Structural and Immunohistochemical Changes in the Hepatic Vascular System in Compensated and Decompensated Stenosis of the Pulmonary Trunk

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Modeling of pulmonary trunk stenosis leads to an increase in hepatic vascular resistance because of veno-arterial and veno-venous reactions. During the compensation phase, bundles of intimal musculature and myoelastic sphincters appear in the arteries, while in the efferent veins hypertrophy of the muscle rolls is observed. The decompensation phase of stenosis is characterized by relaxation of hepatic vascular walls, reduction of the number of arteries with intimal muscles and sphincter structures, and atrophy of muscle rolls in hepatic veins. Sclerotic changes develop in the vascular bed. Failure of the compensatory reactions results in development of chronic hepatic venous plethora with typical morphological manifestations.

Key Words: *pulmonary trunk stenosis; hepatic vessels; electron microscopy; immunohistochemistry*

Pulmonary trunk stenosis (PTS) is one of the most severe congenital heart diseases [1,2,8]. The life span of patients depends on the compensatory potential of the heart and on the structural changes in the visceral vessels, including the hepatic vessels. The hepatic blood vasculature with an appreciable adaptation potential is actively involved in the regulation of the greater circulation. However, the data on the status of the hepatic arteries and veins in PTS are mainly descriptive and have been obtained without using modern methods [3,9]. Studies on autopsy material are rather difficult because of a great variety of concomitant abnormalities and degree of hemodynamic disorders. Hence, we resorted to animal experiments.

We studied structural rearrangement of hepatic vessels under conditions of experimental PTS and evaluated the role of these changes in the development of circulatory compensation and decompensation.

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MATERIALS AND METHODS

The PTS was simulated as described previously [5] in experimental puppies (n=25). The animals were observed for 6-24 months. Eight of them developed signs of heart decompensation with the development of hydrops, anasarca, and congestive visceral plethora. Material from 10 age-matched dogs served as the control. All animals were sacrificed by bleeding from the femoral artery under ether narcosis in accordance with the Helsinki Declaration regulations.

Liver fragments were fixed in 10% neutral formalin and embedded in paraffin. Histological sections were stained with hematoxylin and eosin, after Masson, Hart, van Gieson, and impregnated with silver after Foot. The material for electron microscopy was fixed in 2% glutaraldehyde in phosphate buffer and embedded in epoxy resins. Immunohistochemical reactions were carried out with monoclonal antibodies to α-SMA and CD34. Morphometry of the arteries, portal and hepatic veins was carried out using a MOB-1-15^{x} screw ocular micrometer by measuring the outer diameters and thickness of the media. Branching level

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was evaluated as described previously [6]. Hepatic arteries were divided into 4 groups: large (≥125 μ), medium (124-51 μ), small (50-21 μ), and arterioles (≤20 μ). The portal vein branches were also divided into groups with consideration for the diameters of the accompanied arteries: veins corresponding to the large arteries (≥190 μ), to medium arteries (189-110 μ), to small arteries (109-51 μ), and veins corresponding to arterioles (≤50 μ). Hepatic veins were also divided into 4 groups (large, medium-sized, small, venules), with the diameters similar to those of the respective portal veins. The large hepatic veins in dogs have peculiar muscle rolls; the thickness of these rolls was measured at the level of veins.

The data were statistically processed using Student's *t* test.

