Effects of Atorvastatin on Smoking-Induced Alveolar Injury in Rat Lungs

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ABSTRACT

BACKGROUND: Smoking is one of the most serious health care issues worldwide, as one third to one half of all people who smoke eventually use tobacco habitually. Chronic smoke exposure causes airway and lung parenchymal inflammation and the destruction of alveolar cell walls. Statins may have anti-inflammatory effects that would play a role in preventing the cellular damage associated with smoking.

OBJECTIVE: The aim of this study was to investigate whether atorvastatin protects against smoking-induced inflammation in alveolar epithelial type I (ATI) and type II (ATII) cells in the lungs of rats.

METHODS: Adult male albino Wistar rats (200–250 g) were randomly divided into 3 groups and exposed to cigarette smoke 8 hours per day for 15 days. During that 15-day period, the 2 treatment groups received atorvastatin 0.5 or 1.0 mg/kg/d in 2 mL of methyl cellulose solution and the control group received 2 mL of methyl cellulose solution alone, all via nasogastric catheter. After the 15 days, the lungs were excised and the tissues were examined by transmission electron microscopy.

RESULTS: Thirty rats were divided into 3 groups of 10 rats each. All rats survived the 15 days. In the atorvastatin 0.5-mg group, no changes were found in the ATI cells or in the blood–air barrier. In the atorvastatin 1.0-mg group, we observed hyperplasia in the common basal membranes. Hypertrophy, mitochondrial cristolysis (MC), and intracytoplasmic edema (ICE) were detected in the ATI cells in the 1.0-mg group, while chromatin condensation, atrophic appearance, cell shrinkage, and cytoplasmic vacuolization were observed in the ATII cells. The rough endoplasmic reticulum (rER) tubules of the ATII cells appeared spiral-shaped. In the control group, minimal ICE was detected in the ATI cells. However, microvillus deformation, pseudopod formation, edema, mitochondrial swelling, and MC were observed in the ATII cells. We also observed MC, several pinocytic vesicles, and normal rER tubules in the endothelial cells of the control group.

CONCLUSIONS: The administration of atorvastatin 0.5 mg/kg/d was associated with some attenuation of lung injury caused by smoke inhalation in these rat lungs.
However, atorvastatin 1.0 mg/kg/d was associated with lung damage. Future studies are needed to evaluate the dose–response relationship of atorvastatin to smoking-induced alveolar damage. (Curr Ther Res Clin Exp. 2009;70:366–376) © 2009 Excerpta Medica Inc.

**KEY WORDS:** lung, atorvastatin, ultrastructural changes, rat.

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**INTRODUCTION**

Smoking is one of the most serious health care issues worldwide, as one third to one half of all people who smoke eventually use tobacco habitually. Tobacco use was found to be associated with 1 in 10 deaths among adults, amounting to >5 million deaths annually worldwide. Between 2002 and 2030, tobacco-attributable deaths are projected to decline by 9% in high-income countries and to increase by 50% to 6.8 million deaths in low- and middle-income countries. Unless urgent action is taken, the annual death toll from tobacco use may rise to >8 million worldwide by 2030.

Chronic smoke exposure causes airway and lung parenchymal inflammation and destruction of alveolar cell walls. Cigarette smoke is associated with chronic obstructive pulmonary disease (COPD), cancer, chronic bronchitis, and asthma, as well as suboptimal lung growth in preadolescent and adolescent smokers. Cigarette smoking is the most common risk factor for developing COPD, which is a disease associated with an abnormal inflammatory response of the lungs to noxious particles or gases.

There are 2 types of lung tissue alveolar epithelial cells: type I (ATI) and type II (ATII) (the latter includes alveolar epithelial stem cells). Repair of the damaged alveolar surface depends on the ability of ATII cells to differentiate into pre-ATI cells. The alveoli constitute a major trafficking site of inflammatory cells that can ultimately migrate through the airways and into parenchymal tissue.

Statins, or 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors, are used clinically for their cholesterol-lowering properties. The roles of statins in coronary artery disease are well reported. Some studies have focused on the mechanisms involved in the anti-inflammatory effects of statins.

