7. The role of taurine in acute lung injury, oxidative stress and apoptosis by endoxin and cigarette smoke

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1.1. Introduction

In comparison to other vital organs such as the liver, kidney and brain, the lung is at a greater risk of developing oxidative stress and oxidative injury as a result of its continuous exposure to higher oxygen tensions, to other sources of oxidative stress in the form of environmental pollutants, irritants and free radical-generating carcinogens, and of having a large epithelial surface area [Crapo 2003; Kinnula et al., 2003]. Fortunately, the lung lining fluids, airways, alveolar septa and extracellular spaces are equipped with antioxidant defenses represented by antioxidant enzymes, high levels of glutathione, and hydrogen peroxide-consuming enzymes [Crapo, 2003]. Under conditions of high oxidative stress these antioxidant defenses may work in unison and their physiological levels may, in some instances, become genetically upregulated in intracellular and extracellular spaces to protect the lung against inflammatory reactions by reducing the influx of inflammatory...
cells as well as the magnitude and progression of the accompanying oxidative stress [Crapo, 2003; MacNee, 2001]. In the event that these defenses fail to control the inflammation at a local level, then the process can become systemic and be a cause for organ dysfunction, failure, and even mortality [Lang et al., 2002].

Acute lung injury (ALI) is a critical illness syndrome consisting of acute hypoxemic respiratory failure with bilateral pulmonary infiltrates, and precipitated by a wide variety of predisposing pulmonary and non-pulmonary risk factors but not by left atrial hypertension [Ware and Matthay, 2000]. As a clinical entity, ALI is a major cause of acute respiratory failure with a high morbidity and mortality in critically ill patients [MacNee, 2009]. While the sequestration of neutrophils in airways and lung tissue, intravascular coagulation, disruption of capillary integrity leading to increased alveolar capillary permeability, pulmonary edema [Chabot et al., 1998], increased intrapulmonary shunt, diffuse lung inflammation and injury of pulmonary vascular endothelium are the predominant pathological findings for ALI [Brigham and Meyrick, 1986; Katzenstein et al., 1976], oxidative stress [Brigham and Meyrick, 1986; Chabot et al., 1998; Lang et al., 2002] and apoptosis [Kawasaki et al., 2000] are additional important events responsible for the cellular and pulmonary dysfunction associated with this condition.

A common feature of ALI resulting from an exposure to environmental insults such as bacterial endotoxin, ozone, silica and cigarette smoke [Leikauf et al., 2002] is the development of an inflammatory-immune response that starts with the recruitment and activation of neutrophils, monocytes, macrophages, eosinophils and CD4+ lymphocytes in lung airspaces and parenchyma [Lang et al., 2002; Rahman and MacNee, 1996] and which, in conjunction with activated epithelial cells and resident macrophages, will secrete inflammatory cytokines, chemokines, adhesion molecules and bioactive eicosanoids [Smith, 1994; Takao et al., 2005]. In addition, these cells may be activated to release reactive oxygen (ROS) and reactive nitrogen (RNS) species which can enhance the initial inflammatory response by activating redox-sensitive transcriptions factors regulating the genes for proinflammatory mediators in various lung cells [MacNee, 2000; Rahman, 2002]. Since intracellularly generated ROS and RNS are known to mediate pathophysiological responses such as apoptosis and to act as second messenger for receptors activated by tumor necrosis factor-α (TNF-α) and lipopolysaccharide (LPS) [Rochelle et al., 1998], oxidative stress and alteration of the intracellular redox status could enhance the sensitivity as well as the response to these proinflammatory stimuli [Rahman, 2008]. The same ligand-receptor interactions may initiate apoptotic signals for the recruitment and activation of the initiator caspase-8, which, by activating effector caspases
such as caspase-3, will trigger apoptosis [Kawasaki et al., 2000; Tang et al., 2008]. The relevance of caspase-3 to oxidative stress-stimulated apoptosis of alveolar and pulmonary endothelial cells has been established both in vivo and in vitro. The role of caspase-3 have become apparent from two types of experiments in rats, one demonstrating an increase in caspase-3 activity and in apoptosis among alveolar septal cells after a treatment with a blocker of the vascular endothelial growth factor receptor for the trophic factor, which ensures the survival of epithelial lung cells by suppressing the activation of the key apoptotic mediators Bax and caspase-3 [Kasahara et al., 2000], and another in which these changes were prevented by a pretreatment with a caspase-3 inhibitor [Zhuang et al., 2007]. Further evidence of the role caspase-3 to lung cell apoptosis has come from a study in which α-1 antitrypsin, a potent inhibitor of neutrophil-derived serine proteases, was shown to promote the survival of cultured primary lung endothelial cells to apoptosis induced by ultraviolet light, TNF-α or staurosporine by inhibiting the activity of caspase-3 through a direct noncovalent interaction [Petrache et al., 2006]. On the other hand, oxygen radicals generated close to a cell membrane by interacting with polyunsaturated fatty acids of membrane phospholipids can initiate lipid peroxidation (LPO) leading to the formation of hydperoxides along with malondialdehyde (MDA), 4-hydroxy-2-nonenal (4-HNE), and other long lived aldehydes that are detectable in bronchoalveolar lavage fluid (BALF) and in the circulation [Rahman, 1996].

The imbalance in oxidant-antioxidant equilibrium in favor of oxidants that is observed in patients with ALI and with chronic obstructive pulmonary disease (COPD) associated with and exacerbated by cigarette smoking has suggested the possibility of using antioxidants to control the activation of transcription factors interacting with genes encoding for inflammatory factors, to regulate glutathione (GSH) biosynthesis genes [Rahman, 2006], to boost the levels of endogenous antioxidants, to prevent lung injury and apoptotic cell death, and to improve pulmonary function [Rahman, 2008]. Besides dietary antioxidant vitamins, carotenoids and polyphenols [Grievink et al., 1999; Kelly et al., 2003; Rahman et al., 2006; Samet et al., 2001], pharmacological agents [Rahman and Kilty, 2006], antioxidant enzymes such as catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD) [Chabot et al., 1998], and special diets supplemented with natural antioxidants [Gadek et al., 1999] have been tested in humans and laboratory animals for the ability to protect the lung against oxidative stress by a variety of environmental insults. One of the compounds that have been the subject of extensive evaluation for this purpose is taurine (TAU). However, in spite of the existence of considerable evidence in support of a protective role for this
conditional amino acid in the lungs against oxidant-induced inflammation and oxidative stress, work on its effects on ALI due to an exposure to bacterial endotoxin and cigarette smoke appear to be rather limited. Since this laboratory has previously verified that TAU can attenuate LPS-induced lung inflammation, oxidative stress and apoptosis, it appeared of interest to determine in hamsters whether the same pattern of effects is observable when cigarette smoke (CS) is the causative factor. This comparison was suggested by the results of work carried out in mice suggesting that certain differences exist in the oxidative stress that develops from an exposure to each of these environmental factors [Valença et al., 2008].

