveolar macrophages but

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is primarily expressed in the lungs. Recent studies have shown that Nrf2 protects the lungs from oxidative insults such as high oxygen stress and particulates in the air. The activation of ARE-regulated antioxidant genes, whose products work directly or indirectly to restrict ROS-mediated pulmonary pathogenesis, is thought to be the mechanism of Nrf2-mediated defense [96]. In our study. we confirmed that following exposure to inhaled ATR, cells carry out the physiological response that involves increasing the expression of Nrf2 levels, as well as HO-1, SOD, CAT, and GPx in lungs and plasma. Autophagy can operate as a degradative pathway for oxidatively modified substrates such as proteins and phospholipids, acting as a general cellular protective mechanism against oxidative stress. Since mitochondria are both an intracellular source of ROS and a functional target for ROS generation, autophagy's role in mitochondrial homeostasis may be crucial during oxidative stress [97-99]. For this reason, in the pathogenesis of inflammatory diseases, autophagic clearance may be a critical host response mechanism [100]. Beclin 1 and LC3 are specific marker proteins of autophagy, as well as important regulators of autophagy [101-103]. By Western blot analysis, first, we investigated two of the most common markers used to understand the autophagic process, Beclin 1 and LC3, and the apoptotic process with Bax and Bcl-2 expression. Our results indicate that occasional inhaled exposure to atrazine stimulates autophagy by inducing autophagosome formation and also induces apoptosis by increasing the expression of the pro-apoptotic protein Bax and by reducing the expression of the anti apoptotic protein Bcl-2. Recently, different studies have focused their attention on the effect of occasional pesticide exposure and respiratory pathology. In fact, it has been demonstrated that people who deal with pesticides have been found to have deteriorated pulmonary function [104]. However, until today, this mechanism has remained unclear. Wheezing, airway inflammation, dry/sore throat, cough, breathlessness, and chest tightness are some of the respiratory symptoms linked to pesticide exposure, which are linked with modifications in the tissue architecture of the lungs [105-107]. We demonstrated that, following ATR exposure, the lungs undergo a series of structural changes with an increase in mucus secretion.

Conclusion

Taken together, our data significantly increase the understanding of the mechanisms of immunotoxicity of ATR in the lungs. In particular, we demonstrate, for the first time, that as well as oral administration of ATR can induce serious ROS-related damage, also inhalation can have a similar effect. This could also represent the first step for recognizing that this substance is a problematic air pollutant.

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Disclosure Statement

The authors declare no conflict of interests exists.

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