Mol Cell Biochem
DOI 10.1007/s11010-009-0265-4

Enhanced death signaling in ozone-exposed ischemic-reperfused hearts

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Received: 11 March 2009 / Accepted: 15 September 2009

Abstract Although numerous advancements made in the field of human health have resulted in reduced deaths due to cardiovascular diseases (CVD), many patients with cardiac disease show no established risk. Therefore, other unknown factors may be responsible for the pathophysiology of CVD. Out of 350,000 sudden cardiac deaths each year in the United States, 60,000 deaths have been related to air pollution, suggesting a detrimental role of environmental pollutants in the development of CVD. The present study tested our hypothesis that chronic ozone exposure enhances the sensitivity to ischemia–reperfusion (I/R) injury in isolated perfused hearts. Sprague-Dawley rats were continuously exposed for 8 h/day for 28 and 56 days to filtered air or 0.8 ppm ozone. Isolated hearts were subjected to 30 min of global ischemia followed by 60 min of reperfusion. Cardiac function after I/R measured as left ventricular developed pressure (LVDP), $+dP/dt$, $-dP/dt$, and left ventricular end diastolic pressure (LVEDP) was significantly decreased and increased respectively in ozone-exposed I/R hearts compared to I/R hearts exposed to filtered air. The enhanced sensitivity to I/R injury upon ozone exposure was associated with increased myocardial TNF-α levels and lipid peroxidation and decreased myocardial activities of superoxidase dismutase (SOD) and IL-10. These data suggest that ozone-induced sensitivity to myocardial I/R injury may be due to promoting levels of oxidative stress as well as inflammatory mediators.

Keywords Environmental pollutants · Ozone · Cardiovascular disease · Cardiac function · Ischemia–reperfusion · Oxidative stress · Inflammation · Malondialdehyde · Superoxide dismutase

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Introduction

Environmental pollutants and cardiovascular disease

Cardiovascular disease (CVD) is a major contributor to the mortality rate in North America resulting in approximately 1 million people per year dying of CVD in the United States [1]. Mortality due to CVD appears to be increasing with a disturbing number of cases reporting no obvious established risk [2, 3]. In addition, when genetically similar populations migrate to a new environment, the CVD risks have been shown to be altered [2]. Environmental factors may play an important role in the development of heart disease since out of the approximately 350,000 sudden cardiac deaths each year in the United States, 60,000 deaths could be related to air pollution [4].

Ozone and cardiovascular disease

Ozone, a significant component of air pollution, has been identified as negatively impacting the respiratory and cardiovascular system in humans and laboratory animals.

Published online: 03 October 2009

Although the underlying mechanisms responsible for the ozone-related detrimental effects on cardiovascular health are incompletely understood, some of the proposed mechanisms include; (a) ozone mediated compensatory cardiovascular adjustments due to an impaired alveolar–arterial oxygen transfer and a potential reduction in oxygen supply to the myocardium [5]; (b) ozone-induced oxidation of plasma membrane cholesterol resulting in apoptotic cell death in cardiomyocytes [6]; (c) ozone interactions with synapse of neurons that innervate the lung, resulting in alterations in the autonomic nervous response that can lead to electrophysiological changes in the heart [7]; (d) direct bradycardiac effects due to ozone exposure [8]; (e) ozone-induced pulmonary inflammation and edema, indirectly leading to damaging effects on the cardiovascular system [9]. However, in vitro and in vivo studies using rat model of ischemia–reperfusion (I/R) human injury have reported prevention or reduction of myocardial tissue damage, as well as improved myocardial function subsequent to acute short-term exposure to ozone [10, 11].

In humans, the massive integumant could be exposed for hours to ozone and may be a contributing factor to overall toxicity. People living in warm climates and particularly during the summer months, may become particularly susceptible to ozone exposure. A recent human study reported that short-term exposure to ozone within a 1–2 day period may be linked to acute coronary events in adults without heart disease, suggestive of a detrimental role of ozone in the pathophysiology of CVD [9]. The epidemiological data suggests a significant impact of air pollution on cardiovascular mortality effecting humans from all over the world [12–17]. Additionally, earlier reports have suggested that the release of circulating pro-oxidative and/or pro-inflammatory mediators from the lungs into systemic circulation following pollutant exposure can indirectly mediate CV responses [18, 19].

Despite all the current scientific data, the effects of continuous exposure to environmental pollutants such as *ozone* on CVD have not been systematically studied. In addition, the mechanisms responsible for the ozone-induced cardiovascular dysfunction are not fully understood. We believe there is an important immediate need to address a number of unanswered scientific questions to clarify the role of ozone in the pathogenesis of cardiovascular (CV) injury.