RESULTS

Compensated PTS was associated with a drastic stenosis of the hepatic arteries, thickening of their wall, and plication of the inner elastic membrane (Fig. 1, *a*). The number of vessels with intimal muscles and myoelastic sphincters increased. Signs of wall myoelastosis were found in the portal vein branches. The sinusoids had normal lumen, without CD34 expression in their walls (Fig. 1, *b*); the perisinusoidal spaces were hardly discernible. The portal veins were plethoric, with development of the muscle roll hypertrophy, because of which their lumen was stenosed. Morphometry showed that the thickness of large arteries media increased 1.7 times in comparison with the control, of medium and small arteries 1.2 times, while in arterioles it increased just negligibly (Table 1). The expression of smooth muscle actin α-SMA increased in the tunica media of these vessels (Fig. 1, *c*). The thickness of the walls of portal veins accompanying large arteries increased by 2.3 times, that of medium arteries by 1.4 times, and that of small arteries by 1.2 times; the walls of the arteriolar level veins thickened by 1.5 times (Table 1). More significant changes developed in the hepatic veins. The media thickness in large veins increased 5-fold, of medium veins by 2.1 times, of small veins by 1.6 times, and of venules by 1.7 times (Table 1). The mean thickness of the rolls of the large hepatic veins increased from 36.0±3.5 to 50.0±3.4 μ ($p<0.001$).

In decompensated PTS, hepatic arteries had a wide lumen, thin tunica media, and their inner elastic membrane was less plicated (Fig. 1, *d*). Vessels with intimal musculature and sphincters were rare. Coarse sclerosis was seen in these structures and in the arterial walls. The expression of α-SMA was reduced in the media (Fig. 1, *e*). The portal vein branches were plethoric. Sinusoids were hyperemic. Collagen fibrils were found in the perisinusoidal space (Fig. 1, *f*), which also contained electron-dense substance, making the structure of these vessels similar to that of other organs' capillaries, which normally had a continuous basal plate. The sinusoidal wall started to express CD34 (Fig. 1, *h*). The lumen of hepatic veins was sharply dilated, the vessels were hyperplethoric. Smooth muscle atrophy and signs of muscle roll sclerosis were found in large veins. Morphometry showed changes in the thickness of the arterial tunica media: it decreased by 1.7 times in the large vessels, by 1.3 times in medium vessels, by 1.4 times in small arteries, and by 1.8 times in arterioles in comparison with the parameter under conditions of compensated PTS. Wall thickness of the portal veins accompanying large arteries decreased by 2.3 times, that of veins corresponding to medium arteries and arterioles decreased by 1.7 times, and in the veins accompanying the small arteries the thickness decreased by 1.6 times. The thickness of the media in large hepatic veins decreased 5-fold, in medium veins by 3.6 times, in small veins by 1.9 times, and in venules by 2.4 times. The mean thickness of muscle rolls in hepatic veins decreased from 50.0±3.4 to 31±5 μ ($p<0.001$).

Hence, simulation of PTS leading to overload of the right heart compartments was paralleled by inhibition of the efferent blood flow from the liver with dilatation of hepatic vein branches, thickening of their walls, and muscle roll hypertrophy. Their function was to resist venous congestion fraught with homeostasis disturbances. Adaptation to circulatory disorders in the hepatic arteries consisted in high expression of α-SMA, reflecting myofibroblast proliferation and predominance of their contractile potential over collagenogenesis. This was paralleled by reflex contractures of smooth muscles in these vessels and thickening of their tunica media. This universal mechanism is known in relevant literature as veno-arterial reaction [5], preventing, along with activation of the hepatic vein muscle rolls, sinusoidal plethora. We found that the portal vein walls were also hypertrophic. Presumably, this led to reduction of not only arterial, but also venous blood flow to the liver (veno-venous reaction). In addition to these compensatory vascular reactions constituting the functional component of adaptation, specialized structures regulating blood flow under conditions of circulatory disorders formed in the hepatic arteries. They included bundles of intimal muscles and myoelastic sphincters developing from the medial myocytes migrating into the intima through gaps in the internal elastic membrane [6,7]. Active "cooperation" of hepatic arteries and veins under conditions of threatened venous congestion led to normalization of the structure and function of the microcirculatory system, *i.e.* no perisinusoidal fibrosis or sinusoidal

