The vascular corrosion casting (VCC) and scanning electron microscopy study on changes of vascular networks arrangement in the organs undergoing cyclic volume changes

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The modern research of the venous and arterial plexuses and networks based primarily on the technique of vascular corrosion casts. This specific technique allows to produce a very precise replica of the vascular beds and provides much details of the three-dimensional arrangement of blood vessels in normal or pathological tissues in any organ, both parenchymatous as well as tubular.

In our study the VCC technique has been used to observe changes in the arrangement of the blood vessels in the wall of the gallbladder and urinary bladder in pig, which undergoes a variable change in the volume during their cyclical filling of urine or bile. The changes in the angioarchitecture of the gallbladder and urinary bladder has been describe in a few species of laboratory animals, but the studies mostly concentrate on description of spatial microvascularisation in particular layers.

In the research in domestic pig was conducted the detailed analysis of the arterial and venous systems in the wall of the gallbladder and urinary bladder with particular emphasis on the structure of the blood vessels which undergo in the wall of the organs cyclically stretching and folding. The results show that both the vascular superficial and mucosal networks and the supplying and draining vessels, have features that allow to efficient change their arrangements i.e., the dense subepithelial vascular networks, that assume the shape of mucosal folds; the vascular loops lying under the epithelium; the looping arteries and veins in the lamina propria of mucosa; the blood vessels running at a slant across the muscle and the mucosa layer; the undulating course of the superficial and subserosal arteries and veins.

Keywords: vascular corrosion casting; SEM; gallbladder; urinary bladder

1. Introduction

The vascular corrosion cast is the most adequate and effective technique to examine the angioarchitecture of normal and pathological tissues. This technique involves the creation of replica of vascular bed and associated with scanning electron microscopy (SEM) provides details of the three-dimensional arrangement of the blood vessels in various tissues [5] [19]. The vascular microcorrosion casts precisely reflect the course and connections of blood vessels and also allow to observe arrangement of arterial networks and venous plexuses. What more vascular cast are so detailed that they allow to observe the microanchoarchitecture of capillaries that create the terminal ways of functional and nourishing blood circulation. The surfaces of the corrosion casts are perfect replicas of the internal structure of blood vessels wall and observed in SEM allow the identification of the type of blood vessel based on the shape and orientation of endothelium nuclei and also allow to locate, among others, venous valves.

The method of producing casts from vessels lumen has been known since Leonardo da Vinci made the first vascular casts injected by wax in the 16th century [17] [18]. During the next centuries the method was significantly improved mainly by using the more effective casting media. Before the 20th century the scientists used variety casting media e.g. low melting point metal alloy, cellodin dissolved in organic solvents or celluloid [12] [18]. This injection media allowed to produce the replica of large blood vessels but were not suitable for filling small blood vessels and capillaries networks. The breakthrough came in the twenties of the 20th century when Hinman suggested that lowering the viscosity of the casting medium cause the filling even very small blood vessels [18]. The initiation of methods of obtaining vascular casts similar to that used today was in 1950s when scientists started to use synthesized resins to fill the blood vessels [12]. Since 1971 the semi-polymerized methyl methacrylate resin is commonly used in microcorrosion vascular cast technique because of its proper physicochemical properties, like low viscosity or rapid polymerization [15] [16].

In the research the methyl methacrylate resin (MERCOX) was used to fill blood vessels in pig gallbladder and urinary bladder. The common feature of both organs is the ability to change the volume, which is possible by the plasticity of the components that build the wall of these organs. Both organs have an extensive vascular system consisting of mucosal and superficial venous plexuses and arterial networks and also subepithelial and subserosal capillaries networks. So far, the knowledge of the gallbladder and urinary bladder microvasculature comes from the studies in man, and a very few species of carnivores and laboratory animals [1] [4] [6] [7] [9] [11] [14]. There are some descriptions of ways of supplying these organs in blood, and also the angioarchitecture of their wall or the specific to
the vascular system characteristics resulting from the specification of species. In the literature there is little data on the variable arrangement of vascular networks depending on the degree of filling of both organs.