Changes in I/R injury affords an appropriate paradigm to begin to elucidate the CV effects of chronic ozone exposure and will add to understanding the underlying toxic mechanism of action of ozone-induced CV injury. The purpose of this study was two-fold: (a) test the hypothesis that compared to hearts from rats exposed to filtered air, hearts from rats exposed to ozone will demonstrate attenuated cardiac function and increased tissue damage subsequent to an I/R insult and; (b) ozone enhanced damage to the myocardium, is due to increased oxidative stress levels as well as inflammatory mediator production.

The present study demonstrates an ozone-induced enhanced sensitivity to cardiac injury, subsequent to ischemic insult, and sheds significant insight into the possible mechanism of action that results in a decrease in tolerance to myocardial ischemia. We believe this clinically significant study is timely and will help better understand the mechanisms underlying the pathology of ozone-induced cardiac injury. The long-term goal is to contribute relevant data useful in guiding regulatory policies regarding atmospheric levels of ozone resulting in decreased mortality and reduced environmental related health costs.

Materials and methods

Materials

Chloride salts of sodium, potassium, magnesium, and calcium were obtained from Sigma Chemical Co. and were analytical quality or better. Protein assay kits were purchased from Bio-Rad Laboratories. All media were prepared fresh daily from stock solutions with distilled, deionized water. Concentrations of all substrates, ions, and added media are presented as final concentrations.

Experimental animals

All experimental and animal procedures were in compliance with the principles of the American Physiological Society and with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). The work conforms to the IACUC–TAMUK guidelines concerning the care and use of experimental animals. *Sprague–Dawley rats (50–75 gm) were used. Rats were housed in a room maintained at 22 ± 1°C with a 12:12 h light:dark cycle, and fed Purina rat chow and tap water ad libitum.*

Experimental protocol

Control Group-1: Rats were placed in a chamber and exposed for 8 h/day to filtered air (0.0 ppm ozone) for 28 days; Control Group-2: placed in a chamber and exposed for 8 h/day to filtered air (0.0 ppm ozone) for 56 days; Experimental Group-1: Rats were placed in the chamber and exposed for 8 h/day to 0.8 ppm ozone mixed with air for 28 days; Experimental Group-2: Rats were placed in the chamber and exposed for 8 h/day to 0.8 ppm ozone mixed with air for 56 days.

Ozone exposure

Rats were kept within a common Plexiglas environmental chamber supplied with a constant air flow and subjected to ozone as described previously [20]. Rats were habituated to these new conditions by placing them in the environmental chamber for 5 days prior to ozone exposure. Ozone was generated by passing filtered air across an ultraviolet light. The concentration of ozone was regulated by adjusting the inlet flow of air to an ozonator, thus controlling the quantity of ozone produced. The ozone concentration was continuously measured using a calibrated UV photometric ozone monitor connected to the outlet line of the chamber. For control exposures, rats were simultaneously kept in a same sized chamber provided with filtered room air at the same flow rate. During all experimental procedures, animals were monitored closely to insure there was no unusual distress, discomfort or pain. In order to minimize the involvement of ammonia produced by rat urine and feces, sodium based Zeolites were used and the rat cages were cleaned on a daily basis.

Experimental model

Twenty-four hours after the completion of filtered air or ozone exposure, i.e., on the 27th and 57th day of the experiment, the rats were sacrificed by decapitation. The hearts were quickly excised, mounted on Langendorff apparatus and perfused with Krebs–Henseleit (K–H) buffer (120 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$, 1.25 mM $CaCl_2$, and 11 mM glucose aerated with 95% O_2:5% CO_2, 37°C, pH 7.4) at a constant flow of 10 ml/min [21]. The hearts were electrically stimulated at 300 beats/min via a square wave current of 1.5 ms by using a Phipps and Bird stimulator. The left ventricular systolic pressure (LVSP), the rate of change of pressure development ($+dP/dt$), and rate of change of pressure decay ($-dP/dt$) was measured via a transducer (Model 1050 BP-Biopac System Inc., Goleta, Calif.), which was connected to a water-filled latex balloon inserted into the left ventricle (LV). At the beginning of each experiment, left ventricular end diastolic pressure (LVEDP) was adjusted to approximately 10 mmHg by inflating the balloon. All data were recorded and stored in a computer program (Acqknowledge 3.5.3) by using a Biopac Data Acquisition System (Biopac Systems Inc., Goleta, Calif.). Left ventricular developed pressure (LVDP) was taken as the difference between the LVSP and LVEDP. Hearts from all groups were stabilized for a period of 30 min and I/R was induced by stopping the coronary blood flow for 30 min followed by a 60 min reperfusion. At the end of the perfusion, hearts were carefully removed from the apparatus, washed with cold saline solution, flash frozen in liquid nitrogen, and then stored at −80°C for later biochemical estimations.