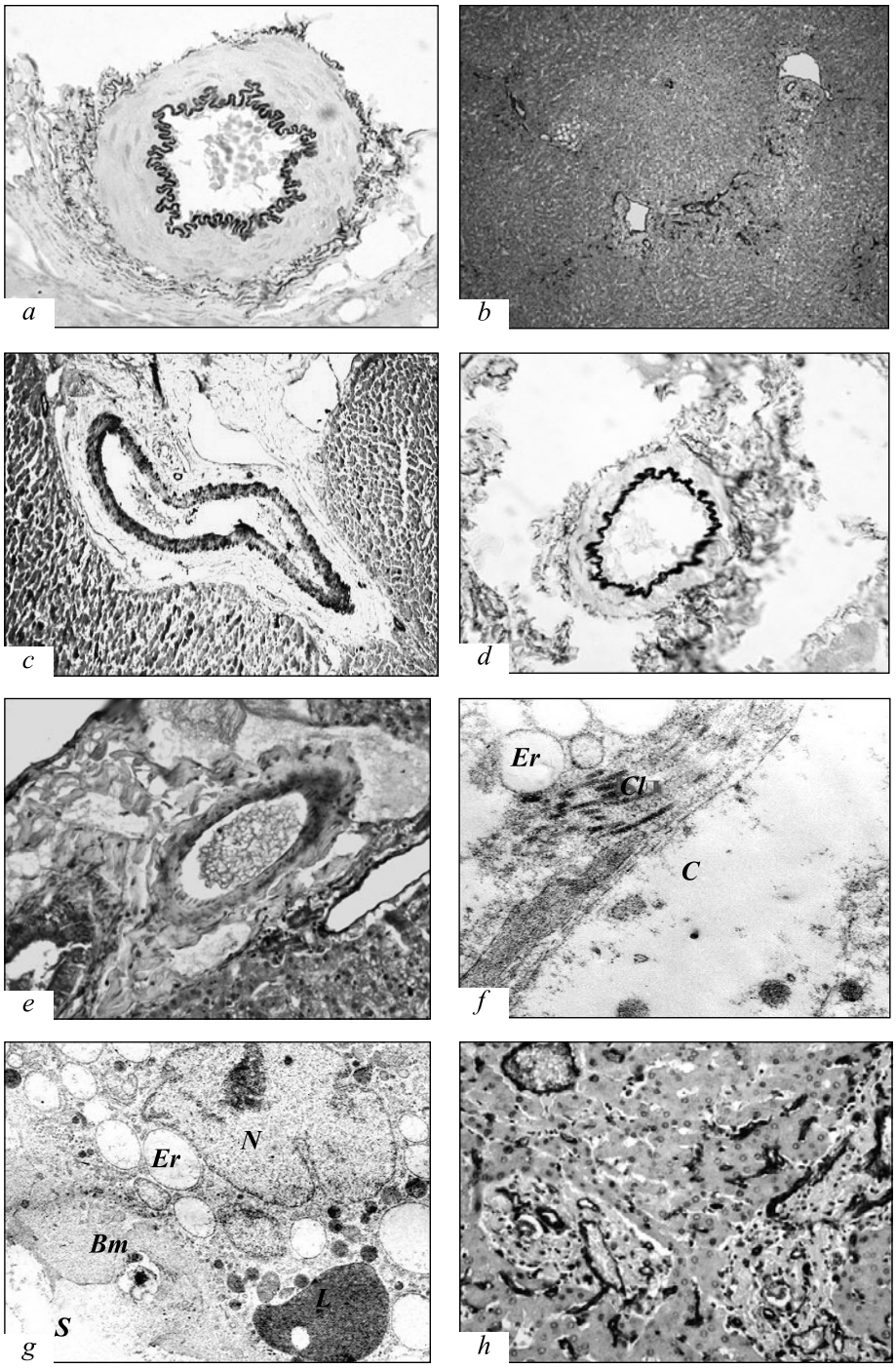

Fig. 1. Hepatic vessels in compensated (*a*-*c*) and decompensated pulmonary trunk stenosis (*d*-*h*). *a*) thick tunica media and increased plication of inner elastic membrane in medium-sized artery; *b*) no CD34 expression in sinusoids; *c*) high α-SMA expression in a large hepatic artery; *d*) thinned tunica media of a small artery membrane; *e*) lower expression of α-SMA in a small hepatic artery; *f*) growth of collagen fibrils (Cl) into perisinusoidal space and formation of membrane-like substance (S: sinusoid; Er: endoplasmatic reticulum); *g*) formation of membrane-like substance – basal membrane (Bm) in perisinusoidal space (N: nucleus; L: lysosomes); *h*) high expression of CD34 in sinusoids. Staining: *a*, *d*: Hart's method. ×200 (*a*, *d*, *h*); ×100 (*b*, *c*); ×160 (*e*); ×18,000 (*f*, *g*).

"capillarization" developed, which was confirmed by the absence of CD34 expression in them.

Decompensated PTS was associated with right-ventricular insufficiency, sharp disorders of efferent blood flow from the liver, and development of hypoxia, leading to reduction of the smooth muscle tone in the afferent and efferent vessels [5]. This led to failure of the above defense vascular reactions. Hypoxic factor is also involved in relaxation of the hepatic vein muscle rolls, and they did not develop to the degree observed

TABLE 1. Thickness of Tunica Media in Hepatic Vessels (μ) in the Control and in Compensated and Decopensated PTS (*M*±*m*)

Bloodflow	Vessels		Control	Compensated PTS	Decompensated PTS
Afferent	Arteries	large	24.0±1.1	42.7±2.8*	25.1±3.6^{+}
		medium	13.2±0.8	16.0±0.8*	12.6±0.7^{+}
		small	6.5±0.1	8.1±0.2*	5.5±0.3**$^{+}$
		arterioles	3.7±0.1	3.9±0.1**	2.2±0.1*$^{+}$
	Portal veins of the levels of	large arteries	6.0±0.2	13.9±1.2*	5.9±0.6^{+}