The aim of the study was to use the vascular corrosion cast technique to show the arrangement of vascular networks in the wall of gallbladder and urinary bladder and to characterize the blood vessels structural adaptations to the changes that occur in the gallbladder and urinary bladder during their cyclical filling up with urine and bile.

2. Vascular corrosion casting technique

2.1 Material
The study used gallbladders and urinary bladders collected after slaughter from five domestic pigs. The organs were immersed for transportation in saline. The main arteries of all organs were injected by a small volume of saline with heparin to prevent blood clots formations in perfused blood vessels.

2.2 Precasting treatment (preparation of tissues and casting medium)
As an injection medium in studies used the methyl-methakrylate Mercox resin (Dainippon) whose physico-chemical properties meet all the standards which have the media used for creating the microscopic castings of blood vessels. These properties include mainly the low viscosity of resin, allowing it the flow through the smallest capillaries, as well as its rapid curing, a small degree of contract and resistance to corrosive substances and the influence of electrons in a vacuum environment in the SEM. Prior to injection, in each organ were prepared main supplying artery which was cannulated. Immediately before injection a cannula was connected to a syringe filled with a synthetic resin containing a polymerization catalyst.

2.3 Resin injection and polymerization
Methakrylate resin injection into the vascular system of gallbladders and urinary bladders were made manually using syringes with a capacity of 20 ml. The resin injected under steady pressure and filled all the vascular bed. The total time of injection did not exceed 15 minutes. The injected organs allowed to stand for about 30 min in room temperature and then placed in a water bath at 60° C for about 20 hours. High temperature enabled to complete the process of polymerization of the resin in the blood vessels. To soften and loosen tissues surrounding the vascular castings, the water bath was added a small amount of detergent.

2.4 Tissue maceration and vascular casts cleaning, freezing and drying
After polymerization of resin in blood vessels and after the pre-maceration in detergent solution, the gallbladders and urinary bladders were immersed in 15% sodium hydroxide solution to get rid of the soft tissues surrounding the vascular casts. Preparations immersed in the NaOH were kept in an incubator at 60 ° C. After about 24 hours there was a first exchange of sodium hydroxide and after 4 days the samples were placed in a lower concentration of hydroxide (about 10%). The total time of tissue maceration was about 7-8 days. Then vascular casts were washed under the slow flow of running water, what allowed rinsing of sodium hydroxide, without damaging the structure of the preparations. Several days rinsing in water with detergent also helped to get rid of the deposits, which were created during the maceration due to the reaction of lipids with sodium hydroxide. After the process of purification of preparations, the castings were washed in distilled water, and then placed in plastic containers and frozen in water. For drying preparations in ice used lyophilizer CHRIST ALPHA 1-2 LD.

2.5 Corrosion casts preparation and SEM observations
Vascular casts of whole organs divided into smaller fragments, of which cut samples with a diameter of not more than 3cm. The final preparation of vascular casts to SEM observation consisted in making preparations capable of electrons conduction. For this purpose vascular casts were covered with a layer of gold of 30 nm using a coater Edwards S150B Sputter Coater. The samples then were mounted on aluminum plaques using a carbon tape. Vascular casts at the edge of the preparations combined with the surface of plaques by copper strands and colloidal silver. After mounting the samples preparations were re-covered with a layer of gold. The observations of microcorrosion vascular casts carried out in a scanning electron microscope Zeiss LEO 435VP under high vacuum (<6x10-4PA). Vascular corrosion casts of gallbladders and urinary bladders were mounted on aluminum plaques in such a way as to permit observation of both the course of large superficial blood vessels and architecture of mucosal and subserosal arterial networks and venous plexuses.
3. Results

3.1 Angioarchitecture of the wall of pig gallbladder and urinary bladder

The wall of pig gallbladder and urinary bladder consists of three basic layers: mucosa, muscular membrane and subserosa or adventitia in urinary bladder (Fig. 2A; 3A). The vascular system in both organs is quite similar and consists of two complex vascular networks, the first of which is located in the mucous layer and the second in the subserosal layer (Fig. 2A; 3A).