Biochemical estimations

Measurement of superoxidase dismutase (SOD)

The heart tissue was homogenized in 50 mM Tris–HCL buffer (pH 8.2) containing 1 mM diethylenetriaminepentaacetic acid and the homogenate was centrifuged at 20,000*g* for 20 min. Superoxide dismutase activity was assayed by quantifying pyrogallol auto-oxidation. Pyrogallol (24 mM) was prepared in 10 mM HCl and stored at 4°C. Catalase (CAT) 30 μM stock solution was made in an alkaline buffer (pH 9.0). SOD activity was measured spectrophotometrically by tracking the auto-oxidation of pyrogallol. The incubation medium contained aliquotes of supernatant (150 μg protein), Tris–HCl buffer containing 25 μl pyrogallol, and 20 μl CAT stock solution in a final volume of 3 ml. Auto-oxidation of pyrogallol was monitored by measuring absorbance at 420 nm at 1 min intervals for 5 min. SOD activity was determined from a standard curve of percent inhibition of pyrogallol auto-oxidation with a commercially available SOD with known activity. SOD specific activity was expressed as units/mg protein where 1 unit of SOD is defined as the amount that shows 50% inhibition at room temperature and pH 7.8 [22].

Measurement of malondialdehyde (MDA) content

The formation of MDA, an indicator of the lipid peroxidation, was measured in the plasma by the thiobarbituric acid method as described elsewhere [23].

Myocardial levels of TNF-α and IL-10

Hearts were washed with PBS. Viable ventricular tissue was flash frozen in liquid nitrogen. Frozen tissue (0.5–1.0 g) was homogenized, and membrane-bound fractions of TNF-α and IL-10 proteins were collected and analyzed by ELISA using a commercially available kit (R & D Systems, Minneapolis, MN) [22].

Statistical analysis

Values are means ± SE. The difference between control and experimental groups was evaluated statistically by one-way ANOVA followed by the Newman–Keuls test. Differences were considered significant at $P < 0.05$.

Results

Ozone effect on hemodynamic changes subsequent to myocardial ischemia–reperfusion

Ischemic-reperfused hearts from rats exposed to 0.8 ppm ozone for 28 or 56 days produced a significant decrease in LVDP and a significant increase in LVEDP as compared to the hearts of the rats exposed to clean air (Figs. 1, 2). In addition, there was a significant depression of both $+dP/dt$ and $-dP/dt$ values as demonstrated by a percent of control decrease in the ozone-exposed I/R hearts (Fig. 3). Overall, compared to the ozone-exposed I/R hearts, the potentiation and attenuation of LVEDP and LVDP, $+dP/dt$ or $-dP/dt$, respectively, was significantly reduced in I/R hearts from air exposed animals.

The elevation in LVEDP and depression in LVDP was significantly greater in the 56 day ozone-exposed I/R hearts compared to I/R hearts from the 28 day ozone exposure group. Since the hemodynamic values for both the 28 and 56 day air exposed groups were not significantly different from each other, only the 56 day air exposure data is shown.

Status of anti- and pro-oxidant levels in ozone-exposed I/R hearts

Ischemic-reperfused hearts from rats exposed to 0.8 ppm ozone for 28 or 56 days produced a decrease in myocardial antioxidant status indicated by the decreased levels of the antioxidant enzyme SOD (Fig. 4). In addition, myocardial MDA levels, an indicator of lipid peroxidation, were increased in the ozone-exposed I/R hearts (Fig. 5). Compared to I/R hearts from ozone-exposed rats, the potentiation and attenuation of oxidant and anti-oxidant status, respectively, was significantly less in air exposed I/R hearts.

The myocardial MDA values for the 28 day ozone-exposed I/R hearts were not significantly different from the values for I/R hearts exposed to ozone for 56 days. However, the attenuation in SOD levels was significantly greater in the 56 day ozone-exposed I/R hearts compared to I/R hearts from the 28 day ozone exposure group. Since these values in the 28 and 56 day air exposed group were not significantly different from each other only the 56 day data is shown for the air exposure group.

Status of anti- and pro-inflammatory proteins in ozone-exposed I/R hearts

Ischemic-reperfused hearts from rats exposed to 0.8 ppm ozone for 28 or 56 days produced a decrease in myocardial levels of the anti-inflammatory protein IL-10 (Fig. 6). In addition, increased inflammation indicated by augmented myocardial production of TNF-α; a pro-inflammatory protein, was observed in the ozone-exposed I/R hearts (Fig. 7). Compared to I/R hearts from ozone-exposed rats, the potentiation and attenuation in pro- and anti-inflammatory proteins, respectively, was significantly less in air exposed I/R hearts.

