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Intimal Ultrastructure of Human Umbilical Arteries

OBSERVATIONS ON ARTERIES FROM NEWBORN CHILDREN OF SMOKING AND NONSMOKING MOTHERS

By Inger Asmussen and Knud Kjeldsen

ABSTRACT

The umbilical artery was chosen as a possible model for evaluating the vascular injury provoked by tobacco smoking in humans. Cords from newborn children delivered by 15 nonsmoking and 13 smoking mothers were studied in the transmission and the scanning electron microscope. Pronounced intimal changes were seen in the arteries from smoking mothers; the most important findings were degenerative changes of the endothelium such as swelling, blebbing, contraction, and subsequent opening of the endothelial junctions with formation of subendothelial edema. Other observations included dilation of the endoplasmic reticulum in the endothelium and reparative changes such as a considerable widening of the basement membrane. Since similar changes can be induced in arteries of animals by exposure to carbon monoxide or perfusion with nicotine, we conclude that the present study supports the concept that tobacco smoking is harmful to the vascular endothelium. This study also contributes to an understanding of the mechanism through which vascular injury is provoked in heavy smokers.

KEY WORDS pregnancy placenta carbon monoxide nicotine tissue hypoxia vascular injury

Clearly, a close connection exists between tobacco smoking and development of arterial diseases, especially coronary heart disease and peripheral arterial disease (1). The question is how does tobacco smoke provoke arterial damage. Evidence accumulated during the last decade indicates that the carbon monoxide in tobacco smoke is harmful to the arterial wall (2). Exposure to low doses of carbon monoxide accelerates atherogenesis in cholesterol-fed animals (3-5) and produces significant ultrastructural changes in the aortic and the coronary endothelium of rabbits and primates that are indistinguishable from early atherosclerosis (6, 7). For obvious reasons similar exposure studies and subsequent vascular biopsies cannot be performed in humans. Moreover, a comparison of vascular biopsy studies in human smokers and nonsmokers has not been published to date. As a possible model for evaluating the vascular damage provoked by tobacco smoking in humans, the umbilical artery of babies of smoking and nonsmoking mothers was used for ultrastructural studies in the present paper.

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Methods

PATIENTS

Pregnant women admitted to the Department of Obstetrics and Gynecology at Rigshospitalet were selected for the study. All patients were examined by the same investigator, and each subject completed a questionnaire on smoking habits before and during pregnancy. Twenty-eight patients took part in the study, 13 smokers and 15 nonsmokers. All smokers were inhaling cigarette smokers. One subject smoked 40-60 cigarettes daily.

Women suffering from hypertension, diabetes, and other diseases and those with Rh negative blood types were not used in this study. The patients chosen for the study were normal before and during the pregnancy: all laboratory investigations (hemoglobin, blood, sugar, etc.) including urinary analyses for sugar, protein, and estriol were normal. Therefore, the only difference in the two experimental groups was smoking habits.

The clinical data for these women are presented in Table 1. All of the patients were white, had normal pregnancies, and delivered babies at full term. All of the children were mature, and malformations were not found.

The average weight of children born to smokers was 3,370 g and that of children born to nonsmokers was 3,695 g, a difference of 325 g. A similar difference of 123 g was found in the weights of the placentas. Macroscopically, placentas from smokers were less loose and more fibrotic in appearance than were those from the control group. Neonatal icterus was found in five cases—four in the control group. Ten of the 13 children born to smokers were female.

ARTERIAL BIOPSIES AND PREPARATION FOR MICROSCOPY

All specimens were selected and handled through the initial preparation by Dr. Asmussen. Immediately after
delivery of the placenta, the umbilical cord was cut about 10 cm from the placenta. A soft plastic catheter was placed in one of the umbilical arteries, and about 50 ml of cold Ringer's solution was infused from a syringe with a light pressure. Perfusion was continued with 4.5% cold glutaraldehyde containing 2% acrolein and buffered to pH 7.4 with 0.2M phosphate buffer for at least 5 minutes to prevent contraction during the following cutting procedures.