SEM observations of the three-dimensional vascular corrosion casts showed that the subserosal arterial network in gallbladder consists of the superficial branches of main artery which gives arterioles that divide into capillaries of the subserosal capillary network. Some of the superficial arteries change their course and penetrate deep into the wall of the gallbladder and reach the mucosa. The mucosal arterioles divide into well-developed subepithelial capillary bed (Fig. 2B). In pig urinary bladder arterioles of the subserosal arterial network pierce the muscular layer and reach the lower part of lamina propria of the mucosa, where they give off perpendicular branches in similar intervals running to mucosal folds (Fig. 3B). Subepithelially the terminal arterioles create the dense capillary network (Fig. 3B). In both organs the arrangement of the subepithelial capillary networks corresponds to the structure of the lamina propria of the mucosa, which may be folded or stretched.

Veins in pig gallbladder and urinary bladder may run independently or create the vascular pairs or vascular triads with arteries (Fig. 2C). In gallbladder the functional blood from the lamina propria of the mucosa is drained first by the perpendicular collecting venules and then by the horizontal veins of the mucosal venous plexus (Fig. 2B). These veins change the course and as the venous trunks pass obliquely through the muscular layer. In the subserosa the blood vessels create the second venous plexus that drained blood from the muscular and subserosal layer (Fig. 2C). In urinary bladder blood from the mucosal folds is drained by the small perpendicular venules that in the lower part of lamina propria connect with veins of the mucosal venous plexus (Fig. 3B). These veins pass obliquely through the thick muscular layer and on the surface of the urinary connect with veins of subserosal venous plexus.
3.2 Changes of vascular networks arrangement in pig gallbladder and urinary bladder

The SEM observations of the vascular microcorrosion casts showed that the vascular systems in the pig gallbladder and urinary bladder are characterized by varied features undergoing cyclic volume changes, that enable the single blood vessels and whole vascular network in particular layers varied course and arrangement.

3.2.1 Variable course of superficial and subserosal blood vessels

SEM observations on pig gallbladders and urinary bladders showed that the superficial and subserosal blood vessels (Fig.4A) may have variable course which depends on the degree of filling the organs with bile and urine. In fully filed organs with a thin wall the superficial arteries and veins have usually arcuate course, and in the organs partially or totally deprived of urine or bile the blood vessels change the course and became undulating (Fig.4C) or spiral (Fig.4B).
Fig. 4  Vascular cast of superficial blood vessels. A: vascular cast of pig urinary bladder. B: vascular corrosion cast of pig urinary bladder (SEM). C: vascular corrosion cast of pig gallbladder (SEM). SV- superficial vein; SA- superficial artery; yellow arrow show superficial blood vessels; green arrow show subserosal blood vessels.

3.2.2 Alternating arrangement of subepithelial capillary networks

In pig urinary bladder and gallbladder was observed the changeable arrangement of subepithelial capillary networks, dependent on shape of the mucosa. In shrunken organs the lamina propria of the mucosa create high and complex folds, in which the subepithelial capillary networks form vascular constructions corresponding to the structure of mucosal folds (Fig.6A; 6B). The subepithelial capillary networks along with the lamina propria of the mucosa folds and stretch.

Fig. 5  Vascular corrosion casts of subepithelial capillary networks. A: vascular corrosion cast of subepithelial capillary networks in pig urinary bladder (SEM). B: vascular corrosion cast of subepithelial capillary networks pig gallbladder (SEM). Mf- mucosal fold.

3.2.3 Vascular loops in the mucosa

The corrosion cast of shrunk urinary bladder revealed the presence of vascular loops in lower parts of lamina propria mucosae (Fig. 5A). The mucosal vascular loops consist of tightly convoluted arterioles and venules which pass in the short perpendicular blood vessels running to the mucosal folds (Fig. 5B). The vascular loops were not observed in pig gallbladder.
4. Discussion

In the organs of variable volume as gallbladder and urinary bladder, the tissues of these organs’ wall are characterized by a variable arrangement. Variable, internal structure of gallbladder and urinary bladder is closely linked with function performed by these organs, involving the accumulation of urine draining from the ureters or bile flowing from the liver. During storage of substances the wall of organs is stretched, and with the outflow of urine via the urethra and the outflow of bile through the bile duct, it shrinks causing apparent organs volume reduction. It should be noted that, as in other animals in the pig thin-walled gallbladder is subject to more subtle than the urinary bladder shrinkage due to the slower and more prolonged outflow of bile.