Compared to I/R hearts from rats, which were exposed to ozone for 28 days, the degree of attenuation in myocardial IL-10 levels was significantly greater in the 56 day ozone-exposed hearts. However, the production of TNF-α was similar in all ozone-exposed I/R hearts. Since the pro- and anti-inflammatory proteins values for the 28 and 56 day air exposed group were not significantly different from each other, only the 56 day air exposure data is shown.

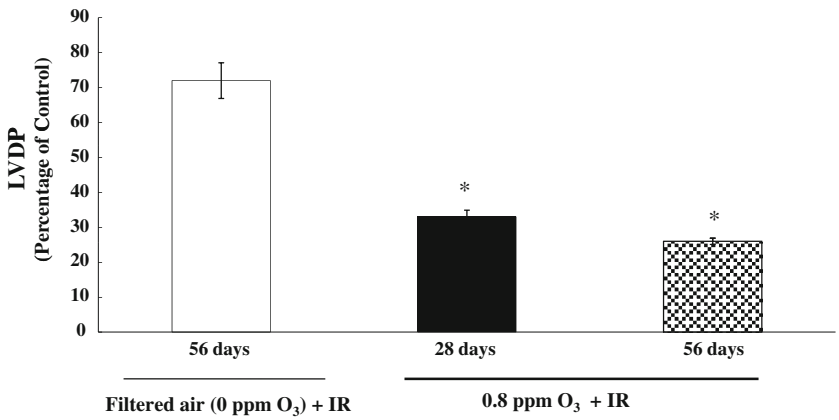

Fig. 1 Left ventricular developed pressure (LVDP) in ischemic-reperfused hearts extracted from 28 and 56 days of air and ozone (O_3) exposed rats. Since the LVDP values for both the 28 and 56 day air exposed group were not significantly different from each other, only the 56 day air exposed data is shown. Values represent the mean ± SEM of six animals in each group. LVDP was the difference between LV systolic pressure and LVEDP. Mean values marked by an *asterisk* are significantly different from corresponding controls at * $P < 0.05$. *O_3* ozone; *IR* ischemia reperfusion

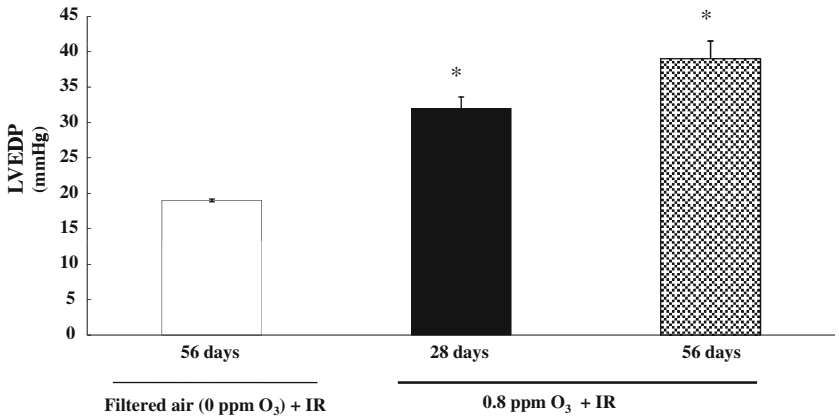

Fig. 2 Left ventricular end diastolic pressure (LVEDP) in ischemic-reperfused hearts extracted from 28 and 56 days of air and ozone (O_3) exposed animals. Since the LVEDP values for both the 28 and 56 day air exposed group were not significantly different from each other, only the 56 day air exposed data is shown. Each value is a mean ± SEM of six animals in each group. * $P < 0.05$-significantly different from respective air exposed group. *O_3* ozone; *IR* ischemia reperfusion