Lee et al., in their controlled study of rats administered oral simvastatin 5 mg/kg once per day for 16 weeks, found that simvastatin was associated with a dose-related decrease in cigarette smoking–induced structural and functional derangement of the lungs of rats, possibly by reducing inflammatory infiltration, inhibiting matrix metalloproteinase-9 induction, and preventing pathologic changes in pulmonary vasculature. Simvastatin has been reported to inhibit lung cell destruction caused by 16 weeks of cigarette smoking.

We conducted this study to assess whether atorvastatin protects against smoking-induced inflammation in ATI and ATII cells in the lungs of rats.

**MATERIALS AND METHODS**

Adult male albino Wistar rats weighing 200 to 250 g were used in this experimental study. All rats were given standard rat chow and tap water ad libitum. This study was carried out according to the principles of the Declaration of Helsinki.
Current Therapeutic Research

Experimental Conditions
On the first study day, the rats were randomly divided into 3 groups using the envelope method: the atorvastatin 0.5- and 1.0-mg groups and the control group. All the rats were housed in the same room, which was 7 m³ in size, and were kept at 20°C (±2°C) under a 12-hour light/dark regimen in stainless steel cages.

Atorvastatin Preparation and Administration
All 3 groups were exposed to cigarette smoke 8 hours a day for 15 days (equivalent to 10 standard cigarettes). During the same 15-day period, the atorvastatin groups received 0.5 or 1.0 mg/kg/d of atorvastatin dissolved in 2 mL methyl cellulose solution and the control group received 2 mL of methyl cellulose solution alone, all via a nasogastric catheter in the morning.

Procedure
The rats were euthanized using ether anesthesia. The lungs were excised and fixed in 2.5% phosphate-buffered glutaraldehyde for histopathologic evaluation. Samples of semithin cross-sections of the lung tissues were prepared with an ultramicrotome, stained with toluidine blue, and examined using a photomicroscope. After the selection of appropriate specimens, the tissues, which were stained with uranyl citrate and lead acetate, were screened by transmission electron microscopy (TEM) (JEOL 1010, JEOL Ltd., Tokyo, Japan). The same tissue samples were examined in 2 different centers by 2 different independent histologists, both of whom were blinded to the treatment the rats received. The findings of the 2 histologists were consistent with each other.

RESULTS
Thirty adult male albino Wistar rats were divided into 3 groups of 10 rats each. All rats survived the 15-day study period.

Atorvastatin 0.5-mg Group
No changes were detected in the ATI cells, the blood–air barrier, or the cellular organelles of the rats in the atorvastatin 0.5-mg group. The common basal membranes of the ATI and capillary endothelial cells were normal (Figure 1A). Minimal increases in collagen fibril structure were observed in the interstitial areas (Figure 1B). Only minimal changes in the mitochondria and microvilli in the ATII cells were noted on examination of the ATI and ATII cells located beneath the collagen matrix. The lysosomes were normally shaped, and minimal intracellular edema was detected in the ATI cells (Figure 1C).

Atorvastatin 1.0-mg Group
Hyperplasia in the common basal membranes of the ATI and capillary endothelial cells was observed in the lung tissue of the atorvastatin 1.0-mg group. Hypertrophy, mitochondrial cryolysis (MC), and intracytoplasmic edema (ICE) were detected in the ATI cells (Figure 2A). A widespread increase of collagen fibrils was seen in the interalveolar septa (Figure 2B). Chromatin condensation, atrophy, cell shrinkage, and
Figure 1A. High magnification of lung tissue of rats in the atorvastatin 0.5-mg group showing alveolar epithelial type I (T1) cells with normal common basal membrane (BM), intracytoplasmic edema (\(*\) ), mitochondrial cristolysis (\(\pm\) ) and endothelial cells (En) (uranyl acetate-lead citrate; magnification x12,000).

Figure 1B. Low magnification of interstitium of lung tissue of rats in the atorvastatin 0.5-mg group showing minimal increases in the interstitial collagen fibrils (Co) and vacuoles (Va) (uranyl acetate-lead citrate; magnification x4400).
cytoplasmic vacuolization were seen in the ATII cells (Figure 2C). The rough endoplasmic reticulum (rER) tubules in the ATII cells were spiral-shaped, closely resembling cells undergoing apoptosis (Figure 2D).