1.2. TAU as an antioxidant

TAU, 2-aminoethanesulfonic acid, is a nonprotein amino acid of ubiquitous occurrence within the animal phyla [Smith et al., 1987], having a widespread distribution in human and animal tissues, and demonstrating a particular abundance in excitatory secretory organs and tissues such as the brain, heart and skeletal muscles [Awapara, 1956; Awapara et al., 1950; Warskulat et al., 2004; Yoshikawa and Kuriyama, 1976].

Two correlations, one between low intracellular and circulating levels of TAU with decreased organ function [Awapara, 1956; Eppler and Dawson, 2001] and increased organ susceptibility to oxidative stress [Eppler and Dawson, 2001]; and another between increased urinary levels and enhanced hepatic cytotoxicity by xenobiotics [Timbrell and Waterfield, 1996], have led to the conclusion that this sulfur-containing compound is a natural cellular protector against a myriad of extracellular insults whose pathogenicity is closely linked to oxidative stress. As a result, TAU has been extensively investigated for its antioxidant properties in a wide range of pathological situations, notably diabetes [Pop-Busui et al., 2001; Obrosova and Stevens, 1999], lung damage [Men et al., 2010], myocardial infarction [Shiny et al., 2005], ischemia/reperfusion injury and apoptosis [Oriyanhan et al., 2005], calcium- [Azuma and Schaffer, 1995; McBroom and Welty, 1977] and iron-overload [Oudit et al., 2004] cardiomyopathy, experimental hypothyroidism [Taş et al., 2004], and acute [Ahn et al., 2001] and chronic pancreatitis [Mas et al., 2006]. In addition, it has also proven of benefit in counteracting the oxidative stress associated with conventional medication [Cetiner et al., 2005; Ohta, et al., 1988] and an exposure to environmental factors such as ozone [Schuller-Levis et al., 1995], nitric dioxide [Gordon et al., 1986], metals [Ghosh et al., 2009; Gürer et al., 2001; Sinha et al., 2008], fluoride [Das et al., 2008], silica [Shi et al., 1997], and paraquat [Izumi et al., 1989].
In comparison to hypotaurine, its sulfinate metabolic precursor in mammalian cells, TAU is a less effective direct antioxidant when added to cell-free systems capable of generating reactive oxygen- and nitrogen-centered species [Aruoma et al., 1998]. While hypotaurine exhibited excellent scavenger affinity towards the hydroxyl radical (•OH) and hypochlorous acid (HClO) and was found capable of interfering with the iron ion-dependent formation of this radical, TAU showed poor reactivity towards •OH and, in common with hypotaurine, was ineffective in binding superoxide anion radical (O2•-) and in reacting with hydrogen peroxide (H2O2), and the product of its interaction with HClO was still sufficiently oxidizing to inactivate α1-antiproteinase [Aruoma et al., 1998]. The same difference in •OH scavenging efficiency between these two compounds has been observed in vitro during the LPO of linoleic acid mediated by either silica or the Fenton reaction [Shi et al., 1997]. The same difference between TAU and hypotaurine was established by comparing their effects on the different stages of LPO, since only hypotaurine decreased Fe(II) autoxidation, the oxidation of Fe(II) by the alkoxyl radical generator cumene hydroperoxide, and LPO induced by lipid hydroperoxide [Tadolini et al., 1995]. Furthermore, in vitro TAU has been found to effectively attenuate nitroprusside (a source of NO) and Fe(III)-stimulated formation of quinone compounds from the oxidation of catecholamines [Biasetti and Dawson, 2002], but to fail to interfere with peroxynitrite (ONOO−)-mediated oxidation of the same amines due to a weak affinity for ONOO− [Biasetti and Dawson, 2002; Mehta and Dawson Jr., 2001]. Likewise, at equimolar concentrations hypotaurine has proven to be a better scavenger of the highly cytotoxic singlet oxygen (1O2) than TAU [Pitari et al., 2000]. However, work in this laboratory have suggested that in vivo conditions the antioxidant potency differences between TAU and its metabolic precursor are less marked than would have been expected based on in vitro reactivities [Pohkrel and Lau-Cam, 2002], possibly because of the conversion of TAU to TAU chloramine (Tau-Cl) by myeloperoxidase (MPO)-containing cells and of the positive role of TAU in sparing glutathione and the enzymes associated with the redox cycling and biosynthesis of this major endogenous antioxidant from the detrimental effects of oxidative stress [Acharya and Lau-Cam, 2010]. In this respect, neutrophils are the major producers of Tau-Cl due to a high content in MPO. Upon becoming activated, these cells generate and release H2O2 [Jang et al., 2009], most of which is used to peroxidize chloride ions (Cl−) to form the more stable and less reactive HClO under the catalytic mediation of the halide-dependent MPO, quite abundant in neutrophils [Jang et al., 2009]. In turn, HClO will chlorinate TAU, the most abundant free amino acid in
neutrophils, to yield Tau-Cl [Klamt and Schacter, 2005]. Since HClO is a more reactive and less stable oxidant than Tau-Cl, the formation of Tau-Cl at inflammatory sites may be viewed as a way to detoxify HOCl and, hence, to protect the lung against neutrophil-induced cytotoxicity [Jang et al., 2009].

In addition to data supporting a limited activity for TAU as a radical scavenger in vitro, there is also evidence that this sulfur-containing amino acid can protect cells against the deleterious effects of oxidant through a membrane stabilizing effect. This indirect antioxidant effect has been demonstrated by incubating intact erythrocytes with TAU and 2,2’-azobis(2-amidinopropane) dihydrochloride (AAPH), a water-soluble generator of peroxy radicals (ROO•), and observing a significant reduction in hemolysis by AAPH relative to samples not containing TAU [Koyama et al., 1992]. The same work also showed that while TAU was able to prevent the leakage of glucose from glucose-loaded egg yolk lecithin liposomes incubated with AAPH, it did not prevent the lipid peroxidative degradation of the same phospholipid when present as a thin film. Additional evidence in support of an indirect antioxidant effect have come from work examining the ability of TAU and retinol to maintain the integrity of bovine rod outer segment glycerophospholipids, formulated as liposomes, from the lipid peroxidizing action of AAPH [Keys and Zimmerman, 1999]. In comparison to retinol, TAU was ineffective in preventing the loss of polyunsaturated fatty acids from the liposomes. However, when both compounds were present along side, the antioxidant action of retinol was enhanced two-fold. This mechanism of protection may account, at least in part, for the protective action of TAU against lipid peroxidation seen in vivo. Thus, supplementation of an animal diet with TAU has been found to reduce LPO and the formation of indices of LPO such as MDA, diene conjugates, lipofuscin and hydroperoxides in the aorta of rats maintained on a high fructose diet [Nandhini et al., 2005]. However, these direct and indirect antioxidant actions of TAU may only account for a part of the protective actions that it can display during situations of oxidative stress and, hence, do not reflect additional interactions with cells susceptible to or undergoing oxidative stress, notably its modulatory role on ROS- and RNS-triggered biochemical events leading to acute inflammation and apoptotic cell death, two pathological events of common occurrence among lung disorders due to inhalation of gaseous or particulate matter from environmental sources [Buttke and Sandstrom, 1994].