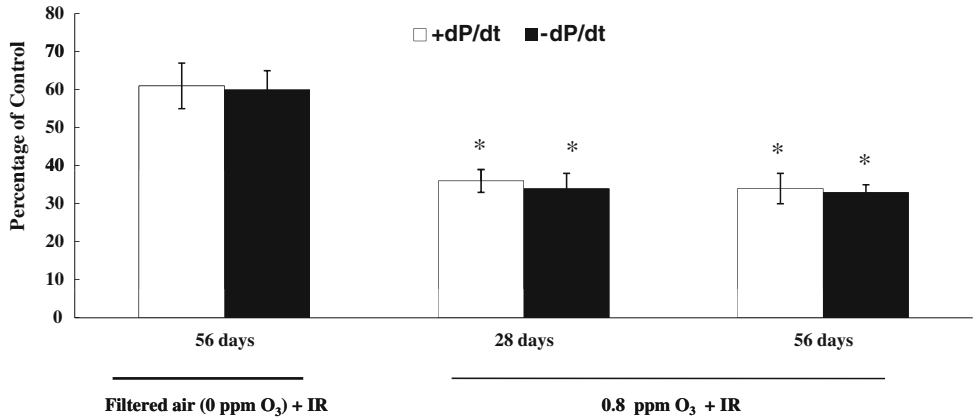

Fig. 3 Rate of pressure development (+d*P*/d*t*) and rate of pressure decay (−d*P*/d*t*) in ischemic-reperfused hearts extracted from 28 and 56 days of air and ozone (O_3) exposed animals. Since the +d*P*/d*t* and –d*P*/d*t* values for both the 28 and 56 day air exposed group were not significantly different from each other, only the 56 day air exposed data is shown. Values represent the mean ± SEM of six animals in each group. Mean values marked by an *asterisk* are significantly different from corresponding controls at * $P < 0.05$. *O_3* ozone; *IR* ischemia reperfusion

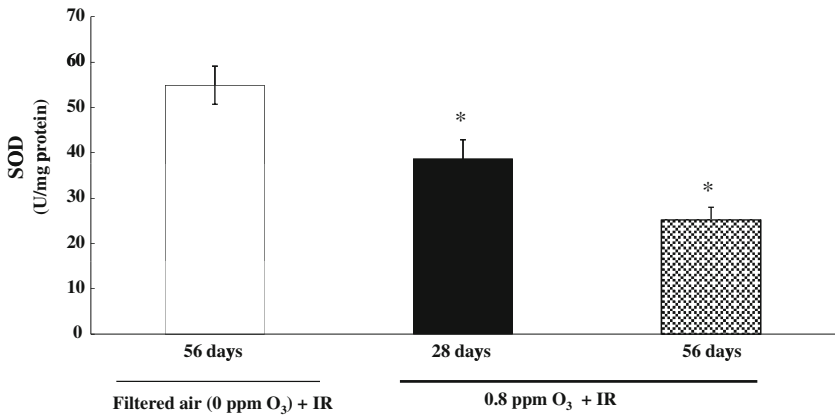

Fig. 4 Superoxide dismutase (SOD) levels in ischemic-reperfused hearts extracted from 28 and 56 days of air and ozone (O_3) exposed animals. Since the SOD values for both the 28 and 56 day air exposed group were not significantly different from each other, only the 56 day air exposed data is shown. Values represent the mean ± SEM for triplicate samples from 6 animals in each group as described under "Materials and methods" section. * $P < 0.05$—significantly different from respective air exposed group. *O_3* ozone; *IR* ischemia reperfusion

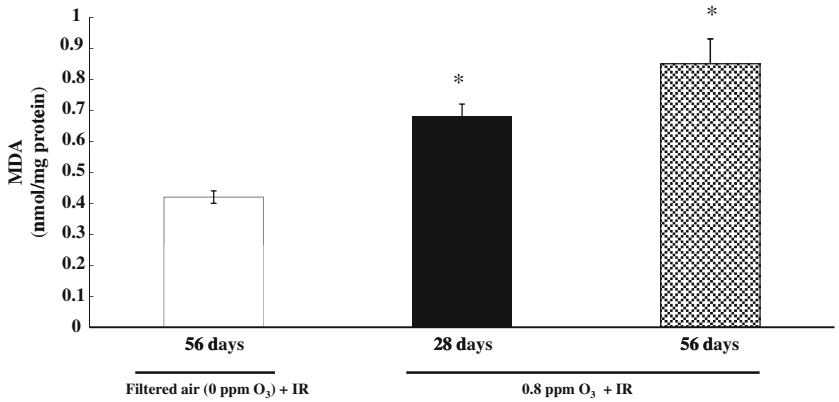

Fig. 5 Malondialdehyde levels in ischemic-reperfused hearts extracted from 28 and 56 days of air and ozone (O_3) exposed animals. Since the MDA values for both the 28 and 56 day air exposed group were not significantly different from each other, only the 56 day air exposed data is shown. Values represent the mean ± SEM for triplicate samples from six animals in each group as described under "Materials and methods" section. Mean values marked by an *asterisk* are significantly different from corresponding controls at * $P < 0.05$. O_3 ozone; *IR* ischemia reperfusion

