

HISTOLOGICAL CHARACTERISTICS OF HEALTHY ANIMAL PERITONEUM

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The aim of this study was to investigate the morphological and histological characteristics of healthy animal peritoneum, with special references to its microvasculature.

Biopsies of parietal peritoneum from the front abdominal wall were collected from 5 healthy rabbits (2 male and 3 female) and 8 persons (2 males and 6 females) mean age $52,50 \pm 5,18$ years. Semi-thin sections were fixed in Sorensen's phosphate buffer and stained with toluidin blue for light microscopy with Opton Photomikroskope III. Ultrathin sections for transmission electron microscopy were fixed in glutaraldehyde, postfixed in osmium tetroxyde, contrasted with uranyl acetate and viewed with EM Philips M208S transmission electron microscope.

Normal rabbit and human parietal peritoneum is composed of a sheet of flat mesothelial cells, separated by a continuous basement membrane of connective tissue containing collagen and elastin fibers, fibroblasts, mononuclear phagocytes, lymphocytes, adipose tissue, small blood vessels, lymphatic vessels and nerves. Only continuous capillaries were observed. Preponderance of euchromatin over heterochromatin was found in the nuclei of endothelial cells. Endothelial citoplasm shows prominent rough endoplasmic reticulum, well-developed Golgi apparatus, numerous mitochondria and ribosomes. Numerous pinocytotic vesicles were found free within the cytoplasm or forming transendothelial channels. The peritoneal structure is similar in humans, rabbits and other rodents making them suitable models for research on peritoneal morphology and physiology.

Key words: blood vessels, human, peritoneum, rabbit, rodent

INTRODUCTION

Until 1980 little research has been done on the morphology of the peritoneal membrane (Di Paolo and Sacchi 2000). In the past decades, during which

peritoneal dialysis (PD) has been increasingly utilized as a long-term treatment of end-stage renal failure, the interest in the structure and functioning principles of the peritoneal membrane increased significantly. Research during the past years provided enough evidence to characterize the peritoneum not just as an inert dialyzing sheet, but as a living and reusable membrane for dialysis (Gotloib and Shostak 1992).

The efficacy of peritoneal dialysis and its success depend on preserving the structural integrity and dialyzing capacity of the peritoneal membrane. Membrane structure and function can change in uremia (Trpinac *et al.*, 2002, Obradović *et al.*, 2000) and with time on PD (Stojimirović, Obradović *et al.*, 2001; Stojimirović, Trpinac *et al.*, 2001), resulting in decreased membrane performance and possible cessation of PD as a treatment modality. More recently, investigators have focused on changes within the peritoneal vascular bed, because it is presumed that changes in vessel density and morphologic features might directly affect membrane function (Honda *et al.*, 1999; Gotloib *et al.*, 2000; Williams *et al.*, 2002). To understand better the changes in the peritoneal membrane during PD, it is necessary to recognize the morphology and physiology of a healthy peritoneum.

So far, most researches of the peritoneum have been conducted on animal models of PD (Mortier *et al.*, 2005). To make adequate conclusions, it is therefore important to recognize the level of comparability between human and animal peritoneum.

The aim of this study was to investigate the morphology of healthy animal peritoneum, with special reference to its microvasculature.

MATERIAL AND METHODS

Subjects

Biopsies of parietal peritoneum from the front abdominal wall were taken from 5 healthy rabbits, 2 male and 3 female, and from 8 persons, 2 males and 6 females, mean age $52,50 \pm 5,18$ years, with no history of previous abdominal surgery. Tissue samples from humans were obtained during elective cholecystectomies, due to cholelithiasis, with no relevant peritoneal involvement. Detailed instructions were provided to the surgeon to ensure uniformity of sampling, in both animals and humans, and to minimize fixation-related or artifactual changes in the specimens. All biopsies were performed immediately following the opening of the abdominal cavity, in order to avoid tissue damage from exposure to air and surgical instruments. All persons included in the study gave written informed consent.