1.3. Endotoxemia, acute lung injury and taurine

Lipopolysaccharide (LPS), an endotoxin present in the outer membrane of Gram-negative bacteria, is an immunogenic virulence factor responsible
for sepsis, a complex syndrome initiated by bacterial infection and regarded as a leading cause of morbidity and mortality among hospitalized patients, especially those that are immunocompromised [Heidemann and Glibetic, 2007; Cruz and Dellinger, 2002]. In addition to causing a systemic inflammatory response that is responsible for multiple organ dysfunction, including lung injury, and a multitude of signs and symptoms, the endotoxemia of sepsis is also a determining factor for life-threatening hemodynamic changes, including vascular hyporeactivity, hypotension and depressed myocardial contractility [Liu et al., 1997]. When endotoxin is administered to experimental animals [Netea et al., 1996; Takao et al., 2005] or to human volunteers [Andreasen et al., 2008; Lowry, 2005], a physiological and clinical picture resembling sepsis is achieved.

The intraperitoneal [Kabir et al., 2002] or intravenous infusion [Lowry, 2005] of endotoxin causes acute lung injury (ALI) in animal models of endotoxin-induced ALI. Numerous humoral and cellular inflammatory/immune mediators are involved in the initiation and perpetuation of endotoxin-induced ALI [Sethi et al., 2001]. Neutrophils appear to be key players in the pathogenesis of this process by releasing proinflammatory cytokines, chemokines and bioactive arachidonic acid metabolites along with ROS, NOS and proteases in response to several cytokines, chemokines, and leukotrienes [Kabir et al., 2002; Sethi and Waxman, 2001; Takao et al., 2005]. Alveolar macrophages residing in the lungs also undergo activation to release another load of proinflammatory and oxidizing factors and whose accumulation will eventually lead to alveolar-capillary damage with high permeability pulmonary edema, alteration of lung mechanics, and severe abnormalities in pulmonary gas exchange [Cheng and Matthay, 2003]. The significance of neutrophils to the interstitial edema and intense inflammatory response that are hallmarks of endotoxemia has been investigated in mice by artificially inducing neutropenia with cyclophosphamide or antineutrophil antibodies and observing a decrease in lung edema, the activation of NF-κB, and the expression of interleukin-1β (IL-1β) and macrophage inflammatory protein-2 (MIP-2), but not of TNF-α, proteins in the lungs [Abraham et al., 2000].

Results from a previous study with LPS in hamsters [Bhavsar et al., 2009], coupled with those reported for other environmental toxicants such as cigarette smoke [MacNee, 2005; Rahman and MacNee, 1996, 2000] and ozone [Laskin et al., 1998; Laskin and Pendino, 1995; Pendino et al., 1993] indicate that the increase in pulmonary cytokine expression and in the activity of intracellular signaling pathways initiated by endotoxin and effected by neutrophils proceed through common steps and represent the major source of
proinflammatory cytokines in the lungs. The lack of effect of neutropenia on TNF-α mRNA suggest that TNF-α does not have a central role in producing ALI after endotoxemia [Abraham et al., 2000] and confirm the results previously gathered in humans with acute respiratory distress syndrome that IL-1β is a more potent proinflammatory mediator than TNF-α [Pugin et al., 1996]. A pivotal role for interleukin-8 (IL-8) in the recruitment of neutrophils into the lungs following an exposure to LPS has also been demonstrated in vivo by using a neutralizing antibody. In this case, rabbits that had received anti-IL-8 antibody prior to an intratracheal priming with heat-killed Streptococcus pyogenes, followed 36 hours later by an intravenous sublethal dose of LPS, were protected from the pulmonary edema, destruction of pulmonary architecture, and impairment in gas exchange caused by the endotoxin. In addition, the influx of neutrophils into the lungs and the rate of acute lethality were also decreased [Yokoi et al., 1997].

In this laboratory, the role of TAU in endotoxemia was investigated by administering this compound, as a solution in phosphate buffered saline (PBS) pH 7, by the intraperitoneal route to Golden Syrian hamsters (100.0 ± 15.0 g) as a single, (50 mg/kg/0.5mL) dose, for 3 consecutive days, either before or after the intratracheal instillation of bacterial LPS (0.2 mL of a 0.1 mg/mL solution in PBS pH 7.4). Following sacrifice of the animals with a high intraperitoneal dose (240 mg/kg/0.7 mL) of pentobarbital sodium at 24 hr from either a posttreatment with TAU or with LPS, bronchoalveolar lavage fluid (BALF) and lung samples were collected for biochemical and histological assessments. This study and that entailing CS received the approval of the Institutional Animal Care and Use Committee of St. John’s University, and the animals were cared and treated according to the guidelines established by the United States Department of Agriculture under the Animal Welfare Act.

1.3.1. Effects of LPS and TAU-LPS on indices of lung inflammation

a) The instillation of LPS increased the inflammatory index above the control value by at least 17-fold (p<0.001) (Figure 1A). A 3-day treatment with TAU, before or after a challenge with LPS, resulted in significant attenuation of the lung injury caused by LPS, with the effect being greater when TAU was given as a pretreatment rather than as a posttreatment. Using the histopathology scoring of inflammation reaction scale given by Szarka et al. [1997], the mean inflammatory index of animals receiving TAU before LPS (1.783) was found ~42% lower (p<0.01 vs. LPS) than that of animal receiving only LPS (3.083, p<0.001 vs. control), an effect that was lower by ~21% (2.450, p<0.05 vs. LPS)
Figure 1. To assess the effect of TAU on the inflammatory response (A) and accumulation of leukocytes in the lung (B) induced by a lung irritant such as LPS or CS, this amino acid was given before (TAU+LPS, TAU+CS) or after (LPS+TAU) the lung irritant. In (A) TAU is shown to attenuate the increase in inflammatory index while in (B) it is shown to lower the accumulation of leukocytes. Each bar represents the mean ± SEM for n = 6. Comparisons were significantly different at *p<0.05 and ***p<0.001 vs. control; at †p<0.05 and ††p<0.01 vs. LPS; at °°p<0.01 vs. CS.
when TAU was given after LPS (Figure 1A). In both instances, the inflammatory response appeared to be limited to the parenchymal tissue and not to involve the airways. The mean inflammatory index value for control animals receiving only PBS (i.e., 0.17) probably reflects a transient inflammatory response brought about by the intratracheal instillation procedure itself.

b) A 3-day treatment with TAU, either before or after LPS administration, had a significant lowering effect on the total number of leukocytes seen in BALF from animals treated with LPS alone (mean of 26.5 x 10^3 cells/mm³, p<0.001 vs. control), especially when TAU was given before (7.7 x 10^3 cells/mm³, 72% decrease, p<0.001) rather than after (14.5 x 10^3/mm³, 44% decrease, p<0.01) LPS and relative to values for animals on LPS alone (Figure 1B).

