THE DEVELOPMENTAL AND AGING PROCESSES OF SCHLEMM’S CANALS

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Received for publication June 15, 1979

Schlemm’s canal (Sinus venous sclerae) of human fetuses and postnatal monkeys (Macaca fuscata) were observed with the light and electron microscope.

Between the 21st and 24th week of gestation of the fetuses, many branches of Schlemm’s vascular plexus at the sclerocorneal junction, which is immature form of Schlemm’s canal, were connected to each other by young fibroblasts invading from the surrounding connective tissue. Our observations indicate that the main pathway of aqueous humor across endothelium of the canal is converted from “intercellular” into “intracellular” (transported by vacuoles and pores) in these stages.

In the postnatal monkeys, large vacuoles were observed in the differentiating endothelium at the inner wall of the canal. Our electron micrographs indicate that these vacuoles are formed by a successive fusion of adjacent pinocytotic vesicles in the endothelial cells and take an important role for aqueous humor transport.

INTRODUCTION

Aqueous humor in the anterior chamber flows into Schlemm’s canal through the trabecular meshwork, and the disturbance of the physiological passage by various causes induces an abnormal increase of the intraocular pressure.

Many morphological investigations concerning this pathway have been made by transmission and scanning electron microscopic levels. Tripathi (1971) and Inomata et al. (1972) described the existence of so-called “giant vacuoles” in the endothelial cells lining at the inner wall of Schlemm’s canal (the wall facing on the trabecular meshwork) and proposed them as the transcellular vacuolar channels. They insisted that intracellular vacuoles, the first originated from invaginations of the plasma membrane, are expanded to giant vacuoles across the cells by an aberrant inflow of the fluid. Thus, free passage of aqueous humor through such intracellular open channels can be possible. On the other hand, Wulle (1968, 1972) observed the developmental process of Schlemm’s canal and the trabecular meshwork of human fetuses with the special reference to differentiation processes of the endothelial lining of the canal.

This paper mainly deals with the differentiation process of Schlemm’s canals of human fetuses especially in ages between the 21st and 24th week of gestation when aqueous humor begins to flow into the canal (Wulle, 1967, 1972), and also with that of the postnatal monkeys. Although the present observations had no fundamental in-
consistencies with the previous papers, a few additional findings especially on the formation process of the canal were obtained. Furthermore, a new hypothesis as to the origin of "giant vacuoles" is proposed.

MATERIALS AND METHODS

Five human fetuses ranging in ages from 21 weeks to 24 weeks of gestation, and 7 Japanese monkeys (Macaca fuscata) ranging in ages from 6 months to 13 years old were used for the present observations.

The materials were perfused from the left ventricle by 2% glutaraldehyde-2.5% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.2-7.4). After the perfusion, the eyes were excised and cut into pieces at the equator. The anterior parts were fixed in a ice cold 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2-7.4) and the regions of Fontana space at the iridocorneal angle were dissected into several pieces in the fixative.

The specimens were refixed in a ice cold 2% osmium tetroxide in the buffer for one hour, dehydrated in graded concentrations of acetone, and then devided into two groups, i. e. specimens for the transmission electron microscopy (TEM) and those for the scanning electron microscopy (SEM).

After dehydration, the specimens for TEM were embedded in epoxy resins. The thin sections were made by a Porter-Blum Ultramicrotome, stained by uranyl acetate and lead hydroxide, and observed either with a Hitachi H-500 or a HU-12AS type electron microscope.

The specimens for SEM were dried by a critical point method using carbon dioxide. They were coated with gold by an ion sputter coating method and observed with Hitachi HFS-2 field emission scanning electron microscope.

RESULTS

In the light microscopy of the thick sections stained with toluidine blue, branches of Schlemm's vascular plexus have already been seen near the sclerocorneal junction at the 21st week of gestation, and the tips of adjacent branches appear to join to each other (Fig. 1). With TEM observations, these branches of the vascular plexus mainly consist of immature endothelial cells with a large nucleus. Each endothelial cell is rather round in shape, and the connection between the adjacent cells has not been well-developed. Many young fibroblasts exist in a very closed apposition to the vessels and produce abundant collagen fibers in the surrounding connective tissue. Some young fibroblasts invade into the plexus and are in contact with the vessels or to each other. These cells appear quite immatured in form and retain many mesenchymal features (Fig. 2).