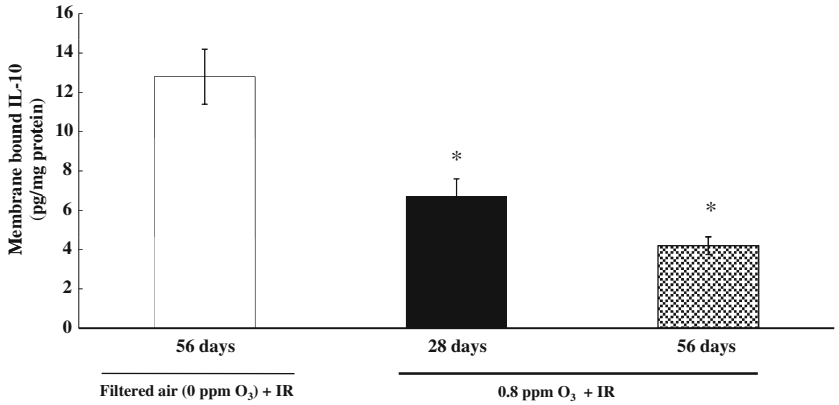

Fig. 6 Levels of membrane-bound IL-10 in ischemic-reperfused hearts extracted from 28 and 56 days of air and ozone (O_3) exposed animals. Since the membrane-bound IL-10 values for both the 28 and 56 day air exposed group were not significantly different from each other, only the 56 day air exposed data is shown. Values represent the mean ± SEM for samples from six animals in each group by ELISA kit. * $P < 0.05$—significantly different from respective air exposed group. O_3 ozone; *IR* ischemia reperfusion

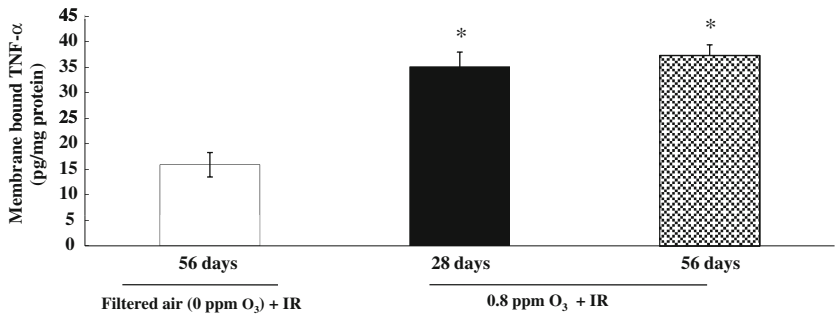

Fig. 7 Levels of membrane-bound TNF-α in ischemic-reperfused hearts extracted from 28 and 56 days of air and ozone (O_3) exposed animals. Since the membrane-bound TNF-α values for both the 28 and 56 day air exposed group were not significantly different from each other, only the 56 day air exposed data is shown. Values represent the mean ± SEM for samples from six animals in each group by ELISA kit. Mean values marked by an *asterisk* are significantly different from corresponding controls at * $P < 0.05$. O_3 ozone; *IR* ischemia reperfusion

Since the extent of ischemic injury in I/R hearts from all air exposed groups were similar, we propose that the significant differences in some of the parameters observed within the ozone group (28 and 56 day exposure) in our study are not due to differences in the weight of these rats among these two groups.

Discussion

Air pollution consists of gaseous and particulate-matter pollutants. The current study follows reports by Bocci [18] and Brooke [19] suggesting that the release of circulating pro-oxidative and/or pro-inflammatory mediators from the lungs into systemic circulation following pollutant exposure can indirectly mediate CV responses.

Exposure to particulate matter, which causes lung inflammation and injury [24], has also been shown to increase myocardial I/R injury [25, 26]. Similarly ozone, a highly reactive oxidant present in photochemical smog, and the gaseous component of air pollution, is known to be involved in pulmonary injury [27]. The cardiovascular detrimental effects appear to be directly linked to an impaired alveolar–arterial oxygen transfer, resulting in reducing oxygen supply to the myocardium [5]. In addition, studies have speculated that interactions of nerve endings in the lung with ozone can result in alterations in the autonomic nervous response that can lead to electrophysiological changes in the heart [7]. Ozone exposure has also been demonstrated to produce alterations in heart rate [8], as well as differential effects on distinct components of the sympatho-adrenal system in rats, both of which could lead to heart arrhythmias [28].

Exposure to toxic levels of ozone has been implicated to increased generation of inflammatory mediators and reactive oxygen species (ROS) [29, 30]. Compared to I/R hearts from 4 and 8 weeks air exposed animals, our results demonstrate ozone-induced enhanced sensitivity to myocardial I/R injury along with significantly increased myocardial oxidative stress levels (enhanced lipid peroxidation as indicated by increased MDA levels) as well as increased synthesis of pro-inflammatory (TNF-α) mediators and decreased production of anti-inflammatory (IL-10) proteins. To the best of our knowledge, these effects of ozone on I/R hearts are novel.