The tissue elements prepared for the cyclic shrinking and stretching in urinary bladder and gallbladder wall are the mucous membrane, adapted to create a high and complex folds and muscle membrane of smooth muscle bands of elongated, circular or oblique course. In the gallbladder degree of stretch of the wall is limited and the maximum physiological stretching occurs when the mucosal folds almost completely disappear. Therefore the epithelium lining the mucosa is not suitable for stretching and in both shrunk and stretched organs it maintains a constant structure. Urinary bladder has a much higher than gallbladder the physiological possibilities of the wall stretching. In urinary bladder the mucosal folds stretch, as well as the overlying epithelium in which the superficial cells from the high and narrow pass in the low and broad cells.

Analyzing the angioarchitecture of the pig gallbladder and urinary bladder observed that the vascular system has features for adaptation to changes in these organs volume, which affect the varied course of the blood vessel, and the variable structure of the vascular networks in the organs being to varying degrees filled with urine or bile. In our study, three different types of variation in blood vessels arrangement were found. These varied vascular features observed in gallbladders and urinary bladders are: 1) spiral, undulating or arcuate course of the superficial and subserosal blood vessels; 2) presence or absence of vascular loops in the mucosa; 3) folded or stretched constructions of subepithelial capillary networks. All of these features were observed in the pig urinary bladder, while in the pig gallbladder the vascular loops of the mucosa were absent.

In both types of organs was observed a distinct tendency of the superficial and subserosal blood vessels to the formation of vascular spirals. Undulating and then spiral arrangement of arteries and veins occurs during the shrinking the gallbladder and the urinary bladder wall. In the organs of filling up with the urine and bile arteries and veins tend to spread and take the slightly undulated or arched course. The changes in the blood vessels course from the straight to the undulated and further the spiral are the result of transferring the effect of shrinkage from the adjacent muscle membrane to the fibrous skeleton encircles the blood vessels. Such a course of blood vessels has been described among others in the urinary bladder in humans [14], the dog [7] and the rabbit [6] and in the gallbladder of dog [12], raccoon dog, fox and cat [9] [10].

Both in the gallbladder and in the urinary bladder was observed the dense subepithelial capillary network, which structure is subject to changes with the folding of the lamina propria of the mucosa. In both organs, the capillary networks are in very close contact with the epithlia, which in the gallbladder is essential for absorption of water and concentrating the bile [9] [11], and in the urinary bladder is necessary for the absorption of potassium ions from the lumen of bladder [3]. The arrangement of capillary networks directly under the epithelium has been described among others in the urinary bladder in humans [14], rat [2] [8] [13], rabbit [6] and mice [20] and in the gallbladder of guinea pigs [1], rabbit, [11] and cat [9]. In all described cases there was a tendency of the subepithelial capillary networks to deform according to the pattern created by the folding mucosa. In the shrunken gallbladder the constructions of subepithelial capillary networks are high and correspond to the arrangement and structure of the dense and complex
mucosal folds. In turn in shrunken urinary bladder the subepithelial capillaries map the arrangement of the low and wide folds of the mucosa. After stretching the wall of organs the capillary networks become flat or slightly wavy.

In the vascular system of the pig urinary bladder are observed different than in the pig gall bladder tends of the mucosal blood vessels to curling into loops. This results among others from the thickness of the urinary bladder mucosa and the occurrence of the looser connective tissue arrangement beneath the folds of the mucosa. Vascular loops in the urinary bladder have been reported, among others, in human [14] and dog [7], and their formation is similar to that in pig. In the shrunken urinary bladder, in the lower parts of the mucosa the arteries and veins running from the subserosa loop and form a band of intensively twisted blood vessels, that became the vascular reserve using when the bladder wall is stretching. Similar structure only that located in the subepithelial parts of the mucosa was observed in gallbladders in guinea pig gall [1] and in cat [9]. In this case, the small blood vessels form low vascular loops extending into the lumen of gallbladder.

References