After the 21st week of gestation, the contact becomes evident between the endothelial cells of the vascular plexus and these young fibroblasts. Thus, adjacent branches of the vascular plexus are gradually connected to each other by such a frequent insertion of young fibroblasts (Fig. 3).

In the 24th week of gestation, Schlemm's canal with a wide lumen has been differentiated by fusion of branches of the vascular plexus, and the endothelial cells at the inner and outer wall of the canal become thinner. It is thought to be interesting that large vacuoles can be seen in the endothelial cells of the canal (Fig. 4).

Fig. 5 shows Schlemm's canal in such a region that the connection between
THE OBSERVATIONS OF SCHLEM'M'S CANAL

adjacent branches of the vascular plexus has been established after the process as shown in Fig. 2 and 3. In such stages, general views of the endothelium are almost the same between the inner and outer wall of the canal. However, the cytoplasm of the endothelial cells contains more numerous pinocytotic vesicles and vacuoles. In some regions of the endothelium, the cell cytoplasm becomes very slender and is fenestrated by pores.

In the 24th week of gestation, some endothelial vacuoles at the inner wall of the canal becomes enlarged in size and increased in number. Between the neighboring endothelial cells, the intercellular spaces are decreased in size and conjugated to each other with electron dense attachments like zonulae adherentes (Fig. 6).

In the observations of the aging process of Schlemm's canal of the postnatal monkeys, no fundamental differences were apparent between the younger and older specimens. However, the present SEM observations at the inner wall of the canal of younger monkey (7 months old, weight ; 1.06 kg.) revealed several interesting findings : spindle shaped projections of the endothelial lining bulging into the lumen can be seen (Fig. 7 and 8). They sometimes end as a long slender tail. The endothelial surface including the projections is opened by pores with varying sizes. Furthermore, the endothelial microvilli are distributed only at the inner wall of the canal. With higher magnified figures by SEM, these microvilli can be classified into two groups, i. e. long slender and short stubbed ones (Fig. 8).

By TEM observations of the specimens obtained from the same monkey, it was confirmed that the endothelial vacuoles at the inner wall of the canal are originated and enlarged by a successive fusion of pinocytotic vesicles (Fig. 9). Some endothelial microvilli form a loop and appear to make intracellular vacuoles by a fusion between the tip and the main cell surface (Fig. 10).

DISCUSSION

It has been observed that Schlemm's canal is the first originated as small venous canaliculi near the sclerocorneal junction about at the end of the 3rd month of gestation (Barber, 1955; Badtke, 1958; Duke-Elder and Cook, 1963; Mann, 1964). In the 5th month, these canaliculi are anastomosed to each other and form the circumferential canal consisting of one or more channels (Badtke, 1958). Wulle (1968, 1972) called these canaliculi as "branches of Schlemm's vascular plexus" and using the human fetuses of 4th, 5th, 6th and 8th month of gestation, observed the developmental process of the canal especially of the morphological changes of the endothelial cell structures under the electron microscope. However, no detailed descriptions have been made on the maturation and enlargemental process of the canal by the previous workers.

On the other hand, according to the previous reports from our laboratory (Mohri, 1974; Yoshitomi, 1977; Saiki, 1978; Yoshizuka, 1979; Yoshizuka et al., 1979), young fibroblasts in mesenchymal origin play a principle role for the neovascularization. These papers have supported a consideration that young fibroblasts transform to vessel forming cells. This is also true for the present observation. Our electron micrographs also reveal that the invasion of abundant young fibroblasts from the surrounding connective tissue, their increase in number by mitotic diversions, and
their incorporation to the pre-existing vascular plexus are the essential to the enlargement and extension of the canal. Thus, between the 21st and 24th week of gestation, branches of Schlemm’s vascular plexus have been connected to each other by young fibroblasts which will further differentiate to immatured endothelial cells.

In the intercellular region of the endothelium, the junctions have not been developed at the 21st week yet, but are observed at the 24th week, and pores can be identified in the endothelial cells at the 24th week. Furthermore, endothelial vacuoles at the inner wall of the canal have been enlarged in size and increased in number at this stage. Thus, our observations indicate that the fenestration of the endothelial cells occur in earlier stages than in those determined by Wulle (1968, 1972). Based on the findings, we concluded that the main pathway of aqueous humor across the endothelium of Schlemm’s canal is changed from “intercellular” into “intracellular” (transport across the cell by large vacuoles or pores) around the 24th week of gestation.