Control Group

Minimal ICE and partial separation were observed in the ATI nuclear membranes of rats in the control group. MC and several pinocytic vesicles were seen in the capillary endothelial cells. Furthermore, the rER tubules of the capillary endothelial cells were of normal shape and size (Figure 3A). Microvillus deformation, pseudopod formation, edema, mitochondrial swelling, and MC were detected in the ATII cells; all intracellular lipid droplets were clear, mature lamellar bodies were absent, and the rER tubules were inactive (Figure 3B).

Discussion

In the present study, administration of atorvastatin 0.5 mg/kg/d was associated with decreased ATI and ATII cellular inflammation. Therefore, atorvastatin may protect against smoking-induced alveolar inflammation at this dosage. However, the administration of atorvastatin 1.0 mg/kg/d was associated with ATI and ATII cellular degeneration and apoptotic processes. We observed ultrastructural changes and an inflammatory response in the ATI and ATII cells in the control group. These findings suggest that atorvastatin 1.0 mg/kg/d may bypass an inflammatory response, leading
Figure 2A. Low magnification of lung tissue of rats in the atorvastatin 1.0-mg group showing alveolar epithelial type I (T1) cells with hyperplasia of the endothelial cells (En) and common basal membrane (BM), mitochondrial cristolysis (+), and intracytoplasmic edema (•) (uranyl acetate-lead citrate; magnification x4400).

Figure 2B. Low magnification of lung tissue of rats in the atorvastatin 1.0-mg group showing interalveolar septa with an increase in collagen fibrils (Co) (uranyl acetate-lead citrate; magnification x4400).
Figure 2C. High magnification of lung tissue of rats in the atorvastatin 1.0-mg group showing alveolar epithelial type II (T2) cells with chromatin condensation (CC), atrophy, cell shrinkage (CS), vacuoles (Va), pseudopods (Ps), mitochondrial cristolysis (+), and collagen fibrils (Co) (uranyl acetate-lead citrate; magnification x7000).

Figure 2D. Low magnification of lung tissue of rats in the atorvastatin 1.0-mg group showing alveolar epithelial type II (T2) cells with spiral-shaped rough endoplasmic reticulum (rER), nucleus (N), and mitochondrial cristolysis (+) (uranyl acetate-lead citrate; magnification x4400).
Figure 3A. High magnification of lung tissue of rats in the control group showing alveolar epithelial type I (T1) cells with minimal intracytoplasmic edema (*), mitochondrial crystolysis (+), and normal rough endoplasmic reticulum (rER) (uranyl acetate-lead citrate; magnification x12,000).

Figure 3B. High magnification of lung tissue of rats in the control group showing alveolar epithelial type II (T2) cells with deformed microvilli (MV), pseudopods (Ps), edema, mitochondrial swelling (Ms), mitochondrial crystolysis (+), and clear intracellular lipid droplets (uranyl acetate-lead citrate; magnification x12,000).
Some studies have indicated that statins are associated with apoptosis in different cancer types and that simvastatin (5 mg/kg) decreased lung parenchymal destruction, inflammatory infiltration, and pulmonary hypertension induced by chronic cigarette smoking and concluded that simvastatin prevented smoking-induced lung damage via pleiotropic effects. Our findings regarding atorvastatin 0.5 mg/kg/d were consistent with the findings of Lee et al.

**Limitations**

One limitation was the small sample size. Our study did not include a group that was not exposed to smoke and did not receive atorvastatin. Quantitative assessment could not be performed for the ultrastructural examination and comparison of the tissues because of the TEM technique.

Although apoptosis in the 1.0-mg group suggests that the higher dose of atorvastatin may be associated with cellular damage, this study was not designed or powered to evaluate atorvastatin apoptosis causality. Comprehensive, well-designed, and adequately powered studies are needed to investigate the dose–response relationship of atorvastatin with cellular damage.
CONCLUSIONS
The administration of atorvastatin 0.5 mg/kg/d was associated with some attenuation of lung injury caused by smoke inhalation in these rats. However, atorvastatin 1.0 mg/kg/d was associated with lung damage. Future studies are needed to evaluate the dose-response relationship of atorvastatin to smoking-induced alveolar damage.

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