c) The administration of TAU, either before or after LPS, was also able to lower the percentage neutrophil count in BALF from animals receiving LPS (mean of 95.3%, p<0.001 vs. control) relative to the value from animals treated only with LPS (by ~14% and ~11% when given before and after LPS, respectively, p<0.05 in both instances vs. LPS) (Figure 2A).

d) An exposure to LPS raised the BALF total neutrophils count by an average of 2.55 x 10^4 cells relative to the control value (~0.02 x 10^4, p<0.001). TAU was able to lower the increase in total neutrophil count induced by LPS, with the effect being greater when it was given before (by 84%, p<0.001) rather than after (by ~53%, p<0.001) LPS, with the effect being clearly significantly different from LPS alone (Figure 2B).

e) The instillation of LPS into the lungs elevated the expression of TNFR1 on BALF cells by an average of ~38-fold (p<0.001 vs. control). This effect was significantly attenuated by TAU, both when given before (only ~15.5-fold increase, p<0.01) or after (only 23.5-fold increase, p<0.01) LPS in comparison to values for animals on LPS alone (Figure 2C).

1.3.2. Effects of LPS and TAU-LPS on apoptotic death of lung cells

a) An exposure to LPS elevated the number of labeled alveolar cells per high power field (HPF) in BALF samples detected by the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay (by ~10-fold over the control value (0.8 cells/HPF vs. 0.08 cells/HPF, p<0.001 vs. control). A co-treatment with TAU was effective in reducing this number to a significant extent. Although the number of labeled alveolar cells per high power field (HPF) was lower when TAU was given before LPS (0.4 cells/HPF vs. 0.8 cells/HPF, p<0.01) than when given
Figure 2. The administration of TAU before a lung irritant such as LPS or CS was effective in reducing the accumulation of neutrophils (as a percentage (A) or as a total count (B)) and of the expression of TNFRI (C) on BALF cells when given before (TAU+LPS, TAU+CS) or after (LPS+TAU) the lung irritant. Each bar represents the mean ± SEM for n = 6. Comparisons were significantly different at *p<0.05, **p<0.01 and ***p<0.001 vs. control; at †p<0.05, ††p<0.01 and †††p<0.001 vs. LPS.
after (0.5 cells/HPF vs. 0.8 cells/HPF, p<0.05), the effect was still significant (Figure 3A). Because of the limitations imposed by the procedure used to stain the alveolar cells, only cells occupying the septa were amenable to counting.

**Figure 3.** The effect of TAU on pulmonary apoptotic cell death by a lung irritant such as LPS or CS was investigated based on the results of the TUNEL assay (A) and the activity of caspase-3 (B) in BALF samples. TAU was able to attenuate apoptosis by a lung irritant when given before (TAU+LPS, TAU+CS) or after (LPS+TAU) the lung irritant. Each bar represents the mean ± SEM for n = 6. Comparisons were significantly different at **p<0.01 and ***p<0.001 vs. control; at ††p<0.01 and †††p<0.001; at °°p<0.01 vs. CS.
b) LPS caused a 10-fold increase in caspase-3 activity of BALF cells, as inferred from difference in absorbance value at 405 nm relative to the control BALF (0.02 vs. 0.002 Abs units/μg of protein, p<0.001). The administration of TAU either as a pretreatment or as a pretreatment to LPS led to reductions in absorbance values at 405 nm equal to 55% (p<0.001) and 50% (p<0.001) of the values observed with LPS alone, respectively (Figure 3B).

1.3.3. Effects of LPS and TAU-LPS on indices of oxidative stress

a) A stimulatory effect for LPS on LPO was inferred from the increase (by an average of 107.5%, p<0.001) in BALF MDA level, measured as thiobarbituric acid reactive substances (TBARS), relative to the control values. TAU lowered the formation of MDA induced by LPS, more effectively when given as a pretreatment (by 44%, p<0.01) than as a post-treatment (by 31% decrease, p<0.01) (Figure 4A).

b) While LPS caused a significant decrease in the control lung GSH level (by 20%, p<0.05), a pretreatment with TAU made this value 18% higher (p<0.05) than with LPS alone. In contrast, giving TAU after LPS did not correct for the decrease in GSH induced by LPS to any appreciable extent (Figure 4B).

c) Since the oxidizing nature of LPS can affect the activity of intracellular antioxidant defenses, the effects of this endotoxin on the activities of the antioxidant enzymes CAT, SOD and GPx in BALF cells were measured in the presence and absence of a 3-day treatment with TAU. Although LPS elevated the CAT activity relative to the control value (by 9%), the effect was not significant (Figure 5A). In contrast, the administration of TAU ei-ther before or after LPS raised this activity to a value above that seen with LPS alone, with the effect being greater when TAU was given before (2-fold increase, p<0.01) rather than after (1.5-fold increase, p<0.5) LPS (Figure 5A). By itself, LPS also lowered the SOD activity of BALF cells (by about 29% vs. control value, p<0.01) (Figure 5B), and a pretreatment or posttreatment with TAU raised this activity to a value above that seen with LPS alone (by ~1.95-fold, p<0.05, and by 1.3-fold, p<0.05, respec-tively) (Figure 5B). In contrast, LPS was found to increase, rather than decrease, the activity of GPx in BALF cells (by 98%, p<0.001 vs. control) (Figure 5C). A treatment with TAU was able to reduce this effect by about 57% when given before LPS (P < 0.001) and by about 49% when given af-ter LPS (p< 0.001) (Figure 5C).
Figure 4. Evidence of the effect of TAU on pulmonary oxidative stress by a lung irritant such as LPS or CS was gathered from the results for TBARS formation (A) and changes in the levels of GSH (B) in BALF samples. While TAU reduced the formation of TBARS when given before the lung oxidant (TAU+LPS, TAU+CS), it was ineffective when given after (CS+TAU) the lung irritant. Each bar represents the mean ± SEM for n = 6. Comparisons were significantly different at *p<0.05, **p<0.01 and ***p<0.001 vs. control; †p<0.05 and ††p<0.01 vs. LPS; °°p<0.01 vs. CS.

1.3.4. Histopathology of lungs that were exposed to LPS and to TAU-LPS

Histopathological examination of lung sections from animals treated only with PBS and stained with H&E demonstrated a normal appearance. Lung
Figure 5. TAU was able to counteract the alterations in BALF CAT, GPx and SOD activities induced by LPS or CS when given before (TAU+LPS, TAU+CS) or after (LPS+TAU) the lung irritant. Each bar represents the mean ± SEM for n = 6. Comparisons were significantly different at *p<0.05, **p<0.01 and ***p<0.001 vs. control; †p<0.05 and †††p<0.01 vs. LPS; at °°p<0.01 and °°°p<0.001 vs. CS.
sections from animals that had been exposed to LPS showed evidence of an inflammatory response affecting the parenchymal tissue but without involving the airways. In contrast, a pretreatment with TAU nullified the altering effect of LPS on lung morphology.

