Maternal Diesel Exhaust Exposure Damages Newborn Murine Brains

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To examine pathologically the influence of diesel exhaust (DE) exposure on fetal nervous system development, brain tissue (cerebral cortex and hippocampus) was collected from newborn mice whose mothers were exposed to DE during pregnancy. After DE exposure, these brain tissues showed evidence of numerous caspase 3-positive cells (a common enzymatic biomarker of apoptosis). Some cells were found to contain crescent-shaped spaces, which are suggestive of apoptotic processes. Granular perithelial (GP) cells, scavenger cells surrounding cerebral vessels of the blood-brain barrier, showed signs of apoptosis; furthermore, the GP cytoplasmic granules had degenerated and showed evidence of what appeared to be ultrafine, DE particles. Additionally, the swelling of astrocyte endfoot that surround capillaries showed degenerative changes similar to myelin figures. Furthermore, the apoptosis of endothelial cells and stenosis of some capillaries were observed. These findings varied in severity, depended on DE concentration, and were not observed in the control group. These observations suggest that exposure of pregnant mice to DE might carry a risk of cellular atrophy and might affect fetal brain development. Our findings also reveal that inhalation of DE might be hazardous to the general health of fetuses.

Key words —— diesel exhaust, brain, apoptosis, caspase-3, endothelial cell, granular perithelial cell

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INTRODUCTION

Diesel exhaust (DE), a major air pollutant in urban areas, is a complex mixture of gases and particles. Most reports on the health impact of DE inhalation have focused on respiratory diseases, such as asthma; however, a connection to central nervous system diseases has been recently identified.[1)] Moreover, maternal inhalation of DE during pregnancy impacts the fetus, particularly in regard to growth and sexual maturation.[2)] The smallest DE particles (DEPs) are nanostructures (< 0.1 μm), composed of various chemical substances that coat a carbon core. Inhaled, ultrafine, air-borne particles are not only found in the respiratory tract but also in extrapulmonary tissues.[3,4)] Therefore, it is extremely likely that maternally inhaled DEPs can pass through the placenta, enter the fetal circulation, and cause cellular damage. Since the development of the blood-brain barrier (BBB) in the fetal brain is incomplete, the fetal brain is impacted by blood-borne substances to a much greater extent than in the adult brain. In this study, we found that exposure of pregnant mice to DE severely impacts fetal brain development.

MATERIALS AND METHODS

Pregnant Institute of Cancer Research (ICR) mice were exposed to DE [3 groups of either 0.3, 1, or 3.0 mg particles/m^3 (n = 10 per group)] during a 12-hr light/12-hr dark cycle (19:00–07:00) from 2 days post-coitus to 16 days post-coitus. Some of the ICR mice (n = 10) were exposed to clean air as a comparative control group. After DE exposure, the pregnant mice were housed in a clean cage in a clean room until delivery; at 11 weeks of age, the brain

tissue was harvested from the newborn mice. The cerebral cortex (frontal and temporal lobes) and the hippocampus were examined with light and electron microscopy. To detect apoptosis in these tissues under a light microscope, the immunohistochemical staining for caspase 3 (a common enzymatic biomarker of apoptosis) was performed. All experimental animals were handled in accordance with institutional and national guidelines for the care and use of laboratory animals.

RESULTS

After DE exposure, both the cerebral cortex and the hippocampus showed evidence of numerous caspase-3-positive cells (a common enzymatic biomarker of apoptosis) under light microscopy. Some cells were found to contain crescent-shaped spaces (CSS), which are specific features of the apoptotic formation process.[5] In addition, the aforementioned cells had a condensed ultrastructure. The granular perithelial (GP) cells, which are scavenger cells that surround cerebral vessels and plays a role in the BBB, showed signs of apoptosis; furthermore, the GP cytoplasmic granules had degenerated and showed evidence of what appeared to be ultrafine DE particles (Fig. 1, panels A and B). Furthermore, the swelling of astrocyte endfoot that surround capillaries had degenerative changes similar to myelin figures (Fig. 1, panels C and D). Furthermore, endothelial cell apoptosis and stenosis of some capillaries were observed (Fig. 1, panels C and D). The findings varied in severity, depended on DE concentration, and were not observed in the control group.

DISCUSSION

Our findings represent strong evidence that prenatal exposure to DE has diffuse and extensive pathological effects on the brains of newborn mice. The following processes may occur: DEPs are inhaled by pregnant mice, pass through the placenta, and enter the fetal circulation; These particles pass through the under-developed fetal BBB, are taken up in the GP cells, and induce apoptosis of both endothelial cells and GP cells; The DEPs are taken up by the GP cells and gradually affect the cerebral parenchyma; and Apoptosis of the endothelial cells results in capillary stenosis and occlusion, which presents a severe risk of widespread cellular atrophy.

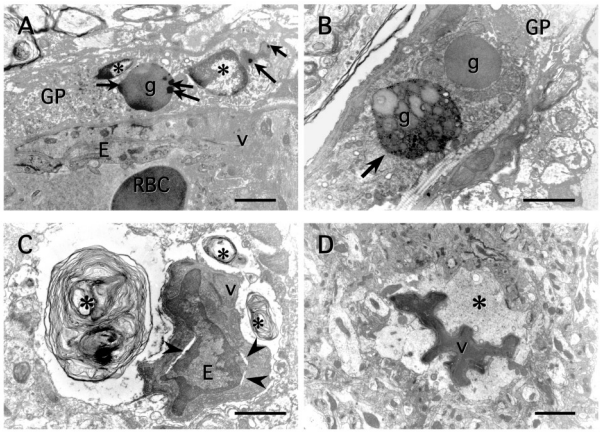

Fig. 1. Electron Micrographs of DE-Exposed Newborn Murine Brain (Cerebral Cortex and Hippocampus)

Scale marker: 3 μm. [A] Apoptosis (*: nuclear fragment) of GP with up-take of particles (arrow) in cytoplasmic granules (g). v: vessel, E: endothelial cell, RBC: red blood cell. [B] GP with up-take of particles and degenerative changes (arrow) of cytoplasmic granules (g). [C] Apoptosis (arrowhead: CSS) of endothelial cells (E), stenosis of vessels (v), and swelling of astrocyte endfoot with degenerative changes similar to myelin figures (*). [D] Stenosis of vessels (v) and swelling of astrocyte endfoot (*).

These phenomena were derived from our observations on the brains of newborn mice whose mothers were exposed to DE during pregnancy; however, the possibility exists that the same phenomena may occur in human infants secondarily to maternal inhalation of DE during pregnancy. The human placenta consists of two layers while the mouse placenta consists of four layers. Furthermore, the length of human gestation is considerably longer than that of the mouse. Therefore, the possibility exists that more severe damage could occur in the developing brains of human infants than in the newborn mice analyzed in this study. Inasmuch as apoptosis of the GP and endothelial cells that surround cerebral vessels promotes a malfunction of the BBB, the brains of these postnatal mice would have diminished protection from various toxins and would thus result in direct damage of cerebral parenchymal cells.

Our findings indicate the existence of a severe health hazard from DE inhalation. We are compiling additional data to further substantiate the toxic effects of DE. The present study proposes the necessity of thorough and extensive examination of the effects of DE inhalation, especially in fetal development.

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