In order to prevent or limit oxidative damage, cells possess relatively high levels of antioxidants such as SOD, CAT, and glutathione (GSH) [31–33]. However, during states of increased oxidative stress, antioxidants become depleted, increasing tissue injury [30]. SOD is generally considered one of the first lines of antioxidant defense [31]. The most prominent and widely distributed form of SOD is Cu/Zn-SOD [29]. Transgenic mice over expressing Cu/Zn-SOD (SOD+/+) have been reported to be resistant to ozone-induced lung inflammation along with increases in TNF-α [29]. In addition, increased ozone-induced airway neutrophilic inflammation has been reported in SOD null mice [34]. Administration of anti-TNF-α prior to ozone can significantly reduce the inflammation and epithelial injury as evidenced by the reduction in PMNs and BALF albumin levels [35]. Based on the current findings, it is reasonable to speculate that SOD, an antioxidant enzyme may play a critical role in ozone-induced cardiac tissue injury as well as in ozone derived increases in inflammatory mediator production. Accordingly, a modulatory role of anti-oxidative enzyme such as SOD may be exerted through an attenuation of TNF-α, which in turn may lead to the down regulation of other inflammatory mediators such as IL-1 and IL-6 leading to decreased inflammation. This sequence of events may explain the role of SOD over expression in reducing ozone-induced inflammation of the I/R heart and vice versa. Additional work is needed to elucidate the effects of altered oxidant/antioxidant balance in ozone-induced enhanced sensitivity to cardiac injury as well as inflammatory mediator production in ozone-exposed animals.

Proinflammatory cytokines are not constitutively expressed in the normal heart [36], but their expression especially that of TNF-α is enhanced in various pathophysiological states associated with increased oxidative stress as seen with ozone exposure [35]. This, in turn, promotes the induction of various other cytotoxic substances on endothelial cells and myocytes. This has profound pathological significance, in terms of development of muscle dysfunction, cell death as well as overall attenuation of heart function [36–38].

The overreaching objective of the current study was to examine the effects of ozone on cardiac function subsequent to an ischemic insult. Further work is needed to evaluate the ozone effects on cardiac function immediately after the 4 or 8 weeks of exposure. Additional parameters such as, simultaneous evaluation of the pattern of expression of diverse oxidant enzymes and inflammatory cytokines, evolution of cardiac dysfunction, and the development of cardiac remodeling could be investigated.

Protective effects of IL-10 have been reported to be mediated through TNF-α blockade as well as inhibition of IL-1 and IL-6 [39]. Studies have reported that both IL-10 and anti IL-12 pretreatment inhibit I/R-induced production of TNF-α, but only IL-10 has protective activities against I/R-induced oxidative stress in the early period [40]. These results suggest that the protective effect of IL-10 could be related to its inhibitory effect of ROS, which reduces the lipid peroxidation and protein carbonyl compounds, and

increases the SOD, CAT activities, and GSH levels. The potential role of exogenous and endogenous IL-10 in experimental myocardial infarction has recently been investigated with results suggesting IL-10 plays a protective role against I/R injury. Despite a growing interest in the role of cytokines in the induction of myocardial injury, not much is known about the effects of ozone on the production and release of IL-10 in the myocardial tissue. The role of IL-10 in ozone-induced cardiac injury had not been examined until now. Our results are the first to report an ozone-induced attenuation of myocardial function subsequent to ischemia due to decreased myocardial levels of IL-10.

A mechanism proposed for explaining the regulation of death and survival signal subsequent to myocardial I/R injury is the differential interaction of p38MAPKα and p38MAPKβ with caveolin-1 and caveolin-3, respectively [41]. Generally, the binding of p38MAPKα with caveolin-1 is linked with death signaling, whereas the survival signal is linked to p38MAPKβ binding with caveolin-3. In recent studies, rapid translocation and activation of p38MAPKs in the caveolin fraction has been reported in I/R hearts. In other words, compared to sham hearts, the I/R hearts were shown to have less p38MAPKα bound to caveolin-1, whereas more p38MAPKβ was reported to bind to caveolin-3 suggesting greater p38MAPKα activity in the I/R myocardium to induce the death signal. Caveolin-1 expression has been reported to be down regulated in ischemic hearts [42] and ozone inhalation has been demonstrated to cause a dramatic reduction in caveolin-1 expression [43]. Cells, which are treated with TNF-α, showed suppressed caveolin-1 expression [43], and re-expression of caveolin-1 in the caveolin-1 knockout mice has been shown to attenuate cardiac defects [44]. This suggests that TNF-α is required for this ozone-induced suppression of caveolin-1 resulting in enhanced death signaling mediated attenuated cardiac function.