1.4. Cigarette smoking, acute lung injury and taurine

Inhalation of cigarette smoke (CS), whether through active or passive smoking, is the major risk factor for the pathogenesis of chronic obstructive pulmonary disease in developed countries and one of the leading cause of death worldwide [Barnes et al., 2002; Pauwels and Rabe, 2004]. An acute or chronic exposure to CS leads to a chemokine-directed recruitment of innate and adaptive inflammatory cells into the airways and lung parenchyma, predominantly neutrophils, but also including macrophages, dendritic cells, CD8+ and CD4+ T lymphocytes, and B lymphocytes, an event that is followed by the activation of and the release by these cells of multiple chemical mediators of inflammation [D’hulst et al., 2005; Yoshida and Tuder, 2007]. In the cigarette smoker, this situation is compounded by the highly oxidizing components present in the gas phase and particulate matter of CS [Church and Pryor, 1985; Kinnula, 2005], and by the involvement of several elastolytic enzymes, including neutrophil serine proteases, proteinase 3 and cathepsins, and macrophage-derived metalloproteinases [Barnes et al., 2002]. In addition, airway and alveolar epithelial cells may also contribute with inflammatory mediators, including neutrophil serine proteases, proteinase 3 and cathepsins, and macrophage-derived metalloproteinases [Barnes et al., 2002], and with the profibrotic cytokine tranforming growth factor-β (TGF-β) [Takizawa et al., 2001]. Furthermore, inflammatory and structural cells, including neutrophils, macrophages, eosinophils and epithelial cells, are activated to produce ROS in excess of the antioxidant defenses available to lung cells and as a result of which, they are capable of oxidizing proteins, DNA and lipids to cause direct lung injury [MacNee, 2001], of increasing the formation of biomarkers of LPO in the lung and microvascular endothelial cells, and of accentuating the inflammatory response through the activation of redox-sensitive transcription factors, notably nuclear factor-κB (NF-κB) and activator protein-1 (AP-1), known to regulate gene expression of proinflammatory cytokines, inflammatory enzymes, adhesion molecules, and antiapoptotic proteins [Ahn and Aggarwal, 2005].

Since smoking cigarettes contributes to an increased cumulative exposure of the lung to ROS from exogenous and endogenous sources, to the lowering of pulmonary stores of endogenous natural antioxidants, especially ascorbate, α-tocopherol, ubiquinol-10, urate, α-lipoic acid and GSH [Bruno et al., 2005;
Eiserich et al., 1995; Elsayed and Bendich, 2001], and to the loss of activity of certain antioxidant enzymes, especially of those associated with GSH [Kinnula, 2005], it appeared appropriate to examine the effect of an antioxidant amino acid like TAU, which has been previously found by this [Bhavsar et al., 2009] and other laboratories [Abdih et al., 2000; Schuller-Levis et al., 1994] to exert a protective action against oxidant-induced acute lung injury and apoptosis [Jeon et al, 2009], and to modulate the release of proinflammatory cytokines [Grimble, 2006]. More importantly, in view of the differences [Valença et al., 2008] and similarities [Hasday et al., 1999] that have been observed in the ALI caused by LPS and by CS, it would be appropriate to determine whether differences or similarities exist in the pattern of protection afforded by TAU in each instance.

All the experiments with CS were carried with groups of 6 female Golden Syrian hamsters of the same age and weight characteristics as those indicated in the LPS study. In this case, the animals were exposed to a combination of sidestream and mainstream second hand passive cigarette smoking in a TE-10c inhalation exposure cigarette smoking machine (Teague Enterprises, Davis, CA). The cigarettes used in the study were 2R4F research grade and were obtained from Tobacco Health Research Institute (Lexington KY). An automatic metered puffer was used to smoke the cigarettes under Federal Trade Commission conditions (i.e., 35 mL of puff volume, a 2 sec puff duration, a puff frequency of 1 puff every 1 min, and a tobacco smoke pollution value of 93.50 ± 7.00 mg/m3). The animals were divided into groups of 3, with each group being placed in an individual cage. After positioning a cage in the smoking chamber of the smoking machine, the animals were allowed free access to food and water. The animals were subjected to whole body second hand passive CS for 2 hr, after which they were returned to their living quarters until needed for a new CS exposure 18-20 hr later. All the experimental groups received a total of 5 smoke exposures, once daily, for 5 consecutive days. Following euthanasia of the animals with intraperitoneal pentobarbital, their lungs were exposed and rinsed three times with normal saline.

To determine the effects of TAU on CS-induced lung injury, the hamsters were treated twice daily with this sulfur-containing compound (as a solution in phosphate buffered saline (PBS) pH 7.4, at a dose of 50 mg/kg/0.5 mL) by the intraperitoneal route, with a 12 hr interval between treatments, for a total of 5 days, and then exposed to cigarette smoke for 5 consecutive days. Controls were treated with: (a) PBS pH 7.4 for 5 days followed by an exposure to CS (positive control), and (b) only PBS pH 7.4 daily for 5 days followed by an exposure to ambient air (negative control). On day 11, at 24 hr after the last smoke exposure, the animals were sacrificed with a high
dose of pentobarbital sodium (240 mg/kg/0.7 mL, intraperitoneally), and bronchoalveolar lavage fluid (BALF) samples were collected by rinsing the bronchoalveolar surface with several portions of PBS pH 7.4, and bringing the volume of the pooled washings to 10 mL with additional PBS pH 7.4. This solution was centrifuged at 1000 x g for 5 min, and the resulting pelleted cells were resuspended in 1.0 mL of PBS pH 7.4. The total leukocyte count was determined with a hemocytometer. Differential counts were obtained on cytospin preparations treated with Wright’s stain. Immediately after collecting the BALF samples, the lungs were surgically removed, rinsed with ice-cold physiologic saline, patted dry with filter paper, and used for histopathological evaluation. The occurrence of pulmonary inflammation, ALI, apoptotic cell death and oxidative stress was ascertained as described earlier for experiments with LPS.

1.4.1. Effects of CS and TAU-CS on indices of lung inflammation

a) Treating hamsters with TAU for 5 days before an acute (5 days) exposure to CS resulted in a significant attenuation of the increase in the inflammatory index value due to CS Thus, the mean inflammatory index was only 2-fold higher than control (0.6 vs. 0.3, p<0.01) in the BALF of animals pretreated with TAU, but 4.3-fold higher than control (1.3 vs. 0.3, p<0.001) in animals only exposed to CS. (Figure 1A). The mean inflammatory index of control animals (0.3) probably reflects a transient inflammatory response brought about by the stress of the intraperitoneal injections of PBS.

b) From the results shown in Figure 1B, it is evident that a short term exposure to CS caused a drastic (4.3-fold) elevation in the mean number of total leukocytes present in BALF relative to the mean control value (17.3 x 10^3 vs. 4.0 x 10^3 cells, p<0.001). A 5-day pretreatment with TAU reduced this elevation to only ~2.1-fold over control (8.3 x 10^3 cells, p<0.001 vs. CS).

c) An acute exposure to CS led to a small and nonsignificant increase in the percentage number of neutrophils in the BALF relative to the control value (7.7% vs. 1.7%). A pretreatment with TAU raised the percentage neutrophil count (to 3.3%) but the effect was not significant (Figure 2A).

d) CS increased the BALF total neutrophils count relative to the control value to nonsignificant extent (1.3 x 10^3 cells vs. 0.1 x 10^3 cells). A pretreatment with TAU led to an attenuation of the increase caused by CS, but again the effect was not significant (0.3 x 10^3 cells) (Figure 2B).

e) Following an exposure to CS, the percentage number of BALF cells expressing TNFR1 rose 32-fold over the control value (19.3% vs. 0.6%,
p<0.05). A pretreatment with TAU was able to reduce this increase (by 1.3% increase, p<0.001 vs. CS) (Figure 2C).