For transmission electron microscopy, the middle portion of the fixed artery was carefully dissected out, cut into small blocks, and immediately transferred to the same glutaraldehyde for 1 hour. Postfixation was performed in 1% buffered osmium tetroxide (pH 7.4) for 1 hour at 4°C. Tissue blocks were dehydrated in graded ethanols, cleared in propylene oxide, and embedded in Epon 812 or Araldite (Durecupan ACM). Sections 0.5-1.0 μm thick were stained with toluidine blue for light microscopy. Ultrathin sections were cut on glass knives with an LKB Ultrotome III, mounted on uncoated copper grids, and contrasted with magnesium uranyl acetate (8) and lead citrate (9). These sections were examined and photographed in a Zeiss EM 9S-2 electron microscope.

Tissue for scanning electron microscopy was obtained in the following way. After perfusion, the artery was cut longitudinally and immediately transferred to a flat vessel containing 4.5% cold glutaraldehyde. Samples measuring about 3 × 3 mm were cut out, fixed with needles to a cork plate, and placed with the endothelium downward in a small beaker with the same glutaraldehyde for 1 hour at 4°C. Stretching, bending, and touching of the inner surface were carefully avoided. Postfixation was performed in 1% buffered osmium tetroxide (pH 7.4) at 4°C. After dehydration with acetone, the specimens were taken through graded mixtures of acetone and benzene to pure benzene, freeze-dried, and mounted on aluminum stubs with silver paste; a conduction coat of gold was deposited in a vacuum with an Edwards 306 coater. The endothelial surface was studied in a Cambridge Steroscan S-600 scanning electron microscope and photographed with a Hasselblad camera.

Results

NONSMOKERS

In the scanning electron microscope, the endothelial surface of the umbilical arteries from nonsmokers showed a regular pattern of spindle-shaped cells (Fig. 1) oriented longitudinally and following the spiraling course of the vessel. With the present technique, the width of the cells was...
approximately 5 µm and the length varied considerably, probably depending on the degree of contraction in the individual cells. The shortest endothelial cells measured about 50 µm and the longest about 100 µm.

In the transmission electron microscope, the endothelium lining the luminal surface was of the continuous type with closed intercellular junctions. The cytoplasm was rich in the organelles usually found in arterial endothelium: rough endoplasmic reticulum, mitochondria, and Golgi complexes (Fig. 2). Although the number of mitochondria was moderate, the Golgi complexes and rough endoplasmic reticulum were often highly developed. The nuclei were usually crenated and contained well-developed nucleoli.

Pinocytic vesicles were seen dispersed in the cytoplasm; they were especially numerous at the luminal surface and at the base of the endothelial cells. A regular feature in this area was the presence of small bundles of fine filaments oriented in the longitudinal axis of the cells; these filaments resembled the myofilaments of the underlying smooth muscle cells. Centrosomes and lysosomes were also observed.

No continuous endothelial basement membrane was present. The basal lamina appeared to consist of fragments of homogeneous material with a medium electron density.

The subendothelial area contained a sparse ground substance and smooth muscle cells, often in close apposition to the endothelial lining, and was rich in bundles of collagen fibers. Since no internal elastic membrane was present, there was no sharp demarcation between the intima and the tunica media. Occasionally, lymphocytes were seen just beneath the endothelial cells.

**SMOKERS**

Many pathological changes in the intima were observed in the arterial samples from smokers. In all of the specimens observed in the scanning electron microscope, the most conspicuous finding was large areas of swollen and irregular endothelial cells with a peculiar cobblestone appearance (Fig. 3). At higher magnifications, small cytoplasmic processes (blebs) were often seen protruding from the surface of these cells (Fig. 4).

In the transmission electron microscope, edematous swelling and degenerative changes were characteristic features. Endothelial swelling was seen in areas of the cobblestone endothelium. As a rule, the rough endoplasmic reticulum in such cells was also enormously dilated by a fluid with a moderate electron density (Fig. 5). Lysosomes of two types were often seen: one type contained marginally arranged, electron-dense, round bodies with opaque dots, giving the organelle a wheellike appearance (Fig. 5) and the other type exhibited a characteristic striation (Fig. 6). In some areas, the basal myoid filaments were considerably contracted, giving the endothelial cells a balloon-like appearance.