Additionally, the p38MAPK protein has been linked to ozone-induced airway hyper-responsiveness with inhibition of p38MAPK reducing ozone-induced inflammation and airway hyper-responsiveness [45]. Based on the current findings, it can be speculated that the significant increases in TNF-α levels in ozone-exposed I/R hearts may lead to decreased caveolin-1 expression, which then causes reduced p38MAPKα interaction with caveloin-1 resulting in enhanced death signaling by significantly increasing the levels of p38MAPKα. In addition as suggested by others, it is also possible that phosphorylation of caveolin-1 by TNF-α results in conformational change in caveloin-1 making it less available for binding [43]. Therefore, the interaction of p38MAPKs with caveolin might play a significant role in enhancing the ozone-induced death signal in the I/R hearts in this study.

Important considerations

Ozone effects

It is possible that animals may acclimate to the effects of chronic ozone, which could play a role in the outcome. Therefore, the length of time after exposure may have an impact on the results. If the hearts are studied immediately after ozone exposure, then the acute effects of ozone cannot be excluded. Peak increases in markers of injury and inflammation after ozone exposure were seen 12–24 h after the end of ozone exposure [46]. Studying the hearts 12–24 h after the final ozone exposure would provide a more accurate assessment of the chronic effects. Therefore, in this study, the ozone effects on cardiac function were evaluated after 24 h of the final exposure to ozone.

The ex vivo model

We are aware that the utility of isolated perfused hearts may be limited from a pathophysiological point of view. Constituents such as platelets, neutrophils, and systemic inflammation or plasma lipids may indirectly affect the heart resulting in increased damage to the myocardium. Changes in these processes are lost in the isolated rat heart preparation and hence several potential sources of cardiovascular injury due to ozone exposure may be missed. We anticipate a greater extent of ozone-induced injury in the in vivo model of I/R injury and studies utilizing an in vivo model of I/R injury are warranted to further establish this dose dependent relationship.

Extrapolation of ex vivo preliminary results to in vivo research

Although future studies comparing the isolated heart model with in vivo findings are required, there has been enough data confirming that ex vivo results have a good correlation with in vivo studies [47].

Extrapolation of animal exposure data to humans

Interspecies comparisons of ozone exposure have led to the following: (1) the total uptake efficiency in rats is larger than in humans; (2) there is a similarity between rodents and humans for the dose–response pattern for both acute inflammation and chronic alveolar interstitial thickness; and (3) that humans may be more responsive than rodents [48, 49]. These findings indicate that rodents may be considered as a sensitive human model for ozone toxicity

and that effects observed in rodents may very well occur in humans, even at lower ozone levels [48, 49].

We are aware of concerns regarding species scaling and the extrapolation of data obtained from animal models to clinical settings, especially when the responses to ischemia can be different between species with a higher heart rate compared to species with a slower heart rate as in humans [50]. Nonetheless, based on our experiences with animal and human functional data with therapeutic agents such as pyridoxal-5-phosphate [47, 51], we believe that this study will help us better understand and increase our knowledge regarding the sensitivity of ozone-exposed hearts to post ischemic contractile dysfunction and increased cardiovascular mortality.

The ozone concentration

When exposed to ozone, rodents remove a smaller fraction of the inhaled amount of ozone (40–47%) [35, 36] than humans (75% with large inter-individual variations) [52, 53]. Hence, the toxicity of ozone observed for a given concentration in rodents may underestimate the effect observed for the same dose in humans. The effects observed in exercising humans are very similar to those produced in rodents exposed at rest to a five-fold higher ozone concentration [48]. Hence, the concentrations chosen in the present study mimic the actual levels of pollution commonly encountered in urban areas.

Equipment

Chamber studies are adequate in providing a method by which to pursue the acute mechanisms of individual air pollutants. However, we do realize they do not reproduce either the mixtures or temporal variation that occurs during ozone alert days. On the other hand, since it is known that individual air pollutants such as ozone, oxides of nitrogen, and suspended particulates all share a common property of being potent oxidants; we believe that our work will form a basis which would initiate further studies to extend our knowledge and understanding of the underlying mechanisms responsible for the cardio-toxic effects of other pollutants possessing similar properties as ozone.

Acknowledgments This work was supported by grants from Texas A&M Health Science Center (TAMHSC), Texas A&M University Kingsville (TAMUK) and US Environmental Protection Agency. We thank Dr. Kuruvilla John and Mr. Don Marek from the Department of Environmental Engineering at TAMUK for their technical support and advice. We are also grateful to TAMUK-Ronald McNair Scholars—Miss Patricia Hinojosa and Mr. Daniel Diaz, TAMHSC-Pharm D student—Ms. BaAimee Nguyen and the King High School-AP Biology Student—Mr. Vishal Sethi for laboratory assistance.

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