		medium arteries	5.1±0.3	6.9±0.4*	4.0±0.2*$^{+}$
		small arteries	4.1±0.2	5.0±0.2*	3.0±0.2*$^{+}$
		arterioles	2.8±0.1	4.2±0.2*	2.4±0.3^{++}
Efferent	Hepatic veins	lage	6.2±0.3	31.1±4.1*	4.5±0.9^{+}
		medium	4.4±0.1	9.5±1.1*	2.6±0.1*$^{+}$
		small	3.2±0.1	5.3±0.3*	2.7±0.1**$^{+}$
		venules	2.7±0.1	4.7±0.3*	1.9±0.1*$^{+}$

Note. *p<0.001, **p<0.05 in comparison with the control; ^{+}p<0.001, ^{++}p<0.05 in comparison with compensated PTS.

in compensated PTS. Migration activity of myocytes was also reduced under these conditions [5]. Adaptation structures in arteries under these conditions were significantly less numerous than during the compensation stage of experimental PTS. α-SMA expression in hepatic arteries media decreased with time because of hypoxemia, myofibroblast activity decreased, their contractile function was replaced by fibroplastic, this being paralleled by sclerotic changes in these vessels. These changes, according to our data, involved afferent and efferent veins and adaptation structures, which failed to maintain regulation of disordered hemodynamics in the hepatic vascular bed under these conditions. Importantly, sclerosed (not functioning) adaptive formations protruding into the vascular lumen become a serious mechanical obstacle for blood flow augmenting the circulatory disorders in the liver. All these events resulted in homeostasis failure at the microcirculatory level consisting in perisinusoidal fibrosis and sinusoidal "capillarization", which was confirmed by ultrastructural and immunohistochemical changes (appearance of CD34 expression). The above changes resulted in inhibition of transcapillary exchange and development of chronic hepatic venous plethora.

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