Even in areas without swelling of the endothelium, extensive edema of the subendothelial space was a regular finding (Fig. 7). The basement membrane was always considerably thickened—often equal in width to the endothelial lining; it consisted of a fine reticular material with a feltlike texture (Figs. 6-8). The smooth muscle cells in the edematous subendothelial space often showed vacuolization. Cytoplasmic protrusions of the cell surface (blebs) were also seen in the transmission electron microscope (Figs. 5, 8). In edematous areas, focal loss of closed intercellular junctions was a characteristic finding (Fig. 6). In addition to these observations, a decreased amount of collagen fibers and an absence of lymphocyte infiltration of the luminal part of the arteries were seen.

The morphological findings have been correlated with tobacco consumption in Table 2.

**Discussion**

Sheppard and Bishop (10) recently described the fine structure of sheep umbilical vessels, but the
only report on the ultrastructure of human umbilical vessel endothelium at term seems to be that of Parry and Abramovich (11). Although double clamping of the selected arterial segments and immersion in cold fixative was used in both of these studies to prevent artifacts due to arterial contraction, Sheppard and Bishop (10) also injected cold fixative into the lumen to retain the fine structure of the intima. In our experience, satisfactory intimal ultrastructure can also be obtained by perfusing glutaraldehyde containing formaldehyde or acrolein to ensure fast penetration of the fixative.

The findings of Sheppard and Bishop (10) and of Parry and Abramovich (11) of a metabolically active arterial endothelium with closed junctions, a thin fragmented basal lamina, and sparse elastic tissue in the intima are very similar to the observations in the nonsmoking controls of the present study. No reports of ultrastructural pathological changes in the umbilical vessels can be found in the literature.

Pathological intimal changes identical to those seen in the specimens of the umbilical arteries from smoking mothers have previously been reported from our laboratory in rabbits exposed for 2 weeks to a moderate degree of carbon monoxide or arterial hypoxia (7, 8). An edematous reaction of the endothelium has also been described by other authors following exposure to severe grades of hypoxia (12-14) and to various compounds such as catecholamines, angiotensin, endotoxin, and certain amines (15, 16). Therefore, it seems reasonable to assume that edematous swelling or blebbing and contraction with subsequent opening of the endothelial junctions is a characteristic reaction of the endothelium to injury.

The oxygen saturation of fetal hemoglobin in the umbilical vein is about 70%; in the umbilical artery...
ULTRASTRUCTURE OF UMBILICAL ARTERIES IN SMOKERS

it is about 26% (17). Fetal carboxyhemoglobin saturations due to maternal smoking vary between 2.5% and 10% depending on the degree of inhalation of the mother (18, 19). On the average, fetal carboxyhemoglobin saturations are about 1.8 times higher than the corresponding maternal values and are in agreement with the values calculated according to Haldane's original equation with due regard to the fetal and maternal blood oxygen tensions.

Due to the low fetal oxygen saturation, the presence of carboxyhemoglobin has a more serious effect on fetal blood oxygen transport than it does on maternal blood oxygen transport. For example, a maternal carboxyhemoglobin level of 6%, corresponding to a fetal carboxyhemoglobin level of about 11%, will reduce fetal blood oxygen transport approximately 15% and therefore significantly interfere with fetal tissue oxygenation.

In the wall of the umbilical artery, the competition between carbon monoxide and oxygen due to the low oxygen tensions in the blood will be more pronounced than it is in the wall of the maternal arteries and may subsequently induce greater ultrastructural changes. Although the intra-arterial pressure is only 50 mm Hg in the human umbilical artery, the ultrastructural findings reported in this study show striking similarities to those reported in the aorta and the coronary arteries of animals after light in vivo exposure to carbon monoxide for 2 weeks (6) or perfusion for 40 seconds with a solution of 100 g/ml of nicotine in buffer (20). Endothelial swelling and degeneration along with opening of endothelial cell junctions and formation of a large edema in the subendothelial space were characteristic features in these two animal studies. However, the pronounced dilation of the endoplasmic reticulum and the wide basement membrane reported in the present study were not observed in the animal experiments; these morphological dissimilarities may reflect differences in metabolism and adaptation in fetal and adult endothelium that render the fetal tissue more sensitive to this type of hypoxia. With these distinctions in mind, the observation that practically no overlapping of the pathological findings was present in this study suggests that the umbilical artery may prove to be a useful model of the vascular injury produced not only by tobacco smoking in humans but also by other agents toxic to the vascular system.