Light microscopy (LM)

Tissue samples for light microscopy were fixed in Sorensen's phosphate buffer, pH 7.4, for 24h at room temperature, and then washed and stored at 4°C before processing. Fixed samples were dehydrated in 96% ethanol and embedded in paraplast. Semi-thin sections were stained routinely with toluidin blue and viewed at 10x and 25x magnification with Opton Photomikroskop III.

Transmission electron microscopy (TEM)

The samples for TEM were fixed in 4% glutaraldehyde in Sorensen's buffer for 24h. For better preservation of membrane structures tanine acid was added to the fixative. Samples were then washed with Sorensen's buffer (3 times for at least 10 minutes), and then postfixed in 1% osmium tetroxyde for 1 hour and in 4% uranyl acetate overnight. After dehydration through graded ethanol mixtures, samples were embedded in Epon and contrasted with uranyl acetate and lead citrate (Hayat 1986). They were viewed with EM Philips M208S transmission electron microscope.

RESULTS

In the assessment of the biopsies attention was paid to the morphologic features of the mesothelial surface and the underlying interstitium, with special attention to blood vessels.

Normal rabbit and human parietal peritoneum is composed of a sheet of flat mesothelial cells, separated by a basement membrane from a thin compact zone of mature fibrous tissue containing collagen and scattered elastin fibres. Deep to this is loose connective tissue with widely spaced collagen fibres, occasional fibroblasts, scattered mononuclear phagocytes, lymphocytes and adipose tissue. In this zone of loose connective tissue small blood vessels, lymphatic vessels and nerves were present. These structures in humans actually represent elements of the peritoneal barrier through which PD is performed: stagnant fluid films in the peritoneal cavity, the mesothelium, the peritoneal interstitium, basement membrane of the capillary endothelium, endothelium and stagnant fluid films in the peritoneal capillary (Flessner, 2005).

Under light microscope mesothelial cells appear flat with a centrally positioned nucleus. TEM shows that the mesothelial cells in the rabbit and human peritoneum are polygonal, often elongated and have numerous microvilli of various lengths on their luminal surface. Mesothelial cell cytoplasm contains numerous oval or roundish pinocytotic vesicles, mitochondria, and rough endoplasmic reticulum. We also observed lamellar bodies, with concentric electron-dense lamellae, within the cytoplasm of mesothelial cells, on their apical surface (Figure 1), and even in the lamina propria. The mesothelial basement membrane is continuous, single and clearly visible in both rabbit and human peritoneum (Figure 1, 2).

The submesothelial lamina propria contains fibroblasts, adipocytes, macrophages, mast cells and lymphocytes. The connective matrix is composed mainly of collagen and elastic fibres (Figure 2).

Blood vessels in rabbit and human peritoneum are mainly true capillaries of the continuous type and postcapillary venules. In the observed capillaries, the vessels' wall is made of a single layer of endothelial cells on a continuous, single basal membrane. In postcapillary venules, besides endothelial cells, pericytes are also present in the vessels' wall (Figures 3, 4). Endothelial cells are elongated, with large, centrally positioned nucleus. Preponderance of euchromatin over

heterochromatin is present in endothelial cells nuclei in both rabbits and humans (Figures 3, 4). Endothelial cells cytoplasm contains the usual organelles characteristic for metabolically active cells: mitochondria, Golgi apparatus, prominent rough endoplasmic reticulum. We also observed numerous round or oval pinocytotic vesicles adhering to the abluminal surface, free in the cytoplasm or adhering to the luminal surface. Vesicles adhering to the surface of endothelial cells increase the cell's exchange area. Some vesicles were seen to flow together, possibly forming channels that pass from one side of the cell to the other, creating a communication between the blood vessel's lumen and the connective tissue of peritoneal lamina propria.