1.4.2. Effects of CS and TAU-CS on apoptotic death of lung cells

a. The effect of CS in causing alveolar cell apoptosis in lung sections was assessed using the TUNEL assay method on lung sections. From the results presented in Figure 3A, it is apparent that a pretreatment with TAU was effective in lowering the number of TUNEL positive (apoptotic) alveolar cells that followed an exposure to CS. While in the absence of TAU, CS caused a 3.75-fold increase in the number of TUNEL positive cells relative to the control value (0.6/HPF vs. 0.16.HPF, p<0.001), in the presence the increase was only 2.5-fold increase over control (0.4/HPF, p<0.01 vs. CS). Because of the limitations imposed by the procedure used to stain the alveolar cells, only those occupying the septa were amenable to counting.

b. Caspase-3 is a commonly used as an indicator of apoptosis. As shown in Figure 3B, an acute exposure to CS caused a 5-fold increase in the absorbance at 405 nm, serving as an index of this enzyme in BALF samples, over the control value (0.1 vs. 0.02 absorbance units/μg protein, p<0.001). In contrast, a 5-day pretreatment with TAU reduced this increase to only 3-fold over control (0.06 absorbance units/μg protein, p<0.01 vs. CS) (Figure 3B).

1.4.3. Effects of CS and TAU-CS on indices of oxidative stress

a) The promoting effect of CS on LPO was inferred from the significant increase in BALF MDA formation, measured as TBARS, which amounted to ~39% above control (1.8 nmol/mg protein, p<0.01) (Figure 4A). The same study indicated that a pretreatment with TAU was not only able to reduce the formation of MDA induced by CS by 40% (~1.1 nmol/mg protein, p<0.01) but to bring it below the control value (by 17%, p<0.05) (Figure 4A).

b) The GSH levels were found to be reduced significantly in BALF samples from animals exposed to CS (by 35%, 0.53 μmol/mg protein, p<0.01 vs. control) (Figure 4B). Under the same conditions, a pretreatment with TAU was found to virtually abolish the loss of GSH caused by CS (by only 2.5%, p<0.001 vs. CS).

c) CAT, GPx and SOD are three antioxidant enzymes which, by virtue of their protective role in the lung against injury by hyperoxia and oxygen-centered free radicals, may become altered by CS and other factors
capable of inducing oxidative stress. In the present study, CS was found to elevate the BALF activity of GPx by 194% over the control value (0.5 vs. 0.17 U/min/mg protein, p<0.001) activity while lowering those of CAT (by ~63%, 1.5 x 10^-2 vs. 0.04 U/min/mg protein, p<0.001) and SOD (by 25%, 1.2 vs. 1.6 U/min/mg protein, p<0.05) relative to the respective control values (Figures 5A-5C). In contrast, a pretreatment with TAU was found to attenuate all these CS-induced enzyme alterations. Thus, TAU reversed the actions of CS on the BALF CAT (75% above control, 0.07 U/min/mg protein, p<0.001) and SOD (44% above control, 2.3 U/min/mg protein, p<0.01) activities, and limited the increase in GPx activity (up by only ~77% above control, 0.3 U/min/mg protein, p<0.001) (Figures 5A-5C).

1.4.4. Histopathology of lungs exposed to CS and TAU plus CS

Histopathological examination of lung sections stained with H&E found that those from animals that had been exposed to ambient air (the control group) showed intact and non-inflamed alveolar septa and non-enlarged alveolar spaces. Lung sections from animals exposed to CS contained alveolar cells that were moderately inflamed, whose septa were mildly thickened, and containing several macrophages in the alveoli. In contrast, lung sections from animals that were exposed to CS following a treatment with TAU were less inflamed and their septa were not as thick as those receiving TAU.

1.5. Discussion

Although CS and LPS represent two independent causes of ALI, they appear to induce common pathologic changes that include diffuse alveolar damage, infiltration by neutrophils and macrophages, and accumulation of protein-rich edema fluid in alveolar spaces [Ritter et al., 2006]. Following an initial inflammatory response that is initiated and amplified by a combination of pro-inflammatory molecules, notably cytokines, chemokines, eicosanoids and intracellular adhesion molecules released by proinflammatory cells [Chabot et al., 1998; Takano et al., 2002] and lung structural cells (including epithelial, endothelial and interstitial cells) [Zhang et al., 1999], several factors may play a contributory role to ALI by CS or LPS, including the oxidant-antioxidant imbalance created by the accumulation of tissue-damaging ROS and RNS [Pendino et al., 1993; Wizemann et al., 1994], membrane LPO, the depletion of intracellular GSH stores, alterations in the activities of antioxidant enzymes, the release of proteases, and the
inactivation of antiproteases [Chabot et al., 1998; Driscoll et al., 1993; van der Vaart et al. 2004]. At the molecular level, increased ROS/RNS levels have been implicated in initiating inflammatory responses in the lungs through the activation of transcription factors such as NF-κB and AP-1, signal transduction, chromatin remodeling and gene expression of pro-inflammatory mediators [Biswal and Rahman, 2009]. The present results not only confirm these findings but also suggest that CS and LPS induce inflammatory responses that are manifested by rather similar cellular and biochemical alterations. Furthermore, the conditionally essential amino acid TAU is found to protect against these alterations, especially when administered as a pretreatment to either insult.

While the increases in the number of neutrophils, alveolar macrophages and total leukocytes in BALF samples in the lung is a common consequence to an acute exposure to an environmental pollutant, the extent and duration of the increase appear to vary according to both the type of and the length of exposure to an environmental pollutant. Thus, the treatment of rats with ozone is reported to cause a transient increase (2-fold) in the number of adherent vascular neutrophils in the lung, which reached a maximum at 2 hr after an exposure, and returned to baseline values by 12 hr postexposure [Lavnikova et al., 1998]. In contrast, following a treatment with LPS, a 5-fold higher number of vascular neutrophils were recovered from the lung than after ozone, with a 3-fold increase over baseline value still being observed after 48 hr. In the same study, unstimulated neutrophils produced more O2•- and nitric oxide (NO) after a challenge with endotoxin than after one with ozone. Although the present study did not attempt to assess the extent of neutrophil accumulation in the lung with respect to time, it, however, was able to verify that at 24 hr after an exposure to a pulmonary irritant a greater number of pro-inflammatory cells had accumulated in BALF after LPS (being mostly neutrophils) than after CS (being mostly macrophages), and that this difference correlated well with the respective increases in inflammatory index values and expression of TNFR1 by alveolar macrophages, and with the extent of lung damage. Without exceptions, all these cellular alterations were effectively attenuated by a three-day treatment with TAU. Since a pretreatment with TAU was somewhat more effective than a posttreatment to LPS, only a pretreatment was used in the case of CS.