In our opinion, the results of this study support the concept that tobacco smoking is harmful to the vascular endothelium. These findings also contribute to an understanding of the mechanism through which vascular injury is provoked in heavy smokers.
Endothelial cell from an umbilical artery from a child of a smoking mother (60 cigarettes/day). Note the swelling of the cell with formation of luminal blebs and the dilation of the endoplasmic reticulum. Beneath the nucleus is a wheellike lysosome. L = lumen, B = cytoplasmic process forming a surface bleb, ER = endoplasmic reticulum, Ly = lysosome, and IJ = intercellular junction. Bar indicates 1 \( \mu m \).
Intima of an umbilical artery from a child of a smoking mother (20 cigarettes/day). Photomontage showing two endothelial cells with focal loss of closed intercellular junctions. Note the wide, continuous basement membrane. L = lumen, E = endothelial cell, IJ = intercellular junction, BM = basement membrane, F = filaments, G = Golgi apparatus, Ly = lysosome, ER = endoplasmic reticulum, and M = mitochondrion. Bar indicates 1 μm.
Intima of an umbilical artery from a child of a smoking mother (25 cigarettes/day). Note the edematous subendothelial space containing smooth muscle cells and the wide basement membrane. L = lumen, E = endothelium, BM = basement membrane, SES = subendothelial space, and SM = smooth muscle cells. Bar indicates 5 μm.
Intima of an umbilical artery from a child of a smoking mother (25 cigarettes/day). Note blebbing of the cell surface and the wide basement membrane. L = lumen, E = endothelium, B = cytoplasmic bleb, M = mitochondrion with condensation of the matrix, BM = basement membrane, C = collagen fibers, and SM = smooth muscle cell. Bar indicates 1 μm.
TABLE 2

Morphological Changes

<table>
<thead>
<tr>
<th>Cigarettes/day</th>
<th>Continuous basement membrane</th>
<th>Basement membrane average width (μm)</th>
<th>Edema</th>
<th>Opening of junctions</th>
<th>Pathological myocytes</th>
<th>Dilation of endoplasmic reticulum</th>
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</thead>
<tbody>
<tr>
<td>0</td>
<td>-</td>
<td>0.1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>(+)</td>
<td>0.6</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
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<td>0.8</td>
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<tr>
<td>20</td>
<td>+</td>
<td>0.9</td>
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</tr>
<tr>
<td>20</td>
<td>+</td>
<td>1.0</td>
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<tr>
<td>20</td>
<td>+</td>
<td>1.3</td>
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<td>+</td>
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<tr>
<td>20</td>
<td>+</td>
<td>1.5</td>
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<td>+</td>
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<td>+</td>
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<tr>
<td>20</td>
<td>+</td>
<td>1.7</td>
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<td>+</td>
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<td>+</td>
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<td>20</td>
<td>+</td>
<td>2.2</td>
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<td>+</td>
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<td>+</td>
</tr>
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<td>2.5</td>
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</tr>
<tr>
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<td>+</td>
<td>3.0</td>
<td>+++</td>
<td>+</td>
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</tr>
</tbody>
</table>

The morphological changes were graded on a scale from + to +++. The most marked difference between the smokers and the nonsmokers was the reparative changes in the basement membrane, which exhibited a considerable widening, and the formation of a continuous membrane. (+) implies that only short patches of continuous basement membrane were seen in the artery. Edema includes both intimal and subintimal edema. The degree of opening of the junctions refers to the focal loss of tight intercellular junctions. The changes in the smooth muscle cells were classified according to the degree of vacuolization, ameboid polymorphism, and "woolly" outer surface. The dilation of the endoplasmic reticulum due to an electron-dense homogeneous mass was only pronounced in the one smoker who consumed 60 cigarettes/day.

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