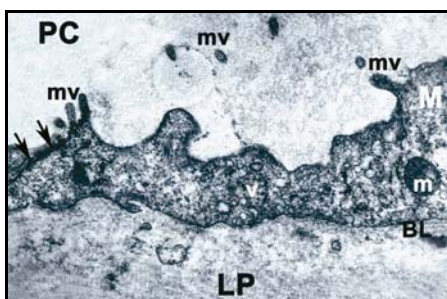


Figure 1. Mesothelium of healthy rabbit peritoneum: PC - peritoneal cavity, M - mesothelial cell, mv - microvilli, ↑ - intercellular junction, BL - basal lamina, LP - lamina propria (TEM)

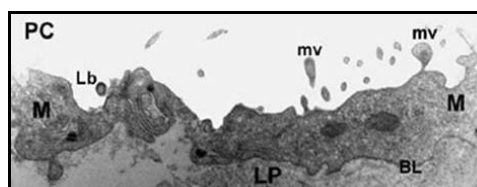


Figure 2. Mesothelium of healthy human peritoneum: PC - peritoneal cavity, Lb - lamellar body, M - mesothelial cell, mv - microvilli, BL - basal lamina, LP - lamina propria (TEM)

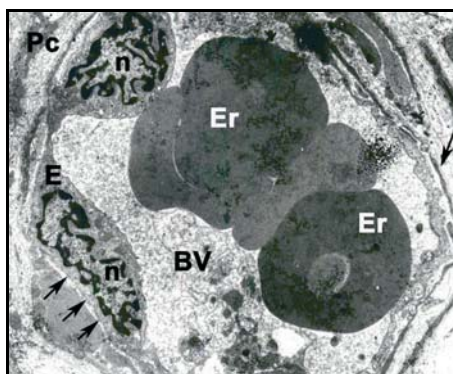


Figure 3. Venous capillary in healthy rabbit peritoneum: Er - red blood cell; BV - blood vessel, E - endothelial cells, n - nucleus, ↑ - basement membrane, Pc - pericyte (TEM)

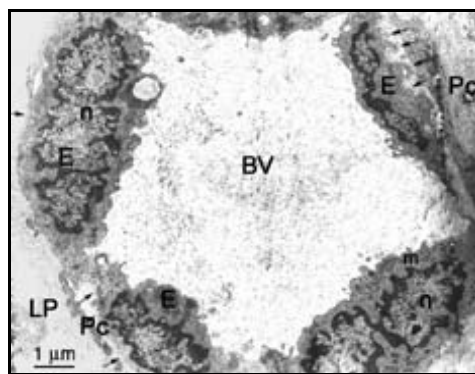


Figure 4. Venous capillary in healthy human peritoneum: BV - blood vessel, E - endothelial cells, n - nucleus, m - mitochondria, ↑ - basement membrane, Pc - pericyte (TEM)

DISCUSSION

The major limitation in the collection of peritoneal biopsy samples has been access to the peritoneum. Due to ethical reasons there is a quite limited number of studies dealing with healthy human peritoneum (Williams *et al.*, 2002). Most research on the healthy peritoneum has been done on animals, mainly rats and rabbits. This is one of the rare studies that includes human material.

The scarcity of literature on peritoneal morphology in humans is compensated by the relatively abundant literature dealing with this subject in animals.

The overall structural organization of peritoneal membrane is similar in rodents and humans (Gotloib *et al.*, 1983; Garosi and di Paolo, 2001). By light microscopy, we observed that human peritoneum consists of a single layer of mesothelial cells over a continuous basement membrane. The basement membrane in the parietal peritoneum overlies loose connective tissue consisting of fibroblasts, collagen fibres, adipocytes, leukocytes and an abundant supply of microvessels and lymphatics. A similar structure was observed in the rabbit peritoneum in our study and it is in concordance with literature data concerning rodent, rat and rabbit, peritoneum (Gotloib *et al.*, 1983; Khanna and Nolph, 1986).