Regardless of the order of its administration relative to that of a lung irritant, TAU was able to reduce the influx of neutrophils, macrophages and other leukocytes into the lung airspaces, to attenuate the ensuing alveolar inflammatory response, and to limit the expression of TNFR1 on macrophages and other granulocytes present in BALF a result of a challenge with LPS. A similar pattern of protection was observed in BALF samples
from animals that were subsequently exposed to CS. Overall, these findings confirm the previously held view that TAU is a potent inhibitory modulator of the pro-inflammatory and immune response in the lung [Marcinkiewicz et al., 1995; Redmond et al., 1998] and other major organs [Redmond et al., 1996; Wei et al., 2008], possibly because of its attenuating action on the transcriptionally regulated production of pro-inflammatory and chemoattracting cytokines by alveolar macrophages [Liu and Quinn, 2002; Park et al., 2002] and other activated leukocytes [Park et al., 1998; Wei et al., 2008], which, in turn, will promote the interaction of pro-inflammatory blood monocytes and neutrophils responsible for ALI, as well as their recruitment to the lungs [Wei et al., 2008]. In this situation, part of the effect TAU could be related to its ability to prevent LPS-induced transendothelial migration of neutrophils into the lung by shortening their rolling velocity and adhesion [Egan et al., 2001b]. Further protection by TAU against ALI may stem from its ability to reduce the release of TNF-α from BALF cells such as neutrophils [Egan et al., 2001a; Wei et al., 2008]. From a mechanistic point of view, the antiinflammatory and injury-protecting actions of TAU appears to be dependent on its conversion to TAU chloramine (TAU-Cl) upon its interaction with HClO since only TAU-Cl, but not TAU, is found to inhibit cytokine production by LPS-stimulated human peripheral mononuclear cells [Chorąży et al., 2002] and murine BALF or peritoneal neutrophils [Marcinkiewicz et al., 1998; Schuller-Levis et al., 1994]. In addition to representing a mechanism for the removal of toxic HClO from the lungs, the formation of TAU-Cl will enable TAU to serve as a negative effector on the signaling pathway for the activation of nuclear transcription factors associated with cytokine synthesis, particularly on the signaling pathway responsible for the activation of NF-κB, by mediating the oxidation of Met45 of IκB kinase-α (IKK-α) [Barua et al., 2001; Kanayama et al., 2002]. This effect of TAU could also translate in a negative effect on the influx of proinflammatory cells into the lungs inasmuch as the NF-κB signaling pathway has a regulatory role on the production of monocyte chemotactic proteins -1 (MCP-1) and -2 (MCP-2), two chemokines involved in macrophage recruitment, by activated neutrophils [Liu and Quinn, 2002].

LPS has been shown to increase the expression of TNF-α and of LPO (measured as MDA and 4-HNE) in the lung in part through a β-adrenoceptor-activated and cAMP-mediated mechanism [Zhang et al., 1999] or a prostaglandin-NO mediated process in rats [Sahan-Firat et al., 2009]; and to stimulate the formation of lipid peroxides [Takano et al., 2004] and MDA and 4-hydroxy-alkenals [Crespo et al., 1999] in the lung when given intratracheally or by the intraperitoneal route to rodents. Likewise, CS, by virtue of its content in ROS and RNS and of its ability to induce the
formation of these reactive species at the pulmonary level, is capable of inducing oxidative stress and, thereby, of causing oxidative lung damage by promoting LPO [Biswal and Rahman, 2009; Koul et al., 2001]. In addition, both LPS and CS can deplete the lung GSH content, alter the activities of antioxidant enzymes (CAT, GPx, SOD) and of enzymes participating in glutathione redox cycling (i.e., glutathione reductase) and biosynthesis (i.e., \(\gamma\)-glutamylcysteine synthetase) [Biswal and Rahman, 2009; Chabot et al., 1998; Koul et al., 2001].

Under basal conditions, the GSH content in alveolar epithelial lining fluid is high, but it becomes depleted upon conversion to its disulfide form (GSSG) during the removal of H2O2 and hydroperoxides by GPx, its conversion by free radicals to glutathiyl and, to a lesser extent, to thiol peroxy radicals [Buettner, 1993], and because of the inability of lung cells to effect its replenishment in the face of the inhibitory effect of oxidants and proinflammatory mediators on \(\gamma\)-glutamylcysteine synthetase (\(\gamma\)GCS), the key enzyme for GSH synthesis [Rahman and MacNee, 2000]. While CS lowered the BALF GSH to a slightly greater (~10%) extent than LPS, TAU was able to counteract the effect of these lung irritant very effectively and to about the same extent. In view of the correlation that appears to exist between a decline in mitochondrial GSH, an increase in ROS, a reduction in procaspase 3 protein levels, the appearance of caspase-3 cleavage products, and the development of apoptotic signals in the absence of any external apoptotic stimuli, it is conceivable that the preserving role of TAU on the mitochondrial GSH stores could also be precluding apoptotic cell death [Armstrong et al., 2002]. Possible mechanisms that may account for the actions of TAU in preserving the physiological GSH/GSSG ratio are its limited and, often, weak free radical scavenging action, and its ability to maintain the patency of antioxidant enzymes and of enzymes required for the biosynthesis and redox cycling of GSH [Nandhini et al., 2005].

Although evidence on the occurrence of LPO and on changes in the activities of antioxidant enzymes as a result of and exposure to either CS or LPO are extensive, there is, however, divergence of results among laboratories. For example, under the present experimental conditions, both CS and LPS were shown to promote LPO by increasing the formation of MDA in BALF relative to control values, results that agree with those reported by most laboratories [Biswal and Rahman, 2009; Chabot et al., 1998; Koul et al. 2001; SahJan-Firat et al., 2009; Stark and Jackson 1990]. However, a study comparing the effect of LPS and CS in the mouse found that while the inhalation of endotoxin lowered, rather than increased, the formation of TBARS in the lung and plasma, but not in BALF, a 5-day exposure to CS increased the TBARS level in both the lung and BALF, but
not in the plasma [Valença et al., 2008]. In contrast, in the present study TAU was found to significantly reduce the formation of TBARS in the BALF of animals exposed to either CS or LPS. Such an effect may reflect the ability of TAU to facilitate the removal of ROS and RNS by attenuating the losses in enzymatic and nonenzymatic antioxidant defenses and in intracellular free calcium brought about by oxidant-generate ROS [Hagar, 2004; Jeon et al., 2009].