In the observation of the aging process of Schlemm’s canal in the postnatal monkeys, the existence of endothelial microvilli was observed exclusively in the younger specimens (7 months old). It was also confirmed by TEM that large vacuoles in the endothelial cells at the inner wall of the canal are formed by a successive fusion of pinocytotic vesicles.

The pathway of aqueous humor between the intertrabecular spaces and Schlemm’s canal has been studied by several workers. Holmberg (1965) suggested that the fluid in the intertrabecular spaces is the first transported into so-called giant vacuoles of the endothelial cells at the inner wall of the canal, and then flows into the lumen. Based on their electron microscopy, Inomata et al. (1972) also insisted that “giant vacuoles” perforating the endothelial cells lining the inner wall of the canal are initiated by invaginations in the basal part of the cell. According to their assumption, these giant vacuoles are produced when a large volume of the fluid inflows into these invaginations. The similar hypothesis which explain vacuolization of the endothelial cells was also proposed by Tripathi (1971). He suggested that the most important factor for the endothelial vacuolization is the onset of the pressure gradient invagination by the fluid inflow.

Basically, we argue for these previous assumptions. However, the fusion of pinocytotic vesicles observed in the postnatal monkey specimens is supposed to be a primary causative factor for the formation of the endothelial vacuoles. We also considered that such fusions do not depend on the pressure gradient of the fluid as suggested by Tripathi (1971). Small vacuoles are the first formed with repetitions of fusion between adjacent pinocytotic vesicles. Then, the expansion of these small vacuoles by the fluid inflow induces fenestrations of the thinner cytoplasmic portion in the endothelial cells. Thus, aqueous humor can be transported across the cell by such open channel system pointed out by Tripathi (1971) and Inomata et al. (1972).

The existence of endothelial microvilli has been reported by many investigators. Recently, Fujimoto et al. (1975) observed them on the endothelial lining of the developing large arteries. However, no descriptions of these structure have been made on Schlemm’s canal. More detailed observations are necessary to clarify whether they have a function for the cytoplasmic vacuoli-
zation or distribute with another function.

REFERENCES


Fig. 1. Branches of Schlemm's vascular plexus (SV) are connected to each other. Human fetuses in the 21st week of gestation. (×530).

Fig. 2. In the Juxtacanalicular areas, many young fibroblasts (YF) in mesenchymal origin are seen. Endothelial cells (EC) of the vessels are immatures, and the fibroblasts (FB) in a closed apposition to the vessels produce abundant collagen fibers (CO). Human fetuses in the 21st week of gestation. (×9,000).
**Fig. 3.** After the 21st of gestation, new junctions are formed between the endothelial cells and young fibroblasts (arrow). Human fetuses in the 21st week of gestation. (×15,000).

**Fig. 4.** Schlemm's canal (SC) in the 24th week of gestation. The structural differences of its inner wall (trabecular meshwork side) (IW) and outer wall (scleral side) (OW) become more apparent than in the previous stages. Human fetuses. (×520).
**Fig. 5.** The connection between the adjacent vessels has been completed. Vacuoles (V) and pores (P) can be seen in the endothelial cells, and pinocytotic vesicles (PV) have increased in number. Human fetuses in the 24th week of gestation. (×12,000)

**Fig. 6.** In the junctional areas of the endothelium at the inner wall of the canal, the neighboring cells are conjugated by electron dense attachments and vacuoles are also observed. Abundant collagen fibers and elastic fibers (EL) exist in the trabecular meshwork. Human fetuses in the 24th week of gestation. (×10,000).
Fig. 7. The inner wall of Schlemm’s canal under the scanning electron microscope. The endothelial projection (EP) and endothelial microvilli are observed. 7 months old monkey. (×2,000).

Fig. 8. The higher magnified figure reveals that these microvilli can be classified into long slender (LMV) and short stubbed ones (SMV). 7 months old monkey. (×23,000).
Fig. 9. The formation of large vacuoles by fusion of pinocytic vesicles is illustrated in an endothelial cell at the inner wall (arrow). Endothelial microvilli (MV) are distributed. 7 months old monkey. (×34,000).

Fig. 10. Endothelial microvillus forms a loop (LO) in a similar manner to marginal fold. 7 months old monkey. (×85,000).