The mesothelial layer shows little variation between species. Mesothelial cells show the same shape and ultrastructural organization in rodents and humans, even in the dimensions of microvilli (Gotloib *et al.*, 1983, Gotloib *et al.*, 2000, Obradović *et al.*, 2001). Microvilli can increase the peritoneal surface area by 40-fold. According to some reports, these microvilli have specific transport functions serving to increase the surface area where exchange in the process of PD occurs. Others maintain that their function is primarily mechanical, providing protection against friction between organs (Fang *et al.*, 2004). Numerous vesicles were found present in rat and rabbit mesothelial cells, where they are believed to contribute to transmesothelial transport between the peritoneal cavity and the connective tissue that supports the mesothelium. The same vesicles were found in mesothelial cells of human peritoneum in our study. The submesothelial basement membrane in healthy mice and rabbits is homogenous, one-layered and continuous, as is in humans (Gotloib *et al.*, 1983).

Lamellar bodies which we observed in the cytoplasm of mesothelial cells, on their apical surface and in the lamina propria, are structures characteristic for serous membranes, their function being to alleviate friction between visceral and parietal peritoneal sheets (Stojimirović *et al.*, 2002).

Some authors showed that 1,7% of the capillaries in rabbit and human diaphragmal peritoneum are fenestrated (Gotloib *et al.*, 1989). We, however, observed only continuous capillaries on our specimen of healthy rabbit and human parietal peritoneum from the front abdominal wall. Endothelial cells forming the blood vessels wall have same ultrastructure in rodents and humans (Gotloib *et al.*, 2000). They are highly active structures, serving not only as a permeability barrier and an effective thromboresistant surface, but also as the location of important synthetic and other metabolic activities (Gotloib *et al.*, 2000). Numerous plasmalemmal vesicles that were observed on our samples from

rabbits and humans are also reported in mice and rats. In the mouse diaphragm, true capillaries show 900 vesicles/ μm^2 , venular segments of capillaries 1200 vesicles/ μm^2 and postcapillary venules 600 vesicles/ μm^2 (Simionescu *et al.*, 1979). Although there are still no data on this subject for humans, such observation points at their possible role in exchange of substances between blood and connective tissue of peritoneum, which is especially important in the process of PD.

CONCLUSIONS

Histological structure of rabbit and human peritoneum is similar, allowing conclusions made based on research on animal peritoneum to be applied in humans.

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HISTOLOŠKE KARAKTERISTIKE PERITONEUMA ZDRAVIH ŽIVOTINJA

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SADRŽAJ

Cilj ovog istraživanja bio je da se ispituju osnovne morfološke i histološke karakteristike zdravog životinjskog peritoneuma, sa posebnim osvrtom na osobine njegove mikrovaskularne mreže.

Ispitani su uzorci tkiva peritoneuma 5 kunića (2 mužjaka i 3 ženke) i 8 osoba, 2 muškarca i 6 žena, srednjeg starosnog doba $52,50 \pm 5,18$ godina. Polutanki isečci tkiva fiksirani su u Sorensenovom puferu i bojeni toluidin plavim za svetlosnu mikroskopiju (Opton Photomikroskop III). Tanki isečci za transmisionu elektronsku mikroskopiju (EM Philips M208S) fiksirani su u glutaraldehidu, postfiksirani osmium tetroksidom i kontrastirani uranil acetatom.

Normalni parijetalni peritoneum kod kunića i ljudi čini sloj pljosnatih mezotelnih ćelija koji je kontinuiranom bazalnom membranom odvojen od vezivnog tkiva sa kolagenim i elastičnim vlaknima, fibroblastima, mononuklearnim fagocitima, limfocitima, masnim tkivom, malim krvnim sudovima, limfnim sudovima i nervnim vlaknima. Zapaženi su kontinuirani kapilari i postkapilarne venule. U je-

drima endotelnih ćelija krvnih sudova dominira euhromatin, a u njihovoj citoplazmi zapaženi su granulirani endoplazmatski retikulum, dobro razvijen Goldžijev kompleks, brojne mitohondrije i ribozomi. Mnogobrojne vezikule nalaze se slobodne u citoplazmi endotelnih ćelija, ili se organizuju u transćelijske kanale. Sličnost strukture peritoneuma kod glodara (kunića i pacova) i ljudi čini ove životinje adekvatnim modelima za proučavanje morfologije i fiziologije peritoneuma.