A greater degree of variability seems to surround the effects of these environmental pollutants on the activities of the antioxidant enzymes CAT, GPx and SOD. As reported here, both LPS and CS significantly lowered the activities of CAT and SOD while increasing that of GPx in BALF samples, with the magnitude of the effects being greater with CS than with LPS. The trend of these results is in good agreement with that reported for erythrocytes from human smokers [Yildiz et al., 2002], but differ partially or completely from those emanating from other laboratories. Indeed, in a study conducted in rats it was determined that within 6 hr of an intraperitoneal treatment with LPS the genes coding for antioxidant enzymes in the lungs were expressed in a divergent manner, with that for Mn-SOD being increased, that of CAT being decreased, and that of either Cu,Zn-SOD or GPx remaining unchanged [Redmond et al., 1996]. Moreover, in another laboratory the daily inhalation of a LPS solution by mice for 5 days within an inhalation chamber was shown to lower the CAT, GPx and SOD activities in BALF, and to a lower the CAT activity without affecting those of SOD or GPx in lung homogenates [Valença et al., 2008]. Additional conflicting data also exists regarding the changes in lung SOD activity following a challenge with LPS. Thus, the intraperitoneal injection of LPS (20 mg/kg) to mice was found to induce a massive (>80%) decrease in extracellular SOD, the most abundant form of SOD in the lungs, but without affecting the cytoplasmic (Cu,Zn-SOD) and mitochondrial (Mn-SOD) SOD isoforms [Wei et al., 2008].

By analogy to the results gathered for LPS, there is also considerable variation in the results reported by different laboratories for the activities of lung antioxidant enzymes from animal models exposed to CS. For example, in the adult rat CS was found to exert either no effect [Gupta et al., 1988], to decrease the activities of SOD and CAT without affecting that of GPx [Luchese, 2009], and to increase the GPx activity without altering those of Cu,Zn-SOD and CAT [Gilks et al., 1998]. In mice, CS is reported to affect the activities of antioxidant enzymes according to the sample tested, with BALF samples exhibiting an increased activity of both CAT and SOD and lower activity of GPx relative to control values; and lung homogenates demonstrating an increase in all three activities [Valença et al., 2008]. To complicate matters further, there are reports indicating that an exposure to CS
induced an increase in SOD and CAT, but not in GPx, activity in hamster alveolar macrophages [McCusker and Hoidal, 1990]; and that the activity of all antioxidant enzymes were increased in the albino rat as a protective measure against CS-induced free radicals [Baskaran et al., 1999]. Although the exact reasons for the discrepancy in antioxidant enzyme activities associated with the inflammatory response to an exposure to LPS or CS among laboratories will necessitate a more detailed and systematic evaluation than that described here, especially as it relates to CS, they could stem from factors such as the type of sample analyzed (plasma, BALF, tissue homogenate), the duration of the exposure to the lung irritant [Clerch and Massaro, 1993; Valença et al., 2009], endotoxin-related factors such as concentration of the endotoxin solution [Carter et al., 2006], the dose of the endotoxin and the route of administration used [Carter et al., 2006], the type of animal model [Dirami et al., 1999], the experimental conditions used to carry out the exposure to CS, including the length of exposure [Schick and Glantz, 2006; Zacyn et al., 1987], the type of CS generator used to generate tobacco smoke, the type of cigarette used [Houdi et al., 1995], the dose of smoke inhaled [Santiago et al., 2009], the number of cigarettes burned [Luchese et al., 1999], and the volume of smoke puffed [Zacyn et al., 1987].

A 3-day treatment with TAU, before or after an exposure to LPS, resulted in a significant reversal of the losses in CAT and SOD activities and a lesser increase in GPx activity brought about by LPS in BALF samples. A similar trend of effects was observed in BALF and lung samples of animals given TAU before an exposure to CS. Irrespective of differences in antioxidant enzyme responses to oxidative stress by CS or LPS noted among laboratories, the present results are indicative of a protective action for TAU against the changes in antioxidant enzymes brought about by the inflammatory state that follows an exposure to CS or LPS. In accord with the findings of other laboratories, the protective actions of TAU on lung antioxidant enzymes verified here may be ascribed, at least in part, to antioxidant properties whereby this amino acid can spare the intracellular GSH content, counteract LPO, and/or limit the formation of ROS and NOS [Men et al., 2010].

In the present study, the occurrence of apoptosis by CS and LPS was assessed on the basis of the increased number of TUNEL-positive cells and of cleaved caspase-3 immunoreactivity that were observed in BALF (CS, LPS) and lung homogenate (CS) samples relative to control samples. LPS is reported to cause disseminated endothelial apoptosis as a preamble to endothelial and alveolar epithelial tissue damage, and that TNF-α can serves as activating signal for the proapoptotic caspase-3 and acid-sphingomyelinase [Kawasaki et al., 2000]. In turn, the apoptosis of parenchymal cells may be a major cause of widespread lung inflammation, of ALI and of chronic
obstructive pulmonary disease (Schmidt and Tuder, 2010). In common with LPS, an exposure to CS can lead to a neutrophil-dependent inflammation, to airspace enlargement, to oxidative stress and protease-antiprotease imbalance, and to histological changes consonant with ALI [Brass et al., 2008]. Interestingly, while chronic smoking is found to down regulate endotoxin-induced neutrophil-mobilizing cytokine production by inhibiting AP-1 activation in the airways [Laan et al., 2004], there is also evidence suggesting that a short-term exposure to CS can potentiate endotoxin-induced lung inflammation [Kulkarni et al., 2007]. An earlier study in cultured MRC-5 cells, a fetal cell line of pneumocytes, demonstrated the ability of a pretreatment with TAU to reduce LPS-induced production of ROS, intracellular Ca2+ accumulation, the activation of mitogen-activated protein kinases, and the translocation of Bax protein, changes that jointly can contribute to apoptotic cell death [Jeon et al., 2009]. In this regard, it is believed that the decrease in the viability of pneumocytes as a result of an exposure to LPS is related to increases in the levels of Ca2+ and ROS, both of which can induce cell death by damaging DNA, causing membrane LPO, and/or directly activating the expression of genes/proteins responsible for apoptosis [Buttke and Sandstrom, 1994]. In addition, high levels of intracellular Ca2+ are associated with oxidative stress, mitochondrial damage and cell death [Jeon et al., 2009]. In line with these results, the present study finds that TAU can reduce the number of TUNEL-positive cells and the activation of caspase-3 in BALF cells and in the lung when given as a pretreatment or posttreatment to the intratracheal instillation LPS or as a pretreatment to CS exposure.

In summary, the results of the present study clearly suggest that an acute exposure to CS or LPS can lead to histological and biochemical alterations in the lung and BALF that are consistent with those of acute inflammation, oxidative stress, LPO and apoptosis; and that an antioxidant compound such as TAU, given as a pretreatment or posttreatment, can be of help in preserving the integrity and viability of the lung during an exposure to a lung irritant by attenuating the influx of inflammatory cells, the development of inflammation and the oxidant-antioxidant imbalance, and the cell injury and apoptotic cell death associated with the exposure.

1.6. Reference


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